Agronomy Research

Established in 2003 by the Faculty of Agronomy, Estonian Agricultural University

Aims and Scope:

Agronomy Research is a peer-reviewed international Journal intended for publication of broad-spectrum original articles, reviews and short communications on actual problems of modern biosystems engineering incl. crop and animal science, genetics, economics, farm- and production engineering, environmental aspects, agro-ecology, renewable energy and bioenergy etc. in the temperate regions of the world.

Copyright & Licensing:

This is an open access journal distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0).
Authors keep copyright and publishing rights without restrictions.

Agronomy Research online:

Agronomy Research is available online at: https://agronomy.emu.ee/

Acknowledgement to Referees:

The Editors of *Agronomy Research* would like to thank the many scientists who gave so generously of their time and expertise to referee papers submitted to the Journal.

Abstracted and indexed:

SCOPUS, EBSCO, DOAJ, CABI Full Paper and Clarivate Analytics database: (Zoological Records, Biological Abstracts and Biosis Previews, AGRIS, ISPI, CAB Abstracts, AGRICOLA (NAL; USA), VINITI, INIST-PASCAL.)

Subscription information:

Institute of Forestry and Engineering,
Estonian University of Life Sciences
Fr.R. Kreutzwaldi Str. 56,
51006 Tartu,
ESTONIA
e-mail: timo.kikas@emu.ee

Journal Policies:

Estonian University of Life Sciences, Latvia University of Life Sciences and Technologies, Vytautas Magnus University Agriculture Academy, Lithuanian Research Centre for Agriculture and Forestry, and Editors of *Agronomy Research* assume no responsibility for views, statements and opinions expressed by contributors. Any reference to a pesticide, fertiliser, cultivar or other commercial or proprietary product does not constitute a recommendation or an endorsement of its use by the author(s), their institution or any person connected with preparation, publication or distribution of this Journal.

CONTENTS

Assessing criteria for adopting sustainable subsoil management practices: an application of multinomial logit model

M. Aghabeygi[1,*], Z. Kiresiewa[2], H. Gerdes[2] and C. Schneider[2]

[1]Thünen Institute of Farm Economics, Bundesallee 63, DE38116 Braunschweig, Germany
[2]Ecologic Institute, Pfalzburger Str. 43-44, DE10717 Berlin, Germany
*Correspondence: Mona.Aghabeygi.21@gmail.com

Abstract. Subsoil management practices are crucial for improving agricultural sustainability by enhancing resource use efficiency and soil health. This study focuses on two key methods: (a) the cultivation of deep-rooted crops like alfalfa, which enhances the accessibility of subsoil resources for subsequent crops, and (b) strip-wise loosening of the subsoil combined with compost addition (UEK)[1], which is being developed and tested within the framework of the Soil³ project. A comprehensive model is developed to assess factors influencing the adoption of these practices using a Multinomial Logit model and survey data from stakeholders in Anhalt-Bitterfeld, Rhein-Sieg-Kreis, and Teltow-Fläming in Germany. Stakeholder responses are categorized into four groups: stakeholder characteristics, adoption factors, alfalfa cultivation and UEK method features. Statistical and econometric analyses reveal three critical factors: insufficient soil water storage capacity, business orientation, and ownership relationships. Through the identification of marginal effects, it appears that 'business orientation' is more influential in the adoption of the alfalfa cultivation method compared to the UEK method, whereas 'ownership relationships' have a greater marginal effect in the UEK method compared to alfalfa cultivation. Findings suggest that policy interventions should address method-specific barriers, promoting business incentives for alfalfa cultivation and resolving land ownership issues for the UEK method. Tailored, region-specific approaches are essential for promoting subsoil management practices, contributing to resilient and resource-efficient farming systems.

Key words: deep-rooted crop method, econometric analysis, Germany, marginal effects, strip-wise loosening method.

INTRODUCTION

With a growing global population and climate change impacts placing greater pressure on food production, the need for yield stability (Guedioura et al., 2023) and effective soil management practices have become increasingly important (Ahrends et al., 2021; Botta et al., 2024). The subsoil, often neglected by policy makers and other social

---

[1] UEK is used here as an abbreviation for strip-wise loosening of the subsoil combined with compost addition. However, in general, this method is known in Germany as the Soil³ method or technology.

stakeholders, holds substantial reserves of water and nutrients, which could play a crucial role in enhancing crop yields, particularly during dry seasons (Kautz et al., 2013, Schneider at al., 2017, Frelih-Larsen et al., 2018).

Notably, subsoil amelioration has a long history (Schneider et al., 2017). Techniques such as deep ploughing and mechanical subsoiling have been employed for many years. Deep ploughing involves turning the soil at depths of 50 cm or more, which inverts the soil profile by bringing subsoil to the surface and burying the topsoil (Gailis et al., 2017). This method aims to break up compacted layers and improve crop growing conditions but requires considerable energy and heavy machinery (Alcántara et al., 2016). Recent advancements have focused on optimizing the operational efficiency of tillage machinery to address these high energy requirements. For instance, (Askari et al., 2021) applied the Response Surface Methodology (RSM) to model and predict key tractive performance parameters of agricultural tractors during semi-deep tillage. By systematically varying factors such as tractor speed, tillage depth, and loading, the study developed accurate predictive models of draft force and tractive efficiency. This approach enables identification of optimal operating conditions that minimize wheel slip and fuel consumption while maintaining effective soil loosening. Such modeling contributes to improving the energy efficiency and sustainability of mechanical subsoil management practices. Mechanical subsoil loosening, also known as subsoiling or deep ripping, focuses on alleviating subsoil compaction without turning the soil (Frelih-Larsen et al., 2018). By allowing deeper and wider root growth, this technique enhances water infiltration and nutrient uptake, ultimately aiming to boost crop yields (Cai et al., 2014). Moreover, differences in tillage systems have been shown to substantially affect soil moisture distribution and crop productivity. Conservation-oriented systems, such as subsoiling with ridgers or chisel-rotary combinations, generally preserve more soil moisture in both the topsoil and deeper profiles compared to conventional plough tillage, especially during dry periods. These practices enhance root development, reduce runoff losses, and improve overall water availability, resulting in higher and more stable yields (Abo-habaga et al., 2022).

Since both techniques mentioned above have certain limitations, such as being too labor-intensive and highly disruptive methods, and even reducing soil fertility in the long term (Frelih-Larsen et al., 2018), a new approach has been developed and tested as part of the Soil³ project[2]. The suggested new method involves subsoil loosening combined with the incorporation of organic matter into the loosened subsoil. This approach is more effective when applied in alternating furrows (e.g., spaced one meter apart) and when the topsoil and subsoil layers remain undisturbed and are not mixed or turned (Schmittmann et al., 2021; Bauke et al., 2024).

In addition to mechanical methods, cultivating deep-rooting plants, such as alfalfa, provides a biological approach to subsoil amelioration. This method uses deep-rooting crops like alfalfa to create vertical root channels (bio pores) in the subsoil, improving access to water and nutrients for subsequent crops (Lynch & Wojciechowski, 2015) and was also further tested in the context of the Soil³ project.

As subsoil amelioration needs to be region-specific, it is important to identify regions within Germany that can benefit most from both subsoil management measures

---

[2] For more details about the Soil³ project, see 'www.soil3.de'

tested in the frame of the Soil³ project. As part of the project, the identification of suitable regions was based on the consideration of a) favorable geogenic, pedogenic and climatic factors and b) socio-economic and political factors. With regard to the first set of factors, (Schneider, 2020) found that mechanical amelioration is most suitable for relatively dry regions with sandy soils in Northeastern Germany. In contrast, biological amelioration can have the strongest positive effects in Central and Southern Germany (Fig. 1).

From a socio-economic and political point of view, a number of region-specific factors may facilitate the uptake of subsoil management measures such as business orientation, regional compost availability, number of mixed farms in a certain region, and others (Hinzmann et al., 2021).

While subsoil management practices have been widely studied, existing research often lacks a comprehensive comparison between biological and mechanical amelioration methods in terms of their practical implementation, stakeholder preferences, and perceived barriers. Most studies focus on subsoil, frequently in connection with issues of subsoil compaction

Figure 1. Map of Germany showing the need for subsoil amelioration. Green areas show that there is no need for subsoil amelioration. Red areas show that there is a need of subsoil amelioration (Schneider et al., 2024).

(e.g., Schjønning et al., 2015), carbon storage and sequestration (e.g. Rumpel et al., 2012), the role of subsoil in plant nutrition (Gaiser et al., 2012, Kautz et al., 2013; Lynch & Wojciechowski, 2015; Schneider & Don, 2019a, 2019b) but pay limited attention to the socio-economic factors influencing adoption of subsoil management techniques. While recent research has begun to examine stakeholders' perceptions of subsoil amelioration, identifying relevant factors like biophysical conditions, economic feasibility, and barriers to implementation (Frelih-Larsen et al., 2018, Hinzmann et al., 2021; Schneider et al., 2024), it is important to employ targeted qualitative and quantitative statistical methods to gain a deeper understanding of how these perceptions align with diverse agricultural and socio-economic contexts. To address this, our study utilizes the Multinomial Logit Model to answer two main questions: (1) What are the key factors influencing the adoption of biological versus mechanical subsoil management practices, and how do these factors vary across different agricultural contexts? (2) How do stakeholders perceive and prefer these methods, and what barriers hinder their implementation?

In line with the context of stakeholders' decision-making processes, we use data collected from a survey conducted between March and June 2023. A Multinomial Logit model is applied to analyse how stakeholders differ in their perceptions and preferences regarding subsoil management methods.

MATERIALS AND METHODS

Analytical framework of the study

The analytical framework employed in this study serves as the foundation for analysing and interpreting the research data, allowing for a comprehensive exploration of the key variables and their interrelationships. By delineating the structure and methodology of our analysis, this framework establishes a clear roadmap for understanding the intricate aspects of the research topic and drawing meaningful conclusions (Fig. 2).

Data source and study site

This study examines data from an online survey conducted between March and June 2023, focusing on German stakeholders' perspectives regarding subsoil management and deep-rooting practices, particularly the use of the Soil³ technology and alfalfa cultivation. The Soil³ technique, developed by (Schmittmann et al., 2021) involves the strategic opening of subsoil layers and the placement of organic material to enhance nutrient capacity. As part of the implementation of this technology, it is crucial to understand the perspectives of stakeholders, especially stakeholders, who may be directly impacted by its adoption. Additionally, the long-standing practice of deep-rooting crops, particularly alfalfa, was analysed to assess its potential for supporting sustainable soil management. In particular, the survey aimed to gather insights on factors influencing the adoption of both subsoil amelioration practices, such as financial resources of the farm, land ownership structure, business orientation, availability and quality of compost in the region, and soil properties. The surveys also explored the willingness of farmers and stakeholders to adopt these techniques, as well as the interest in integrating policy incentives, such as humus-building premiums, into agricultural practices.

While other subsoil amelioration techniques exist, the focus of the Soil3 project was on improving subsoil properties in central field studies through the combined growth of deep-rooting pre-crops (alfalfa cultivation) and technical subsoil heterogenization via rotary cutters and economic organic matter injection (UEK). Both techniques were considered to be highly suitable solutions that provide attractive options for plants to invest into roots, thereby elevating nutrient and water uptake from the subsoil.

The survey was administered using the open-source application LimeSurvey Cloud Version 6.6.5. Major agricultural organizations and local stakeholder associations across Germany were contacted by email and asked to distribute the survey link to their members.

The survey distribution targeted the federal states of North Rhine-Westphalia, Brandenburg, and Saxony-Anhalt. The final respondent distribution in regions was as follows: 6 from Rhein-Sieg-Kreis (14.63%), 14 from Teltow-Fläming (34.15%), 8 from Anhalt-Bitterfeld (19.51%), and 13 from other regions (31.71%). These regions were selected due to their proximity to field experiments and demonstration farming sites for the Soil³ technology. It was expected that stakeholders in these areas, having practical experience with the Soil³ technique, would offer valuable insights into its effectiveness.
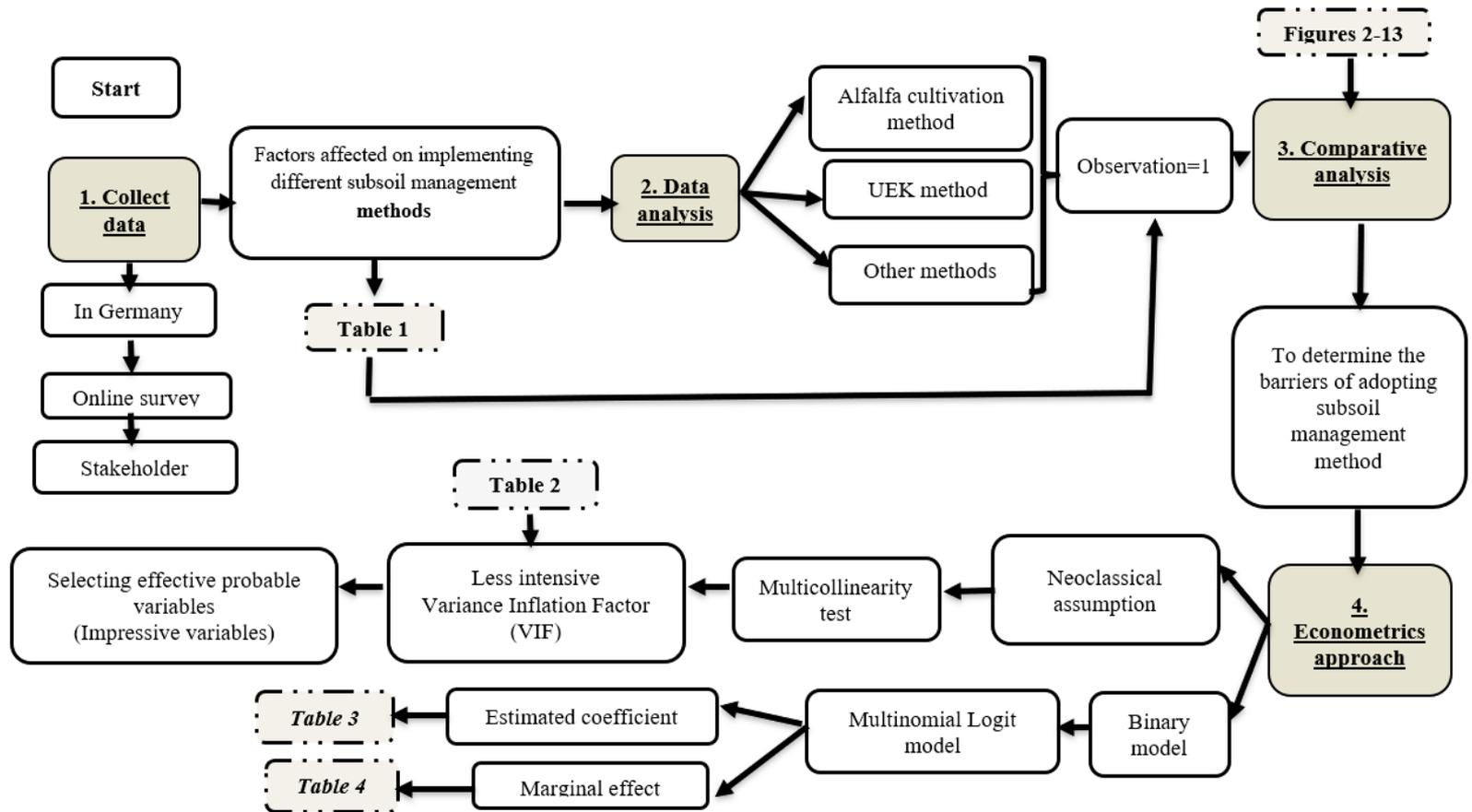
Figure 2. Analytical framework of the study.

The survey was organized into four main groups of questions. The first group focused on stakeholder characteristics, gathering demographic and professional information about the respondents. The second group aimed to identify and analyze the key factors that differentiate the implementation of subsoil management practices. The third group examined the features of the alfalfa cultivation method, investigating its implementation and impact. The fourth group explored the details of the UEK method, including its application and outcomes (see Table 1 for details).

Since the focus shifts to assessing and identifying factors that either facilitate or act as barriers to the adoption and implementation of subsoil management methods, attention was given to a second group of factors related to the specific influences on the implementation of these methods, namely, the Alfalfa cultivation method and the UEK method. Forty-one stakeholders were initially asked separate questions regarding Alfalfa cultivation and the UEK method. Responses were collected independently for each method. These responses were then pooled (82 answers), enabling the definition of independent variables for model estimations.

To assess the most impactful factors influencing the implementation of different subsoil management techniques, an econometric model is required. Given that the data collected from stakeholders is quantitative and discrete, a suitable approach for estimation is the binary model, such as the Logit model. However, since there are multiple implementation scenarios-specifically the Alfalfa and UEK methods - multinomial logit model is more appropriate.

The initial step in modelling involves defining the dependent and independent variables. The dependent variable in this analysis is categorized into two methods: Alfalfa cultivation and the UEK method. Five key elements define this variable: low yield levels, insufficient soil water storage capacity, existing soil compaction, financial resources of the farm, and business orientation (e.g., arable farming, horticulture, fodder farming, livestock farming, mixed business), along with farming form (organic vs. conventional) and ownership relationships (e.g., lease agreements, land ownership). Each element is rated on a five-point scale from 'not relevant' to 'very relevant,' with values ranging from 1 to 4 and 0 for 'no opinion.' The summation of these values for each respondent creates a new scale ranging from 0 (all variables rated as 'not relevant') to 35 (all variables rated as 'very relevant'), reflecting their likelihood of adopting different subsoil management techniques. To convert these continuous values into discrete groups, thresholds are established based on average scores: 23 for the Alfalfa method and 24 for the UEK method. Respondents scoring above these thresholds are categorized into the respective groups, while those scoring below are placed in a third group. It should be noted that other groups are not related to the subsoil management methods not considered in this study. These groups are associated with stakeholders who provided lower scale values (primarily selecting 'not relevant') for most of the factors in both the Alfalfa cultivation and UEK methods. As a result, their responses fall below the defined threshold based on the average scale values. This indicates that these stakeholders did not perceive most factors as effective or influential enough to distinguish between the implementation of the Alfalfa cultivation method and the UEK method.

Table 1. Stakeholder survey: categorization of inquired variables

Group	Variables	Variable name
First Group: Stakeholder characteristics	Which group of actors would you place yourself in? (Stakeholder, Chambers of agriculture, Local politics, Environmental association, Other)	Actor_Group
	How do you manage your business? (Conventional, Organic, Tillage-plough, Conventional/Tillage-plough, Organic/Tillage-plough, Conventional/organic/tillage, No answer)	Business_Type
	Which region do you work in? (Teltow-Fläming, Rhein-Sieg-Kreis, Anhalt-Bitterfeld, Others)	Region_Work
	Which groups would you assign yourself to? (Group1, Group2, Group3)[3]	Assigned_Groups
	How can politics facilitate the practical application of knowledge in subsoil management? (Financing information campaigns for stakeholders and agricultural advisors (Info_Campaigns_Stakeholders), Financing information campaigns to educate the general public (Info_Campaigns_Public), Promoting the exchange of experiences between stakeholders/agricultural advisors (Experience_Exchange_Stakeholders), Promotion of consulting services (Consulting_Services_Promotion), Promoting the exchange of knowledge among stakeholders (e.g. field schools, field days) (Knowledge_Exchange_Stakeholders), Establishment of region-specific test areas under real conditions, Additional (Regional_Test_Areas), targeted and long-term research funding (Research_Funding_Targeted), Other)	Politics_Knowledge_Application
	Do humus-building bonuses provide incentives for adopting mechanical or biological subsoil management practices? (Yes, No)	Humus_Bonus_Incentive
	How would you evaluate stakeholder s' interest in implementing humus-building bonuses in agriculture within your region? (Very low, Low, Neutral, High, very high, No opinion)	Stakeholder_Interest_Humus_Bonus

---

[3] Group 1: The subsoil should be improved to reduce yield fluctuations. I support UEK, especially for low-yield or degraded soils, and have few concerns about its practical application. I also believe biological methods like growing alfalfa are effective for subsoil enhancement. Group 2: To reduce yield fluctuations, the subsoil should be utilized and improved. I am convinced of new measures like UEK only if backed by reliable research. Generally, stakeholder s will adopt measures only if they offer a favorable cost-benefit ratio, including organic practices such as growing alfalfa. Group 3: Subsoil should be incorporated into agricultural management under certain conditions. I favor biological methods, such as alfalfa cultivation, over mechanical approaches like UEK. I believe that organic measures offer long-term benefits and can achieve similar results without compromising soil health.

Table 1 (continued)

Group	Variables	Variable name
	How would you evaluate the interest of local politicians in introducing humus-building bonuses for agriculture in your region? (Very low, Low, Neutral, High, very high, No opinion)	Politician_Interest_Humus_Bonus
	How would you assess civil society's interest in the introduction of humus-building bonuses for agriculture in your region? (Very low, Low, Neutral, High, very high, No opinion)	Civil_Society_Interest_Humus_Bonus
Second Group: What factors influence the implementation of subsoil management; Alfalfa cultivation method & UEK method (Not relevant, hardly relevant, neutral, relevant, very relevant, no opinion)	Low yield level of the area	Low_Yield_Level
	Insufficient water storage capacity of the soil	Water_Storage_Capacity
	Existing soil compaction in the subsoil	Soil_Compaction
	Financial resources of the company	Financial_Resources
	Orientation of the business (e.g., Arable farming, horticulture, fodder farming, livestock farming, mixed business)	Business_Orientation
	Form of farming (organic, conventional)	Farming_Form
	Ownership relationships (term of lease agreements, land ownership)	Ownership_Relationships
Third Group: The features of the alfalfa cultivation method	Readiness to grow Alfalfa (Very low, Low, Neutral, High, very high, No opinion)	Alfalfa_Readiness
	How should politics promote the cultivation of alfalfa? (Increased support for the cultivation of alfalfa, e.g. B. via the Common Agricultural Policy (CAP) (Alfalfa_CAP_Support), Integration of alfalfa cultivation into contractual nature conservation (Alfalfa_Nature_Conservation), Promote the development of local marketing initiatives for alfalfa-based feed (Alfalfa_Local_Marketing), Promote feeding strategies (particularly soy replacement) for ruminants, pigs, poultry and horses based on alfalfa (Alfalfa_Feeding_Strategy), Promote fertilizer production based on alfalfa (Alfalfa_Fertilizer_Production), Funding of experimental facilities for protein production from alfalfa(Alfalfa_Protein_Facilities), Promotion of biogas substrate from alfalfa through premiums) (Alfalfa_Biogas_Premium)	Politics_Alfalfa_Support

Table 1 (continued)

Group	Variables	Variable name
Fourth group: The features of the UEK cultivation method How do you assess the availability and quality of compost for the implementation of UEK in your district? (Very low, Low, Neutral, High, very high, No opinion)	Available quantity-farm own compost	Farm_Own_Compost_Quantity
	Available quantity-purchase from compost plants	Compost_Plant_Quantity
	Compost quality based on the pollutants contained -farm own compost	Farm_Own_Compost_Quality
	Compost quality based on the pollutants contained purchase from compost plants	Compost_Plant_Quality
	Stakeholder s' openness in implementing technical innovations such as UEK	Stakeholder_Openness_UEK

This approach results in three distinct groups: 34 stakeholder s implementing the Alfalfa method (41.46%), 37 stakeholder s using the UEK method (45.12%), and 11 stakeholder s employing other methods (13.42%). Based on this definition of the dependent variables and the main categories for the subsoil management methods, the following section reports the percentage contributions of each variable, while maintaining them within their respective subsoil management groups.

Data analysis
In this study, the independent variables are binary (coded as 1 and 0), meaning the calculation of mean and standard deviation does not provide meaningful insights for such data. Additionally, the dependent variable is discrete, with more than two possible values (1, 2, and 3). Given these characteristics, we opted for a Multinomial Logit Model, which is specifically designed to handle discrete dependent variables with more than two categories. This approach allows us to appropriately model the relationship between the independent variables and the likelihood of adopting different subsoil management methods. Therefore, in this section, we focus on figures to better illustrate the distribution of independent variables (factors) across the different categories of the dependent variable (subsoil methods), providing a clearer understanding of how these factors influence adoption decisions.

Factors perceived as barriers or influencers for adopting each method are detailed in Table 1. Additionally, Fig. 3 through 14 provide a visual representation of the percentage distribution of these factors across the different subsoil management methods. This detailed breakdown highlights the distinct considerations and barriers associated with each method, based on the respondents' feedback.

The analysis of respondent distribution identified five categories: stakeholders, chambers of agriculture, local politicians, environmental associations, and others. The results revealed that stakeholders constituted the majority group, accounting for 71% of all respondents. Among these, 68% reported considering the alfalfa cultivation method, while 70% indicated interest in the Soil³ technique. The second largest group, classified as others, represented 19% of the sample, with 21% showing adoption tendencies for both the alfalfa and UEK methods. The chambers of agriculture ranked third, comprising 10% of the respondents, of which 12% applied the alfalfa method and 8% employed the UEK method. Local political actors and environmental associations did not contribute responses to the survey (Fig. 3).
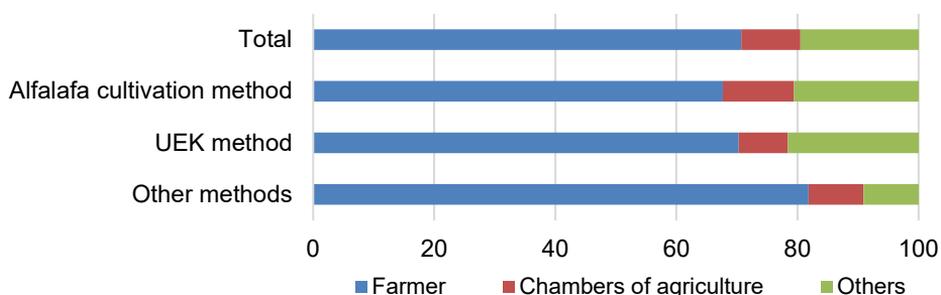
Figure 3. The first group: Stakeholder characteristics: Which group of actors would you place yourself in? (Actor_Group).

The analysis of farming system types among respondents revealed that conventional systems represented the predominant category, accounting for 27% of all respondents. Within this group, 23% were associated with the alfalfa cultivation method and 27% with the UEK method. The conventional/tillage plough system followed closely, comprising 24% overall, with 26% corresponding to the alfalfa method and 24% to the UEK method. Respondents who did not specify their farming system constituted approximately 31% of the total sample. The remaining categories, including organic, tillage/plough, organic/tillage plough, and conventional/organic/tillage systems, each accounted for less than 10% across all methods (Fig. 4).
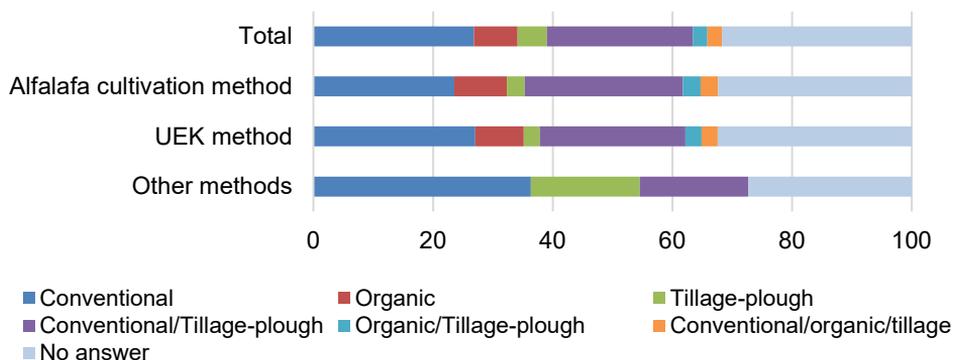
Figure 4. The first group: Stakeholder's feature: How do you manage your business? (Business_Type).

The regional analysis identified Teltow-Fläming as the leading area, representing 34% of the total distribution. Within this region, the alfalfa cultivation method accounted for 35%, while the UEK method comprised 30%. Anhalt-Bitterfeld and Rhein-Sieg-Kreis emerged as the second and third most represented regions overall and specifically for the UEK method. In contrast, for the alfalfa cultivation method, both regions exhibited percentage shares below 15%, with no significant difference observed between them (Fig. 5).
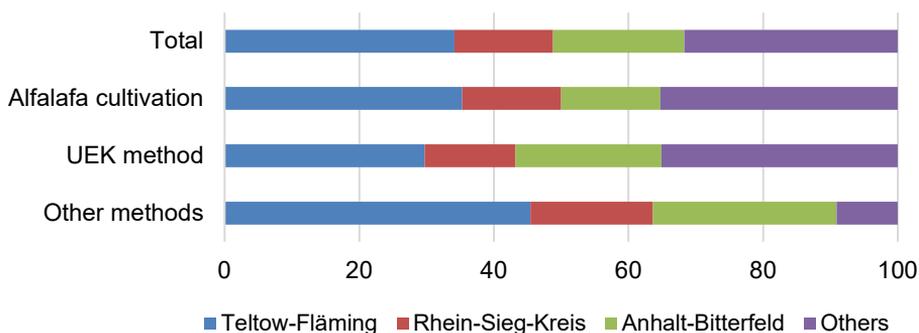
Figure 5. First group: Stakeholder's feature: Which region do you work in? (Region_Work).

The survey identified three distinct respondent groups based on their attitudes toward subsoil management practices. Group 1 emphasized improving subsoil conditions to reduce yield fluctuations. Respondents in this group expressed strong support for the UEK method, particularly for low-yield or degraded soils, and reported minimal concerns regarding its practical implementation. They also recognized the effectiveness of biological approaches, such as alfalfa cultivation, in enhancing subsoil quality. The percentage contributions were comparable within this group, with both the alfalfa cultivation and UEK methods accounting for approximately 34–35%.

Group 2 similarly prioritized yield stabilization through subsoil improvement. Respondents in this group were receptive to innovative techniques like IEC but emphasized the importance of scientific validation before adoption. Stakeholders generally favored practices with a positive cost–benefit balance, including organic methods such as alfalfa cultivation. The distribution of responses in this group mirrored that of Group 1, with no notable variation in percentage shares.

Group 3 proposed integrating subsoil management into agricultural practices only under specific conditions. These respondents preferred biological methods, particularly alfalfa cultivation, over mechanical approaches such as UEK, citing the long-term benefits of organic techniques for soil health and sustainability. While the overall contribution rates in this group were similar to those in Groups 1 and 2, the share of both alfalfa and UEK methods was approximately 6% lower (Fig. 6).
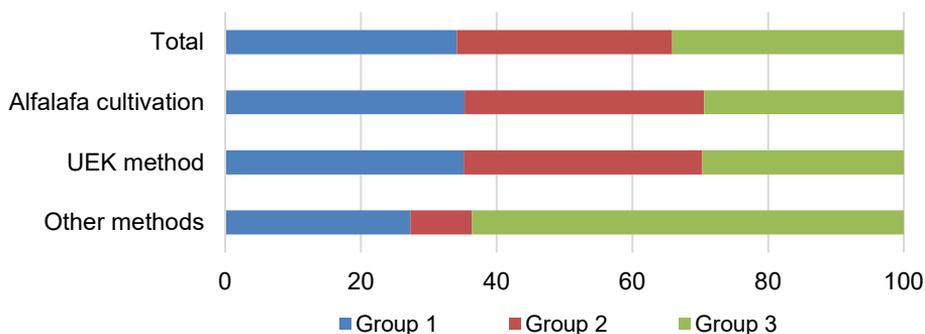
Figure 6. First group: Stakeholder's feature: Which groups would you assign yourself to? (Assigned_Groups).

The analysis of respondents' perspectives on policy measures supporting subsoil management identified seven key instruments: (1) financing information campaigns for stakeholders and agricultural advisors, (2) financing information campaigns for the general public, (3) promoting the exchange of experiences between stakeholders and agricultural advisors, (4) promoting consulting services, (5) encouraging the exchange of knowledge among stakeholders (e.g., field schools, field days), (6) establishing region-specific test areas under real conditions, and (7) providing additional, targeted, and long-term research funding.
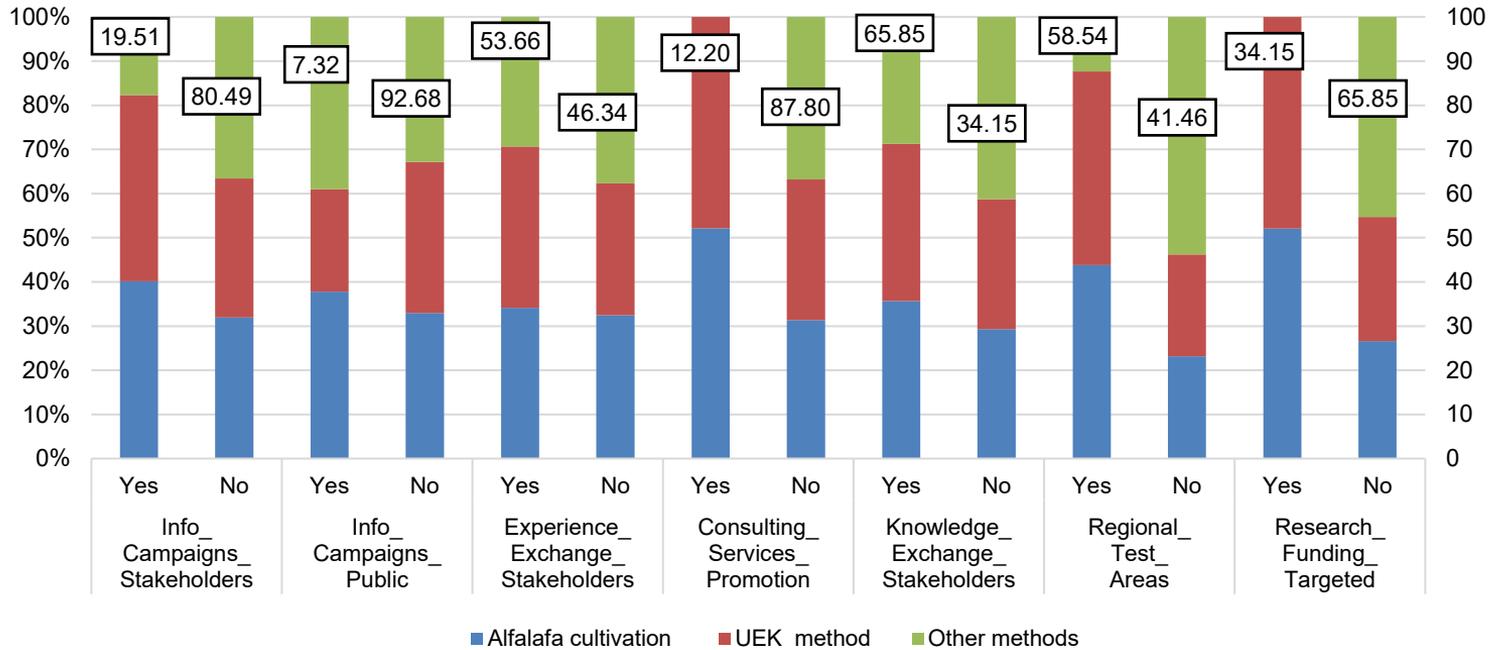
Figure 7. First group: Stakeholder's feature: How can policy-makers facilitate the practical application of knowledge in subsoil management? (Politics_Knowledge_Application).

The findings revealed that knowledge exchange among stakeholders - for instance, through field schools and field days - was perceived as the most effective policy measure, supported by approximately 66% of all respondents. This perception was consistent among both users of the alfalfa cultivation and UEK methods, with around 68% in each group endorsing this option. The establishment of region-specific test areas ranked second, receiving 58% overall support and roughly 65% from both method groups. The promotion of experience exchange between stakeholders and agricultural advisors was identified as the third most effective measure, cited by 53% of respondents overall, including 57% of UEK users and 53% of alfalfa users.

In contrast, measures such as financing information campaigns for the general public (93%), promoting consulting services (88%), and financing information campaigns for stakeholders and agricultural advisors (80%) were regarded as less effective for facilitating the practical application of knowledge in subsoil management (Fig. 7).

The analysis revealed that more than 65% of respondents – across both the alfalfa cultivation and UEK methods – perceived humus-building bonuses as an effective incentive for encouraging the adoption of mechanical and biological subsoil management practices. This finding highlights a strong consensus among stakeholders regarding the role of financial and policy-based incentives in promoting sustainable subsoil management approaches (Fig. 8).
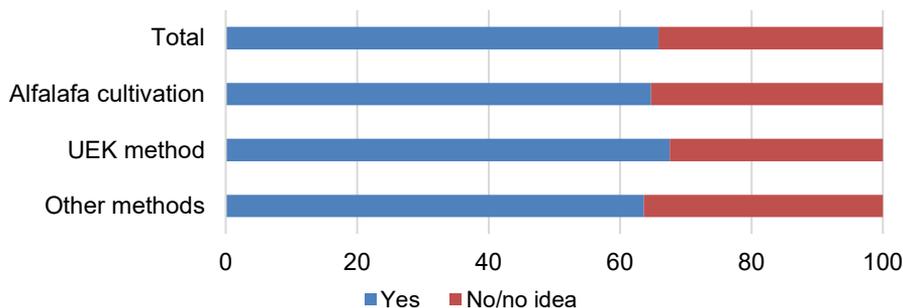
Figure 8. First group: Stakeholder's feature: Do humus-building bonuses provide incentives for adopting mechanical or biological subsoil management practices? (Humus_Bonus_Incentive).

The assessment of respondents' interest in implementing humus-building bonuses within their regions focused on three main target groups: local politicians, civil society representatives, and stakeholders. The analysis showed that stakeholders demonstrated the strongest interest, with 44% reporting high interest and 22% indicating very high interest in introducing humus-building bonuses. In contrast, approximately 32% of civil society respondents expressed a neutral position or no opinion, while around 37% of local politicians reported very low interest in implementation.

Across the two subsoil management methods, the pattern remained consistent with the overall results. Among stakeholders, the highest level of engagement was observed – 41% showing high interest and 23% very high interest for the alfalfa cultivation method, and 51.35% high interest and 19% very high interest for the UEK method (Fig. 9).

The study evaluated seven key factors influencing the implementation of subsoil management methods, specifically the alfalfa cultivation and UEK methods. These factors included the low yield level of the area, insufficient water storage capacity of the soil, existing soil compaction in the subsoil, financial resources of the enterprise, business orientation (e.g., arable farming, horticulture, fodder farming, livestock farming, mixed operations), form of farming (organic or conventional), and ownership relationships (such as the duration of lease agreements and land ownership).
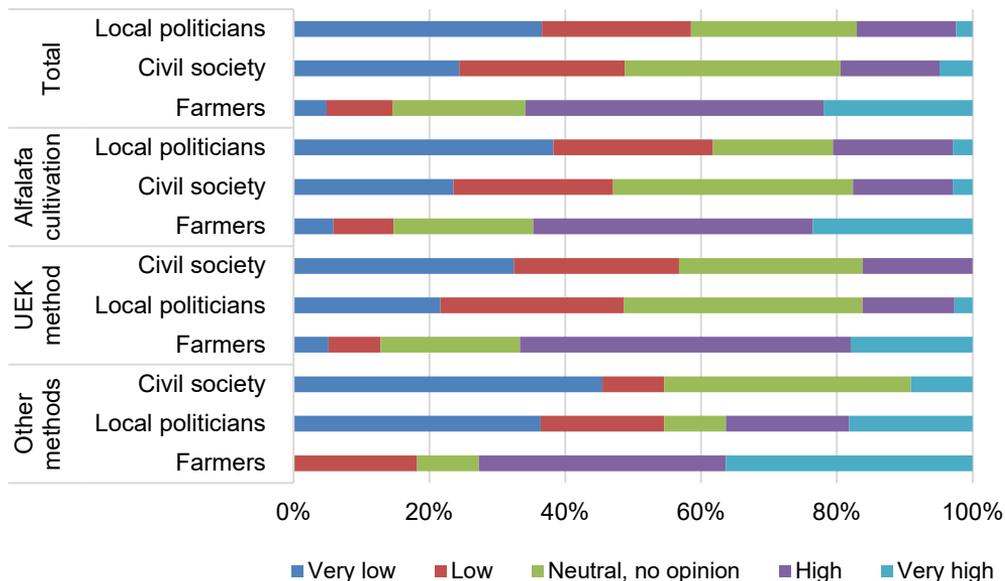
Figure 9. First group: Stakeholder's feature: How would you assess stakeholder s' interest in implementing humus-building bonuses in agriculture within your region? (Stakeholder_Interest_Humus_Bonus).

The analysis revealed that respondents were most neutral about the effects of low yield level (46%), insufficient water storage capacity (39%), and ownership relationships (37%) on subsoil management implementation. Nonetheless, over half of respondents indicated that low yield level and insufficient water storage capacity exert a high or very high influence on adoption decisions. Existing soil compaction (24% high, 37% very high) and business orientation (27% high, 32% very high) also emerged as significant determinants. In contrast, financial resources were generally perceived as having limited relevance for implementation outcomes.

When disaggregated by management method, the results showed a more differentiated pattern. For the alfalfa cultivation method, respondents identified existing soil compaction and business orientation as the most influential factors, while financial resources were rated as having a low or very low impact. Conversely, among respondents using the UEK method, financial resources and ownership relationships were recognized as having a substantial influence on implementation decisions (Fig. 10).
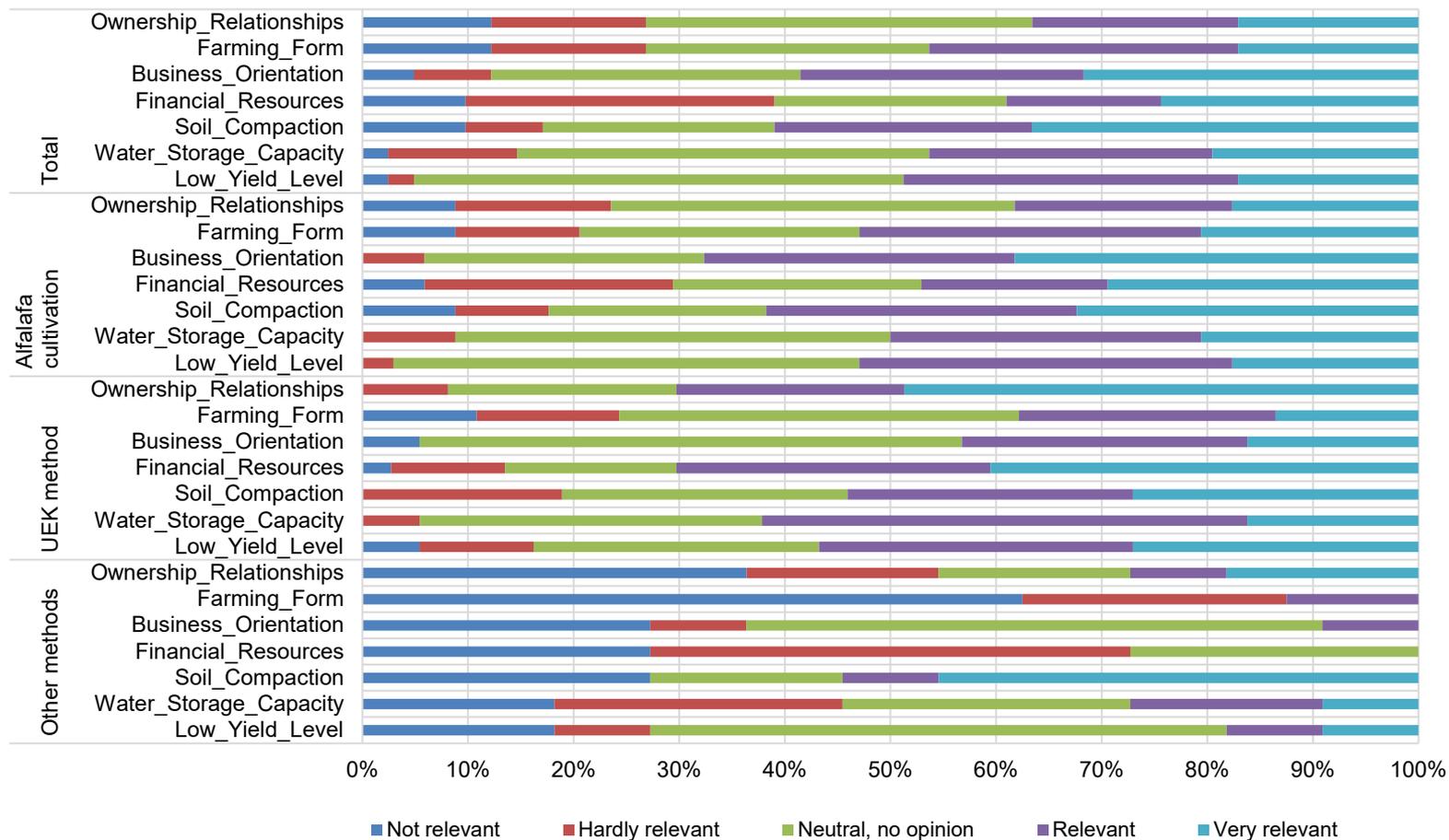
Figure 10. Second group: What factors influence the implementation of subsoil management; Alfalfa cultivation method, UEK method and other.

The analysis of respondents' readiness to grow alfalfa revealed generally low levels of willingness across all groups. More than half of the respondents – both overall and within the two subsoil management methods (alfalfa cultivation and UEK) – reported very low or low readiness to engage in alfalfa cultivation. This finding suggests that, despite the recognized agronomic benefits of alfalfa for subsoil improvement, significant reservations remain among stakeholders regarding its practical adoption (Fig. 11).
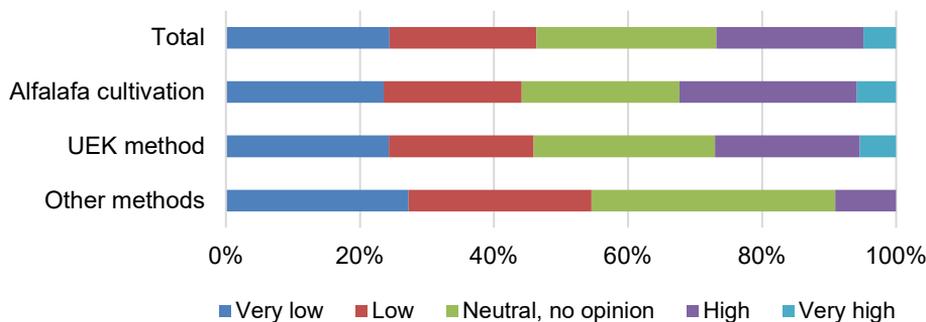
Figure 11. Third group: Alfalfa cultivation method features: Readiness to grow Alfalfa (Alfalfa_Readiness).

The evaluation of policy measures to promote alfalfa cultivation considered a range of potential strategies, including: (1) increasing support for alfalfa cultivation through the Common Agricultural Policy (CAP), (2) integrating alfalfa into contractual nature conservation schemes, (3) promoting local marketing initiatives for alfalfa-based feed, (4) encouraging feeding strategies that use alfalfa as a substitute for soy in ruminant, pig, poultry, and horse diets, (5) supporting fertilizer production derived from alfalfa, (6) funding experimental facilities for protein extraction from alfalfa, and (7) promoting alfalfa as a biogas substrate through financial premiums. Respondents evaluated each option using a binary (yes/no) scale, with both current users of alfalfa cultivation methods and users of alternative subsoil management approaches participating in the assessment.

The results indicated that enhanced CAP support for alfalfa cultivation was the most widely favored policy option, endorsed by 51% of respondents as an effective strategy. The promotion of local marketing initiatives followed, receiving 41% support. Moderate approval levels – around 36% – were recorded for measures such as developing feeding strategies, funding experimental protein production facilities, and promoting alfalfa-based biogas substrates through premiums. In contrast, 76% of respondents considered fertilizer production based on alfalfa an ineffective approach, yielding the highest share of 'no' responses among all options.

Importantly, the response patterns were consistent across both respondent groups – those applying alfalfa cultivation and those using other methods – indicating a shared perception of which strategies are most and least effective in promoting alfalfa cultivation (Fig. 12).
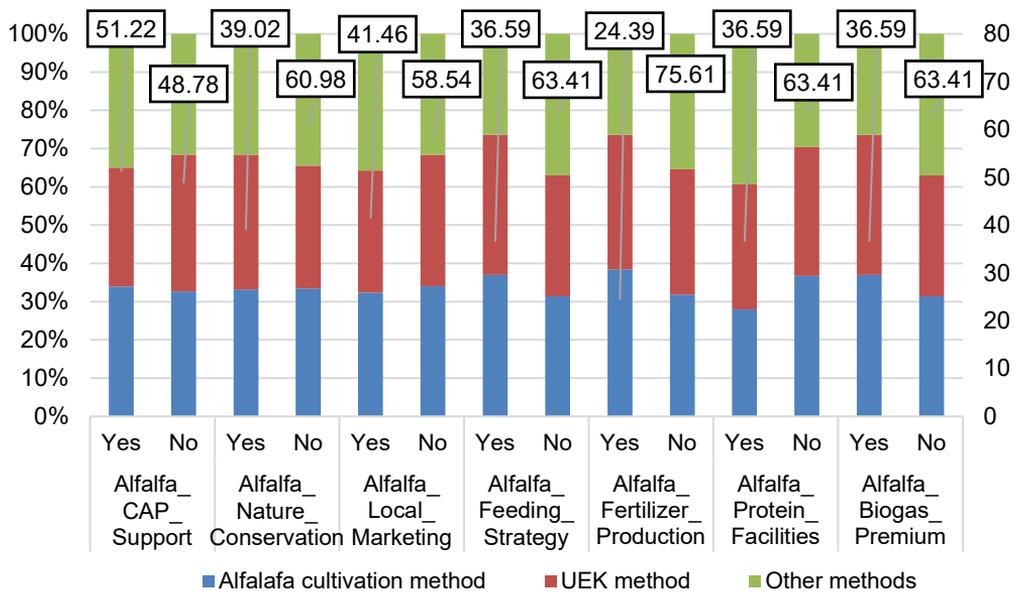
Figure 12. Third group: Alfalfa cultivation method features: How should politics promote the cultivation of alfalfa?

The analysis of respondents' openness to adopting technical innovations, such as the UEK method, revealed notable differences among stakeholder groups. The findings indicated that most respondents expressed neutral or uncertain attitudes toward the adoption of such innovations, with this uncertainty more prevalent among those practicing alfalfa cultivation. In contrast, respondents already implementing the UEK method demonstrated a higher level of willingness to embrace technical advancements. This pattern is both logical and expected, as the UEK approach inherently requires a greater degree of technical adaptation and familiarity with mechanized subsoil management practices (Fig. 13).
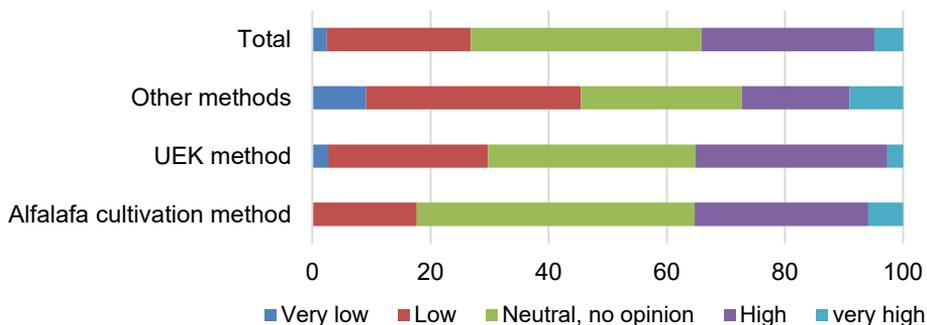
Figure 13. Fourth group: UEK method features: Stakeholder s' openness in implementing technical innovations such as UEK (Stakeholder_Openness_UEK).
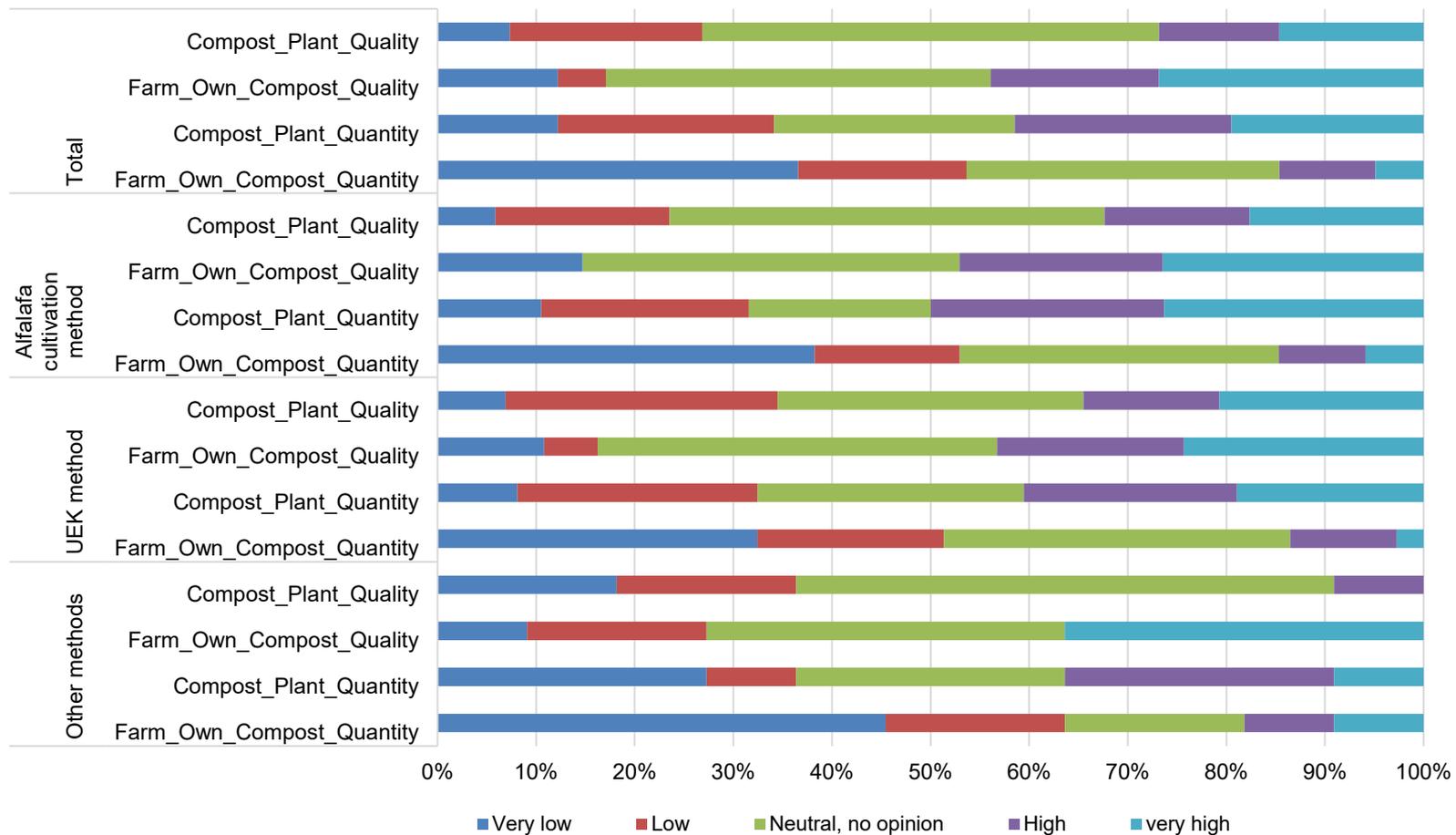
Figure 14. Fourth group: UEK method features: How do you assess the availability and quality of compost for the implementation of UEK in your district?

The evaluation of compost availability and quality for implementing the UEK method considered four main aspects: (1) the available quantity of farm-own compost, (2) the available quantity of compost purchased from compost plants, (3) the quality of farm-own compost with respect to pollutant content, and (4) the quality of purchased compost based on pollutant levels. Respondents rated these factors on a five-point scale ranging from very low to very high, with an additional no opinion option.

The results indicated that more than 40% of respondents perceived both the availability of purchased compost and the quality of farm-own compost as having a high to very high impact on the implementation of the UEK method. When analysed separately for each management approach, these two aspects consistently received the highest importance ratings. In contrast, the available quantity of farm-own compost recorded the largest share of very low and low assessments, both overall and within the alfalfa cultivation and UEK groups. These findings suggest that respondents generally regard farm-own compost availability as less influential for the successful application of the UEK method compared to externally sourced compost and quality-related factors (Fig. 14).

Data analysis techniques and model specification

The multinomial logit (MNL) model is a statistical technique used to model choice behavior among multiple discrete alternatives (Train, 2002). The MNL model is an extension of the binary logit model to accommodate multiple categorical outcomes (Cameron & Trivedi, 2016). This model is particularly useful in understanding the factors that influence decision-making processes and widely used in the field of econometrics and social sciences for analysing choice data where the dependent variable is categorical and comprises more than two categories (Hensher et al., 2015). The MNL model assumes that individuals select the option that maximizes their utility, which is influenced by the attributes of the alternatives and the characteristics of the individuals (Maddala & Lahiri, 2009). In this study, the MNL model is employed to understand the factors influencing how stakeholders differ in their perceptions on preferences and barriers in the subsoil management practices. The multinomial logit model is formulated under the assumption that the choices are mutually exclusive and exhaustive (Li, 2011). Let y_i be the categorical dependent variable representing the choice made by individual i from J alternatives (Eq. 1). The probability that individual i choose alternative j (where j=1, 2, …, J) is given by:

$$P(y_i = j) = \frac{e^{X_i \beta_j}}{\sum_{k=1}^{J} e^{X_i \beta_k}} \qquad (1)$$

Here X_i is a vector of independent variables for individual i and β_j is vector of coefficient associated with alternative j.

The assumptions for the MNL model include the following: Independence of Irrelevant Alternatives (IIA), which states that the relative odds of choosing between any two alternatives are unaffected by the presence of other alternatives; Mutually Exclusive and Exhaustive Choices, meaning each individual chooses exactly one alternative from the set (Train, 2002); and No Perfect Multicollinearity, which requires that the independent variables must not be perfectly correlated (Wooldridge, 2016).

The parameters β_j are estimated using the maximum likelihood estimation (MLE) method. The likelihood function for the multinomial logit model is given by (Greene, 2018) (Eq. 1):

$$L(\beta) = \prod_{i=1}^{N} \prod_{j=1}^{J} P(y_i = j)^{y_{ij}} \tag{2}$$

In Eq. 2, y_{ij} is an indicator variable that equals 1 if individual i choose alternative j and 0 otherwise. The log likelihood function is:

$$\ln L(\beta) = \sum_{i=1}^{N} \sum_{j=1}^{J} y_{ij} \ln P(y_i = j) \tag{3}$$

The coefficient β_j are estimated by maximizing the log-likelihood function (Eq. 2).

Marginal effects in the multinomial logit model indicate how a change in an independent variable affects the probability of choosing a particular alternative relative to a baseline alternative (Greene, 2018). In Eq. 4, the marginal effect of variable X_k on probability of choosing alternative j is given by:

$$ME_j(X_k) = \frac{e^{X_i \beta_j} \beta_{jk}}{\sum_{k=1}^{J} e^{X_i \beta_k}} \tag{4}$$

β_{jk} is the coefficient of X_k associate with alternative j in Eq. 4 (Heckman & Leamer, 2007). Marginal effects can be computed for each independent variable to understand their impact on choice probabilities across all alternatives. They provide insights into the direction and magnitude of the effect of explanatory variables on the likelihood of choosing different alternatives (Hensher et al., 2015).

The estimated coefficients β_j in Eq. 4, represent the log odds of choosing alternative j relative to the baseline alternative. In simpler terms, βj tells us how the odds of picking alternative j change when a predictor variable increases by one unit. A positive βj means the odds of choosing alternative j go up, while a negative βj means the odds go down. The log odds help us understand these changes in a way that keeps the probabilities between 0 and 1. For interpretation purposes, the coefficients can be exponentiated to obtain odds ratios (Winkelmann, 2008). Marginal effects can also be computed to understand the impact of a unit change in an independent variable on the probability of choosing a particular alternative (Li et al., 2005).

Goodness-of-fit and model validation for the MNL model are assessed using several measures. The Likelihood Ratio Test compares the fitted model with a null model (a model with only intercepts) to evaluate improvement in fit. The Pseudo-R-squared provides an indication of the model's explanatory power. Additionally, a Confusion Matrix and Predictive Accuracy are used to evaluate the model's predictive performance (Friedman et al., 2019).

The MNL model is employed using data gathered from an online survey to investigate influential factors affecting the adoption of various subsoil methods in specific regions. The dependent variable categorically represents stakeholders' preferences and perceived barriers toward methods such as alfalfa cultivation, UEK, and others. Independent variables, detailed in Table 1, are included to ascertain substantial predictors of stakeholders' perceptions and preferences regarding subsoil management methods, quantifying their respective impacts (Greene, 2018).

To ensure the reliability and stability of our estimates, we applied a penalized multinomial logit (MNL) model. This method is particularly useful when working with a relatively small sample size, as it applies a penalty to the model's coefficients, preventing overfitting and improving estimation accuracy. By incorporating this penalty,

the model adjusts for potential issues such as collinearity or the influence of outliers, which could distort results in smaller datasets. Penalized MNL models are widely recognized for their ability to enhance the robustness of choice models, ensuring more reliable and consistent predictions, even in the presence of limited observations. This approach is well-suited for our study, where the goal is to identify key factors influencing the adoption of subsoil management practices while mitigating potential biases associated with small sample sizes. The multinomial logit model provides a robust framework for analyzing choice data with multiple categories. Its flexibility in handling categorical dependent variables and multiple predictors makes it an ideal choice for this study. The results obtained from the MNL model offer valuable insights into the determinants of factors and help in understanding the decision-making process of implementing subsoil management practices (De Jong et al., 2018; Rainey & MacCaskey, 2021).

Although binary logistic regressions could be used to model each subsoil management method separately, we opted for the Multinomial Logit Model (MNL) due to the nature of our dependent variable, which includes three distinct and mutually exclusive categories: Alfalfa cultivation, the UEK method, and other methods. The MNL is particularly suited for modeling situations where there are multiple choice outcomes, as it allows for the simultaneous estimation of the relative probabilities of each choice being adopted, based on the influencing factors. Using separate binary logistic regressions would not account for the interdependencies between the choices and would limit our ability to compare the likelihood of adopting each method in a single framework. Therefore, the MNL provides a more comprehensive and appropriate approach for analyzing the factors influencing the adoption of different subsoil management methods.

In consideration of econometric neoclassical assumptions and degrees of freedom, it is not feasible to incorporate all factors (variables) into the model. To discern the primary influential variables that could impact the adaptation barriers in implementing diverse subsoil management practices, a multicollinearity test was conducted following (Midi et al., 2010), utilizing the Variance Inflation Factor (VIF) as outlined in Table 2.

This test was undertaken because, despite explicit separation of subsoil management practices in the model, there exists implicit and indirect interdependence among them. This phenomenon, often referred to as variables with high correlation values, can undermine the meaningfulness and significance of estimated parameters. Such high correlation may hinder variables from demonstrating their individual impacts. The VIF is employed at two levels: VIF = 1 indicates no multicollinearity, signifying that the variable lacks correlation with other predictors; VIF between 1 and 5 suggests acceptable multicollinearity, indicating high correlation with other predictors, potentially leading to unreliable estimates. To guarantee the model's robustness, factors with varying VIF values were systematically added and removed in the logit model. Variables exhibiting high multicollinearity VIF values were excluded, enhancing the reliability of the model by avoiding variables with intertwined correlations. This process ensured the stability and significance of coefficient values and strengthened the reliability of the model (Bianco & Martinez, 2009). As a result of these tests, the most distinguishing variable between subsoil management were determined by grey color in Table 2 as follows: 'Insufficient water storage capacity of the soil,' 'Orientation of the business,' and 'Ownership relationships'.

RESULTS

To identify the key factors influencing the implementation of different subsoil management methods, the analysis was organized into four distinct groups: Stakeholder Characteristics, Factors Influencing SubSoil Management Implementation, Features of the Alfalfa Cultivation Method, and Features of the UEK Cultivation Method. These groups were designed to identify the primary determinants of stakeholders' preferences for various subsoil management techniques.

For more precise modeling and clearer interpretation, the independent variables, which were assessed on various levels, were aggregated into three main categories. The first group combines responses rated as 'Not relevant' and 'Hardly relevant,' the second group includes 'Neutral' and 'No opinion,' and the third group consists of 'Relevant' and 'Very relevant.' A similar aggregation was applied to the variable categories: 'Very low' and 'Low' were grouped together, 'Neutral' and 'No opinion' formed a second group, and 'High' and 'Very high' were combined into the third group.

After addressing multicollinearity and completing the variable selection process, the coefficients were estimated using a multinomial logit model. This model incorporates dummy variables for each category within the independent variables and provides outputs for each variable category and region. The multicollinearity tests identified three key distinguishing variables: 'Insufficient water storage capacity of the soil,' 'Orientation of the business,' and 'Ownership relationships.' The results are presented based on these selected variables, which are expected to notably impact the perception and adoption of different subsoil management methods. The results include: (1) the coefficients and their significance levels, and (2) the Marginal Effects of each variable. These outputs are detailed below, offering insights into how these factors influence the adoption of various subsoil management techniques.

Synthesis of differences in perceptions towards subsoil management practices at regional level

To estimate a multinomial logit model (Eqs 2 and 3) aimed at assessing the critical factors influencing the implementation of various subsoil management techniques, we conducted an analysis using both dependent and independent variables. The dependent variable consists of three distinct subsoil categories: Alfalfa Method, UEK Method, and Others. Within the framework of the multinomial logit model, the 'Others' category serves as the reference group, against which the interpretations of the Alfalfa and UEK methods are compared.

Independent variables were selected following a thorough evaluation of their statistical characteristics and distribution percentages across the three subsoil management categories. To mitigate multicollinearity, a diagnostic procedure was performed to finalize the choice of independent variables. Consequently, variables such as 'Insufficient water storage capacity of the soil,' 'Orientation of the business,' and 'Ownership relationships' were identified as pertinent factors influencing the subsoil management methods under study.

In this analysis, the 'Other method' is designated as the base outcome for the dependent variable (Subsoil methods), while 'not relevant/hardly relevant' is the reference category for the independent variable. Additionally, Anhalt-Bitterfeld is chosen

as the base region[4]. The estimated coefficients and their statistical significance are presented in Table 2.

In the multinomial logit model estimation conducted using Stata, the statistical analyses were performed with Stata 18, developed and distributed by StataCorp LLC, College Station, Texas, USA. This software provides a reliable environment for estimating discrete choice models such as the multinomial logit (MNL), ensuring accurate computation of coefficients, standard errors, and *p*-values while allowing for robust diagnostic testing and model validation.

The first output is related to the iteration log. This log tracks the likelihoods at each iteration of multinomial logistic regression. It's an iterative process aiming to maximize likelihood. Iteration begins with a null model, then predictors are added. Convergence, signaled by minimal differences between iterations, halts the process, displaying results.

In the multinomial logit model estimation using Stata, the iteration log records the likelihood values at each step. This iterative process aims to maximize the likelihood function, starting with a null model and adding predictors until convergence is achieved.

Table 2. variance inflation factor (VIF) results for identifying key factors with differential preferences among subsoil management methods

N	Variable name	VIF	1/VIF
1	Alfalfa_Local_Marketing	9.66	0.10
2	Alfalfa_Protein_Facilities	7.36	0.14
3	Farm_Own_Compost_Quality	6.56	0.15
4	Humus_Bonus_Incentive	6.56	0.15
5	Politician_Interest_Humus_Bonus	6.24	0.16
6	Alfalfa_Fertilizer_Production	6.16	0.16
7	Stakeholder_Openness_UEK	6.11	0.16
8	Info_Campaigns_Public	5.9	0.17
9	Knowledge_Exchange_Stakeholders	5.51	0.18
10	Business_Type	5.67	0.18
11	Civil_Society_Interest_Humus_Bonus	5.36	0.19
12	Low_Yield_Level	5.28	0.19
13	Alfalfa_Readiness	4.58	0.22
14	Farm_Own_Compost_Quantity	4.44	0.23
15	Alfalfa_Nature_Conservation	4.41	0.23
16	Alfalfa_CAP_Support	4.34	0.23
17	Actor_Group	4.08	0.25
18	Water_Storage_Capacity	4.03	0.25
19	Assigned_Groups	3.63	0.28
20	Consulting_Services_Promotion	3.61	0.28
21	Regional_Test_Areas	3.57	0.28
22	Alfalfa_Biogas_Premium	3.49	0.29
23	Experience_Exchange_Stakeholders	3.41	0.29
24	Info_Campaigns_Stakeholders	3.21	0.31
25	Research_Funding_Targeted	3.07	0.33
26	Alfalfa_Feeding_Strategy	2.71	0.37
27	Soil_Compaction	2.6	0.38
28	Financial_Resources	2.51	0.40
29	Stakeholder_Interest_Humus_Bonus	2.47	0.40
30	Farming_Form	2.43	0.41
31	Compost_Plant_Quantity	2.41	0.42
32	Ownership_Relationships	2.31	0.43
33	Business_Orientation	2.17	0.46
34	Compost_Plant_Quality	2.12	0.47

VIF = 1: No multicollinearity. The variable has no correlation with other predictors. VIF between 1 and 5: multicollinearity acceptable. The variable is highly correlated with other predictors, and its estimates may be unreliable. Source: Result of model.

---

[4] Any region other than Anhalt-Bitterfeld could also be chosen as the baseline region in the multinomial logit model estimation. The choice of baseline region does not affect the relative comparisons between alternatives, but it will change the interpretation of the coefficients, as they are expressed relative to the chosen baseline.

Convergence, indicated by minimal changes between iterations, was reached after 6 iterations. This shows the model's parameters stabilized, suggesting a successful estimation where further iterations would not significantly alter the coefficients. This outcome is typical in multinomial logit models, reflecting the process of finding optimal parameter values that best fit the observed data.

The multinomial logit model was estimated, and the model's overall significance was confirmed by a likelihood ratio chi-squared test (LR chi2(18) = 51.97, $p < 0.001$). This indicates that at least one predictor in the model has a statistically significant effect on the outcome. The model demonstrated a moderate level of explanatory power, explaining approximately 31.89% of the variance in the outcome variable, as indicated by the pseudo-R-squared value. Additionally, the model exhibited a good fit to the data with a log likelihood of -55.490. These results collectively suggest that the multinomial logit model effectively predicts or explains the categorical outcome variable, with the included predictors contributing significantly to the model's performance and explanatory capacity (Table 3).

The results of the multinomial logit model indicate that the relevance of insufficient water storage capacity of the soil notably influences the choice of method. When 'insufficient water storage capacity of the soil is neutral compared to not relevant/hardly relevant, the log odds[5] of choosing the Alfalfa method over the 'Other method' increase by 3.53 (p-value = 0.08), and for the 'UEK method' over the 'Other method', the increase is 3.91 (p-value = 0.05). Similarly, when 'insufficient water storage capacity of the soil 'is relevant compared to not relevant/hardly relevant, the log odds of choosing the 'Alfalfa method' over the 'Other method' increase by 3.69 (p-value = 0.04), and for the 'UEK method', the increase is 3.88 (p-value = 0.03). These results are statistically significant at the 10% level, indicating a strong influence of insufficient water storage capacity of the soil (insufficient water storage capacity of the soil) relevance on method choice. However, the coefficients for the regional variables related to 'insufficient water storage capacity of the soil' are not statistically significant (p-values > 0.10). This indicates that regional differences in insufficient water storage capacity of the soil do not significantly influence the choice of method in this sample. While the positive coefficients suggest that the likelihood of choosing 'Alfalfa or UEK methods' over the 'Other method' may increase in regions like 'Rhein- Sieg- Kreis' and 'other regions' compared to 'Anhalt-Bitterfeld', the lack of statistical significance means we cannot confidently assert these differences. These findings highlight the importance of insufficient water storage capacity of the soil relevance in influencing method choice and suggest that factors like regional differences may require further investigation with larger samples or additional variables to better understand their potential impacts (Table 3).

The analysis suggests that the relevance of the 'Orientation of the business' significantly impacts the choice of both the Alfalfa and UEK methods over the other method. Specifically, when business orientation is considered relevant, the odds of choosing the Alfalfa method are significantly higher (6.38 times the odds, p-value = 0.01), and the odds of choosing the UEK method are also significantly higher

---

[5] Odds refer to the ratio of the probability of an event occurring (e.g., choosing a specific method) to the probability of it not occurring (e.g., choosing the 'Other method').

(5.99 times the odds, *p*-value = 0.01). Even when business orientation is neutral, the odds of choosing the UEK method over the other method are significantly increased (3.79 times the odds, *p*-value = 0.02). These findings underscore the importance of business orientation in method selection, particularly when it is perceived as relevant. The statistically significant results for the relevant orientation levels highlight its crucial role in influencing decisions between the methods, suggesting that businesses that consider their orientation as relevant are more likely to choose the Alfalfa or UEK methods over the other method (Table 3).

The analysis shows that the relevance of 'Ownership relationships' has a notable impact on the choice of methods, particularly for the UEK method when they are 'relevant/ very relevant'. For the UEK method, when ownership relationships are 'relevant/ very relevant', the odds of choosing the 'UEK method' over the 'Other method' increase substantially (5.43 times the odds, *p*-value = 0.005), indicating a strong and significant influence. Although the coefficient for 'relevant/ very relevant' ownership relationships for the 'Alfalfa method' is just above the conventional threshold for significance (*p*-value = 0.06), it still indicates a notable trend that 'relevant/ very relevant' ownership relationships may influence method choice. Similarly, the coefficient for 'neutral/no opinion' ownership relationships for the 'UEK method', with a *p*-value of 0.09, suggests a marginally significant effect. These findings highlight the importance of ownership relationships in method selection, particularly when they are perceived as 'relevant/ very relevant'. The statistically significant results for 'neutral/no opinion' and 'relevant/ very relevant' ownership levels underscore their role in influencing decisions between methods, suggesting that ownership relationships are a critical factor in determining the choice of Alfalfa or 'UEK methods' over the 'Other method'.

The variable 'Insufficient Water Storage Capacity of the Soil' shows that both 'neutral/no opinion' and 'relevant/very relevant' levels notably increase the likelihood of choosing the Alfalfa and UEK methods over other methods, with a slightly stronger significance observed for the UEK method. Regional differences do not significantly alter this effect. Similarly, 'Business Orientation' indicates that a 'relevant/very relevant' business orientation strongly and significantly raises the likelihood of selecting both the Alfalfa and UEK methods. A 'neutral/no opinion' orientation also proves significant for the UEK method. Regarding 'Ownership Relationships', 'relevant/very relevant' ownership relationships significantly influence the choice of the UEK method. 'Neutral/no opinion' ownership relationships show marginal significance for both methods. Overall, while the factors of insufficient water storage capacity and business orientation significantly impact the choice of subsoil management methods, regional differences do not notably affect these outcomes. Ownership relationships are also crucial, especially for the UEK method when deemed 'relevant/very relevant.' This comparative analysis highlights the importance of these factors in the selection of subsoil management methods across different regions (Table 3).

Table 3. Multinominal logit model estimation

SubSoil Management Method			Coefficient	Standard error	z	P>|z|	[95%'Conf.'Interval]	
Alfalfa cultivation method	Water_Storage_Capacity	Neutral/ no opinion	3.53	2.07	1.71	0.09	-0.53	7.59
		Relevant/very relevant	3.70	1.86	1.99	0.05	0.06	7.34
	Business_Orientation	Neutral/ no opinion	2.29	1.76	1.30	0.19	-1.16	5.75
		Relevant/very relevant	6.39	2.47	2.59	0.01	1.55	11.23
	Ownership_Relationships	Neutral/ no opinion	2.60	1.37	1.90	0.06	-0.08	5.27
		Relevant/very relevant	3.49	1.90	1.84	0.07	-0.22	7.21
	Region_Work	Teltow-Fläming	1.19	1.30	0.92	0.36	-1.36	3.75
		Rhein-Sieg-Kreis	2.24	1.80	1.25	0.21	-1.29	5.77
		Other	3.49	2.40	1.45	0.15	-1.22	8.20
	Constant		-8.31	3.58	-2.32	0.02	-15.32	-1.30
UEK method	Water_Storage_Capacity	Neutral/ no opinion	3.92	2.07	1.89	0.06	-0.14	7.98
		Relevant/very relevant	3.88	1.86	2.09	0.04	0.25	7.52
	Business_Orientation	Neutral/ no opinion	3.79	1.71	2.22	0.03	0.44	7.15
		Relevant/very relevant	6.00	2.48	2.42	0.02	1.13	10.86
	Ownership_Relationships	Neutral/ no opinion	2.35	1.41	1.66	0.10	-0.42	5.12
		Relevant/very relevant	5.44	1.93	2.82	0.01	1.65	9.22
	Region_Work	Teltow-Fläming	1.13	1.30	0.88	0.38	-1.41	3.67
		Rhein-Sieg-Kreis	2.15	1.83	1.18	0.24	-1.43	5.73
		Other	4.08	2.41	1.69	0.09	-0.64	8.80
	Constant		-9.99	3.65	-2.74	0.01	-17.15	-2.84
Other method	Constant (base come)							

Iteration 0: log likelihood=	-81.47	Iteration 4: log likelihood= -55.49		Number of observations=	82
Iteration 1: log likelihood=	-60.12	Iteration 5: log likelihood= -55.49		LR chi2(18)=	51.97
Iteration 2: log likelihood=	-56.23	Iteration 6: log likelihood= -55.49		Prob>chi2=	0
Iteration 3: log likelihood=	-55.53	Pseudo R2=	0.32	Log likelihood=	-55.49

Source: result of model.

Marginal effects of key factors influencing stakeholders' preferences and perceptions for implementing various subsoil management methods

When examining the marginal effects of the independent variables on the choice of three different categories of the dependent variable (subsoil methods) while also considering the three different levels of the dependent variable(not relevant/ hardly relevant, neutral/no opinion, relevant/very relevant) and four regions simultaneously in a multinomial logit model (Eqs 3 and 4), the interpretation focuses on how changes in the independent variables ('insufficient water storage capacity of the soil ', Orientation of the business,' and 'Ownership relationships') impact the probability of choosing each category of the dependent variable over the base outcome (often set as one of the categories). The outcomes of marginal effects pertaining to both subsoil management methods and regions are detailed in Tables 4. This helps us understand how variations in independent variables affect the likelihood of choosing one subsoil method over others by examining the marginal effects across the three dependent variable categories, revealing the factors driving method selection.

The marginal effects analysis provides insight into how a one-unit increase in perceived 'insufficient water storage capacity of the soil' influences the selection of subsoil management methods. Across all three methods – Alfalfa, UEK, and Others – a higher perceived relevance of water storage capacity corresponds to increased probabilities of method selection. Specifically, for the Alfalfa method, each one-unit increase in perceived relevance leads to a 0.31 percentage point increase ($p = 0.02$) for not relevant/hardly relevant, 0.40 percentage point increase ($p = 0.00$) for neutral/no opinion, and 0.44 percentage point increase ($p = 0.00$) for relevant/very relevant levels. Similarly, for the UEK method, a one-unit increase results in a 0.28 percentage point increase ($p = 0.05$) for not relevant/hardly relevant, 0.49 percentage point increase ($p = 0.00$) for neutral/no opinion, and 0.46 percentage point increase ($p = 0.00$) for relevant/very relevant levels. The Others method also responds to perceived relevance levels, albeit with weaker effects, indicating a 0.41 percentage point increase ($p = 0.00$) for not relevant/hardly relevant, 0.11 percentage point increase ($p = 0.00$) for neutral/no opinion, and 0.10 percentage point increase ($p = 0.00$) for relevant/very relevant levels (Table 4).

To compare the marginal effects of three primary factors across two subsoil management practices, we analyzed the results presented in Table 4. The marginal effects of 'insufficient water storage capacity of the soil' for both the 'relevant' and 'very relevant' statuses in alfalfa cultivation and the UEK method are similar, with values of 0.44 and 0.46, respectively. In contrast, the marginal effects for the other two factors show notable differences between the two methods. For the 'orientation of the business' factor, the marginal effect in the alfalfa cultivation method is 0.61, while in the UEK method, it is approximately 0.35, which is roughly half of the alfalfa value. Similarly, for the 'ownership relationships' factor, the marginal effect in the UEK method is 0.67 for both the 'relevant' and 'very relevant' statuses, whereas in the alfalfa cultivation method, this value is less than half, at 0.27. Overall, it appears that 'orientation of the business' is more influential in the alfalfa cultivation method compared to the UEK method, whereas 'ownership relationships' has a greater marginal effect in the UEK method compared to alfalfa cultivation.

Table 4. Marginal effects of key factors influencing stakeholders' preferences and perceptions for implementing various subsoil management methods

Factor	Subsoil management method	Status	Margin	Standard error	z	P>|z|	[95%'Conf.'Interval]	
Water_Storage_Capacity	Alfalfa cultivation method	Not relevant/hardly relevant	0.31	0.14	2.21	0.03	0.04	0.59
		Neutral/ no opinion	0.40	0.09	4.58	0.00	0.23	0.58
		Relevant/ very relevant	0.44	0.07	6.37	0.00	0.31	0.58
	UEK method	Not relevant/hardly relevant	0.28	0.14	1.94	0.05	0.00	0.56
		Neutral/ no opinion	0.49	0.09	5.51	0.00	0.32	0.66
		Relevant/ very relevant	0.46	0.07	6.62	0.00	0.32	0.59
	Other method	Not relevant/hardly relevant	0.41	0.12	3.29	0.00	0.17	0.65
		Neutral/ no opinion	0.11	0.04	2.35	0.02	0.02	0.19
		Relevant/ very relevant	0.10	0.04	2.61	0.01	0.03	0.18
Business_ Orientation	Alfalfa cultivation method	Not relevant/hardly relevant	0.25	0.15	1.60	0.11	-0.06	0.55
		Neutral/ no opinion	0.21	0.06	3.27	0.00	0.08	0.34
		Relevant/ very relevant	0.61	0.07	8.77	0.00	0.48	0.75
	UEK method	Not relevant/hardly relevant	0.27	0.12	2.14	0.03	0.02	0.51
		Neutral/ no opinion	0.62	0.07	8.84	0.00	0.48	0.76
		Relevant/ very relevant	0.35	0.07	5.18	0.00	0.22	0.48
	Other method	Not relevant/hardly relevant	0.49	0.14	3.48	0.00	0.21	0.76
		Neutral/ no opinion	0.17	0.05	3.72	0.00	0.08	0.26
		Relevant/ very relevant	0.04	0.02	1.68	0.09	-0.01	0.08
Ownership_ Relationships	Alfalfa cultivation method	Not relevant/hardly relevant	0.44	0.09	4.73	0.00	0.26	0.63
		Neutral/ no opinion	0.61	0.09	6.74	0.00	0.43	0.79
		Relevant/ very relevant	0.27	0.06	4.21	0.00	0.14	0.39
	UEK method	Not relevant/hardly relevant	0.19	0.09	2.10	0.04	0.01	0.36
		Neutral/ no opinion	0.24	0.08	2.96	0.00	0.08	0.40
		Relevant/ very relevant	0.67	0.07	10.07	0.00	0.54	0.80
	Other method	Not relevant/hardly relevant	0.37	0.09	4.33	0.00	0.20	0.54
		Neutral/ no opinion	0.15	0.06	2.55	0.01	0.03	0.27
		Relevant/ very relevant	0.06	0.02	2.64	0.01	0.02	0.11

Source: Result of model.

DISCUSSION

Our analysis has shown that the variables 'Insufficient water storage capacity of the soil', 'Orientation of the business', and 'Ownership relations' play a key role when it comes to the preferences and perceptions related to the implementation of subsoil management methods in the four case study regions. To some extent, these findings can be explained by the results of previous sociological research, which has been carried out in the frame of the Soil[3] project.

In times of climate change, nutrient capacity of the soil has become a focus point of stakeholders and policy-makers. Climate scientists predict and increasing sequence of extreme weather events in Germany, such as droughts or heavy rainfalls, with potentially detrimental effects for stakeholder s but, in the case of flooding events, also for the surrounding communities (World Weather Attribution, 2024). The acceptance analysis among agricultural stakeholders in Germany carried out by (Hinzmann et al., 2021) showed that a majority of the interviewed stakeholders was well aware of the challenges that the agricultural sector in Germany will have to face when those predictions become reality. At the same time, the acceptance analysis revealed a high awareness among agricultural stakeholders with regard to the role that subsoil and related management strategies can play in mitigating the effects of droughts and, to some extent, also flooding events. Overall, the importance of water storage capacity of the soils has been highly ranked across the three groups of stakeholders identified in the context of the acceptance analysis. Pedological research carried out in the frame of the Soil[3] project supports the assumption that subsoil, when managed appropriately, have positive effects on the hydrologic balance of the managed area, with water (and nutrients) being increasingly absorbed by the crops, suggesting that crop yields can be stabilized in times of drought (Bauke et al., 2024). Similar evidence has been reported in recent studies demonstrating that tillage systems significantly affect soil moisture distribution and retention. Conservation-oriented approaches, such as subsoiling with ridgers or chisel-rotary combinations, enhance soil water storage in both surface and deeper layers, thereby improving crop resilience and yields during dry periods (Abo-habaga et al., 2022). These findings underline the crucial link between soil structure management and hydrological balance, reinforcing the role of subsoil-related practices in mitigating drought effects.

Given that the marginal effect values of the statuses 'relevant' and 'very relevant' are similar for the alfalfa cultivation method and for the UEK method (see Table 4), one can hold that stakeholders in the four case study regions perceive the potentials, which the methods hold for increasing the water storage capacity of the soil, are relatively high for both methods, implying also that the implementation potential is equally high for both methods in view of this variable. For other methods, our results suggest that this variable is not relevant or hardly relevant.

The orientation of the business is a highly relevant determinant when it comes to the application of subsoil management techniques. Farms that focus predominantly on open livestock farming and have mainly grassland under management, are not usually suitable candidates for the application of either the alfalfa cultivation method or the UEK method, as the conversion of grassland into cropland is restricted in Germany. Among the farm types that can be considered for application of both methods, however, one can find a broad range of business orientations, including arable farming, horticulture, fodder

farming, livestock farming, and mixed business (i.e. crop and livestock farming). Mixed farming is particularly suitable for alfalfa cultivation, as alfalfa can be used as cattle fodder (Schneider et al., 2024). Overall, the existence of utilization opportunities for alfalfa has emerged as a significant acceptance factor for its cultivation (Hinzmann et al., 2021), which may either be realized in the form of an on-farm circular economy approach (utilizing alfalfa as fodder and, subsequently, the resulting manure as natural fertilizer), or by creating new, regional alfalfa-based value chains. As implied by the different marginal effect values of the statuses (relevant / very relevant' in the case of the alfalfa cultivation method versus 'neutral / no opinion in the case of the UEK method', see Table 4), it becomes apparent that the above-mentioned considerations play an important role when it comes to deciding between application of the two methods. The orientation of the business and existing utilization potentials are key factors when it comes to alfalfa cultivation. The application of the UEK method, on the other hand, is not dependent on related considerations and can thus be applied in a broader set of business orientations. For other methods, our results suggest that this variable is not relevant or hardly relevant.

Finally, our results suggest that ownership relationships play an important role when it comes to the implementation of subsoil management strategies. As implied by the marginal effect value of the status 'relevant/ very relevant' in the case of the UEK method, one can hold that land ownership can be considered a decisive factor on the side of the stakeholder s. This means that it makes a difference if a stakeholder owns the land which is considered for subsoil management, or if the land is leased. Although lease contracts usually run over a longer period of time (6–12 years in Germany), which could lead to the conclusion that this is not a relevant factor, stakeholder s and other agricultural stakeholders who participated in our survey seem to consider this to be a relevant aspect. One reason for this could be the perceived invasive nature of the UEK method (altering subsoil conditions for a longer period of time), the costs related to the application of the UEK method (approx. 750€ per hectare) as well as possible investment in new technology, or the perceived uncertainty with regard to the expected yield effect. Recent research addressing the optimization of tillage machinery operation also points to the potential for improving the cost-effectiveness and energy efficiency of such interventions. For instance, studies using response surface methodology (RSM) to model tractive performance of agricultural tractors during semi-deep tillage have identified optimal combinations of speed, depth, and loading that minimize fuel use and wheel slip while maintaining soil loosening effectiveness (Askari et al., 2021). Such advances highlight opportunities to reduce operational barriers associated with subsoil management techniques like the UEK method.

These are obviously relevant considerations when access to the land is limited until a certain point of time, and renewal of the lease contract is uncertain. While alfalfa cultivation also requires land access for a longer period of time due to its perennial cultivation, the perception among agricultural stakeholders in the four case study regions seems to be that land ownership is not a decisive factor. For other methods, our results suggest that this variable is not relevant or hardly relevant.

As regards the statistical analysis of the collected data, the multinomial logit (MNL) model serves as a widely adopted method for analysing categorical outcome data, particularly in predicting probabilities associated with various choices within discrete choice contexts. Its primary strengths include straightforward interpretation and a

relatively simple estimation process, making it an effective tool for examining individual choice behaviour across multiple alternatives. Additionally, the MNL model demonstrates computational efficiency, which is advantageous for handling datasets, and it offers valuable insights into how predictor variables influence outcome probabilities (Jong et al., 2019). However, a limitation of the MNL model is its assumption of independence of irrelevant alternatives (IIA). This assumption posits that the relative odds of choosing between any two alternatives remain unaffected by the presence of other options (Tutz, 2021). Such a premise may not reflect real-world scenarios where choices can be similar or substitutable. Furthermore, the model's linearity in the logit of probabilities limits its flexibility, which can reduce predictive accuracy in more complex choice situations (Grange et al., 2024).

To enhance the robustness of our findings, increasing the number of surveyed stakeholder s would provide a more comprehensive assessment. The study's narrow focus on only three regions in Germany may restrict the generalizability of its findings to broader international contexts. The unique cultural, economic, and policy landscapes of Germany could limit the applicability of these results to other agricultural settings. Moreover, the lack of longitudinal data constrains our ability to observe changes in stakeholders' perceptions and practices over time, thereby limiting the establishment of causal relationships. This restriction hinders our capacity to capture long-term trends and responses to policy shifts, complicating efforts to draw robust conclusions about the sustained impact of such changes on stakeholders' decision-making processes. Another limitation is that we only focus on the data collected from the survey, and there is not any analysis of geophysical conditions and relevant socio-economic criteria within the selected German regions. Additionally, the study does not address the implications of any mismatch between geophysical conditions and socio-economic factors, which could notably impact the effectiveness of subsoil management efforts. Addressing these aspects can remarkably contribute to advancing subsoil management practices and promoting agricultural sustainability.

In summary, this study provides valuable insights into the key factors influencing the acceptance and implementation of subsoil management methods in Germany, particularly the roles of Water Storage Capacity, Orientation of the Business, and Ownership Relations. Stakeholders recognize the value of these methods in improving soil health and mitigating climate-related water scarcity, particularly in drought-prone regions. Specifically, we highlight the significant importance of water storage capacity in improving crop yields during drought conditions, the relevance of farm business orientation for adopting certain management techniques, and the critical influence of land ownership relationships on the implementation of subsoil management strategies. Our findings indicate that these factors are crucial when stakeholders decide on adopting techniques like alfalfa cultivation and the UEK method. However, barriers to implementation include soil conditions, the business orientation of farms, and land ownership. While alfalfa cultivation is preferred by mixed farming operations due to its dual utility, its adoption is limited by restrictions on converting grassland to cropland and lack of market opportunities. The more flexible UEK method can be applied across a broader range of farming systems, yet it still requires technical knowledge and infrastructure. Landowners are more likely to invest in these methods, while tenants face challenges due to uncertain land tenure and lack of long-term control over land. This study adds to the growing body of knowledge by providing empirical evidence

supporting the adoption of these methods in the face of climate change challenges, offering new insights into stakeholder perceptions, and drawing on sociological and pedological research from the Soil³ project. Addressing these barriers will be crucial for enhancing the uptake of subsoil management strategies.

While this study provides substantial insights into stakeholder preferences, it has limitations. First, the focus is on only four case study regions in Germany, meaning the findings may not be fully representative of the broader agricultural landscape. The perceptions and practices of stakeholders in other regions or countries may differ, limiting the generalizability of the results. Second, our study does not explore in depth the economic or financial barriers to implementing subsoil management techniques, which could provide additional context for understanding stakeholders' decisions. Finally, while we have considered a variety of stakeholders, the study could benefit from a more diverse sample, including a broader representation of farm types, scales, and management practices to strengthen the validity of the conclusions.

CONCLUSIONS

The analysis of subsoil management methods using a multinomial logit model has revealed critical factors influencing stakeholders' decisions to adopt specific methods, such as alfalfa cultivation and the UEK method, compared to other alternatives. The study identified 'insufficient water storage capacity of the soil,' 'orientation of the business,' and 'ownership relationships' as notable determinants of method choice. Our findings indicate that the perceived relevance of 'insufficient water storage capacity of the soil' substantially increases the likelihood of choosing both alfalfa and UEK methods, with a slightly stronger effect observed for the UEK method. This underscores the importance of water management in influencing subsoil management decisions. Similarly, 'business orientation' emerges as a strong factor, particularly for the alfalfa method, where a relevant business orientation notably raises the probability of its adoption. In contrast, 'ownership relationships' play a more decisive role in the adoption of the UEK method, suggesting that land tenure and ownership considerations are pivotal in this context. The analysis of marginal effects further highlights the nuanced differences between the two subsoil management methods. While the orientation of the business is more influential for alfalfa cultivation, ownership relationships have a greater impact on the choice of the UEK method. These insights suggest that tailored interventions focusing on business incentives may promote alfalfa cultivation, while addressing ownership and land tenure issues could enhance the adoption of the UEK method. In conclusion, the study provides valuable evidence that can inform policy and decision-making aimed at increasing the uptake of sustainable subsoil management practices (Gerdes et al., 2024). By addressing the specific barriers identified in this research, stakeholders can more effectively promote practices that enhance soil health and agricultural productivity.

The research results are relevant for policymakers in the agricultural domain and beyond. As summarized by Gerdes et al. (2024), key policy recommendations for the implementation of subsoil management in Germany include the expansion of support for alfalfa cultivation due to its multifunctional benefits, the development of value chains for innovative alfalfa products, the enhancement of collaboration for better knowledge transfer between researchers and agricultural practitioners, the clarification of legal

frameworks for subsoil intervention, and increased research funding to further explore subsoil management techniques across diverse conditions (Gerdes et al., 2024).

Future research should focus on increasing sample sizes to enhance statistical power and reliability. Additionally, expanding the diversity of regions and nations included in the analysis will facilitate a more comprehensive understanding of subsoil management practices across various contexts. Building on the results of this study, future investigations can explore how specific findings can be applied to improve subsoil management strategies. Incorporating geophysical conditions and relevant socio-economic criteria into these studies will yield valuable insights into the factors influencing the effectiveness of subsoil management approaches. By pursuing these avenues, subsequent research can significantly advance our understanding of sustainable agricultural practices and their broader implications for policy development and implementation.

REFERENCES

Abo-Habaga, M.M., Ismail, Z.E. & Okasha, M.H. 2022. Effect of tillage systems on soil moisture content and crops productivity. *Journal of Soil Sciences and Agricultural Engineering* **13**(8), 367–378. doi: 10.21608/jssae.2022.138432.1077

Ahrends, H.E., Siebert, S., Rezaei, E.E., Seidel, S.J., Hüging, H., Ewert, F., Döring, T., Rueda-Ayala, V., Eugster, W. & Gaiser, T. 2021. Nutrient supply affects the yield stability of major European crops – a 50-year study. *Environmental Research Letters* **16**, 014003. doi: 10.1088/1748-9326/abc849

Alcántara, V., Don, A., Well, R. & Nieder, R. 2016. Deep ploughing increases agricultural soil organic matter stocks. *Global Change Biology* 22. doi: 10.1111/gcb.13289

Askari, M., Abbaspour-Gilandeh, Y., Taghinezhad, E., El Shal, A.M., Hegazy, R. & Okasha, M. 2021. Applying the Response Surface Methodology (RSM) approach to predict the tractive performance of an agricultural tractor during semi-deep tillage. *Agriculture* **11**, 11043. doi: 10.3390/agriculture11111043

Bauke, S.L., Seidel, S.J., Athmann, M., Berns, A.E., Braun, M., Gocke, M.I., Guigue, J., Kautz, T., Kögel-Knabner, I., Ohan, J., Rillig, M., Schloter, M., Schmittmann, O., Schulz, S., Uhlig, D., Schnepf, A. & Amelung, W. 2024. Short-term effects of subsoil management by strip-wise loosening and incorporation of organic material. *Soil & Tillage Research* **236**, 105936. doi: 10.1016/j.still.2023.105936

Bianco, A. & Martínez, E. 2009. Robust testing in the logistic regression model. *Computational Statistics & Data Analysis*. doi: 10.1016/j.csda.2009.04.015

Botta, G.F., Ezquerra-Canalejo, A., Romay, C.Rivero, D. & Hidalgo, R.J. 2024. The agricultural traffic effects on soil and maize (Zea mays L.) yields under two different tillage systems. *Agronomy Research* **22**(3), 1098–1110. doi: 10.15159/AR.24.054

Cai, H., Ma, W., Zhang, X., Ping, J., Yan, X., Liu, J., Yuan, J., Wang, L. & Ren, J. 2014. Effect of subsoil tillage depth on nutrient accumulation, root distribution, and grain yield in spring maize. *Crop Journal* **2**, 297–307. doi: 10.1016/j.cj.2014.04.006

Cameron, A.C. & Trivedi, P.K. 2016. *Microeconometrics: Methods and Applications*. Cambridge University Press.

De Jong, V.M., Eijkemans, M., Van Calster, B., Timmerman, D., Moons, K., Steyerberg, E. & Van Smeden, M. 2018. Sample size considerations and predictive performance of multinomial logistic prediction models. *Statistics in Medicine*. doi: 10.1002/sim.8063

Frelih-Larsen, A., Hinzmann, M. & Ittner, S. 2018. The 'invisible' subsoil: an exploratory view of societal acceptance of subsoil management in Germany. *Sustainability* **10**, 3006. doi: 10.3390/su10093006

Friedman, J., Hastie, T. & Tibshirani, R. 2019. *The Elements of Statistical Learning: Data Mining, Inference, and Prediction* (2nd Ed.). Springer. doi:10.1007/978-0-387-84858-7

Gailis, J., Turka, I. & Ausmane, M. 2017. Soil tillage and crop rotation differently affect biodiversity and species assemblage of ground beetles inhabiting winter wheat fields. *Agronomy Research* **15**(1), 94–111.

Gaiser, T., Perkons, U., Küpper, P.M., Puschmann, D.U., Peth, S., Kautz, T., Pfeifer, J., Ewert, F., Horn, R. & Köpke, U. 2012. Evidence of improved water uptake from subsoil by spring wheat following lucerne in a temperate humid climate. *Field Crops Research* **126**, 56–62. doi: 10.1016/j.fcr.2011.09.019

Gerdes, H., Kiresiewa, Z., Schneider, C., Dengler, F. & Ittner, S. 2024. Empfehlungen für die Umsetzung eines nachhaltigen und gesellschaftlich akzeptierten Unterbodenmanagements in Deutschland. *Ecologic Institute*, Berlin. https://www.ecologic.eu/de/19850

Grange, L.D., González, F., Marechal, M. & Troncoso, R. 2024. Estimating multinomial logit models with endogenous variables: Control function versus two adapted approaches. *Transportation Research Part A: Policy and Practice* **183**, 104068. doi: 10.1016/j.tra.2024.104068

Greene, W.H. 2018. *Econometric Analysis* (8th Ed.). Pearson, 1168 pp. doi:99.150005/9781292231150

Guedioura, I., Rahmoune, B., Khezzaren, A., Dahoumane, A. & Laouar, N. 2023. Combined effect of soil practices and chemical treatments on weeds growth, soil features, and yield performance in field wheat crop under Mediterranean climate. *Agronomy Research* **21**(1), 62–77. doi: 10.15159/AR.22.079

Heckman, J.J. & Leamer, E. 2007. *Handbook of Econometrics*. 1st Edition, Volume 6B. https://shop.elsevier.com/books/handbook-of-econometrics/heckman/978-0-444-53200-8

Hensher, D.A., Rose, J.M. & Greene, W.H. 2015. *Applied Choice Analysis: A Primer* (2nd Ed.). Cambridge University Press. doi: 10.1017/CBO9781316136232

Hinzmann, M., Ittner, S., Kiresiewa, Z. & Gerdes, H. 2021. An acceptance analysis of subsoil amelioration amongst agricultural actors in two regions in Germany. *Frontiers in Agronomy* **3**, 660593. doi: 10.3389/fagro.2021.660593

Jong, V.M., Eijkemans, M.J.C., Van Calster, B., Timmerman, D., Moons, K.G.M., Steyerberg, E.W. & Van Smeden, M. 2019. Sample size considerations and predictive performance of multinomial logistic prediction models. *Statistics in Medicine* **38**(9). doi: 10.1002/sim.8063

Kautz, T., Amelung, W., Ewert, F., Gaiser, T., Horn, R., Jahn, R., Javaux, M., Kemna, A., Kuzyakov, Y., Munch, J.-C., Pätzold, S., & Köpke, U. 2013. Nutrient acquisition from arable subsoils in temperate climates: A review. *Soil Biology and Biochemistry* **57**, 1003–1022. doi: 10.1016/j.soilbio.2012.09.014

Li, B. 2011. The multinomial logit model revisited: A semiparametric approach in discrete choice analysis. *Transportation Research Part B: Methodological* **45**(3), 461–473. doi: 10.1016/j.trb.2010.09.007

Li, G., Song, H. & Witt, S.F. 2005. Recent developments in econometric modelling and forecasting. *Journal of Travel Research* **44**(1), 82–99. doi: 10.1177/0047287505276594

Lynch, J.P. & Wojciechowski, T. 2015. Opportunities and challenges in the subsoil: Pathways to deeper rooted crops. *Journal of Experimental Botany* **66**, 2199–2210. doi: 10.1093/jxb/eru508

Maddala, G.S. & Lahiri, K. 2009. *Introduction to Econometrics* (4th Ed.). Wiley & Sons Publishers. https://www.researchgate.net/publication/263188812_Introduction_to_Econometrics_4th_edition

Midi, H., Sarkar, S.K. & Rana, S. 2010. Collinearity diagnostics of binary logistic regression model. *Journal of Interdisciplinary Mathematics*. doi: 10.1080/09720502.2010.10700699

Rainey, C. & McCaskey, K. 2021. Estimating logit models with small samples. *Political Science Research and Methods* **9**(3), 549–564. doi: 10.1017/psrm.2021.9

Rumpel, C., Chabbi, A. & Marschner, B. 2012. Carbon storage and sequestration in subsoil horizons: Knowledge, gaps and potentials. In: Lal, R., Lorenz, K., Hüttl, R., Schneider, B. & von Braun, J. (eds). *Recarbonization of the Biosphere*. Springer, Dordrecht. doi: 10.1007/978-94-007-4159-1_20

Schjønning, P., Van den Akker, J.H., Keller, T., Greve, M.H., Lamandé, M., Simojoki, A., Stettler, M., Arvidsson, J. & Breuning-Madsen, H. 2015. Chapter Five - Driver-Pressure-State-Impact-Response (DPSIR) analysis and risk assessment for soil compaction - a European perspective. In: Sparks, D.L. (ed.). *Advances in Agronomy* **133**, 183–237. Academic Press. doi: 10.1016/bs.agron.2015.06.001

Schmittmann, O., Christ, A. & Schulze Lammers, P. 2021. Subsoil melioration with organic material - principle, technology and yield effects. *Agronomy* **11**(10), 1970. doi: 10.3390/agronomy11101970

Schneider, C., Kiresiewa, Z., Gerdes, H., Pazmino Murillo, J., Skadell, L., Sakhaee, A., Schneider, F. & Don, A. 2024. Regionalised factors for successful implementation of subsoil management: Evidence from three case studies in Germany. *BonaRes Series*. doi: 10.20387/BonaRes-n2ta-v096

Schneider, F. 2020. *Root-restricting layers in German agricultural soils: Extent, cause and management strategies.* Dissertation, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn. Online-Ausgabe in bonndoc. https://nbn-resolving.org/urn:nbn:de:hbz:5-60043

Schneider, F. & Don, A. 2019a. Root-restricting layers in German agricultural soils. Part I: Extent and cause. *Plant and Soil* **442**, 433–451. doi: 10.1007/s11104-019-04185-9

Schneider, F. & Don, A. 2019b. Root-restricting layers in German agricultural soils. Part II: Adaptation and melioration strategies. *Plant and Soil* **442**, 419–432.

Schneider, F., Don, A., Hennings, I., Schmittmann, O. & Seidel, S.J. 2017. The effect of deep tillage on crop yield – what do we really know? *Soil & Tillage Research* **174**, 193–204. doi: 10.1016/j.still.2017.07.005

Train, K.E. 2002. *Discrete Choice Methods with Simulation* (3rd Ed.). Cambridge University Press. https://eml.berkeley.edu/books/train1201.pdf

Tutz, G. 2021. Uncertain choices: The heterogeneous multinomial logit model. *Sociological Methodology* **51**(1), 86–111. doi: 10.1177/0081175020979689

Winkelmann, R. 2008. *Econometric Analysis of Count Data* (5th Ed.). Springer-Verlag, Berlin Heidelberg. ISBN 978-3-540-77648-2. doi: 10.1007/978-3-540-78389-3

Wooldridge, J.M. 2016. *Introductory Econometrics: A Modern Approach* (6th Ed.). Cengage Learning. https://www.scirp.org/reference/referencespapers?referenceid=3086091

World Weather Attribution (WWA). 2024. 10 years of rapidly disentangling drivers of extreme weather disasters. Otto, F., Clarke, B., Barnes, C., Kimutai, J., Zachariah, M., Merz, N., Vrkic, D., Philip, S., Kew, S., Pinto, I., Vahlberg, M., Singh, R., Horne, Z., Arrighi, J., Sparks, N., Giguere, J. & Gilford, D. *Grantham Institute for Climate Change*. doi: 10.25561/115431

Liming alone is not enough: The role of phosphorus and boron in supporting legume pasture growth

P. Aguiar[1,2], M. Arrobas[2,3], C. Aguiar[2,3], N. Rodrigues[4], M. Jongen[4,5],
R.F.M. Teixeira[5], T. Domingos[5] and M.Â. Rodrigues[2,3,*]

[1]Instituto Superior Politécnico do Cuanza Sul (ISPCS), Rua 12 de Novembro, Sumbe P.O. Box 82, Angola
[2]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[3]Laboratório para a Sustentabilidade e Tecnologia em Regiões de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[4]Terraprima – Serviços Ambientais, Centro de Negócios do Porto Alto, Fracção S, Avenida das Nações Unidas, nº 97, 2135-199 Samora Correia, Portugal
[5]MARETEC − Marine, Environment and Technology Centre, LARSyS, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1, 1049-001 Lisbon, Portugal
[*]Correspondence: angelor@ipb.pt

Abstract. Soil fertility management is a critical factor for ensuring the persistence of the legume component in improved pastures in Mediterranean regions. To promote legume growth, the most common practices include the application of lime to correct Soil acidity and phosphate fertilizers to address phosphorus (P) deficiencies. This study examined two acidic soils and evaluated the need for P and boron (B) supplementation following liming. A factorial pot experiment (Soil type × vegetation type × fertilizer treatment) was conducted using subterranean clover (*Trifolium subterraneum* L.), annual ryegrass (*Lolium multiflorum* Lam.), and their mixture. Results revealed significantly higher dry matter yield (DMY) for the legume (18.1 g pot^{-1}) compared to the grass (5.9 g pot^{-1}), due to the legume's ability to access atmospheric nitrogen (N). Soil 2, which had higher initial P availability than Soil 1, and P fertilization both enhanced N accumulation in legume tissues, the percentage of N derived from the atmosphere (%Ndfa), and DMY. These outcomes indicate that liming alone was insufficient to increase P availability and eliminate the need for P fertilization. For instance, DMY increased from 15.0 g pot^{-1} with liming alone to 19.4 g pot^{-1} when P was supplemented. Additionally, B application increased N uptake and %Ndfa in the legume, with the latter rising from 48.9% in the liming-only control to 77.5% with B supplementation. These results suggest that liming, by increasing Soil pH, may reduce B bioavailability, highlighting the need for B supplementation following lime application.

Key words: annual ryegrass, biological nitrogen fixation, dry matter yield, Mediterranean pastures, subterranean clover, %Ndfa.

INTRODUCTION

Grasslands play a central role in animal nutrition and form the foundation of livestock production systems across vast regions of the world. In recent decades, improved pastures have been expanding globally as a response to the growing need for more productive agricultural systems, both in intensive and extensive forms of pasture-based livestock farming (Hayes et al., 2017; Badgery et al., 2024). The concept of improved pastures is primarily based on the introduction of high-yielding species, with particular emphasis on legumes, due to their capacity to access atmospheric N, thereby reducing reliance on synthetic fertilizers (Ovalle et al., 2006; Teixeira et al., 2015; Monjardino et al., 2022).

The N introduced into the system by legumes can also benefit associated non-leguminous species, thus enhancing the overall productivity of the pasture (Riday & Albrecht, 2012; Monjardino et al., 2022). However, ensuring the persistence of legume species remains a challenge, requiring grazing management strategies that support successful seed production and natural reseeding (Nyfeler et al., 2009; Hayes et al., 2017). In addition to grazing strategies, several Soil management practices have also been recommended to enhance legume persistence, with liming and P application being the most prominent (Moir et al., 2016; Morton, 2020; McLachlan et al., 2024). Nonetheless, under specific conditions, the role of other nutrients should not be underestimated (Bellaloui et al., 2014; Hamilton et al., 2015; Zhou et al., 2023).

Soil acidity represents a major constraint on plant productivity, given the widespread prevalence of acidic soils globally, with liming being a widely adopted corrective practice (Upjohn et al., 2005; Holland et al., 2018; Hackney et al., 2019). Soil acidity impairs plant growth primarily due to the toxicity of aluminum (Al^{3+}), and in some cases, manganese (Mn^{2+}), which are present in high concentrations in the Soil solution. Additionally, deficiencies in essential cations such as calcium (Ca^{2+}), and in some cases magnesium (Mg^{2+}), also contribute to reduced plant performance under acidic conditions (Kochian et al., 2004; Weil & Brady, 2017). Once applied, agricultural lime reacts with carbon (C) dioxide in the Soil atmosphere and with water, forming calcium (Ca) and magnesium (Mg) bicarbonates. These compounds neutralize both exchangeable and residual acidity. Acidic ions in the Soil solution and adsorbed onto Soil colloids, namely H^+, Al^{3+}, and Mn^{2+}, are displaced by basic cations (Ca^{2+} and Mg^{2+}), leading to the precipitation of aluminum (Al) and manganese (Mn) as their respective hydroxides [$Al(OH)_3$ and $Mn(OH)_2$], thereby increasing Soil pH (Weil & Brady, 2017). In improved pastures enriched with legumes, liming, in conjunction with appropriate grazing management, is a key strategy to enhance both productivity and the persistence of the legume component (Moir et al., 2016; Morton, 2020; McLachlan et al., 2024). Legumes are particularly sensitive to Soil acidity, as they tend to have higher Ca requirements than grasses, and nodulation is highly susceptible to Al toxicity and Ca deficiency (Morton, 2020; Yang et al., 2021; Hungria & Nogueira, 2023).

In legume-rich pastures, P application is a key management practice, as liming may correct Soil acidity for several years, while P typically needs to be applied annually (Olson-Rutz & Jones, 2015; Teixeira et al., 2015; McLachlan et al., 2024). P availability in the Soil is crucial not only for plant growth, but also for the development and

functioning of root nodules, thereby facilitating biological N fixation and providing legumes with a competitive advantage over grasses (Maxwell et al., 2013; Mitran et al., 2018). Acidic soils are often associated with low levels of available P (Upjohn et al., 2005; Arrobas et al., 2023a). P availability in highly acidic soils is reduced due to its strong affinity for iron (Fe) and Al oxides, leading to its precipitation as insoluble compounds such as $AlPO_4$ and $FePO_4$ (Weil & Brady, 2017). Thus, alleviating Soil acidity through liming may be sufficient to mobilize previously precipitated P, potentially rendering additional P fertilization redundant in the years following lime application.

B has been reported as one of the most significant plant nutritional disorders in inland regions of Portugal (Portela et al., 2015; Arrobas et al., 2024). This issue is most prevalent in dicot species, which generally have higher B requirements than monocots. B plays a critical role in cell wall biosynthesis, particularly through its binding with cis-diol-containing compounds, which are more prevalent in dicots than in monocots (Behera et al., 2023; Cakmak et al., 2023). Since pasture systems often aim to maintain a balance between legumes (dicots) and grasses (monocots), B may represent a key factor in effective pasture management. The importance of B in the growth and development of legume root nodules, and thus in biological N fixation, has also been well documented (Bellaloui et al., 2014; Hamilton et al., 2015; Hackney et al., 2019). Conversely, lime application can induce B deficiency in plants (Arrobas et al., 2023b), as at higher pH levels, B becomes more tightly bound to Soil particles, reducing its availability. Furthermore, plants may exhibit an increased demand for B when Ca is abundant (Weil & Brady, 2017), a situation that may compromise the persistence and productivity of the legume component in pasture systems.

Since liming is generally regarded as a routine practice in acidic soils, this study seeks to determine whether liming is sufficient to enhance P availability to the point of eliminating the need for its direct application. Additionally, the study examines whether liming interferes with the bioavailability of other nutrients, particularly B, as previous research in other crops (Arrobas et al., 2023b) has shown that liming can induce severe B deficiency. Thus, the experimental design included liming as the control treatment, with three additional treatments: lime with P addition, lime with B addition, and lime with both P and B additions. The experiment was conducted under controlled conditions using pots and included three vegetation types: subterranean clover, annual ryegrass, and a mixture of both species. Two acidic soils of different origins were tested.

The main working hypotheses were as follows: i) the addition of P is not necessary when Soil is limed, due to increased P availability; and ii) the supply of B is essential, particularly for the legume component, due to the risk of B deficiency induced by liming.

MATERIALS AND METHODS

General experimental conditions

The pot experiment was conducted at the agricultural experimentation unit of the Polytechnic Institute of Bragança, located at coordinates 41°47'49.96" N, 6°45'42.90" W, at an altitude of 670 m above sea level. Sowing was done on September 19, 2023, and harvest was on April 29, 2024. The region is characterized by a Mediterranean climate with Atlantic influence. According to the Köppen-Geiger climate classification, the region is categorized as Csb, which denotes cold, rainy winters and hot, dry summers

(IPMA, 2025). The mean annual temperature is 12.9 °C, and the average annual precipitation amounts to 790.0 mm. The monthly average temperature and precipitation values recorded during the experimental period are presented in Table 1.

Table 1. Monthly mean air temperature and precipitation values during the experimental period, alongside the regional climatological normal (1991–2020) (IPMA, 2025)

		Temperature (ºC)		Precipitation (mm)	
		Normal	2023/2024	Normal	2023/2024
2023	September	18.3	17.0	43.9	87.6
	October	13.4	14.7	106.9	212.8
	November	8.2	10.2	91.8	126.8
	December	5.4	4.5	112.3	70.0
2024	January	4.8	6.3	106.6	107.0
	February	6.3	8.1	62.4	86.2
	March	9.3	8.6	68.6	122.2
	April	11.3	11.8	68.0	57.4
	May	14.8	13.4	63.4	35.6
	June	19.0	18.4	32.3	85.8

Experimental design, description, and trial management

The experiment was arranged as a factorial design comprising three factors: Soil type, vegetation type, and fertilisation treatments. Each combination of factors was replicated three times. Two Soil types were used: Soil 1, collected from a seminatural lowland pasture in central Portugal, and Soil 2, sourced from a seminatural mountain pasture in northern Portugal. The plant species selected for this study were subterranean clover (*Trifolium subterraneum* ssp. *subterraneum*, cv. Denmark), annual ryegrass (*Lolium multiflorum* Lam., cv. Falladino), and a mixture of the two species. Calcium carbonate was applied as a liming treatment (Lime) to address the high Soil acidity, and in this experiment, it was considered the control treatment. The additional treatments included: Lime plus P (Lime + P), Lime plus B (Lime + B), and Lime combined with both P and B (Lime + P + B). In total, the experiment comprised 72 pots, resulting from the combination of two soils, three vegetation types, four treatments, and three replicates.

Soil 1 is classified as a Dystric Leptosol, while Soil 2 corresponds to an Umbric Dystric Leptosol (WRB, 2022). Selected physical and chemical properties of the soils, determined from samples collected at a depth of 0–15 cm shortly before the onset of the trial, are presented in Table 2.

Laboratory-grade calcium carbonate (neutralizing value 100% as $CaCO_3 \approx 56\%$ as CaO) was applied to each Soil at rates adjusted according to initial pH to reach pH 6.5. This value is considered optimal for maximizing the bioavailability of key Soil nutrients, including P (Weil & Brady, 2017). The estimation of the required calcium carbonate dose was based on Soil pH, texture, and organic matter content, following the standardized procedures outlined by Veloso et al. (2022). For Soil 1, the application rate was estimated at 4,000 kg ha⁻¹, whereas for Soil 2 it was 7,000 kg ha⁻¹. To translate these hectare-based values into the amounts required for pot experiments, it was considered that each pot contained 3 kg of oven-dried fine soil, while one hectare corresponds to 2,000 t of soil. Accordingly, the final application rates per pot were determined as 7 g for Soil 1 and 12 g for Soil 2, respectively. P was applied at a rate equivalent to 80 kg P_2O_5 ha⁻¹

(37.4 kg P ha^{-1}). Considering the surface area of each pot (0.022 m²), this corresponded to 0.076 g of P (0.174 g P$_2$O$_5$) per pot, supplied as 1 g pot^{-1} of single superphosphate (18% P$_2$O$_5$). Using a similar calculation, B was applied at a rate of 1.5 kg ha^{-1}, corresponding to 0.02 g pot^{-1} of Neobor (14.9% B as disodium tetraborate pentahydrate).

Table 2. Selected physical and chemical Soil properties (mean ± standard deviation, $n = 3$) of Soil samples collected at a depth of 0–15 cm shortly before the onset of the trial, from the lowland pasture (Soil 1) and the mountain pasture (Soil 2)

	Soil 1	Soil 2
[1]Clay (g kg^{-1})	12.4 ± 0.08	14.8 ± 0.27
[1]Silt (g kg^{-1})	12.4 ± 0.23	10.7 ± 2.07
[1]Sand (g kg^{-1})	75.3 ± 0.31	74.5 ± 1.89
Texture (USDA)	Sandy loam	Sandy loam
[2]pH (H2O)	5.4 ± 0.06	5.0 ± 0.10
[3]Organic carbon (g kg^{-1})	17.7 ± 0.29	50.8 ± 3.32
[4]Total nitrogen (g kg^{-1})	1.4 ± 0.13	3.04 ± 0.25
[5]Extractabe phosphorus (mg kg^{-1})	33.4 ± 1.54	106.4 ± 9.32
[5]Extractable potassium (mg kg^{-1})	108.4 ± 2.94	312.5 ± 78.59
[6]Exchangeable calcium (cmol$_c$ kg^{-1})	1.87 ± 0.14	4.40 ± 0.49
[6]Exchangeable magnesium (cmol$_c$ kg^{-1})	0.61 ± 0.03	1.25 ± 0.12
[6]Exchangeable potassium (cmol$_c$ kg^{-1})	0.45 ± 0.02	1.20 ± 0.24
[6]Exchangeable sodium (cmol$_c$ kg^{-1})	0.13 ± 0.01	0.25 ± 0.11
[6]Exchangeable acidity (cmol$_c$ kg^{-1})	0.70 ± 0.00	0.43 ± 0.12
[7]CEC (cmol$_c$ kg^{-1})	3.76 ± 0.15	7.54 ± 0.79
[8]Extractable boron (mg kg^{-1})	0.72 ± 0.47	1.05 ± 0.22

[1]Robinson pipette method; [2]Potentiometry; [3]Walkley-Black; [4]Kjeldahl; [5]Ammonium lactate (Egner- Riehm); [6]Ammonium acetate; [7]Cation exchange capacity; [8]Hot water, azomethine-H.

The fertilizers corresponding to each treatment were thoroughly mixed with 3 kg of air-dried fine Soil (< 2 mm) per pot. The seeding rate for each pot was based on the recommended field application rates for the selected species, namely 20 kg ha^{-1} for subterranean clover and 40 kg ha^{-1} for annual ryegrass. The seed quantities per pot were estimated based on the surface area of the pots, resulting in the application of 44 mg and 88 mg of subterranean clover and annual ryegrass seeds, respectively. In the mixed-species treatment, half of each seed dose was used. Seeds were sown at a depth of 1 cm.

Irrigation was provided whenever rainfall was insufficient to support regular plant growth. During the initial weeks, weeds were manually removed whenever distinguishable from the sown species, to maintain uniformity across the vegetation samples to be analysed. Pots were surrounded by wooden stakes to shield their sides from direct sunlight and prevent overheating. To ensure uniform exposure to solar radiation, pot positions were rotated weekly.

Sampling and laboratory analyses

Plants were harvested near ground level at the advanced flowering stage of the legume and the early heading stage of the grass, 212 days after sowing. After harvesting, the samples were briefly washed in clean water to remove any Soil or mineral debris attached to the plants. Subsequently, the samples were dried in a forced-air oven set to 70 °C, weighed, and ground using a 1 mm mesh sieve.

The samples were subjected to elemental analysis. N was determined by the Kjeldahl method, while B and P were determined by colorimetry. Potassium (K), Ca, Mg, Mn, Fe, copper (Cu), and zinc (Zn) were determined by atomic absorption spectrophotometry following digestion of the samples with nitric acid in a microwave oven (Temminghoff & Houba, 2004).

Initial Soil samples were air-dried and passed through a 2 mm sieve. Soil texture was analysed using the Robinson pipette method. Soil pH was determined potentiometrically in a 1:2.5 soil-to-solution ratio. Organic C content was assessed by wet oxidation following the Walkley-Black method. Soil N was determined using the Kjeldahl method. Extractable P and K were determined using the ammonium lactate method (Egner-Riehm), while exchangeable bases were accessed with the ammonium acetate method. B was determined using the hot-water extraction method followed by azomethine-H colorimetry. All these analytical procedures are fully described in van Reeuwijk (2002).

Data analysis

The data analysis was conducted using the statistical software SPSS Statistics (version 25, IBM SPSS, Armonk, NY, USA). Normality and homogeneity of variances were initially assessed using the Shapiro-Wilk test and Levene's test, respectively. The effects of the treatments were compared using a three-way analysis of variance (ANOVA), with factors Soil type, vegetation type, and fertilisation treatment. When significant interactions were observed for relevant results, a two-way ANOVA was applied separately for each vegetation type, considering only the factorial combination of Soil type × fertiliser treatment. When significant differences between treatments were found ($P < 0.05$), means were separated using the Tukey *HSD* test ($\alpha = 0.05$).

The %Ndfa was estimated using the difference method, which compares the N content in legume tissues to that found in the non-legume species (Unkovich et al., 2008):

$$\%Ndfa = \frac{N_{legume} - N_{non-legume}}{N_{legume}} \cdot 100 \qquad (1)$$

RESULTS

Dry matter yield

The results showed a significant interaction among most of the factors under study, although within each factor, significant differences were also observed (Fig. 1). DMY was significantly higher in Soil 2 (14.8 g pot^{-1}) compared to Soil 1 (10.7 g pot^{-1}). Regarding vegetation types, the legume exhibited the highest DMY (18.1 g pot^{-1}), significantly surpassing that of the mixture (14.3 g pot^{-1}), which in turn was significantly greater than that of the grass (5.9 g pot^{-1}). Among treatments, those that received P showed significantly higher values compared to the control (11.2 g pot^{-1}), particularly the Lime + P (13.2 g pot^{-1}) and Lime + P + B (14.2 g pot^{-1}) treatments.

Figure 1. Dry matter yield (DMY) as a function of Soil type, vegetation type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (P) values from a three-way ANOVA and interactions between all factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.
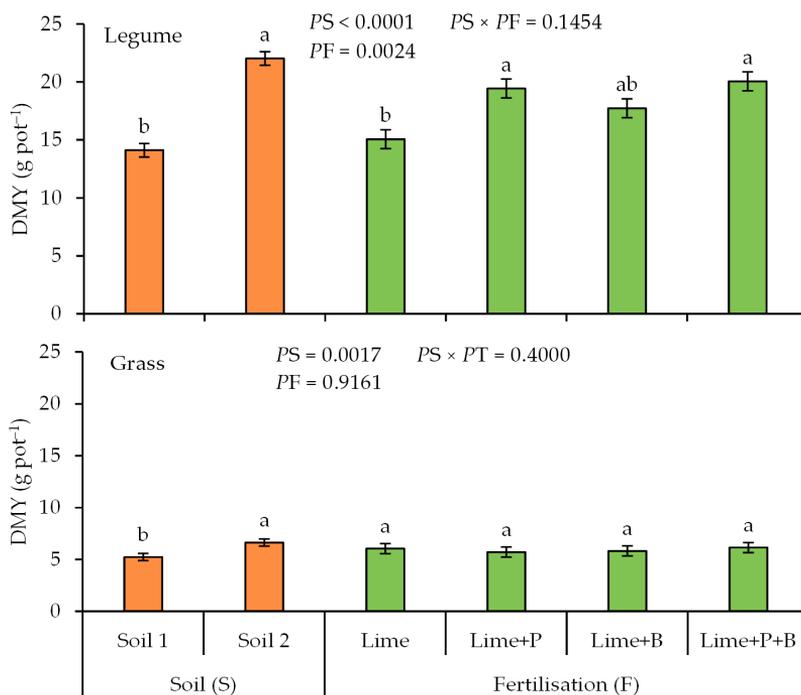
Figure 2. Dry matter yield (DMY) of legume and grass as a function of Soil type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P+B]. The probability (P) values from a two-way ANOVA and interactions between factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

Nitrogen concentration and uptake in plant tissues

The analysis of N concentration in plant tissues also revealed significant interactions among some of the factors studied, although not in the three-way combination (Fig. 3). Soil type did not influence tissue N concentration. Legume exhibited the highest N concentration (24.1 g kg⁻¹), followed by the mixture (22.2 g kg⁻¹), and grass (19.0 g kg⁻¹). This finding suggests that vegetation type had a greater impact on N acquisition than Soil type, likely due to the legume's ability to access atmospheric N. Fertilizer treatments significantly affected tissue N concentration, with the control showing the lowest values

Figure 3. Tissue nitrogen (N) concentration as a function of Soil type, vegetation type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (P) values from a three-way ANOVA and interactions between all factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

Due to the significant interaction observed and to allow for a clearer discrimination among factors and factor levels, a two-way ANOVA was conducted separately for each vegetation type (Fig. 4). Soil type did not significantly influence N concentration in the tissues of either species. In legume, fertilizer treatments that included P, B, or both nutrients led to significantly higher tissue N concentrations compared to the control. In grass, no significant effect of fertilizer treatments on N concentration was observed

The amount of N recovered in the aboveground plant biomass exhibited a response pattern distinct from that observed for tissue N concentration (Fig. 5). Soil had a marked effect on N uptake, with Soil 2 showing the highest mean value, possibly due to the greater DMY observed under cultivation in this soil. Differences among vegetation types were further accentuated, reflecting the combined and congruent effects of both tissue N concentration and DMY. Regarding fertilizer treatments, the contrast with the control became more pronounced, with treatments receiving P standing out as having the highest N uptake.
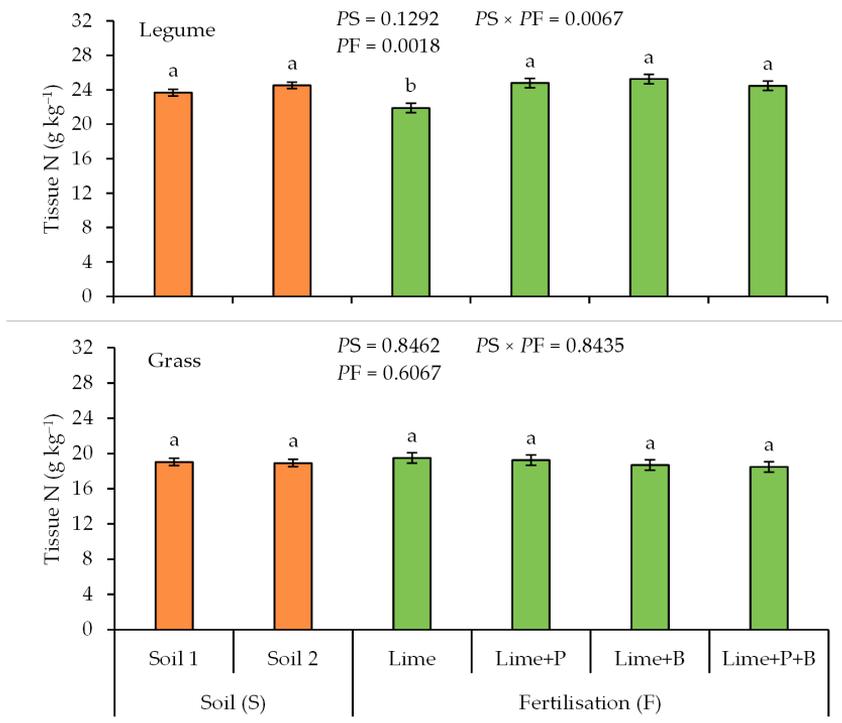
Figure 4. Tissue nitrogen (N) concentration in legume and grass as a function of Soil type and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (P) values from a two-way ANOVA and interactions between factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.
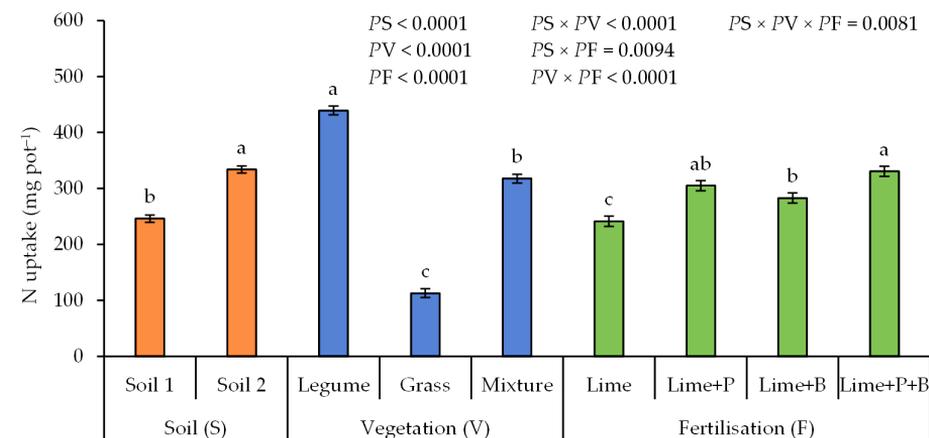
Figure 5. Nitrogen (N) uptake as a function of Soil type, vegetation type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (P) values from a three-way ANOVA and interactions between all factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

The %Ndfa was higher in Soil 2 compared to Soil 1 when the results were expressed in mg pot^{-1} (Table 3). Treatments that received P, B, or both nutrients also showed higher values of the %Ndfa compared to the control. Specifically, in the case of B, the %Ndfa values were particularly higher in Soil 1, which initially had lower levels of B and organic matter.

Table 3. Nitrogen derived from the atmosphere in legume pots subjected to various fertiliser treatments [Lime, Lime + Phosphorus (P), Lime + Boron (B), and Lime + P + B], expressed in mg per pot (mg pot^{-1}) and as a percentage (%)

	Lime	Lime + P	Lime + B	Lime + P + B	Average
	mg pot^{-1}				
Soil 1	109.0	225.9	299.7	308.8	235.8
Soil 2	324.2	533.7	371.1	438.6	416.9
	%				
Soil 1	48.9	68.6	77.5	75.7	70.0
Soil 2	72.5	82.0	73.6	77.5	76.9

Phosphorus concentration and uptake in plant tissues

No significant interaction among factors was observed for tissue P concentration (Fig. 6). P concentration in plant tissues was higher in Soil 1 than in Soil 2. Among vegetation types, the grass exhibited the highest P concentration, followed by the mixture, and then the legume. Fertilizer treatments did not significantly influence tissue P concentration.

PS = 0.0492 PS × PV = 0.3774 PS × PV × PF = 0.4242
PV < 0.0001 PS × PF = 0.7944
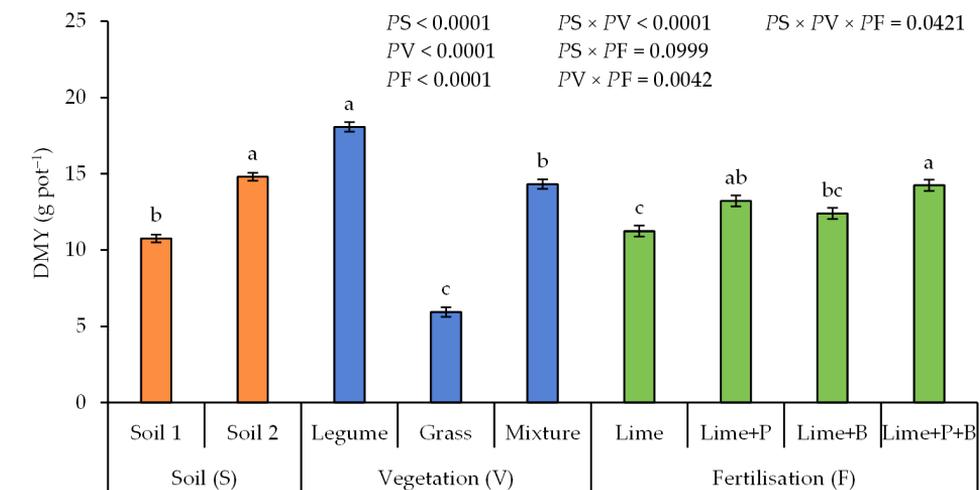PF = 0.4161 PV × PF = 0.6236
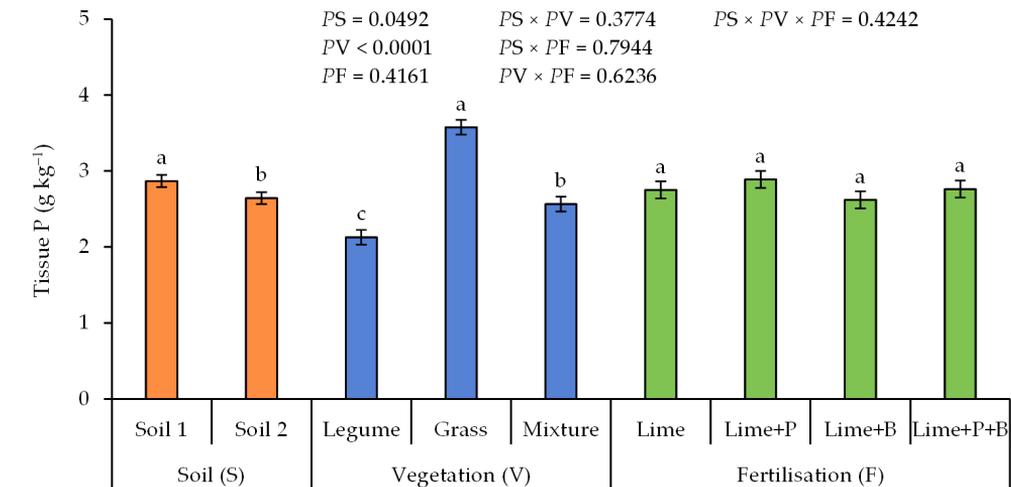
Figure 6. Tissue phosphorus (P) concentration as a function of Soil type, vegetation type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (*P*) values from a three-way ANOVA and interactions between all factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test (α = 0.05). Error bars represent standard errors.

The amount of P recovered in the aboveground plant biomass was higher in Soil 2, displaying an inverse trend relative to tissue P concentration (Fig. 7). This was attributed to the greater DMY observed in Soil 2. P uptake was also higher in the legume than in the grass, again contrasting with the pattern of tissue P concentration, due to the higher DMY of the legume. Among fertilizer treatments, P application, with or without B, stood out as having the most pronounced effect on P recovery in plant biomass.
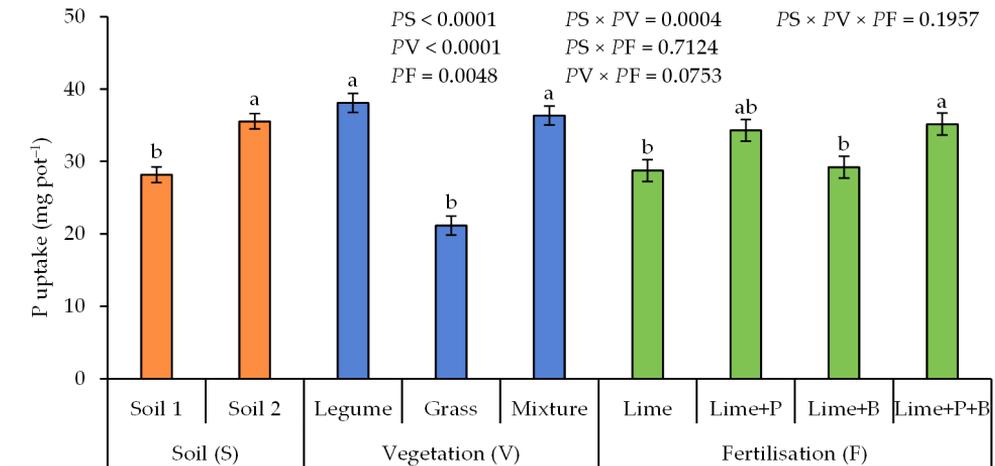
Figure 7. Phosphorus (P) uptake as a function of Soil type, vegetation type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (*P*) values from a three-way ANOVA and interactions between all factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

Boron concentration and uptake in plant tissues

Tissue B concentration was significantly higher in plants grown in Soil 1 compared to those grown in Soil 2 (Fig. 8). The legume exhibited significantly higher tissue B levels than the mixture, which in turn had higher levels than grass. Treatments that included B resulted in higher tissue B concentrations than those that did not receive B.
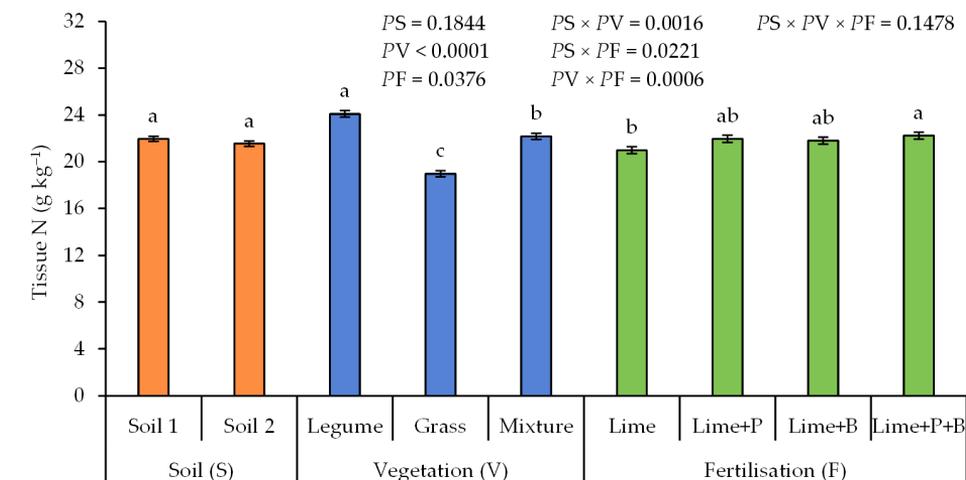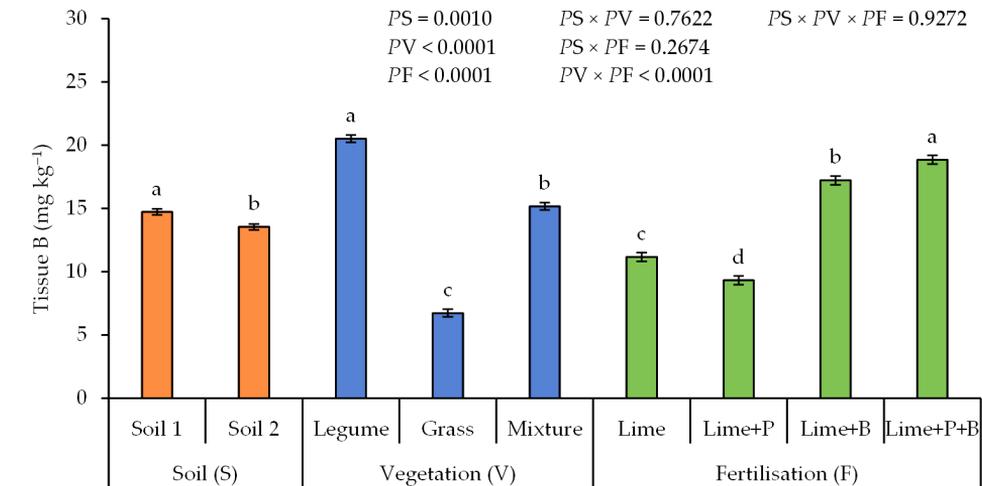

Figure 8. Tissue boron (B) concentration as a function of Soil type, vegetation type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (*P*) values from a three-way ANOVA and interactions between all factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

The total amount of B recovered by the vegetation was higher in Soil 2 compared to Soil 1 (Fig. 9), contrary to the pattern observed for tissue B concentration. In contrast, the patterns observed for vegetation type and fertilizer treatments were maintained; however, the differences among factors were amplified due to their combined effect with DMY.
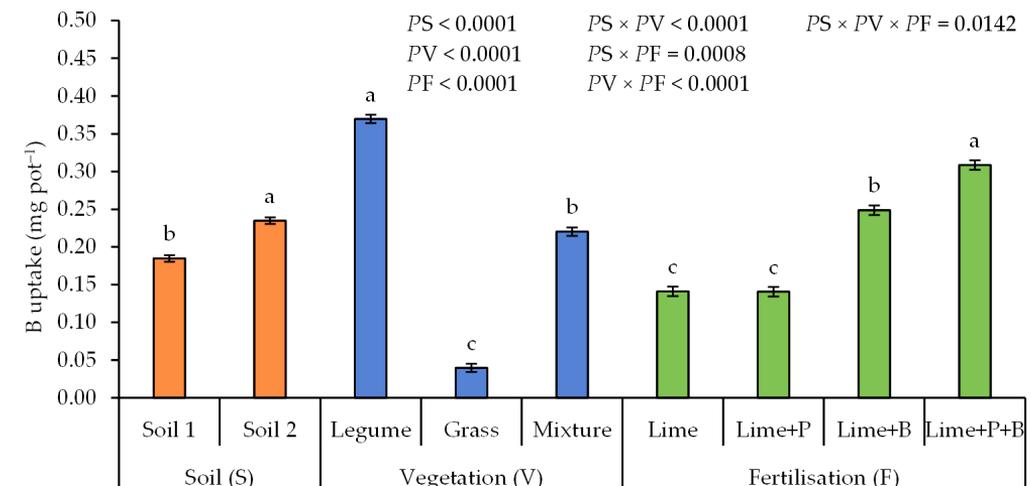
Figure 9. Boron (B) uptake as a function of Soil type, vegetation type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (*P*) values from a three-way ANOVA and interactions between all factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

DISCUSSION

Effect of Soil type and plant species on dry matter yield

Soil type had a significant effect on the DMY of both grass and legume species, with consistently higher yields recorded in Soil 2 (Fig. 2). At the start of the experiment, Soil 2 exhibited superior fertility properties known to support plant productivity, including higher levels of organic matter, P, K, Ca, and Mg. Although Soil 2 was more acidic than Soil 1, the application of lime across all pots as a baseline (and control treatment) likely minimized pH-related differences. Thus, nutrient availability, rather than Soil pH, appears to have been the dominant factor influencing plant growth. Plants grown in Soil 2 recovered more N than those in Soil 1, likely reflecting the higher total N and organic matter contents, which may have enhanced N availability through mineralization. The soils used in the experiment were sieved, a process that improves aeration but also disrupts Soil aggregates, often increasing mineralization rates (Weil & Brady, 2017). The increased N release from organic matter in Soil 2 likely explains the greater DMY observed in the grass species, which typically depend more heavily on soil-available N (Dordas et al., 2019; Dimande et al., 2024).

For the legume species, a higher %Ndfa was observed when grown in Soil 2 (Table 3), which may indicate enhanced biological N fixation in this soil. Both species produced more biomass in Soil 2 than in Soil 1; however, the legume substantially outperformed the grass in terms of DMY. These findings highlight the importance of N availability as a key limiting factor for plant productivity, an established constraint in many agricultural soils (Weil & Brady, 2017; Hawkesford et al., 2023). Legumes, through their symbiotic association with N-fixing microorganisms of the Rhizobiaceae family, often produce greater biomass than non-legume species under comparable growth conditions, as consistently demonstrated in intercropping systems (Dordas et al., 2019; Dimande et al., 2024). Generally, the lower the availability of mineral N in the soil, the greater the competitive advantage of legumes over grasses, provided that essential conditions for nodulation and nodule activity are met (Aguiar et al., 2024; Akshit et al., 2024). This ability to access atmospheric N enables legumes to grow and achieve high productivity even in the absence of N fertilization (Rodrigues et al., 2015; Enriquez-Hidalgo et al., 2018; Aguiar et al., 2024).

Plant response to Soil phosphorus and phosphorus fertilization

P application increased DMY exclusively in the legume species (Fig. 2). Furthermore, P supplementation led to higher N concentrations in legume tissues, whereas no such effect was observed in the grass. Notably, P application also enhanced the %Ndfa in the legume across both Soil types when compared to the control (Table 3). When comparing soils, legumes cultivated in Soil 2 exhibited a higher %Ndfa. Overall, these results suggest that both native Soil P and P applied as fertilizer may have influenced nodule formation and functionality, thereby enhancing biological N fixation. This, in turn, indirectly contributed to the observed increase in DMY of the legume component. The fact that grass DMY did not respond to P application reinforces this interpretation. It is well-established that nodulated legumes have significantly higher P requirements than non-symbiotic plants due to P's critical role in various stages of the symbiotic N fixation process (Chen et al., 2023).

P is essential for maintaining energy metabolism within root nodules, supporting the energetically demanding reduction of atmospheric dinitrogen to ammonia and the subsequent assimilation into amino acids and ureides (Hawkesford et al., 2023; Hungria & Nogueira, 2023). Moreover, P is crucial for the biosynthesis of nucleic acids and phospholipids (O'Hara, 2001; Hungria & Nogueira, 2023). As a result, nodules function as strong P sinks and often contain P concentrations up to three times higher than those found in roots or shoots, particularly under P-limited conditions (Chen et al., 2023).

In response to P deficiency, many legumes have evolved adaptive strategies to access sparingly soluble P pools, including a range of morphological, biochemical, and metabolic adaptations that collectively enhance P acquisition (Buoso et al., 2022; Lambers, 2022). Remarkably, rhizobia mirror their host plant's response by upregulating genes involved in P uptake and metabolism (Sadowsky, 2005; Hungria & Nogueira, 2023).

Although Soil acidity was corrected in this study, a factor known to enhance P bioavailability (Moir et al., 2016; Morton, 2020; Bouray et al., 2022), and both soils, particularly Soil 2, were initially well-supplied with P, the legume still responded positively to P fertilization in terms of increased DMY. This finding underscores the

essential role of P fertilisation and corroborates previous studies that highlight its importance in promoting legume growth, an effect typically attributed to enhanced biological N fixation (Moir et al., 2016; Somavilla et al., 2021; McLachlan et al., 2024).

Plant response to boron application

B application had a modest effect on DMY, although in the case of the legume, the increase was consistent (Fig. 2). Supporting this observation, B supplementation significantly increased N concentration in legume tissues, but not in the grass. Furthermore, B application clearly enhanced the %Ndfa compared to the control, with this effect being particularly pronounced in Soil 1, which had lower initial B content and lower organic matter levels. Although nodule counts were not performed and nodule activity was not assessed, this sequence of results provides strong evidence that B favored nodule formation and/or functionality, thereby enhancing the plant's access to atmospheric N and subsequently promoting vegetative growth, a relationship that has been well documented (Cakmak et al., 2023; Hungria & Nogueira, 2023).

B plays a critical role in the establishment and functionality of legume-rhizobia symbiosis. It is essential for the formation of infection threads and the successful invasion of root cortical cells by rhizobia. Under B-deficient conditions, the adhesion of rhizobial cells to the infection thread wall is impaired, hindering bacterial progression through the thread and ultimately preventing colonization of the host plant (Cakmak et al., 2023; Hungria & Nogueira, 2023). B is also vital for maintaining proper nodule architecture. Legumes require B concentrations that are four to five times higher for nodule development than for root elongation (Carpena et al., 2000). Furthermore, studies have shown that nitrogenase activity is significantly greater in B-fertilized plants compared to unfertilized controls (Santachiara et al., 2019). Consequently, B deficiency leads to reduced nodulation and compromised nodule function (Reguera et al., 2010; Cakmak et al., 2023; Hungria & Nogueira, 2023).

Given that initial B levels in the soils ranged from medium (Soil 1) to high (Soil 2), according to the classification by Veloso et al. (2022), a significant response to B fertilization was not expected, despite the observed increases in B concentration in plant tissues following its application. Indeed, several studies have reported that in soils with B concentrations exceeding 0.5 mg kg^{-1}, DMY responses to B fertilization are typically negligible (Castagnara et al., 2012; Das et al., 2020). Furthermore, the relatively high levels of organic matter in both soils, known to enhance B availability (Weil & Brady, 2017), further complicate the interpretation of the positive effects observed in this study on both DMY and biological N fixation.

However, when these findings are considered alongside the significant differences in tissue B concentrations between the non-supplemented treatments (Lime and Lime + P) and the B-supplemented ones (Lime + B and Lime + B + P), a plausible explanation emerges. It is likely that liming reduced B bioavailability, thereby inducing a functional B deficiency in treatments without B supplementation. This effect would be particularly pronounced in legumes, which have a substantially higher B requirement (Cakmak et al., 2023; Hungria & Nogueira, 2023). A similar phenomenon was reported by Arrobas et al., (2023b) in olive. At elevated Soil pH, B tends to become less available due to increased adsorption onto inorganic and organic colloids (Weil & Brady, 2017).

Moreover, B demand in plants tends to increase in Ca-rich environments (Weil & Brady, 2017). Therefore, joined together, these results suggest that liming may reduce B bioavailability, underscoring the necessity of B supplementation following lime application

CONCLUSIONS

The combined results for dry matter yield (DMY), total nitrogen (N) uptake, and the percentage of N derived from the atmosphere (%Ndfa) across different soils and treatments demonstrated that the legume accessed atmospheric N, which resulted in substantially higher DMY compared to the grass. This combination of findings, particularly the differences between treatments with and without phosphorus (P), also indicated that both the initial Soil P and the P applied as fertilizer enhanced biological N fixation. These results support the hypothesis that, even when lime is applied, an amendment known to increase P availability, P fertilization remains necessary to increase N fixation and DMY in legumes. Moreover, the data suggest that liming increases the need for boron (B) supplementation. Even in soils with initially adequate B levels and organic matter content, B application led to increased N uptake, higher %Ndfa, and a slight increase in DMY, underscoring its importance following liming.

These findings have important implications for pasture management in acid soils. They highlight the agronomic advantage of including legumes in mixed swards to enhance N availability and reduce reliance on synthetic N fertilizers. Fertilizer strategies should be tailored to meet the specific nutritional demands of legumes, particularly following lime application, with attention to potential B limitation. To build on these findings, future studies should quantify nodulation and assess nodule function directly, as well as investigate Soil N dynamics over time. Such data would improve understanding of how P and B availability, in interaction with liming, influence N fixation and long-term pasture productivity under field conditions.

REFERENCES

Aguiar, P., Arrobas, M., Nharreluga, E.A. & Rodrigues, M.Â. 2024. Different species and cultivars of broad beans, lupins, and clovers demonstrated varying environmental adaptability and nitrogen fixation potential when cultivated as green manures in Northeastern Portugal. *Sustainability* **16**, 10725. https://doi.org/10.3390/su16231072512

Akshit, S.K., Sheoran, N., Devi, P., Sharma, K., Kamboj, E. & Kumar, P. 2024. Legumes in cropping systems: a way toward agricultural sustainability and diversification. Commun. *Soil Sci. Plant Anal.* **55**(4), 596–608. https://doi.org/10.1080/00103624.2023.2274035

Arrobas, M., Raimundo, S., Correia, C.M. & Rodrigues, M.Â. 2024. Omitting the application of nitrogen or potassium reduced the growth of young chestnut (*Castanea sativa*) trees, while a lack of boron decreased fruit yield. *Soil Syst.* **8**, 104. doi: 10.3390/soilsystems8040104

Arrobas, M., Conceição, N., Pereira, E., Martins, S., Raimundo, S., Brito, C., Correia, C.M. & Rodrigues, M.Â. (2023a). Dolomitic limestone was more effective than calcitic limestone in increasing Soil pH in an untilled olive orchard. *Soil Use Manage.* **39**, 1437–1452. https://doi.org/10.1111/sum.12948

Arrobas, M., Raimundo, S., Conceição, N., Moutinho-Pereira, J., Correia, C.M. & Rodrigues, M.Â. 2023b. On sandy, boron-poor soils, liming induced severe boron deficiency and drastically reduced the dry matter yield of young olive Trees. *Plants* **12**, 4161. doi: 10.3390/plants12244161

Badgery, W., Broadfoot, K., Millar, G., Harris, C. & Boschma, S. 2024. Tropical pastures add value to grazing systems in temperate environments of Eastern Australia. *Field Crops Res.* **312**, 109402. https://doi.org/10.1016/j.fcr.2024.109402

Behera, B., Kancheti, M., Raza, M.B., Shiv, A., Mangal, V., Rathod, G., Altaf, M.A., Kumar, A., Aftab, T., Kumar, R., Tiwari, R.K., Lal, M.K. & Singh, B. 2023. Mechanistic insight on boron-mediated toxicity in plants vis-à-vis its mitigation strategies: A review. *Int. J. Phytoremediation* **25**(1), 9–26. https://doi.org/10.1080/15226514.2022.2049694

Bellaloui, N., Mengistu, A., Abdelmajid, M.A., Abel, C. & Zobiole, L.H.S. 2014. Role of boron nutrient in nodules growth and nitrogen fixation in soybean genotypes under water stress conditions. In: Price, A. (Ed.). *Improving Crop Productivity in Sustainable Agriculture* (Chapter 10, pp. 237–258). InTech. https://doi.org/10.5772/56994

Bouray, M., Moir, J.L., Condron, L.M. & Paramashivam, D. 2022. Early effects of surface liming on Soil P biochemistry and dynamics in extensive grassland. *Nutr. Cycl. Agroecosys.* **124**, 173–187. https://doi.org/10.1007/s10705-021-10163-4

Buoso, S., Zamboni, A., Franco, A., Commisso, M., Guzzo, F., Varanini, Z., Pinton, R., Tomasi, N. & Zanin, L. 2022. Nodulating white lupins take advantage of the reciprocal interplay between N and P nutritional responses. *Physiol. Plant.* **174**(1), 13607. doi: 10.1111/ppl.13607

Cakmak, I., Brown, P., Colmenero-Flores, J.M., Husted, S., Kutman, B.Y., Nikolic, M., Rengel, Z., Schmidt, S.B. & Zhao, F.J. 2023. Micronutrients. In: Rengel, Z., Cakmak, I. & White, P.J. (Eds.), *Marschner's Mineral Nutrition of Plants.* 4th Ed., pp. 283–385. Chennai Ltd., India: Elsevier. https://doi.org/10.1016/B978-0-12-819773-8.00006-X

Carpena, R.O., Esteban, E., Sarro, M.J., Peñalosa, J., Gárate, A., Lucena, J.J. & Zornoza, P. 2000. Boron and calcium distribution in nitrogen-fixing pea plants. *Ciênc. Veg.* **151**(2), 163–170. https://doi.org/10.1016/S0168-9452(99)00210-1

Castagnara, D.D., Krutzmann, A., Zoz, T., Steiner, F., Conte, E., Castro, A.M., Neres, M.A. & Oliveira, P.S.R.D. 2012. Effect of boron and zinc fertilization on white oats grown in Soil with average content of these nutrients. *Rev. Bras. Zootec.* **41**(7), 1598–1607. https://doi.org/10.1590/S1516-35982012000700007

Chen, Z., Wang, L., Cardoso, J.A., Zhu, S., Liu, G., Rao, I.M. & Lin, Y. 2023. Improving phosphorus acquisition efficiency through modification of root growth responses to phosphate starvation in legumes. *Front. Plant Sci.* **14**, 1094157. doi: 10.3389/fpls.2023.1094157

Das, H., Devi, N.S., Singh, R.K., Singh, A.H., Singh, L.N. & Singh, N.G. 2020. Influence of boron and lime on boron concentration and dry matter yield of green gram (Vigna radiata L.). *Int. J. Chem. Stud.* **8**(6), 631–634. https://doi.org/10.22271/chemi.2020.v8.i6i.10843

Dimande, P., Arrobas, M. & Rodrigues, M.Â. 2024. Intercropped maize and cowpea increased the land equivalent ratio and enhanced crop access to more nitrogen and phosphorus compared to cultivation as sole crops. *Sustainability* **16**, 1440. doi: 10.3390/ su16041440

Dordas, C.A., Lithourgidis, A.S. & Galanopoulou, K. 2019. Intercropping of faba bean with barley at various spatial arrangements affects dry matter and N yield, nitrogen nutrition index, and interspecific competition. *Not. Bot. Horti Agrobot. Cluj-Napoca* **47**(4), 1116–1127. https://doi.org/10.15835/nbha47411520

Enriquez-Hidalgo, D., Gilliland, T.J., Egan, M. & Hennessy, D. 2018. Production and quality benefits of white clover inclusion into ryegrass swards at different nitrogen fertilizer rates. *J. Agric. Sci.* **156**(3), 378–386. https://doi.org/10.1017/S0021859618000370

Hackney, B., Jenkins, J., Powells, J., Edwards, C., Meyer, S.D.D., Howieson, J., Yates, R. & Orgill, S. 2019. Soil acidity and nutrient deficiency cause poor legume nodulation in the permanent pasture and mixed farming zones of south-eastern Australia. *Crop Pasture Sci.* **70**, 1128–1140. https://doi.org/10.1071/CP19039

Hamilton, L.J., Reed, K.F.M., Leach, E.M.A. & Brockwell, J. 2015. Boron deficiency in pasture based on subterranean clover (*Trifolium subterraneum* L.) is linked to symbiotic malfunction. *Crop Pasture Sci.* **66**(11), 1197–1212. https://doi.org/10.1071/CP14300

Hawkesford, M.J., Cakmak, I., Coskun, D., De Kok, L.J., Lambers, H., Schjoerring, J.K. & White, P.J. 2023. Functions of macronutrients. In: Rengel, Z., Cakmak, I. & White, P.J. (Eds.), *Marschner's Mineral Nutrition of Plants.* 4th Ed., Elsevier, Ltd., Chennai, India, pp. 201–281. doi: 10.1016/B978-0-12-819773-8.00019-8.

Hayes, R.C., Li, G.D., Sandral, G.A., Swan, T.D., Price, A., Hildebrand, S., Goward, L., Fuller, C. & Peoples, M.B. 2017. Enhancing composition and persistence of mixed pasture swards in southern New South Wales through alternative spatial configurations and improved legume performance. *Crop Pasture Sci.* **68**(12), 1112–1130. doi: 10.1071/CP16395

Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A. & Hayes, R.C 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. *Sci. Total Environ.* **610–611**, 316–332. https://doi.org/10.1016/j.scitotenv.2017.08.020

Hungria, M. & Nogueira, M.A. 2023. Nitrogen fixation. In: Rengel, Z., Cakmak, I. & White, P.J. (Eds.), *Marschner's Mineral Nutrition of Plants*. 4th Ed. India, Chennai, pp 615–650. Elsevier, Ltd. DOI: https://doi.org/10.1016/B978-0-12-819773-8.00006-X.

IPMA (Instituto Português do Mar e a Atmosfera). 2025. *Normais Climatológicas* (in Portuguese) [Climate Normals]. Available online: https://www.ipma.pt/pt/oclima/normais.clima/ (accessed on 15 May 2025).

Kochian, L.V., Hoekenga, O.A. & Piñeros, M.A. 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorus efficiency. *Annu. Rev. Plant Biol.* **55**, 459–493. https://doi.org/10.1146/annurev.arplant.55.031903.141655

Lambers, H. 2022. Phosphorus acquisition and utilization in plants. *Annu. Rev. Plant Biol*. **73**, 17–42. DOI: https://doi.org/10.1146/annurev-arplant-102720-125738.

Maxwell, T.M.R., Moir, J.L. & Edwards, G.R. 2013. Phosphorus response and efficiency of four adventive annual clovers grown in a New Zealand high country Soil under glasshouse conditions. *N. Z. J. Agric. Res*. **56**, 203–214. doi: 10.1080/00288233.2013.809775

McLachlan, J.W., Flavel, R.J. & Guppy, C.N. 2024. Preferential phosphorus placement improves the productivity and competitiveness of tropical pasture legumes. *Legum. Sci.* **6**(2), e235. https://doi.org/10.1002/leg3.235

Mitran, T., Meena, R.S., Lal, R., Layek, J., Kumar, S. & Datta, R. 2018. Role of Soil phosphorus on legume production. In: Meena, R., Das, A., Yadav, G., Lal, R. (eds), *Legumes for Soil Health and Sustainable Management*, pp. 487–510. Springer. doi:10.1007/978-981-13-0253-4_15

Moir, J., Jordan, P., Moot, D. & Lucas, R. 2016. Phosphorus response and optimum pH ranges of twelve pasture legumes grown in an acid upland New Zealand Soil under glasshouse conditions. *J. Soil Sci. Plant Nutr.* **16**, 438–460. doi: 10.4067/S0718-95162016005000038

Monjardino, M., Loi, A., Thomas, D.T., Revell, C.K., Flohr, B.M., Llewellyn, R.S. & Norman, H.C. 2022. Improved legume pastures increase economic value, resilience and sustainability of crop-livestock systems. *Agric. Syst.* **203**, 103519. doi: 10.1016/j.agsy.2022.103519

Morton, J.D. 2020. A review of research on the effect of lime on New Zealand soils and pastures. *N. Z. J. Agric. Res.* **63**(2), 189–201. https://doi.org/10.1080/00288233.2018.1537293

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J. & Lüscher, A. 2009. Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *J. Appl. Ecol.* **46**(3), 683–691. doi: 10.1111/j.1365-2664.2009.01653.x

O'Hara, G.W. 2001. Nutritional constraints on root nodule bacteria affecting symbiotic nitrogen fixation: A review. *Aust. J. Exp. Agric.* **41**, 417–433. DOI: 10.1071/EA00087

Olson-Rutz, K. & Jones, C. 2015. Soil *Nutrient Management for Forages: Phosphorus, Potassium, Sulfur and Micronutrients.* Department of Land Resources and Environmental Sciences, Montana State University. Bozeman, MT 59717

Ovalle, C., Urquiaga, S., Pozo, A.D., Zagal, E. & Arredondo, S. 2006. Nitrogen fixation in six forage legumes in Mediterranean central Chile. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **56**(4), 277–283. https://doi.org/10.1080/09064710500310246

Portela, E., Ferreira-Cardoso, J., Louzada, J. & Gomes-Laranjo, J. 2015. Assessment of boron application in chestnuts: nut yield and quality. *J. Plant Nutr.* **38**(6), 973–987. doi: 10.1080/01904167.2014.963116

Reguera, M., Bonilla, I. & Bolanos, L., 2010. Boron deficiency results in induction of pathogenesis-related proteins from the PR-10 family during the legume-rhizobia interaction. *J. Plant Physiol.* **167**(8), 625–632.

Riday, H. & Albrecht, K.A. 2012. Combining Kura clover with forage legumes and grasses to optimize pasture forage legume content. *Agron. J.* **104**(2), 353–362. doi: 10.2134/agronj2011.0088

Rodrigues, M.Â., Dimande, P., Pereira, E.L., Ferreira, I.Q., Freitas, S., Correia, C.M., Moutinho-Pereira, J. & Arrobas, M. 2015. Early-maturing annual legumes: An option for cover cropping in rainfed olive orchards. *Nutr. Cycl. Agroecosys.* **103**(2), 153–166. https://doi.org/10.1007/s10705-015-9730-5

Sadowsky, M.J. 2005. Soil stress factors influencing symbiotic nitrogen fixation. In: Werner, D. & Newton, W.E. (Eds.), *Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment*. Springer. Dordrecht, The Netherlands, pp. 89–112

Santachiara, G., Salvagiotti, F. & Rotundo, J.L. 2019. Nutritional and environmental effects on biological nitrogen fixation in soybean: A meta-analysis. *Field Crops Res*. **240**, 106–115.

Somavilla, A., Ramos Marques, A.C., Caner, L., Oliveira, L.B., Quadros, F.L.F., Chabbi, A., Tiecher, T. & Santos, D.R. 2021. Phosphate fertilization and liming in a trial conducted over 21 years: A survey for greater forage production and Pampa pasture conservation. *Eur. J. Agron*. **125**, 126259. https://doi.org/10.1016/j.eja.2021.126259

Teixeira, R.F., Proença, V., Crespo, D., Valada, T. & Domingos, T. 2015. A conceptual framework for the analysis of engineered biodiverse pastures. *Ecol. Eng.* **77**, 85–97. doi: 10.1016/j.ecoleng.2015.01.002

Temminghoff, E.E. & Houba, V.J. 2004. *Plant Analysis Procedures*, 2nd ed.; Kluwer Academic Publishers: London, UK. https://doi.org/10.1007/978-1-4020-2976-9

Unkovich, M., Herridge, D., Peoples, M., Cadish, G., Boddey, B., Giller, K., Alves, B. & Chalk, P. 2008. *Measuring Plant-Associated Nitrogen Fixation in Agricultural Systems* (ACIAR Monograph **136**, 258 pp.). Australian Center for International Agricultural Research.

Upjohn, B., Fenton, G. & Conyers, M. 2005. *Soil Acidity and Liming*, 3rd ed.; Agfact AC.19. Department of Primary Industries, Wellington, Australia, 24 pp.

Van Reeuwijk, L.P. 2002. *Procedures for Soil Analysis*, 6th ed., Technical Paper 9. International Soil Reference and Information Centre: Wageningen, The Netherlands, 101 pp.

Veloso, A., Sempiterno, C., Calouro, F., Rebelo, F., Pedra, F., Castro, I.V., Gonçalves, M.C., Marcelo, M.E., Pereira, P., Fareleira, P., Jordão, P., Mano, R. & Fernandes, R. 2022. *Manual Fertilização das Culturas* [Crop Fertilization Manual]. INIAV-Instituto Nacional de Investigação Agrária e Veterinária, I.P. Oeiras, Portugal, 468 pp. (in Portuguese).

Weil, R.R. & Brady, N.C. 2017. *Nature and Properties of Soils*, 15th ed.; Pearson: London, UK, 1086 pp.

WRB (World Reference Base for Soil Resources). 2022. *International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*. (4th ed.). International Union of Soil Sciences (IUSS), Working Group. Austria, Vienna, 236 pp.

Yang, Q., Lin, Y., Jin, L., Ren, X., He, C. & Liu, Q. 2021. Responses of mineral nutrient contents and transport in red clover under aluminum stress. *Legum. Sci.* **3**(4), 94. https://doi.org/10.1002/leg3.94

Zhou, J., Sun, X., Chen, C. & Chen, J. 2023. The effect of molybdenum fertilizer on the growth of grass-legume mixtures related to symbiotic rhizobium. *Agronomy* **13**, 495. https://doi.org/10.3390/agronomy13020495

Agrivoltaics: a paradigm for sustainable
dual land use - an overview

C.A. Arredondo[1,*], A. Guerrero Arredondo[2], C.E. Arrieta Gonzalez[1],
V. Rajamani[3] and S. Sarkar[4]

[1]Universidad de Medellín, Facultad de Ingenierías, Grupo de Investigación en Energía - GRINEN, Carrera 87 N°30 – 65, Medellín, Colombia
[2]Universidad de Medellín, Facultad de Ingenierías, Grupo de Investigación en Energía - GRINEN, Estudiante Ingeniería en Energía, Carrera 87 N°30 – 65, Medellín, Colombia
[3]Fachhochschule Dortmund, Mechanical Engineering, Sonnenstraße 96, DE44139, Dortmund, Germany
[4]RV College of Engineering, Department of Electrical and Electronics Engineering, Mysore Rd, RV Vidyaniketan, Post, Bengaluru, IN560059 Karnataka, India
[*]Correspondence: caarredondo@udemedellin.edu.co

Abstract. Agrivoltaic systems is an emerging solution that combines agricultural production with photovoltaic energy generation on the same area. This paper synthesizes findings from approximately 251 peer-reviewed studies, technical reports, and real-world applications to explore the classification, benefits, and implementation of agrivoltaics systems globally. Case studies from Japan, France, Africa, and Latin America reveal yield increases above 10% for certain crops under partial shading and energy production outputs of up to 1.5 MW ha^{-1}, depending on the panel type and configuration. The overview also examines key technological developments, such as bifacial modules and smart irrigation, which improve efficiency and resource management. Additionally, it discusses the economic, environmental, and social benefits and identifies the main barriers to widespread adoption. By evaluating current challenges and future perspectives, this overview provides a comprehensive synthesis of how agrivoltaic systems contribute to sustainable energy and food production, highlighting their global relevance, integrating emerging technologies, and emphasizing the policy frameworks that support successful deployment.

Key words: agrivoltaics, dual land use, photovoltaic systems, sustainable agriculture, energy transition, climate resilience.

INTRODUCTION

In recent years, there has been a surge of interest in novel solutions at the nexus of climate change, renewable energy generation, and sustainable food production. Agrivoltaics has emerged as a key area of focus, representing an integrative solution that combines photovoltaic energy production with agricultural practices. This dual land-use

strategy not only harnesses solar energy but also upholds the principles of sustainable agriculture by enhancing land efficiency and fostering ecological balance.

Agrivoltaic system offer a broad spectrum of benefits, including renewable energy generation, greenhouse gas emissions reductions, and contributions to rural economic growth in line with Green New Deal objectives (Proctor et al., 2021). Additionally, it provides solutions to land-use conflicts by enabling concurrent agricultural and photovoltaic utilization, thereby reducing competition for land resources (Tomich et al., 2011).

Unlike conventional photovoltaic installations, which often displace agricultural activities, agrivoltaics maintains agricultural productivity while contributing to renewable energy goals. This dual approach supports food-energy-water nexus objectives by creating favourable microclimatic conditions, conserving water, and fostering rural energy self-sufficiency.

Despite its initial slow adoption, agrivoltaics has recently gained significant traction globally, driven by increasing interest and advancements in research (Touil et al., 2021). The concept, also referred to as agrophotovoltaics, agrovoltaics, agrisolar, or dual-use solar, was first proposed by proposed by Adolf Goetzberger in Germany around 1982 (Goetzberger & Zastrow, 1982). Since then, it has evolved into an innovative strategy for sustainable energy and agricultural practices, offering a complementary solution that optimizes land use and transforms both the energy and agricultural sectors (Mamun et al., 2022; Wagner et al., 2023).

Agrivoltaic systems (AVS) combine photovoltaic (PV) energy generation with agricultural production on the same land, addressing both energy transition goals and agricultural productivity challenges (Dupraz et al., 2011; Barron-Gafford et al., 2019). Initially conceptualized to optimize land use efficiency, the approach now encompasses diverse configurations, technological innovations, and integration models tailored to different crops, climates, and socio-economic contexts (Dinesh & Pearce, 2016; Weselek et al., 2019). By integrating agricultural productivity with renewable energy generation, agrivoltaic systems represent a versatile approach capable of addressing multiple sustainability challenges, from resource efficiency to climate adaptation, while aligning with broader policy and development objectives (van de Ven et al., 2021; Lu, 2024). These systems can also play a role in rural electrification and decentralized energy supply, as evidenced in related renewable energy initiatives (Dunmade, 2021), expanding their socio-economic relevance in both developed and developing regions.

Agriculture is a considerable energy-consuming sector, contributing significantly to global greenhouse gas emissions (Bumbiere et al., 2023). Implementing energy management strategies is therefore fundamental for achieving sustainability. Agrivoltaic systems offer an innovative solution to enhance energy efficiency while maintaining agricultural productivity, directly supporting global emission reduction and land optimization goals.

While several reviews have addressed the technical and environmental benefits of agrivoltaic systems, few have provided a comprehensive synthesis that captures their global applicability, long-term economic viability, and region-specific implementation dynamics. There is also limited discussion on social acceptance, policy integration, and infrastructure readiness (factors crucial for widespread adoption). This qualitative overview aims to fill those gaps and offer a broader understanding of how agrivoltaic systems connect with diverse agricultural and energy contexts.

This paper synthesizes key qualitative findings on agrivoltaics, exploring the classification, benefits, case studies, emerging technologies, and challenges. It is based on a structured literature review of 251 peer-reviewed articles and technical reports selected using clear inclusion criteria, as described in the Methodology section. By delving into the interdependencies of energy and agricultural production, also seeks to identify a path forward for agrivoltaics, underscoring its potential as a key component of sustainable development and a driving force for global change. The results are structured thematically to highlight key trends, case studies, benefits, and challenges in the implementation of agrivoltaics systems worldwide.

METHODOLOGY

This overview is based on a systematic synthesis of scientific literature designed to capture the global landscape of agrivoltaic systems. A multi-faceted methodological approach was used to examine the classification, implementation, benefits, and emerging technologies associated with agrivoltaics across various regions and agricultural contexts. The review integrates comprehensive literature analysis, global case studies, and selected experimental findings to evaluate the environmental, technical, and policy dimensions of agrivoltaic systems.

The methodological framework followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021), and involved a multi-stage process of literature identification, screening, and eligibility assessment.

The literature search was conducted across four academic databases: Scopus, Web of Science, Google Scholar, and MDPI, targeting publications between 2010 and 2024. In addition to the 251 studies included through the formal selection process, a small number of relevant 2025 publications, identified during the final manuscript revision stage, were cited to incorporate the most recent developments in the field. These publications were not part of the systematic pool and are therefore not reflected in the PRISMA counts. The search utilized a combination of keywords, including 'agrivoltaic systems', 'solar energy in agriculture', 'dual land use', and 'sustainable agriculture' to identify a broad spectrum of relevant studies. The initial search yielded an initial pool of 2087 records.

All retrieved records were compiled, and 396 duplicates were identified and removed, resulting in 1691 documents. These were screened by title and abstract screening based on predefined inclusion criteria: (1) the document must be a peer-reviewed journal article or a technical report from a recognized institution; (2) its primary focus must be the integration of solar energy and agriculture; and (3) it must directly discuss themes such as system performance, observed benefits, implementation challenges, or emerging technologies. Based on this screening, 1123 records were excluded as they did not meet the scope of our review.

The remaining 568 articles proceeded to a full-text evaluation to assess relevance and methodological transparency. Studies that lacked methodological transparency, provided no empirical insights, or did not substantively address agrivoltaic systems were excluded, resulting in a final selection of 251 publications. Additional documents from

institutions such as NREL, NSEFI and Fraunhofer ISE were included to support case-specific discussions. Geographic diversity was also considered to reflect the wide adaptability of agrivoltaic systems.

The selected sources were thematically categorized to inform the structure of this overview. Case studies from diverse regions were analyzed to highlight technical performance, crop compatibility, and institutional frameworks. Recent developments in agrivoltaics technologies were reviewed to assess their effectiveness and practical applicability. This multidisciplinary and structured approach ensured a comprehensive assessment of the agrivoltaics landscape. The review process is summarized in the PRISMA flow diagram in Fig. 1.
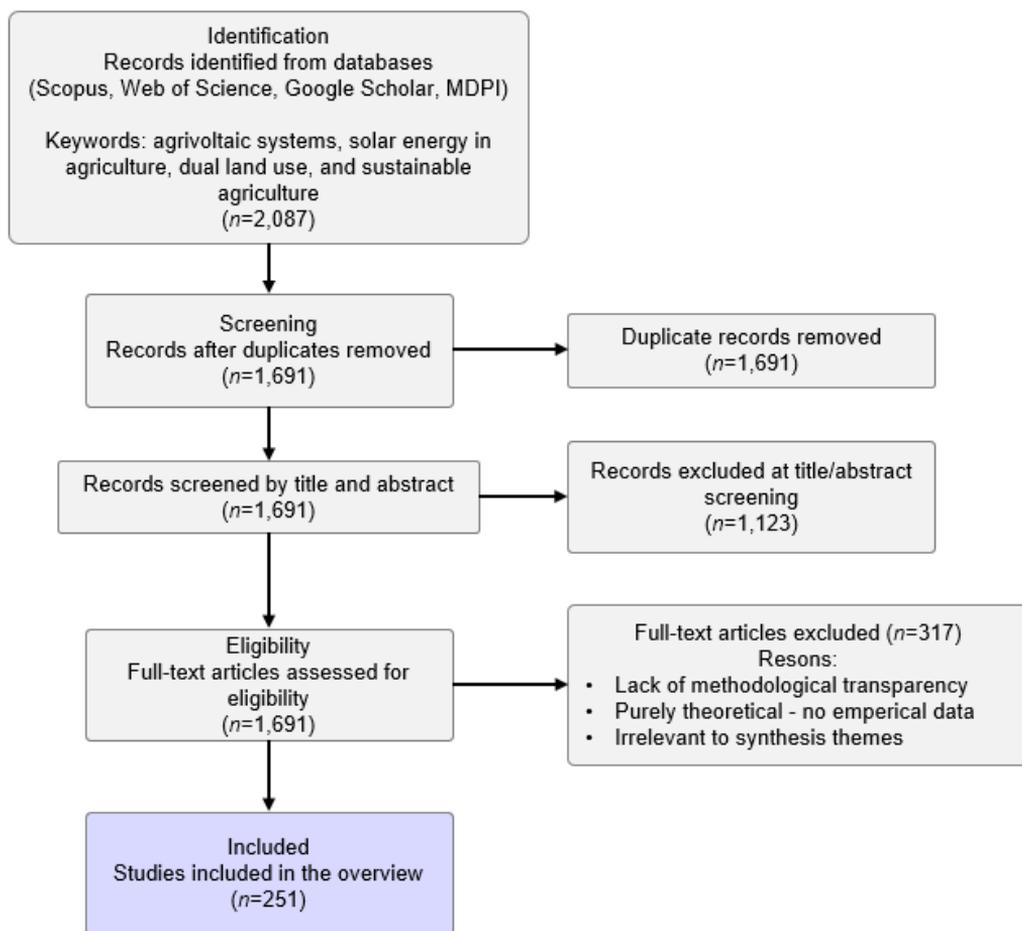
Figure 1. Overview of the research methodology applied in the agrivoltaic systems review. Source: Own elaboration.

The findings from this literature synthesis are presented in the subsequent sections, which details the key themes and insights identified through the methodological process described above.

CLASSIFICATION OF AGRIVOLTAICS

Agrivoltaic systems are classified according to several criteria in order to facilitate a deeper understanding of their implementation and to enable more effective optimization (SETO. 2022), (Sekiyama & Nagashima, 2019). Notable classifications include configuration type, application type, and system type. The National Renewable Energy Laboratory (NREL) provides a detailed explanation of the application type classification as follows (Macknick et al., 2022).

Classification by Configuration Type

Elevated Systems: Photovoltaic modules are positioned over crops at heights above 1.8 meters, protecting against severe weather and creating a controlled microenvironment. This setup benefits regions with high temperatures and intense sunlight but requires careful management to balance shading with sunlight needs for high-value crops like berries and grapes, optimizing energy production and crop yield.

Inter-row Systems: Vegetation is grown between rows of photovoltaic modules, allowing for larger farming equipment and extensive operations. Although these systems offer less direct weather protection and ensure sufficient sunlight for photosynthesis and growth. Typically, these systems are used for lower-value crops like grass, cereals, and hardy vegetables, which are resilient to light and environmental variations (Macknick et al., 2022).

Classification by Application Type

Crop and Food Production: The integration of agrivoltaics into crop production confers benefits regarding microclimate management. This is achieved through the provision of partial shade, which serves to reduce water evaporation, lower soil temperature, and protect plants from extreme heat. This enhances water efficiency and can increase crop yields, particularly in arid and semi-arid regions (Macknick et al., 2022).

Livestock Production: Integrating animals like sheep, cows, poultry, bees, and rabbits with solar panels offers significant benefits. Sheep manage vegetation by feeding, reducing maintenance needs. The shade provided by the panels can also reduce heat stress in animals, improving animal welfare and productivity (Macknick et al., 2022).

Ecosystems Services Provision: These systems are crucial for environmental conservation, enhancing biodiversity by creating habitats for pollinators and wildlife, enhancing biodiversity. Such a system enhances soil health through the deposition of organic matter and microbial activity, which are essential for sustainable agriculture. Furthermore, they facilitate the provision of clean water, climate regulation, and recreational opportunities, thereby linking renewable energy with environmental protection (Macknick et al., 2022).

Solar Greenhouses: This approach combines energy production with the creation of optimal agricultural environments. The integration of photovoltaic panels into greenhouses enables farmers to effectively manage light and temperature, thereby promoting optimal plant growth. This approach enables the year-round cultivation of high-value crops and reduces dependency on external energy sources (Macknick et al., 2022).

In Fig. 2, a classification of agrivoltaics systems according to their intended application is presented.

According to Fraunhofer Institute for Solar Energy Systems (ISE), agrivoltaics can also be classified by system type (Fraunhofer Institute for Solar Energy Systems ISE. 2022), as follows.

Classification by System Type

Open Systems: Including ground-level, interspace, and aerial / overhead modules (Fig. 2), are designed to adapt to a variety of agricultural practices (Fig. 3). Ground-level systems are well-suited for permanent grasslands and grazing animals, while interspace systems are ideal for use between crop rows. Aerial / overhead systems improve air circulation and environmental protection, with solar tracking enhancing energy capture and efficiency without disrupting existing farming practices.

a)   b)
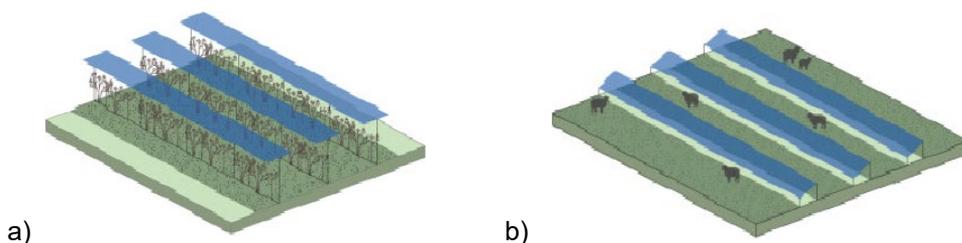
Figure 2. Types of open agrivoltaic systems: a) Overhead system with fixed modules, and b) Interspace system with fixed modules (Biró-Varga et al., 2024).
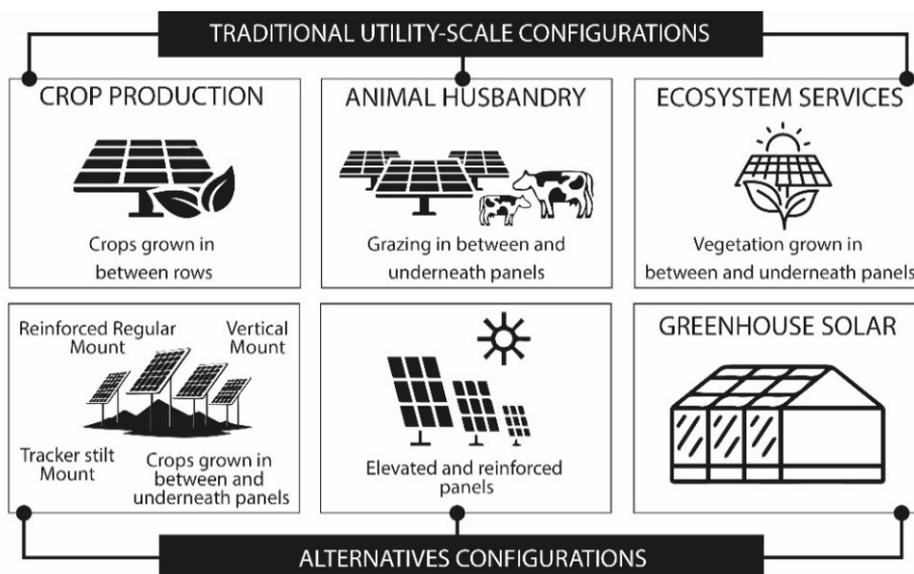
Figure 3. Classification of agrivoltaics systems by application. Adapted from (Dreves, 2022).

Closed Systems: Which can be photovoltaic greenhouses, combine solar panels with greenhouse technology to create controlled microclimates that enhance crop production. The aforementioned systems provide shading, thereby reduce the need for artificial cooling, and allow for the creation of specific growing conditions. The electricity generated is used to power greenhouse operations, making the system highly energy-efficient and sustainable (Dreves, 2022).
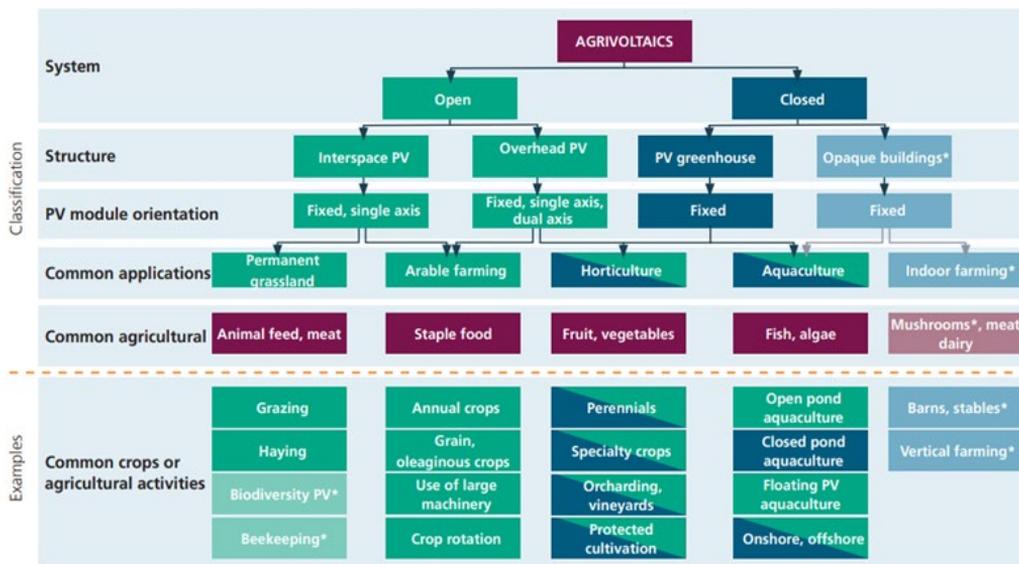
Figure 4. Classification by type of agrivoltaic system (Fraunhofer Institute for Solar Energy Systems ISE (2022)).

Fig. 4 illustrates the classification by type of agrivoltaic system. The classification system allows agrivoltaic systems to be adapted to a variety of agricultural and environmental contexts, enhancing efficiency and sustainability. By optimizing land use, agrivoltaics enhance crop and livestock productivity while improving the performance and benefits of photovoltaic systems (Dreves, 2022). This approach represents a transformative shift in sustainable agriculture and energy production.

BENEFITS OF AGRIVOLTAIC SYSTEMS

Agrivoltaic systems offer farmers a valuable opportunity to diversify their income streams. They facilitate the simultaneous production of electricity and crops, providing an additional revenue stream. This dual capability is especially valuable in rural areas with limited economic opportunities, as it provides a means of financial stability through the sale of both energy and agricultural products (Walston et al., 2022; PV Tech Power, 2023; Wydra et al., 2023). In the United States, several states have identified the potential of agrivoltaics and implemented financial incentives to encourage the development of these projects. For instance, the state of Massachusetts offers a tariff add-on of $0.06/kWh for agrivoltaic projects through its SMART program (Pascaris,

2021), while the state of New Jersey has authorized a pilot agrivoltaic program of up to 200 MW on non-preserved farmland, alongside funding a research system at Rutgers University (PV Tech Power, 2023; Kirto et al., 2024). Furthermore, Colorado has also invested in agrivoltaic research with the objective of optimizing its economic, technical, and environmental benefits (PV Tech Power, 2023; Uchanski et al., 2023). These initiatives highlight the significant economic potential of agrivoltaic systems for a broader adoption and for energy production (Pascaris et al., 2023).

While the benefits of agrivoltaic systems are substantial, it is important to recognize the potential trade-offs that may emerge during implementation. For instance, although agrivoltaics can generate additional income through energy production, the initial capital investment for elevated mounting structures are higher than for ground-mounted PV systems (Trommsdorff et al., 2022). Also, the use bifacial modules, or smart irrigation systems may offset short-term economic gains, particularly in small-scale or resource-constrained farms (see Case studies and emerging technologies sections for detailed analysis). The higher capital expenditure and the operation and maintenance costs need to be considered to determine costs. On the other hand, the sale of both electricity and agricultural products needs to be considered for the calculation of Return on Investment (ROI) (Sturchio & Knapp, 2023). Similarly, while crop yields may benefit from improved microclimatic conditions, not all plant species respond positively to partial shading, and site-specific adaptation is required to ensure long-term productivity. In some cases, land-use efficiency might also be affected if the solar infrastructure limits access for machinery or harvesting activities. These trade-offs do not negate the benefits of agrivoltaics but make clear the importance of tailored system designs, proper crop-panel matching, and supportive policy frameworks to maximize net benefits. A critical understanding of these limitations can enhance decision-making and promote the sustainable scaling of agrivoltaic practices.

From an environmental perspective, agrivoltaic systems provide numerous benefits that significantly contribute to sustainability and environmental conservation. The integration of solar panels with crops aids in soil conservation and improving water quality. Vegetation beneath the panels mitigates soil erosion and reduces water run-off (Walston et al., 2022; Wydra et al., 2023). This is of particular importance in areas prone to desertification and soil degradation (Dreves, 2022). Moreover, agrivoltaic systems can enhance the efficiency of solar panels by reducing the temperatures around them, which improves their performance and decreases water evaporation, thereby reducing the irrigation needs (Marrou et al., 2013; Dreves, 2022). Furthermore, these systems promote biodiversity by providing habitats for pollinators and other species. This biodiversity boost benefits local ecosystems and agricultural production by improving pollination services (Dreves, 2022), (The potential of agrivoltaics for the US). Overall, the environmental benefits demonstrate the potential of agrivoltaic systems to foster more sustainable and resilient agriculture, while simultaneously advancing renewable energy goals and reducing carbon emissions (Wagner et al., 2023). These benefits serve to illustrate the transformative impact on modern agricultural and environmental practices.

From a technical perspective, agrivoltaic systems offer significant benefits for photo-voltaic systems. The presence of crops beneath the solar panels provides a natural cooling effect, which is particularly advantageous in warm climates where high temperatures can reduce solar panel efficiency (Jerome et al., 2022). Studies have indicated that lowering the temperature around the panels enhances their performance and increases energy output (Sheik et al., 2022), (Kumari et al., 2022). In addition, the partial shading provided by the panels helps to retain soil moisture, thus reducing the need for frequent irrigation and conserving water resources (Omer et al., 2022).

The integration of vegetation with solar installations represents an effective means of controlling weed growth. This results in a reduction in the necessity for mechanical or chemical weed control methods, thereby lowering the maintenance costs associated with photovoltaic systems. Furthermore, the vegetation beneath the panels contributes to soil health by adding organic matter and promoting beneficial microbial activity (Jiufu et al., 2024). These factors not only enhance the technical viability of agrivoltaics systems but also support eco-friendly agricultural practices, thereby their sustainability (Time et al., 2024). These benefits illustrate how agrivoltaic systems can optimize the performance of solar panels, reduce operational costs, and enhance energy production.

While the benefits of agrivoltaic systems are widely acknowledged, distinguish between theoretical claims and those substantiated by empirical evidence remains crucial. For example, pilot projects in Japan and France have demonstrated concrete outcomes, such as increased farmer income, enhanced land-use efficiency, and improved energy output stability. Similarly, studies in Kenya confirm benefits in water conservation and energy access for off-grid farming. However, in regions like Brazil, most benefits remain conceptual, pending long-term implementation data. A table that summarizes these comparative outcomes by highlighting the location, main applications, technologies, challenges, and policy support for agrivoltaic systems can be found in the Case Studies section (Table 1). This serves as a useful guide for future scalability and adoption strategies.

CASE STUDIES OF AGRIVOLTAIC SYSTEMS

Agrivoltaic systems have been adopted in diverse geographical contexts, offering insights into their adaptability, socio-economic impact, and integration with local agricultural practices. Notable case studies include countries such as Japan, France, Africa, China, and others, where pilot projects have been implemented to study the impacts and advantages of agrivoltaics systems. The case studies included in this section have been selected based on several criteria, including geographical diversity, variety of crops and agricultural practices, scale of implementation, and the specific socio-economic and environmental challenges addressed by each project. While many case studies reveal common benefits, such as increased land-use efficiency and improved crop yields, each also demonstrates distinct strategies, challenges, and policy settings that inform context-specific and scalable deployment of agrivoltaic systems.

Japan has been a pioneer in the implementation of agrivoltaic systems since the introduction of its feed-in tariff scheme in 2012. This approach has significantly augmented the country's renewable energy supply, with a notable growth in renewable energy between 2012 and 2019. In the country, agrivoltaic systems have been employed with over 120 different crop species, including rice, tea, and blueberries. Re-search indicates that these systems can provide shade that benefits certain crops by reducing water stress and improving the local microclimate, thereby enhancing agricultural productivity (Gonocruz et al., 2021; Nakata & Ogata, 2023; Chalgynbayeva et al., 2024). A case study conducted at the agrivoltaic experimental farm operated by the CHO Institute of Technology in Ichihara City demonstrated the effectiveness of a photovoltaic system with a total output capacity of 4.5 kW. The system secured a feed-in tariff rate of 48 yen (approximately 0.44 USD) per kWh (Sekiyama & Nagashima, 2019). Another study suggests that agrivoltaic systems in Japan could generate approximately 284 million MWh per year, which is equivalent to approximately 29% of the country's total electricity demand (Gonocruz et al., 2021). These studies may position Japan as a leader in this field.

France exemplifies how the adoption of agrivoltaic systems constitutes a key element of the country's energy transition efforts. A case study in the Pyrénées-Atlantiques region underscores the critical role of social acceptance and effective governance in the implementation of these projects. Researchers found that the integration of energy production with agriculture on the same land requires active community participation and a clear political vision. This collaborative approach serves to mitigate conflicts between different land uses, thereby fostering a harmonious coexistence of food and energy production (Carrausse & Arnauld de Sartre, 2023; DW News (2024)). The Pyrénées-Atlantiques case study highlights that the success of agrivoltaic systems relies on engaging local stakeholders and the establishment of supportive governance frameworks. By addressing potential conflicts and promoting community involvement, agrivoltaic systems can effectively contribute to the goals of agricultural sustainability and renewable energy. This illustrates the potential of agrivoltaic systems to enhance agricultural productivity and energy genera-tion, in alignment with France's broader objecttives for sustainable development.

A study conducted in India examines the potential for the deployment of agrivoltaic systems on existing grape farms. The results indicate that this implementation on a national scale could generate over 16,000 GWh of electricity, potentially meeting the energy demands of more than 15 million people (Malu et al., 2017).

One such interesting project is Cochin International Airport Limited (CIAL) in Kerela, India. The world's first airport to operate entirely on solar power, had previously conducted an experiment with organic farming on one of its solar plant sites within the airport premises. This Airport features an impressive setup of eight solar plants within its premises with total capacity of solar plants in Feb. 2020 of 42 MWp. It was noted that the project yields an annual organic produce output of approximately 60–80 tons. Additionally, the airport has established strong market linkages, enabling direct sales of its agricultural products to regular consumers and passengers, thereby creating an additional revenue stream. The success of this initiative is largely attributed to interspace

cultivation techniques. Over 20 variety of vegetables are grown under this project (National Solar Energy Federation of India (2023)).

Several agrivoltaic pilot programs are being conducted across Africa in collaboration with European research centers. In Algeria, the Watermed 4.0 project has shown promising encouraging outcomes, with a significant increase in the yield and size of potato crops cultivated under agrivoltaic installations in comparison to uncovered fields. Furthermore, projects involving agrivoltaic systems can assist in restoring the fertility of agricultural areas that have been adversely affected by progressive infertility due to climate change or land aridity. This is achieved through the use of solar technologies for irrigation and water management, thereby enhancing agricultural productivity, (Randle-Boggis et al., 2021; Macdonald et al., 2022; DW News (2024)).

A case study conducted in Hungary on apple production revealed the economic potential of agrivoltaic systems. The utilization of agrivoltaic technologies in intensive and super-intensive apple orchards not only enhanced fruit production but also facilitated the coverage of high fixed costs associated with the installation of these systems. The study concluded that agrivoltaic systems are economically viable and can enhance the competitiveness of farmers by efficiently utilizing space and implementing sustainable agricultural practices (Chalgynbayeva et al., 2022, 2024).

The integration of agrivoltaics has the potential to advance Canada towards net-zero power generation and greenhouse gas emissions (Jamil et al., 2023a). A case study demonstrated that the installation of racking structures on grape farms could enable the country to generate over 10 GW of renewable electricity (Jamil et al., 2023b). This substantial increase in sustainable energy production underscores the transformative potential of agrivoltaic systems to contribute to the nation's energy and environmental goals.

The United States has several incentives and research programs that have promoted the implementation of agrivoltaic systems. A noteworthy example is Jack's Solar Garden in Colorado, which integrates solar energy generation with agricultural practices. This project has facilitated sheep grazing and the creation of habitats for pollinators, showcasing the multifunctional and sustainable benefits of agrivoltaic systems for both the local community and the environment (Dreves, 2022; PV Tech Power (2023)).

Germany has been a pioneer in the research and implementation of agrivoltaic systems. The Fraunhofer Institute for Solar Energy Systems (ISE) conducted a study on a 50 MWp project in Maharashtra, India, demonstrating economic viability with a competitive levelized cost of electricity (LCOE). This project highlighted the importance of institutional structures and international cooperation to maximize the benefits of agrivoltaic systems, underscoring their potential for large-scale applications (Wydra et al., 2023; Chalgynbayeva et al., 2024.

Italian agrivoltaic systems have been employed to address the decline in soil fertility resulting from climate change. A collaborative project with Green Cross International and the public research agency ENEA (Italian National Agency for New Technologies, Energy and Sustainable Economic Development) led to the construction of a five-megawatt photovoltaic plant in an agricultural region of Morocco. This initiative demonstrated that agrivoltaic systems can improve the local microclimate, boost agricultural productivity, and provide energy for irrigation and water desalination

systems, benefiting local communities and mitigating the effects of climate change (Di Francia & Cupo, 2023; DW News (2024)).

South Korea's experience highlights the dual benefits of agrivoltaics, with improved agricultural yields and reduced environmental impact facilitated by supportive government policies. A study demonstrated that the implementation of these systems on agricultural lands can improve energy efficiency and agricultural productivity while reducing environmental impact. These projects in South Korea have been supported by government policies that promote renewable energy adoption and the integration of agrivoltaic technologies (Kim, M. et al., 2021; Kim, Y. et al., 2023; Chalgynbayeva et al., 2024).

China has been a pioneer in the integration of agriculture and photovoltaic energy through various agrivoltaic projects. A study conducted between 2007 and 2016 has evaluated the development levels and coordination between the photovoltaic industry and agriculture. The results proven significant improvements in land use efficiency and agricultural productivity. The projects in China have highlighted the im-portance of resource coordination and optimized agricultural practices in order to fully realize the benefits of agrivoltaic systems (Xiao et al., 2021; Hu, 2023; Chalgynbayeva et al., 2024).

Latin American research focuses on agrivoltaic systems with the objective of enhancing agricultural productivity and renewable energy generation. These projects showcase their potential of agrivoltaics systems to address environmental challenges, improve land use efficiency, and support local economies through sustainable practices.

Brazil demonstrates how agrivoltaics can address both energy generation and food security in semi-arid regions. The Ecolume Agrivoltaic System, for instance, has demonstrated the dual benefits of generating renewable energy and improving agricultural productivity. The integration of aquaponic systems beneath solar panels, this project not only increased solar power production through the cooling effect of plant transpiration but also enhanced crop yields and provided protein sources through fish farming. These initiatives underscore the potential of agrivoltaic systems to address both energy and food security in degraded or desertified areas (IDB Invest; Mongabay News (2022)).

In Chile, agrivoltaic projects are designed to optimize the efficiency of land use in regions that are experiencing water scarcity and high energy demands. These projects, which are supported by organizations such as IDB Invest of Inter-American Development Bank, combine goat farming with solar energy production. They demonstrate how agrivoltaic systems can enhance agricultural resilience and renewable energy generation. This approach not only optimizes land use but also supports local economies by integrating sustainable agricultural practices with energy production (IDB Invest). A study focused on a 100 kWp east-west vertical bifacial agrivoltaic facility in Chanco suggests potential benefits for energy production and water conservation. The results indicate that vertical agrivoltaic systems can reduce the water demand of irrigated crops by 1410 m³/ha while simultaneously generating renewable energy (Bruhwyler et al., 2023).

Argentina is implementing agrivoltaic systems with the dual objective of supporting sustainable agriculture and renewable energy goals. Projects in regions such as Patagonia are being implemented with the goal of integrating solar panels with

traditional farming practices. The objective is to improve land use efficiency and provide stable energy supplies for irrigation and other agricultural needs. These initiatives are part of broader efforts to promote sustainable development and reduce the carbon footprint of agricultural activities (IDB Invest).

The case studies collectively demonstrate the transformative potential of agrivoltaic systems in improving agricultural productivity, addressing environmental challenges, and advancing renewable energy objectives across diverse geographical contexts.

On the other hand, it can be said that an important number of countries are actively promoting agrivoltaic systems through a combination of fiscal incentives, regulatory frameworks, and supportive policies. France leads with a robust legal framework under the 2023 Act APER (loi relative à l'accélération de la production d'énergies renouvelables), which recognizes agrivoltaics as a key component of its national renewable energy strategy. The French government provides financial support through the 'Plan de Relance', offering subsidies and streamlined permitting processes to facilitate the adoption of agrivoltaics (Masons, 2024). Germany also provides support for agrivoltaics through the Renewable Energy Sources Act (EEG), which offers feed-in tariffs and low-interest loans through the KfW (Kreditanstalt für Wiederaufbau) Renewable Energy Program. This makes it easier for farmers to manage the costs associated with these systems. In Japan, the Ministry of Agriculture, Forestry, and Fisheries (MAFF) provides subsidies and tax deductions to encourage the integration of solar energy into agricultural practices, with a stable income guaranteed through the country's feed-in tariff system (MAFF Japan (2021)). In the United States, states like Massachusetts offer additional financial incentives through programs like the SMART initiative, thereby in-creasing the viability of agrivoltaics as a local farming option (Pascaris, 2021).

The case studies reviewed reveal a set of shared patterns that help explain the success of agrivoltaic systems across different regions. Countries such as France, Germany, and Japan have advanced more rapidly due to supportive policies, financial incentives, and clear regulatory guidelines. Technological innovations have also played a key role. In Japan, bifacial panels help optimize light distribution, while in Brazil, systems that combine solar panels with aquaponics have shown improved productivity and better use of land. Climate conditions shape outcomes as well. Projects in arid regions such as Algeria and Chile benefit from water savings and improved microclimates created by solar shading. In addition, local engagement has proven essential. In France and the United States, strong community participation and inclusive governance have contributed to long term project acceptance. These experiences suggest that successful implementation of agrivoltaic systems depends on a combination of context specific technologies, active local involvement, and well aligned policy frameworks.

Table 1. presents a comparative summary of the global agrivoltaic case studies discussed in this section.

Table 1. Comparative Agrivoltaics Case Studies by Country

Country/ Region	Main Application	Key Crops	Technology Used	Outcomes	Challenges	Policy Support
Japan	Maximizing land-use efficiency in mountainous and rural regions	Rice, leafy greens, fruit trees	Fixed-tilt and elevated PV systems	Enhanced income, stable energy generation	Crop compatibility, shading issues	Government subsidies and research funding
France	Enhancing vineyard productivity and energy generation	Grapes (vineyards)	Dynamic PV panels with tracking	Reduced drought stress, stable yields	Regulatory complexity	Agri-PV pilot programs and national strategy
Kenya	Improving rural electrification and water access	Vegetables and grains	Fixed-tilt PV systems with water pumps	Reduced water loss, better irrigation	Cost, limited technical capacity	NGO and donor-supported pilot projects
Germany	Integrating agrivoltaics in temperate climates	Potatoes, beets, vegetables	Bifacial modules, smart sensors	Yield preservation and CO_2 reduction	Public resistance, zoning laws	EU support and national climate plans
China	Rehabilitating degraded land, boosting energy	Goji berries, corn	Bifacial modules on elevated structures	Increased land productivity	High installation cost	National dual-use land policy
USA	Research and demonstration for commercial farms	Lettuce, kale, pollinator plants	Adjustable-height PV panels	Improved biodiversity, extended growing season	Lack of standardization	DOE-funded projects and local initiatives
India	R&D, governmental supported projects and commercial projects	Vineyards, Vegetables (20+ varieties e.g Tomatoes, onions, etc.	Mix of approaches using mono facial and bifacial Fixed panels above crops	Water saving and energy security	Small land holdings, awareness	KUSUM scheme and renewable incentives
Brazil	Feasibility analysis and early-stage pilots	Coffee, pastures	Experimental PV arrays	Theoretical benefits identified	Lack of long-term data	Limited, mostly academic support
Africa (Regional)	Supporting food-energy-water nexus	Various regional crops	Simple PV systems, often fixed	Community-level access gains	Low technical capacity, funding gaps	NGO-led support, minimal policy integration

Source: Own elaboration from the reviewed case studies.

EMERGING TECHNOLOGIES IN AGRIVOLTAICS

One of the most significant innovations in agrivoltaic technology is the use of bifacial solar panels. These panels are capable of capturing sunlight from both sides, increasing their efficiency. The light reflected from the ground and plants can be absorbed by the underside of the panel, thereby increasing the total energy produced. Furthermore, these panels facilitate the management of light and shade over crops, optimizing the microclimate for plants (Nakata & Ogata, 2023; Chalgynbayeva et al., 2024).

The performance of bifacial modules in agrivoltaic applications reveal substantial variability depending on environmental and design parameters. Field studies conducted in diverse agricultural contexts demonstrate that energy yield improvements from 10% to 30% compared to monofacial systems (Badran & Dhimish, 2024; GrowingSolarMist, 2025) with bifaciality factors ranging from 70% to 95%, depending on the cell technology used (Katsikogiannis et al., 2022; Mohd Zaki, 2024). Surface characteristics beneath the panels play a critical role in determining rear-side irradiance gains. For example, grass-covered soils can add approximately 5.2% additional energy, sandy surfaces about 10.8%, and highly reflective white surfaces up to 21.9% (Guarino et al., 2025). Advanced heterojunction cell technologies further push bifaciality values toward 95% to 100%, offering enhanced benefits in elevated agrivoltaic configurations where modules are installed positioned above crop canopies (Buari & Kumari, K., 2023; Yakubu et al., 2024).

Economic analysis of bifacial agrivoltaic systems present a trade-off between higher upfront costs and improved long-term performance. While bifacial modules typically cost $0.05/W to $0.15/W more than monofacial ones, representing a higher capital cost of 10% to 15% (Mouhib et al., 2022; Almarshoud et al., 2024), comprehensive lifecycle assessments indicate LCOE reductions of 8% to 10% due to energy output (Hussain & Ghosh, 2024). Global optimization analyses suggest that bifacial systems achieve economic viability through careful design choices, such as ground coverage ratios between 0.3 and 0.5, and site-specific tilt angle adjustments (Zhong et al., 2023). The financial demand is further enhanced by the ability to generate higher energy output using the same mounting infrastructure, avoiding proportional increases in balance-of-system costs.

Design optimization for bifacial agrivoltaic systems introduces distinctive challenges not present in traditional PV installations. Ground coverage ratio must be carefully balanced to manage inter-row shading while maximizing albedo capture (Muñoz García et al., 2024). Additionally, agronomic compatibility requires elevated mounting, typically 1 to 2 meters above the crop canopy, to ensure adequate sunlight for photosynthesis and to enhance rear-side irradiance.

Albedo management is another distinctive operational consideration in bifacial agrivoltaic systems. Since, rear-side energy gain depends directly on ground reflectivity, effective system planning must consider seasonal crop cycles, vegetation maintenance, and the potential use of reflective ground covers. These factors interact dynamically over time and should be integrated into the system's agronomic and energy performance strategies to maximize year-round efficiency.

The technical and economic advantages of bifacial solar panels outlined above have significant implications for agrivoltaic deployment worldwide, particularly in regions where agricultural productivity and renewable energy expansion are both national priorities. India exemplifies this convergence, where the government's ambitious solar energy targets and the country's substantial agricultural sector create ideal conditions for bifacial agrivoltaic adoption.

India's bifacial PV panel market reflects this growing potential, with projections indicating expansion at a CAGR of 13.60% during the forecast period from FY2025 to FY2032, increasing from USD 488.11 million in FY2024 to USD 1,353.78 million by FY2032. Recent technological developments further support this growth trajectory. In September 2024, Sharp announced the launch of a 450 W bifacial TOPCon solar module with an efficiency of 22.52%, certified under IEC61215 and IEC61730 standards. The panel features an operating temperature coefficient of -0.29% per °C and is designed for both solar farms and rooftop installations, with compact dimensions enabling space-efficient deployment (Markets & Data, 2024).

These market developments are being translated into practical applications through several commissioned pilot projects that demonstrate the real-world implementation of bifacial agrivoltaic systems. The following case studies illustrate how the theoretical advantages of bifacial technology are being realized in operational agrivoltaic installations across India (GIZ, 2024).

The Parbhani installation in Maharashtra's Marathwada region represents one of the most comprehensive bifacial agrivoltaic demonstrations to date. This 5-acre facility, with a capacity of 1.4 MW as part of a larger 50 MW solar project, was commissioned on November 12, 2022, through a collaborative effort between SunSeed APV, Kanoda Energy, and GIZ. The project's design incorporates four distinct APV configurations alongside a control farming area, enabling systematic comparison of different approaches to agrivoltaic implementation. The installation's most notable feature is a 6000 m² elevated structure housing a shade net system for trellised vegetables, which demonstrates the practical application of albedo management principles discussed earlier. This elevated configuration enhances rear-side irradiance capture while providing optimal growing conditions for crops including watermelon, capsicum, muskmelon, and spinach. Lower-elevation configurations at 1.25 m and 1.75 m heights offer alternative cost-effective approaches for different agricultural applications. The project's sophisticated water management system, incorporating drip irrigation and soil moisture sensors, exemplifies how panel shading can be leveraged to reduce water consumption while maintaining crop productivity (Mongabay, 2024). With extensive instrumentation, the farm supports R&D in agrivoltaics, informing future designs and crop strategies shown in Fig. 5.

Complementing these agricultural applications, the National Institute of Solar Energy (NISE) has pioneered a distinct approach through its vertically-installed bifacial PV panel pilot project in Haryana. This 5 kW experimental installation positions bifacial panels vertically with east-west orientation, addressing the challenge of energy generation balance throughout the day while maximizing agricultural land availability. This configuration represents an innovative solution to the space competition between solar installations and crop cultivation (National Solar Energy Federation of India (2023)).

Figure 5. Green capsicum at elevated section I at agrivoltaics plant near Parbhani, India (Mongabay. 2024).

The Nashik facility in Maharashtra further demonstrates the scalability of bifacial agrivoltaic systems. Initially developed as a 250 kWp installation on a one-acre plot owned by Sahyadri Farms, an FPO, the project's success led to expansion to 500 kWp capacity. The installation features an elevated structure at 4-meter height with bifacial panels positioned at 6-meter pitch intervals, allowing cultivation of high-value crops including grapes, oranges, raspberry, tomato, and strawberry beneath the solar canopy. This project, developed through collaboration between Sunseed APV, Kanoda Energy, and GIZ, exemplifies the economic viability potential discussed in the technical analysis above. (Rahman et al., 2023).

Despite these successful demonstrations, pilot project experiences have revealed several implementation challenges that must be addressed for broader adoption. The higher capital costs associated with bifacial panels, typically 10% to 15% above monofacial alternatives, require careful economic optimization to achieve favorable lifecycle cost outcomes. Design complexity increases significantly as engineers must balance solar irradiance requirements for energy generation with photosynthetic light needs for crop productivity, necessitating site-specific optimization approaches. Furthermore, the dual-technology nature of agrivoltaic systems demands enhanced technical expertise from operators, requiring training programs that bridge agricultural and photovoltaic knowledge domains. Regulatory frameworks remain underdeveloped, with policy guidelines needed to provide clear pathways for project approval, grid interconnection, and agricultural land use compliance. These challenges underscore the need for continued research and development efforts to optimize system designs and crop selection strategies specifically adapted to diverse Indian agricultural contexts (National Solar Energy Federation of India. 2023).

Another significant technological advancement is the use of solar trackers, which adjust the orientation of the solar panels to follow the sun's movement throughout the day. This technology optimizes solar light capture and can be integrated with agrivoltaic

systems to ensure that crops receive the optimal amount of light and shade, enhancing both energy production and agricultural yield (Casares de la Torre et al., 2022; Di Francia & Cupo, 2023).

The study on the Even-lighting Agrivoltaic System (EAS) introduces innovative concepts and potential future developments for agrivoltaics. The EAS confers economic benefits upon farmers, by increasing their income, providing uniform illumination for crops, and boosting the daily irradiation received by crops by 47.38% compared to conventional agrivoltaic systems (Zheng et al., 2021).

Smart irrigation systems represent another emerging technology in agrivoltaic applications, offering quantifiable benefits in water conservation, energy efficiency, and crop productivity. These systems integrate IoT sensors, automated controllers, and real-time data analytics to optimize water delivery based on soil moisture content, weather conditions, and crop-specific requirements (Tajima & Iida, 2021). Recent studies demonstrate that sensor-controlled irrigation systems, when implemented under photovoltaic panel structures, can cut irrigation water requirements by 20 to 35% (Elamri et al., 2018; Mohammedi et al., 2023), while simultaneously increase crop water-productivity by up to approximately 90 kg m^{-3} (AL-agele et al., 2021). In water limited regions, these systems have shown the potential to recover the initial investment, estimated at less than €6,000 per hectare, within three to five growing seasons, (Champness et al., 2023; Di Francia & Cupo, 2023; Di Gennaro et al., 2024); Zidane et al., 2025).

The integration of smart irrigation with agrivoltaic systems lies in the complementary relationship between solar panel microclimate effects and precision water management. Agrivoltaic canopies reduce net solar radiation, canopy temperature and soil-evaporation, thereby lowering crop water demand. Smart irrigation systems enhance these effects by delivering water efficiently based on real time sensor data. Studies in dryland regions of Arizona report midday leaf-water potential 40% less negative, and thus lower stress, inside agrivoltaic plots than in full sun, even when irrigation is halved (Barron-Gafford et al., 2025). Furthermore, modeling work conducted in Montpellier on lettuce crops showed that combining panel tilt adjustment with soil moisture thresholds could achieve 20 to 30% seasonal water savings while maintaining yield losses at or below 10% (Elamri et al., 2018).

From an economic perspective, the integration of smart irrigation systems within agrivoltaic installations presents favorable cost-benefit profiles. Commercial Internet of Things platforms designed specifically for agrivoltaics report total hardware and installation costs below €6,000 for a two-hectare block. This includes soil moisture probes, LoRa gateway and solar powered pump upgrades (Di Gennaro et al., 2024), representing only 3 to 5% of the total system cost associated with elevated single-axis agrivoltaic configurations (Zidane et al., 2025). A multi-region cost–benefit analysis in Italy estimated that the additional capital expenditure for precision irrigation reduces the levelized cost of electricity by 1 to 2 €/MWh, primarily due to lower panel soiling and improved photovoltaic efficiency. While water conservation yields a net present benefit of €800 to 1,500 ha^{-1} yr^{-1}, achieving payback periods of 3 to 4 years for wheat and solar energy combination (Di Francia & Cupo, 2023). The overall economic feasibility is further enhanced through energy integration strategies. For example, smart pumps powered directly from the DC output of agrivoltaics system can operate without

relying on the power grid. The pumps can be scheduled during periods of reduced energy dispatch, thereby increasing the site's internal energy use by around 12% percent in simulation studies (Al Mamun et al., 2025).

Furthermore, few deployments have implemented combined strategies that integrate panel-tilt schedules with irrigation schedules. The development of integrated optimization frameworks that use environmental sensors alongside photovoltaic tracking remains an area of active investigation (Elamri et al., 2018; Navarro-González et al., 2023). Successful implementation depends on site specific features such as soil type, crop selection, and climate conditions, all of which influence sensor placement and irrigation control. The scalability from demonstration projects to commercial agricultural operations requires progress in standardizing technologies and cost reduction strategies.

Figure 6. Fish Pond set-up underneath PV-modules in Bhaloji, Rajasthan, India (National Solar Energy Federation of India. 2023).

Future developments in smart irrigation for agrivoltaics applications include the use of machine learning models capable of forecasting irrigation needs. These models rely on historical data, weather predictions, and real-time plant monitoring to guide water application with greater precision (Saikai et al., 2023; Umutoni & Samadi, 2024). Policy frameworks that include water-pricing and environmental incentives programs that reward verified water savings, could help improve the financial returns and encourage wider implementation (Giannoccaro et al., 2022; (Mooney et al., 2022). These technological and policy advances are expected to further improve water use efficiency while maintaining the productive combination of farming and solar energy, positioning smart irrigation as a key element in the evolution of precision agriculture in photovoltaic environment. An innovative approach in agrivoltaic systems is the 'Fish Pond Agri-PV System' located in Bhaloji, Rajasthan, India shown in Fig. 6. This 30 kW solar power project is integrated with a fish pond with total site capacity of 1MW and is likely part of the Prime Minister's Kisan Urja Suraksha evam Utthaan Mahabhiyan (KUSUM) scheme, which supports farm-based solar power initiatives. The system involves installing solar panels over or around the fish pond, enabling simultaneous

electricity generation and aquaculture operations. The system likely serves a dual function: harnessing solar energy for electricity generation while potentially boosting fish production in the pond beneath. Research indicates that the shading provided by solar panels can help regulate water temperature, creating favorable conditions for aquaculture. While agrivoltaic is still an emerging technology, its potential to address food and energy security challenges, particularly in water-stressed regions, is making it an increasingly attractive option for sustainable development (National Solar Energy Federation of India (2023)).

CHALLENGES AND MITIGATION STRATEGIES IN IMPLEMENTING AGRIVOLTAIC SYSTEMS

One of the primary obstacles to the implementation of agrivoltaic systems is the necessity of ensuring compatibility between crop types and solar panels. Not all crops exhibit the same response to the partial shading provided by solar panels. The selection of appropriate crops is crucial to achieve the optimal balance between food pro-duction and energy generation. It is therefore essential that ongoing research be conducted to identify the most optimal crop-panel combinations and configurations (Chalgynbayeva et al., 2024). To address these compatibility challenges, research institutions have developed systematic approaches based on the 'five C's' framework: (1) Climate, soil and environmental conditions, (2) Configurations, (3) Crop selection, (4) Compatibility and flexibility, (5) Collaboration and partnerships (Macknick et al., 2022). The InSPIRE project identified these five key factors that contribute to the success of agrivoltaics initiatives. It provides a standardized yet adaptable methodology for guiding both crop selection and system design. By applying this approach, farmers can make evidence-based decisions about optimal crop-panel combinations suitable for their specific agricultural and climatic conditions (Macknick et al., 2022; Soto-Gómez, 2024).

The initial installation costs of agrivoltaic systems can be prohibitive for many farmers. The necessity for additional infrastructure, such as elevated structures for panels and enhanced irrigation systems, can significantly increase project costs. This financial burden may act as a deterrent for farmers, particularly those in regions with limited financial resources, from adopting this technology (Tajima & Iida, 2021). For example, a study conducted in Germany indicates that the implementation costs of agrivoltaic systems can be considerably higher than those of conventional ground-mounted PV systems. The use of bifacial modules has been found to result in an average cost increase of €326 per kWp. The costs associated with mounting systems can vary considerably. Tilted and overhead agrivoltaic systems installed at heights exceeding four meters have been found to cost, on average, €372 per kWp, in comparison to a mean cost of just €76 per kWp for standard ground-mounted systems. The aforementioned costs can range from €240 to €500 per kWp, depending on the specific system configuration. Furthermore, the costs associated with site preparation and installation of agrivoltaic systems can range from €190 to €260 per kWp, which is considerably higher than the €60 to €100 per kWp typically associated with ground-mounted systems (Pascaris, 2021). However, recent technological developments have increasingly focused on cost reduction through modular agrivoltaic systems designed for incremental deployment and

reduced upfront investment. Research demonstrates a modular design approach, where each unit is prefabricated independently and assembled using interlocking components and bolted connections, facilitating both construction and scalability (Toledo & Scognamiglio, 2021; Zhang et al., 2025). This approach enables farmers to begin with small-scale installations and gradually expand capacity as financial conditions improve, thereby reducing the barrier to entry without technical performance. Additionally, collaborative financing models, such as peer-to-peer lending schemes, have been shown to accelerate the adoption of renewable energy technologies. These mechanism offer alternative funding pathways that bypass conventional banking systems, making agrivoltaic adoption more accessible, especially in underserved or credit-constrained contexts (Zhang et al., 2025).

Moreover, regulations and policies can also act as impediments to the implementation of agrivoltaic systems. Land use regulations, including restrictions on the height and placement of solar panels, can limit the viability of these systems. Additionally, the absence of specific incentives for agrivoltaics can impede their large-scale adoption. It is essential that governments develop policies that actively support the integration of agrivoltaic systems into current agricultural and energy practices. Likewise, studies on renewable energy expansion (Karapidakis et al., 2023) underscore that the lack of robust grid infrastructure and energy storage solutions can lead to further complications in the integration of intermittent energy sources, including agrivoltaics, particularly in remote or isolated regions.

In addition to the economic and regulatory challenges associated with the implementation of agrivoltaics systems, the limited access to reliable electricity infrastructure in rural areas poses a significant barrier to the widespread implementation of such systems. In many developing regions, where electrification rates remain low, agrivoltaics could serve as a decentralized energy solution, offering both electricity and agricultural benefits. However, as highlighted in previous rural electrification studies (Dunmade, 2021), successful adoption requires significant investment in grid stability, storage solutions, and technical expertise. Without appropriate infrastructure and policy frameworks to support energy distribution, agrivoltaic projects may struggle to deliver their full benefits, particularly in areas where agricultural communities face economic and logistical constraints. To overcome rural infrastructure constrains and limited technical expertise, demonstration projects and community-based learning have proven to be effective strategies for transferring agrivoltaic technology. Studies highlight the importance of partnerships between governmental agencies, solar energy providers, and local farming communities to facilitate the successful implementation of these systems. Such collaborations provide the necessary resources and assistance (Dupraz et al., 2011; Fraunhofer Institute for Solar Energy Systems ISE (2022); Zeddies et al., 2025)).

Furthermore, the implementation of supportive policies and the provision of specific financial incentives for agrivoltaic projects can help offset high initial costs associated with such projects (Schindele et al., 2020; Wagner et al., 2024). Subsidy programs, feed-in tariffs, and tax credits can facilitate the accessibility of these systems to farmers (Pascaris et al., 2021; Jamil & Pearce, 2023). Financial incentives can play a pivotal role in promoting the adoption of agrivoltaic technologies and fostering their integration into sustainable agriculture.

Finally, fostering collaboration between the public and private sectors can facilitate the implementation of agrivoltaic systems. Partnerships between governmental entities, solar energy companies, and farmers can facilitate the development of successful agrivoltaic projects by providing the necessary resources and assistance. Pilot programs and demonstration projects can also serve to showcase the benefits of agrivoltaic technology on a large scale, encouraging broader adoption.

In summary, the findings from the case studies, emerging technologies, and challenges discussed in this paper provide comprehensive answers to the research questions. The global applicability and effectiveness of agrivoltaic systems are clearly demonstrated with regard to diverse geographical and socio-economic contexts. Technological innovations are playing a critical role in improving system efficiency, while the identified challenges emphasize the importance of supportive policies and collaborative efforts in overcoming barriers to implementation. Together, these findings contribute to a more accurate understanding of how agrivoltaic systems can be successfully integrated into sustainable agricultural and energy practices.

CONCLUSIONS

Agrivoltaic systems offer a promising solution for integrating renewable energy generation with agricultural production, providing economic, environmental, and technical benefits. Case studies from Japan, France, Africa, and Latin America demonstrate diverse applications and successful implementations. These systems improve agricultural productivity by creating favorable microclimates, optimizing light and water use, and supporting higher crop yields. For the purpose of scalability, the economic implications in terms of capital, and operation and maintenance costs, as well as the technology costs (for example, tracking) need to be understood. There is also the need for establishing standardised systems designs as well as the documentation of best practices to enable widespread application of agrivoltaics.

Technological advancements, including bifacial solar panels, solar trackers, and smart irrigation systems, have played a key role in enhancing the efficiency and effectiveness of agrivoltaic systems. These innovations enable better resource management, improve solar panel performance, and encourage the adoption of sustainable agricultural practices.

Despite these promising benefits, several challenges remain, including the compatibility of crops with solar panels, high initial installation costs, and regulatory barriers. To address these challenges, further research is required to identify the most effective crop-panel configurations to develop supportive policies and financial incentives. Public-private collaborations are crucial for ensuring the availability of the necessary resources and support for the successful project implementation. Additionally, fostering interdisciplinary cooperation among researchers, policymakers, agricultural experts, and energy sector stakeholders will be essential to drive innovation and develop solutions adaptable to different environmental and socio-economic scenarios.

In conclusion, the integration of agrivoltaic systems holds significant potential for advancing sustainable agriculture and driving the energy transition efforts. Technical advantages for solar photovoltaic systems in agrivoltaics include enhanced panel performance due to natural cooling from crop transpiration, decreased vegetation-related

maintenance, and improved soil conditions through organic matter deposition. Tackling the existing challenges and capitalizing on these innovations is essential for agrivoltaic systems to meaningfully contribute to climate change mitigation, rural development, and long-term sustainability.

Based on these findings, this integrative qualitative overview contributes to the field by combining global case evidence, emerging technologies, and policy perspectives to provide a context-adapted and scalable roadmap for the implementation of agrivoltaic systems.

This qualitative overview provides a comprehensive synthesis of agrivoltaic systems to deepen the understanding of their relevance across diverse contexts. However, it also acknowledges certain limitations that need further investigation. These include variability in crop responses under different panel configurations or panel shading, economic modeling over extended timeframes, and the scalability of systems across differing geographical and policy environments. Additionally, social acceptance and land tenure dynamics complexities remain underexplored areas with implications for widespread adoption. To advance the field, future research should emphasize region-specific design optimization, crop-panel compatibility assessments, full-lifecycle environmental impact analyses, and adaptable policy frameworks. Policymakers are encouraged to implement incentive structures, revise zoning regulations, and support pilot projects to foster adoption. Industry actors should prioritize modular designs, cost-reduction strategies, and collaborative research. Integrating agrivoltaic development with broader sustainability agendas, including water management, biodiversity preservation, and rural resilience, will be crucial for maximizing their societal and environmental value.

In summary, this overview highlights the significant potential of agrivoltaic systems to improve land use efficiency, strengthen climate resilience and adaptation, and enhance food and energy security across diverse geographical and socio-economic contexts. Technological innovations, including bifacial solar panels, solar tracking mechanisms, and integrated smart irrigation have demonstrated their effectiveness in optimizing both energy generation and agricultural productivity. To ensure broader adoption and scalability, the presence of supportive policy frameworks remain essential. These include targeted investment incentives, adaptative regulatory mechanisms, and collaborative efforts across research institutions, industry stakeholders, and government entities. Collectively, these findings reinforce the relevance of agrivoltaics as a practical and adaptable solution that contributes meaningfully to the goals of sustainable development.

REFERENCES

AL-agele, H.A., Proctor, K., Murthy, G & Higgins, C. 2021. A case study of tomato (solanum lycopersicon var. legend) production and water productivity in agrivoltaic systems. *Sustainability* **13**, 2850. doi: 10.3390/su13052850

Almarshoud, A.F., Abdel-halim, M.A., Almasri, R.A & Alshwairekh, A.M. 2024. Experimental study of bifacial photovoltaic module performance on a sunny day with varying backgrounds using exergy and energy analysis. *Energies* **17**, 5456. doi: 10.3390/en17215456

Al Mamun, M.R., Ahmed, A.K., Upoma, S.M., Haque, M.M & Ashik-E-Rabbani, M. 2025. IoT-enabled solar-powered smart irrigation for precision agriculture. *Smart Agricultural Technology* **10**, 100773. doi: 10.1016/j.atech.2025.100773

Badran, G & Dhimish, M. 2024. Comprehensive study on the efficiency of vertical bifacial photovoltaic systems: a UK case study. *Scientific Reports* **14**, 18380. doi: 10.1038/s41598-024-68018-1

Barron-Gafford, G.A., Murphy, P., Salazar, A., Lepley, K., Rouini, N., Barnett-Moreno, I. & Macknick, J.E 2025. Agrivoltaics as a climate-smart and resilient solution for midday depression in photosynthesis in dryland regions. *Sustainable Agriculture* **3**, 32. doi: 10.1038/s44264-025-00073-1

Barron-Gafford, G.A., Pavao-Zuckerman, M.A., Minor, R.L., Sutter, L.F., Barnett-Moreno, I., Blackett, D.T., Thompson, M., Dimond, K., Gerlak, A.K., Nabhan, G.P. & Macknick, J.E. 2019. Agrivoltaics provide mutual benefits across the food–energy–water nexus in drylands. *Nat Sustain* **2**, 848–855. doi: 10.1038/s41893-019-0364-5

Biró-Varga, K., Sirnik, I & Stremke, S. 2024. Landscape user experiences of interspace and overhead agrivoltaics: A comparative analysis of two novel types of solar landscapes in the Netherlands. *Energy Research & Social Science* **109**, 103408. doi: 10.1016/j.erss.2023.103408

Buari, O.K & Kumari, K. 2023. Optimizing agrivoltaics electricity generation in Sweden: A techno-economic analysis of latitude-dependent design systems. Mälardalen University. Available at: https://urn.kb.se/resolve?urn=urn:nbn:se:mdh:diva-63547

Bumbiere, K., Sereda, S., Pubule, J & Blumberga, D. 2023. The role of energy management in the agricultural sector: key prerequisites and impacts. *Agronomy Research* **21**(S2), 439–450. doi: 10.15159/AR.23.034

Bruhwyler, R., Sánchez, H., Meza, C., Lebeau, F., Brunet, P., Dabadie, G., Dittmann, S., Gottschalg, R. & Negroni, J.J. 2023. Vertical agrivoltaics and its potential for electricity production and agricultural water demand: A case study in the area of Chanco, Chile. *Sustainable Energy Technologies and Assessments* **60**. doi: 10.1016/j.seta.2023.103425

Carrausse, R. & Arnauld de Sartre, X. 2023. Does agrivoltaism reconcile energy and agriculture? Lessons from a French case study. *Energ. Sustain Soc* **13**(1). doi: 10.1186/s13705-023-00387-3

Casares de la Torre, F.J., Varo, M., López-Luque, R., Ramírez-Faz, J. & Fernández-Ahumada, L.M. 2022. Design and analysis of a tracking / backtracking strategy for PV plants with horizontal trackers after their conversion to agrivoltaic plants. *Renewable Energy* **187**. doi: 10.1016/j.renene.2022.01.081

Chalgynbayeva, A., Balogh, P., Szőllősi, L., Gabnai, Z., Apáti, F., Sipos, M. & Bai, A. 2024. The Economic Potential of Agrivoltaic Systems in Apple Cultivation – A Hungarian Case Study. *Sustainability* **16**, 232. doi: 10.3390/su16062325

Chalgynbayeva, A., Mizik, T. & Bai, A. 2022. Cost–Benefit Analysis of Kaposvár Solar Photovoltaic Park Considering Agrivoltaic Systems. *Clean Technol* **4**, 1054–1070. doi: 10.3390/cleantechnol4040064

Champness, M., Vial, L., Ballester, C & Hornbuckle, J. 2023. Evaluating the performance and opportunity cost of a smart-sensed automated irrigation system for water-saving rice cultivation in temperate australia. *Agriculture* **13**, 903. doi: 10.3390/agriculture13040903

Di Francia, G. & Cupo, P. 2023. A Cost–Benefit Analysis for Utility-Scale Agrivoltaic Implementation in Italy. *Energies* **16**, 2991. doi: 10.3390/en16072991

Di Gennaro, S.F., Cini, D., Berton, A & Matese, A. 2024. Development of a low-cost smart irrigation system for sustainable water management in the Mediterranean region. *Smart Agricultural Technology* **9**, 100629. doi: 10.1016/j.atech.2024.100629

Dinesh, H. & Pearce, J.M. 2016. The potential of agrivoltaic systems. *Renewable and Sustainable Energy Reviews* **54**, 299-308. doi: 10.1016/j.rser.2015.10.024

Dreves, H. 2022. Growing Plants, Power, and Partnerships Through Agrivoltaics. *NREL Transforming Energy*. https://www.nrel.gov/news/program/2022/growing-plants-power-and-partnerships.html

Dunmade, I. 2021. Community/shared solar power option: a pathway to sustainable rural electrification in Nigeria. *Agronomy Research* **19**(4), 1734–1476. doi: 10.15159/AR.21.150

Dupraz, C., Marrou, H., Talbot, G., Dufour, L., Nogier, A & Ferard, Y. 2011. Combining solar photovoltaic panels and food crops for optimising land use: Towards new agrivoltaic schemes. *Renewable Energy* **36**(10), 2725–2732. doi: 10.1016/j.renene.2011.03.005

DW News. 2024. Climate change puts agrivoltaics projects in Northern Africa in the spotlight. https://www.dw.com/en/climate-change-puts-agrivoltaic-projects-in-northern-africa-in-the-spotlight/a-61972022

Elamri, B., Cheviron, J.M., Lopez, C., Dejean, G & Belaud, G. 2018. Water budget and crop modelling for agrivoltaic systems: Application to irrigated lettuces. *Agricultural Water Management* **208**, 440–453. doi: 10.1016/j.agwat.2018.07.001

Fraunhofer Institute for Solar Energy Systems ISE. 2022. Agrivoltaics: Opportunities for Agriculture and the Energy Transition. Freiburg, Germany.

Giannoccaro, G., Roselli, L., Sardaro, R & de Gennaro, B.C. 2022. Design of an incentive-based tool for effective water saving policy in agriculture. *Agricultural Water Management* **272**, 107866. doi: 10.1016/j.agwat.2022.107866

GIZ. 2024. *Agrivoltaics in India*. https://energyforum.in/fileadmin/india/media_elements/publications/20240502_AgriPV_in_India/Agrivoltaics_in_India.pdf

Goetzberger, A. & Zastrow, A. 1982. On the Coexistence of solar-energy conversion and plant cultivation. *Int. J. Sol. Energy* **1**(1), 55–69.

Gonocruz, R., Nakamura, R., Yoshino, K., Homma, M., Doi, T., Yoshida, Y. & Tani, A. 2021. Analysis of the Rice Yield under an Agrivoltaic System: A Case Study in Japan. *Environments* **8**, 65. doi: 10.3390/environments8070065

GrowingSolarMist 2025. Bifacial Solar Panels Benefits for Farming Operations. Avlaible at: https://growingsolarmist.com/bifacial-solar-panels-benefits-for-farming-operations Retrieved on July 30th, 2025

Guarino, S., Buscemi, A., Chiaruzzi, C & Lo Brano, V. 2025. Modelling and analysis of V-shaped bifacial PV systems for agrivoltaic applications: A Python-based approach for energy optimization. *Applied Energy* **389**, 125785. doi: 10.1016/j.apenergy.2025.125785

Hu, Z. 2023. Towards solar extractivism? A political ecology understanding of the solar energy and agriculture boom in rural China. *Energy research and social science* **98**, 102988. doi: 10.1016/j.erss.2023.102988

Hussain, S.N & Ghosh, A. 2024. Evaluating tracking bifacial solar PV based agrivoltaics system across the UK. *Solar Energy* **284**, 113102. doi: 10.1016/j.solener.2024.113102

IDB Invest. 2023. Agriphotovoltaics double opportunity - Latin America and Caribbean. https://www.idbinvest.org/en/blog/agribusiness/agrophotovoltaics-double-opportunity-latin-america-and-caribbean

Jamil, U., Bonnington, A. & Pearce, J.M. 2023a. The Agrivoltaic Potential of Canada. *Sustainability* **15**(4), 3228. doi: 10.3390/su15043228

Jamil, U., Vandewetering, N. & Pearce, J.M. 2023b. Solar photovoltaic wood racking mechanical design for trellis-based agrivoltaics. *PLoS ONE* **18**(12), e0294682. doi: 10.1371/journal.pone.0294682

Jamil, U. & Pearce, J.M. 2023. Energy Policy for Agrivoltaics in Alberta Canada. *Energies* **16**(1), 53. doi: 10.3390/en16010053

Jerome, W., Chew, B., Shiddalingeshwar, C.D., Soon Tay, R.H. & Jusuf, S.K. 2022 Effects of Agrivoltaic Systems on the Sur-rounding Rooftop Microclimate. *Sustainability* **14**(12), 7089. doi:10.3390/su14127089

Jiufu, L., Zhongxin, L., Wen, Li, Shi, W. & Sui, X. 2024. The Early Effects of an Agrivoltaic System within a Different Crop Cultivation on Soil Quality in Dry–Hot Valley Eco-Fragile Areas. *Agronomy* **14**(3), 584. doi:10.3390/agronomy14030584

Karapidakis, E., Kikaki, A. & Mozakis, J. 2023. Sustainable power generation expansion with RES and energy storage. *Agronomy Research* **21**(3), 1154–1160. doi: 10.15159/AR.23.063

Katsikogiannis, O.A., Ziar, H & Isabella, O. 2022. Integration of bifacial photovoltaics in agrivoltaic systems: A synergistic design approach. *Applied Energy* **309**, 118475. doi: 10.1016/j.apenergy.2021.118475

Kim, M., Oh, S-Y. & Jung, J.H. 2021. History and legal aspect of agrivoltaics in Korea. Nucleation and Atmospheric Aerosols. *AIP Conf. Proc*. **2635**(1), 050001. doi: 10.1063/5.0127822

Kim, Y., On, Y., So, J., Kim, S. & Kim, S. 2023. A Decision Support Software Application for the Design of Agrophotovoltaic Systems in Republic of Korea. *Sustainability* **15**, 8830. doi: 10.3390/su15118830

Kirto, E., Woodruff, C. & Wu, S. 2024. *Environmental Benefits and Market Potential of Agrivoltaics*. Masters project submitted for the Master of Environmental Management degree of Duke University, USA, 34 pp.

Kumari, S., Pandit, A., Bhende, A. & Rayalu, A. 2022. Thermal Management of Solar Panels for Overall Efficiency Enhancement Using Different Cooling Techniques. *Int. J. Environ Res.* **16**, 53. doi:10.1007/s41742-022-00431-8

Macdonald, J., Probst, L. & Cladera, J.R. 2022. Opportunities and challenges for scaling agrivoltaics in rural and Urban Africa. *AIP Conf. Proc* **2635**(1), 070002. doi: 10.1063/5.0105526

Macknick, J., Hartmann, H., Barron-Gafford, G., Beatty, B., Burton, R., Seok-Choi, C, Davis, M., Davis, R., Figueroa, J., Garret, A., Hain, L., Herberth, S., Janski, J., Kinzer, A., Knapp, A., Lehan, M., Losey, J., Marley, J., MacDonald, J., McCall, J., Nebet, L., Ravi, S., Schmidt, J., Staie, B. & Waltson, L. 2022. The 5 Cs of Agrivoltaic Success Factors in the United States: Lessons from the InSPIRE Research Study. *National Renewable Energy Laboratory*. doi: 10.2172/1882930

MAFF Japan. 2021. Subsidies for Renewable Energy in Agriculture.

Malu, P.R., Sharma, U.S. & Pearce, J.M. 2017. Agrivoltaic potential on grape farms in India. *Sustainable Energy Technologies and Assessments* **23**. doi: 10.1016/j.seta.2017.08.004

Mamun, M.A.A., Dargusch, P., Wadley, D., Zulkarnain, N.A. & Aziz, A.A. 2022. A review of re-search on agrivoltaic systems. *Renew. Sustain Energy Rev.* **161**, Article 112351. doi: 10.1016/j.rser.2022.112351

Markets & Data. 2024. India Bifacial Solar Market Assessment, Opportunities and Forecast, FY2018-FY2032F https://www.marketsandata.com/industry-reports/india-bifacial-solar-market

Marrou, H., Dufour, L. & Wery, J. 2013. How does a shelter of solar panels influence water flows in a soil–crop system? *European Journal of Agronomy* **50**, 38–51. doi: 10.1016/j.eja.2013.05.004

Masons, P. 2024. Rising opportunities for agrivoltaic energy projects under France's regulatory strategy. https://www.pinsentmasons.com/out-law/analysis/rising-opportunities-agrivoltaic-energy-projects-under-regulatory-strategy

Mohammedi, S., Dragonetti, G., Admane, N & Fouial, A. 2023. The Impact of Agrivoltaic Systems on Tomato Crop: A Case Study in Southern Italy. *Processes* **11**, 3370. doi: 10.3390/pr11123370

Mohd Zaki, N.A. 2024. Bifacial PV modules for agrivoltaic applications. University Sains Malaysia. Available at: https://erepo.usm.my/handle/123456789/21648

MONGABAY. 2022. In Brazil's semiarid region agrivoltaics show promise for food energy security. https://news.mongabay.com/2022/07/in-brazils-semiarid-region-agrivoltaics-show-promise-for-food-energy-security/

MONGABAY. 2024. Agrivoltaics in India get a fresh boost from tech and design innovations. https://india.mongabay.com/2024/08/agrivoltaics-in-india-get-a-fresh-boost-from-tech-and-design-innovations/

Mooney, D.F., Hoag, D.L.K., Rasul, Z.I & Gao, S. 2022. More risk, more money: When are payments for water savings from limited irrigation profitable for farmers? *Water Resources and Economics* **40**, 100212. doi: 10.1016/j.wre.2

Mouhib, E., Micheli, L., Almonacid, F.M & Fernández, E.F. 2022. Overview of the Fundamentals and Applications of Bifacial Photovoltaic Technology: Agrivoltaics and Aquavoltaics. *Energies* **15**, 8777. doi: 10.3390/en15238777

Muñoz García, M.A., Nieto Morone, B., Moreda Cantero, G.P., Alonso García, M.C., Fialho, L & Baptista, F. 2024. Bifacial panels for agrivoltaics and crop influence: expected benefits. In: *41st European Photovoltaic Solar Energy Conference*, 23–27 September, Vienna, Austria.

Nakata, H. & Ogata, S. 2023. Integrating Agrivoltaic Systems into Local Industries: A Case Study and Economic Analysis of Rural Japan. *Agronomy* **13**, 513. doi: 10.3390/agronomy13020513

National Solar Energy Federation of India (NSEFI). 2023. Agrivoltaics in India – Overview of projects and relevant policies. https://energyforum.in/fileadmin/india/media_elements/Photos_And_Gallery/20201210_SmarterE_AgroPV/20201212_NSEFI_on_AgriPV_in_India_1__01.pdf

Navarro-González, F.J., Pardo, M., Chabour, H.E. & Alskaif, T. 2023. An irrigation scheduling algorithm for sustainable energy consumption in pressurised irrigation networks supplied by photovoltaic modules. *Clean Technologies and Environmental Policy* **25**, 2009–2024. doi: 10.1007/s10098-023-02486-3

Omer, A., Liu, W., Li, M., Zheng, J., Zhang, F., Zhang, X., Mohammed, S.O.H., Fan, L., Liu, Z., Chen, F., Chen, Y. & Ingenhoff, J. 2022. Water evaporation reduction by the agrivoltaic systems development. *Solar Energy* **247**. doi: 10.1016/j.solener.2022.10.022

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., Chou, R., Glanville, J., Grimshaw, J.M., Hróbjartsson, A., Lalu, M.M., Li, T., Loder, E.W., Mayo-Wilson, E. McDonald, S., McGuinness, L.A., Stewart, L.A., Thomas, J., Tricco, A.C., Welch, V.A., Whiting, P. & Moher, D. 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* **372**, n71. doi: 10.1136/bmj.n71

Pascaris, A.S. 2021. Examining existing policy to inform a comprehensive legal framework for agrivoltaics in the U.S. *Energy Policy* **159**. doi: 10.1016/j.enpol.2021.112620

Pascaris, A.S., Gerlak, A.K. & Barron-Gafford, G.A. 2023. From niche-innovation to mainstream markets: Drivers and challenges of industry adoption of agrivoltaics in the U.S. *Energy Policy* **181**. doi: 10.1016/j.enpol.2023.113694

Pascaris, A.S., Schelly, C., Burnham, L. & Pearce, J. 2021. Integrating solar energy with agriculture: Industry perspectives on the market, community, and socio-political dimensions of agrivoltaics. *Energy Research & Social Science* **75**. doi: 10.1016/j.erss.2021.102023

Proctor, K., Murthy, G. & Higgins, C. 2021. Agrivoltaics Align with Green New Deal Goals While Supporting Investment in the US' Rural Economy. *Sustainability* **13**, 137. doi: 10.3390/su13010137

Rahman, A., Sharma, A., Postel, F., Goel, S., Kumar, K. & Laan, T. 2023. Agrivoltaics in India: Challenges and opportunities for scale-up. International Institute for Sustainable Development. *IISD*, Winnipeg, Canada, 1–31.

Randle-Boggis, R.J., Lara, E., Onyango, J., Temu, E.J. & Hartley, S.E. 2021. Agrivoltaics in East Africa: Opportunities and Challenges. *AIP Conf. Proc.* **2361**, 090001. doi: 10.1063/5.0055470

Saikai, Y., Peake, A & Chenu, K. 2023. Deep reinforcement learning for irrigation scheduling using high-dimensional sensor feedback. *PLOS Water* **2**(9), e0000169. doi: 10.1371/journal.pwat.0000169

Schindele, S., Trommsdorff, M., Schlaak, A., Obergfell, T., Bopp, G., Reise, C., Braun, C., Weselek, A., Bauerle, A., Högy, P., Goetzberger, A. & Weber, A. 2020. Implementation of agrophotovoltaics: Techno-economic analysis of the price-performance ratio and its policy implications. *Applied Energy* **265**. doi: 10.1016/j.apenergy.2020.114737

Sekiyama, T. & Nagashima, A. 2019. Solar Sharing for Both Food and Clean Energy Pro-duction: Performance of Agrivoltaic Systems for Corn, A Typical Shade-Intolerant Crop. *Environments* **6**(6), 65. doi: 10.3390/environments6060065

Sheik, M., Kakati, P., Dandotiya, D., Ravi, U. & Ramesh, C. 2022. A comprehensive review on various cooling techniques to decrease an operating temperature of solar photovoltaic panels. *Energy Nexus* **8**. doi: 10.1016/j.nexus.2022.100161

Silvia Ma Lu. 2024. *Solar Irradiance Assessment in Agrivoltaic Systems: Understanding Photo-synthetically Active Radiation Separation Models and Dynamic Crop Albedo Effect in Agri-voltaic Systems Modelling*. Mälardalen University Licentiate Thesis, 94 pp.

Soto-Gómez, D. 2024. Integration of Crops, Livestock, and Solar Panels: A review of agrivoltaic systems. *Agronomy* **14**, 1824. doi: 10.3390/agronomy14081824

Sturchio, M.A & Knapp, A.K., 2023. Ecovoltaic principles for a more sustainable, ecologically informed solar energy future. *Nature Ecology & Evolution* **7**(11), 1746–1749. doi: 10.1038/s41559-023-02174-x

Tajima, M. & Iida, T. 2021. Evolution of agrivoltaic farms in Japan. *AIP Conf. Proc*. 2361, 030002. doi: 10.1063/5.0054674

The potential of agrivoltaics for the US. 2023. Solar industry, farmers and communities. *PV Tech Power* **34**. https://www.pv-tech.org/technical-papers/the-potential-of-agrivoltaics-for-the-us-solar-industry-farmers-and-communities/

The Solar Energy Technologies Office (SETO). 2022. Market Research Study Agrivoltaics. Rochester, NY.

Time, A., Gomez-Casanovas, N., Mwebaze, P., Apollon, W., Khanna, M., DeLucia, E.H. & Bernacchi, C.J. 2024. Conservation agrivoltaics for sustainable food-energy production. *Plants, People, Planet*. doi:10.1002/ppp3.10481

Toledo, C & Scognamiglio, A. 2021. Agrivoltaic systems design and assessment: a critical review, and a descriptive model towards a sustainable landscape vision (Three-Dimensional Agrivoltaic Patterns). *Sustainability* **13**, 6871. doi: 10.3390/su13126871

Tomich, T.P., Brodt, S., Ferris, H., Galt, R., Horwath, W.R., Kebreab, E., Leveau, Johan H.J., Liptzin, D., Lubell, M., Mere, P., Michelmore, R., Scow, K., Six, J., Williams, N. & Yang, L. 2011. Agroecology: A Review from a Global-Change Perspective. *Annu. Rev. Environ. Resour*. **36**,193–222. doi: 10.1146/annurev-environ-012110-121302

Touil, S., Richa, A., Fizir, M. & Bigwa, B. 2021. Shading effect of photovoltaic panels on horticulture crops production: a mini review. *Rev Environ Sci Biotechnol* **20**, 281–296. doi: 10.1007/s11157-021-09572-2

Trommsdorff, M., Dhal, I.S., Özdemir, Ö.E., Ketzer, D., Weinberger, N & Rösch, C. 2022. Chapter 5 - Agrivoltaics: Solar Power Generation and Food Production. In: *Solar Energy Advancements in Agriculture and Food Production Systems.* Gorjian, S & Campana, P.E. (eds.). Academic Press, Cambridge, MA, USA, 159–210.

Uchanski, M., Thomas. H., Jennifer, B. & Kurt, L. 2023. Characterization of Agrivoltaic Crop Environment Conditions Using Opaque and Thin-Film Semi-Transparent Modules. *Energies* **16**(7), 3012. doi:10.3390/en16073012

Umutoni, L & Samadi, V. 2024. Application of machine learning approaches in supporting irrigation decision making: A review. *Agricultural Water Management* **294**, 108710. doi: 10.1016/j.agwat.2024.108710

van de Ven, D.J., Capellan-Peréz, I., Arto, I., Cazcarro, I., de Castro, C., Patel, P. & Gonzalez-Eguino, M. 2021. The potential land requirements and related land use change emissions of solar energy. *Sci. Rep.* **11**(1), 2907. doi: 10.1038/s41598-021-82042-5

Wagner, J., Bühner, C., Gölz, S., Trommsdorff, M. & Jürkenbeck, K. 2024. Factors influencing the willingness to use agrivoltaics: A quantitative study among German farmers. *Applied Energy* **361**. doi: 10.1016/j.apenergy.2024.122934

Wagner, M., Lask, J., Kiesel, A., Lewandowski, I., Weselek, A., Högy, P., Trommsdorff, M., Schnaiker, M.A. & Bauerle, A. 2023. Agrivoltaics: The Environmental Impacts of Combining Food Crop Cultivation and Solar Energy Generation. *Agronomy*. doi: 10.3390/agronomy13020299

Walston, L.J., Barley, T., Bhandari, I., Campbell, B., McCall, J., Hartmann, H.M. & Dolezal, A.G. 2022. Opportunities for agrivoltaic systems to achieve synergistic food-energy-environmental needs and address sustainability goals. *Frontiers In Sustainable Food Systems* **6**. doi: 10.3389/fsufs.2022.932018

Weselek, A., Ehmann, A., Zikeli, S., Lewandowski, I., Schindele, S. & Högy, P. 2019. Agrophotovoltaic systems: applications, challenges, and opportunities. *A review. Agron. Sustain. Dev*. **39**, 35. Doi: 10.1007/s13593-019-0581-3

Wydra, K., Vollmer, V., Busch, C. & Prichta, S. 2023. Agrivoltaic: Solar Radiation for Clean Energy and Sustainable Agriculture with Positive Impact on Nature. doi: 10.5772/intechopen.111728

Xiao, Y., Zhang, H., Pan, S., Wang, Q., He, J. & Jia, X. 2021. An agrivoltaic park enhancing ecological, economic and social benefits on degraded land in Jiangshan, China. Nucleation and Atmospheric Aerosols. *AIP Conf. Proc*. **2635**(1), 020002. doi: 10.1063/5.0106454

Yakubu, R.O., Mensah, L.D., Quansah, D.A & Adaramola, M.S. 2024. A systematic literature review of the bifacial photovoltaic module and its applications. *The Journal of Engineering* **8**, e12421. doi: 10.1049/tje2.12421

Zeddies, H.H., Parlasca, M & Qaim, M. 2025. Agrivoltaics increases public acceptance of solar energy production on agricultural land. *Land Use Policy* **156**, 107604. doi: 10.1016/j.landusepol.2025.107604

Zhang, Y., Chen, T., Gasparri, E & Lucchi, E. 2025. A modular agrivoltaics building envelope integrating thin-film photovoltaics and hydroponic urban farming systems: a circular design approach with the multi-objective optimization of energy, light, water and structure. *Sustainability* **17**, 666. doi: 10.3390/su17020666

Zheng, J., Meng, S., Zhang, X., Zhao, H., Ning, X., Chen, F., Omer, A.A.A., Ingenhoff, J. & Liu, W. 2021. Increasing the comprehensive economic benefits of farmland with Even-lighting Agrivoltaic Systems. *PLoS ONE* **16**(7), e0254482. doi: 10.1371/journal.pone.0254482

Zhong, J., Zhang, W., Xie, L., Zhao, O., Wu, X., Zeng, X & Guo, J. 2023. Development and challenges of bifacial photovoltaic technology and application in buildings: A review. *Renewable and Sustainable Energy Reviews* **187**, 113706. doi: 10.1016/j.rser.2023.113706

Zidane, T.E.K., Zainali, S., Bellone, Y., Guezgouz, M., Khosravi, A., Lu, S.M., Tekie, S., Amaducci, S & Campana, P.E. 2025. Economic evaluation of one-axis, vertical, and elevated agrivoltaic systems across Europe: a Monte Carlo Analysis. *Applied Energy* **391**, 125826. doi: 10.1016/j.apenergy.2025.125826

Development and analysis of initial material of winter spelt wheat (*Triticum aestivum* L. ssp. *spelta*) for productivity breeding

I. Diordiieva[1,*], L. Riabovol[1], Ya. Riabovol[2], O. Serzhyk[1], S. Maslovata[3], L. Musienko[4], S. Karychkovska[5] and M. Fesko[1]

[1]Uman National University, Faculty of Agronomy, Department of Genetics, Plant Breeding and Biotechnology, 1 Institytska Str., UA 20301 Uman, Ukraine
[2]Uman National University, Faculty of Agronomy, Department of Plant Growing, 1 Institytska Str, UA 20301 Uman, Ukraine
[3]Uman National University, Faculty of Foresty and Landscape Management, Department of Foresty, 1 Institytska Str., UA 20301 Uman, Ukraine
[4]Uman National University, Uman, Ukraine, Faculty of Agronomy, Department of Agrochemistry and Soil Science, 1 Institytska Str, UA 20301 Uman, Ukraine
[5]Uman National University, Faculty of Management, Department of Ukrainian and Foreign Languages, 1 Institytska Str., UA 20301 Uman, Ukraine
*Correspondence: diordieva201443@gmail.com

Abstract. In the studies of 2019–2024, the productivity indicators of the starting material of winter spelt wheat, created by hybridization with soft winter wheat at Uman National University (Right-Bank Forest-Steppe of Ukraine), were analyzed. In the process of research, spelt wheat samples were identified that can be used in practical selection as donors of genes for certain traits, in particular, high yield (5.25–5.82 t ha^{-1}); semi-dwarfism and low-stemming in breeding for plant height reduction (samples 1786, 1817, 1559, 1674 and 1755; improved grain threshing (91%) and optimal spike structure (samples 95, 155, 1725); high grain protein content (23.8–28.7%), gluten (49.1–57.2%), alveograph indicator (340–425 alveograph units), grain hardness (60.8–68.2 instrument units) in breeding for grain quality (samples 13, 40 and 128). The correlation between productivity indicators was analyzed and it was found that the greatest influence on spelt yield is the quality of grain threshing ($r = 0.89 \pm 0.00$).

Key words: spelt wheat, plant height, threshing quality, yield, grain quality.

INTRODUCTION

Spelt (*T. aestivum* L. ssp. *spelta*) is one of the oldest species of emmer wheat, known as early as 7–8 millennia BC (Babenko et al., 2018). The phylogeny of spelt is a controversial issue. By origin, it is divided into two subspecies of independent origin: European (supraconvar. *spelta*) and Asian (supraconvar. *kuckuckianum* Gökg. ex Dorof.) (Nesbitt, 2001; Poltoretskyi et al., 2018). Scientists consider the Asian subspecies of

spelt to be the oldest hexaploid species of wheat and, probably, the ancestor of soft wheat, which was formed in the 6th millennium BC in Iraq by spontaneous hybridization of the species *Triticum turgidum* ssp. *dicoccon* i *Aegilops Tauschii* ssp. *strangulata* (Kuckuck & Schiemann, 1957; Fans et al., 2006). The emergence of European spelt subspecies in Central and Eastern Europe dates back to the Bronze Age (3rd millennium BC) (Poltoretskyi et al., 2018).

With the advent of naked-grain wheat species, spelt gradually disappeared from cultivated crops. The reason for this was its significantly lower yield, which is due to the low quality of threshing. Currently, the demand for spelt is growing and spelt ranks third among wheats in terms of sown areas. It began to be intensively grown in France, Austria, Israel, Italy, the USA, Australia, Germany, etc. (Alvarez & Guzmán, 2018; Bradna et al., 2019; Suchowilska, 2020). The interest in spelt is due to a number of positive characteristics, in particular, it is a valuable dietary nutrient, characterized by a high grain protein content (up to 25%) balanced in amino acid composition (Dvořáček et al., 2002; Arzani & Ashraf, 2017; Diordiieva et al., 2024). Spelt has the highest grain protein content among hexaploid wheat species, exceeding in this indicator the species *T. aestivum* L. ssp. *aestivum* by 5–10%, *T. aestivum* L. ssp. *sphaerococum* Persiv. by 3–8%, *T. aestivum* L. ssp. *compactum* Host. by 4–5%, *T. petropavlovskyi* Udacz. et Migusch by 2–6% (Arzani & Ashraf, 2017). It is characterized by a high biological value of protein, exceeding soft and durum wheat in the content of essential amino acids, in particular, tryptophan by 10–15% and 15–20%, respectively (Dvořáček et al., 2002).

Spelt wheat breeding is currently being carried out in Ukraine, Switzerland, Austria, Hungary, Germany and Serbia. However, the crop range is limited by a small number of varieties and forms of local selection. Spelt remains a rare species that requires selection improvement. The relatively low yield (approximately 4.0–4.5 t ha^{-1}) and poor threshability of the grain (about 70–75%) are the main limitations for the large-scale adoption of spelt in agricultural production (Babenko et al., 2018; Diordiieva et al., 2024). In this regard, the current task of crop breeding is to increase the yield and quality of grain threshing while maintaining a high content of protein and gluten in the grain. This can be achieved by involving related species of the genus *Triticum* L., in particular *T. Aestivum* L. ssp. *aestivum*, in the hybridization system as donors of genes for individual productivity indicators. By hybridizing the species *T. aestivum* ssp. *spelta* and *T. aestivum* L. ssp. *aestivum*, new transgressive forms of spelt with improved quantitative and qualitative productivity indicators can be obtained.

The purpose of the research was to create and analyze the starting material of winter spelt wheat by hybridization with soft wheat to isolate promising genotypes and involve them in the breeding process of obtaining highly productive crop varieties.

MATERIALS AND METHODS

The research was conducted in the conditions of the Right-Bank Forest-Steppe of Ukraine (Uman town), subzone with unstable moisture conditions (there are droughts once in 2–3 years). The climate of the region is temperate continental. The average long-term precipitation rate for the region is 586 mm, the average annual air temperature is + 8.8 °C. The studies used the generally accepted technology for growing winter grain crops. Sowing was carried out at the optimal time for the zone – the third decade of September. A systematic method of plots placement was used in the research. The

numbers were arranged in blocks with every fifth number being a standard. The standard was the winter spelt wheat variety Zoria Ukrainy.

Reciprocal crosses were performed between high-yielding, regionally adapted soft winter wheat varieties (Kryzhynka, Favorytka, Podolianka, Panna, Kharus, Farandol) with winter spelt wheat (varieties Zorya Ukrainy and Europa). In the F_{2-5} generations, splitting was recorded according to a number of characteristics, in particular, by spike morphology. In the fifth generation (F_5), when the progeny showed stabilization across all traits and no further splitting was observed, taking into account the general plant habit and spike morphology, all created materials were divided into soft wheat types (a medium-dense spike with normal ear scales and loose grain threshing), spelt wheat types (a long, lax spike with coarse ear scales and difficult grain threshing) and spelt-like forms (an elongated, lax or medium-dense spike with improved grain threshing quality). The selection of samples for research was carried out directly among spelt and spelt-like forms. Based on the analysis of the developed progeny and repeated selections according to productivity traits, a genetic collection of initial material was established, comprising over 200 winter spelt wheat samples differing in morphological, varietal, and economically valuable indicators. Among them, 19 best samples were selected and used as the object of research during 2019–2024. Their brief characteristics are given in Table 1.

The soil of the experimental field is podzolized heavy loamy chernozem. The content of humus in the arable layer was 3.2−3.4%, the level of saturation of the bases in the range of 90−93%, the reaction of the soil solution was medium acid (pHKCl 5.7), hydrolytic acidity was 1.9−2.3 smmol kg^{-1} of soil, the content of mobile compounds of phosphorus and potassium – 125–150 mg kg^{-1}.

All phenological observations, analysis of yield structure indicators was carried out according to the methodology for qualification testing of cereal, groat, and legume crop varieties for suitability for distribution in Ukraine (2016). Grouping of wheat samples by plant height was carried out according to the modified scale of A.P. Orliuk and co-authors (2006). Biometric indicators (plant height, number of grains per spike, length, density and weight of grain per spike) were determined on 50 plants selected in two non-adjacent replications. Quality indicators (protein, gluten, starch content, alveograph indicator, sedimentation, grain hardness) were determined by the infrared spectroscopy method using the Infratec™ Nova device (FOSS Analytical, Sweden), vitreousness - using a diaphanoscope.

The samples were ranked by alveograph indicator (a.u.) on the following scale: > 500 a.u. – excellent improver, 400–500 a.u. – good improver, 280–400 a.u. – satisfactory improver, 260–280 a.u. – valuable wheat, 240–260 a.u. – good filler, 180–240 a.u. – satisfactory filler, < 180 a.u. – weak wheat. According to the grain hardness, the wheat samples were divided into three categories: > 60 instrument units (i. u.) – hard grain type, 54–60 i. u. -medium-hard grain type, < 54 i. u. -soft grain type.

After all the calculations and analyses, the grain was threshed and the yield was determined. The threshing quality was determined by the proportion of completely threshed grain to the total amount of grain, in percent. The reliability of research, correlation-regression analysis and the significance of differences between indicators were determined at the level of significance $P < 0.05$ using Statistica 12 program (StatSoft, USA).

Table 1. Origin and description of the studied genotypes of winter spelt wheat

Sample	Parental forms ♀	♂	Description
13	Panna	Zoria Ukrainy	Short-growing ($h^* = 100$ cm), with an awnless, long (over 15 cm), loose white spike with coarse spike scales.
40	Panna		Medium-growing ($h = 113$ cm), with an awnless, long, lax, red spike, with tough glumes and poor threshing quality.
66	Panna		Short-growing ($h = 95$ cm), with strong straw and high lodging resistance. The spike is awned, long, loose, white in color, with coarse spike scales and poor threshing quality.
76	Kryzhynka		Medium-growing ($h = 110$ cm), awned genotype. Spike scales are softened, and the spike stem is strong, which contributes to improved grain threshability.
86	Panna		Tall-growing ($h = 122$ cm) genotype with an awnless, elongated spike (about 13 cm) of low density. It shows improved grain threshability.
95	Podolianka		Tall-growing ($h = 120$ cm) awned specimen with spiny speltoid spike of low density. Has improved grain threshing quality.
128	Zoria Ukrainy	Panna	Tall-growing ($h = 125$ cm) genotype with an awnless spike of typical spelt morphology.
155	Panna	Zoria Ukrainy	Medium-growing ($h = 109$ cm) specimen with awned long (over 16 cm), loose spike, has softened spike scales and improved grain threshing quality.
179	Podolianka		Short-growing ($h = 100$ cm) specimen with typical spelt spike morphology except for length (about 13 cm).
202	Zoria Ukrainy	Panna	Short-growing ($h = 98$ cm) specimen with awnless red long spike and poor threshing quality.
1559	Kryzhynka	Zoria Ukrainy	Short-growing ($h = 87$ cm), has increased individual plant productivity and improved grain threshability.
1674	Farandol		Short-growing ($h = 89$ cm), has increased individual plant productivity and improved grain threshability.
1691	Krasnodarska 99		Tall-growing ($h = 120$ cm) specimen with an awned, spelt-like spike of medium length and low density.
1695	Farandol		Tall-growing ($h = 129$ cm) specimen with a high tillering capacity and improved grain threshability.
1721	Panna		Medium-growing ($h = 106$ cm) specimen with an awned spike of typical spelt morphology.
1725	Kopylivchanka		Medium-growing ($h = 110$ cm) specimen with high tillering coefficient and improved grain threshing quality.
1755	Panna		Short-growing ($h^* = 98$ cm) specimen with high individual plant productivity and improved grain threshability.
1786	Favorytka		Semi-dwarf ($h = 82$ cm) specimen with long loose awnless spike of spelt type.
1817	Kharus		Semi-dwarf ($h = 75$ cm) specimen with high individual plant productivity and improved grain threshability.

Note: h – plant height.

RESULTS AND DISCUSSION

Spelt is a tall-stemmed species ($h = > 110$ cm). Despite the presence of strong straw, its tall-stemmed forms are prone to lodging. In order to reduce the height of plants, semi-dwarf (Kopylivchanka, Panna, Kharus) and low-stem (Favorytka, Podolianka, Kryzhynka) varieties of soft winter wheat were involved in the breeding process to create the initial material for spelt wheat, which allowed to obtain a significant range of variability in plant height and select highly productive semi-dwarf and low-stem genotypes.

Genetic variability of species of the genus *Triticum* L. in plant height is provided by more than 20 specific genes Rht_1–Rht_{20} (Grant et al., 2018; Yakymchuk, 2018). At the same time, the scientific literature does not describe the genetics of plant height control in the *T. aestivum* ssp. *spelta*. When hybridizing tall-stemmed spelt and common wheat varieties with dominant or recessive dwarfism, various types of gene interaction (complementary, epistatic, polymeric) and the formation of offspring with a wide range of variability in plant height are observed (Sichkar et al., 2016; Yakymchuk, 2018).

In the conducted studies, plant height varied over a wide range from 56 to 125 cm. According to the classification by A.P. Orlyuk and co-authors, all the developed materials were classified by plant height as follows: dwarfs ($h < 60$ cm), semi-dwarfs ($h = 60$–84 cm), short-stemmed ($h = 85$–104 cm), medium-stemmed ($h = 105$–119 cm), and tall-stemmed ($h > 120$ cm) forms (Table 2). From the point of view of practical selection, semi-dwarf and low-stem genotypes are valuable, since reducing the height of spelt plants is an urgent task of breeding. The semi-dwarf sample 1817 and low-stem samples 1559, 1674 and 1755 were distinguished, which are characterized by a high grain weight per main spike (2.24–2.48 g) and yield (5.25–5.82 t ha^{-1}). It is worth noting the high-stem sample 1695, which was characterized by the highest yield in the experiment (6.35 t ha^{-1}) and medium- and high-stem samples 95, 155, 1691 and 1725, which significantly exceeded the standard in terms of yield (5.41–5.81 t ha^{-1}).

By hybridizing soft wheat with spelt, an urgent unresolved issue of spelt breeding was addressed – the development of forms with an optimal spike structure and enhanced grain threshability. Spelt spike is long and lax, with a brittle rachis, that, when mechanically damaged, breaks into individual segments and a coarse ear scale, which tightly covers the seed and makes it difficult to thresh grain from the ear.

The length and density of the spike of hexaploid wheat species is regulated by the genes *S/s*, which determines a long, lax spike, and *C/c*, which has a pleiotropic effect and leads to a significant shortening of the spike, spike scale, and grain (Johnson, 2008). The length of the wheat spike is also regulated by the lengthening genes *L1/l1/, L2/l2*.

When spelt, which has the genotype $SSL_1L_1L_2L_2$, is hybridized with soft wheat varieties containing the *C/c* gene and differing in the allelic state of the $L_1/l_1/$ and L_2/l_2 genes, the resulting progeny occupies an intermediate position between the original forms or approach spelt in terms of ear length and density. This explains the elongation of the spike in the resulting progeny to 13.2–17.0 cm, which exceeds the original varieties of soft winter wheat by 1.8–2.5 cm. The spike density in all studied samples remains low (< 16.0 pcs. spikelets 10 cm^{-1} of spikelet stem).

The nature of grain threshing from a wheat spike is controlled by the *Q/q* gene (Kerber & Rowland, 1974; Kato et al., 2003). The nature of the spikelet scales (coarse or optimal spikelet scales) is controlled by the dominant *Tg* allele of the *Tg/tg* gene in

the homozygous state (Sood et al., 2009). To form a phenotype with free grain threshing, it is necessary to have dominant *QQ* alleles and recessive *tgtg* alleles in the genotype. This genotype is characteristic of soft wheat. The genotype of spelt wheat is qqTgTg.

Table 2. Analysis of economically valuable traits of winter spelt wheat samples, 2019–2024

Sample	Grain weight per main spike, g	Spike length, cm	Grain number per spike, pcs.	Spike density, pcs 10 cm^{-1}	Yield, t ha^{-1}	Threshing quality, %
Zoria Ukrainy (St)	1.78 [d]	16.1 [b]	45 [b]	14.4 [c]	5.12 [d]	73 [e]
Semi-dwarfs (60–84 cm)						
1786	2.05 [c]	15.6 [c]	44 [b]	15.4 [a]	5.25 [d]	84 [b]
1817	2.48 [a]	16.1 [b]	47 [a]	14.4 [c]	5.82 [b]	87 [a]
Low-growing (85–104 cm)						
13	1.45 [f]	13.7 [e]	43 [c]	15.3 [a]	3.87 [f]	81 [c]
66	1.71 [e]	14.3 [d]	41 [d]	14.5 [b]	4.31 [e]	73 [e]
179	1.42 [f]	13.2 [e]	47 [a]	15.5 [a]	4.16 [f]	77 [d]
202	1.40 [f]	18.2 [a]	41 [c]	13.6 [d]	4.30 [e]	75 [e]
1559	2.34 [a]	15.3 [c]	48 [a]	15.7 [a]	5.65 [b]	87 [b]
1674	2.06 [c]	14.6 [d]	44 [b]	15.1 [b]	5.28 [c]	86 [b]
1755	2.24 [b]	17.0 [a]	46 [a]	14.5 [b]	5.25 [c]	89 [a]
Medium-growing (105–119 cm)						
40	1.22 [g]	14.3 [d]	39 [d]	14.5 [c]	4.36 [e]	77 [d]
76	1.87 [d]	15.1 [c]	41 [c]	14.8 [b]	5.18 [d]	86 [b]
155	1.83 [d]	16.2 [b]	44 [b]	13.8 [d]	5.41 [b]	91 [a]
1721	1.54 [f]	16.2 [b]	42 [c]	13.8 [d]	4.68 [d]	78 [d]
1725	1.48 [f]	16.1 [b]	44 [b]	13.6 [d]	5.78 [b]	91 [a]
Tall-growing (> 120 cm)						
86	1.84 [d]	13.3 [e]	44 [b]	14.8 [b]	5.18 [d]	86 [b]
95	1.61 [e]	13.5 [e]	43 [b]	14.6 [c]	5.58 [b]	90 [a]
128	1.42 [f]	16.6 [b]	43 [c]	13.4 [d]	4.85 [d]	76 [d]
1691	2.02 [c]	15.6 [c]	42 [c]	14.3 [c]	5.81 [b]	82 [c]
1695	1.74 [e]	16.0 [b]	44 [b]	15.5 [a]	6.35 [a]	87 [a]

Note: different letters indicate values that are significantly different within one line according to results of the Tukey' test ($P < 0.05$).

Various variants of recombination of these genes during hybridization of soft wheat with spelt ensure the formation of hybrids with different grain threshing characteristics: from free to complicated with the formation of intermediate forms with improved grain threshing. In the conducted studies, 17 samples significantly exceeded the standard for grain threshing quality. Samples 86, 95, 155, 1559, 1674, 1695, 1725, 1755, 1786, and 1817 showed superior performance for this trait, demonstrating improved grain threshability (84–91%).

The value of spelt for breeding and production is in its high quality characteristics and technological properties of grain. When hybridizing it with other wheat types, it is important to select parental pairs in such a way that there are no significant differences in quality indicators, in particular, the content of protein and gluten in the grain,

since these indicators are inherited from the worst of the parents. In the studies conducted, most samples were significantly inferior to spelt in the content of protein and gluten in the grain. However, mid-stem samples 40 and 76 were isolated, which significantly exceeded the standard in terms of protein (25.6–27.8%) and gluten (53.2–57.2%) content (Table 3).

Table 3. Technological properties of the grain of winter spelt wheat samples, 2019–2024

Sample	Content, %			Alveograph indicator, a. u.	Grain hardness, i. u.	Sedimentation, mm	Vitreousness, %
	protein	gluten	starch				
Zoria Ukrainy (St)	24.2 [b]	52.1 [a]	50.8 [d]	320 [d]	62.2 [b]	64.7 [a]	88 [a]
Semi-dwarfs (60–84 cm)							
1786	19.7 [d]	41.6 [d]	53.4 [c]	285 [e]	58.1 [c]	62.2 [c]	83 [b]
1817	20.9 [c]	43.5 [d]	52.8 [c]	340 [c]	60.7 [b]	63.1 [b]	86 [a]
Low-growing (85–104 cm)							
13	24.5 [b]	51.8 [b]	50.2 [e]	345 [a]	65.5 [a]	65.2 [a]	88 [a]
66	19.7 [d]	39.9 [e]	53.2 [b]	280 [e]	57.7 [c]	63.5 [b]	87 [a]
179	21.5 [c]	44.5 [d]	51.2 [d]	295 [d]	61.2 [b]	65.4 [a]	85 [b]
202	21.9 [c]	43.2 [d]	51.0 [d]	340 [c]	59.5 [c]	63.7 [b]	87 [a]
1559	20.9 [c]	42.8 [d]	51.8 [d]	325 [c]	61.7 [b]	58.8 [d]	81 [c]
1674	16.8 [g]	35.1 [f]	58.1 [a]	300 [d]	53.2 [e]	59.2 [d]	68 [f]
1755	17.8 [e]	37.3 [e]	55.6 [b]	325 [c]	56.8 [d]	64.1 [a]	76 [d]
Medium-growing (105–119 cm)							
40	28.7 [a]	57.2 [a]	50.0 [e]	425 [a]	65.4 [a]	68.2 [a]	90 [a]
76	25.6 [a]	53.2 [a]	50.7 [e]	385 [b]	64.8 [a]	62.2 [c]	88 [a]
155	17.8 [f]	37.5 [e]	55.4 [c]	375 [b]	57.6 [c]	60.1 [c]	80 [c]
1721	21.4 [c]	45.4 [c]	52.7 [c]	355 [b]	59.1 [c]	60.8 [c]	85 [b]
1725	16.8 [e]	36.3 [f]	56.7 [b]	250 [f]	57.7 [c]	51.1 [e]	72 [e]
Tall-growing (> 120 cm)							
86	19.2 [d]	42.4 [d]	52.3 [c]	295 [d]	58.8 [c]	61.6 [c]	87 [a]
95	17.8 [f]	36.3 [f]	56.3 [b]	295 [d]	57.6 [c]	60.5 [c]	81 [c]
128	23.8 [b]	49.1 [b]	51.2 [d]	340 [c]	61.3 [b]	60.8 [c]	80 [c]
1691	21.7 [c]	44.5 [d]	51.8 [d]	325 [c]	61.7 [v]	63.4 [b]	82 [c]
1695	18.8 [e]	41.0 [e]	54.4 [c]	325 [c]	57.5 [c]	58.9 [d]	74 [e]

Note: different letters indicate values that are significantly different within one line according to results of the Tukey' test ($P < 0.05$).

Flour strength is the main determining element of its baking properties. The greatest influence on this indicator is the amount and quality of gluten, the state of the protein-proteinase complex, the activity of proteolytic enzymes, the presence of activators and inhibitors of proteolysis, etc. The studies have shown that the alveograph indicator varied depending on the genotype within the range from 285 to 425 a.u. It was the highest in samples 40 (425 a.u.), 76 (385 a.u.) and 155 (375 a.u.). According to the classification by alveograph indicator, sample 40 was identified as a good improver, sample 1725 – as a good filler, and the other studied genotypes were identified as satisfactory improvers.
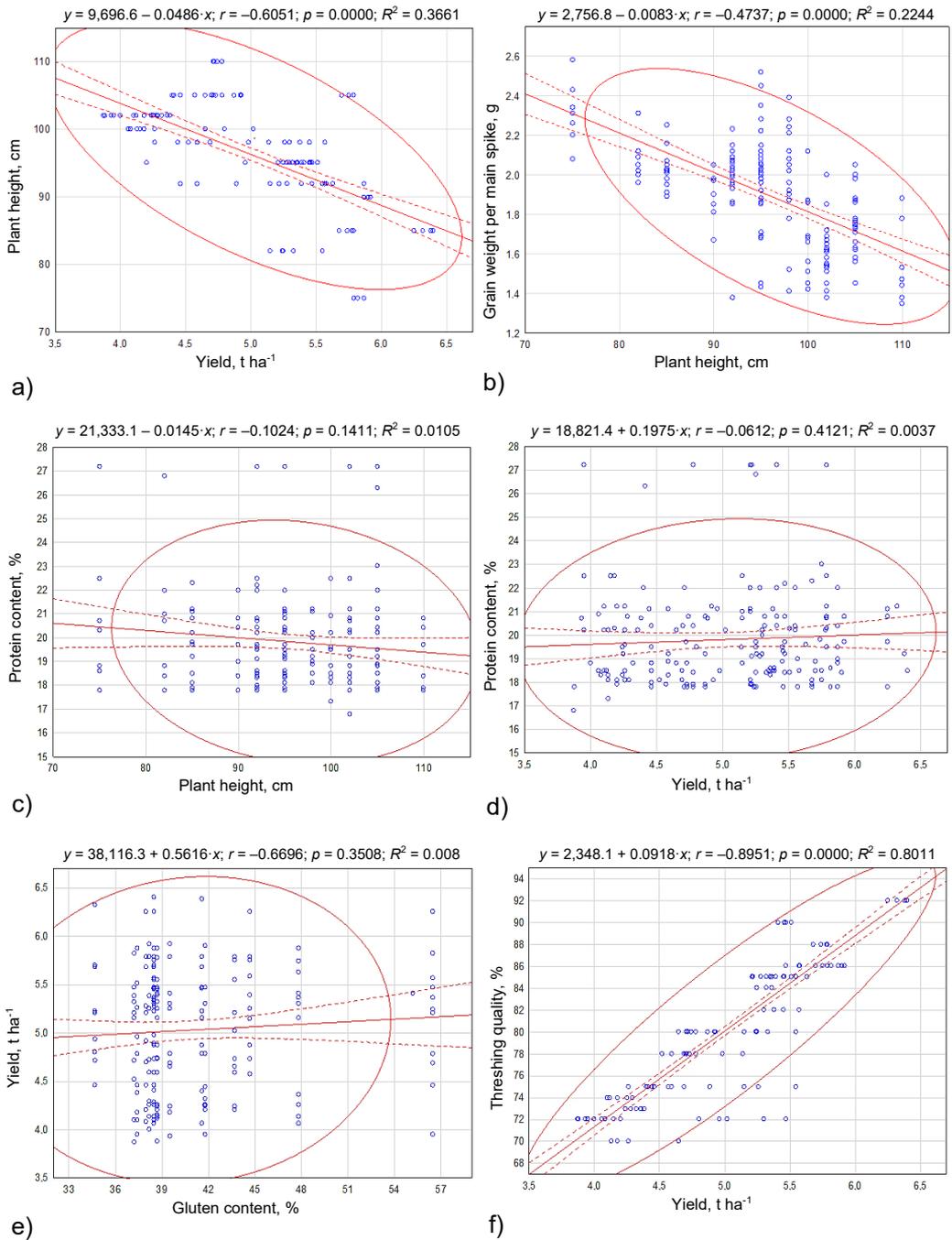
Figure 1. Dot plots and the theoretical lines of regression of the relationships according to the linear correlation between the plant height and grain weight per main spike of spelt wheat (a); plant height and yield of spelt wheat (b); protein content and plant height of spelt wheat (c); protein content and yield of spelt wheat (d); gluten content and yield of spelt wheat (e); threshing quality and yield of spelt wheat (f).

1472

The characteristic 'hard grain/soft grain' is used for technological specialization of wheat varieties. Hard-grained genotypes are optimally suitable for the bread and bakery product production. Soft-grained genotypes are often called biscuit and are used in the confectionery industry. Among the created materials, samples 13, 40, 76, 128, 179, 1559, 1691, 1817 were identified as hard-grained, sample 1674 - as soft-grained, and the rest – as semi-soft grained.

Scientists point out that with a decrease in plant height, the morphological and anatomical features of spelt change, which significantly affects the development of other traits (Zečević et al., 2005; Kochmarskyi, 2012). The conducted correlation-regression analysis indicates a negative correlation of medium strength between yield and height of spelt wheat plants (Fig. 1, a) $r = -0.60 \pm 0.00$; $y = 6,696.1 – 0.0486 \cdot x$, where x is plant height (cm), y – is yield, (t ha^{-1}).

A negative correlation of medium strength was observed between grain weight per spike and plant height (Fig. 1, b): $r = -0.47 \pm 0.00$; $y = 2,756.8 – 0.0083 \cdot x$, where x is plant height (cm), y – is grain weight per spike (g). A weak negative correlation was found between plant height and protein content (Fig. 1, c): $r = -0.10 \pm 0.14$; $y = 21,333.1 - 0.0145 \cdot x$, where x is protein content (%), and y – is plant height (cm). A weak correlation was observed between yield and protein content in the grain (Fig. 1, d): $r = 0.06 \pm 0.41$; $y = 18,821.4 + 0.1975 \cdot x$, where x is yield (t ha^{-1}), and y – is protein content (%). It was found that spelt wheat yield is significantly influenced by threshing quality (Fig. 1, f): $r = 0.89 \pm 0.00$; $y = -2,348.1 + 0.0918 \cdot x$, where x is threshing quality (%), and y – is yield (t ha^{-1}).

CONCLUSIONS

1. By hybridizing soft winter wheat and winter spelt, a new starting material of winter spelt wheat was created, which is characterized by a high level of manifestation of economic-valuable characters and can be used in breeding schemes for creating high-yielding crop varieties.

2. Spelt wheat samples were identified that can be used in practical breeding as donors of genes for individual traits, in particular, high-yielding (yield 5.25–5.82 t ha^{-1}) semi-dwarf and low-stem samples 1786, 1817, 1559, 1674 and 1755 - in breeding to reduce plant height; samples 95, 155, 1725 with improved grain threshing (90–91%) - to create forms with optimal ear structure; samples 13, 40 and 128 with a high protein content in the grain (23.8–28.7%), gluten (49.1–57.2%) - in breeding for grain quality.

3. Consistently high yielding samples of spelt wheat 1559 and 1691 were distinguished, which successfully combine high yield (5.65–5.81 t ha^{-1}) with high grain quality (protein content - 20.9–21.7%, gluten - 42.8–44.5%).

4. It was established that the yield of spelt wheat is significantly affected by the threshing quality ($r = 0.89 \pm 0.00$) and weight of 1,000 grains ($r = 0.72 \pm 0.00$). No strong relationship was found between yield and grain protein and gluten content. This makes it possible to conduct selective and genetic improvement of spelt simultaneously for yield and grain quality without reducing the level of mutually exclusive indicators.

REFERENCES

Alvarez, J.B. & Guzmán, C. 2013. Spanish Ancient Wheat: A genetic resource for wheat quality breeding. *Advances in Crop Science and Technology* **1**(1), 1–7. doi: 10.4172/2329-8863.1000101

Arzani, A. & Ashraf, M. 2017. Cultivated ancient wheats (*Triticum* spp.): a potential source of health-beneficial food products. *Comprehensive reviews in food science and food safety* **3**, 477–488. doi: 10.1111/1541-4337.12262.

Babenko, L.M., Hospodarenko, H.M., Rozhkov, R.V., Pariy, Y.F., Pariy, M.F., Babenko, A.V. & Kosakivska, I.V. 2018. *Triticum spelta*: Origin, biological characteristics and perspectives for use in breeding and agriculture. *Regulatory Mechanisms in Biosystems* **9**(2), 250–257. doi:10.15421/021837

Bradna, J., Šimon, J., Hájek, D., Vejchar, D., Polišenská, I. & Sedláčková, I. 2019. Comparison of a 1 t and a 55 t container when storing spelt grain in mild climate of the Czech Republic. *Agronomy Research* **17**(6), 2203–2210. https://doi.org/10.15159/AR.19.200

Diordiieva, I.P., Riabovol, I.S., Riabovol, L.O., Babii, M.M., Fedorenko, S.V., Serzhuk, O.P., Maslovata, S.A., Liubchenko, A.I., Novak, Z.M. & Liubchenko, I.O. 2024. Breeding and genetic improvement of spelt wheat (*Triticum spelta*) by interspecific hybridization. *Regulatory Mechanisms in Biosystems* **15**(3), 463–468. doi: 0.15421/022465

Dvořáček, V., Čurn, V. & Moudrý, J. 2002. Evaluation of Amino Acid Content and Composition in Spelt Wheat Varieties. *Cereal Research Communications* **30**(1), 187–193. doi:10.1007/BF03543407.

Fans, J.D., Simons, K.J., Zhang, Z. & Gill, B.S. 2006. The wheat super domestication gene *Q*. *Frontiers of Wheat Bioscience* **100**, 129–148.

Grant, N.P., Mohan, A., Sandhu, D. & Kulvinder, S. 2018. Gill Inheritance and Genetic Mapping of the Reduced Height (*Rht18*) Gene in Wheat. *Plants (Basel)* **7**(3), 58–65. doi: 10.3390/plants7030058

Johnson, E.B., Nalam, V.J., Zemetra, R.S. & Riera-Lizarazu, O. 2008. Mapping the compactum locus in wheat (*Triticum aestivum* L.) and its relationship to other spike morphology genes of the *Triticeae*. *Euphytica* **163**(2), 193–201. doi:10.1007/s10681-007-9628-7

Kato, K., Sonokawa, R., Miura, H. & Sawada, S. 2003. Dwarfing effect associated with the threshability gene *Q* on wheat chromosome 5A. *Plant Breed*ing **122**, 489–492. https://doi.org/10.1111/j.1439-0523.2003.00886.x

Kerber, E.R. & Rowland, G.G. 1974. Origin of the free threshing character in hexaploid wheat. *Can J Genet Cytol*. **16**, 145–154. doi:10.1139/g74-014

Kochmarskyi, V.S. 2012. Evaluation of soft winter wheat samples of the world gene pool by plant height and ear spines. *Tavria Scientific Bulletin* **78**, 33–38 (in Ukrainian).

Kuckuck, H. & Schiemann, E. 1957. Über das Vorkommen von Speltz und Emmer (*Triticum spelta* L. und *Tr. dicoccum* Schübl.) im Iran. Z. Pflazenzücht **38**, 383–396.

Methodology for examination of plant varieties of the cereal, grain and leguminous group for suitability for distribution in Ukraine 2016. Kyiv: Ukrainian Institute of Expertise of Plant Varieties. (in Ukrainian).

Nesbitt, M. 2001. Wheat evolution: Integrating archaeological and biological evidence. *Wheat taxonomy: The legacy of John Percival* (Special Issue 3), 37–59.

Orliuk, A.P., Gonchar, O.M. & Usik, L.O. 2006. *Genetic markers of wheat*. Kyiv: Alefa, 144 pp. (in Ukrainian).

Poltoretskyi, S., Hospodarenko, H., Liubych, V., Poltoretska, N. & Demydas, H. 2018. Toward the theory of origin and distribution history of *Triticum spelta* L. *Ukrainian Journal of Ecology* **8**, 263–268. doi: 10.15421/2018_336

Sood, S., Kuraparthy, V., Bai, G. & Gill, B.S. 2009. The major threshability genes soft glume (sog) and tenacious glume (Tg), of diploid and polyploid wheat, trace their origin to independent mutations at non-orthologous loci. *Theor. Appl. Genet.* **119**(2), 341–351. doi: 10.1007/s00122-009-1043-0

Suchowilska, E., Wiwart, M., Krska, R. & Kandler, W. 2020. Do *Triticum aestivum* L. and *Triticum spelta* L. Hybrids Constitute a Promising Source Material for Quality Breeding ofNew Wheat Varieties? *Agronomy* **10**(1), 43. doi: 10.3390/agronomy10010043

Sichkar, S.M., Morgun, V.V. & Dubrovna, O.V. 2016. Inheritance of morphological traits of F_1–F_2 *T.spelta × T. aestivum* hybrids. *Physiology of plants and genetics* **48**(4), 344–355 (in Ukrainian).

Yakymchuk, R.A. 2018. Character of inheritance of stem length in dwarf mutants of soft winter wheat obtained in the area of the Chernobyl nuclear power plant. *Physiology of plants and genetics* **50**(1), 46–58 (in Ukrainian).

Zečević, V., Knežević, D., Mićanović, D., Pavlović, M. & Urošević, D. 2005. The inheritance of plant height in winter wheat (*Triticum aestivum* L.). *Genetika* **37**(2), 173–179. doi:10.2298/GENSR0502173Z

The effects of microalgae (*Tetradesmus obliquus*, *Spirulina platensis* and *Chlorella vulgaris*) on the nutritional profile of broiler meat

A. Freiberga[1], A. Ilgaza[1], S. Jonova[1], S. Eglite[1], D. Gorbacevska[1], A. Plivca[1], K. Neiberts[2], M. Zolovs[3,4] and P. Semjonovs[2]

[1]Latvia University of Life Science and Technologies, Faculty of Veterinary Medicine, K. Helmana street 8, LV-3004 Jelgava, Latvia
[2]University of Latvia, Institute of Biology, Laboratory of Industrial Microbiology and Food Biotechnology, O. Vaciesa street 4, LV-1004 Riga, Latvia
[3]Statistics Unit, Riga Stradins University, Balozu street 14, Riga, Latvia
[4]Daugavpils University, Institute of Life Sciences and Technology, Department of Biosystematics, Vienibas street 13, LV-5401 Daugavpils, Latvia
[*]Correspondence: Correspondence: anete.freiberga@lbtu.lv

Abstract. This study investigated the effect of dietary supplementation with three edible microalgae species (*Tetradesmus obliquus*, *Spirulina platensis*, and *Chlorella vulgaris*) on the nutritional profile of broiler chicken meat. Ross 308 broilers were fed a standard diet enriched with 0.5% of one of the microalgae species over a 43-day fattening period. Meat samples from the breast and thigh muscles were collected on days 35 and 43 to analyse its nutritional profile. Although no statistically significant differences were observed in growth performance, several biologically differences became apparent. *C. vulgaris* administration was associated with an initial increase in thigh meat fat content on day 35, followed by a notable reduction by day 43, and led to higher protein and polyunsaturated fatty acid levels. However, it resulted in the highest omega-6/omega-3 ratio among the groups. *S. platensis* contributed to a more favorable fatty acid profile, with the lowest omega-6/omega-3 ratio and increased protein levels, particularly in breast meat. *T. obliquus* supplementation produced leaner meat, improved vitamin B$_{12}$ content, and maintained a more balanced omega-6 to omega-3 ratio by day 43, suggesting a cumulative benefit with prolonged feeding. These findings indicate that each microalga has species-specific effects on broiler meat quality, supporting their potential use as sustainable functional feed ingredients tailored to specific nutritional goals in poultry production.

Key words: broiler, chickens, edible microalgae, *Spirulina platensis*, *Chlorella vulgaris*, *Tetradesmus obliquus*, meat quality, fatty acids.

INTRODUCTION

Poultry meat is a globally important source of high-quality protein due to its affordability, safety and short production cycle. (Wahyono et al., 2018; Biesek et al.,

2020). However, rising costs of conventional feed ingredients, such as soybean meal and cereals, coupled with sustainability concerns, have driven interest in alternative feed sources. (Babatunde et al., 2021). Microalgae are considered a promising alternative because they contain proteins, polyunsaturated fatty acids (PUFAs), vitamins, minerals and pigments, which could enhance both animal performance and the nutritional quality of meat (Abdel-Wareth et al., 2024). Microalgae are distinguished from land plants by their high content of plastid pigments and diverse carotenoids, and they also synthesize a wide variety of bioactive compounds such as polysaccharides and polyunsaturated fatty acids (Bazarnova et al., 2020; Šefcova et al., 2021). The quantitative and qualitative composition of the intestinal microbiota in broiler chickens can be altered by stressors in industrial poultry farming (Eglite et al., 2024). Gut microbiota plays a key role in the health, growth and performance of poultry. While probiotics are well known to enhance these parameters, other feed additives, such as microalgae, also offer beneficial effects (Akinyemi et al., 2020; Šefcová et al., 2020; Šefcová et al., 2021). Positive outcomes also have been achieved through the administration of algae, regarding animal performance and immunity. (El-Bahr, et al., 2020; Liu, et al., 2021). A key advantage of algae supplementation is its ability to modulate the poultry gut microbiome by supporting beneficial bacteria and microbial diversity. This improves digestion and nutrient uptake (Abdel-Wareth et al., 2024). Studies have demonstrated that microalgae supplementation can enhance growth performance and meat quality in various livestock species, including poultry, although these effects largely depend on the algal species and inclusion level in the diet (Madeira et al., 2017). Microalgae are microscopic photosynthetic organisms that are found in both marine and freshwater environments (Priyadarshani & Rath, 2012). *T. obliquus* contains significant levels of proteins, lipids, and carbohydrates, with a particularly high protein content under mixotrophic conditions; it is rich in essential amino acids (though limited in methionine and cysteine) and has a lipid profile dominated by polyunsaturated fatty acids, including omega-3 and omega-6 (Piasecka et al., 2020). The effects of Tetradesmus supplementation have been tested on both laying hens and pre-starter broilers, with changes observed in egg yolk colour, immune cells and gut microbiota (Rim et al., 2022; Kim et al., 2023). Spirulina (*Arthrospira spp.*) is a blue-green microalga with over 60% easily digestible protein, essential amino acids, vitamins, minerals, and bioactive compounds such as β-carotene, phycocyanin, phenolics, and polysaccharides, which contribute to its antioxidant, anti-inflammatory, hypolipidemic, and immunomodulatory properties, making it a valuable functional feed ingredient (Finamore et al., 2017; Stunda-Zujeva & Berele, 2023; Stunda-Zujeva et al. 2023). The findings also show that spirulina supplementation increases the levels of antioxidants and n-3 polyunsaturated fatty acids (PUFAs) in meat, while concurrently lowering the levels of α-tocopherol and increasing the levels of saturated fatty acids (Costa et al., 2024). *Spirulina* is one of the most widely studied and in-demand species for food and feed applications. Its cultivation potential under natural light conditions in mid-latitude regions such as the Baltic States has been evaluated positively (Stunda-Zujeva et al., 2018). *Chlorella vulgaris* is a nutrient-rich green microalga containing high-quality protein, essential minerals and a broad spectrum of vitamins, including B-complex and fat-soluble vitamins (A, D, E and K). These compounds, along with carotenoids, chlorophyll, and polysaccharides, confer antioxidant, anti-inflammatory, immunomodulatory, and metabolic benefits, making

C. vulgaris a sustainable source of alternative protein with potential applications in human and animal nutrition (Spolaore et al., 2006; Alfaia et al., 2021; Orusmurzaeva et al., 2022; Maurício et al., 2023; Mendes et al., 2024a). Including *C. vulgaris* in the diet enhances the oxidative stability of broiler meat by increasing antioxidant levels, boosting carotenoid deposition and improving the balance between pro- and antioxidants (Mendes et al., 2024b). When included in poultry diets at appropriate low levels, seaweeds can enhance meat and eggs with valuable bioactive compounds (Michalak & Mahrose, 2020). Although previous studies have shown that algae in the diet can affect growth performance and meat composition, there is still limited comparative data on different species of microalgae. Furthermore, the mechanisms by which algal nutrients and percentage of inclusion level enrich meat have not been fully determined. The present study therefore aimed to evaluate the effects of low-dose supplementation with *S. platensis*, *C. vulgaris* and the lesser-known *T. obliquus* on the growth performance, fatty acid composition, and vitamin and mineral content of broiler chicken meat.

MATERIALS AND METHODS

The experimental part of the study was conducted at the Clinical Research Centre, Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, Jelgava, Latvia, from mid-April to the end of May 2024. Permit No. 152/2024 was obtained from the Food and Veterinary Service to conduct the experiment 'The effect of feeding microalgae on the health, productivity and production quality of laying hens and broiler chickens.

Microalgae biomass. Biomass of *Spirulina* and *Chlorella* for poultry experiments was purchased from Buxtrade.de., in Germany. *T. obliquus* (OM02) is an isolate from the Laboratory of Industrial Microbiology and Food Biotechnology (LIMFB) that was obtained from waterbodies in Riga, Latvia. The maintenance of microalgae culture prior to utilisation in a photobioreactor (PBR) and throughout the entirety of the experimental stages was facilitated by employing the standard HP medium procured from VariconAqua (United Kingdom). Inoculations were prepared from stock culture. The initial inoculum was constituted of 10% of the total volume of the starting culture, which was derived from the original stock cultures. The inoculum was gradually increased in scale (250 mL, 500 mL, 1 L, 2 L Erlenmayer flasks and 10 L airlift PBR) until it reached 10 L, which was designated as the final inoculum stage prior to its transfer to an air lift PBR of 140 L capacity. Each stage of inoculum was cultivated for a period of seven days. The first four stages were cultivated statically under a 12:12 hour day: night cycle at room temperature (20 °C). The final stage was cultivated under a 10:10 hour day: night cycle in an airlift PBR. In conclusion, a total of 10 litres of culture medium was utilised to initiate the 140-litre airlift of the PBR. The cultivation of the green microalga *T. obliquus* was performed in a 140 L Phyco-Lift vertical tubular air-lift photobioreactor (Varicon Aqua, UK) under semi-continuous operation. The photoperiod employed was 10 h of light followed by 10 h of darkness, the light intensity of which was gradually increased over time. The light source used was LED panels emitting red and white light. The cultivation process was initiated with an inoculum of 10 L, which possessed an initial optical density (OD540) of approximately 0.2. The aeration process was continuous, with a rate of approximately 5 L min⁻¹. The

air was enriched with CO_2 and the pH was maintained within the range of 7.5 to 9.5, with the CO_2 supply being adjusted in real time based on continuous pH monitoring. The mean temperature of the culture medium was sustained within the range of 25–30 °C. The growth medium employed throughout the experiment was HP medium. On a weekly basis, 50% of the reactor volume was harvested for the collection of biomass, followed by replenishment with fresh medium. At each harvest, samples were examined by light microscopy to assess microbial contamination. The biomass was harvested and subsequently concentrated through a combination of elevated pH and centrifugation.

Table 1. Summary of fatty acid profile, nutrient composition, vitamins, minerals, and antioxidant content of microalgae biomass (*C. vulgaris*, *S. platensis*, and *T. obliquus*)

	Parameter	Unit	*C. vulgaris*	*S. platensis*	*T. obliquus*
Fatty acids	Total saturated fatty acids (SAFA)	g/100 g	2.5	2.8	1.9
	Total monounsaturated fatty acids (MUFA)	g/100 g	0.7	0.4	1.9
	Total polyunsaturated fatty acids (PUFA)	g/100 g	6.1	2	3.1
	Sum of trans fatty acids isomers	g/100 g	< 0.1 (0.1 ± 0.1)	< 0.1 (0.1 ± 0.1)	0.7
	Total Omega-3 fatty acids	g/100 g	0.4	< 0.1 (0.1 ± 0.1)	2
	Total Omega-6 fatty acids	g/100 g	4.5	2	1
	Total Omega-9 fatty acids	g/100 g	0.4	0.1	1.7
Amino acids	Aspartic acid	mg/100 g	3,550	4,340	2,260
	Glutamic acid	mg/100 g	6,100	7,840	3,850
	Serine	mg/100 g	1,800	2,940	1,740
	Histidine	mg/100 g	960	1,000	662
	Glycine	mg/100 g	2,520	3,020	2,400
	Arginine	mg/100 g	3,580	3,940	2,140
	Threonine	mg/100 g	1,890	2,950	1,970
	Alanine	mg/100 g	3,670	4,600	3,470
	Proline	mg/100 g	2,110	2,310	2,020
	Tyrosine	mg/100 g	1,340	2,490	1,350
	Valine	mg/100 g	2,450	3,490	2,200
	Methionine	mg/100 g	1,010	1,550	947
	Cysteine	mg/100 g	360	340	318
	Isoleucine	mg/100 g	1,580	3,130	1,440
	Leucine	mg/100 g	3,950	5,270	3,460
	Phenylalanine	mg/100 g	2,170	2,770	2,120
	Lysine	mg/100 g	3,530	2,940	2,050
Vitamins	Vitamin E (α-tocopherol)	mg/100 g	17.3	2.8	8.3
	Vitamin B1 (thiamine)	mg/100 g	0.08	1.15	nd
	Vitamin B2 (ryboflavin)	mg/100 g	3.86	2.19	nd
	Vitamin B3 (niacin)	mg/100 g	22.9	16.2	nd
	Vitamin B5 (pantothenic acid)	µg/100 g	1.23	96.1	nd
	Vitamin B6 (pyridoxine)	mg/100 g	1.52	0.61	0.38
	Vitamin B7 (biotin)	µg/100 g	21.5	1.62	nd
	Vitamin B9 (folic acid)	µg/100 g	1030	108	nd
	Vitamin B12 (cyanocobalamin)	µg/100 g	1.54	168	105

Table 1 (continued)

Minerals and nutrients					
Sodium (Na)	mg/100 g	434	322	nd
Salt as sodium chloride (NaCl)	mg/100 g	1,085	805	nd
Iron (Fe)	mg/100 g	14,026	75.4	28.3
Zinc (Zn)	mg/100 g	0.99	1.34	26.2
Magnesium (Mg)	mg/100 g	258	254	357
Moisture	g/100 g	2	4.4	nd
Protein (N*6.25)	g/100 g	56.9	69.6	54.8
Dietary fiber	g/100 g	10.4	7.8	nd
Energy value	kcal/100 g	406	372	nd
Carbohydrates	g/100 g	15.8	6.7	28.5
Fat	g/100 g	10.5	5.7	9.4
Ash	g/100 g	4.38	5.78	nd
Total sugars	g/100 g	0.22	0	nd
Total polyphenols as gallic acid	mg/100 g	528.03	188.93	nd
Total Carotenoids	mg/100 g	13.63	15.41	11.45

Symbols such as '<' indicate values below the detection limit of the accredited method; nd – not detected.

Following this, the biomass was washed in distilled water and dried at 45 °C to prevent the degradation of bioactive compounds. Microalgae biomass samples (*C. vulgaris*, *S. platensis*, and *T. obliquus*) were submitted to the Hamilton Laboratory for detailed compositional analysis. The evaluation included quantification of fatty acid profiles, amino acid composition, vitamins, minerals, proximate nutritional content, and antioxidant compounds such as polyphenols and carotenoids.

Experimental design and animal management. A total of 140 unsexed Ross 308 broiler chicks were obtained from a commercial hatchery within two hours of hatching, to minimise environmental exposure and reduce the risk of pathogen contamination strict biosecurity measures were implemented throughout the study, including restricted access to the experimental facility and the use of protective equipment, such as disposable clothing, gloves and footwear. Personnel visited the site twice daily for feeding purposes to monitor the health and welfare of the birds. During the study, the broiler chickens were not given any vaccinations. After being brought to the Clinical Research Centre, the chickens were weighed and then randomly divided into four study groups (control (BK), *S. platensis* (BS), *C. vulgaris* (BC) and *T. obliquus* (BT)), each containing day-old Ros 308 broiler chickens (*n* = 35, total *n* = 140). The broilers were reared for 43 days under controlled conditions abided by the Ross Broiler Management Guide (Aviagen Ross Broiler Guide, 2024). For body weight measurements, animals were weighed at day 1; 8; 14; 22; 29; 35 and 43 and the averaged values were used for analyses. To examine the fatty acid, vitamin and mineral profile of the breast and thigh meat, we randomly selected and euthanised five chicks from each group on day 35 and 7 chicks from each group on day 43. The broiler chickens were stunned with a blowgun and euthanised by bleeding. All chickens were fed basal diets free of probiotics, antibiotics, or anticoccidiostats. All four study groups were given the same amount of food on the feeding tables twice a day. The poultry-based diet for all groups was specifically formulated for Ross 308 broiler chickens aged 1–35 days and above and consisted of three phases. Starter (from day 0 to day 18: 14.5 MJ kg^{-1}), Grower (from day 18 to day 35: 14.46 MJ kg^{-1}) and Finisher (from day 35 until the end of the study on day 43: 14.39 MJ kg^{-1}). The primary

protein sources in the feed were wheat grains, soybean meal (GMO), corn, and rapeseed cake. The chemical composition of the feed is detailed in Table 2.

Table 2. Feed components and proximate composition of starter (days 1–18), grower (days 18–35) and finisher (above 35th day) diets for the control and experimental groups, granulated (crushed)

Starter	Grower	Finisher	
Wheat	Wheat	Wheat	
Soybean meal (GMO)	Soybean meal (GMO)	Soybean meal (GMO)	
Corn	Corn	Corn	
Rapeseed cake	Rapeseed cake	Rapeseed cake	
Rapeseed oil	Rapeseed oil	Rapeseed oil	
Ca carbonate	Ca carbonate	Ca carbonate	
Monocalcium phosphate	Monocalcium phosphate	Monocalcium phosphate	
Na chloride	Na chloride	Na chloride	

Nutritional additives	Starter	Grower	Finisher
Vitamins (3a):			
A (3a672a), IU kg^{-1}	14,853.96	14,853.96	14,853.96
D_3 (3a671), IU kg^{-1}	2,983.86	2,983.86	2,983.86
E (3a700, all-rac-alfatoko.acet), mg kg^{-1}	41.5	42.62	43.16
B_1 (thiamine 3a821), mg kg^{-1}	6.25	6.11	6.03
B_2 (riboflavin 3a826), mg kg^{-1}	7.85	7.72	7.63
B_5 (Ca-d-pantothenic acid 3a841), mg kg^{-1}	46.87	46.33	46.07
B_4 (choline chloride 3a890), mg kg^{-1}	1,599.07	1,475.36	1,397.6
B_3 (niacin 3a315), mg kg^{-1}	44.37	44.01	44.3
B_6 (pyridoxine 3a831), mg kg^{-1}	8.8	8.59	8.42
B_{12} (cyanocobalamin 3a835), mcg	23.74	23.74	23.74
K_3 (methadone 3a711), mg	2.3	2.37	2.37
B_7 (H-biotin 3a880), mg	0.05	0.05	0.05
B_c (folic acid 3a316), mcg	0.59	0.59	0.59
Micronutrients (3b):			
Iron sulfate (3b103), (Fe), mg kg^{-1}	126.04	116.32	111.71
Copper sulfate (3b405), (Cu) mg kg^{-1}	18.77	18.1	17.67
Manganese oxide (3b502), (Mn) mg kg^{-1}	112.26	110.14	109.79
Zinc oxide (3b603), (Zn) mg kg^{-1}	89.55	87.91	86.94
Sodium selenite (3b801), (Sn) mg kg^{-1}	0.32	0.32	0.31
Calcium iodate (3b202), (J) mg kg^{-1}	1.22	1.22	1.22
Cobalt carbonate (3b304), (Co) mg kg^{-1}	0.67	0.66	0.66
Analytical components:			
ME (metabolizable energy), MJ	14.5	14.46	14.39
crude protein, %	22.79	20.31	19.1
Crude fat, %	4.54	4.73	4.79
Fiber, %	4.89	5.38	5.5
Ash, %	4.76	4.48	4.28
Starch, %	36.31	39.78	41.82
Calcium (Ca), %	0.99	0.90	0.93
Phosphorus (P), %	0.54	0.52	0.51
Sodium (Na), %	0.15	0.15	0.14
Methionine, %	0.38	0.37	0.33
Lysine, %	1.32	1.6	1.06

All experimental diets were provided with the same dose of biomass (5 g kg^{-1}) of the respective microalgal supplement, which was added to the BS, BC and BT groups. All groups were housed in four identical bio-chambers (total floor area of the room 20.76 m^2, divided in four equal parts - 5.19 m^2), equipped with a comprehensive microclimate control system. This system regulated temperature, humidity and air supply, as well as controlling the composition of incoming and outgoing air and light cycles. Each bio-chamber was also fitted with video surveillance equipment to enable continuous monitoring. The chambers were equipped with stationary automatic watering systems and movable feeding tables which were adjusted according to the age of the chicks prior to placement. Clean wood shavings covered the flooring of each chamber to maintain hygiene and ensure animal comfort. The broiler chickens were raised until the 43rd day of life. The lighting and temperature regime was established based on prior research and Ross 308 breeder guidelines (Aviagen Ross broiler guide, 2024). During the first week of the experiment, the ambient temperature in the chambers for all groups was kept between 27 and 30 °C. As the birds grew, the temperature was gradually reduced until it reached 20–22 °C by the end of the study. On the first day, the light/dark cycle consisted of 23 hours of light and 1 hour of darkness (23/1). Subsequently, the darkness period was progressively increased to six hours, with a regime of 18 hours of light and six hours of darkness (18/6) from day eight to 26th. During the final week, the dark period was gradually shortened to create a light/dark cycle of 20 hours of light and 4 hours of darkness (20/4). The switch from light to dark occurred through the red light.

Body weight. All birds were weighed, and the averaged values were used for analyses. This was carried out on day 1; 8; 14; 22; 29; 35 and 43.

Meat quality parameters. Samples of breast and thigh meat were analysed on days 35 and 43. Five samples were taken from each group on day 35, and seven samples from each group on day 43. The pooled meat samples were prepared in accordance with laboratory requirements for minimum sample volume providing a representative overview of the overall meat composition. These samples were examined for their fatty acid, vitamin and mineral content. Only vitamins and minerals were determined on day 43. The samples were frozen at -22 °C for later examination. To determine the nutritional composition of chicken breast and thigh meat, the samples were sent to the J.S. Hamilton Poland Sp. z o.o. laboratory for testing. The results of the analysis of the fatty acid profile of the chicken breast and thigh meat samples were expressed in g/100 g of fresh weight. To detect minerals in the meat samples, they were sent to Eurofins Labtarna Lietuva, JSC laboratory. Mineral concentrations (Fe, Se, K, Mg) were expressed in mg kg^{-1}, vitamin E in mg kg^{-1}, and vitamin B$_{12}$ in µg/100 g, all calculated on a fresh weight basis.

Statistical analyses. Generalised linear models (GLMs) were used to assess the effects of different dietary treatments (Control, *Tetradesmus, Spirulina*, and *Chlorella*) on chicken weight, with chicken age and initial weight included as covariates. The chi-squared test was applied to evaluate differences in categorical meat quality parameters. Descriptive statistics (mean, standard deviation, minimum, and maximum) were calculated. Statistical data analysis was performed with Jamovi (v.2.5). The results were considered statistically significant when the *p value* was < 0.05.

RESULTS AND DISCUSSION

Effects of diet on body weight. After adjusting for chicken age, no statistically significant differences in body weight were observed between treatment groups ($p = 0.777$; see Table 3). All groups exhibited consistent weight gain from day 1 to day 43, indicating normal growth trajectories regardless of dietary treatment. By day 43, the BT group reached the highest individual weight (4,029 g), followed by the BC group (3,903 g).

Table 3. Average body weight (g) of broiler chickens by treatment group from day 1 to day 43

Age	Group	Weight			
		Average	SD	Min	Max
Day 1	Control (BK)	61.49	1.43	59.90	63.40
	Tetradesmus obliquus (BT)	60.55	2.03	57.30	63.00
	Spirulina platensis (BS)	61.72	1.27	59.80	63.00
	Chlorella vulgaris (BC)	60.67	1.07	59.00	61.60
Day 8	Control (BK)	181.09	6.03	169.80	189.80
	Tetradesmus obliquus (BT)	176.57	7.86	166.80	191.20
	Spirulina platensis (BS)	180.17	4.99	172.40	187.80
	Chlorella vulgaris (BC)	170.56	6.46	160.20	180.60
Day 14	Control (BK)	454.07	26.86	421.60	513.30
	Tetradesmus obliquus (BT)	431.29	30.64	375.30	483.00
	Spirulina platensis (BS)	437.22	25.06	396.60	467.30
	Chlorella vulgaris (BC)	429.13	34.50	377.60	508.60
Day 22	Control (BK)	895.68	28.29	829.00	933.50
	Tetradesmus obliquus (BT)	882.16	74.97	761.50	1,068.00
	Spirulina platensis (BS)	881.46	79.89	726.50	1,009.00
	Chlorella vulgaris (BC)	884.32	82.17	731.50	1,084.00
Day 29	Control (BK)	1,556.92	184.91	1,298.00	2,028.00
	Tetradesmus obliquus (BT)	1,514.84	167.01	1,179.00	1,889.00
	Spirulina platensis (BS)	1,473.36	143.81	1,241.00	1,760.00
	Chlorella vulgaris (BC)	1,521.88	173.53	1,,245.00	1,881.00
Day 35	Control (BK)	2,225.25	289.01	1770.00	2,890.00
	Tetradesmus obliquus (BT)	2,034.36	319.11	1,203.00	2,624.00
	Spirulina platensis (BS)	2,185.20	197.94	1,936.00	2,563.00
	Chlorella vulgaris (BC)	2,145.56	242.37	1,690.00	2,607.00
Day 43	Control (BK)	3,061.00	468.75	2,122.00	3,903.00
	Tetradesmus obliquus (BT)	3,032.89	462.71	1,676.00	4,029.00
	Spirulina platensis (BS)	3,046.00	276.40	2,509.00	3,566.00
	Chlorella vulgaris (BC)	2,964.85	306.37	2,403.00	3,506.00

Includes mean, standard deviation (SD), minimum and maximum weight values for each group at different time points.

Although the differences were not statistically significant the BS and BK groups consistently showed higher average body weights compared to the BC and BT groups throughout the rearing period. Standard deviation values increased across all groups over time particularly after day 22 suggesting greater variability in growth as the birds aged.

Meat quality parameters. It should be noted that pooled meat samples were used for the analysis of fatty acids, vitamins and minerals. While this approach provided sufficient material for accurate laboratory testing and offered a representative overview

of the group's composition, it may have reduced statistical power and limited the ability to detect differences between groups. This should be considered a methodological limitation of the study. Although no statistically significant differences in meat quality parameters were observed between the treatment groups ($p > 0.05$), various nutritional patterns were identified that may have biological or practical significance, see Table 4.

By day 35, the *Spirulina* (BS) group exhibited the most favorable fatty acid profile (MUFA and PUFA at 0.9 and 05 g/100 g), with the lowest omega-6 to omega-3 ratio (4.3), which matched the lower PUFA level of the Spirulina biomass (2.0 g/100 g), see Table 1. Meanwhile, the *Chlorella* (BC) group had the highest total fat (2.8 g/100 g) and energy content (115 kcal/100 g) including the highest levels of monounsaturated (1.3 g/100 g) and polyunsaturated (0.7 g/100 g) fatty acids, as well as omega-6 (0.6 g/100 g) and omega-9 (1.1 g/100 g) fatty acids. This corresponded with the algae biomass profile of *Chlorella*, which had highest PUFA content (6.1 g/100 g) and omega-6 (4.5 g/100 g) see Table 1. The *Tetradesmus obliquus* group had a lower breast fat content (2.4 g/100 g) with MUFA and PUFA levels at 1.1 and 0.6 g/100 g, respectively. The algae biomass for *Tetradesmus* also contained moderate PUFA (3.1 g/100 g) and the lowest SAFA content (1.9/100 g). Similar trends persisted up to day 43. The BC group

Table 4. Fatty acid profile, proximate composition, energy value, and micronutrient content of broiler breast meat at days 35 and 43

Day 35 Breast meat				
Fatty acids g/100 g	BK	BT	BS	BC
Total (SAFA)	0.5	0.7	0.6	0.8
Total (MUFA)	0.9	1.1	0.9	1.3
Total (PUFA)	0.5	0.6	0.5	0.7
Total Omega-3	0.1	0.1	0.1	0.1
Total Omega-6	0.4	0.5	0.4	0.6
Total Omega-9	0.8	0.9	0.8	1.1
Water	75.7	75.8	75.4	75.1
Total ash	1.29	1.24	1.29	1.17
kcal/100 g	106	111	106	115
Protein	21.9	22.3	22	22.4
Fat	2	2.4	2	2.8
Day 43 Breast meat				
Fatty acids g/100 g	BK	BT	BS	BC
Total (SAFA)	0.8	0.5	0.5	1.2
Total (MUFA)	1.2	0.7	0.8	2.1
Total (PUFA)	0.6	0.4	0.4	1
Total Omega-3	0.1	0.1	0.1	0.1
Total Omega-6	0.5	0.4	0.4	0.9
Total Omega-9	1.1	0.6	0.7	1.9
Water	74.3	75.8	76.1	74.6
Total ash	1.14	1.28	1.15	1.04
kcal/100 g	116	106	104	121
Protein	23	22.6	21.8	20.2
Fat	2.7	1.7	1.9	4.5
Minerals and vitamins				
Fe mg kg^{-1}	4.1	3.8	4.2	3.5
Se mg kg^{-1}	0.33	0.28	0.27	0.26
K mg kg^{-1}	3,120	3,250	3,060	2,990
Mg mg kg^{-1}	220	212	191	206
E vit. mg kg^{-1}	0.391	0.375	0.472	0.855
B12 vit. µg/100 g	0.325	0.412	0.357	0.350

Includes Saturated Fatty Acids (SAFA), monounsaturated fatty acids (MUFA - omega-9), polyunsaturated fatty acids (PUFA - omega-3, -6), fat, protein, water, ash, and energy values.

Includes iron, selenium, potassium, magnesium, vitamin E, and vitamin B12 concentrations.

exhibited the highest levels of MUFA (2.1 g/100 g), PUFA1.0 g/100 g), omega-9 (1.9 g/100 g) (and total fat (4.5 g/100 g), as well as the highest calorie content (121 kcal/100 g). This mirrored *Chlorella's* high PUFA and omega-6 values in the feed. In contrast, the *Tetradesmus* (BT) and *Spirulina* (BS) groups exhibited leaner profiles, with lower fat content (1.7 and 1.9 g/100 g, respectively) and energy value (106 and

104 kcal/100 g, respectively), corresponding with its lower fat, PUFA and MUFA levels in feed. Additionally, the *Tetradesmus* group showed the highest vitamin B_{12} (0.412 µg/100 g) and potassium levels (3,250 mg kg^{-1}), which were also among the highest in the algal biomass. The control group had the highest protein content (23 g/100 g), followed by the BT group (22.6 g/100 g). In terms of mineral and vitamin content on day 43, all groups had similar levels of iron, selenium, and magnesium. However, the *Chlorella* group had the highest concentration of vitamin E (0.855 mg kg^{-1}), almost double that of the control group.

Although no statistically significant differences in thigh meat quality parameters were observed between the dietary treatment groups ($p > 0.05$), several noteworthy nutritional trends emerged in the context of broiler meat enrichment and functional feed development. See Table 5. On day 35, the *C. vulgaris* (BC) group had the highest fat content (7.7 g/100 g), total monounsaturated fatty acids (MUFA; 3.7 g/100 g), PUFA (1.8 g/100 g), and omega-6 (1.6 g/100 g), aligning with *Chlorella's* high PUFA and omega-6 content in the algae composition. The *Spirulina* group showed a lower fat content (6.2 g/100 g) with MUFA at 3.0 g/100 g and PUFA at 1.5 g/100 g. The *T. obliquus* (BT) group had slightly lower fat content (6.8 g/100 g), MUFA (3.2 g/100 g), and PUFA (1.7 g/100 g). Across all groups, omega-3 values in meat remained at 0.2 g/100 g, matching the modest omega-3 presence in the algal biomass. *S. platensis* group had

Table 5. Fatty acid profile, proximate composition, and micronutrient content of broiler thigh meat at days 35 and 43

Day 35 thigh meat				
Fatty acids g/100 g	BK	BT	BS	BC
Total (SAFA)	1.8	1.8	1.6	2.1
Total (MUFA)	3.7	3.2	3	3.7
Total (PUFA)	1.8	1.7	1.5	1.8
Total Omega-3	0.2	0.2	0.2	0.2
Total Omega-6	1.5	1.4	1.3	1.6
Total Omega-9	3.4	2.9	2.7	3.4
Water	73.5	73.4	74.1	73.1
Total ash	1.11	1.21	1.22	1.04
kcal/100 g	141	136	133	145
Protein	18.6	18.7	19.4	18.9
Fat	7.4	6.8	6.2	7.7
Day 43 thigh meat				
Fatty acids g/100 g	BK	BT	BS	BC
Total (SAFA)	1.5	1.5	2	1
Total (MUFA)	2.6	2.7	3.5	1.6
Total (PUFA)	1.5	1.3	1.7	0.9
Total Omega-3	0.2	0.2	0.2	0.1
Total Omega-6	1.2	1.1	1.5	0.8
Total Omega-9	2.3	2.4	3.1	1.5
Water	74.9	74.7	74.3	75
Total ash	1.09	1.03	0.97	1.14
kcal/100 g	129	128	142	117
Protein	19.3	19.4	19.1	21.1
Fat	5.8	5.6	7.3	3.6
Minerals and vitamins				
Fe mg kg^{-1}	6.1	6.9	6.3	6.5
Se mg kg^{-1}	0.31	0.3	0.3	0.28
K mg kg^{-1}	2,860	2,850	2,820	2,980
Mg mg kg^{-1}	191	190	184	195
E vit. mg kg^{-1}	0.220	0.221	0.141	0.152
B_{12} vit. µg/100 g	0.368	0.414	0.383	0.390

Includes Saturated Fatty Acids (SAFA), monounsaturated fatty acids (MUFA - omega-9), polyunsaturated fatty acids (PUFA - omega-3, -6), fat, protein, water, ash, and energy values.

Includes iron, selenium, potassium, magnesium, vitamin E, and vitamin B_{12} concentrations.

the highest protein content (19.4 g/100 g), which also is the highest in algal biomass. By day 43, the trends in fatty acids had changed. The *Spirulina* (BS) group showed the highest concentrations of MUFA (3.5 g/100 g), PUFA (1.7 g/100 g), omega-6

(1.5 g/100 g), and omega-9 (3.1 g/100 g) in thigh meat, corresponding to the algae's composition, where *Spirulina* had moderate levels of omega-6 and higher antioxidant content. Meanwhile, the *Chlorella* (BC) group exhibited the lowest total fat content (3.6 g/100 g) and a notably higher protein content (21.1 g/100 g). MUFA and PUFA levels were 1.6 g/100 g and 0.9 g/100 g, respectively, which reflected *Chlorella's* higher PUFAS and protein content in the biomass. Water and ash content remained relatively constant across all groups. In terms of micronutrients, the highest iron (Fe) content was in BT group (6.9 mg kg^{-1}) and the concentrations of selenium were very similar between the groups (0.28–0.31 mg kg^{-1}) in thigh meat. Potassium and magnesium levels were relatively uniform, with the *Chlorella* group showing a slightly higher potassium (K, 2,980 mg kg^{-1}) and magnesium (Mg, 195 mg kg^{-1}) content. Regarding vitamin concentrations, *Tetradesmus* (BT) group had the highest levels of vitamin B_{12} (0.414 µg/100 g), and vitamin E content (0.221 mg kg^{-1}) and moderate thigh fat (5.6 g/100 g), again aligning with the nutrient composition of its algal supplement. Despite having a lower fat content, BC meat maintained a relatively high B_{12} level (0.390 µg/100 g).

This study examined how dietary supplementation with three edible microalgae species – *T. obliquus*, *S. platensis* and *C. vulgaris* – affects the growth and meat quality of broiler chickens. Although no statistically significant differences in body weight or meat quality were observed, several biologically relevant trends emerged that support existing research and shed insight on the functional properties of these microalgae. While body weight did not differ significantly between groups, the numerically highest final weight was observed in the *Tetradesmus* group (4,029 g) see Table 3, followed closely by the control and *Spirulina* groups. This aligns with previous findings indicating that microalgal supplementation can support growth performance without negatively impacting overall productivity (El-Bahr et al., 2020; Šefcová et al., 2021). The *Spirulina* group showed a favorable omega-6 to omega-3 ratio (4:1) and increased PUFA levels in both breast and thigh meat. Based on findings from (Simopoulos et al., 2002) and (World Health organization, 2003), a lower dietary omega-6 to omega-3 ratio is associated with improved health outcomes. While our results showed that *Spirulina* supplementation improved the fatty acid profile of broiler meat by increasing n-3 polyunsaturated fatty acids (PUFAs) and lowering the n-6/n-3 ratio, other studies have reported no significant effects on fatty acid methyl esters in breast meat (Yalçınkaya et al., 2025). In contrast, Spínola et al. (2024) observed enhanced fatty acid profiles and antioxidant capacity, with higher n-3 PUFA deposition and reduced lipid oxidation, likely due to Spirulina's β-carotene and other carotenoids, which protect cells from oxidative stress by preventing oxygen-induced damage (Tinkler, 1994). However, these effects were not strictly linear at higher inclusion levels, suggesting that dosage and dietary context play an important role in determining the impact of Spirulina on meat quality. Supplementation with *Chlorella* vulgaris was associated with increased concentrations of monounsaturated and polyunsaturated fatty acids, along with higher total fat and protein content in broiler meat. Boskovic Cabrol et al. (2022) reported that replacing soybean meal with *C. vulgaris* improved protein digestibility and amino acid availability, further supporting the role of *Chlorella* in enhancing meat protein deposition. In our study, breast and thigh meat from the *C. vulgaris* group showed higher fat content compared with the other treatments. This differs from previous findings where *C. vulgaris* supplementation

reduced or had little effect on meat lipid levels (Alfaia et al., 2021; Varzaru et al., 2024). The discrepancy may be related to differences in diet formulation, algal inclusion level and composition, or broiler strain.

T. obliquus demonstrated the most beneficial fatty acid profile, achieving omega-6/omega-3 ratios close to 4:1 or lower, especially on day 43. This improvement can be attributed to its ability to synthesise omega-3 fatty acids, including ALA, and possibly DHA under mixotrophic cultivation conditions (Piasecka et al., 2020). The trend toward improved fatty acid ratios over time suggests a cumulative effect, supporting previous research indicating that longer feeding durations promote omega-3 deposition in tissues, and decrease the n-6 to n-3 ratio (Shahid et al., 2019). Notably, the omega-6/omega-3 ratio in the *Tetradesmus* and *Spirulina* fed groups approached or fell below 4:1 on day 43, which aligns with the optimal range recommended by (Simopoulos et al., 2002) and the (World Health organization, 2003) for reducing inflammation and lowering chronic disease risk. The variation observed between breast and thigh meat is likely due to differences in lipid metabolism and muscle composition. Thigh meat has a higher fat content and may therefore allow for greater accumulation of dietary fatty acids. However, the distribution patterns were consistent across both tissue types (Crespo & Esteve-García, 2001). This finding supports the systemic availability and metabolic utilisation of algal-derived fatty acids. In terms of micronutrients, the concentrations of iron (Fe), selenium (Se), potassium (K) and magnesium (Mg) in broiler meat were relatively uniform across the different treatment groups, with only minor differences observed. Potassium levels were slightly higher in breast meat from *the Tetradesmus* group and in thigh meat from the *Chlorella* group. This is consistent with the nutrient profile of microalgae, which provide vitamins (A, C, E and B complex) and minerals such as iron, potassium, magnesium and calcium (Becker, 2013). Notably, C. vulgaris is characterised by higher phosphorus content and substantial potassium levels (Tokuşoğlu & Ünal, 2003). Therefore, the modest enrichment of meat potassium plausibly reflects dietary mineral contributions from the algae, at least in part, and aligns with reports that potassium is the predominant mineral in chicken muscle, followed by phosphorus and sodium (Demirbaş, 1999). In addition to differences between species, the mineral composition of algae varies with growth conditions (Santhakumaran et al., 2020), which may further influence mineral deposition in meat. In addition to modulating lipid composition, supplementation with microalgae affected the vitamin profile of broiler meat, particularly vitamin B_{12}. It should be noted that the analytical method used in this study measured the total amount of vitamin B_{12} present and did not distinguish between its active (methylcobalamin and adenosylcobalamin) and inactive (pseudovitamin B_{12}) forms. Future studies using advanced analytical methods are required to validate the specific forms deposited in poultry meat.

Notably, supplementation with *C. vulgaris* resulted in elevated vitamin B_{12} concentrations in both breast and thigh muscles by day 43, despite the low cobalamin content measured in the algal biomass. This is consistent with previous research showing that *Chlorella* contains bioavailable forms of vitamin B_{12}, such as methylcobalamin and adenosylcobalamin (Kittaka-Katsura et al., 2002), which are efficiently absorbed and deposited in animal tissues. By contrast, Spirulina platensis exhibited a high total vitamin B_{12} concentration in the biomass. However, previous studies have shown that most of its corrinoid compounds are pseudovitamin B_{12} analogues with low intrinsic factor binding affinity and no bioactivity in humans (Watanabe et al., 1999; Watanabe et. al., 2014).

Therefore, while *Spirulina* supplementation led to increased total vitamin B_{12} levels in meat, its nutritional value for human consumers remains uncertain. No significant data on the vitamin B_{12} content of *T. obliquus* currently exist in the literature. However, in this study, birds supplemented with *Tetradesmus* also showed elevated B_{12} levels in meat tissues. Whether this reflects true dietary contribution or indirect metabolic effects remains unclear and warrants further investigation. Regarding vitamin E (α-tocopherol), supplementation with Spirulina led to the highest concentrations in thigh meat, which is consistent with its well-documented abundance of tocopherols and carotenoids (Khan et al., 2005). In contrast, *Chlorella* vulgaris resulted in the highest α-tocopherol content in breast meat by day 43, indicating effective tissue deposition and confirming its classification as a rich natural source of fat-soluble vitamins, including α-tocopherol (Del Mondo et al., 2020). Although the biomass of *T. obliquus* used in our study contained a substantial amount of vitamin E (8.3 mg/100 g), this did not result in an increase in α-tocopherol levels in the broiler meat. On day 43, the vitamin E content of the breast meat in the *Tetradesmus* group (0.375 mg kg^{-1}) was slightly lower than in the control group (0.391 mg kg^{-1}), and much lower than in the Chlorella group (0.855 mg kg^{-1}). These results imply that, despite its inherent α-tocopherol content, the bioavailability or metabolic utilisation of vitamin E from *T. obliquus* may be limited. This aligns with previous findings by (Chronopoulou et al., 2019), who demonstrated that the extraction and bioavailability of fat-soluble vitamins from *T. obliquus* can depend on specific processing methods.

CONCLUSION

This study demonstrated that microalgae supplementation alters broiler meat composition in a species-specific manner. *Spirulina* improved the n-6/n-3 ratio and increased n-3 PUFAs, *Chlorella* enhanced protein and vitamin E but also increased fat, and *Tetradesmus* produced leaner meat with higher vitamin B_{12} level and a more balanced fatty acid ratio. These findings highlight the potential of different microalgae as functional feed ingredients to improve poultry meat quality. These results demonstrate that microalgae modulate the nutrient composition of broiler meat in species-specific ways.

REFERENCES

Abdel-Wareth, AAA., Williams, A.N., Salahuddin, M., Gadekar, S. & Lohakare, J. 2024. Algae as an alternative source of protein in poultry diets for sustainable production and disease resistance: present status and future considerations. *Front Vet Sci.* **11**, 1382163. doi: 10.3389/fvets.2024.1382163

Akinyemi, F.T., Bello, S.F., Uyanga, V.A., Oretomiloye, C. & Meng, H. 2020. *International Journal of Poultry Science* **19**(7), 294–302.

Heat Stress and Gut Microbiota: Effects on Poultry Productivity. *International Journal of Poultry Science.* **19**, 294–304. 10.3923/ijps.2020.294.302

Alfaia, C.M., Pestana, J.M., Rodrigues, M., Coelho, D., Aires M.J., Ribeiro, D.M., Major, V.T., Martins, C.F., Santos, H., Lopes, P.A., Lemos, J.P.C., Fontes, C.M.G.A., Lordelo, M.M. & Prates, J.A.M. 2021. Influence of dietary Chlorella vulgaris and carbohydrate-active enzymes on growth performance, meat quality and lipid composition of broiler chickens, *Poultry Science* **100**(2), 926–937. https://doi.org/10.1016/j.psj.2020.11.034

Aviagen Ross Broiler Guide. Available online https://aviagen.com/assets/Tech_Center/Ross_PS/Ross_PS_PocketGuide Production 2024 EN.pdf (accessed on April 2024)

Babatunde, O.O., Park, C.S. & Adeola, O. 2021. Nutritional Potentials of Atypical Feed Ingredients for Broiler Chickens and Pigs. *Animals* (Basel). **11**(5), 1196. doi: 10.3390/ani11051196

Bazarnova, J., Kuznetsova, T., Aronova, E., Popova, L. & Pochkaeva, E. 2020. A method for obtaining plastid pigments from the biomass of *Chlorella* microalgae. *Agronomy Research* **18**(4), 2317–2327. https://doi.org/10.15159/AR.20.175

Becker, E.W. 2013. Microalgae for human and animal nutrition. In A. Richmond & Q. Hu (Eds.). *Handbook of microalgal culture: Applied phycology and biotechnology* (2nd ed., pp. 461–503). Wiley-Blackwell. https://doi.org/10.1002/9781118567166.ch25

Biesek, J., Kuźniacka, J., Banaszak, M., Kaczmarek, S., Adamski, M., Rutkowski, A., Zmudzińska, A., Perz, K. & Hejdysz, M. 2020. Growth performance and Carcass quality in broiler chickens fed on legume seeds and rapeseed meal. *Animals* (Basel). **10**(5), 846. doi: 10.3390/ani10050846

Boskovic Cabrol, M., Martins, J.C., Malhão, L.P., Alfaia, C.M., Prates, J.A.M., Almeida, A.M., Lordelo, M. & Raymundo, A. 2022. Digestibility of Meat Mineral and Proteins from Broilers Fed with Graded Levels of *Chlorella vulgaris*. *Foods* **11**(9), 1345. doi: 10.3390/foods11091345

Chronopoulou, L., Dal Bosco, C., Di Caprio, F., Prosini, L., Gentili, A., Pagnanelli, F. & Palocci, C. 2019. Extraction of Carotenoids and Fat-Soluble Vitamins from *Tetradesmus Obliquus* Microalgae: An Optimized Approach by Using Supercritical CO_2. *Molecules* **24**(14), 2581. https://doi.org/10.3390/molecules24142581

Costa, M.M., Spínola, M.P., Tavares, B., Pestana, J.M., Tavares, J.C., Martins, C.F., Alfaia, C.M., Carvalho, D.F.P., Mendes, A.R., Ferreira, J.I., Mourato, M.P., Lordelo, M.M. & Prates, J.A.M. 2024. Effects of high dietary inclusion of *Arthrospira platensis*, either extruded or supplemented with a super-dosing multi-enzyme mixture, on broiler growth performance and major meat quality parameters. *BMC Veterinary Research* **20**, Article 176. https://doi.org/10.1186/s12917-024-04144-1

Crespo, N. & Esteve-Garcia, E. 2001. Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens, *Poultry Science* **80**(1), 71–78. https://doi.org/10.1093/ps/80.1.71

Del Mondo, A., Smerilli, A., Sané, E., Sansone, C. & Brunet, C. 2020. Challenging microalgal vitamins for human health. *Microbial Cell Factories* **19**, Article 201. doi: 10.1186/s12934-020-01445-6

Demirbaş, A. 1999. Proximate and heavy metal composition in chicken meat and tissues. *Food Chemistry* **67**(1), 27–31. https://doi.org/10.1016/S0308-8146(99)00103-X

Eglite, S., Jonova, S., Gorbačevska, D., Zolovs, M. & Ilgaza, A. 2024. Effects of *Lactobacillus farciminis* and *Lactobacillus rhamnosus* on the duodenal development of specific-pathogen-free broiler chickens. *Veterinary World* **17**(11), 2517–2526. doi: 10.14202/vetworld.2024.2517-2526

El-Bahr, S., Shousha, S., Shehab, A., Khattab, W., Ahmed-Farid, O., Sabike, I., El-Garhy, O., Albokhadaim, I. & Albosadah, K. 2020. Effect of Dietary Microalgae on Growth Performance, Profiles of Amino and Fatty Acids, Antioxidant Status, and Meat Quality of Broiler Chickens. *Animals* **10**(5), 761. https://doi.org/10.3390/ani10050761

Finamore, A., Palmery, M., Bensehaila, S. & Peluso, I. 2017. Antioxidant, Immunomodulating, and Microbial-Modulating Activities of the Sustainable and Ecofriendly Spirulina. *Oxidative Medicine and Cellular Longevity.* https://doi.org/10.1155/2017/3247528

Khan, Z., Bhadouria, P. & Bisen, P.S. 2025. Nutritional and therapeutic potential of Spirulina. *Curr Pharm Biotechnol*. **6**(5), 373–9. doi: 10.2174/138920105774370607

Kim, Y.B., Park, J., Heo, Y.J., Lee, H.G., Kwon, B.Y., Joo, S.S., Joo, S.Y., Kim, M., Kim, Z.H. & Lee, K.W. 2023. Effect of Dietary *Chlorella vulgaris* or *Tetradesmus obliquus* on Laying Performance and Intestinal Immune Cell Parameters. *Animals: an open access journal from MDPI* **13**(10), 1589.

Kittaka-Katsura, H., Fujita, T., Watanabe, F. & Nakano, Y. 2002. Purification and characterization of a corrinoid compound from Chlorella tablets as an algal health food. *J. Agric Food Chem*. **50**(17), 4994–7. doi: 10.1021/jf020345w

Liu, W.C., Zhu, Y.R., Zhao, Z.H., Jiang, P. & Yin, F.Q. 2021. Effects of Dietary Supplementation of Algae-Derived Polysaccharides on Morphology, Tight Junctions, Antioxidant Capacity and Immune Response of Duodenum in Broilers under Heat Stress. *Animals* **11**(8), 2279. https://doi.org/10.3390/ani11082279

Madeira, M.S., Cardoso, C., Lopes, P.A., Coelho, D., Afonso, C., Bandarra, N.M. & Prates, J.A. 2017. Microalgae as feed ingredients for livestock production and meat quality: A review. *Livestock science* **205**, 111–121. https://doi.org/10.1016/j.livsci.2017.09.020

Maurício, T., Couto, D., Lopes, D., Conde, T., Pais, R., Batista, J., Melo, T., Pinho, M., Moreira, A.S.P., Trovão, M., Barros, A., Cardoso, H., Silva, J., Domingues, P. & Domingues, M.R. 2023. Differences and Similarities in Lipid Composition, Nutritional Value, and Bioactive Potential of Four Edible Chlorella vulgaris Strains. *Foods* **12**(8), 1625. doi: 10.3390/foods12081625

Mendes, A.R., Spínola, M.P., Lordelo, M. & Prates, J.A.M. 2024a. Chemical Compounds, Bioactivities, and Applications of Chlorella vulgaris in Food, Feed and Medicine. *Applied Sciences* **14**(23), 10810. https://doi.org/10.3390/app142310810

Mendes, A.R., Spínola, M.P., Lordelo, M. & Prates, J.A.M. 2024b. Assessing the Influence of Cumulative *Chlorella vulgaris* Intake on Broiler Carcass Traits, Meat Quality and Oxidative Stability. *Foods (Basel, Switzerland)* **13**(17), 2753. https://doi.org/10.3390/foods13172753

Michalak, I. & Mahrose, K. 2020. Seaweeds, Intact and Processed, as a Valuable Component of Poultry Feeds. *Journal of Marine Science and Engineering* **8**(8), 620. doi: 10.3390/jmse8080620

Orusmurzaeva, Z., Maslova, A., Tambieva, Z., Sadykova, E., Askhadova, P., Umarova, K., Merzhoeva, A., Albogachieva, K., Ulikhanyan, K. & Povetkin, S. 2022. Investigation of the chemical composition and physicochemical properties of Chlorella vulgaris biomass treated with pulsed discharges technology for potential use in the food industry. *Potravinarstvo Slovak Journal of Food Sciences* **16**, 777–789. https://doi.org/10.5219/1803

Piasecka, A., Nawrocka, A., Wiącek, D. & Krzemińska, I. 2020 Agro-industrial by-product in photoheterotrophic and mixotrophic culture of Tetradesmus obliquus: Production of ω3 and ω6 essential fatty acids with biotechnological importance. *Sci. Rep.* **10**, 6411. doi: 10.1038/s41598-020-63184-4

Priyadarshani, I. & Rath, B. 2012. Commercial and industrial applications of micro algae – A review *J. Algal Biomass* **3**(4), 89–100.

Rim, C.Y., Jung, H., An, S.H., Joo, S.S., Kim, Z.H., Kong, C. & Kim, M. 2022. Supplementation of microalgae (*Tetradesmus* sp.) to pre-starter diet for broiler chickens. *Korean Journal of Poultry Science* **49**(2), 125–137. https://doi.org/10.5536/KJPS.2022.49.2.125

Santhakumaran, P., Ayyappan, S.M. & Ray, J.G. 2020. Nutraceutical applications of twenty-five species of rapid-growing green microalgae as indicated by their antibacterial, antioxidant, and mineral content. *Algal Research* **47**, 101878. doi: 10.1016/j.algal.2020.101878

Šefcová, M.A., Santacruz, F., Larrea-Álvarez, C.M., Vinueza-Burgos, C., Ortega-Paredes, D., Molina-Cuasapaz, G., Rodríguez, J., Calero-Cáceres, W., Revajová, V., Fernández-Moreira, E. & Larrea-Álvarez, M. 2021. Administration of Dietary Microalgae Ameliorates Intestinal Parameters, Improves Body Weight, and Reduces Thawing Loss of Fillets in Broiler Chickens: A Pilot Study. *Animals* **11**(12), 3601. doi: 10.3390/ani11123601

Šefcová, M., Larrea-Álvarez, M., Larrea-Álvarez, C., Revajová, V., Karaffová, V., Koščová, J., Nemcová, R., Ortega-Paredes, D., Vinueza-Burgos, C., Levkut, M. & Herich, R. 2020. Effects of Lactobacillus Fermentum Supplementation on Body Weight and Pro-Inflammatory Cytokine Expression in Campylobacter Jejuni-Challenged Chickens. *Veterinary Sciences* **7**(3), 121. https://doi.org/10.3390/vetsci7030121

Shahid, M.S., Wu, Y., Xiao, Z., Raza, T., Dong, X. & Yuan, J. 2019. Duration of the flaxseed diet promotes deposition of n-3 fatty acids in the meat and skin of Peking ducks. *Food Nutr. Res.* **63**. doi: 10.29219/fnr.v63.3590

Simopoulos, A.P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy* **56**(8), 365–379. doi: 10.1016/S0753-3322(02)00253-6

Spínola, M.P., Costa, M.M. & Prates, J.A.M. 2024. Effect of Cumulative Spirulina Intake on Broiler Meat Quality, Nutritional and Health-Related Attributes. *Foods* **13**(5), 799. doi: 10.3390/foods13050799

Spolaore, P., Joannis-Cassan, C., Duran, E. & Isambert, A. 2006. Commercial applications of microalgae, *Journal of Bioscience and Bioengineering* **101**(2), 87–96. doi: 10.1263/jbb.101.87

Stunda-Zujeva, A. & Berele, M. 2023. Algae as a Functional Food: A Case Study on Spirulina. In: Abomohra, A., Ende, S. (eds) *Value-added Products from Algae*. Springer, Cham, pp. 563–594. https://doi.org/10.1007/978-3-031-42026-9_17

Stunda-Zujeva, A., Berele, M., Lece, A. & Šķesters, A. 2023. Comparison of antioxidant activity in various spirulina containing products and factors affecting it. *Sci Rep.* **13**(1), 4529. doi: 10.1038/s41598-023-31732-3

Stunda-Zujeva, A., Zuteris, M. & Rugele, K. 2018. Sunlight potential for microalgae cultivation in the mid-latitude region the Baltic states. *Agronomy Research* **16**(3), 910–916. doi: 10.15159/AR.18.126

Tinkler, J.H., Böhm, F., Schalch, W. & Truscott, T.G. 1994. Dietary carotenoids protect human cells from damage. *Journal of Photochemistry and Photobiology B: Biology* **26**(3), 283–285. https://doi.org/10.1016/1011-1344(94)07049-0

Tokuşoğlu, Ö. & Ünal, M.K. 2003. Biomass nutrient profiles of three microalgae: *Spirulina platensis*, *Chlorella vulgaris*, and *Isochrisis galbana*. *Journal of Food Science* **68**(4), 1144–1148. https://doi.org/10.1111/j.1365-2621.2003.tb09615.x

Varzaru, I., Untea, A.E., Panaite, T.D., Turcu, R., Saracila, M., Vlaicu, P.A. & Oancea, A.G. 2024. *Chlorella vulgaris* as a Nutraceutical Source for Broilers: Improving Meat Quality and Storage Oxidative Status. *Foods* **13**(15), 2373. https://doi.org/10.3390/foods13152373

Wahyono, N.D. & Utami, M.M.D. 2018. A review of the poultry meat production industry for food safety in Indonesia. *Journal of Physics:* Conference Series, **953**, 012125. https://doi.org/10.1088/1742-6596/953/1/012125

Watanabe, F., Katsura, H., Takenaka, S., Fujita, T., Abe, K., Tamura, Y., Nakatsuka, T., Nakano, Y. 1999. Pseudovitamin B(12) is the predominant cobamide of an algal health food, spirulina tablets. *J. Agric. Food Chem.* **47**(11), 4736–41. doi: 10.1021/jf990541b

Watanabe, F., Yabuta, Y., Bito, T. & Teng, F. 2014. Vitamin B_{12}-containing plant food sources for vegetarians. *Nutrient* **6**(5), 1861–1873. https://doi.org/10.3390/nu6051861

World Health Organization. 2003. Diet, nutrition and the prevention of chronic diseases (WHO Technical Report Series 916). https://www.who.int/publications/i/item/924120916X

Yalçınkaya, H., Yalçın, S., Ramay, M.S., Onbaşılar, E.E., Bakır, B., Elibol, F.K.E., Yalçın, S., Shehata, A.A. & Basiouni, S. 2025. Evaluation of *Spirulina platensis* as a Feed Additive in Low-Protein Diets of Broilers. *International Journal of Molecular Sciences* **26**(1), 24. https://doi.org/10.3390/ijms26010024

Integrating AI and sustainable materials: machine learning approaches to wood structural behavior

M. González-Palacio[1,*], JM. García-Giraldo[2] and L. González-Palacio[3]

[1]Universidad de Medellín, Faculty of Engineering, Department of Computer Science, Carrera 84 #30-65, CO 050026 Medellín, Colombia
[2]Universidad de Medellín, Faculty of Engineering, Department of Civil Engineering, Carrera 84 #30-65, CO 050026 Medellín, Colombia
[3]Universidad EAFIT, Faculty of Engineering, Department of Computer Science, Carrera 49 N° 7 Sur - 50, CO 050022 Medellín, Colombia
[*]magonzalez@udemedellin.edu.co

Abstract. Wood is a potential construction material that provides a renewable source for this crucial task compared to other classical materials, such as steel or concrete, with high carbon fingerprinting levels. This suitable material minimizes energy use and adds more sustainability to ecological consciousness. Tree planting promotes the balance of the carbon dioxide ecosystem and captures and stores greenhouse gas emissions. Wood also has peculiar characteristics in terms of its structural strength and thermal insulation, optimizing energy consumption by reducing the need for cooling or heating needs. To use this material in construction, it is mandatory to study the resistance parameters like compressive, tensile, and shear strengths, enabling it for great-span structural projects. The traditional modeling strategies used for characterizing stress-strain performances usually simplify the assumptions, overpassing the complex mechanical behavior of the wood under different physical conditions. Nonetheless, previous analyses have shown that the traditional models may exhibit significant deviations from the actual resistance parameters since they can be limited in predicting non-linear and anisotropic properties inherent in wood. To address these limitations, this study proposes using machine-learning-based regressors to predict the mechanical properties of wood. Notably, we propose Multiple Linear Regression models to preserve the model's interpretability while preserving the ability to model the linear properties in the studied scenarios. Furthermore, we use metaheuristic models based on deep learning and ensemble methods to increase the goodness of fit of the predictions. We used an experimental campaign with a widespread type of wood characterization of different parameters under tension parallel to the grain, compression parallel and perpendicular to the grain, and shear conditions. The results showed a lower root mean square error (RMSE) and a higher determination index (R^2). Preliminary results demonstrated the ability of machine-learning-based modeling to obtain more accurate and reliable mechanical behavior of renewable construction materials like wood.

Key words: artificial neural networks, machine learning, random forest, sustainable materials.

INTRODUCTION

A challenge for the global construction industry is related to the growing infrastructure requirements that cause minimal environmental degradation. The sector is responsible for 37% of total global CO_2 emissions and plays a critical role in contributing to climate change (Ding et al., 2022). To achieve this, it is necessary to use more sustainable, cost-effective, high-performance materials (Kumar et al., 2023) using the tools provided by Industry 4.0 (Giraldo & Palacio, 2020), such as the Internet of Things (Palacio et al., 2017; Gonzalez-Palacio et al., 2018), and Artificial Intelligence (AI) (Kim et al., 2024). However, the study of these materials has been mainly based on concrete and steel that have formed the backbone of construction over many years, and their environmental impacts, such as carbon footprint and resource depletion, have scarcely been studied using cutting-edge AI-based tools (Rane, 2023).

In that way, researchers continue searching for alternative materials that would serve in a more sustainable direction using the paradigms of Industry 4.0. Among them, wood is one of the alternatives that presents the advantage of renewal, carbon sequestration, and mechanical properties comparable to traditional materials in many uses (Peng et al., 2023). Wood is one of the few construction materials that requires low energy consumption, resulting in lower CO_2 emissions during production. Its versatility enables shorter construction times and reduces the need for specialized tools while providing an aesthetically pleasing appearance with an attractive visual design (Rodríguez-Grau et al., 2022). Despite such favorable factors, complete structural characterization of wood regarding mechanical resistance parameters, i.e., compressive, tensile, and shear strengths, remains a significant challenge. This is a limitation in the wide acceptability of wood for structural purposes, where there is a necessity for reliability and predictability of mechanical performance.

Traditional methods for characterizing wood's mechanical properties rely on extensive experimental testing, which can be time-consuming and costly. Standardized testing procedures have used different recommendations to characterize the wood performance for construction, like those provided by the American Society for Testing and Materials and the International Organization for Standardization (ASTM, 2021), which demand large samples and special instruments in harsh environments with unfavorable conditions (M. González-Palacio et al., 2024) to measure all the resistance parameters of wood accurately. Many tests involve destructive procedures, resulting in material loss and higher research expenses. Besides, the need for competent personnel and extreme testing conditions may also raise the cost and duration of the characterization studies. Hence, there is an increasing interest in alternative approaches that can efficiently predict the mechanical properties of wood with a few experimental inputs.

Several studies have explored different methodologies for modeling and predicting the mechanical properties of wood materials. Particular research efforts have been directed toward developing empirical models and advanced computational techniques to understand the relationships between different mechanical parameters better. Unlike materials such as steel and concrete, which have numerous studies on their mechanical properties and the correlations between them, wood has a more limited number of studies aimed at determining its mechanical properties (Arriaga et al., 2023). This limited

information is primarily due to the wide variety of structural wood species used in the construction sector and the significant variability in mechanical properties among wood species across different production regions worldwide.

However, some open issues are a matter of research nowadays. A crucial gap concerns diminishing the number of experiments performed to characterize the wood performance under different conditions. The current methodologies require large datasets containing stress and strain data, which demand extensive measurement campaigns. Thus, the current study fills this gap by introducing a novel methodology that uses parallel-to-the-grain stresses and strains to forecast perpendicular-to-the-grain stress using Machine Learning (ML) strategies, aiming to diminish the need for performing extensive measurement campaigns in different directions to characterize the wood behavior. The contributions of this research are threefold:

1. We analyze variable correlations among the predictors to find relations between parallel and perpendicular grain stresses and strain.

2. We model these relationships using different parametric and nonparametric strategies like Multiple Linear Regression (MLR) (Yan & Su, 2009), Artificial Neural Network (ANN) (Kim et al., 2024), and Random Forest (RF) (Sharma et al., 2024) using only a minimum experimental dataset required for wood property prediction.

3. We improve the accuracy of the predictions using RF models that yield an R^2 up to 0.91 for the elastic and inelastic regions. The R^2 was obtained from the test set once we randomly split the database into training and test sets. We used the training set to tune different hyperparameters according to the chosen methods, and the test set was used to measure the ability of the trained methods to generalize the results since this particular subset was unknown for the method in the training phase. Thus, we computed the R^2 using the actual measured values from the test set and the forecasted values using our models. As a consequence, we show that machine learning algorithms effectively enhance predictive accuracy performance.

The rest of this paper is organized as follows: Section II establishes the theoretical foundations that support the research problem addressed in this study. Section III describes the database used and provides insights about the collected data. Section IV provides the methodology to fit diverse machine-learning-based models from the Extraction, Transformation, and Load (ETL) life cycle to topics related to grid search and cross-validation of the model's hyperparameters. Section V presents and discusses the results. Finally, Section VI concludes.

THEORETICAL FRAMEWORK

Wood is one of the most abundant biomaterials on Earth and has been a cornerstone in construction throughout history (Ding et al., 2022). In that way, wood has great potential as a construction material. Nonetheless, it has anisotropic mechanical properties, meaning that its strength changes depending on the grain direction. Besides, the anatomical structure of wood significantly influences its structural properties. Its microstructure considerably impacts parameters such as anisotropy, porosity, density, and mechanical strength (Arriaga et al., 2023). Thus, the mechanical properties of wood primarily depend on the direction in which loads are applied, with significant variation observed between samples from the same wood batch due to different growth conditions and variations in the trunk position during the cutting process. As a cultural and

engineering tradition, wood construction has been passed down through generations, embodying the skills, techniques, and knowledge developed by our ancestors throughout history (Véliz-Fadic et al., 2024). Given its lightness, high strength-to-weight ratio, and aesthetic appeal, wood has seen a greater interest by engineers and architects in the last decades for applications from residential buildings to large-scale commercial projects. The applications of wood in such services have not yet been fully explained concerning the mechanical behavior for various loading conditions based on the resistance parameters that come in compressive, tensile, and shear strengths. The complexities associated with wood's natural variability, including moisture content, species differences, and growth conditions, further complicate its characterization.

The structure of wood can be divided into three levels: macrostructure, microstructure, and sub-microstructure (Niemz & Sonderegger, 2017). Each level directly impacts its mechanical properties (Toumpanaki et al., 2021). Wood is classified as an orthotropic material, which exhibits different mechanical properties along each of its principal axes, i.e., longitudinal, radial, and tangential, which are perpendicular to each other (Fig. 1). To determine its mechanical properties under elastic and inelastic conditions, at least twelve resistance parameters must be identified, including elasticity moduli, shear moduli, and Poisson's ratios, nine of which are independent. However, its structural performance is affected over time by phenomena such as creep and relaxation (Senalik & Farber, 2021).
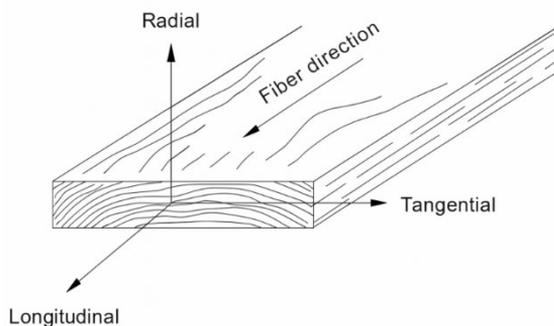
Figure 1. Principal axes of wood of mechanical properties (Senalik & Farber, 2021).

The mechanical properties of wood refer to its ability to withstand loads while maintaining its structural integrity. Determining these properties requires laboratory testing using specialized testing equipment (Record, 1914). To determine the mechanical properties of a wood sample, homogeneous specimens are generally used, preferably free from defects such as knots, cross grains, cracks, or splits. Despite selecting test specimens to be as homogeneous as possible, variations in the obtained mechanical properties may be observed. While this variability is common in most materials used in the construction industry, wood tends to exhibit a high standard deviation (Senalik & Farber, 2021) due to its natural composition, which is influenced by a wide range of external factors that can alter its structure. Due to its cellular composition, wood often exhibits nonlinear behavior, making the parameters for assessing its mechanical performance in this inelastic range more complex (Holmberg et al., 1999). Inelastic behavior factors and changes in moisture content manifest over time as creep and stress relaxation in wood (Senalik & Farber, 2021).

It has been evidenced that density, fiber angle, and ring angle influence the mechanical properties of wood and the microfibril angle. These factors affect properties such as the modulus of elasticity and strength (Arriaga et al., 2023). The apparent density of wood is one of the most influential variables in determining its modulus of elasticity

and mechanical strength. Another parameter that influences wood's mechanical properties and structural behavior is its hygroscopic moisture content. This property allows the wood to exchange moisture with the surrounding air, with values ranging from 30% to over 200% for green wood and below 12% for dry wood (Glass & Zelinka, 2021). Moisture variation in wood, influenced by different drying processes, significantly correlates with its mechanical properties. During adsorption processes, wood conditioned from a dry state exhibits a higher modulus of elasticity and modulus of rupture compared to wood conditioned from a water-saturated state with the same moisture content (Ishimaru et al., 2001). ASTM D198 establishes a standard wood moisture content of 12% for the evaluation of mechanical properties as the standard value for structural wood. This percentage is used to standardize the determination of mechanical properties across most wood species, allowing for comparison between results obtained from different specimens. The 12% value represents the typical hygroscopic equilibrium of wood under average indoor environmental conditions, where its dimensional stability and mechanical properties remain relatively consistent and reliable for structural application (ASTM, 2021). An increase in wood density is directly associated with higher mechanical strength (Soares et al., 2021). In contrast, moisture content has the opposite effect: values below 12% result in low variability in mechanical properties, whereas moisture levels above 12%, particularly those approaching the fiber saturation point (28-30%) lead to a significant reduction in the strength of the wood (Yau et al., 2024).

DATABASE DESCRIPTION

The research was supported by experimental data performed by (Loss, 2023) in which an extended measurement campaign conducted in the Department of Wood Science of the University of British Columbia to determine the mechanical material properties of Canadian small clear spruce-pine-fir wood, widely adopted in North America for constructing cross-laminated timber panels. A total of 690 specimens classified as visually graded No. 2 (sawn wood graded visually based on visible defects like knots, slope of grain or wane), and machine stress-rated 2100fb 1.8E (sawn wood evaluated for structural quality through non-destructive machine). Specimens were tested under compression, tension, and shear loads, in both parallel and perpendicular directions to the grain. The wood test samples were manufactured and treated by a high-precision Homag Centateq P-300 CNC machine to turn the specimens into standardized ones, which would be left in a conditioning room to reach a stable weight with a relative humidity at 20 °C and 65% relative humidity for a period of 15 to 30 days according to the ASTM D143-22 standard to reach a stable weight. Moisture content in test specimens was then measured moisture content using a Sartorius Extend ED6202S-CW digital balance with a precision of 0.01% to be as accurate as possible. Each wood test sample was weighed just before testing. Mechanical tests were carried out using an MTS 810 universal testing machine, and the calibration was performed according to the ASTM E4-01 standard. Other tests performed on the wood include compression and tension parallel and perpendicular to the wood grain, executed at controlled speeds. Real-time data with an acquisition rate of 2 Hz up to the failure of each specimen displays force and deformation. Test results of 690 specimens provide an essential database that will enhance the understanding of the structural performance of Spruce-Pine-Fir and

enable the design and construction of larger-scale structures using this sustainable material.

Table 1. Descriptive statistics of the parallel-to-the-grain experiment

	Mean	Min	Q1	Median	Q3	Max	Std. Dev.
Strain (%)	1.14	0	0.26	1.04	1.88	3.77	0.88
Stress Parallel (MPa)	30.16	0	27.25	32.95	37.93	53.46	11.90

For modeling purposes in the current paper, we present the descriptive statistics of the subsets related to parallel-to-the-grain and perpendicular-to-the-grain stresses and the corresponding strains, as shown in Tables 1 and 2. The results of these tables show a significant difference in the mechanical behavior of the material under these loading conditions. Regarding strain, the perpendicular-to-the-grain experiment shows much higher values according to all statistical measures: the mean value of strain is 4.82% in the case of perpendicular-to-the-grain versus 1.14% in the parallel-to-the-grain case. This means that the material deforms more when loaded perpendicular, supported by the broader range in the experiment for perpendicular, where the maximum strain reached 9.99% compared to 3.77% from the parallel experiment. All these variations are correlated with the anisotropic structure of wood. Its load-bearing capacity varies depending on the direction in which the load is applied and primarily depends on the fiber orientation (Unsal & Candan, 2008). The standard deviation of strain in the perpendicular test was higher, showing more variation in the material's response.

Table 2. Descriptive statistics of the perpendicular-to-the-grain experiment

	Mean	Min	Q1	Median	Q3	Max	Std. Dev.
Strain (%)	4.82	0	2.2	4.79	7.38	9.99	2.95
Stress Perpendicular (MPa)	7.30	0	5.68	7.55	9.19	14.92	2.91

Conversely, the stress values show the opposite behavior. Thus, it can be noticed that the material resists more when loaded parallel to the grain, e.g., the average stress in the parallel experiment is 30.16 MPa compared with the perpendicular direction that achieved up to 7.30 MPa. Also, the interquartile range is more significant in the parallel case because of the increased spread of stress values within the sample. Furthermore, the maximum achieved stress in the parallel direction is higher at 53.46 MPa compared to the maximum of 14.92 MPa in the perpendicular experiment, highlighting the material's superior strength when aligned with the grain. The observed difference between tensile and compressive behavior was expected due to the anatomical differences in wood that result in its anisotropy. Consequently, this difference in strength is significant for the different elastic symmetry axes of wood, making its analytical understanding complex. However, various analytical models theoretically represent this behavior, establishing a possible relationship between wood strength in the principal orthotropic axes (Mascia et al., 2013).

Although different in magnitude, the standard deviations for stress in both directions are relatively consistent proportionally with their respective means, suggesting similar degrees of relative variability in both cases. Overall, data indicate that material is significantly stronger but less deformable when subjected to parallel-to-the-grain loading, whereas it is more compliant but weaker in perpendicular loading

conditions. These differences agree with the expected anisotropic character of wood or similar material, where internal structure confers greater strength in the direction of the grain and flexibility across the grain. Finally, since the dimensions in strain, perpendicular stress, and parallel stress are in different scales, it suggests that we have to homogenize the input data of the proposed models to avoid possible bias.

METHODOLOGY

The process of obtaining the prediction models is depicted in Fig. 2 and comprises four stages. First, we perform the Extraction, Transformation, and Load (ETL) (Khan et al., 2024) to homogenize the data according to the modeling needs. Second, we conduct the feature extraction (Ehtisham et al., 2024) to check the correlations between the predictors and the objective variable. Third, we fit the models. Finally, we analyze the results. The following subsections comprehensively show each stage. Our goal is to find a set of models as

$$\text{Stress}_{\text{per}} = f(\text{Stress}_{\text{par}}, \text{Strain}), \tag{1}$$

where Stress_{per} is the perpendicular-to-the-grain stress, $\text{Stress}_{\text{par}}$ is the parallel-to-the-grain stress, and Strain is the strain.

Data extraction/transformation	Feature extraction
- Data fusion from parallel-to-the-grain and perpendicular-to-the-grain datasets - Homogenize data with the equispaced strain values for both datasets using cubic spline interpolation - Merge both datasets with strain and parallel-to-the-grain as predictor variables, and perpendicular-to-the-grain as dependent variable	- Visual assessment of the relationship between predictors and dependent variable - Determination of linear and nonlinear zones for modelling - Calculation of Pearson and Spearman indexes for both conditions estimator
Modelling	**Analysis of results and insights**
- Random split of the dataset into Training and Test subsets - Model initialization for Multiple Linear Regression, Multilayer Perceptron (MLP), and Random Forest (RF) - Hyperparameter diversity for MLP and RF for grid search tuning strategy - Models fitting using five-fold cross-validation - Model election according to RMSE and R^2 - Model performance evaluation using the test set	- Performance evaluation of the chosen model - Material anisotropic behavior interpretation - Structural safety assessment - Validation of the reduction of experiments using the fitted models

Figure 2. Modeling process.

Data extraction and transformation

The process of data extraction and transformation is depicted in Fig. 3. The first step we followed to obtain the models was to conduct a data fusion between the diverse experiments. The database generally included 43 experiments for parallel-to-the-grain compression and 100 experiments for perpendicular-to-the-grain compression. In that way, the first step was to merge all the data into two files for each experiment. This fusion provides a well-established and repeatable way to capture the behavior of these two standardized experiments to capture the mechanical properties of the wood.

The second step was to limit both experiments to the same strain range since the database characterization shown in Table 1. and Table 2. allowed us to understand that the parallel-to-the-grain campaign was limited to a lower value than the perpendicular-to-the-grain campaign. It was performed to have a uniform range and ease the model fitting phase. In that way, the maximum value of both datasets was 3.77% strain.

| Data fusion per experiment | → | Define operation ranges | → | Interpolation using cubic splines | → | Data fusion for both experiments |
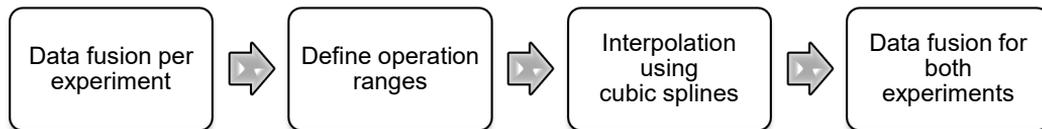
Figure 3. Data extraction and transformation.

The third step was to run cubic splines interpolation to have uniform and equispaced rows in both datasets. To this end, we define a range from 0 to 3.77% strain with steps of 0.01%. Cubic spline interpolation approximates smooth, continuous functions through the measurement campaign using cubic polynomials. First, it divides the data points into intervals and fits a cubic polynomial in each interval. These polynomials are determined to satisfy continuity conditions at each given point: continuity of the function, first derivative, and second derivative of the function. To uniquely determine the cubic spline, additional boundary conditions must be imposed. This is commonly accomplished by specifying the second derivative at the endpoints (the natural splines), or the first derivative equals the slope of the data at the ends, which results in clamped splines. The system of equations obtained from these conditions is solved for the coefficients of the cubic polynomials that are then used to interpolate new values within the range and give smooth, accurate approximations of the underlying function. An example of the accuracy of this method is depicted in Fig. 4.
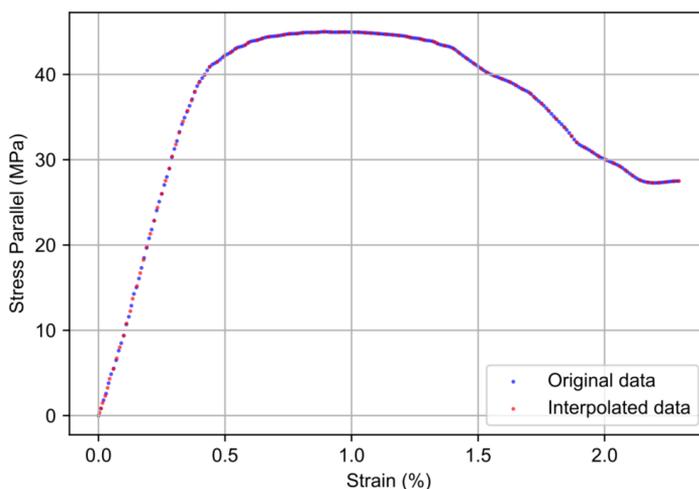
Figure 4. Interpolation process using cubic splines for the first trial of the parallel-to-the-grain experiment. The red curve is the original data, and the blue curve is the interpolated data.

Finally, in the fourth step, we merged the resulting datasets to obtain a three-column database with the variables strain, parallel-to-the-grain stress, and perpendicular-to-the-grain stress.

Feature extraction

The second stage of our framework is determining how the predictor variables correlate with the dependent variable. To this end, we propose the following steps. First, we plotted both independent variables versus the perpendicular-to-the-grain stress to identify linear and nonlinear zones. Then, we delimited these zones to calculate the Pearson and Spearman indices for both zones. The Pearson correlation index helps determine if there are linear relationships between the variables bounded in the interval [-1, 1]. Specifically, *i)* if the values are close to 1, it can be concluded that there is a positive correlation; ii) if the values are close to -1, the correlation is negative; and *iii)* if the values are close to zero, there is no correlation. Furthermore, the Spearman correlation index determines if a nonlinear monotonical function exists to perform regression and can be obtained by ranking the data in ascending order, then assigning a sequential order to each row in the database, subsequently calculating the difference d between the predictor and the dependent variable for each rank; and finally, obtaining the correlation rank ρ.

Modeling

In this section, we propose different parametric and nonparametric models to predict the perpendicular-to-the-grain stress using the results of another experiment (parallel-to-the-grain stress versus strain), improving the time to characterize the wood and reducing the costs associated with the destructive trials.

Multiple linear regression. After obtaining the correlations between the predictors and the dependent variable, we propose a classical Multiple Linear Regression (MLR) model as a baseline to compare the results of different ML-based models. The proposed MLR model is

$$\text{Stress}_{\text{per}} = b_0 + b_1 \text{Strain} + b_2 \text{Stress}_{\text{par}} + \epsilon, \tag{2}$$

where b_0 is the independent term, b_1 is the weight for the Strain, b_2 is the weight for $\text{Stress}_{\text{par}}$, and ϵ is the error that is assumed as independent and identically distributed (i.i.d) zero-mean gaussian variable. The weights' interpretation will help determine the effect of each predictor (positive/negative) on the dependent variable and its relative importance according to the magnitude of the corresponding coefficient.

Artificial neural network. We propose an ANN-based regressor to improve the prediction ability of the fitted models to forecast the perpendicular-to-the-grain stress. In particular, we used a multilayer perceptron (MLP) regression architecture, a type of ANN that aims to approximate complex relationships between the input features and continuous target variables. In the first stage, we apply a max-min scaler to homogenize all the predictor variables in the same orders of magnitude. For instance, the scalization of the ith sample of the strain variable can be calculated as

$$\text{Strain}_i = \frac{\text{Strain}_i - max(\text{Strain})}{max(\text{Strain}) - min(\text{Strain})} \tag{3}$$

The exact process is performed for the variable Stress$_{\text{per}}$. The scaling process must be performed to avoid a specific variable dominating the other due to the difference in

magnitudes. The architecture of an MLP includes an input layer, one or more hidden layers, and an output layer, as shown in Fig. 5. In our case, we have two input neurons (Strain and Stress$_{par}$), a set of n hidden layers with different numbers of neurons h$_{ij}$ (where i indicates the number of the hidden layer and j indicates the neuron number of the corresponding layer), and an output layer (Stress$_{per}$). The number of neurons in each hidden layer is an essential hyperparameter since it influences the capability of the model to learn the pattern of the data directly, so it is mandatory to find the most suitable configuration through hyperparameter tuning.
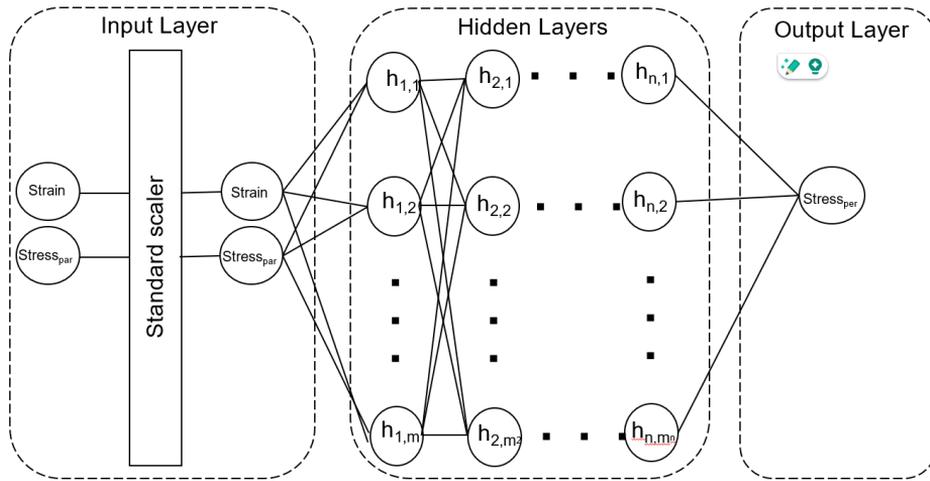
Figure 5. Multilayer Perceptron Architecture for the regressor.

The neurons h$_{ij}$ are part of the fundamental computing unit. They receive weighted inputs, with an activation function applied to an output propagating to subsequent layers. Each neuron sums all the inputs based on an optimized weight, adds a bias, and transforms the obtained calculation using an activation function that introduces the non-linearity needed to learn complex relationships. We used the rectified linear unit (ReLU) activation function due to its computational efficiency and involvement in preventing vanishing gradient problems. Furthermore, we varied different learning rates since this hyperparameter regulates the optimization algorithm's step size for updating the model weights during backpropagation. The learning rate is a crucial hyperparameter since too high values cause the model to oscillate around an optimal solution, and too low values generate slow or suboptimal convergence. Often, the choice of learning rate is empirical or dynamically adjusted during training using optimization strategies that adaptively modify the learning rate, such as Adam or Stochastic Gradient Descent (SGD). In that way, we generated a grid varying the MLP architecture regarding the number of hidden layers, the number of neurons per layer, the learning rate values, and the adaptative algorithm to adequately balance the learning rate variation.

Once the hyperparameters grid is configured, we divide the dataset into training and test sets. This split was performed with a proportion of 80% for training and 20% for testing. We used the training set to find how a particular configuration of hyperparameters learns the patterns and measure the goodness-of-fit using the Root Mean Square Error (RMSE) for the corresponding network configuration. Furthermore,

we implemented a five-fold cross-validation strategy as follows. First, we divided the training set into five subsets, namely S_1, S_2, S_3, S_4, and S_5. Then, we trained with four subsets, e.g., S_1, S_2, S_3, and S_4, and measured the RMSE with S_5. Subsequently, we performed the same procedure, varying the subset to calculate the RMSE; that is, we trained four models with the same hyperparameters. Finally, with the obtained RMSEs, we calculated the standard deviation to assess the generalization ability of each configuration. We chose the hyperparameters which exhibited the lowest RMSE and best R^2. With the fitted MLP model, we used the test set to find the RMSE and R^2 to assess how the fitted model performs in the presence of unknown data.

Random Forest. We fitted a Random-Forest-based model to improve the accuracy in the prediction of the perpendicular-to-the-grain stress, as depicted in Fig. 6. From that figure, it can be noticed that this architecture has different stages, from the scalization of the variables, the regression using Decision Trees (DTs), and the aggregation of the prediction of the DTs to obtain the final prediction.
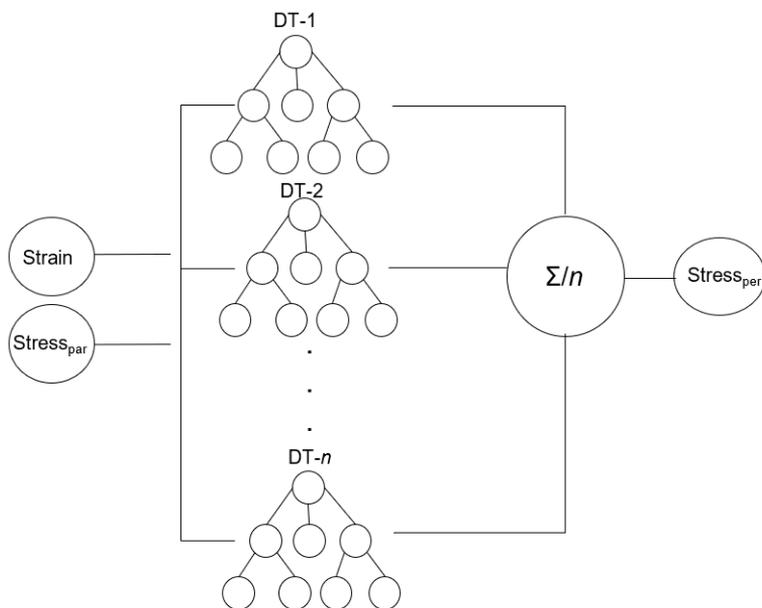
Figure 6. Random Forest architecture.

Subsequently, we train a set of n DTs. The DTs are machine-learning strategies that can be used for regression using the Classification and Regression Trees (CART) algorithm. The operation principle behind the CART algorithm is to partition the input space recursively into different regions to minimize a cost function based on the Mean Square Error (MSE). To this end, the algorithm CART computes all the possible partitions for the predictor variables, calculates the MSE for each, scores the partitions, sorts them to find the lowest MSE, and configures a set of nested if-else clauses verifying if the current value of the predictor variables meets any of the criteria in the conditionals. The partitions finish when a stop criterium is met, like the number of nested conditionals (number of branches) or the minimum number of points per each conditional (number

of leaves per branch). Both stop criteria are hyperparameters for this strategy and must be fine-tuned using grid search and cross-validation, as previously discussed in the MLP section. Finally, the DT delivers the mean of the values for the corresponding region.

However, the DTs are prone to cause overfitting, an undesirable balance between the prediction performance when the training is performed, usually high, versus the performance when the test set is used, usually low. Because of that, a set of n DTs is fitted using bootstrapping and aggregation (bagging), as shown in Fig. 6. Thus, the bootstrapping randomly splits the training set using fewer rows and augments the size of the corresponding subsets, repeating some rows. Each DT uses a different subset to incorporate a random character into the random forest. Finally, the results of all the DTs are aggregated by computing the mean of each prediction. The value for n is also a hyperparameter that has to be fine-tuned using grid search and cross-validation.

RESULTS AND DISCUSSION

In this section, we present the results of the feature extraction stage, where we determined if the predictor variables could predict the dependent variable's variability. After fitting all the proposed models in Section III, we present the obtained models and performance metrics.

Feature extraction

The first step to validate the relationships between the predictor variables and the perpendicular-to-the-grain stress was to plot the different trials for the same experiments. Figs 7 and 8 provide a graphical compilation of all the original data used in the analysis conducted in this study. They highlight the contrast in data behavior when plotting the stress–strain relationship (Fig. 7) versus the relationship between stresses perpendicular and parallel to the grain (Fig. 8).
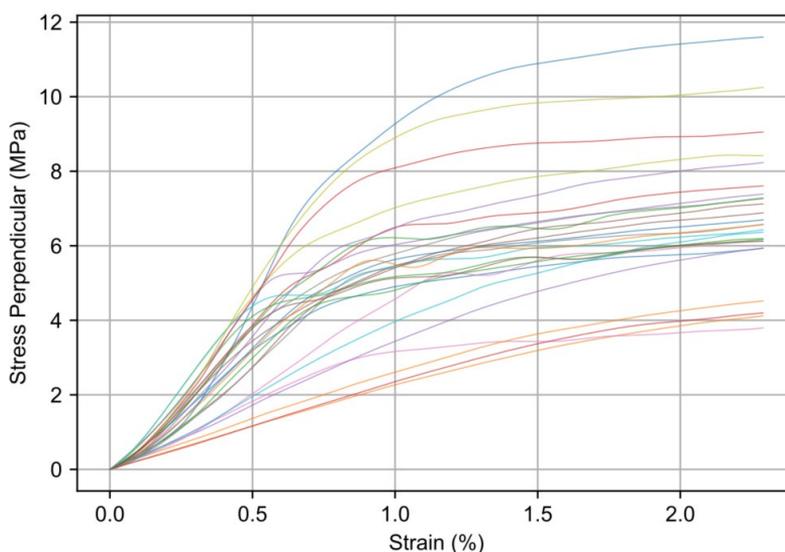
Figure 7. Trends for different trials for perpendicular-to-the-grain stress versus strain and.

1503

For the case of Fig. 8, the following conclusions can be drawn. First, it can be noticed that there is a linear region for each trial that exhibits the elastic material behavior, i.e., when the load is applied and subsequently released, and when the wood can recover the original shape without alterations. This linear relationship is maintained up to strain values close to 0.5% for the higher-strength samples, and up to approximately 1% for the lower-strength specimens. It can also be noticed that there is a nonlinear region, i.e., an inelastic region, where the wood cannot recover its original shape after a load-release operation, and after the unloading of the structural element, permanent residual deformations remain. Although there is a clear relationship between the strain and the perpendicular-to-the-grain stress, we can remark that there is considerable variability among the diverse trials since some of them stabilize at 4 MPa while the most resistant stabilize within the range of 6 MPa to 12 MPa. These variations are associated with the specimen's heterogeneity, caused mainly by grain orientation, density, moisture content, and natural defects like knots or fiber misalignment. Specifically, it can be noticed that this variability does not follow a single universal pattern. In that way, machine-learning-based models can predict this nonlinear behavior and capture the variability efficiently.
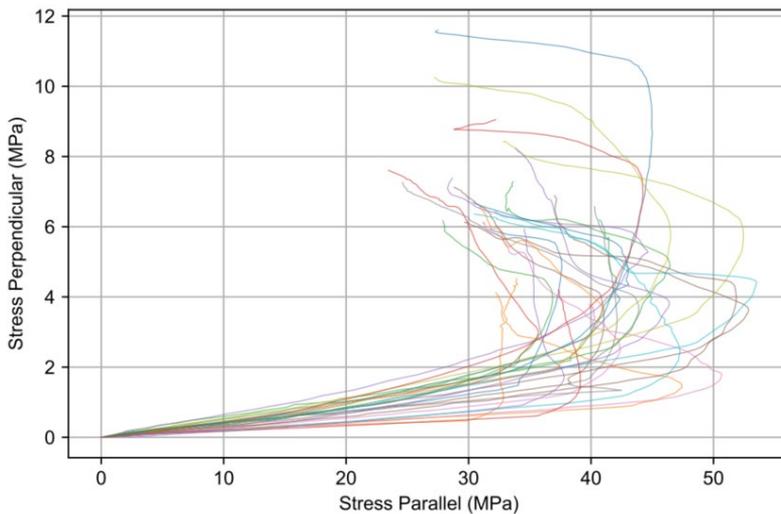
Figure 8. Trends for different trials for perpendicular-to-the-grain stress versus parallel-to-the-grain stress.

This accurate prediction of material behavior in both its linear elastic range and plastic performance region provides a significant advantage of Machine Learning over traditional analytical and numerical finite element models commonly used to predict the structural performance of various materials, including wood. The nonlinear behavior of materials is primarily influenced by parameters such as plasticity, anisotropy, and material defects, which are, in most cases, complex to model using conventional mathematical approaches.

In addition, the following can be noticed in Fig. 8, which presents the trend between the perpendicular-to-the-grain stress versus the parallel-to-the-grain stress. First, a relatively linear trend is presented, where a proportional increase as the parallel-to-the-

grain stress increases. All specimens, regardless of their maximum load-bearing capacity, exhibit linear elastic behavior up to approximately 30 MPa of stress parallel to the wood grain. Again, this behavior corresponds to the elastic region where the material deforms predictably under different loads. Nonetheless, the curves become highly nonlinear as the predictor variable increases, exhibiting a complex and scattered distribution. Since wood is an anisotropic material, the microstructural failures become noticeable, mainly explained by different phenomena like cracking, fiber buckling, and delamination. Furthermore, the same effect of high variability in different specimens is presented due to differences in moisture content and density, among others. Finally, the nonlinear behavior can also be attributed to the microcracks and shear deformations caused when the parallel-to-the-grain load is applied, affecting the perpendicular-to-the-grain stress. Machine Learning algorithms, deep learning models, and regression techniques may identify highly complex patterns in the mechanical behavior of wood under different stress conditions. This capability may enable accurate predictions of wood behavior under both parallel and perpendicular loading to the fiber. These models efficiently handle large volumes of data, perform multivariable analysis, and adapt to different behavioral models to effectively predict mechanical properties, failure mechanisms, and long-term performance of materials such as wood with high precision.

The nonlinear relationship between stress and strain shown in Fig. 7 exhibits a trend with a stable and well-defined transition. In contrast, Fig. 8 shows that the nonlinear relationship between stress perpendicular to the grain and stress parallel to the grain does not follow a clear pattern, as each test displays an independent behavior. Despite the observed nonlinearity in both the stress-strain relationship and the interaction between parallel and perpendicular grain stresses, machine learning techniques can effectively predict the mechanical behavior of wood in the perpendicular-to-grain direction based on its structural performance in the parallel-to-grain direction.

On the other hand, we used the Pearson and Spearman indices to determine the relationship among the strain, the parallel-to-the-grain stress, and the perpendicular-to-the-grain stress, as shown in Table 3, from the following can be noticed. First, both indices show that the strain influences the perpendicular-to-the-grain stress,

Table 3. Correlations between predictor variables and the perpendicular-to-the-grain Stress. We used the Pearson correlation factor R^2 and the Spearman index ρ

	R^2 (%)	ρ (%)
Strain (%)	0.58	0.62
Stress Parallel (MPa)	0.299	0.52

particularly on the other hand, we used the Pearson and Spearman indices to determine the relationship among the strain, the parallel-to-the-grain stress, and the perpendicular-to-the-grain stress, as shown in Table 3**Error! Reference source not found.**, from the following can be noticed. First, both indices show that the strain influences the perpendicular-to-the-grain stress, particularly in the elastic zone, where a simple linear regression can be effective and computationally inexpensive to predict this variable. Nonetheless, the inelastic region, characterized by a nonlinear behavior, shows that the parallel-to-the-grain could be used to find a monotonic parametric or nonparametric function to improve the quality of the prediction. In that way, both variables contribute to predicting the variability of the dependent variable and validate our hypothesis previously depicted in Eq. (1).

Multiple linear regression model

The following stage of the proposed framework was fitting the different parametric and nonparametric models. To this end, we randomly divided the collected data into training and test sets with a proportion of 80%–20%, using the Python library sklearn.model_selection and the method train_test_split. Subsequently, we fitted the model in Eq. (2) and found the coefficients and 95% Confidence Intervals (CIs) shown in Table 4. Our modeling was conducted for the elastic region, where the parallel-to-the-grain stress is less than 30 MPa, and the strain is less than 0.8% since the results from Table 3 and Fig. 7 showed that an MLR model could be suboptimal in the inelastic region. Notice that the independent term obtained a value of -0.296 MPa with a CI in the range [-0.473, -0.121]. This suggests that when no loads are parallel or perpendicular, the load has a slightly negative value, which can be attributed to a measurement error without a physical interpretation. Regarding b_1, note that a 1% increase in the strain means that the perpendicular-to-the-grain stress also increases by 3.411 MPa, which is expected for this material that is prone to deflect due to its nature. Besides, the narrow confidence interval shows that this variable adequately approximates the dependent variable behavior. Besides, the estimation for b_2 suggests that for 1 MPa increase in the parallel-to-the-grain stress, an increase of 0.042 MPa occurs in the dependent variable.

Table 4. Coefficients and 95% CIs for the MLR model

Variable	Symbol	Value	95% CI	Units
Independent term	b_0	-0.296	[-0.473, -0.121]	MPa
Strain (%)	b_1	3.411	[3.297, 3.526]	MPa/%
Stress Parallel (MPa)	b_2	0.042	[0.031, 0.052]	Adim

This value can be explained since a more pronounced deformation arises in parallel deformation, while perpendicular behavior is more resilient under stress conditions. This behavior is primarily due to the anisotropic structure of wood and the arrangement of its cells, which influence how stresses are transmitted within the material's internal structure. Since the cellulose fibers are aligned parallel to the applied load, stress transmission occurs efficiently without significant interruptions. In contrast, in the perpendicular direction, wood exhibits lower load-bearing capacity due to its anatomical structure. The application of load in this direction tends to separate the fibers, leading to faster failure. In the same way, the narrow confidence interval suggests that this variable accurately predicts the perpendicular-to-the-grain stress in the elastic zone.

On the other hand, we present the numerical performance of the fitted MLR model in Table 5. Notice that the results of the training and test sets are similar, indicating that the model has adequate generalization ability and a low risk of overfitting. Regarding the

Table 5. MLR model performance for training and test sets

Subset	R^2	RMSE (MPa)
Training	0.909	1.039
Test	0.916	1.036

R^2 and the RMSE, the model can explain about 91% of the variability of the wood behavior and exhibit about 1 MPa of error for the elastic zone, indicating that for normal operational conditions, an MLR-based model is a computationally effective and highly interpretable model, even considering that wood is anisotropic and depends on uncontrollable variables like moisture and density.

Artificial neural network

We fitted the MLP-based regression model in Fig. 5 using the library sklearn.neural_network and the class MLPRegressor. First, we used the same database split described in the previous section, with 80%–20% proportion. However, our aim in fitting this model is to find a regressor that can accurately forecast the elastic and inelastic regions, different from the MLR-based model that was fitted only for the elastic region. In that way, we conducted a grid search and cross-validation to find the best configuration to achieve the lowest bias, variance, RMSE, and the highest R^2. The summary of the hyperparameters is shown in Table 6. Specifically, we tested the following network architectures: *i)* a single hidden layer with 50 neurons, *ii)* a single layer with 100 neurons, and *iii)* two hidden layers with 50 neurons in each layer. Regarding the activation function responsible for introducing the nonlinear behavior to the regressor, we tested the ReLU and hyperbolic tangent options since they are commonly used for regression tasks. Regarding the learning rate, we used a static strategy with values of 0.0001 and 0.01, aiming to have slow and fast responses in convergence. Furthermore, we also set a dynamic scheme to adjust the network weight updates using well-known algorithms like Adam and SGD. Finally, we performed the five-fold cross-validation scheme to avoid overfitting, controlling the standard deviation of the fitted models. In that way, we tested three network configurations, two activation functions, two learning rates, two weight optimizers, and five-fold cross-validation, so we trained 120 models.

Table 6. Hyperparameter ranges and best configurations for the MLP model

Hyperparameter	Configurations tested	Best configuration
Network architecture	(2, 50, 1), (2, 100, 1), (2, 50, 50, 1)	(2, 50, 50, 1)
Activation functions	ReLU, hyperbolic tangent	ReLU
Learning rate	0.0001 and 0.01	0.0001
Optimizer	Adam and SGD	Adam

The best model achieved the results shown in Table 7 using the following configuration: *i)* a ReLu activation function, *ii)* a learning rate of 0.0001, *iii)* a network architecture with two layers with 50 neurons per layer, and *iv)* the weight optimizer Adam. With the selected configuration, the following observations can be drawn. First, the MLP-based achieved a considerably high R^2 when comparing the predictions versus the actual data from the test subset since we achieved a capacity to predict variability up to 70% compared to the obtained correlations up to 0.62 in Table 3, which denotes a noticeable improvement of the nonparametric machine-learning-based regressors to learn nonlinear patterns like those exhibited by the anisotropic behavior of wood. Besides, the standard deviation

Table 7. MLP-based model performance for training and test sets considering elastic and non-elastic regions. The training includes the standard deviation *s* to show the model's generalization ability

Subset	R^2	RMSE (MPa)
Training	0.729	1.311, $s = 0.104$
Test	0.708	1.378

of the cross-validation process was 0.104 MPa, a relatively negligible value considering the high variability of the material. Finally, the RMSE ranges from 1.3 to 1.4 MPa, which can be high considering the scale of the experiments. However, a visual exploration

performed in Fig. 9. showed that the MLP-based regressor can predict with acceptable accuracy the behavior of the dependent variable when using the strain and the parallel-to-the-grain stress. Although these results seem less accurate than those obtained by the MLR-based model, it is essential to note that this model was fitted to predict the material performance in the inelastic zone, which is highly variable.

(a) Trial 9   (b) Trial 15   (c) Trial 21
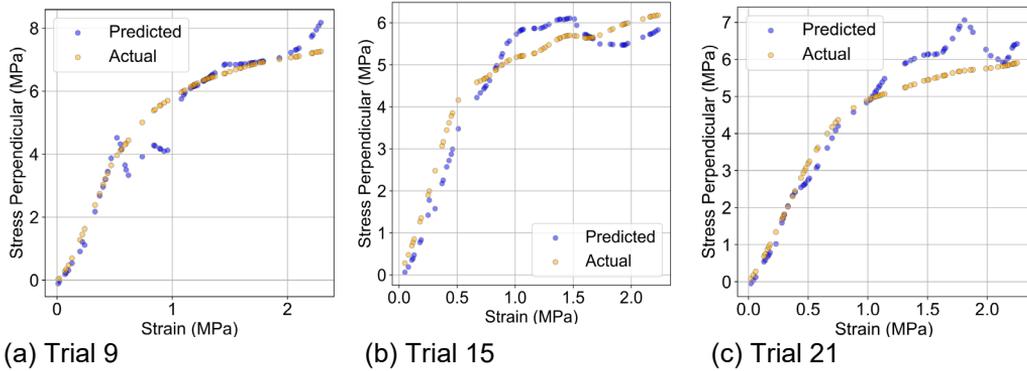
Figure 9. Prediction of the perpendicular-to-the-grain stress versus strain for some experiment trials using the MLP-based model. The dark purple scatters represent the actual data, and the blue scatters represent the predictions.

Random forest

The last model we fitted was based on the Random Forest according with the architecture presented in Fig. 6 using the library sklearn.ensemble and the class RandomForestRegressor. As discussed in the previous section, we kept the same database split into 80%–20% proportions for the training and test sets. We also performed the grid search and five-fold cross-validation procedures to preserve low bias, variance, and high accuracy. In the same way as the MLP-based regressor, we aimed to predict the behavior of the perpendicular-to-the-grain stress based on the elastic and inelastic zones. The summary of the considered hyperparameters is shown in Table 8. .

Table 8. Hyperparameter ranges and best configurations for the RF model

Hyperparameter	Configurations tested	Best configuration
Forest size	50, 100, 200	100
Tree Depth	No restriction, 10, 20	10
Min number of simples per split	2, 5, 10	5

Specifically, we tested different forest sizes, with values of 50, 100, and 200 trees in the forest. Regarding the trees' depths, we limited the DTs with no restrictions and constrained this hyperparameter to 10 and 20 levels. This configuration allowed us to examine how deeper trees influence the model's performance regarding overfitting. Finally, we also varied the hyperparameter related to the minimum number of samples required to split a node with values of 2, 5, and 10, ensuring that these settings allow the trees to grow sufficiently while preserving the generalization capacity of the random forest. Since we set three different forest sizes, three tree depths, and three values for the minimum number of samples per split and used five subsets for the cross-validation process, we trained a total of 135 models. The selected model was the one that provided

the best tradeoff between accuracy and generalization, aiming to capture the linear and nonlinear behavior of the perpendicular stress of the wood.

The best model achieved the results in Table 9. using the following configuration: *i)* a forest size of 100 DTs, *ii)* a tree depth of 10, and *iii)* a minimum number of samples to split a node of 5. From this table, it can be noticed that the RF-based model achieved the best performance compared to the MLP-based model and the MLR model since it could predict the data variability up to 91% in the test set; that is, our fitted model could adequately forecast the inelastic behavior of the wood in the perpendicular-to-the-grain stress when using the strain and the parallel-to-the-grain stress. Note also that the RMSE achieved was considerably lower than

Table 9. RF-based model performance for training and test sets considering elastic and non-elastic regions. The training includes the standard deviation s to show the model's generalization ability

Subset	R^2	RMSE (MPa)
Training	0.95	0.58, $s = 0.082$
Test	0.91	0.62

that obtained using the MLP-based regressor with values up to 0.62 MPa. Finally, the standard deviation was 0.082 MPa, indicating an adequate generalization ability of the model in the presence of unknown data. The visual exploration of some experiment trials in Fig. 10 confirms the fitted model's accuracy since most predicted values are close to the actual ones when using the test set.

(a) Trial 9   (b) Trial 15   (c) Trial 21

Figure 10. Prediction of the perpendicular-to-the-grain stress versus strain for some experiment trials using the RF-based model. The orange scatters represent the actual data, and the blue scatters represent the predictions.

CONCLUSIONS

This study showed how machine learning techniques can enhance the prediction of mechanical behavior in wood, a sustainable construction material that is becoming increasingly important in engineering. Conventional modeling approaches in the characterization of wood, like multiple linear regression, have demonstrated a lack of capability in representing nonlinear and anisotropic material properties, particularly in the inelastic region. We further proposed applying ANN and RF models to balance interpretability and computational complexity in improving predictive accuracy and generalization capability. The MLR model, computationally efficient and interpretable,

showed an adequate performance for the elastic region but limited capabilities for the inelastic zone. The MLP-based model was trained using hyperparameter tuning with cross-validation to result in a better nonlinear relationship capture capability, especially around the transition between elastic and inelastic behavior. While this provided a higher capability in learning complex patterns, the model had higher RMSEs: 1.31 MPa for training and 1.38 MPa for testing. That may indicate that neural networks are struggling with wood's inherently variable and anisotropic nature and could require more extensive feature engineering or larger datasets to achieve optimal performance. However, the random forest model had the best performance among all three techniques, with the most generalizable and closest predictions in elastic and inelastic regions. Giving a high R^2 of 0.95 for training and 0.91 for testing, with a significantly lower RMSE at 0.58 MPa for training and 0.62 MPa for testing, the RF-based approach showed the best adaptability to learn linear and nonlinear behaviors. It also seemed that the variance in this model, when cross-validated, was relatively low, pointing toward its robustness and strong generalization capabilities. This result now points toward ensemble learning, especially tree-based models, for modeling such mechanical responses for complicated materials like wood.

As expected, the data observed in the experimental tests show a strong correlation that can be predicted using ML-based models. This prediction of the main mechanical properties along the different anisotropic axes is primarily attributed to the anatomy of the wood, which consists mainly of elongated cells organized in a predominant direction. The orientation of these cells plays a crucial role in determining the structural performance of the material. Although the fitted models achieved an adequate prediction capacity with high accuracy, this research also outlines essential conclusions regarding wood characterization and sustainable construction since it showed that the use of machine learning techniques can reduce the need for extensive experimental setups and campaigns, reducing the associated costs, since the experiments associated with the perpendicular-to-the-grain stress can be forecasted using a different experiment, in this case, the characterization of the parallel-to-the grain stress versus the strain. These findings can be extrapolated for different structural methodologies with high reliability in civil engineering.

USE OF GENERATIVE AI DECLARATION. The authors confirm that they used ChatGPT to fix typo errors, improve grammar, translate some excerpts from Spanish, and enhance the readability of the manuscript. However, they declare that the manuscript is original, and the improved texts obtained from the language models have been carefully reviewed.

REFERENCES

Arriaga, F., Wang, X., Íñiguez-González, G., Llana, D.F., Esteban, M. & Niemz, P. 2023. Mechanical properties of wood: A review. *Forests* **14**(6), 1202. doi: 10.3390/f14061202

ASTM International. 2021. ASTM D198-21: Standard Test Methods of Static Tests of Lumber in Structural Sizes. West Conshohocken, PA: ASTM International. https://doi.org/10.1520/D0198-21

ASTM International. 2021. ASTM D5457-21a Standard Specification for Computing Reference Resistance of Wood-Based Materials and Structural Connections for Load and Resistance Factor Design. West Conshohocken, PA: ASTM International. doi: 10.1520/D5457-21A

Ding, Y., Pang, Z., Lan, K., Yao, Y., Panzarasa, G., Xu, L., Lo Ricco, M., Rammer, D.R., Zhu, J.Y. & Hu, M. 2023. Emerging engineered wood for building applications. *Chemical Reviews* **123**(5), 1843–1888. https://doi.org/10.1021/acs.chemrev.2c00588

Ehtisham, R., Qayyum, W., Camp, C.V., Plevris, V., Mir, J., Khan, Q.Z. & Ahmad, A. 2024. Computing the characteristics of defects in wooden structures using image processing and CNN. *Automation in Construction* **158**, 105211. doi: 10.1016/j.autcon.2023.105211

Giraldo, J.M.G. & Palacio, L.G. 2020. The fourth industrial revolution, an opportunity for Civil Engineering. *2020 15th Iberian Conference on Information Systems and Technologies (CISTI)*, 1–7. https://doi.org/10.23919/CISTI49556.2020.9141138

Glass, S.V. & Zelinka, S.L. 2021. Moisture relations and physical properties of wood. In *Wood Handbook—Wood as an Engineering Material* (FPL-GTR-282), Chap. 4, 4–1–4–22.

Gonzalez-Palacio, M., Moncada, S.V., Luna-delRisco, M., Gonzalez-Palacio, L., Montealegre, J.J.Q., Orozco, C.A.A., Diaz-Forero, I., Velasquez, J.-P. & Marin, S.-A. 2018. Internet of things baseline method to improve health sterilization in hospitals: An approach from electronic instrumentation and processing of steam quality. *2018 13th Iberian Conference on Information Systems and Technologies (CISTI)*, 1–6. https://doi.org/10.23919/CISTI.2018.8398648

González-Palacio, M., Tobón-Vallejo, D., Sepúlveda-Cano, L.M., Luna-delRisco, M., Roehrig, C. & Le, L.B. 2024. Machine-Learning-Assisted Transmission Power Control for LoRaWAN Considering Environments With High Signal-to-Noise Variation. *IEEE Access* **12**, 54449–54470. https://doi.org/10.1109/ACCESS.2024.3387457

Holmberg, S., Persson, K. & Petersson, H. 1999. Nonlinear mechanical behaviour and analysis of wood and fibre materials. *Computers & Structures* **72**(4–5), 459–480. https://doi.org/10.1016/S0045-7949(98)00331-9

Ishimaru, Y., Arai, K., Mizutani, M., Oshima, K. & Iida, I. 2001. Physical and mechanical properties of wood after moisture conditioning. *Journal of Wood Science* **47**, 185–191. https://doi.org/10.1007/BF01171220

Khan, B., Jan, S., Khan, W. & Chughtai, M.I. 2024. An overview of ETL techniques, tools, processes and evaluations in data warehousing. *Journal on Big Data* **6**(1), 1–20. https://doi.org/10.32604/jbd.2023.046223

Kim, J.-H., Park, W.-G. & Kim, N.-H. 2024. Performance of convolutional neural network (CNN) and performance influencing factors for wood species classification of *Lepidobalanus* growing in Korea. *Scientific Reports* **14**(1), 18141. doi: 10.1038/s41598-024-69281-y

Kumar, R., Gupta, S.K., Wang, H.-C., Kumari, C.S. & Korlam, S.S.V.P. 2023. From efficiency to sustainability: Exploring the potential of 6G for a greener future. *Sustainability* **15**(23), 16387. https://doi.org/10.3390/su152316387

Loss, C. 2023. Experimental campaign on the mechanical properties of Canadian small clear spruce-pine-fir wood: Experimental procedures, data curation, and data description. *Data in Brief* **48**, 109064. https://doi.org/10.1016/j.dib.2023.109064

Mascia, N.T., Todeschini, R. & Nicolas, E.A. 2013. Criterion assessment of strength of anisotropic materials applied to wood using uniaxial and biaxial tests. *Revista Sul-Americana de Engenharia Estrutural* **10**(2), 5–30 (in Portuguese).

Niemz, P. & Sonderegger, W. 2017. *Holzphysik: Physik des Holzes und der Holzwerkstoffe* [Wood physics: Physics of wood and wood-based materials]. München: Carl Hanser Verlag. 580 pp. (in German). https://doi.org/10.3139/9783446445468

Palacio, M.G., Palacio, L.G., Montealegre, J.J.Q., Pabón, H.J.O., Del Risco, M.A.L., Roldán, D., Salgarriaga, S., Vásquez, P., Hernández, S. & Martínez, C. 2017. A novel ubiquitous system to monitor medicinal cold chains in transportation. *2017 12th Iberian Conference on Information Systems and Technologies (CISTI)*, 1–6. doi: 10.23919/CISTI.2017.7975685

Peng, L., Searchinger, T.D., Zionts, J. & Waite, R. 2023. The carbon costs of global wood harvests. *Nature* **620**(7972), 110–115. https://doi.org/10.1038/s41586-023-06187-1.

Record, S.J. 1914. *The mechanical properties of wood: Including a discussion of the factors affecting the mechanical properties, and methods of timber testing*. New York, NY: John Wiley & Sons, 165 pp.

Rodríguez-Grau, G., Marín-Uribe, C.R., García-Giraldo, J.M. & Estay, C. 2022. Flexural strength characterisation of oblique radiata pine splice joints. *Wood Material Science & Engineering* **17**(6), 1010–1019. https://doi.org/10.1080/17480272.2021.1990405

Senalik, C.A. & Farber, B. 2021. Mechanical properties of wood. In *Wood Handbook—Wood as an Engineering Material* (FPL-GTR-282), Chap. 5: pp. 5–1; 5–44.

Sharma, A., Garg, S. & Sharma, V. 2024. ATR-FTIR spectroscopy and machine learning for sustainable wood sourcing and species identification: Applications to wood forensics. *Microchemical Journal* **200**, 110467. https://doi.org/10.1016/j.microc.2023.110467

Soares, L.S.Z.R., Fraga, I.F., de Souza e Paula, L., Arroyo, F.N., Ruthes, H.C., de Moura Aquino, V.B., Molina, J.C., Panzera, T.H., Branco, L.A.M.N., Chahud, E., Christoforo, A.L. & Lahr, F.A.R. 2021. Influence of moisture content on physical and mechanical properties of *Cedrelinga catenaeformis* wood. *BioResources* **16**(4), 6758–6765.

Toumpanaki, E., Shah, D.U. & Eichhorn, S.J. 2021. Beyond what meets the eye: Imaging and imagining wood mechanical–structural properties. *Advanced Materials* **33**(28), 2001613. https://doi.org/10.1002/adma.202001613

Unsal, O. & Candan, Z. 2008. Moisture content, vertical density profile and Janka hardness of thermally compressed pine wood panels as a function of press pressure and temperature. *Drying Technology* **26**(9), 1165–1169. https://doi.org/10.1080/07373930802266306

Véliz-Fadic, F.I., Rodríguez-Grau, G., Marín-Uribe, C.R., García-Giraldo, J.M., González-Palacio, L. & Araya-Letelier, G. 2024. Flexural performance assessment of the effect of the splice length of the Jupiter ray type made of radiata pine using computer-aided design and computer-assisted manufacturing. *Construction and Building Materials* **449**, 138272. https://doi.org/10.1016/j.conbuildmat.2024.138272

Yan, X. & Su, X. 2009. *Linear regression analysis: Theory and computing*. Singapore: World Scientific, 348 pp.

Yau, Y., Sulaiman, T.A., Baba, Z.B., Amartey, B.H.S., Sabari, A.A. & Garba, I. 2024. Influence of density and moisture content on the compressive strength parallel to grain of *Gmelina arborea* timber specie. In *Proceedings of the 12th Annual and International Conference of the Renewable and Alternative Energy Society of Nigeria (RAESON)*, Wudil, Kano State, Nigeria, 2–5 June 2024, 1–12.

Agrophysical condition of chernozem as a criterion for its readiness for soil tillage minimization

Ye. Ihnatiev[1,5], V. Bulgakov[2], O. Demydenko[3], I. Holovach[2], O. Trokhaniak[2], A. Rucins[4] and J. Olt[5,*]

[1]Dmytro Motornyi Tavria State Agrotechnological University, 66 Zhukovsky Str., UA 69600 Zaporizhzhia, Ukraine
[2]National University of Life and Environmental Sciences of Ukraine, 15, Heroyiv Oborony Str., UA03041 Kyiv, Ukraine
[3]Cherkasy State Agricultural Experimental Station National Scientific Centre, Institute of Agriculture of NAAS of Ukraine, 13 Dokuchaieva Str., Kholodnianske village, Cherkassy district, UA20731 Cherkassy region, Ukraine
[4]Latvia University of Life Sciences and Technologies, Institute of Engineering and Energetics, Faculty of Engineering and Information Technologies, Ulbroka Research Centre, 1 Instituta Str., Ulbroka, Ropazu Region, Stopinu Municipality, LV 2130 Latvia
[5]Estonian University of Life Sciences, Institute of Forestry and Engineering, 56 Kreutzwaldi Str., EE51006 Tartu, Estonia
*Correspondence: jyri.olt@emu.ee

Abstract. The objective of this study was to develop an agrophysical criterion for assessing the condition of leached chernozem under systematic shallow tillage for six years, using differential porosity, crop productivity, and energy efficiency as indicators. The results were compared to those obtained under conventional ploughing, with the goal of evaluating the feasibility of further tillage minimization in a short-rotation grain crop rotation system. The study employed standard research methods, including field observations, laboratory analysis, mathematical statistics, and comparative-calculative approaches. Under shallow tillage, the median bulk density in the 0–30 cm soil layer was higher by 0.03 g cm^{-3} compared to ploughing, while the standardized density range remained similar across tillage systems. However, both the upper and lower typical values increased by 0.02 g cm^{-3} under shallow tillage. The coefficient of variation for bulk density was 1.85 times higher under ploughing than under shallow tillage. Differential porosity measurements across five crops in the sixth year of the trial revealed that in spring, shallow tillage resulted in higher bulk density (by 0.06 g cm^{-3}), lower total porosity (by 3%), and a 6.0% reduction in air-filled pore volume compared to ploughing. At the same time, the ratio of water-filled to air-filled pores approached an optimal 1:1 balance under shallow tillage, whereas ploughed soils showed a dominance of air-filled pores. Systematic use of both ploughing and conservation-oriented shallow tillage over five years in a five-field grain-row crop rotation produced similar levels of productivity in terms of yield, energy efficiency, and output of grain and feed units. While productivity indicators under ploughing remained stable relative to the mean, shallow tillage demonstrated a positive trend. This trend is associated with the completion

of the transition period following the cessation of deep ploughing and the shift toward preliminary deep chisel tillage, followed by systematic shallow tillage at a depth of 10–12 cm.

Key words: leached chernozem, bulk density, soil moisture, ploughing, shallow tillage.

INTRODUCTION

The global trend toward the minimization of soil tillage is driven, on the one hand, by the effort to reduce material and labor inputs for cultivation and, on the other hand, by the potential to manage soil-forming processes and achieve the expanded reproduction of soil fertility – something that remains unattainable under continuous conventional plowing. Permanent minimal tillage promotes the development of self-regulation processes similar to those found in virgin soils. It contributes to the accumulation of organic matter, nutrients, and stored energy in the upper soil profile of chernozem, which, when combined with a rational anthropogenic impact, creates a foundation for sustainable enhancement of soil fertility (Sayko & Maliyenko, 2007).

The soil cover of the Cherkasy region is primarily composed of typical chernozems, heavily degraded dark gray podzolized and degraded soils, podzolized chernozems, and light and gray podzolized soils. In terms of texture, the soils range from light loam to medium and heavy loam. Typical and heavily degraded chernozems dominate the soil cover of the region, accounting for over 50%.

Tillage itself does not enrich the soil environment with energy materials necessary for restoring the fertility of chernozem soils. However, it directly affects the agrophysical parameters of the soil, which determine the air and thermal conditions of the soil climate, the degree and depth of plant residue incorporation, and consequently influence the dynamics and balance between the synthesis and mineralization of humus, the formation of available nutrient forms, and their uptake by agricultural crops (Bardgett et al., 2014; Tonkha et al., 2017).

Chernozems, as the main productive resource of agriculture, have long been subjected to conditions that differ significantly from the optimal ones formed in their natural state, making them increasingly prone to degradation processes (Krupenikov, 2008). The primary cause is the inconsistency of applied technologies with the natural principles underlying chernozem formation (Bulgakov et al., 2024b). One such inconsistency is conventional tillage, which disrupts the sod layer and litter, leading to progressive agrophysical degradation of chernozems within agrocenoses (Demydenko, 2013; Philippot et al., 2013; Zhang et al., 2015).

The choice of a tillage system for chernozem soils has always been one of the most controversial and pressing issues in agricultural practice within the Forest-Steppe zone of Ukraine. Ongoing debates concerning the necessity of deep tillage with soil inversion have led to a growing need to minimize tillage. This trend increasingly favors reducing both the depth and frequency of tillage operations (Demydenko & Velychko, 2015).

In recent decades, the most balanced approach to chernozem management has been the differentiated tillage system, which combines various ploughing and non-inversion tillage techniques (Tomnytskyi et al., 2024). Issues related to the deterioration of agrophysical properties following the abandonment of intensive tillage are addressed through the periodic application of ploughing for the most demanding crops (Gordienko, 2004; Bulgakov et al., 2020a). At the same time, substantiated evidence is emerging in

support of tillage minimization on chernozems with naturally high agrophysical properties, which are relatively close to optimal for the cultivation of most crops (Bulgakov et al., 2024a).

One of the most practical approaches to addressing the issue of chernozem degradation is the further improvement of soil tillage technologies through the reduction of production costs. As a result, resource-saving technologies of primary tillage are becoming increasingly widespread (Matyukha & Semenov, 2024). These practices contribute to the improved adaptation of agricultural crops to the challenging modern natural and climatic conditions, helping to maintain agroecological balance within agrocenoses (Dolia & Shevchenko, 2024).

Changes in the depth of primary tillage significantly affect the fertility of chernozem. Generally, less cultivated chernozems require deeper tillage (Shevchenko et al., 2024), whereas as the degree of cultivation and improvement of agrophysical properties increases, the sensitivity of chernozem to intensive tillage decreases. In such cases, fertility under tillage minimization may exceed that achieved through conventional deep tillage (Shykula, 2001; Torma et al., 2017; Vozhegova et al., 2021).

In modern agricultural production, resource-saving technologies – such as combined, minimum, and zero tillage systems – are widely used. Under these systems, agrophysical factors of chernozem fertility require particular attention. Soil bulk density is the key indicator of the physical state of chernozem for successful crop cultivation (Samofalova et al., 2013; Hirte et al., 2017; Bulgakov et al., 2021; Yermakov et al., 2021). Favorable physical properties are the foundation and an essential condition for realizing the potential fertility of chernozem and achieving high crop yields (Jordan et al., 2010; Copec et al., 2015; Nandan et al., 2019; Modak et al., 2020). Creating and maintaining an optimal structure of the arable layer through various tillage systems is a critical task in contemporary agriculture (Lampurlanes et al., 2016; Castellini et al., 2019; Adamchuk et al., 2021).

The current global trend toward soil tillage minimization is driven by two major objectives: reducing energy and labor costs and ensuring sustainable soil fertility reproduction. In Ukraine's Central Forest-Steppe, long-term systematic ploughing of podzolic chernozems has led to severe agrophysical degradation, expressed in excessive compaction, reduced porosity, and disruption of the soil's structural organization. Such conditions significantly limit the potential for effective implementation of minimal or zero tillage systems.

Many long-term studies and meta-analyses have shown that reduction of tillage intensity often results in changes to bulk density, pore size distribution, and aggregate stability, although the magnitude and direction of such changes depend strongly on soil type, climate, and transition period. For example, a global meta-analysis reported that no-tillage generally improves soil structure and porosity, particularly in the upper soil layers, although effects on bulk density are modest and variable (Mondal & Chakraborty, 2022).

Similarly, in a long-term trial, Dam et al. (2005) observed how changes in tillage affected soil bulk density, seedling emergence, and yield in continuous maize systems under differing tillage regimes. These findings underscore that transitions in tillage systems must be examined over multi-year periods, especially in degraded soils, to detect stable trends in soil physical indices.

The scientific problem lies in the absence of clear criteria for assessing when degraded chernozems become agrophysically ready for minimization of tillage. Without this, the transition to reduced tillage depth often leads to unstable soil responses and yield fluctuations, resulting in the discrediting of the concept of tillage minimization in crop rotations. Therefore, there is a need to substantiate agrophysical indicators that can serve as reliable criteria for determining the readiness of podzolic chernozem to shift from conventional ploughing to shallow and, subsequently, zero tillage systems.

Additionally, several works emphasize that soil structural recovery under reduced disturbance is not instantaneous. For instance, short-term investigations suggest that a considerable period (often more than 3–5 years) is needed to develop improved pore continuity and stability (Jensen et al., 2020). This supports our choice of a 6-year trial period and justifies monitoring temporal dynamics of bulk density and porosity during this critical transition window.

However, despite the wealth of such studies, there is still a lack of clearly defined agrophysical readiness criteria for degraded podzolized chernozems in the initial 5–6 years of transition to shallow tillage in temperate Forest-Steppe zones. In many published works, the discussion of bulk density, porosity, and yield is descriptive, and rarely linked to a criterion by which a degraded chernozem can be judged fit for further minimization of tillage.

The dynamic nature of soil processes influenced by tillage, along with their impact on fertility, necessitates the systematic study of changes in the agrophysical parameters of chernozems in the Forest-Steppe zone of Ukraine – both in the short and long term.

This study aims to identify the agrophysical criterion that determine the soil's readiness for tillage minimization based on long-term field observations of bulk density, differential porosity, and productivity under systematic ploughing and surface tillage in a five-field grain-row crop rotation.

MATERIALS AND METHODS

The research was conducted from 2016 to 2021 in a long-term field experiment at the Cherkasy State Agricultural Experimental Station of the National Scientific Center 'Institute of Agriculture of the NAAS'. The soil type is strongly degraded, low-humus, medium-loamy leached chernozem, developed on calcareous loess-like loam according to the national classification (Polupan et al., 2005) or Chernic Phaeozems (Hyperhumic, Siltic, Calcaric, Cutanic, Episiltic, Sodic) according to WRB 2022 (coordinates 49°56'42"N, 32°06'54"E).

In the arable layer, the humus content ranged from 2.76% to 3.03% (by method developed by Tyurin I.V., modified by Symakov V.M. (State standard of Ukraine, DSTU 4289:2004)), the sum of absorbed bases ranged from 24.5 to 28.1 meq per 100 g of soil, hydrolytic acidity ranged from 1.99 to 2.19 meq per 100 g of soil, and the pH of the salt extract ranged from 5.56 to 6.31. The base saturation degree was 92.8–93.3%, the content of available phosphorus (by Chirikov method (State Standard of Ukraine DSTU 4115:2002)) was 9.0 mg per 100 g of soil, and exchangeable potassium (by Brovkina method (State Standard of Ukraine DSTU 4405:2005)) was 12 mg per 100 g of soil.

The study was carried out within a stationary field experiment focused on evaluating the productivity of a five-field grain-row crop rotation system, which included the following crops: maize (Zea mays), barley (Hordeum vulgare), soybean (Glycine max), sunflower (Helianthus annuus) and sugar beet (Beta vulgaris). The tillage system consisted of:

1. Differential tillage based on ploughing.

2. Differential tillage using deep chiseling (applied in 2015), followed by shallow tillage at a depth of 10–12 cm for all crops in the rotation.

The fertilization system included the application of $N_{75}P_{75}K_{82}$ along with 6 tons per hectare of crop residues.

Soil sample analyses, records, and calculations were conducted according to standard procedures. Soil moisture was determined using the thermogravimetric method during the key crop growth stages (State standard of Ukraine, DSTU ISO 11465:2001). Soil bulk density was measured using the cutting ring method in the modification by N.A. Kachynsky during the periods of intensive crop growth and yield formation (State standard of Ukraine, DSTU ISO 11272:2001). When determining bulk density using metal cylinders with a height of 10 cm and a volume of 275 cm³, the measurements were carried out layer by layer (0–10 cm, 10–20 cm, and 20–30 cm), followed by calculating the average value for the 0–30 cm soil layer. Soil moisture samples were simultaneously collected by layers (0–10 cm, 10–20 cm, and 20–30 cm) and later analyzed in the laboratory by weighing the soil samples before and after drying. Measurements were taken three to four times during the growing season across the five fields of the crop rotation over a five-year period. Soil sampling for bulk density and moisture determination was conducted within the same experimental plots (fields). Sampling was performed in five replicates.

To calculate the differential porosity, the main agrophysical characteristics of the podzolized chernozem were used: the particle density, bulk density, maximum hygroscopic moisture, wilting point moisture, and total moisture capacity:

1. Total porosity, defined as the total volume of voids between solid particles as a percentage of the total soil volume (TP, %): $TP = (d - d_v) \cdot 100\%$, where d – is the particle density of the soil, g cm⁻³; d_v – is the bulk density of the soil, g cm⁻³.

2. Volume of pores occupied by tightly bound (maximum hygroscopic) water (P_{mh}, %): $P_{mh} = (W_{mh} \cdot d_v) / 1.5$, where W_{mh} – is the maximum hygroscopic moisture (% of oven-dry soil weight); 1.5 is the density of water at W_{mh}, g cm⁻³.

3. Volume of pores occupied by loosely bound water (P_{lb}, %): $P_{lb} = (W_{wp} - W_{mh}) / 1.25$, where W_{wp} – is the wilting point moisture (% of oven-dry soil weight); 1.25 is the density of loosely bound water, g cm⁻³.

4. Volume of pores occupied by capillary water (CP, %): $CP = (W_{fc} - W_{wp}) \cdot d_v$, where W_{fc} – is the field (total) moisture capacity (%).

5. Volume of pores occupied by water of all categories (WP, %); $WP = P_{mh} + P_{lb} + CP$;

6. Aeration porosity (AP, %): $AP = TP - WP$.

For modeling and calculations, a total of 355 paired determinations of bulk density and soil moisture were used.

Hydrothermal coefficient (HTC), determined by Selyaninov's method – is the sum of precipitation during the period when the average daily air temperature is above 10° C divided by the sum of active temperatures for the same period, reduced ten times. HTC < 0.4 – very severe drought, HTC from 0.4 to 0.5 – severe drought, HTC from 0.6 to 0.7 – moderate drought, HTC from 0.8 to 0.9 – mild drought, HTC from 1.0 to 1.5 – sufficiently moist, HTC > 1.5 – excessively moist.

Statistical data processing was performed using nonparametric statistical methods – that is, methods that do not require assumptions about the type of data distribution. Instead of operating with the raw values, these methods use their ranks or frequencies, which makes them flexible for analyzing data measured on different scales. For sample description, quartile analysis was applied – dividing an ordered data series into four equal parts. The following indicators were calculated during the analysis: mean value (Mean); minimum value (Min); maximum value (Max); median (Med) – the second quartile ($L_{0.50}$), which divides the data in half; amplitude range (Δ_a = Max – Min); lower quartile ($L_{0.25}$) – the value below which 25% of observations lie; upper quartile ($L_{0.75}$) – the value below which 75% of observations lie; quartile range ($\Delta n = L_{0.75} - L_{0.25}$) corresponding to the 50% probability level; probability deciles – lower ($L_{0.10}$) and upper ($L_{0.90}$); coefficient of variation (C_v, %) – characterizing the degree of relative variability of indicators.

The energy efficiency assessment was carried out using the methodology of Kalinichenko (2016). Statistical analysis of research results was performed using the 'Method of Analysis of Variance' with the STATISTICA software, employing nonparametric statistical methods, as well as correlation and factor analysis (Bulgakov et al., 2020b).

RESULTS

The impact of different tillage methods on the bulk density and soil moisture content of the 0–30 cm soil layer of leached chernozem was studied. It was established that the average bulk density, regardless of tillage method, was the same; however, the amplitude range of bulk density under ploughing was 1.75 times greater compared to shallow tillage. The median bulk density under shallow tillage was higher by 0.03 g cm^{-3}, while the standardized range of density was the same for both tillage methods, with an increase of 0.02 g cm^{-3} in both the upper and lower quantiles under shallow tillage. The coefficient of variation of bulk density under ploughing was 1.85 times higher than under shallow tillage (Table 1).

Under different tillage methods, the factor loading of soil moisture was mainly associated with the principal factor F1: R = +0.94 ± 0.03; R^2 = 0.88, whereas bulk density showed a strong direct correlation along factor F2 (R = +0.97 ± 0.02).

The amplitude range of chernozem bulk density was wider under ploughing (Δ = 0.51 g cm^{-3}) compared to shallow tillage (Δ = 0.41 g cm^{-3}). The shift in density under ploughing occurred within a moisture interval of Δ = 18.5%, whereas under shallow tillage, the interval was Δ = 17.8%. In the latter case, the minimum moisture level was 1.32 times higher compared to ploughing.

Regardless of the tillage method, the normalized range of bulk density was within 0.17–0.18 g cm^{-3} for a normalized moisture range of 11.5–12.4% to 21.0–22.0%.

Table 1. Normalized parameters of bulk density and soil moisture content in the 0–30 cm soil layer depending on the tillage system of degraded leached chernozem, 2016–2021

Soil moisture, % Bulk density, g cm⁻³				Soil moisture quantiles, % Bulk density quantiles, g cm⁻³			
Mean	Min / Max	Max / Min	Med., $L_{0.50}$	$L_{0.10}$ / $L_{0.90}$	$L_{0.25}$ / $L_{0.75}$	$L_{0.75}$ / $L_{0.25}$	$L_{0.90}$ / $L_{0.10}$
	Amplitude range: $\Delta_a = Max - Min$			Normalized range: $\Delta_n(50\%) = L_{0.75} - L_{0.25}$; $\Delta_n(10\%) = L_{0.90} - L_{0.10}$			
Ploughing (22–25 cm)							
16.5	7.11	22.6	17.6	8.50	12.7	20.4	21.8
1.19	1.38	0.98	1.18	1.33	1.26	1.11	1.08
Shallow tillage (10–12 cm)							
15.6	6.9	22.5	16.7	9.25	11.1	19.7	20.9
1.19	1.39	1.05	1.22	1.35	1.27	1.13	1.10

The normalized range at a 10% significance level (Δ_n, 10%) for bulk density, regardless of the tillage method, was 0.25 g cm⁻³ under a normalized soil moisture range of 13.3% for ploughing and 11.4% for shallow tillage. In the first case, the minimum value of soil moisture was lower than the wilting point (WP = 8.85%), while in the second case it was higher than WP: 8.5% and 9.25%, respectively.

The bulk density value at the median under ploughing was 0.01 g cm⁻³ lower than the mean, whereas under shallow tillage, it was 0.03 g cm⁻³ higher than the mean. This indicates a stable trend toward increasing bulk density in the latter case, though still within optimal values, optimizing both total and differential porosity.

The coefficient of variation for bulk density under ploughing was 1.15 times higher compared to shallow tillage (Table 1).

According to the general model for different tillage systems, each unit increase in bulk density corresponded to a 0.019% decrease in soil moisture, indicating a consistent pattern in the change of bulk density depending on soil moisture content. However, under shallow tillage, each unit increase or decrease in bulk density corresponded to a 0.018% change in soil moisture (Table 2). Under systematic ploughing, for each unit increase in total porosity, there was a decrease in the volume of pores filled with capillary water and air in a ratio of 1.7 to 1, whereas under shallow tillage, this ratio was 0.25 to 1. Shallow tillage led to the stabilization of capillary water-filled pore volume while maintaining an adequate amount of air-filled pore volume.

Table 2. Influence of the tillage system on changes in bulk density as a function of soil moisture in the 0–30 cm layer of leached, degraded chernozem (2016–2021)

Regression equation	Correlation coefficient, r	Determination coefficient, r²
Ploughing		
$y = 1.52 - 0.019 \cdot x$	r = –0.87	0.75
Shallow tillage		
$y = 1.50 - 0.018 \cdot x$	r = –0.78	0.61
Overall model		
$y = 1.51 - 0.019 \cdot x$	r = –0.77	0.60

The determination of bulk density and calculation of differential porosity under different tillage methods for five crops in a row-crop grain rotation, in the sixth year of the experiment, showed that in spring, bulk density under systematic shallow tillage was 0.06 g cm⁻³ higher than under ploughing. The average total porosity under shallow

tillage was 3 to 4 percentage points lower. The volume of pores filled with water was higher compared to systematic ploughing, which influenced the ratio of pore types: under shallow tillage, the ratio was close to the lower optimal threshold (~1:1), while under ploughing, it favored air-filled pores.

A similar trend was observed in the amplitude and normalized range of porosity categories. The stabilization of differential porosity under systematic shallow tillage was confirmed by a lower coefficient of variation (C_V) for all porosity parameters compared to ploughing (Table 3).

Table 3. Normalized parameters of differential porosity in the 0–30 cm soil layer depending on the tillage system of leached, degraded chernozem, 2016–2021

Agrophysical indicators	Mean	Median	Min	Max	Quantile: $L_{0.25}$	$L_{0.75}$	C_V, %
			Amplitude range: Δ_a=Max − Min		Normalized range: $\Delta_n(50\%)$=$L_{0.75}$ − $L_{0.25}$		
Systematic ploughing (22–25 cm)							
	1.09	1.08	-	-	-	-	7.75
TP, %	58.0	58.0	53.0	64.0	56.0	64.0	5.52
WP, %	24.0	25.	19.0	29.0	21.0	27.0	12.0
CP, %	18.0	18.0	13.0	21.0	15.0	20.0	14.5
AP, %	33.0	33.0	24.0	35.0	35.0	37.0	17.6
WP to AP	0.77	0.77	0.42	0.82	0.60	0.73	27.9
Systematic shallow tillage (10–12 cm)							
	1.14	1.15	-	-	-	-	8.61
TP, %	56.0	55.0	55.0	61.0	52.0	59.0	6.42
WP, %	26.0	27.0	20.0	29.0	24.0	29.0	10.0
CP, %	18.0	18.0	14.0	20.0	17.0	19.0	10.0
AP, %	27.0	28.0	35.0	32.0	28.0	30.0	15.8
WP to AP	~1.0	~1.0	0.57	0.91	0.77	~1.0	21.5

Note: TP – total porosity, %; WP – water-filled pores, %; CP – capillary pores, %; AP – aeration pores, %; C_V – coefficient of variation, %.

Under systematic ploughing, crop yields in the 5-field crop rotation demonstrated a steady upward trend compared to shallow tillage, although the differences were not statistically significant. A significant increase in yield was recorded for maize and sugar beet. The grain unit output per rotation under both systematic ploughing and shallow tillage ranged from 34.4 to 35.1 tonnes, or 6.88 to 7.02 tonnes per hectare. The largest contributions to the total grain unit output came from maize, sunflower, and sugar beet.

The feed unit output was nearly the same regardless of the tillage method, amounting to 34.5–34.6 tonnes, or 6.90–6.91 tonnes per hectare. In terms of feed-protein units, ploughing slightly outperformed shallow tillage: 37.0 tonnes vs. 33.9 tonnes, or 7.40 t ha^{-1} vs. 6.79 t ha^{-1}. The highest contribution to total feed-protein output came from maize (23.3–24.9%) and sunflower (22.6–23.6%) (see Table 4).

The energy content of the harvest under systematic ploughing reached 416.0 MJ per crop rotation, or 83.3 MJ ha^{-1}. Under shallow tillage, the energy content of the harvest was lower by 9 MJ and 1.9 MJ ha^{-1}. The most energy-dense crops were maize and sugar beet, yielding 121–128 MJ ha^{-1}.

Table 4. Productivity of agricultural crops under different soil tillage methods in a five-field grain-row crop rotation during 2016–2021

Productivity indicators	Crops in the 5-field crop rotation system					
	Maize	Spring barley	Soybean	Sunflower	Sugar beet	Average
Shallow tillage						
$N_{75}P_{75}K_{82}$ + 6 t ha^{-1} crop residues						
Yield, t ha^{-1}	7.95	3.74	2.35	3.55	46.7	-
Energy content of yield, GJ ha^{-1}	120.3	61.5	42.6	63.3	119.4	81.4*
						407.0
Grain units, tons	7.95*	2.99	4.25	7.10	12.10	6.88*
	23.1	8.69	12.4	20.6	35.2	34.4
Feed units, tons	10.60	4.52	3.76	4.01	11.60	6.90*
	30.7	13.1	10.9	11.6	33.6	34.5
Energy efficiency coefficient (EEC)	4.80	3.90	3.70	2.41	3.55	3.67
Ploughing						
$N_{75}P_{75}K_{82}$ + 6 t ha^{-1} crop residues						
Yield, t ha^{-1}	8.15	3.56	2.47	3.60	47.50	-
Energy content of yield, GJ ha^{-1}	128	58.5	44.8	64.0	121.4	83.3*
						416.0
Grain units, tons	8.15	2.86	4.49	7.18	12.4	7.02*
	23.2	8.15	12.9	20.5	35.3	35.1
Feed units, tons	10.7	4.35	3.44	4.06	12.0	6.91*
	30.9	12.6	9.94	11.7	34.7	34.6
Energy efficiency coefficient (EEC)	5.71	5.39	5.05	3.01	4.58	4.75

Note: *Numerator – value per hectare (t ha^{-1}); Denominator – total output (t).

The output of crop residues was similar under both tillage systems, amounting to 40.7–41.3 tonnes per crop rotation (7–8 t ha^{-1}), with energy content in residues reaching 242–245 MJ, or 48–49 MJ ha^{-1}.

Analysis of the exponential regression equations ($y = a \cdot e^{-bx}$) reflecting the dynamics of changes in grain unit yield under different soil tillage treatments (Fig. 1) revealed that the rate of decline depends on the tillage method and fertilizer application. For the unfertilized variants, the decline coefficient under surface tillage (b = 0.061) was approximately 1.2 times higher compared to plowing (b = 0.051), indicating a more intensive decrease in grain unit yield under surface tillage. In contrast, for the fertilized variants, the opposite tendency was observed: the decline coefficient under plowing with fertilizers (b = 0.067) exceeded that under surface tillage with fertilizers (b = 0.061), which indicates a slower decline in crop yield under surface tillage toward the end of the observation period.

Thus, at the later stages of the experiment, surface tillage combined with fertilizer application provided more stable grain unit yield values compared to plowing. This effect may be attributed to the gradual recovery of the soil's natural fertility mechanisms: structure formation, accumulation of organic matter, and activation of soil biota, which become evident only after several years of implementing surface tillage technology.

The determination coefficients ($R^2 = 0.32 – 0.39$) indicate a moderate fit of the models to the experimental data; however, the obtained dependencies make it possible to assess general trends in yield changes depending on tillage practices and fertilization.
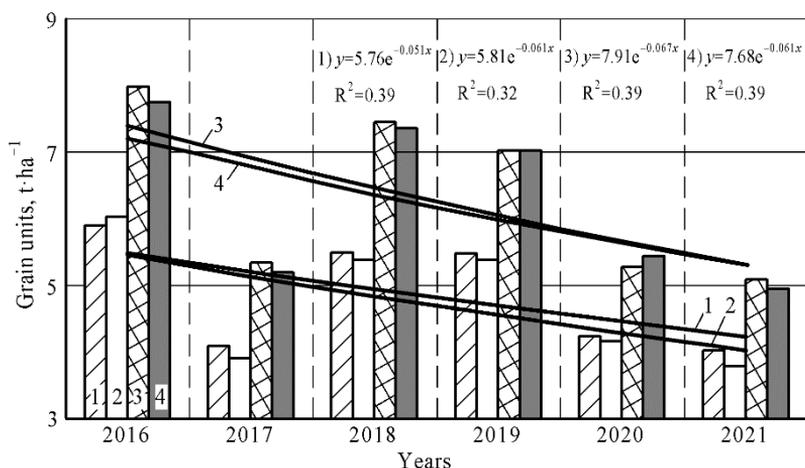
Figure 1. The effect of different tillage methods on the dynamics of grain unit yield in a five-field crop rotation during 2016–2021.

An agro-energy assessment of the input costs associated with the formation of productivity in a five-field crop rotation under different tillage systems showed that, regardless of the soil tillage method, a high level of production efficiency was ensured (energy efficiency coefficient, EEC > 2.5) for the main output. At the same time, energy profitability under shallow tillage increased by 1.45 times compared to plowing for the main produce and by 1.35 times for biological yield. The energy intensity of crop production within the rotation under average bulk density was found to be similar across tillage systems. However, the amplitude range of bulk density under plowing was 1.75 times greater compared to chisel tillage. The median bulk density in the 0–30 cm layer of chernozem was 0.03 g cm^{-3} higher, while the standardized density range remained the same regardless of tillage system, increasing by 0.02 g cm^{-3} under chisel tillage for both upper and lower typical values. The coefficient of variation of bulk density under plowing was 1.85 times higher compared to shallow tillage.

DISCUSSION

A key criterion for assessing the agroecological status of agricultural land is, first and foremost, the level of soil fertility, which serves as the foundation for the functioning of these land categories. Soil fertility determines land productivity, its economic significance, and value. It is defined as the soil's ability to meet plant needs for nutrients, water, air, and heat in sufficient quantities for normal growth and development – factors that together constitute the principal indicator of soil quality (Medvedev, 2013).

It is particularly important, under the conditions of the Central Forest-Steppe of Ukraine, to develop a criterion for assessing the agrophysical state of degraded podzolized chernozem during the initial years (five to six years) of transition to systematic shallow tillage. This is essential for preventing situations that could lead to the partial or complete discrediting of the core idea of tillage minimization in agrocenoses with diverse crop rotation systems. The need for such an approach is especially critical under sharply deteriorating weather and climate conditions, when the

moisture coefficient (MC), calculated according to Shashko's method, decreases to values around 0.55, and the hydrothermal coefficient (HTC), determined by Selyaninov's method, drops to values between 0.5 and 0.61 or lower for a period of one to one and a half months during the growing season of field crops. Such conditions were observed during the years of research in the Central Forest-Steppe region.

Based on the conducted research, the nature of the potentially unstable response of chernozem as a system becomes clear during the initial years of systematic shallow non-moldboard tillage, particularly when the soil is in an advanced stage of agrophysical degradation and exposed to critical weather and climate conditions. During the first two years of transition, a temporary increase in bulk density and reduced air porosity were observed, reflecting the soil's structural inertia before self-regulation processes became dominant. Under conventional ploughing, the 0–30 cm layer of chernozem does not exhibit differentiation in bulk density or in the structure of the pore space. Partial humus depletion and agrophysical degradation lead to the formation of a cloddy and compacted structure in the horizon, whereas long-term non-moldboard tillage promotes the development of a fine-crumbly, loose soil structure. Following the discontinuation of systematic ploughing, a transition period of five to six years is required to achieve an optimal structure in the 0–30 cm soil layer, characterized by the following profile: loose (0–10 cm) – compacted (10–20 cm) – loose (20–30 cm). In our experiment, the median bulk density under surface tillage reached 1.33 g cm^{-3} compared to 1.30 g cm^{-3} under ploughing, while the coefficient of variation was 1.85 times lower, indicating structural stabilization. The qualitative restructuring of the pore system, aiming at an optimal ratio of pores filled with water and air, serves as a sufficient criterion for the feasibility of further tillage minimization, ultimately allowing the implementation of zero-tillage systems on medium-humus, medium-loam, podzolized chernozem.

Comparison of the obtained results with the data from the literature sources indicates a general trend towards the necessity of minimizing traditional tillage of Chernozem soils. Thus, the findings of Baliuk et al. (2023) highlight a significant decrease in organic matter content and the need for the implementation of restorative practices, which fully aligns with our conclusions regarding the agrophysical degradation of Chernozems under intensive ploughing. The analysis by Lykhovyd (2024) confirmed the advantages of surface tillage over conventional ploughing in terms of soil biological activity, which also correlates with our findings on the improvement of soil physical properties under systematic minimal tillage. The study by Vilde et al. (2012) emphasized the importance of adapting tillage practices to specific soil properties to enhance yield and energy efficiency, supporting our recommendations for optimizing the structural parameters of working bodies to preserve soil porosity and prevent compaction. Therefore, the obtained results complement the overall scientific picture and confirm the relevance of transitioning to soil-conservation tillage technologies.

The stabilization of physical parameters coincided with yield and energy efficiency levels under surface tillage becoming comparable to those under ploughing after the fifth year of observation. Our observation that the median bulk density under systematic surface tillage was 0.03 g cm^{-3} higher than in ploughed soil, yet accompanied by a significantly reduced coefficient of variation, resonates with trends reported in other long-term trials. For example, Alam et al. (2014) found that zero and minimum tillage systems tended to stabilize bulk density with a smaller range of variation, while maintaining or improving porosity and yield over time.

In our study, the optimal 1:1 ratio of water-filled to air-filled pores under surface tillage aligns with the direction of structural shifts reported by Li et al. (2019), who documented that conservation tillage improved pore distribution and water retention while balancing aeration conditions.

The measurable trends only emerged after several years of surface tillage, illustrating a transitional soil response. This pattern is consistent with other findings that soil structural recovery and pore reorganization under reduced tillage require a temporal lag. Jensen et al. (2020) note that conversion from tillage to no-till often initially increases surface bulk density until soil reconfiguration stabilizes.

Moreover, meta-analytical evidence (Nunes et al., 2020) shows that while reductions in tillage intensity may not always lead to immediate gains in soil physical indicators, over a moderate timeframe ($\geq$ 5 years) improvements in aggregate stability and porosity become discernible, reinforcing the general applicability of our 5–6 year findings.

Thus, our results suggest that the convergence toward stabilized bulk density plus a balanced water-air pore volume may function as a practical readiness threshold for degraded chernozems before further tillage minimization. This criterion is strengthened by the fact that yields and energy efficiency under surface tillage became comparable to those under ploughing over time. In this context, the literature supports the viability of such a threshold: authors often caution that until structural equilibrium is achieved, premature adoption of reduced tillage can provoke yield instability or structural setbacks (e.g., poor aeration in topsoil under long-term no-till, as noted by ten Damme et al., 2025).

In sum, our study demonstrates that in strongly degraded podzolized chernozems, a multi-year observation window is critical to identify when the soil's internal structure has shifted sufficiently to enable stable minimal tillage. The criterion we propose – stabilized bulk density combined with optimal differential porosity ratio can guide the timing of agronomic transitions from shallow to even zero tillage, minimizing risks of structural or yield failures.

CONCLUSIONS

Under shallow tillage, the median bulk density of the 0–30 cm layer of chernozem was higher by 0.03 g cm^{-3}, while the standardized range of density remained the same regardless of tillage method. However, under shallow tillage, both the upper and lower typical values of density increased by 0.02 g cm^{-3}. The coefficient of variation of bulk density under ploughing was 1.85 times higher compared to shallow tillage.

Differentiated porosity calculations for different tillage methods under five crops, conducted in the sixth year of the experiment, revealed that in the spring period, the bulk density under systematic shallow tillage was 0.06 g cm^{-3} higher than under ploughing. Total porosity was 3% higher, while the volume of air-filled pores was 6.0% lower. At the same time, the ratio of water-filled to air-filled pores reached an optimal level (1:1), whereas under ploughing, the ratio favored air-filled pores.

The influence of tillage method on the formation of differentiated porosity in the 0–30 cm soil layer demonstrated certain features. Under systematic ploughing, for every unit increase in total porosity, there was a reduction in the volume of both capillary water-filled pores and air-filled pores at a ratio of 1.7:1. In contrast, under shallow tillage, this ratio was 0.25:1, indicating the stabilization of capillary water-filled pore

volume while maintaining a sufficient amount of air-filled pore volume. This provides grounds to argue for the possibility of further tillage minimization.

Systematic application (over five years) of both ploughing and conservation shallow tillage in a five-field grain-row crop rotation ensured comparable productivity in terms of yield, energy efficiency, and output of grain and fodder units. While productivity indicators under ploughing remained stable relative to the average, under conservation tillage they showed a better trend, which can be attributed to the completion of the transition period following the cessation of ploughing and the prior implementation of deep chiseling, followed by the adoption of systematic shallow tillage at a depth of 10–12 cm.

REFERENCES

Adamchuk, V., Bulgakov, V., Ivanovs, S., Holovach, I. & Ihnatiev, Y. 2021. Theoretical study of pneumatic separation of grain mixtures in vortex flow. *Engineering for Rural Development* **20**, 657–664.

Alam, K., Islam, M., Salahin, N. & Hasanuzzaman, M. 2014. Effect of Tillage Practices on Soil Properties andCrop Productivity in Wheat-Mungbean-Rice Cropping System under Subtropical Climatic Conditions. *Scientific World Journal* **2014**, 437283. doi: 10.1155/2014/437283

Baliuk, S., Vorotyntseva, L., Zakharova, M., Panarin, R., Kuts, O. & Mykhailyn, V. 2023. Changes in the properties of Chernozem soils under management and strategic approaches to restore their fertility. *International Journal of Environmental Studies* **81**, 1–8. doi: 10.1080/00207233.2023.2271339

Bardgett, R.D., Mommer, L. & De ries, F.T. 2014. Going underground: root traits as drivers of ecosystem processes. *Trends Ecol. Evol.* **29**, 692–699. doi: 10.1016/j.tree.2014.10.006

Bulgakov, V., Bonchik, V., Holovach, I., Fedosiy, I., Volskiy, V., Ihnatiev, Y. & Olt, J. 2021. Justification of parameters for novel rotary potato harvesting machine. *Agronomy Research* **19**(S2), 984–1007.

Bulgakov, V., Gadzalo, I., Chernovol, M., Demydenko, O., Holovach, I., Ihnatiev, Ye., Trokhaniak, O., Mitkov, V. & Olt, J. 2024a. Structural-aggregate condition and utilization of productive water reserve depending on the tillage method of podzolized chernozem in agrocenosis, *Agronomy Research* **22**(2), 750–773. doi: 10.15159/AR.24.083

Bulgakov, V., Gadzalo, I., Kropivnyi, V., Demydenko, O., Holovach, I., Ihnatiev, Ye., Trokhaniak, O. & Olt, J. 2024b. Assessment of the structural-aggregate composition of podzolized chernozem under various agrogenic impacts and post-agrogenic state. *Agronomy Research* **22**(3), 1111–1127. doi: 10.15159/AR.24.078

Bulgakov, V., Pascuzzi, S., Ivanovs, S., Santoro, F., Anifantis, A.S. & Ihnatiev, I. 2020a. Performance assessment of front-mounted beet topper machine for biomass harvesting. *Energies* **13**(14), 3524.

Bulgakov, V., Sevostianov, I., Kaletnik, G., Babyn, I., Svanovs, S., Holovach, I. & Ihnatiev, Y. 2020b. Theoretical studies of the vibration process of the dryer for waste of food. *Rural Sustainability Research* **44**(339), 32–45.

Castellini, M., Fornaro, F. & Garofalo, P. 2019. Effects of no-tillage and conventional tillage on physical and hydraulic properties of fine textured soils under winter wheat. *Water* **11**, 484. doi:10.3390/ w11030484

Copec, K., Filipovic, D. & Husnjak, S. 2015. Effects of tillage systems on soil water content and yield in maize and winter wheat production. *Plant Soil Environment* **61**, 213–219. doi: 10.17221/156/2015- PSE

Dam, R.F., Mehdi, B.B., Burgess, M.S.E., Madramootoo, C.A., Mehuys, G.R. & Callum, I.R. 2005. Soil bulk density and crop yield under eleven consecutive years of corn with different tillage and residue practices in a sandy loam soil in central Canada. *Soil and Tillage Research* **84**(1), 2005, 41–53. doi: 10.1016/j.still.2004.08.006

Demydenko, O.V. & Velychko, V.A. 2015. Energy reproduction of soil formation of chernozems in agrocenoses. *Agrokhymiya i Gruntoznavstvo* **7**, 19–27 (in Ukrainian).

Demydenko, O.V. 2013. Reproduction of the fertility of typical chernozems in agrocenoses under soil-conserving farming. *Visnyk ahrarnoi nauky* **11**, 47–51. (in Ukrainian).

Dolia, S. & Shevchenko, M. 2024. Influence of primary tillage on some soil fertility indicators and corn yield. *Ukrainian Black Sea Region Agrarian Science* **28**(2), 33–41. doi: 10.56407/bs.agrarian/2.2024.33

Gordienko, V.P. 2004. Minimization of tillage and problems of its application. *Ahrarnyi visnyk Prychornomoria* **26**, 21–25 (in Ukrainian).

Hirte, J., Leifeld, J., Abiven, S. & Oberholzer, H.-R. 2017. Overestimation of crop root biomass in field experiments due to extraneous organic matter. *Front Plant Sci.* **8**, 284. doi: 10.3389/fpls.2017.00284.

Jordan, A., Zavala, L.M. & Gil, J. 2010. Effects of mulching on soil physical properties and runoff under semi-arid conditions in southern Spain. *Catena* **81**(1), 77–85. doi: 10.1016/j.catena.2010.01.007

Jensen, J.L., Schjønning, P., Watts, C.W., Christensen, B.T. & Munkholm, L.J. 2020. Short-term changes in soil pore size distribution: Impact of land use. *Soil and Tillage Research* **199**, 104597. doi: 10.1016/j.still.2020.104597

Kalinichenko, O.V. 2016. Methodological principles of assessing the energy efficiency of crop production. *Oblik i finansy* **2**(72), 150–155. doi: 10.33987/vsed.1(69).2019.40-47 (in Ukrainian).

Krupenikov, I.A. 2008. *Chernozemy. Origin, perfection, tragedy of degradation, ways of protection and revival*. Pontos, Chisinau, 288 pp. (in Russian).

Lampurlanes, J., Plaza-Bonilla, D., Alvaro-Fuentes, J. & Cantero-Martinez, C. 2016. Long-term analysis of soil water conservation and crop yield under different tillage systems in Mediterranean rainfed conditions. *Field Crops Research* **189**, 59–67. doi: 10.1016/j.fcr.2016.02.010

Li, Y., Li, Z., Cui, S., Jagadamma, S. & Zhang, Q. 2019. Residue retention and minimum tillage improve physical environment of the soil in croplands: A global meta-analysis. *Soil and Tillage Research* **194**, 104292. doi: 10.1016/j.still.2019.06.009

Lykhovyd, P. 2024. Biological activity of soils in Ukraine depending on tillage options: A meta-analysis. *Regulatory Mechanisms in Biosystems* **15**, 119-128. doi: 10.15421/022418

Matyukha, V.L. & Semenov, S.S. 2024. Influence of cultivation methods on the soil aggregate state in the context of weed development in winter wheat plantations. *Agrology* **1**(2), 1–8. doi: 10.32819/202402

Medvedev, V.V. 2013. Latest technologies and tillage tools for preserving the physical properties of soils. *Visnyk ahrarnoi nauky* **8**, 5–9 (in Ukrainian).

Modak, K., Biswas, D.R. & Ghosh, A. 2020. Zero tillage and residue retention impact on soil aggregation and carbon stabilization within aggregates in subtropical India. *Soil and Tillage Research* **202**, 104649. doi: 10.1016/j.still.2020.104649

Mondal, S. & Chakraborty, D. 2022. Global meta-analysis suggests that no-tillage favourably changes soil structure and porosity. *Geoderma* **405**, 115443, doi: 10.1016/j.geoderma.2021.115443

Nandan, R., Singh, V. & Singh, S. 2019. Impact of conservation tillage in rice-based cropping systems on soil aggregation, carbon pools and nutrients. *Geoderma* **340**, 104–114. doi: 10.1016/j.geoderma.2019.01.001.

Nunes, M.R., Karlen, D.L. & Moorman, T.B. 2020. Tillage Intensity Effects on Soil Structure Indicators – A US Meta-Analysis. *Sustainability* **12**(5), 2071. doi: 10.3390/su12052071

Philippot, L., Raaijmakers, J.M., Lemanceau, P. & Van der Putten, W.H. 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nat. Rev. Microbiol*. **11**, 789–799. doi: 10.1038/nrmicro3109

Polupan, M.I., Solovei, V.B. & Velichko, V.A. 2005. Classification of soils of Ukraine. Kyiv: *Ahrarna nauka*, 1–300 (in Ukrainian).

Samofalova, I.A., Kamenskih, N.Y. & Alikina, A.N. 2013. Ettect of the Main Treatment Methods on the Qualitative Composition of Humus SodPodzolic Soils in the Perm Region. *Soil-Water Journal* **2**(1), 951–958.

Sayko, V.F. & Maliyenko, A.M. 2007. *Tillage systems in Ukraine*. Publishing House 'EKMO', Kyiv, 44 pp. (in Ukrainian).

Shevchenko, S., Derevenets-Shevchenko, K., Desyatnyk, L., Shevchenko, M., Sologub, I. & Shevchenko, O. 2024. Tillage effects on soil physical properties and maize phenology. *International Journal of Environmental Studies*. doi: 10.1080/00207233.2024.2320032

Shykula, M.K. 2001. Self-regulation of the fertility of chernozem under conditions of soil protection tillage. *Visnyk ahrarnoi nauky* **6**, 5–11 (in Ukrainian).

State Standard of Ukraine DSTU 4115:2002. 2003. Soils. Determination of Mobile Phosphorus and Potassium Compounds by the Modified Chirikov's Method. Introduced on January 1, 2003. Kyiv. 10 p. (in Ukrainian).

State Standard of Ukraine DSTU 4405:2005. 2006. Soil quality. Determination of mobile forms of phosphorus and potassium by the Kirsanov method modified by NSC ISSAR. Introduced on June 1, 2006. Kyiv. 18 p. (in Ukrainian).

ten Damme, L., Goberna, M., Sánchez-Moreno, S., Pulido-Moncada, M., Philippot, L., Ros, M., Bragazza, L., Hallin, S., Feiziene, D. & Munkholm, L. 2025. Long-Term Application of No-Tillage-Induced Greater Risk of Poor Topsoil Aeration Along a European Pedoclimatic Gradient. *European Journal of Soil Science* **76**. doi: 10.1111/ejss.70081

Tomnytskyi, A., Hranovska, L., Lykhovyd, P., Reznichenko, N. & Kozyriev, V. 2024. Tillage influence on agrophysical soil properties and crop productivity in the irrigated conditions of the steppe zone of Ukraine. *Scientific Horizons* **27**(10), 70–78. doi: 10.48077/scihor10.2024.70

Tonkha, O.L., Balayev, A.D. & Vitvitsky, S.V. 2017. *Biological activity and humus status of chernozems of the Forest-Steppe and Steppe of Ukraine*. Vidavnitstvo, Kyiv, 355 pp. (in Ukrainian).

Torma, S., Vilček, J., Lošak, T., Kužel, S. & Martensson, A. 2017. Residual plant nutrients in crop residues – an important resource. *Acta Agriculturae Scandinavica*, 358–366. doi: 10.1080/09064710.2017.1406134

Vilde, A., Lapins, D., Dinaburga, G., Cesnieks, S. & Valainis, O. 2012. Investigations in precise agriculture: Results, problems, perspective development. *Engineering for Rural Development* **11**, 74–80.

Vozhegova, R.A., Malyarchuk, A.S., Reznichenko, N.D. & Kotelnikov, D.I. 2021. Effect of different basic tillage and fertilizer systems on corn grain yield, when irrigating in the south of Ukraine. *Land Reclamation and Water Management* **1**, 1–9. doi: 10.31073/mivg202101-285

Yermakov, S., Hutsol, T., Glowacki, S., Hulevskyi, V. & Pylypenko, V. 2021. Primary assessment of the degree of torrefaction of biomass agricultural crops. *Vide. Tehnologija. Resursi – Environment, Technology, Resources* **1**, pp. 264–267.

Zhang, S., Chen, X., Jia S. & Liang, A. 2015. The potential mechanism of longterm conservation tillage effects on maize yield in the black soil of Northeast China. *Soil and Tillage Research* **154**, 84–90. doi: 10.1016/j.still.2015.06.002

WRB. 2022. *World Reference Base for Soil Resources. International soil classification system for naming soils and creating legends for soil maps. 4th edition*. Vienna, Austria, International Union of Soil Sciences (IUSS), 1–236.

Variable responses of *Ficus carica* genotypes to water deficit: antioxidant and membrane stability insights

M. del R. Jacobo-Salcedo[1], L.Á. González-Espíndola[2], I. Sánchez Cohen[1], R. Valdez-Cepeda[3], R. Trejo-Calzada[2,*], E. Nava-Reyna[1] and J.G. Arreola-Ávila[2]

[1]Centro Nacional de Investigación Disciplinaria en Relaciones Agua Suelo Planta Atmósfera del Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, km 6.5 Canal Sacramento Zona Industrial, C.P. 35140 Gómez Palacio, Durango, México
[2]Universidad Autónoma Chapingo, Unidad Regional de Zonas Áridas, Carretera Gómez Palacio-Cd. Juárez, km 40. C.P. 35230, Bermejillo, Durango, México
[3]Universidad Autónoma Chapingo, Centro Regional Universitario Centro-Norte, Apdo. Postal 196, C.P. 98001, Zacatecas, México
*Correspondence: rtrejo@chapingo.uruza.edu.mx

Abstract. Drought stress is a significant challenge to agricultural productivity, particularly for fig plants, which require robust adaptive mechanisms against water-limited environments. This study aims to assess the biochemical responses of six different fig genotypes to varying soil moisture conditions. The measures of electric conductivity (EC), antioxidant activity (AA), total flavonoid (TFC), total phenolic (TPC), tannins (TT), and total protein (TP) were used as markers of stress tolerance in water deficit (WD) and rehydration conditions. Results showed genotype-specific differences in AA and secondary metabolite production. TFC was associated with enhanced oxidative stress tolerance, particularly under WD conditions, as the Arista genotype showed a 40% increase at 7 days after irrigation suspension. TPC levels indicated a general increase in response to WD, with the Arista genotype exhibiting the most pronounced rise. Conversely, TT decreased by nearly 50% in the Ceballos genotype under field capacity (FC) conditions, likely due to dilution effects from increased growth rates. In addition, TP varied significantly among genotypes, with the San Antonio genotype showing a 25% increase under WD. These findings provide insights into physiological mechanisms underpinning fig plant adaptation to water stress, highlighting the potential of specific genotypes for cultivation in arid and semi-arid regions, offering a framework for selecting young drought-resistant fig varieties.

Key words: DPPH, flavonoids, phenols, tannins, native genotypes.

INTRODUCTION

Ficus carica (fig) production holds significant potential as an alternative crop in water-deficit environments due to its promising adaptability to arid and semi-arid conditions (Ammar et al., 2022). This adaptability is particularly significant for

agricultural production because the main factor affecting productivity in these regions is water deficiency (FAO, 2019). While some studies have examined fig productivity, it is influenced by various factors, including ecophysiological, genetic, and biochemical responses (Gholami et al., 2012). Understanding how figs respond to drought periods, insufficient rainfall, shorter irrigation periods, high temperatures, and low ambient humidity is essential for optimizing water use strategies in fig cultivation. However, evaluating fig tree productivity requires long-term observations. Şahin et al. (2001) reported that fig trees achieve optimal yields after seven years. *Ficus carica* has a long evolution; it has developed extensive genetic diversity, resulting in some varieties exhibiting drought tolerance, whereas others do not. Consequently, the identification of early drought-tolerant genotypes is crucial for introducing resilient fig materials to water-deficient areas (Darwish et al., 2015).

Additionally, early-stage screening programs are necessary to select drought-resistant cultivars efficiently (Hssaini et al., 2020). These selection programs should incorporate physiological indicators such as photosynthesis, vegetative growth patterns, and antioxidant activity to facilitate effective selection and acceptable prediction of drought tolerance (Gholami et al., 2012). By studying the response of young fig plants, we can identify potential genotypes to face changes in climatic patterns, mainly water stress conditions.

In this context, we hypothesize that native *Ficus carica* genotypes possess efficient antioxidant mechanisms to tolerate drought conditions in comparison with a commercial variety. The purpose of the research was to evaluate the drought tolerance of native *Ficus carica* genotypes compared to the Black Mission commercial variety by analyzing their eco-physiological and biochemical responses under water deficit and irrigation recovery conditions. This study aims to identify genotypes with efficient antioxidant mechanisms and adaptive traits in early growing stages for cultivation in arid and semi-arid regions.

MATERIAL AND METHODS

Study site and plant material: This study was conducted at the 'Universidad Autonoma Chapingo, Unidad Regional Universitaria de Zonas Aridas', which is in Bermejillo, Durango, Mexico (103.601944° LN and -25.895277° LO, at an altitude of 1,119 m above sea level) (INEGI, 2015). This region has a desert climate with summer and winter rains, an average annual precipitation of 258 mm, and an annual average evaporation of 2,000 mm (Medina et al., 2005).

To identify native plant materials, backyard or wild fig trees were collected during field visits to various zones of the Comarca Lagunera of Durango and Coahuila, Mexico. These fig trees were selected based on specific adaptation characteristics, including low water availability, good-sized figs (over 4 cm in diameter and 5 cm in length), and resistance to environmental conditions. Five accessions were identified, and layers were obtained from those materials. These were named considering the collection site as Arista (282.864794° N; 65.907639° E, backyard), Ceballos (293.610636° N; 58.931169° E, backyard), Fortuna (293.431864° N; 59.011461° E, wild), Guadalupe Victoria (282.266789° N; 64.968342° E, backyard), and San Antonio (291.565239°N; 56.083531° E, wild). In parallel, plants of the Black Mission variety (commercial cultivar) were included in the experiment, which were obtained from the state of Morelos, Mexico.

Experimental Design: The experimental design was a randomized block design in a split-plot arrangement with three replications (blocks). The main plot factor was water regime, consisting of two levels: water deficit (WD) and field capacity (FC). The sub-plot factor was plant genotypes, with six fig material: Arista, Black Mission, Ceballos, Fortuna, Guadalupe Victoria, and San Antonio. Within each block, water treatments were randomly assigned to main plots, and genotypes were randomly assigned to subplots with each water regime. The experimental unit consisted of four six-month-old fig plants grown in a 10 kg capacity pot containing 9.5 kg of soil. Plants were grown under a green shade mesh that reduced light by approximately 40%, ensuring uniform irradiance. Soil used in all pots was characterized by the following properties: sand 38%, clay 32%, and silt 30%, pH of 8.8, electric conductivity of 3.61 dS m^{-1}, organic matter content of 2.68%, bulk density of 1.41 g cm^{-3}, and nitrogen 24.15 mg kg^{-1}. Field capacity (FC) of 33%, and permanent wilting point (PWP) of 20%, FC and PWP were determined based on the pressure plate Apparatus, reported in gravimetric percentage. In addition, the gravimetric percentage of FC and the PWP were transformed according to the soil texture using the software SPAW Version 6.02, from the Natural Resources Conservation Service. To assess evaporation independently of plant transpiration, three additional pots containing bare soil were included and evaluated using the same weighing protocol and supported by an evaporimeter installed in the experimental area.

The young fig trees were subjected to a three-month adaptation period, during which irrigation was adjusted based on their water consumption (pots were measured daily, and water used is registered in rewater as necessary). The control plants (CC) were kept at field capacity (FC), while the plants under water deficit (WD) were subjected to irrigation restriction. This stage was delimited until the loss of turgor, depigmentation of the leaves, and cyanotic apices and petioles pigments (signs of stress) were detected. The period of days after irrigation suppression (DAIS) was delimited from 1 to the 7th day; on the 7th day, plants were rehydrated, and from the 8th to the 15th day, the plants were returned and preserved at field capacity moisture (DFCM) as shown in Fig. 1.
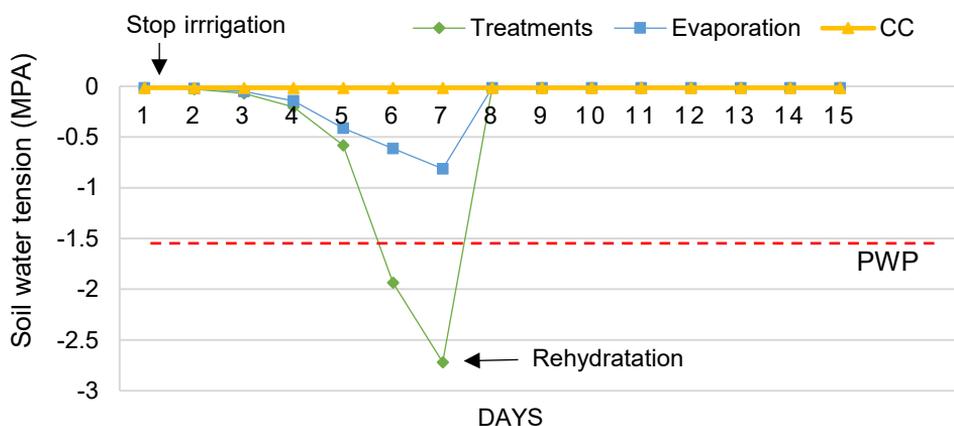
Figure 1. Changes in soil tension (MPa) during the experimental condition in treatments, control plants, and evaporation (water loss in pots without plant water consumption).

The mean soil water tension (SWT) in fig material pots was -2.7 MPa on the seventh day after irrigation suspension (Fig. 1). Notably, in the evaporation pots (without plants), the soil SWT before rehydration was -0.8 MPa. In contrast, control plants (CC) were maintained at field capacity with daily rehydration to preserve soil moisture (0 MPa). The permanent wilting point (PWP, -1.6 MPa) was reached between days 5 and 6 of the irrigation suspension period, even though the plant material did not show physical signs of stress.

Samples from the fig materials were collected for chemical analysis from day one after irrigation suppression until a water deficit was generated. Subsequently, samples were taken on the seventh DAIS, followed by sampling on days one, four, and eight of DFCM. Recently, mature leaves were sampled between 10:00 and 11:00 h on sunny and clear days. The samples were frozen using liquid nitrogen to preserve them before lab measurements were taken. The variables measured were electrical conductivity (EC) in fresh tissue, antioxidant activity (AA, DPPH), total flavonoids (TFL), total phenols (TFE), total tannins (TT), and total proteins (TP).

Electrical conductivity (EC): The changes in EC in the leaf tissue's resuspension medium indirectly quantified the integrity of the membranes. For this purpose, a sample of 4 cm^2 of fresh leaf was taken, weighed (fresh weight), and kept submerged in distilled water for 24 hours. Subsequently, the EC was measured with a conductivity meter (Hanna Instruments) in the tissue resuspension solution (Sánchez-Urdaneta et al., 2003).

Antioxidant activity (AA): The AA was determined by the DPPH method, which is based on the determination of the concentration of 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma Aldrich Co., UK). The plant extract (200 µg of sample in 10 mL of acetone-water 7:3) was shaken for 30 min and centrifuged at 5,000 rpm at 4 °C for 15 min. The reaction mixture was incubated in the dark for 60 min and evaluated by spectrometry at 515 nm. Antioxidant activity (AA) was expressed in µM Trolox (6-hydroxy-2,5,7,8-tetramethylchromium-2-carboxylic acid 97%, Sigma Aldrich, Co., UK) equivalent (TE) of fresh matter (TE µM g^{-1}MF) (Parejo et al., 2000).

Secondary metabolites with antioxidant activity: The extract was used to determine total phenols (TPF), total flavonoids (TFL), and total tannins (TT). The plant extract was prepared by mixing 100 mg of the sample in 1 ml of methanol, which was kept under agitation in ice for 24 h before use.

The total phenol content (TPF) was determined using the Folin-Ciocalteu method with modifications. For the reaction mixture, 50 µl of the plant extract, 3 mL of distilled H_2O, and 250 µl of Folin-Ciocalteau (FC) reagent were used. The mixture was allowed to react for 5 min, then 750 µl of 20% Na_2CO_3 was added to the mixture, and it was homogenized in a vortex for 2 min. Once the mixture was homogenized, it was incubated for 30 min at room temperature. Quantification was performed by spectrometry at 760 nm, using gallic acid as a standard at a concentration of 10 mg ml^{-1} in a methanol solution. The TPF was expressed as mg of gallic acid (GA) eq g^{-1} of FW (Lamuela-Raventós, 2018).

The total flavonoids determination was carried out based on the aluminum chloride method (Barnum, 1977), which consisted of preparing the reaction mixture with 100 µl of extract and 1.4 mL of distilled H_2O. Flavonoid reagent (500 µl) was added to the mixture. The mixture was thoroughly shaken and then left undisturbed for 30 min at room temperature in a dark environment. The samples were read at 415 nm in a

spectrometer. The TFL was determined using a calibration curve with Quercetin (QE, Sigma Aldrich, Co. UK.) at a concentration of 1 mg mL^{-1}. The TFL was expressed as eq QE mg 100 g^{-1} FW.

The total tannins TT method (Harborne, 1984) was carried out using 100 μl of the plant extract, 5 mL of distilled H$_2$O, 500 μl of FC, and 1 ml of 35% Na$_2$CO$_3$. The reaction mixture was homogenized at room temperature and left undisturbed for 30 min. After this time, the samples were read at 724 nm in a spectrometer. For quantification in the calibration curve, gallic acid (GA) was used at a concentration of 1 mg mL^{-1}. The TT is expressed as mg GA eq mg^{-1} FW.

The preparation of the plant extract for the quantification of proteins (TP) by the Bradford method (Bradford, 1976) was carried out using 0.5 g of leaf tissue in 5 mL of 100 mm potassium phosphate buffer, pH 7.5. The extract was incubated on ice in a horizontal shaker for one hour and centrifuged for 30 min at 6,000 rpm. For Pro quantification, 200 μl of the protein extract was added with 800 μl of Bradford reagent. The determination was performed in a spectrometer with readings at 590 nm and 450 nm using bovine serum albumin (BSA) at 1.45 mg ml^{-1} as the calibration curve. Pro was expressed as mg of extracted protein g^{-1} FW.

Statistical Analysis: The statistical analyses developed were one-way ANOVA tests and factorial analysis. Statistical differences were determined with a significance of $p \leq 0.05$, according to the Tukey multiple range test. The data were processed in the PASW statistical program version 18.0.0, Chicago, SPSS Inc.

RESULTS AND DISCUSSION

During the experimental period, the maximum observed temperature was 45 °C, and the minimum temperature was 21 °C. These temperatures indicated intense heat and high evaporation rates. The dynamics of the maximum and minimum temperatures and humidity are shown in Fig. 2.
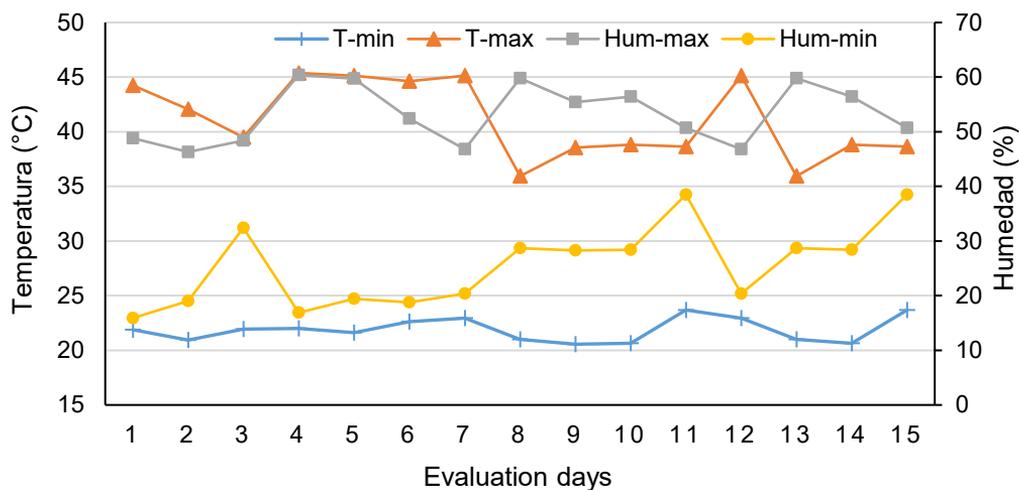
Figure 2. Dynamics of mean minimum and maximum temperature (°C) and mean minimum and maximum relative humidity (%) during the evaluation period.

Table 1 summarizes the evaluated factors (Time (T), Genotypes (G), and Soil Moisture (SM) conditions, and their interactions. The single effects showed significant differences in most of the evaluated variables, leading to interaction responses. Notably, the time and the interaction of T×G were significant for all the evaluated variables. Moreover, all the factors and interactions had important effects on antioxidant activity (AA).

Table 1. Square mean and effects of genotypes (G), time (T), and soil moisture (SM), and their interactions on electric conductivity (EC), and antioxidant parameters (AA, TFC, TPC, TT, and TP) of fig leaves

	Electrical conductivity (EC)	Antioxidant Activity (AA)	Total flavonoids (TFL)	Total phenols (TPF)	Total tannins (TT)	Total protein (TP)
Time (T)	30,633.38***	2,093.58***	42.09***	325.09***	157.36***	1.727***
Genotype (G)	2,923.73^{ns}	2,697.32***	11.63***	189.93**	77.83**	0.044^{ns}
Soil Moisture (SM)	34,311.47**	3,830.80***	15.49***	1,076.56**	445.97***	0.051^{ns}
T×G	5,187.84*	1,005.32***	2.33**	99.76**	66.21***	0.086***
T×SM	12,971.06**	1,719.03***	2.18^{ns}	79.25^{ns}	35.07^{ns}	0.034^{ns}
G×SM	2,279.37^{ns}	298.88**	3.23**	96.40^{ns}	46.18^{ns}	0.097**
T×G×SM	5,169.33^{ns}	504.85***	1.98**	89.63^{ns}	37.20^{ns}	0.051*

Note: Asterisks indicate the level of statistical significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns = not significant.

The electric conductivity (EC) of the leaf tissue incubation solution was evaluated to assess the integrity of cellular membranes in maintaining water and mineral balance. Under normal conditions, apoplast water contains few ions, while symplast water contains a wide variety of ions (Scoffoni et al., 2023). Water deficit disrupts balance, damaging membranes through reactive oxygen species (ROS), leading to ion leakage (Kamanga et al., 2018). The EC evaluation showed significant differences across factors, including T, SM, T×G, and T×SM. This method effectively assessed physiological stress in fig plant tissues under water deficit conditions. The integrity of cellular membranes is essential for maintaining ion gradients and overall cellular function (Abbas et al., 2023). Damage to these membranes causes the leakage of ions, which increases the EC of the surrounding solution (Shcherbakov et al., 2021).

Table 2 presents the dynamics of electric conductivity during the evaluation period for each genotype under WD and FC conditions. The statistical analysis was conducted at each evaluation time among genotypes (horizontal, lowercase), considering the water condition. At the initial condition, the genotype Ceballos showed statistical differences in both water conditions. Although the differences found on the 7th day after irrigation suppression (highest stress stage) were significant, the integrity of the membranes was affected in all genotypes. The electric conductivity showed a significant increase of around 50% compared to the EC of the plants in FC of soil moisture. In optimally irrigated conditions, ions and solutes are diluted, resulting in lower conductivity. During a water deficit, reduced water volumes concentrated ions, increasing EC, an indicator of drought severity (Wu et al., 2022).

Table 2. Interaction T * G mean values of electrical conductivity (EC) in the resuspension of leaf tissue in fig trees subjected to water deficit (WD) and field capacity (FC) during two periods, one of humidity restriction and the second of rehydration

| Genotype | SWC | Electrical Conductivity (EC) µs cm^{-1} | | | | |
| | | DAIS | | DFCM | | |
		1	7	8	11	15
Arista	WD	20.65 [a-d]	39.48 [a]	30.93 [a]	24.58 [ab]	17.65 [b-e]
	FC	23.07 [ab]	24.15 [cde]	25.02 [ab]	30.12 [a]	14.49 [de]
Black Mission	WD	20.16 [a-d]	28.81 [bcd]	26.09 [ab]	20.99 [ab]	15.57 [de]
	FC	21.94 [abc]	20.98 [de]	17.97 [b]	22.98 [ab]	22.62 [a-d]
Ceballos	WD	21.81 [abc]	29.07 [bcd]	26.70 [ab]	16.56 [b]	27.32 [a]
	FC	13.66 [d]	20.29 [de]	18.27 [b]	23.50 [ab]	12.85 [e]
Fortuna	WD	14.39 [cd]	30.93 [abc]	30.00 [ab]	29.48 [ab]	18.71 [b-e]
	FC	16.69 [bcd]	23.47 [cde]	22.29 [ab]	27.12 [ab]	20.78 [a-e]
Guadalupe	WD	21.74 [abc]	37.46 [ab]	28.01 [ab]	23.92 [ab]	17.02 [cde]
Victoria	FC	25.98 [a]	17.91 [e]	22.53 [ab]	25.98 [ab]	25.48 [ab]
San Antonio	WD	20.60 [a-d]	34.19 [ab]	29.72 [ab]	20.35 [ab]	14.92 [de]
	FC	23.58 [ab]	22.06 [cde]	22.63 [ab]	20.36 [ab]	24.44 [abc]

Note: Values are presented as mean EC with standard error, and different letters indicate significant differences between values within each genotype and SWC treatment ($p < 0.05$).

Following rehydration, EC measurements at 24 hours during the days at field capacity moisture (DFCM) stage revealed significant recovery from pick stress conditions. The changes in electric conductivity at 15 days at field capacity moisture showed significant statistical differences between SWC in Ceballos, Guadalupe Victoria, and San Antonio genotypes. Interestingly, a higher EC value was observed for Guadalupe Victoria and San Antonio under FC conditions, possibly associated with waterlogging stress (Wu et al., 2022).

Figure 3. Interaction T and SM mean results of electrical conductivity (EC) in the leaf tissue in fig trees subjected to water deficit (WD) and field capacity (FC) during two periods, one of humidity restriction (DAIS) and the second of rehydration (DFCM).

The interaction between time (T) and soil moisture (SM) showed significant differences at 7 days after irrigation suspension (DAIS) (Fig. 3). Followed by a complete

recovery at 4 days following rehydration to field capacity moisture. By 11 days at field capacity moisture, the EC values were normalized to those of plants under optimal humidity, indicating favorable conditions for selecting materials' tolerance to water deficit. Interestingly, at 11 and 15 days at field capacity moisture, plants in field capacity exhibited higher EC values than those under water deficit. This suggests that plants demonstrated significant osmotic adjustment plasticity, adapting to either water scarcity or abundance. Measurements taken during the experiment revealed that plants experiencing water stress exhibited higher ion concentrations in the solution, leading to increased EC values. Likely, the damage to membrane permeability caused by water stress was not permanent, as recovery was observed in the measurements after 8 days of rehydration.

The assessment of free radical scavenging activity, measured as antioxidant activity (AA), revealed significant differences across factors and their interactions (Table 1). On average, fig genotypes exhibited a 60% increase in AA under water stress as compared to their initial condition. The Black Mission genotype showed the smallest increase in AA. Additionally, the plant's response to oxidative damage was associated with the production of flavonoids (Table 4). Previous research has shown that abiotic stress can enhance the production of secondary metabolites, like antioxidants, which contribute to cellular structural rigidity (Li et al., 2020).

Table 3. Interaction T * G in the Antioxidant Activity (AA) by the DPPH method in leaf tissue of fig trees subjected to water deficit (WD) and field capacity (FC) during two periods, one of humidity restriction and the second of rehydration

Genotype	SWC	AA, DPPH (TE µM g^{-1}FW)				
		DAIS		DFCM		
		1	7	8	11	15
Arista	WD	57.06 [a]	91.68 [a]	110.35 [ab]	82.91 [c]	91.18 [a]
	FC	57.41 [a]	59.00 [bc]	54.70 [def]	92.91 [bc]	80.15 [ab]
Black Mission	WD	39.78 [a]	55.39 [bc]	132.07 [a]	103.56 [b]	80.56 [ab]
	FC	44.25 [a]	45.32 [cd]	58.80 [c-f]	151.64 [a]	85.46 [bc]
Ceballos	WD	56.68 [a]	48.89 [cd]	125.44 [a]	96.04 [bc]	75.81 [b]
	FC	59.84 [a]	33.69 [d]	72.12 [cde]	107.12 [b]	75.19 [b]
Fortuna	WD	53.45 [a]	93.15 [a]	88.64 [bc]	148.92 [a]	83.89 [ab]
	FC	52.97 [a]	37.93 [d]	46.05 [ef]	56.87 [d]	74.68 [b]
Guadalupe Victoria	WD	40.73 [a]	68.29 [b]	88.64 [bc]	37.12 [de]	34.05 [d]
	FC	43.08 [a]	34.19 [d]	34.55 [f]	48.00 [de]	44.63 [cd]
San Antonio	WD	59.84 [a]	100.53 [a]	82.84 [bcd]	33.41 [e]	35.72 [d]
	FC	60.08 [a]	36.29 [d]	51.94 [def]	36.06 [e]	54.24 [c]

Note: Values are presented as mean AA with standard error, and different letters indicate significant differences between values within each genotype and SWC treatment ($p < 0.05$).

Most genotypes presented an increase in AA from 1 to 7 DAIS in the WD condition, except the Ceballos genotype. The AA increased in different proportions among genotypes, even in the recovery period. Most of the evaluated genotypes had high AA at the final evaluation, except Guadalupe Victoria and San Antonio, which returned to the initial condition. In the Black Mission genotype, the AA increase was detected 24 hours after irrigation was reinstated (Table 3). This suggests that upregulation of AA helps fig plants mitigate the harmful effects of water stress, maintain cellular integrity, and sustain

viability. This response is likely linked to the activation of non-enzymatic antioxidants, including flavonoids, phenolic acids, and other substances (Seleiman et al., 2021).

An intriguing observation was the decrease in AA in the Ceballos genotype at 7 DAIS. This could be attributed to metabolic limitations, as severe water deficit restricts photosynthesis, thereby reducing ATP production and carbon availability due to stomatal closure (Jacobo-Salcedo et al., 2024a & 2024b). As well, the subsequent increase in AA observed at 8 days at field capacity moisture may reflect the plant´s ability to perceive changes in soil and leaf water potential through mechanosensitive proteins and shifts in cell turgor pressure (Osmolovskaya et al., 2018; Sparke & Wünsche, 2020). The gradual activation of AA in response to water stress highlights the enhanced tolerance of these fig plants under challenging conditions.

Reports on AA in fig seed oil range from 17 to 35 mg g^{-1} of oil expressed in Trolox equivalent (Hssaini et al., 2020). These values are lower than those found in the leaf tissue of fig accessions under both soil moisture conditions described in this study, as shown in Fig. 4. When dried figs are considered, reported AA ranges from 387 to 825 mol TE 100g DW^{-1} (Hoxha et al., 2015). Differences may arise due to analytical methods, such as figs being taken as analytical samples in dry matter.
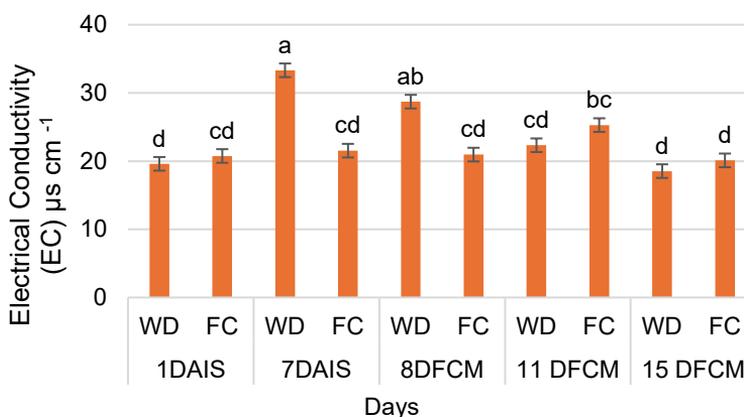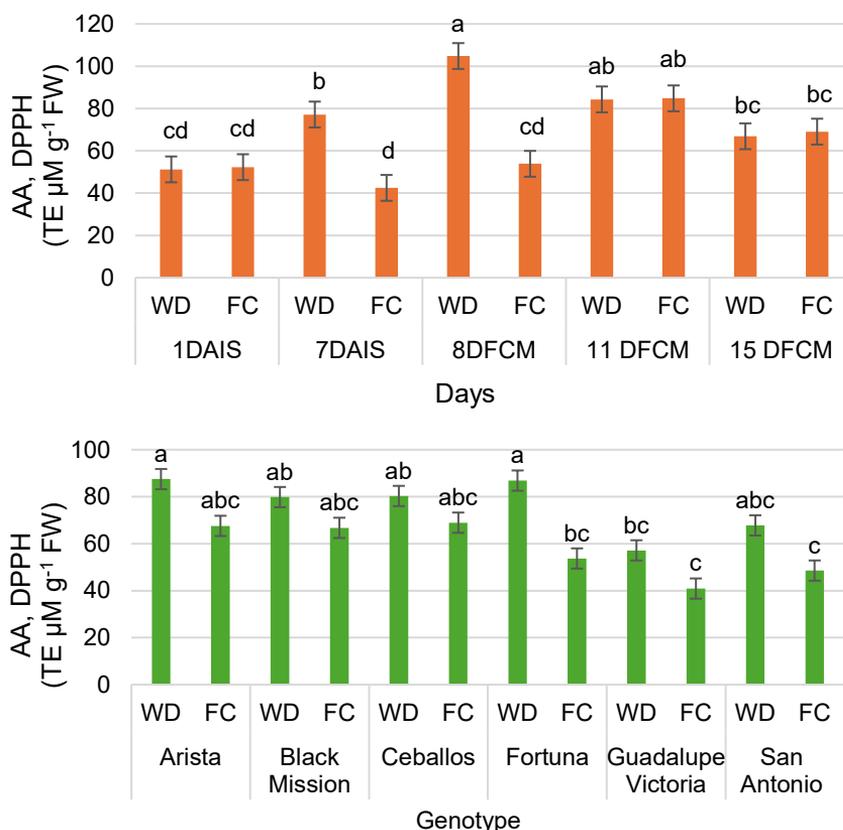

Figure 4. Interaction T*SM and G*SM mean results on antioxidant activity (AA) in leaf tissue in fig trees subjected to water deficit (WD) and field capacity (FC) during two periods, one of humidity restriction (DAIS) and the second of rehydration (DFCM).

The technique employed in this study measures the quantity of free H+ atoms in plant extract that the DPPH radical scavenges. Therefore, the Guadalupe Victoria and San Antonio genotypes exhibited lower AA during the evaluation, in contrast to the fig-evaluated materials. Although these two genotypes presented an increase in AA in both soil moisture conditions, their response was moderate. Our results suggest a relatively low production of free radicals in response to stress. Among the mechanisms by which plants can respond to stress induced by water deficiency, we indicate that these accessions might use other mechanisms. Such characteristics are desirable for these materials in areas with limited water availability.

To better understand the response of the evaluated fig genotype, one of the highlighted antioxidants is total flavonoid content (TFC). TFC acts as an antioxidant by scavenging ROS, thereby protecting the cellular structure. It plays a critical role in plant survival mechanisms by modulating biochemical pathways to adapt to water scarcity, offering valuable insight into the stress tolerance mechanisms of plants. Additionally, TFC accumulation can serve as a biomarker for drought-tolerant plant materials (Shomali et al., 2022).

At 1 DAIS, TFC levels revealed statistical differences among genotypes, which were linked to their origin and the variability among genotypes (Table 4). Plants under field capacity (FC) conditions showed a decrease in TFC from the initial to the final evaluation. A similar performance was observed in Arista and Ceballos genotypes under WD conditions. However, in the Black Mission, Guadalupe Victoria, and San Antonio genotypes, TFC increased significantly under maximum stress conditions at 7 DAIS.

Table 4. Total flavonoid content (TFC) in leaf tissue of fig trees subjected to water deficit (WD) and field capacity (FC) during two periods, one of humidity restriction (DAIS) and the second of rehydration in days at field capacity moisture (DFCM)

Genotype	SWC	Total Flavonoid Content (TFC) (mg EQ g FW^{-1})				
		DAIS		DFCM		
		1	7	8	11	15
Arista	WD	8.61 [abc]	7.54 [abc]	8.52 [a]	5.78 [a-e]	5.19 [cd]
	FC	9.08 [a]	5.14 [d]	6.61 [abc]	6.53 [ab]	6.45 [ab]
Black Mission	WD	5.55 [d]	7.54 [abc]	6.62 [abc]	5.84 [a-d]	3.94 [e]
	FC	7.05 [a-d]	6.12 [bcd]	4.53 [de]	6.21 [abc]	5.76 [abc]
Ceballos	WD	8.27 [abc]	5.52 [d]	4.99 [b-e]	5.42 [b-e]	5.81 [abc]
	FC	7.50 [a-c]	5.50 [d]	6.85 [ab]	4.67 [cde]	5.21 [cd]
Fortuna	WD	7.93 [abc]	7.98 [ab]	6.10 [bcd]	7.41 [a]	6.32 [ab]
	FC	7.02 [a-d]	5.75 [cd]	3.89 [e]	4.17 [de]	4.76 [de]
Guadalupe	WD	6.75 [bcd]	9.04 [a]	4.76 [cde]	4.91 [b-e]	5.23 [cd]
Victoria	FC	8.90 [ab]	5.75 [cd]	4.06 [e]	5.77 [a-e]	5.16 [cd]
San Antonio	WD	7.95 [abc]	9.07 [a]	6.13 [bcd]	6.31 [abc]	6.56 [a]
	FC	6.40 [cd]	5.77 [cd]	5.54 [b-e]	4.02 [e]	5.48 [bcd]

Note: Values are presented as mean EC with standard error, and different letters indicate significant differences between values within each genotype and SWC treatment ($p < 0.05$).

The results observed in Fig. 5 showed a statistical difference in TFC for the Fortuna genotype between WD and FC conditions throughout the evaluation period. This suggests that TFC plays an important role in the plant´s response to adverse environmental conditions.
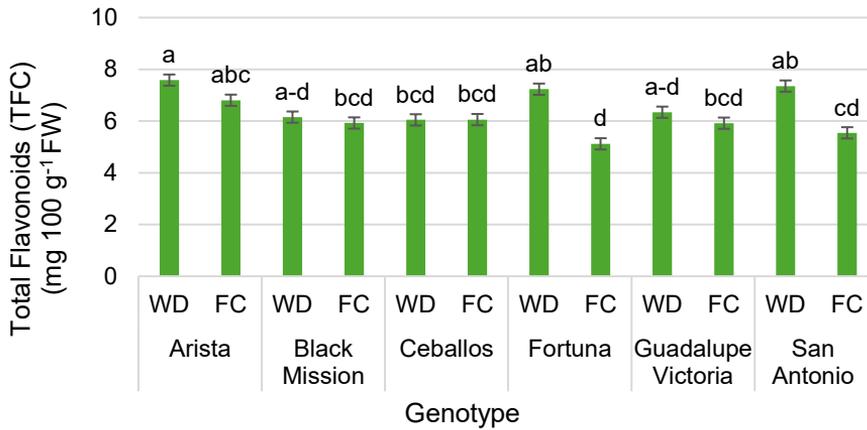
Figure 5. Interaction G*SM mean results of total flavonoid content (TFC) in fig trees subjected to water deficit (WD) and field capacity (FC).

TPC is part of the plant's defense mechanism against oxidative stress caused by drought (Ammar et al., 2015). Under WD conditions, TPC tended to increase in most genotypes (Table 5). TPC responses varied significantly across genotypes and sampling times, indicating genotype-specific reactions to water stress. Measuring TPC provides insight into the plant's capacity to counter oxidative damage caused by stress. Genotypes with higher TPC levels under stress conditions generally exhibit better tolerance to adverse environments. This is the case for the Arista genotype, which presented a significant increase at 7 DAIS from the original condition, even under FC conditions. The performance of TPC in fig genotypes aligns with findings from Stanković (2011) on *Marrubium peregrinum.* Additionally, similar TPC levels have been reported in fig cultivars by Konyalioğlu et al. (2005) and Marrelli et al. (2012).

Table 5. Total Phenolic compounds (TPC) in leaf tissue of fig trees subjected to water deficit (WD) and field capacity (FC) during two periods, one of humidity restriction (DAIS) and the second of rehydration in days at field capacity moisture (DFCM)

| Genotype | SWC | Total Phenolic Compounds (TPC) (mg GA g FW^{-1}) | | | | |
| | | DAIS | | DFCM | | |
		1	7	8	11	15
Arista	WD	2.646 [b]	3.946 [a]	3.352 [a]	2.382 [cd]	3.789 [a]
	FC	2.787 [b]	2.884 [ab]	2.129 [a]	3.681 [abc]	3.749 [a]
Black Mission	WD	2.524 [b]	2.616 [bc]	3.794 [a]	2.526 [cd]	1.731 [d]
	FC	2.702 [b]	2.667 [bc]	2.968 [a]	33.24 [bcd]	3.808 [a]
Ceballos	WD	3.655 [ab]	2.939 [ab]	3.544 [a]	3.423 [bc]	2.007 [cd]
	FC	2.645 [a]	3.039 [ab]	2.323 [a]	2.633 [bcd]	1.847 [d]
Fortuna	WD	4.174 [a]	30.97 [ab]	2.755 [a]	4.984 [a]	1.890 [d]
	FC	2.709 [b]	2.170 [bc]	3.108 [a]	2.056 [d]	2.013 [cd]
Guadalupe Victoria	WD	3.284 [ab]	1.464 [c]	3.520 [a]	3.891 [ab]	2.792 [bc]
	FC	3.195 [ab]	2.952 [ab]	3.322 [a]	2.483 [cd]	1.766 [d]
San Antonio	WD	3.486 [ab]	2.716 [b]	3.355 [a]	3.708 [abc]	3.593 [ab]
	FC	2.984 [ab]	3.101 [ab]	3.108 [a]	2.608 [bcd]	1.758 [d]

Note: Values are presented as mean EC with standard error, and different letters indicate significant differences between values within each genotype and SWC treatment ($p < 0.05$).

Tannins, which are polyphenolic compounds, interact with proteins, cellulose, and lignin to form hydrophobic complexes. These structures reduce water permeability in plant tissues, enhancing water resistance (Yang et al., 2021). In the initial evaluation, tannin content (TT) was consistent across genotypes (Table 6). However, the Ceballos genotype showed differences at 7 days after irrigation suppression, when the TT was almost half at the FC condition. This genotype maintained consistent performance in subsequent evaluations, suggesting an adaptation response of tannin production to environmental conditions.

Interestingly, most genotypes showed decreased TT levels at 7 days after irrigation suppression under the FC condition, contrary to the expected response. This could be linked to a decrease in ambient humidity and an increase in temperature on the day of evaluation and in the previous days (Fig. 2). Moreover, the fig plants at field capacity had adequate water, leading to faster cell expansion and biomass accumulation due to the increased growth rate, which can dilute the concentration of secondary metabolites like tannins (Table 6).

Table 6. Total tannins (TT) in leaf tissue of fig trees subjected to water deficit (WD) and field capacity (FC) during two periods, one of humidity restriction (DAIS) and the second of rehydration in days at field capacity moisture (DFCM)

| Genotype | SWC | Total Tannins (TT) (mg g FW^{-1}) | | | | |
| | | DAIS | | DFCM | | |
		1	7	8	11	15
Arista	WD	2.452 [a]	2.411 [ab]	2.426 [bcd]	2.492 [c]	3.005 [ab]
	FC	2.403 [a]	1.865 [bc]	2.099 [cd]	3.366 [abc]	3.457 [a]
Black Mission	WD	2.337 [a]	2.836 [a]	3.867 [a]	2.437 [c]	1.917 [ab]
	FC	2.109 [a]	2.238 [ab]	3.258 [abc]	3.821 [ab]	3.049 [ab]
Ceballos	WD	2.694 [a]	2.544 [ab]	3.206 [abc]	3.886 [ab]	1.978 [ab]
	FC	2.575 [a]	1.319 [c]	3.316 [abc]	3.595 [abc]	1.696 [b]
Fortuna	WD	3.005 [a]	2.635 [ab]	2.929 [a-d]	4.117 [a]	2.376 [ab]
	FC	2.245 [a]	1.781 [bc]	1.961 [d]	2.573 [c]	1.872 [ab]
Guadalupe	WD	2.585 [a]	1.859 [bc]	2.600 [bcd]	3.036 [abc]	2.493 [ab]
Victoria	FC	2.510 [a]	2.170 [abc]	1.715 [d]	2.733 [ab]	2.153 [ab]
San Antonio	WD	2.978 [a]	2.250 [ab]	3.467 [ab]	3.367 [abc]	3.037 [ab]
	FC	3.085 [a]	2.339 [ab]	2.792 [a-d]	2.954 [ab]	1.937 [ab]

Note: Values are presented as mean EC with standard error, and different letters indicate significant differences between values within each genotype and SWC treatment ($p < 0.05$).

Total proteins, determined by the Bradford method, reflect the soluble protein content in plant extracts. These proteins generally decrease under water deficit conditions because of ROS, which is associated with high toxicity (Ashraf et al., 2018). TP presented statistical differences among genotypes at 1 day after irrigation suppression (Table 7. This difference in initial condition can be related to the capacity of those materials to use soluble proteins as a mechanism to face water deficit conditions, as it is visible in Fortuna and San Antonio accessions. Moreover, the San Antonio genotype at 7 days after irrigation suppression showed statistical differences in soil water content. This increase in TP was observed at WD, and the condition remained high even in the recovery period. This performance could be related to the plant's response by increasing

the synthesis of antioxidant proteins such as superoxide dismutase, catalase, and peroxidase to mitigate oxidative damage (Hasanuzzaman et al., 2013). Also, the production of enzymes involved in the synthesis of compounds related to osmotic adjustment substances, such as proline (Jacobo-Salcedo et al., 2024b).

Table 7. Total Proteins (TP) in leaf tissue of fig trees subjected to water deficit (FD) and field capacity (FC) during two periods, one of humidity restriction and the second of rehydration

Genotype	SWC	Total Proteins (TP) (mg proteína g FW^{-1})				
		DAIS		DFCM		
		1	7	8	11	15
Arista	WD	0.426 [a-d]	0.361 [d]	0.649 [cd]	1.213 [a]	1.576 [a]
	FC	0.508 [a-d]	0.289 [d]	0.625 [cd]	1.107 [abc]	1.026 [bc]
Black Mission	WD	0.739 [a]	0.424 [cd]	0.794 [bcd]	1.008 [abc]	1.255 [ab]
	FC	0.676 [ab]	0.422 [cd]	0.842 [bcd]	1.167 [ab]	0.949 [bcd]
Ceballos	WD	0.569 [abc]	0.398 [cd]	0.757 [bcd]	1.169 [a]	0.954 [bcd]
	FC	0.555 [abc]	0.552 [bcd]	0.677 [cd]	0.900 [abc]	1.097 [bc]
Fortuna	WD	0.290 [cd]	0.808 [b]	1.376 [a]	0.638 [bc]	1.067 [bc]
	FC	0.225 [d]	0.569 [bcd]	0.500 [d]	0.840 [abc]	0.635 [cd]
Guadalupe	WD	0.493 [a-d]	0.688 [bc]	1.100 [ab]	1.045 [abc]	0.991 [bc]
Victoria	FC	0.390 [bcd]	0.821 [b]	0.607 [cd]	0.715 [bc]	0.521 [d]
San Antonio	WD	0.395 [bcd]	1.547 [a]	0.984 [abc]	1.009 [abc]	1.255 [ab]
	FC	0.503 [a-d]	0.771 [b]	0.593 [cd]	0.634 [c]	0.896 [bcd]

Note: Values are presented as mean EC with standard error, and different letters indicate significant differences between values within each genotype and SWC treatment ($p < 0.05$).

During the evaluation, the Fortuna genotype presented statistical differences in soil water content (Fig. 6). As previously mentioned, proline (Pro) plays a substantial role in osmotic regulation. Pro exhibited a strong ability to hydrate, which protected cell structures. Pro formed associations with soluble proteins to create hydrophobic skeletons that stabilize and defend biological macromolecules and cell structures (Yang et al., 2021).
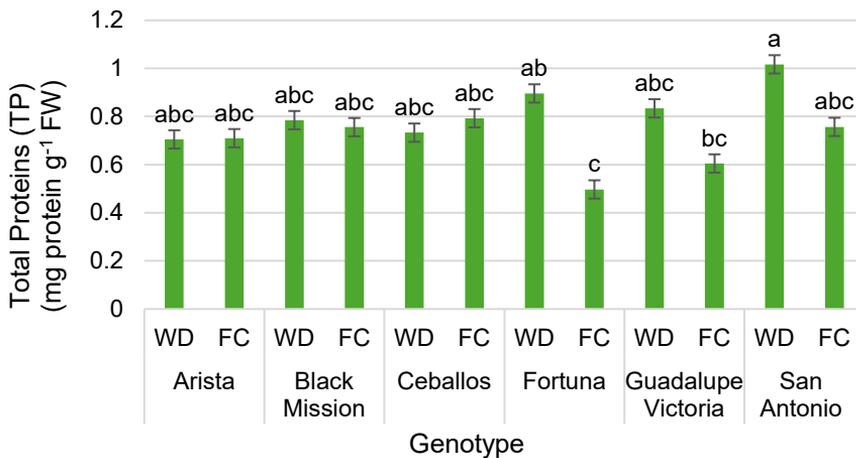
Figure 6. Interaction T*SM and G*SM mean results of total protein (TP) in fig trees subjected to water deficit (WD) and field capacity (FC).

CONCLUSIONS

The PWP reached during 5 to 6 days of the irrigation suspension period presented an absence of physical stress, indicating internal adaptation mechanisms in the evaluated fig genotypes. However, the EC measure revealed an increase in days at the irrigation suspension period evaluation related to membrane damage. This response demonstrates the fig-evaluated genotypes' plasticity. The 60% increase in the AA under drought stress indicates that non-enzymatic antioxidant pathways involving flavonoid and phenolic compounds were activated in young plants. Genotypes demonstrated adaptive processes, including protein synthesis and antioxidant activation, as evidenced by the significant interaction among the examined factors (time, genotype, and soil moisture conditions). Guadalupe Victoria and San Antonio accessions presented an important performance in the evaluation of water stress and water recovery because those present AA response as a critical mechanism for dryland production systems. In most genotypes, TFC and TPC markedly increased under drought conditions, demonstrating their function in scavenging ROS and protecting fig plants. The Arista genotype exhibited the most substantial TPC increase, signifying enhanced drought tolerance. The fig genotypes exhibited unexpected patterns of TT under WD and FC conditions. This response suggests a complex relationship between secondary metabolite production and environmental factors. Notably, the San Antonio genotype maintained elevated protein levels even during the days at field capacity moisture, reflecting a robust stress response mechanism. The evaluations at field capacity moisture indicated a substantial recovery in physiological parameters, considering EC, AA, and SWT. Those variables' responses highlight the potential of Guadalupe Victoria, San Antonio, and Arista genotypes for future breeding programs targeting drought-prone regions. The research highlights the potential of specific *Ficus carica* genotypes for drought tolerance, providing a basis for breeding programs and sustainable agricultural practices in water-limited regions.

REFERENCES

Abbas, K., Li, J., Gong, B., Lu, Y., Wu, X., Lü, G. & Gao, H. 2023. Drought Stress Tolerance in Vegetables: The Functional Role of Structural Features, Key Gene Pathways, and Exogenous Hormones. *International Journal of Molecular Sciences* **24**(18), 1–25. https://doi.org/10.3390/ijms241813876

Ammar, A., Ben, I., Gouiaa, M. & Mars, M. 2022. Fig (*Ficus caric*a L.) vulnerability to climate change : Combined effects of water stress and high temperature on ecophysiological behavior of different cultivars. *South African Journal of Botany* **147**, 482–492. https://doi.org/10.1016/j.sajb.2022.02.014

Ammar, S., Del Mar Contreras, M., Belguith-Hadrich, O., Segura-Carretero, A. & Bouaziz, M. 2015. Assessment of the distribution of phenolic compounds and contribution to the antioxidant activity in Tunisian fig leaves, fruits, skins, and pulps using mass spectrometry-based analysis. *Food and Function* **6**(12), 3663–3677. https://doi.org/10.1039/c5fo00837a

Ashraf, M.A., Akbar, A., Askari, S.H., Iqbal, M., Rasheed, R. & Hussain, I. 2018. Recent advances in abiotic stress tolerance of plants through chemical priming: An overview. In Advances in Seed Priming (pp. 51–79). https://doi.org/10.1007/978-981-13-0032-5_4

Barnum, D.W. 1977. Spectrophotometric determination of catechol, epinephrine, dopa, dopamine and other aromatic vic-diols. In *Analytica Chimica acta.* Vol. **89**(1), 157–166. Scientific Publishing Company. https://doi.org/10.1016/S0003-2670(01)83081-6

Bradford, M.M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. In *Analytical Biochemistry.* Vol. **72**.

Darwish, D.A., El-Berry, I.M., Mustafa, N.S., Moursy, F.S. & Hagagg, L.F. 2015. Detecting drought tolerance of fig (*Ficus carica* L.) cultivars depending on vegetative growth and peroxidase activity. *International Journal of ChemTech Research* **8**(4), 1520–1532.

FAO. 2019. Proactive approaches to drought preparedness - Where are we now and where do we go from here? http://www.fao.org/3/ca5794en/ca5794en.pdf

Gholami, M., Rahemi, M. & Rastegar, S. 2012. Use of rapid screening methods for detecting drought-tolerant cultivars of fig (*Ficus carica* L.). *Scientia Horticulturae* **143**, 7–14. https://doi.org/10.1016/j.scienta.2012.05.012

Harborne, J.B. 1984. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Chapman and Hall, pp. 84–87.

Hasanuzzaman, M., Nahar, K., Alam, M.M., Roychowdhury, R. & Fujita, M. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. In *International Journal of Molecular Sciences* **14**(5), (9643–9684). https://doi.org/10.3390/ijms14059643

Hoxha, L., Kongoli, R. & Hoxha, M. 2015. Antioxidant activity of some dried autochthonous Albanian fig (*Ficus carica*) cultivars. *International Journal of Crop Science and Technology* **1**(2), 20–25.

Hssaini, L., Hanine, H., Charafi, J., Razouk, R., Elantari, A., Ennahli, S., Hernández, F. & Ouaabou, R. 2020. First report on fatty acids composition, total phenolics and antioxidant activity in seeds oil of four fig cultivars (*Ficus carica* L.) grown in Morocco. *OCL - Oilseeds and Fats, Crops and Lipids* **27**(8), 1–10. https://doi.org/10.1051/ocl/2020003

Jacobo-Salcedo, M. del R., Valdez-Cepeda, R.D., Sanchez-Cohen, I., Arreola-Ávila, J.G., González -Espíndola, L.A. & Trejo-Calzada, R. 2024a. Ability of fig tree (*Ficus carica* L.) accessions to thrive under limited and unlimited soil water conditions. *Acta Agrobotanica* **77**, 1–10. https://doi.org/10.5586/aa/183916

Jacobo-Salcedo, M. del R., Valdez-Cepeda, R.D., Sánchez-Cohen, I., González-Espíndola, L., Arreola-ávila, J.G. & Trejo-Calzada, R. 2024b. Physiological mechanisms in Ficus carica L. genotypes in response to moisture stress. *Agronomy Research* **22**(Special Issue II), 685–702. https://doi.org/10.15159/AR.24.047

Kamanga, R.M., Mbega, E. & Ndakidemi, P. 2018. Drought Tolerance Mechanisms in Plants: Physiological Responses Associated with Water Deficit Stress in Solanum lycopersicum. *Advances in Crop Science and Technology* **06**(03), 1–8. https://doi.org/10.4172/2329-8863.1000362

Konyalioğlu, S., Sağlam, H. & Kivçak, B. 2005. α-tocopherol, flavonoid, and phenol contents and antioxidant activity of Ficus carica leaves. *Pharmaceutical Biology* **43**(8), 683–686. https://doi.org/10.1080/13880200500383538

Lamuela-Raventós, R.M. 2018. *Folin-Ciocalteu method for the measurement of total phenolic content and antioxidant capacity*.

Li, Z., Cheng, B., Peng, Y. & Zhang, Y. 2020. Adaptability to abiotic stress regulated by γ-aminobutyric acid in relation to alterations of endogenous polyamines and organic metabolites in creeping bentgrass. *Plant Physiology and Biochemistry* **157**(July), 185–194. https://doi.org/10.1016/j.plaphy.2020.10.025

Marrelli, M., Menichini, F., Statti, G.A., Bonesi, M., Duez, P., Menichini, F. & Conforti, F. 2012. Changes in the phenolic and lipophilic composition, in the enzyme inhibition and antiproliferative activity of *Ficus carica* L. cultivar Dottato fruits during maturation. *Food and Chemical Toxicology* **50**(3–4), 726–733. https://doi.org/10.1016/j.fct.2011.12.025

Medina, G.G., Díaz, P.G., H.J., L., C.J.A., R. & Marín S.M. 2005. B*asic climatological statistics for the state of Durango*. (Period 1961–2003). Technical Book No. 1. Guadiana Valley Experimental Field (in Spanish).

National Institute of Statistics and Geography (INEGI). 2015. 'G*lobal Indicator of Economic Activity*'. Press Release No. 18/15, available at In: http://www.inegi.org.mx/inegi/contenidos/espanol/ prensa/Boletines/Boletin/Comunicados/Indicador%20global%20de%20la%20 actividad%20economica/2015/Enero/comunica.pdf (in Spanish).

Osmolovskaya, N., Shumilina, J., Kim, A., Didio, A., Grishina, T., Bilova, T., Keltsieva, O.A., Zhukov, V., Tikhonovich, I., Tarakhovskaya, E., Frolov, A. & Wessjohann, L.A. 2018. Methodology of drought stress research: Experimental setup and physiological characterization. In *International Journal of Molecular Sciences* (Vol. **19**, Issue 4089, pp. 1–25). MDPI AG. https://doi.org/10.3390/ijms19124089

Parejo, I., Codina, C., Petrakis, C. & Kefalas, P. 2000. Evaluation of scavenging activity assessed by Co(II)/EDTA-induced luminol chemiluminescence and DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical assay. *Journal of Pharmacological and Toxicological Methods* **44**(3), 507–512. https://doi.org/10.1016/S1056-8719(01)00110-1

Şahin, S. 2013. Evaluation of antioxidant properties and phenolic composition of fruit tea infusions. *Antioxidants* **2**(4), 206–215. https://doi.org/10.3390/antiox2040206

Sánchez-Urdaneta, A.B., Peña-Valdivia, C.B., Trejo, C., Aguirre, R., J.R., Cárdenas, E. & Galicia Jiménez, A.B. 2003. Permeability of root membranes of wild and domesticated bean (*Phaseolus vulgaris* L.) seedlings under moisture deficit. *Interciencia* **28**(10), 597–603 (in Spanish).

Scoffoni, C., Albuquerque, C., Buckley, T.N. & Sack, L. 2023. The dynamic multi-functionality of leaf water transport outside the xylem. *New Phytologist* **239**(6), 2099–2107. https://doi.org/10.1111/nph.19069

Seleiman, M.F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., Dindaroglu, T., Abdul-Wajid, H.H. & Battaglia, M.L. 2021. Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants* **10**(2), 1–25. doi: 10.3390/plants10020259

Shcherbakov, V.V., Artemkina, Y.M., Akimova, I.A. & Artemkina, I.M. 2021. Dielectric characteristics, electrical conductivity and solvation of ions in electrolyte solutions. *Materials* **14**(19), 1–26. https://doi.org/10.3390/ma14195617

Shomali, A., Das, S., Arif, N., Sarraf, M., Zahra, N., Yadav, V., Aliniaeifard, S., Chauhan, D.K. & Hasanuzzaman, M. 2022. Diverse Physiological Roles of Flavonoids in Plant Environmental Stress Responses and Tolerance. *Plants* **11**(22). doi: 10.3390/plants11223158

Sparke, M.A. & Wünsche, J.N. 2020. Mechanosensing of plants. In *Horticultural Reviews* (Issue January 2020, pp. 43–83). https://doi.org/10.1002/9781119625407.ch2

Stanković, M.S. 2011. Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac J. Sc.* **33**(1), 63–72.

Wu, J., Wang, J., Hui, W., Zhao, F., Wang, P., Su, C. & Gong, W. 2022. Physiology of Plant Responses to Water Stress and Related Genes: A Review. *Forests* **13**(2). https://doi.org/10.3390/f13020324

Yang, X., Lu, M., Wang, Y., Wang, Y., Liu, Z. & Chen, S. 2021. Response mechanism of plants to drought stress. *Horticulturae* **7**(3), 1–36. https://doi.org/10.3390/horticulturae7030050

Detection of changes in the defence factors of Nicotiana Tabacum plant under the influence of insertion and expression of heterologous transgenes (*des*A, *des*C, *Hu*INF*α*-2*b*)

T. Kyrpa[1,*], A. Potrokhov[1], M. Kharkhota[2] and M. Kuchuk[1]

[1]Institute of Cell Biology and Genetic Engineering of NAS of Ukraine, Academika Zabolotnoho Str.148, UA03143 Kyiv, Ukraine
[2]Institute of Microbiology and Virology named after D.K. Zabolotny, Str. Academika Zabolotnoho, 154, UA03143 Kyiv, Ukraine
[*]Correspondence: t-kirpa@ukr.net

Abstract. Genetically modified plants may have some changes in physiological and biochemical reactions depending on the type of transgene. In this study, we present the results of the analysis of tobacco plants with the insertion and expression of the genes for human interferon alpha (*Hu*INF*α*-2*b*), Δ12-acyl-lipid desaturase (*des*A of the cyanobacterium *Synechocystis sp.* PCC 6803) and Δ9-acyl-lipid desaturase (*des*C of the cyanobacterium *Synechococcus vulcanus*). Wild-type tobacco plants were used as a control. The level of accumulation of polyfructans and changes in the fatty acid spectrum in the leaves of plants under normal physiological conditions and after exposure to low temperatures were tested. It was found that all transgenic plants had some changes in the composition of fatty acids, however, only plants with the *Hu*INF*α*-2*b* gene insertion had an increased content of polyfructans. These data may indirectly indicate a difference in the two defense strategies of the plant organism depending on the insertion and expression of the transferred gene.

Key words: abiotic stress, polyfructans, acyl-lipid desaturases, adaptation, fatty acids.

INTRODUCTION

Adaptation of plants to various stress factors requires the existence and functioning of certain protect mechanisms (Mareri et al., 2022). This can include the storage of essential substances (such as sugars, polysaccharides, etc.), as well as changes in the qualitative composition of certain plant cell structures (for example, changes in the spectrum of fatty acids in phospholipid membranes) (Riseh et al., 2021; Vahalová & Cifra, 2023). Genetic engineering helps accelerate the acquisition of new quantitative or qualitative traits by plant organisms (Dash & Osborne, 2023). Typically, biotechnological approaches reduce the time it would take to produce the same plants through breeding (Bigini et al., 2021). The insertion of a transgene into a plant organism can affect the expression of host genes or create competition for substrate-enzyme bonds, causing the expression of a new protein. Thus, not all aspects of the transformation event

itself remain sufficiently covered in terms of intracellular physiological and biochemical processes. (Müller, 2024). First of all, the phenomenon of transformation events, namely the insertion and expression of a transgene, can affect the accumulation of organic substances that affect metabolism and adaptive characteristics of plants, such as carbohydrates (Hafeez et al., 2023). They make up 85–90% of the substances that make up the plant (Kutzli et al., 2021). Carbohydrates are formed in the plant body as a result of the photosynthetic apparatus and are one of the main aspects in the food chain of humans, animals and microorganisms (Wani et al., 2023). In plants, carbohydrates are found as a support material; substances dissolved in cell sap; reserve deposits; and components of compounds that play an important role in metabolism (van Bel, 2021). The positive role of increasing the amount of sugars in plant tissues under the influence of various adverse environmental factors (salinity, drought, high temperatures, low temperatures, etc.) is known (Mehdi et al., 2024). Sugars increase the resistance of proteins to various physical and chemical influences that cause their coagulation (Ng et al., 2022). Therefore, the important role of sugars in frost, drought, gas and salt resistance is associated with the stabilization of protein molecules (Jahed et al., 2023). One of the main reserve compounds of higher plants is fructans (Márquez-López et al., 2022) (Benkeblia, 2022). There are several main types of fructans: inulin (mainly in dicots), levan, neo-inulin and neo-levan (in monocots), depending on the structure of the polymer molecule (Verma et al., 2021). Another important factor that plays an important role in maintaining the integrity of the cell and its interaction with the surrounding factors is the fatty acid spectrum (Rawat et al., 2021). First of all, fatty acids in membrane phospholipids affect the properties of the membrane itself (Martin & Douliez, 2021). With an increase in the proportion of unsaturated fatty acids in membrane phospholipids, the viscosity and plasticity of membranes increase, which prevents mechanical degradation due to cell desiccation (Kaur et al., 2022). In addition, an increase in the proportion of unsaturated fatty acids in membrane phospholipids reduces the freezing point, which leads to an increase in the adaptive potential of plants to low temperatures and frosts (Zhao et al., 2024). Desaturases are enzymes that promote the formation of double bonds in fatty acids and thus convert them from saturated to unsaturated (Kazaz et al., 2022). These groups have been broadly classified into two evolutionary unrelated groups of soluble acyl-acyl carrier protein (ACP) and membrane-bound desaturases (Halim et al., 2022). This natural mechanism makes it possible to improve the quality of plant resistance to various abiotic stress factors (Xiao et al., 2022). However, the transfer, insertion and expression of transgenes can have both negative and positive effects (Sayed et al., 2022).

In this study, we investigated the content of polyfructans and changes in the proportion of fatty acids in experimental tobacco plants *Nicotiana tabacum* (as a model plant organism) expressing genes of different origin and characterised by different substrate specificity, since the effect of transferred genes on the functioning of a transgenic organism remains a very promising topic for research. In this study, we investigated plants with insertion and stable expression of cyanobacterial desaturase genes (*des*A (gene of Δ12-acyl-lipid desaturase of cyanobacterium *Synechocystis sp.* PCC 6803), *des*C (gene of Δ9-acyl-lipid desaturase of cyanobacterium *Synechococcus vulcanus*) and *Hu*INF*α*-2b (gene coding for recombinant human interferon alpha-2b) on the level of polyfructans accumulation in leaves as one of the indicators of plant

adaptation to low temperatures and frosts and changes in the fatty acid spectrum as another strategy of plant adaptation to cold stress.

Acyl-lipid desaturases also function in the plant organism, so the desaturases that will be caused by transgene expression can be provided with a reaction substrate. Regarding the *Hu*INF*α-2b* transgene, it expresses a protein that is not native to plants.

MATERIALS AND METHODS

Plant material

Transgenic plants of the model object genes *Nicotiana tabacum*, in which the insertion and expression of *des*A (gene of Δ12-acyl-lipid desaturase of cyanobacterium *Synechocystis sp*. PCC 6803), *des*C (gene of Δ9-acyl-lipid desaturase of cyanobacterium *Synechococcus vulcanus*) and *Hu*INF*α-2b* (gene coding for recombinant human interferon *alpha-2b*) were confirmed, were taken from the collection of the Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine. Plants were previously obtained as a result of *Agrobacterium tumefaciens*-mediated transformation. The initial genetic constructs were created on the basis of the pBISN vector with selective *npt*II gene (*des*A, *Hu*INF-*α2b*) and with selective *bar* gene (*des*C) under the control of 35S CaMV promoter. Transgene insertion was confirmed by PCR, and expression was demonstrated indirectly through the activity of the reporter gene for the thermostable lichenase *lic*BM3 in plants with insertion and expression of desaturase genes. A qualitative lichenase assay was performed for the initial detection of expression and a quantitative lichenase assay was performed to determine the level of transgene expression in the plant organism. (Gerasymenko et al., 2015). The expression of interferon in transgenic tobacco plants with human INF-*α2b* gene was determined using ELISA method. Testing of extracts obtained from transgenic plants on cell culture of piglet inoculated textiles (PIT), which was infected with vesicular stomatitis virus (VSV) and have revealed interferon-like activity (Potrokhov et al., 2017). Wild-type tobacco *N. tabacum* plants were used as a control.

Microclonal plant propagation

Plants: *N. tabacum, N. tabacum* with insertion and expression of *des*A and *des*C and *Hu*INF*α-2b* transgenes were grown and propagated on nutrient agar medium MS. All plants were cultured on standard MS medium, *in vitro* at 25 ± 1 °C and 16-h light period (100 quantum mmol m^{2}s). To maintain the culture and increase plant biomass, transplants were carried out once a month. To do this, aseptic scalpels were used to cut off plants that had been cultivated for a month on agar medium and transferred to fresh medium. Plants after one month of cultivation were used for the study.

Determination of the total level of polyfructans by the colourimetric method

For colorimetric studies, extracts from the experimental control plants were obtained. The test material was weighed (100 μg), homogenised with distilled water and centrifuged for 15 minutes at 15,000 g. After extraction, the required aliquot of the supernatant was taken for analysis. For the colorimetric analysis, 100 μl of 0.1% resorcinol aqueous solution and 100 μl of HCl (5:1) were added to 100 μl of the extract. The resulting solution was heated in a water bath for 5 min at +80 °C. After heating for

5 min at +80 °C and the appearance of a characteristic cherry colour, the optical density was measured using an Eppendorf biofotometr plus automatic analyzer at 550 nm. For the blank sample, the reaction solution was used with distilled water 100 µl instead of sample extract.

Chromatographic determination of fatty acids in plants

The fatty acid (FA) spectrum was analysed by gas chromatography and mass spectrometry. The isolation of FACs and the formation of their methyl esters for gas chromatographic analyses was carried out in one step according to the method.

A weight of leaves (200 mg) was cut with grease-free scissors and transferred to glass test tubes with screw caps. Reaction mixture A was prepared from methanol:toluene:sulfuric acid in a volume ratio of 44:20:2. Reaction mixture B contained a solution of the internal standard hexadecanoic acid in heptane 10 mg mL^{-1} and heptane at a ratio of 1:84.

To each tube was added 3.3 mL of reaction mixture (A), followed by 1.7 mL of reaction mixture (B). The tested tubes were tightly sealed with teflon gasketed lids and kept in a water bath at 80 °C for 2 hours. Cooled at room temperature, the mixture separated into two phases. The upper phase was selected, in which the methyl esters of fatty acids formed were concentrated. Selected 300 µl of the upper phase was transferred to a vial with a tightly closed lid. The internal standard was a 20% solution of hexadecanoic acid in heptane. The fatty acid spectrum was studied by GLPC MS using Agilent 6890N/5973inert instrument coupled to DBFFAP capillary column (30 m × 0.25 mm × 0.25 µm) (J&W Scientific, United States). The results were analysed according to the values of graphical absorption peaks calculated by the chromatograph software. FAMEs were identified by comparison of obtained spectra with NIST 02 mass spectrum library entries and with spectra of standard mixture of bacterial FAMEs (47080U, Supelco).

Statistical data processing

For processing of statistical data we used MC Exel 2019 programme package. The level of reliable probability Rndx was 0.95. The data obtained were statistically processed using Statistica 10.02. In of polyfructans studies, 1 analytical replicate and 9 biological replicates were used and in the study of the fatty acid spectrum1 analytical replicate and 6 biological replicates were used.

Plant cultivation, hypothermic stress, analysis of polyfructans, preparation of samples for gas chromatography and mass spectrometry analysis, calculation of the results were carried out at the Institute of Cell Biology and Genetic Engineering of NAS of Ukraine, gas chromatography and mass spectrometry analysis was carried out at the Institute of Microbiology and Virology named after D.K. Zabolotny

RESULTS AND DISCUSSION

Genetically modified organisms, in particular, genetically modified plants, are increasingly being used in agriculture and industry. At the same time, all factors that may affect the environment must be taken into account. While new quantitative or qualitative characteristics are being imparted to plants, the transferred genes can have a

negative impact on important intracellular physiological and biochemical processes of plants. For the study, tobacco plants of the model object *Nicotiana tabacum* were used. Wild-type and transgenic *Nicotiana tabacum* plants were used, in which the insertion and expression of the gene *des*A (encoding the Δ12-acyl-lipid desaturase gene of the cyanobacterium *Synechocystis sp*. PCC 6803) or the *des*C gene (encoding the Δ9-acyl-lipid desaturase gene of the cyanobacterium *Synechococcus vulcanus*). The cyanobacterial desaturase genes were fused in a same reading frame with the *lic*BM3 gene of the *Clostridium thermocellum* thermostable lychenase reporter protein. It should be noted that the transgenic plants did not have any morphological differences with the control plants (Fig. 1).

Figure 1. Plants used in the study: 1 – Control plant *N. tabacum* wild type; 2 – *N. tabacum* with gene coding for recombinant human interferon alpha-2b. 3 – *N. tabacum* with the gene of Δ12-acyl-lipid desaturase of cyanobacterium *Synechocystis sp*. PCC 6803. 4 – *N. tabacum* with the gene of Δ9-acyl-lipid desaturase of cyanobacterium *Synechococcus vulcanus*.

Freezing temperatures and frosts are one of the most common factors that lead to plant damage or death, which negatively affects the yield of important crops. Therefore, research into mechanisms that can positively influence plant resistance to this type of stress is an important task. Plants were tested to hypothermic stress of 0 °C 20 min, -5 °C 60 min. The level of polyfructans and changes in the fatty acid spectrum were analysed under normal physiological conditions and after exposure to low temperature stress.

The research used transgenic plants tobacco in which the products of transgene expression were characterised by different substrate specificity. By studying the effect of the transferred genes on the accumulation of sugars in the plant organism, it was found that in plants expressing the human interferon gene, the level of this substance differed from tobacco plants expressing cyanobacterial desaturase genes under normal physiological conditions and under cold stress. At the usual ambient temperature, the content of polyfructans was higher in plants expressing the interferon gene, lower levels of sugars were found in non-transgenic tobacco plants, and the lowest levels were found in plants expressing desaturase genes.

After exposure to freezing stress, the level of sugar accumulation in wild-type tobacco plants and tobacco expressing the interferon gene increased. It was determined that in control plants the fructans content was 7 ± 0.57 mg g^{-1} wet weight before the onset of cold exposure, and after exposure to low temperatures the fructans content was 15 ± 1.28 mg g^{-1} wet weight. Similarly, in plants with the interferon gene, an increase in the level of fructans accumulation was observed from 15 ± 0.63 mg g^{-1} to 22 ± 3.21 mg g^{-1}. However, in plants expressing cyanobacterial desaturase genes, the level of sugar accumulation remained without statistically significant changes (Fig. 2).
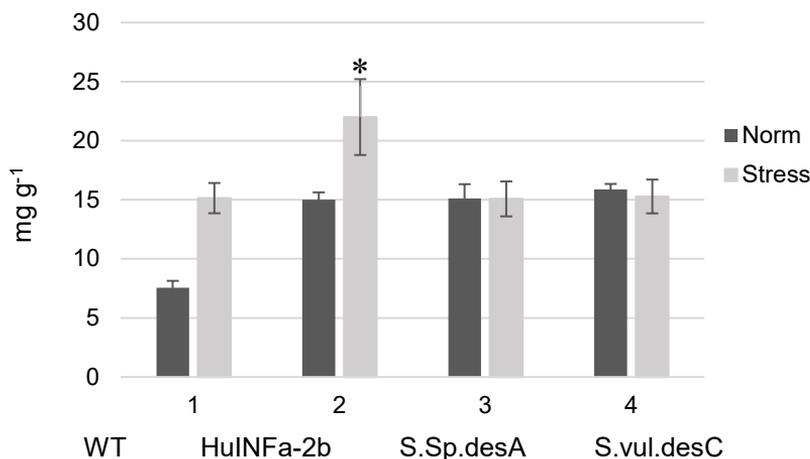
Figure 2. The level of sugars (polyfructans) after exposure to cold stress. WT – *N. tabacum* wild type; HuINF*α-2b* – *N. tabacum* with gene coding for recombinant human interferon alpha-2b. *S.sp.des*A – *N. tabacum* with the gene of Δ12-acyl-lipid desaturase from cyanobacterium *Synechocystis sp.* PCC 6803. *S. vul. des*C – *N. tabacum* with the gene for Δ9-acyl-lipid desaturase from the cyanobacterium *Synechococcus vulcanus.*

Since desaturases contribute to an increase in the proportion of unsaturated fatty acids in membrane phospholipids, the composition of the fatty acid spectrum was checked. The content of fatty acids in transgenic and control plants was analysed by gas chromatography and mass spectrometry. It was determined that under normal physiological conditions, wild-type tobacco had a predominant proportion of palmitic fatty acid (59.5%) in the composition of membrane phospholipids. Tobacco with insertion and expression of the human interferon gene had an increased proportion of palmitic acid (64.35%) with a reduced level of linoleic acid (12.3%) compared to wild-type tobacco (21.26%). Tobacco with the expression of the *des*A gene had an increased proportion of linoleic acid (35.09%) with a decrease in palmitic acid (46.81%) and tobacco with the expression of the *des*C gene had an increased proportion of palmitic acid (62.69%) compared to control non-transgenic tobacco (59.5%), but slightly lower than that of tobacco with the interferon gene (64.35%). These results are explained by the fact that certain proteins of the desaturase genes promote the formation of double bonds in the corresponding positions. The protein of the interferon gene has no direct substrate for binding in the plant organism, so the position of the insertion can affect the biochemical processes of plant metabolism.

After exposure to freezing stress, certain differences in the composition of fatty acids in transgenic plants were found.

In general, the desaturation reaction can be enhanced by an increase in the level of free oxygen and free oxygen radicals in the cell, which arise as a result of negative influences and destructive changes in the cell. In this way, damage signalling is triggered to increase the expression of desaturase genes.

Table 1. *Analysis of fatty acid spectrum data under normal conditions (+) and after temperature stress (-)

No.	Plants	Conditions	Palmitic acid, µg mL^{-1} (% of total fatty acid content)	Linoleic acid, µg mL^{-1} (% of total fatty acid content)	Linolenic acid, µg mL^{-1} (% of total fatty acid content)
1	*N. tabacum* Wild type (control)	+	80.8 ± 7.2 (59.5%)	26.3 + 3.3 (19.37%)	28.7 + 4.2 (21.26%)
		–	80.8 ± 7.3 (59.499%)	26.3 + 1.3 (19.37%)	28.7 + 4.2 (21.13%)
2	*N. tabacum* with *Hu*INFα-2b transgene	+	92.6 + 3.3 (64.35%)	33.6 + 1,3 (23.35%)	17.7 + 0.3 (12.3%)
		–	79.6 + 2.8 (66.83%)	26.9 + 1.96 (22.586%)	12.6 + 0.4 (10.579%)
3	*N. tabacum* with *des*A transgene	+	85.9 + 4.4 (46.81%)	29.9 + 1.5 (16.29%)	64.4 + 9.3 (35.09%)
		–	66.9 + 0.08 (38.187%)	8.7 + 0.9 (4.966%)	97.99 + 9.6 (55.93%)
4	*N.tabacum* with *des*C transgene	+	86.2 + 0.009 (62.69%)	29.5 + 0.1 (21.45%)	21.8 + 0.1 (15.8%)
		–	85.4 + 7.01 (62.84%)	26.6 + 1.4 (19.57%)	23.9 + 3.3 (17.5864%)

Taking into account that desaturase enzymes have a reaction substrate in the plant organism, it can be assumed that the *des*C gene, which causes the expression of Δ9-acyl-lipid desaturase, which promotes the formation of double bonds at the Δ9 position and converts palmitic acid into oleic acid, supplies the substrate for other desaturase species and thus triggers the desaturase cascade.

The protein of the *des*A (Δ12-acyl-lipid desaturase) gene forms a double bond at the Δ12 position and converts oleic acid to linoleic acid. However, the results of the analysis show that there was also an increase in linolenic acid, which indicates the supply of a reaction substrate for further transformations.

As for the absence of changes in the control plants, it can be assumed that they had a different defence system based on an increase in the concentration of polyfructans and other defence mechanisms that were not analysed in this study. However, we did not study cold stress of greater severity. It is possible that with increasing stress levels, we can observe changes in the fatty acid spectrum of wild-type tobacco and/or changes in the level of polyfructans in tobacco with cyanobacterial desaturase gene expression.

CONCLUSION

The introduction and expression of transgenes is an interesting phenomenon that can have both negative and positive effects on the physiological processes of a plant organism. Sometimes it can affect defense systems and adaptation to environmental conditions. Tobacco plants with confirmed insertion and expression of transgenes with different substrate specificity may have some differences in the measurement of certain physiological indicators of adaptation. Desaturase genes (*des*A or *des*C) undoubtedly

enhance the protective properties of membranes (increasing their plasticity) against low temperatures and frost. In this case, we can consider the response to stress of two plant defense systems – an increase in the proportion of unsaturated fatty acids in membrane phospholipids and an increase in the accumulation of polyfructans (plants with the human interferon gene, *Hu*INF-*α2b* gene). It is likely that with increasing stress, plants expressing desaturases will tend to increase the accumulation of polyfructans. This is planned to be tested later.

REFERENCES

Benkeblia, N. 2022. Insights on Fructans and Resistance of Plants to Drought Stress. In *Frontiers in Sustainable Food Systems* **6**, Frontiers Media S.A. doi: 10.3389/fsufs.2022.827758

Bigini, V., Camerlengo, F., Botticella, E., Sestili, F. & Savatin, D.V. 2021. Biotechnological resources to increase disease-resistance by improving plant immunity: A sustainable approach to save cereal crop production. *Plants* **10**(6), MDPI AG. doi: 10.3390/plants10061146

Dash, D.M. & Osborne, W.J. 2023. A systematic review on the implementation of advanced and evolutionary biotechnological tools for efficient bioremediation of organophosphorus pesticides. *Chemosphere* **313**, Elsevier Ltd. doi: 10.1016/j.chemosphere.2022.137506

Gerasymenko, I.M., Sakhno, L.A., Kyrpa, T.N., Ostapchuk, A.M., Hadjiev, T.A., Goldenkova-Pavlova, I.V. & Sheludko, Y.V. 2015. Characterization of Nicotiana tabacum plants expressing hybrid genes of cyanobacterial Δ9 or Δ12 acyl-lipid desaturases and thermostable lichenase. *Russian Journal of Plant Physiology* **62**(3), 283–291. https://doi.org/10.1134/S1021443715030073

Hafeez, A., Ali, B., Javed, M.A., Saleem, A., Fatima, M., Fathi, A., Afridi, M.S., Aydin, V., Oral, M.A. & Soudy, F.A. 2023. Plant breeding for harmony between sustainable agriculture, the environment, and global food security: an era of genomics-assisted breeding. *Planta* **258**(5). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s00425-023-04252-7

Halim, N.F.A.A., Ali, M.S.M., Leow, A.T.C. & Rahman, R.N.Z.R.A. 2022. Membrane fatty acid desaturase: biosynthesis, mechanism, and architecture. *Applied Microbiology and Biotechnology* **106**(18), 5957–5972. https://doi.org/10.1007/s00253-022-12142-3

Jahed, K.R., Saini, A.K. & Sherif, S.M. 2023. Coping with the cold: unveiling cryoprotectants, molecular signaling pathways, and strategies for cold stress resilience. *Frontiers in Plant Science* **14**, Frontiers Media SA. https://doi.org/10.3389/fpls.2023.1246093

Kaur, M., Saini, K.C., Ojah, H., Sahoo, R., Gupta, K., Kumar, A. & Bast, F. 2022. Abiotic stress in algae: response, signaling and transgenic approaches. *Journal of Applied Phycology* **34**(4), 1843–1869. Springer Science and Business Media B.V. doi: 10.1007/s10811-022-02746-7

Kazaz, S., Miray, R., Lepiniec, L. & Baud, S. 2022. Plant monounsaturated fatty acids: Diversity, biosynthesis, functions and uses. *Progress in Lipid Research* **85**, Elsevier Ltd. https://doi.org/10.1016/j.plipres.2021.101138

Kutzli, I., Weiss, J. & Gibis, M. 2021. Glycation of plant proteins via maillard reaction: Reaction chemistry, technofunctional properties, and potential food application. *Foods* **10**(2), MDPI AG. https://doi.org/10.3390/foods10020376

Mareri, L., Parrotta, L. & Cai, G. 2022. Environmental Stress and Plants. *International Journal of Molecular Sciences* **23**(10). MDPI. https://doi.org/10.3390/ijms23105416

Márquez-López, R.E., Loyola-Vargas, V.M. & Santiago-García, P.A. 2022. Interaction between fructan metabolism and plant growth regulators. *Planta* **255**(2), Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s00425-022-03826-1

Martin, N. & Douliez, J.-P. 2021. *Fatty Acid Vesicles and Coacervates as Model Prebiotic Protocells*. https://doi.org/10.1002/syst.202100024ï

Mehdi, F., Cao, Z., Zhang, S., Gan, Y., Cai, W., Peng, L., Wu, Y., Wang, W. & Yang, B. 2024. Factors affecting the production of sugarcane yield and sucrose accumulation: suggested potential biological solutions. *Frontiers in Plant Science* **15**. Frontiers Media SA. https://doi.org/10.3389/fpls.2024.1374228

Müller, G.A. 2024. " Centric" and " Excluding" Conceptions of Biological Inheritance. *Advances in Historical Studies* **13**(02), 122–155. doi: 10.4236/ahs.2024.132007

Ng, J.W., Othman, N. & Yusof, N.H. 2022. Various coagulation techniques and their impacts towards the properties of natural rubber latex from Hevea brasiliensis – a comprehensive review related to tyre application. *Industrial Crops and Products* **181**. doi: 10.1016/j.indcrop.2022.114835

Potrokhov, A, Trokhymenko, A., Zubr Z., Matveeeva, N. 2017. Assessment of antiviral activity extract from transgenic tobacco plants with human interferon α-2B gene. *Scientific Issues Ternopil Volodymyr Hnatiuk National Pedagogical University Series*: Biology **3**. 64–69.

Rawat, N., Singla-Pareek, S.L. & Pareek, A. 2021. Membrane dynamics during individual and combined abiotic stresses in plants and tools to study the same. *Physiologia Plantarum* **171**(4), 653–676. https://doi.org/10.1111/ppl.13217

Riseh, R.S., Ebrahimi-Zarandi, M., Gholizadeh Vazvani, M. & Skorik, Y.A. 2021. Reducing drought stress in plants by encapsulating plant growth-promoting bacteria with polysaccharides. *International Journal of Molecular Sciences* **22**(23). MDPI. https://doi.org/10.3390/ijms222312979

Sayed, N., Allawadhi, P., Khurana, A., Singh, V., Navik, U., Pasumarthi, S.K., Khurana, I., Banothu, A.K., Weiskirchen, R. & Bharani, K.K. 2022. Gene therapy: Comprehensive overview and therapeutic applications. *Life Sciences* **294**. Elsevier Inc. https://doi.org/10.1016/j.lfs.2022.120375

Vahalová, P. & Cifra, M. 2023. Biological autoluminescence as a perturbance-free method for monitoring oxidation in biosystems. *Progress in Biophysics and Molecular Biology* **177**, 80–108. Elsevier Ltd. https://doi.org/10.1016/j.pbiomolbio.2022.10.009

van Bel, A.J.E. 2021. The plant axis as the command centre for (re)distribution of sucrose and amino acids. *Journal of Plant Physiology* **265**. Elsevier GmbH. doi: 10.1016/j.jplph.2021.153488

Verma, D.K., Patel, A.R., Thakur, M., Singh, S., Tripathy, S., Srivastav, P.P., Chávez-González, M.L., Gupta, A.K. & Aguilar, C.N. 2021. A review of the composition and toxicology of fructans, and their applications in foods and health. *Journal of Food Composition and Analysis* **99**. Academic Press Inc. doi: 10.1016/j.jfca.2021.103884

Wani, M.Y., Ganie, N.A., Dar, K.A., Dar, S.Q., Khan, A.H., Khan, N.A., Zahmatkesh, S., Manzar, M.S. & Banerjee, R. 2023. Nanotechnology future in food using carbohydrate macromolecules: A state-of-the-art review. *International Journal of Biological Macromolecules* **239**. https://doi.org/10.1016/j.ijbiomac.2023.124350

Xiao, R., Zou, Y., Guo, X., Li, H. & Lu, H. 2022. Fatty acid desaturases (FADs) modulate multiple lipid metabolism pathways to improve plant resistance. *Molecular Biology Reports* **49**(10), 9997–10011. Springer Science and Business Media B.V. doi: 10.1007/s11033-022-07568-x

Zhao, Y., Li, S., Wu, J., Liu, H., Wang, P. & Xu, L. 2024. Insights into membrane lipids modification in barley leaves as an adaptation mechanism to cold stress. *Plant Growth Regulation* **103**(2), 369–388. https://doi.org/10.1007/s10725-023-01114-w

Review of farm safety and health risk management tools

J. Leppälä[1,*] and R. Rautiainen[2]

[1]Natural Resources Institute Finland (Luke), Latokartanonkaari 9, FI00790 Helsinki, Finland
[2]University of Nebraska, Swanson Hall 3058, Omaha, 68198-5110 Nebraska, USA
[*]Correspondence: jarkko.leppala@luke.fi

Abstract. Risk management aims to prevent intolerable risks that could jeopardize a farm's goals and strategies. Many studies indicate that safety and health risks pose the greatest threats to farm continuity and business sustainability. Even a single injury to a key farmworker could have severe consequences for the whole farm.

The review employs content and thematic analysis to identify and classify safety and health risk management tools to assist farmers in their important risk management efforts. There is also an increasing need in EU to provide information about social conditionality requirements on farms, which include also terms in farm safety and health management.

A total of 62 risk management tools were analysed. The main typical risk factors in agriculture include machinery safety, livestock safety, chemical handling, and health hazards such as animal dust, gases, noise, biological hazards causing skin and respiratory diseases and ergonomic issues. This study is part of the Strengthening Farm Safety and Health Knowledge and Innovation Systems (SafeHabitus) project. The findings will contribute to the development of a farm safety and health risk management tool database in the SafeHabitus project.

Key words: agriculture, safety, health, risk management.

INTRODUCTION

Risk management is essential for every business. It involves planning, goal setting, and identifying the best strategies to achieve objectives in mitigating hazards and risks. A hazard is commonly understood as anything with the potential to cause harm, while a risk is typically defined as the combination of the likelihood of a harmful event occurring and the severity of the consequences of such event. A risk factor increases the probability of a harmful event. So, the hazard become a risk because of particular risk factor. Effective risk management ensures that intolerable risks do not threaten a company's business goals, production, or overall sustainability (Juran & Godfrey, 1998; COSO, 2004; Hardaker et al., 2004; Scarborough et al., 2009; Eastwood et al., 2010; Kirch, 2018; SFS-EN ISO 45001, 2023).

Safety and health risks are among critical threats to farm continuity and business sustainability is safety and health risk. A key reason for this vulnerability is that farms are typically micro-sized enterprises, often operated by just one or two individuals. In

turn, poor business sustainability on farms may cause farmers mental health problems and stress. In Europe, family farms dominate the agricultural sector, accounting for over 90% of all farms. This means that an injury to a key farmworker can have severe, even existential, consequences for the whole farm (Merisalu et al., 2019; Leppälä et al., 2021; Schuh et al., 2022). According to Goetsch (2015), the terms 'occupational safety and health' and 'occupational health and safety' have also been adopted in publications by the International Labour Organization (ILO). Occupational health generally refers to hazards linked to diseases and long-term health effects, whereas occupational safety pertains to hazards that can result in workplace accidents, injuries, or sudden acute conditions.

Management of safety and health risks is a critically important management task on a farm. EU statistics show that 1,500–2,000 in 100,000 agricultural workers experiences an injury each year and about one in ten thousand becomes a victim of an occupational fatality (Merisalu et al., 2019; Eurostat, 2024). Actual rates are estimated to be much higher due to exclusion of self-employed farmers and other difficulties in data collection (Reinvee, 2024). Farming has been number one in the frequency of serious injuries in many parts of the world, including the USA (ILO 2019; BLS, 2025). Farmers have reported that an injury, disease or burnout of a key person is a crucial risk to their farm (Leppälä et al., 2013). Starting 2025, the EU Common Agriculture Policy includes a social conditionality rule, which obliges employers on farms to provide certain social conditions for workers. The farm employers are links agricultural subsidies to providing acceptable conditions to employees including work contracts, proper personal protection equipment and safe working conditions (Laurent & Nguyen 2022; Vinci, 2024).

Governmental records, accident insurance systems, universities and research institutes, and extension service reports are primary sources for identifying potential safety and health risks in agriculture (Leppälä et al., 2021; Rautiainen et al., 2009; Frank et al., 2004). Research data and information from these sources form the foundation for developing farm safety and health risk management services and tools. Common safety issues on farms include physical injuries from working with machinery and animals, as well as slips, trips, and falls and frequent health issues include respiratory diseases, skin conditions, and musculoskeletal disorders caused by heavy, repetitive tasks. Key sources of farm injuries include human error, machinery, livestock, hand tools, and unsafe working surfaces (Donham & Thelin, 2006; Rautiainen et al., 2009; Karttunen, 2014; Leppälä, 2016; McNamara et al., 2020). Additionally, numerous studies have identified structural risk factors that predispose farmers to injuries. In a systematic review and meta-analysis, Jadhav et al. (2015; 2016) found 24 significant demographic, personal/behavioral, environmental, and safety-related risk factors for injury.

A management tool is defined as an aid used to accomplish a management task. While the term 'tool' traditionally refers to physical equipment or machinery, in corporate management, it encompasses software, analytical methods, policies, concepts, processes, communication networks, strategic planning tools, and knowledge management aids (Nedelko et al., 2012; Daft, 2015). Safety and health risk management tools help assess risks and integrate various accident prevention methods and services to reduce known risks and potential hazards (Reason, 1997; Suutarinen, 2004; Leppälä et al., 2012; McNamara, 2014). The hierarchy of controls is one framework for consideration of control measures with five levels ranked from most to least effective:

elimination, substitution, engineering controls, warnings, administrative controls, and personal protective equipment (PPE) (NIOSH, 2024). This framework can be applied to identifying effective strategies for mitigating risks and also for characterizing and evaluating safety and health risk management tools. The fundamental steps in risk management involve establishing the context and then identifying, analyzing, addressing, monitoring, and communicating risks (COSO, 2004; Aloini et al., 2007; Leppälä, 2016; ISO 31000). In the current review, safety and health risk management tools may include farm safety checklists, occupational health screening protocols, operating safety instructions, occupational accident insurance schemes, and safety education (McNamara, 2014; Leppälä, 2016).

This study is part of the Strengthening Farm Safety and Health Knowledge and Innovation Systems (SafeHabitus, 2023) project, which aims to raise awareness of agricultural safety and health management. The findings of this study support the development of a database that will assist farm managers in selecting effective risk management strategies and tools.

MATERIALS AND METHODS

The aim of this study was to review the existing literature related to farm safety and health risk management tools and services designed for identifying and managing safety and health risks on farms. The study employed a mixed methods approach, combining quantitative and qualitative methods, Content and thematic analyses were used to identify and characterize safety and health risk management tools for farms. Content analysis can be either quantitative or qualitative, categorizing data into numerical values, words, or thematic groups. It addresses key questions such as who, where, when, what, and why. Thematic analysis, on the other hand, focuses on qualitative aspects by identifying patterns and themes within the data (Elo & Kyngäs, 2008; Vaismoradi et al., 2013).

The searches for safety and health risk management literature were conducted using Google Advanced Search, Google Scholar, ScienceDirect, and Web of Science databases. The search keywords included farm, agriculture, health, safety, risk management, and tools, with a focus on EU and Western countries' agriculture and farming from 2014 to 2023. Each search engine functions slightly differently, and all search variables are detailed in Table 2. The latest update to the search was completed in May 2024. Only tools specifically designed for practical farm safety and health management were selected. Traditional peer-reviewed articles might not always cover the latest developments and practical tools or references. This was also the case in this study and this is why the grey literature - such as government reports, guidebooks, advisory service and technical paper links, was an important data source for this study.

To capture a broader range of practical tools, Google Advanced Search was used to identify farm occupational safety and health (OSH) risk management tools used by extension services. Since scientific articles on farm management tools used in extension and training are limited, this method helped gather relevant additional information. The search was concluded when no new farm safety risk management tools or services were found in the search results.

ANALYSIS

The content analysis method by Harwood & Garry (2003) was used to categorize the farm risk management tools for Table 2. The tools identified through the search were classified and quantified based on several factors, including: country of origin, organization type, years active, tool administration, tool level, number of users, registration requirements, annual user costs, safety or health risk factors handled and available tools and services. The data coding variables are defined and explained in Table 1, while the full list of included tools is provided in the appendix.

Table 1. Risk management tool classification variables and their definitions

Variable	Definition
Country of origin/use	The original country where the tool is established and used; country/ international (international organisation or many countries involved)
Service provider type	Public / Private / Both public and private organization
Years active	Years in active use: Under 10 years / 10–20 years / Over 20 years
Registration	User registration available or needed? Yes / No
Tool level	Comprehensive farm OSH tool / General farm safety / Specific risk tool
Administration	Administration by Farmer self/ Consultant / Both farmer and consultant
Number of users	N/A information not available / 0–1,000 / 1,000–10,000, Over 10,000 users
Annual user cost	N/A information not available, totally free, partly free/partly cost, cost under 100, cost 100–1,000, cost over 1,000 Euros
Safety or health risk issues (content analysis)	Risk areas covered in the farm health and safety management tool or program
Tools or services provided (thematic analysis)	Safety and health risk management features of services offered in the tool or program

The ChatGPT AI content generator was utilized to identify farm risk areas and tool features from the included OSH risk management tools for farms. The extracted results were added to a database for further content and thematic analysis of the types of risks addressed in the farm health and safety management tool or program is handling. The results found by the AI were checked by the authors on the tools' original web pages. The identified risk types were counted and added to the result table. Following this, a second round of queries was conducted using the AI ChatGPT search tool to identify, safety and health risk management features or services provided by the tool or program. Finally, thematic analysis was applied to define and categorize risk management themes within the safety and health risk management tools and services.

RESULTS

The volume of literature on general farm risk management with a focus on health and safety has significantly increased over the past ten years, as observed through a Google search (Fig. 1). In 2014, a search using the keywords 'farm health and safety risk management' and the exact phrase 'farm risk management' yielded 51 literature hits. By 2023, this number had grown to 242 hits. However, there are uncertainties with Google

searches, as the number of hits can fluctuate over time due to factors such as websites being closed or new ones opening. Additionally, marketing algorithms can influence search results, which should be taken into account when interpreting general Google search outcomes.

A Google search using the keywords 'farm agriculture health safety risk management tool' over the years 2014–2023 yielded 310,000 results. However, after sorting the results manually for relevance to Western-type farm or agriculture health and safety risk management, the relevant hits were limited to 170 items, and beyond that point, the relevant results diminished. In further analysis, only 50 of these items were actual safety and health risk management tools. The rest of the items were about food safety and risk management in other industries, which were excluded in the analysis.
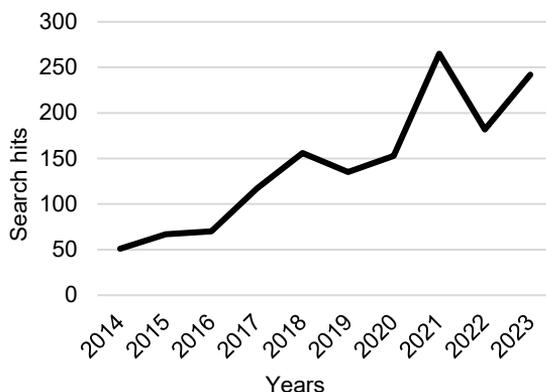
Figure 1. Numbers of farm health and safety and risk management literature in Google search in years 2014–2023.

Table 2. Literature search protocols and results

Source of the search[1]	Farm safety and health risk management search results (hits)	Included farm safety and health risk management tools
Google advanced search Keywords: farm, agriculture, health, safety, risk management, tool	190	50
Google scholar Keywords: farm, agriculture, health, safety, risk management, tool; exact phrase: farm risk management	24	4
Science Direct Keywords: farm, agriculture, health, safety, risk management, tool. 'Farm risk management' term in title, abstract or keywords. Focus on medical, agricultural and social subjects	125	4
Web of Science Keywords: farm, agriculture, health, safety, risk management, tools (All Fields) and Publication Years 2014–2023	34	4
Total	373	62

[1] Search criteria: Years included: 2014–2023/ English language programs, guidance materials, reviews and research articles/

Words in the text/ EU/western country related agriculture/farming / Searches made during March 2024 and May 2024.

Further database searches yielded results described in the following. Google scholar had 24 hits of which 18 concerned farms and only four selected literature hits concerned farm safety and health risk management issues. Science Direct had 125 hits, but only 12 concerned farm safety and health risks and only four were about managing these risks. Web of Science had 34 hits of which only five concerned farming and four were about occupational safety and health (OSH) risk management in practice. Some literature items were included in more than one search but included in the results only once. Altogether there were 62 health and safety risk management tool literature items included in the final analysis (Table 2).

Content analysis of the tools

The identified tools were analyzed and characterized based on several variables including country, service provider type, years active, registration requirements, tool level, administration type, number of users, annual costs for a user, farm safety and health risk factors addressed, and the tools or services included for farm safety and health risk management (Table 3). The majority of the literature hits came from English-speaking countries, particularly from Australia, USA and international. However, there were also contributions from non-English-speaking countries, which have been active in farm safety and health risk management research and publishing their findings in English.

Table 3. Content analysis results of the tools. The counted observation results are in brackets

Variables	Results
Country of origin	Australia (18), USA (13), International (8), New Zealand (7), Ireland (6), Canada (4), UK (3), Finland (2), Italy (1), France (1), Norway (1)
Service provider type	Public (34), Private (23), Public/Private (6)
Years active	Under 10 years (41), 10–20 years (8), Over 20 years (14)
Registration available or needed	Yes (32), No (31)
Tool level	Comprehensive farm OSH tool (40), General farm safety (4), Specific risk tool (19)
Administration	Farmer self- administrated (35), Consultant administration (15), Both farmer and consultant administration (13)
Number of users	N/A (53), 0–1,000 (1), 1,000–10,000 (5), over 10,000 (4)
Annual cost to users, participants	N/A information not available (8), totally free (31), partly free/partly cost (5): cost under 100 (5), cost range 100–1,000 (11), cost range 500–5,000 Euros (6)
Farm safety and health risk types	Machinery handling (52), Chemical handling (49), Livestock handling (44), Environment conditions (+ noise) (37), Slips, trips and falls (+heights) (36), Contamination (35), Emergency situations (25), Mental stress (24), Worker safety (23), Confined spaces (+ suffocation) (23), Family and child safety (9), Safety culture, behavior (8) Ergonomics, MSD's and lifting (7), Biological hazards and diseases (6), Fire (5), Communication, motivation (5), Electric installation (4), Visitor safety (3)

In Australia and New Zealand, many OSH tools are based on a small number of original public farm safety risk management guidebooks. These tools have been widely disseminated through effective stakeholder collaboration, spreading across various geographic areas and nations in the region. The diversity of service providers in these countries has played a significant role in promoting the tools and ensuring their adaptation and adoption (Table 3).

Half of the service providers were from public organizations, which also contribute funding to maintain the services. Both public and private organizations offer paid services, meaning farmers are required to pay for some aspects of the OSH services. Public and private organizations can also collaborate to provide joint services. The general pattern observed is that comprehensive self-managed risk management tools are typically free to download, often provided by government, research organizations, or private associations. These tools are designed for broad use by farmers. However, more specific OSH risk management tools and advisory services tend to be paid services. The more specialized the professional advisory services, the higher the cost.

Additionally, half of the services require or offer the option for registration to create an account. This allows farmers to store and manage farm-specific information, and use a systematic approach for managing risks. Registration and own data account could help in risk identification, handling and monitoring and documenting risk management activities over time.

The search and analysis revealed that there is only in few cases where information is available about the user numbers and service costs of the tools and services (Table 3). Most public and private services do not disclose the number of their users. One possible reason for this lack of transparency is that it may take years for service providers to accumulate user data and nearly 70 percent of the OSH risk management services in this study have been active for less than 10 years. An exception is SafeAgSystems in Australia, which has over 9,000 participants using their tools and website. It is also known that in Ireland, under the Safety, Health, and Welfare at Work Act (2005), farmers are required to complete a Risk Assessment Document or a Safety Statement. Nearly half of the farmers completed the risk assessment document in its initial implementation phase, based on a survey (McNamara, 2008).

Farm work involves numerous hazards and risks that can lead to injuries or ill-health to farmers and farm workers. Most OSH risk management program or service providers offer comprehensive, multi-risk management tools and reviews tailored to farms. The primary goal of these farm safety and health risk management tools and services is to prevent and address the most critical work hazards and risks present on farms. More than half of the service providers focus on managing risks related to the safe handling of machinery, chemicals, and livestock. The handling of machinery includes vehicles such as tractors, quad bikes, and combines, as well as other vehicles and field machinery, each with its own set of safety risks. Livestock safety varies depending on the type of animal being handled - whether they are cows, horses, pigs, poultry, or sheep. Common safety risks across all livestock types include animal dust, biological safety risks, skin and respiratory diseases, and ergonomic safety issues. These risks are relevant to all forms of livestock production and must be addressed to ensure farm workers' health and safety.

Farm worker safety risks and emergency situations are critical issues in risk management, gaining increased attention due to the new social conditionality rules within the European Union Common Agricultural Policy (CAP). These rules aim to improve the overall safety and health standards on farms. Work environment risks are also a key focus, encompassing safety hazards related to changing working conditions, such as temperature fluctuations, cold and warm weather, rain, and the various hazards in the work environment causing slips, trips, falls, struck by, struck against, burn, over-exertion and other types of injuries.

Traditional environmental health hazards on farms include noise, dust, mold, and airborne gases. However, increasing concerns about contamination risks from biological hazards - including the spread of zoonoses - are also emerging. These environmental factors are influenced by policy, social relationships, and the economic challenges faced by farmers, contributing to growing levels of mental stress. Consequently, mental stress has increasingly become a common component of the safety and health management systems provided to farmers, helping them cope with the growing psychological pressures tied to environmental, economic, and social challenges.

Specific risks had fewer risk management tools available, as they may apply to smaller number of farms. While fire risks apply to practically all farms, only five OSH risk management tools specifically address and manage fire risks, likely due to the separation of OSH and fire prevention fields. Some important safety concerns seem to be underrepresented in many farm safety risk management tools. For example, ergonomic and musculoskeletal disorder risks, which are prevalent due to repetitive physical tasks, are not always highlighted. Similarly, issues like poor safety culture, communication problems, and family and child safety are often excluded from available tools. These gaps suggest that while some risks are widely recognized, others may be neglected in the development of safety and health management frameworks for farms, even though they can have significant long-term impacts on farmers' and farm workers' well-being.

Thematic analysis of tools and services

Table 4 presents the results of the thematic analysis of the farm safety and health risk management tools or services identified from the collected data. These tools and services found are listed and categorized according to their function within the standard risk management framework (ISO 31000). The initial phase of establishing a risk management system includes tools for defining safety policies, strategies, objectives, resources, workforce, and activities. This phase may also encompass crisis management or emergency planning. The manager's commitment to safety is crucial for setting the farm's safety policy and fostering a safety culture (Worksafe Australia, 2024). Grimbuhler & Viel (2019) applied the safety climate scale to vineyard farm workers, which assesses safety climate by evaluating the safety culture through the organization's personnel safety practices and health management, revealing their attitudes, behaviors, and perceptions towards safety.

Table 4. Analysis results of the farm safety and health risk management tools

Tools and services	Risk management tool categories
• Safety policy, strategy or safety culture/ climate defined • Mapping activities, inventory, resources, workforce • Safety/crises management plan, objectives and methods defined	Farm safety and health management framework and setting tools
• Risk source or consequence, statistical/ survey analysis • Workplace or job safety analysis, risk/ blind spots analysis factor reviews, identification, checklists, inspections • Customize risk check for own farm, near miss analysis • Safety culture/ behavioral analysis, defining exceptional vents • Family safety check, vulnerability/ disability/ workload check • Risk analysis, evaluation, risk matrices, prioritization	Safety and health risk assessment, review, analysis tools
• Reading instructions, guidebooks, standards, laws, protocols • Improving skills, training, education, learn from videos/podcasts • Taking care of maintenance, fixing, spare parts • Availability of PPE's, relevant working clothes, first aid kits • Using PPE's, safety guards, proper use of working clothes • Eliminating safety risks, task/site development/ management • Taking care of insurances, back up or alternative funding • Have social network, relief workers, contractors available to help • Knowledge of risk handling methods and good practices • Taking care of scheduling, time to do work in safe manner • Making emergency plans, emergency/first aid training • Arrange time for free time, hobbies and well-being, stress handling • Have support available: professional, advisory and peer support • Taking are of cleaning, proper storages, clear walkways/roads • Consider ergonomic development, wearable safety technology	Risk handling tools
• Making risk development/monitoring plan on a farm • Doing schedules, seasonal safety monitoring, checking dates/times • Using remote monitoring, reporting measures, alarms, sensors • Have own/family educational records, worker records • Following news, regulations, research, markets, prices • Organizing documents, terms and contracts, smart software use	Risk monitoring tools
• Taking care of worker, family member, social communication • Doing worker, family member, visitor safety orientation • Getting and handling feedback, have farm safety forum • Use of media channels, networking, stakeholder communication • Use of mobile phones, messages, photos, videos, information board, information cards, clear instructions, materials, comic pictures, visualising for kids and foreigners, multilingual instructions, drones, cloud services	Communication tools

Safety and health risk assessment tools include those for risk identification, review, analysis, and evaluation. These tools aim to identify and document safety and health hazards and their potential consequences. Farm risk management tools often rely on statistics and checklists to identify common sources of farm injuries, such as machinery, livestock, and falls, as well as health hazards like chemicals, dust, and poor ergonomics. Other tools in this category include workplace or job safety analyses, child safety checks, and safety culture evaluations. Tools to assess the probability of risks and prioritize them,

such as risk matrices, also fall under this category. Additionally, tools like customized risk check templates and near-miss reports can be used to identify risks and improve safety awareness (Agricultural Safety and Health Training Portal; Certified Safe Farm; Canada FarmSafe, U.S. Dairy Excellence).

Writing, using and keeping operating instructions available are simple and effective risk management activities for every farm. However, it is often overlooked when using new machinery or handling chemical products in a rush. Exceptional situations that arise during tasks are often the most dangerous, so farmers should be especially cautious in these moments. Being aware of risks costs little and can make a significant difference. Other risk management tools include acquiring new skills, performing machinery maintenance, using appropriate personal protective equipment (PPE), eliminating safety risks, improving ergonomics, task management, scheduling work activities, having relief workers, managing stress, and maintaining clean walkways and work areas (Pork SA: Farm Safety Self-Assessment Guide; U.S. Dairy Excellence; Safe Ag Systems; Farmsafe Australia; Safety Champion).

While some of these tools may seem easy and simple, they can save lives in certain situations, and many do not incur high costs. Regular cleaning is an inexpensive yet effective form of risk management. There is no clear data on which tools are most popular or effective, as the key is to identify the right tools for each individual farm, since every farm is unique. While insurance doesn't prevent risks, it helps enhance farm resilience and ensures continuity in case of an accident. Newer risk management tools, such as the OIRA tool, offer farm-tailored risk management software that provides more detailed information for farm management. Additionally, this data can be utilized within stakeholder networks and for worker social conditionality management (OIRA, 2024).

Risk monitoring involves tracking activities and establishing a few clear measures to follow. Monitoring helps determine whether actions have been completed or not. The challenge lies in identifying the key development areas, selecting the most effective measures to track, and getting the monitoring process started. The list of monitoring tasks should be derived from the risk identification, risk assessment phase, or even the farm safety policy and objectives. New technologies, such as video cameras, smart document handling systems, and sensors, can assist farmers in these monitoring and management tasks (Country Wide Insurance; National Institute of Food and Agriculture (NIFA); Penn State Safety and Health Management Services and Tools; Safety Revolution).

Finally, risk management on a farm cannot be fully effective without communication among farm personnel. It is crucial to communicate safety issues clearly and in the right language to the farm family and workers. Common communication challenges include using outdated or incorrect information and a lack of effective communication. Gathering feedback from others and properly addressing it is also vital. Additionally, maintaining communication with neighbors and other stakeholders can provide valuable information on safety concerns and help foster a community safety culture on farms. Farm safety is largely dependent on how communication is handled and which messaging tools are used for communication (Worksafe Australia; Farm Health and Safety Management Self-Assessment Table, NZ; Safety & Health Guide for Manitoba Farms; Evans & Heiberger, 2015; Durst et al., 2018; Kilanowski et al., 2020; Leppälä et al., 2021).

DISCUSSION

This study gives an overview about safety and health risk management tools provided for farmers. The goal was to give information about different safety and health risk management systems and programs to develop safety and health risk management database for a farmer and consulting use. The overview compiled the results of a literature review, content analysis, and thematic analysis of existing farm safety and health risk management tools at the farm level. Google, Google Scholar, Science Direct and Web of Science search engines were used to search for farm safety and health risk management tools. The scientific databases, including Google Scholar, provided only a few results related to the practical use of farm safety and health risk management tools. Using Google search is not ideal for scientific literature searches due to the vast number of search results. However, the relevant search hits for this study decreased quickly, with no additional matches found after 170 hits. A list of the included tools can be found in Appendix One.

The literature on farm health and safety risk management has increased in recent years. The results were analyzed using variables such as country, service provider type, years active, registration requirements, tool level, administration type, number of users, annual costs per user, farm safety and health risk factors, and the tools or services provided for farm safety and health risk management. The study's findings highlighted many valuable safety and health risk management tools, along with potential services to enhance farm safety risk management.

Half of the service providers and a significant portion of the funding came from public organizations. This is justified, as the direct and indirect costs of farm injuries and diseases are ultimately paid by society and the primary food sector (Rautiainen et al., 2006; Adhikari et al., 2025). Self-managed risk management tools provided for farmers are typically free to download from public organization services and sources, which are then disseminated through advisory services. While comprehensive and general risk tools and checklists are often free to use, specific OSH risk management tools and advisory services for farmers usually come with a fee, ranging from 50 to 5,000 euros. The more specialized the professional advisory services, the higher the cost.

There is limited information available regarding user numbers and service costs for the tools. However, it is known that in Ireland, under the Safety, Health and Welfare at Work Act (2005), farmers are required to complete a Risk Assessment Document or a Safety Statement. According to Mohammadrezaei et al. (2024), nearly 70 percent of Irish farmers use the Irish farm safety code risk assessment document. Some private companies, such as Australian SafeAgSystems, report having nearly 10,000 users. This disclosure may also serve as a marketing strategy to highlight the widespread use and effectiveness of their safety risk management tools and services. It is also worth noting that most OSH risk management service providers have relatively young services or tools.

The main typical risk factors in agriculture addressed by these tools and services include machinery safety, livestock safety, chemical handling, and health hazard exposures such as animal dust, gases, noise, biological safety risks, and ergonomic issues causing skin and respiratory diseases. Farm worker safety risks and emergency situations are gaining more attention, especially in the light of the new EU social conditionality under the Common Agricultural Policy (CAP) regulations for farm employers (Vinci, 2024).

Environmental challenges are also highlighted in safety management, particularly concerning harsh weather conditions, rain and water, temperature fluctuations, and the increasing need for chemicals. Climate change-related risks require future awareness in production, asset, building, and economic risk management. These factors can contribute to stress and mental health issues among farmers, in addition to the traditional environmental health hazards present on farms. However, issues such as ergonomic and musculoskeletal disorders, safety culture, communication, and child safety should still be prioritized in farm risk management development. A list of top safety and health risk topics in farm safety and health management tools and services were:

- Safety and health risks on handling cows and horses
- Safety and health risks in farm machinery and vehicle use or maintenance
- Poor ergonomic and other work environment issues
- Poor chemical safety and storage arrangements
- Slips, trips and falls
- Safety and health risks for farm workers, poor farm worker orientation and management
- Risks caused by stress, work strain and mental health
- Poor electricity installations or other farm building asset maintenance
- Safety and health risks for farm children and farm family
- Lack of safety management and safety culture.

Thematic analysis results indicated that some tools emphasized mapping out safety management settings such as safety policy, strategies, objectives, resources, workforce, and activities. In management literature, it is often noted that a clear vision and well-defined objectives make it easier for a manager to follow a strategy (Strategic Planning, 2009). Risk management is not solely about evaluating risks; it also involves identifying positive opportunities. In essence, effective risk management should distinguish between 'good' risks and 'bad' risks, ultimately leading to better decision-making and management choices (Rasmussen, 1997).

In complex world of risk management tools in general, checklists have proven their effectiveness and quality. When a farm is considered as a complex unit, safety checklists or other risk review tools support the farm manager's job by providing a structured way to evaluate potential risks. This study found that most farm safety management planning guides incorporate checklists, but worksite or job safety analyses are also valuable safety assessment tools.

However, one challenge is that comprehensive checklists have often become too lengthy, which can make them for farmers overwhelming or difficult to use effectively. By tailoring these checklists to the specific needs and conditions of their farm, farmers can create more focused, efficient, and practical tools for managing safety risks. Some services provide paid consultants for doing customized risk reviews for farms. Some tool services have also templates, which guide farmers to make their own customized checklist. To these templates farmers could add issue measures for some activities or workplaces, which they have found risky. However, they may still need advisory support to customize and follow the safety and health risks

Farmers sometimes may claim that managing safety risks is difficult and expensive, but that's not always the case. One of the simplest and most effective risk handling tools is often overlooked: read the manual instructions. For example, reading the guidelines for using a chemical product or taking the time to understand the safety features of new

machinery can prevent accidents. Other simple practices, like not jumping from a tractor or being extra cautious when something unexpected happens, can also make a huge difference. These actions are often forgotten in the rush to get things done, but taking the time to read the instructions or plan ahead is often the quickest and most effective way to get the job done. The best part is, it doesn't cost anything - just a bit of attention and mindfulness before starting a task. And it does not cost much but just thinking.

Risk management in factories focuses on controlling workplace safety conditions (Reason, 1997). However, this becomes more challenging on a farm, where the work environment is constantly changing - whether due to unpredictable outdoor conditions in the fields or the behavior of livestock inside the animal house. Unlike a factory setting, where the environment is more controlled, managing farm safety culture requires a different approach (Leppälä, 2016; Leppälä et al., 2021). If it's not possible to control every factor, the next best solution is to establish clear routines and procedures for how tasks are done. By creating structured, reliable routines, farmers can mitigate the risks posed by these unpredictable elements and ensure safety on the farm, even when conditions are beyond their control.

Following few essential risk measures on a farm serves as a monitoring tool. The advantage of monitoring is that if you have a clear schedule, protocol or checklist to follow, you are more likely to accomplish your safety goals. The biggest challenge, however, is getting started and identifying the areas that need development. The list of monitoring tasks should stem from the farm's management safety policy, objectives, and risk assessment findings. By having these structured guidelines in place, farmers can more effectively track and address safety issues over time.

Effective communication is crucial for creating an organized and safe farm environment. The exchange of information differs between stakeholders, media representatives, and internal farm communications. From a communication perspective, a modern farm should be viewed as an organization. One common mistake made by managers is listening to worker feedback but failing to act on it. This can lead to frustration and missed opportunities for improvement.

The development of new digital tools and online services has greatly enhanced risk management in various industries, including agriculture. Online software services are becoming more prevalent and efficient, enabling a broader range of risks and issues to be addressed in a more organized manner. These intelligent programs can process larger amounts of risk management information, making the system more efficient. The use of media communication tools, such as videos and audio products, could also play a greater role in farm safety risk management, offering new and diverse ways to educate and raise awareness. Using various methods for learning is expected to be beneficial for farmers' learning and knowledge development.

However, despite the growth of technological innovation, the basic need for strong communication skills and regular feedback remains essential between farm workers, farmers, and the broader farm community. The new technologies should be integrated into traditional farm management tasks. For instance, carrying a fully charged mobile phone in the field work or forest should be a standard practice for every farmer and farm worker, but also not to forget the old practices like keeping bottle of drinking water and a first aid kit on the tractor. Another future challenge among farmers is to cope with the stress caused by work activities, market, policy and environmental changes. The numerous shifts occurring in agriculture may increase the risk of burnout,

particularly given the demanding nature of farmers' work (O'Shaughnessy et al., 2022; Geidelina-Lugovska & Cekuls, 2025). Added to this, human behavioral factors, work ability, skills and safety culture development on farms are coming more and more important in the future (Leppälä et al., 2021; Lezdkalne, 2025; Mattila et al. 2025).

CONCLUSIONS

The development of farm safety and health risk management has progressed significantly in recent years. However, these tools and activities often do not become part of the everyday routine for every farmer and farm worker unless there is systematic support for these management practices among farmers and their stakeholders. The key questions driving the background of farm safety and health risk management still remain:

1. How are farm safety and safety culture developing (e.g., are accidents decreasing)?

2. How are farmers' skills in safety and health management improving?

3. How can farmers effectively use safety and health management tools (including new digital tools and devices) to enhance farm safety?

If a safety guide, personal protective equipment (PPE), safety device, policy rule, software, video, or any other safety tool prevents even one injury or saves a single life, the return on investment has been paid back. The value of these tools is not just in their initial cost, but in the long-term benefits they bring by reducing accidents, injuries, and fatalities, thereby ensuring safer working conditions for farm workers and improving overall farm safety.

REFERENCES

Adhikari, S., Wilson, F. & Rautiainen, R. 2024. Cost of agricultural injuries in the United States: Estimates based on surveillance, insurance, and government statistics. *Am. J. Ind. Med*. **67**, 801–812. doi:10.1002/ajim.23628

Aloini, D., Dulmin, R. & Mininno, V. 2007. Risk management in ERP project introduction: Review of the literatuure. *Information & Management* **44**, 547–567. doi: 10.1016/j.im.2007.05.004

BLS. Census of Fatal Occupational Injuries. 2025. Number and rate of fatal work injuries, by private industry sector, 2023. Available at: https://www.bls.gov/charts/census-of-fatal-occupational-injuries/number-and-rate-of-fatal-work-injuries-by-industry.htm (Accessed May 25, 2025).

Cecchini, M., Bedini, R., Mosetti, D., Marino, S. & Stasi, S. 2018. Safety Knowledge and Changing Behavior in Agricultural Workers: an Assessment Model Applied in Central Italy. *Safety and Health at work* **9**(2), 125–238. doi.org/10.1016/j.shaw.2017.07.009.

Cook, A., Fry, R., Desai, Y., Swindall, R., Boyle, J., Wadle, C., Casanova, V., Cloonan, M., Rogers, F., Horst, M., Morgan, M. & Norwood, S. 2022. Agricultural Injury Surveillance Using a Regional Trauma Registry. *J. Surg Res*. **273**(May), 181–191. doi: 10.1016/j.jss.2021.11.018

COSO. 2004. Enterprise risk management – Integrated framework. Application Techniques. Committee of sponsoring Organizations of the Treadway Commission. September 2004. Durham. USA, 105 pp.

Daft, R. 2015. *Management*. South-Western College Pub; 12 edition, 800 pp.

Donham, K.J. & Thelin, A. (ed.) 2006. *Agricultural Medicine: Occupational and Environmental Health for the health professions*. Blackwell. 429 pp.

Durst, PT., Moore, S.J., Ritter, C. & Barkema, HW. 2018. Evaluation by employees of employee management on large US dairy farms. *J. Dairy Sci.* **101**(8), 7450–7462. doi: 10.3168/jds.2018-14592

Eastwood, R., Lipton, M. & Newell, A. 2010. *Farm size*. In: R. Evenson and P. Pingali (eds.) *Handbook of Agricultural Economics IV*, Oxford, UK: North-Holland, Elsevier, pp. 3323–3397.

Elo, S. & Kyngäs, H. 2008. The qualitative content analysis process. *Journal of Advanced Nursing* **62**(1), 107–115. doi: 10.1111/j.1365-2648.2007.04569.x

Eurostat. 2024. Statistics. Published in internet: http://epp.eurostat.ec.europa.eu/portal/page/portal/statistics/themes

EU 2019/1152. 2019. Directive (EU) 2019/1152 on transparent and predictable working conditions in the European Union. The European Parliament and the Council of the European Union.

Evans, J. & Heiberger, S. 2015. Fitting Farm Safety into Risk Communications Teaching, Research and Practice. *Journal of Applied Communications* **99**(3), doi: 10.4148/1051-0834.1060.

Frank, A., McKnight, R., Kirkhorn, S. & Gunderson, P. 2004. *Annu. Rev. Public Health* 2004. **25**:225–45. doi: 10.1146/annurev.publhealth.25.101802.123007

Geidelina-Lugovska, M. & Cekuls, A. 2025. Organizational identification, workaholism, and burnout: a cross-sectional study of age, gender, tenure, and work arrangements. *Agronomy Research* **23**(S1), 45–56, 2025. https://doi.org/10.15159/AR.25.016

Goetsch, D.L. 2015. *Occupational Safety and Health for Technologists, Engineers, and Managers*. 8th Edition, Pearson Education Limited, Harlow,715 pp.

Grimbuhler, S. & Viel, J.F. 2019. Development and psychometric evaluation of a safety climate scale for vineyards. *Environ Res.* **172**(May), 522–528. doi: 10.1016/j.envres.2019.03.007

Harwood, T. & Garry, T. 2003. An Overview of Content Analysis. *The Marketing Review* **3**(4), 479–498.

Hardaker, J.B., Huirne, R.B.M., Anderson, J.R. & Lien, G. 2004. *Coping with Risk in Agriculture*. Cabi Publishing. UK, 332 pp.

Health & Welfare at Work Act 2005. https://www.hsa.ie/eng/Topics/Managing_Health_and_Safety/Safety,_Health_and_Welfare_at_Work_Act_2005/ HSA

ILO. 2019. Statistics and databases. https://www.ilo.org/global/statistics-and-databases/lang--en/index.htm.

ISO 31000. 2018. Risk management – Principles and Guidelines. 2018-11-15.

Jadhav, R., Achutan, C., Haynatzki, G., Rajaram, S., & Rautiainen, R. 2015. Risk Factors for Agricultural Injury: A Systematic Review and Meta-analysis. *J. Agromedicine* **20**(4), 434–49. doi: 10.1080/1059924X.2015.1075450

Jadhav, R., Lander, L., Achutan, C., Haynatzki, G., Rajaram, S., Patel, K., & Rautiainen, R. 2016. Review and Meta-analysis of Emerging Risk Factors for Agricultural Injury. *Journal of Agromedicine* **21**(3), 1–14. http://dx.doi.org/10.1080/1059924X.2016.1179611

Juran, J.M. & Godfrey, A.B. 1998. Juran's quality handbook. New York. McGraw -Hill. 1872p.

Karttunen, J. 2014. *Clustering of occupational injuries, diseases and disability in Finnish farmers - an opportunity for targeted prevention*. PhD Thesis. Department of agricultural sciences. University of Helsinki. Helsinki. http://urn.fi/URN:ISBN:978-952-10-8881-0

Kilanowski, J.F.2020. Agricultural Safety Comic Book for Latinx Migrant Families: Development and Evaluation. *J Pediatr Health Care*. 2020 May–Jun;**34**(3), 230–238. doi: 10.1016/j.pedhc.2019.11.003

Kirch, W. (eds). 2008. *Encyclopedia of Public Health*. Springer, Dordrecht. doi: 10.1007/978-1-4020-5614-7_3038

Kjestveit, K., Aas, O. & Holte, K.A. 2021. Occupational injury rates among Norwegian farmers: A sociotechnical perspective. *J Safety Res.* **77**(Jun), 182–195. doi: 10.1016/j.jsr.2021.03.001

Laurent, C. & Nguyen, G. 2022. Innovation in Labour Organisation and Social Conditionality: Implications for Farm Advisory Services. *EuroChoices* **21**(1). doi: 10.1111/1746-692X.12350

Leonard, J. 2015. Stress Management in Farming in Ireland. A report for NUFFIELD IRELAND Farming Scholarships. 47 pp.

Leppälä, J., Griffin, P., McNamara, J. & Rautiainen, R. (eds.). 2021. *Safety Culture and Risk Management in Agriculture: Sacurima Cost Action CA16123.* Highlights and Conclusions. Natural resources and bioeconomy studies 63/2021. Natural Resources Institute Finland. Helsinki, 81 pp.

Leppälä, J. 2016. *Systematic Risk Management on Farms.* PhD Thesis. Department of Industrial Engineering and Management. Aalto University publication series 17/2016. Espoo. Finland, 130 pp.

Leppälä, J., Kolstrup, CL., Pinzke, S., Rautiainen, R., Saastamoinen, M. & Särkijärvi, S. 2015. Development of a Safety Management Web Tool for Horse Stables. *Animals* (Basel). **5**(4).1136–46. doi: 10.3390/ani5040402

Leppälä, J., Kauranen, I. & Rautiainen, R. 2013. Effectiveness of occupational health service programmes in farmers' safety and security risk management. *International Journal of Agricultural Management* **2**(3), 130–140. doi: 10.5836/ijam/2013-03-02

Leppälä, J., Murtonen, M. & Kauranen, I. 2012. Farm risk map: a contextual tool for risk identification and sustainable management on farms. *Risk Management* **14**(1), 42–59. https://doi.org/10.1057/rm.2011.14

Lezdkalne, J. 2025. Integrating human factors into occupational accident investigation: a literature review of methodologies and their application. *Agronomy Research* **23**(S1), 93–108. https://doi.org/10.15159/AR.25.036

Lipari, M. & Watson, H. 2019. Check your blind spots: 360° of farm risk management. AgEcon Search. *22nd International Farm Management Congress.* Grand Chancellor Hotel, Launceston, Tasmania, Australia. pp. 1–18.

Mattila, T.E.A., Liski, E., Väre, M. & Rautiainen, R.H. 2025. Diminished work ability as a contributing factor for farmer's interest in switching to organic production. *Agronomy Research* **23**(S1), 134–150. https://doi.org/10.15159/AR.25.019

McNamara. 2008. Evaluation of strategies to assist farmers to implement safety and health requirements in Ireland. Available at: https://www.hsa.ie/eng/Your_Industry/Agriculture_Forestry/Research/Evaluation_Strategies.pdf?utm_source=chatgpt.com.

McNamara, J., Kinsella, A., Osborne, A., Blake, C., Meredith, D. & Kinsella, J. 2020. Identifying Farmer Workplace Injury Risk Factors in Ireland Using Farm Accounts Data. *J. Agromedicine* **26**(4), 411–419. doi: 10.1080/1059924X.2020.1837704

McNamara, J.G.A. 2014. *Study of the Effectiveness of Risk Assessment and Extension Supports for Irish Farmers to Improve Farm Safety and Health Management.* UCD School of Agriculture and Food Science, 360 pp.

Merisalu, E., Leppälä, J., Jakob, M. & Rautiainen, R. 2019. Variation in Eurostat and national statistics of accidents in agriculture. *Agronomy Research* **17**(5), 1969–1983, doi: 10.15159/ar.19.190

Mohammadrezaei, M., Meredith, D. & McNamara, J. 2024. Counting Farm Injuries and Fatalities: An Assessment of Irish Occupational Health and Safety Surveillance Data Systems. *Journal of Agromedicine* **29**(2), 289–296. doi: 1080/1059924X.2024.2317862

Nedelko, Z. 2021. What Drives the Usage of Management Tools Supporting Industry 4.0 in Organizations? *Sensors* **21**, 3512. doi: 10.3390/s21103512

NIOSH. 2024. Hierarchy of controls. Available at: https://www.cdc.gov/niosh/hierarchy-of-controls/about/index.html

O'Shaughnessy, B., Hagan, A.D., Burke, A., McNamara, J. & O'Connor, S. 2022. The prevalence of farmer burnout: Systematic review and narrative synthesis. *Journal of Rural Studies* **96**. doi.org/10.1016/j.jrurstud.2022.11.002

Rasmussen, J. 1997. Risk management in a dynamic society: A modelling problem. *Safety Science* **27**(2–3), pp. 183–213. doi: 10.1016/S0925-7535(97)00052-0

Rautiainen, R., Ledolter, J., Ohsfeldt, R., Donham, K. & Zwerling, C. 2009. Risk Factors for Serious Injury in Finnish Agriculture. *Am. J. Ind. Med.* **52**, 419−428. doi: 10.1002/ajim.20688

Rautiainen, R.H., Ohsfeldt, R., Sprince, N.L., Donham, K.J., Burmeister, L.F., Reynolds, S.J., Saarimäki, P. & Zwerling, C. 2006. Cost of Compensated Injuries and Occupational Diseases in Agriculture in Finland'. *Journal of Agromedicine* **10**(3), 21−29. doi: 10.1300/j096v10n03_03

Reason, J. 1997. *Managing the risks of organizational acidents*. Aldershot, England: Ashgate Publishing ltd., 252 pp.

Reinvee, M. 2024. SafeHabitus. Deliverable D3.1 Farm safety in the EU: Which injuries, and who is counted? Estimating the number of farm accidents and fatalities in the EU. Available at: https://www.safehabitus.eu/wp-content/uploads/2024/08/SafeHabitus_D3.1_Farm-safety-in-the-EU.pdf.

Rodriguez, A., Lopez, S.N. & Douphrate, D.I. 2023. On-Farm Health Screening Needs of Immigrant Dairy Workers in the Texas Panhandle and South Plains. *J. Agromedicine* **28**(4), 665–675. doi: 10.1080/1059924X.2023.2200418

Safehabitus. 2023. Strengthening farm safety and health knowledge and innovation systems. Website. https://www.safehabitus.eu/

Sargeant, K. & Paine, M. 2015. Building a robust capability framework to face the fast-growing challenges of the New Zealand dairy industry. *Rural Extension and Innovation Systems Journal* **11**(1), p. 210–217.

Scarborough, N.M., Wilson, D.L. & Zimmerer, T.W. 2009. *Small Business Management: An Entrepreneurial Approach* (9th ed.). New Jersey: Prentice Hall. Jersey: Pearson Education, Inc, Upper Saddle River, 912 pp.

Schuh, B., Gaupp-Berghausen, M., Münch, A., Badouix, M., Hat, K., Brkanovic, S., Dax, T., Machold, I., Schroll, K., Juvančič, L., Erjavec, E., Rac, I. & Novak, A. 2022. *Research for AGRI Committee - The Future of the European Farming Model: Socioeconomic and territorial implications of the decline in the number of farms and farmers in the EU*. European Parliament, Policy Department for Structural and Cohesion Policies, Brussels. doi:10.13140/RG.2.2.15638.63040

SFS-EN ISO 4500:2023. *Occupational health and safety management systems. with guidance for use*. Finnish Standards Association SFS. Helsinki, 97 pp.

Strategic Planning: Why It Makes a Difference, and How to Do It. 2009. *J. Oncol Pract*. **5**(3), 139–143. doi: 10.1200/JOP.0936501

Suutarinen, J. 2004. Management as a Risk Factor for Farm Injuries. *Journal of Agricultural Safety and Health* **10**(1), 39–50. doi: 10.13031/2013.15673

Vaismoradi, M., Turunen, H. & Bondas, T. 2013. Content analysis and thematic analysis: Implications for conducting a qualitative descriptive study. *Nursing and Health Sciences* **15**, 398–405.

Vinci, C. 2024. Improving EU farmers' and agricultural workers' working conditions and mental well-being. In *AT A GLANCE Plenary*. EPRS | European Parliamentary Research Service. PE 767.154 – December 2024.

Worksafe Australia. 2024. Farm safety checklist. Available at: https://www.worksafe.wa.gov.au/system/files/migrated/sites/default/files/atoms/files/farm_safety_checklist_2021.pdf.

APPENDIX 1. Tools included in the study:

Tools	Organisation/Source
Safety management software for agribusiness	SafeAgSystems
Risk Management Tools	Farmsafe
Safety culture and risk management in agriculture	Luke
Safety Champion Agriculture Safety Management Software |	Safety Champion
A Comprehensive Guide to Farm Safety	SafetyCulture
Farm health and safety management self-assessment table	Worksafe
Farmwise - Your essential guide to health and safety in agriculture	Farmwise, HSA, UK
Farm Safety Code of Practice - Risk Assessment Document	HSA, Ireland
Agriculture - Managing risks	Safe Work Australia
Health and Safety on Farm - Risk management	Beef and Lamb New Zealand
Farm risk map - Risk assessment tool for farms	LUKE
Farm safety - risks and hazards	Better health channel
Resources for farmers	AgHealth Australia
Farmers' Guidebook to work health and safety	Safework SA
Farm safety management plan	Beef and Lamb New Zealand
Safety in Farming and Agriculture	WHS Systems
FARM SAFETY SELF ASSESSMENT GUIDE	Pork SA
A guide to developing safety management systems	Worksafe
5 common farm hazards and how to manage them	Onside
Farm safety self-assessment	NSW Government
Child Safety	Gov. Ireland
Online interactive risk-assessment (OiRA)	EUOSHA
4 steps to manage hazards and risk	Work Safe, Tasmania
Farm buildings, equipment and environment	Gov. Ontario
Manage the risks of machinery in your rural workplace	Country Wide Insurance
Farm safety and health	NDSU
Work health safety (WHS) plan for farm	Comcare
SAFETY & HEALTH GUIDE FOR MANITOBA FARMS	Manitoba
Work, Health and Safety	Agforce, Australia
Agricultural safety software	Ideagen
AgrAbility, US	NIFA
Agricultural Safety Health Training Portal, US	Iowa, Great Plains
Safety and Health Management Planning for General Farmers and Ranchers, US	Pennstate
A Guide to Your Farm Safety Plan	Nova Scotia
Canada FarmSafe Plan, Canada	Casa
Certified Safe Farm (CSF), US	Iowa University
Risk management programmes for farm dairies, New Zealand	MPI
Essential farm safety practices, Australia	Digi Clip
Farm safety videos, US	Dairy Excellence
Farm safety check, US	UMASH
Ensuring Child Safety on Farms, Australia	ProcessWorx
Farm health and safety, UK	Safety revolution
Farm Safety: Risk Management, UK	NFU
Managing chemical risk in the agriculture sector	ILO
The Farm Safety, Health & Wellness Toolkit	VCE
Farm safety advise, Ireland	FBD
Health of farmers videos, Ireland	Teagasc

Agritourtism Safety & Risk Management, US	UVM
Vision Zero: Guide for individual farmers	ISSA
Advisory safety expert service	Ag health and safety alliance
Fitting farm safety into risk communications teaching, research and practice	Google Scholar; Evans & Heiberger, 2015
Stress Management in Farming in Ireland	Google Scholar; Leonard, 2015
On-Farm Health Screening Needs of Immigrant Dairy Workers in the Texas Panhandle and South Plains	Google Scholar; Rodriguez et al., 2023
Npr-Check Your Blind Spots: 360â° Of Farm Risk Management	Google Scholar; Lipari & Watson, 2019
Agricultural Injury Surveillance Using a Regional Trauma Registry	Science Direct: Cook et al., 2022
Agricultural Safety Comic Book for Latin Migrant Families: Development and Evaluation	Science Direct: Kilanowski, 2020
Occupational injury rates among Norwegian farmers: A sociotechnical perspective	Science Direct: Kjestveit et al., 2021
Safety Knowledge and Changing Behavior in Agricultural Workers: an Assessment Model Applied in Central Italy	Science Direct: Cecchini et al., 2018
A new web tool for equine activities. The safety section contained a safety checklist, stable safety map and good practices to support human health	Web of Science; Leppälä et al., 2015
Evaluation by employees of employee management on large US dairy farms	Web of Science; Durst et al., 2018
Development and psychometric evaluation of a safety climate scale for vineyards:	Web of Science; Grimbuhler & Viel, 2019
Building a robust capability framework to face the fast-growing challenges of the New Zealand dairy industry	Web of Science; Sargeant & Paine, 2015

Advancing precision agriculture: a case study of open source autosteering with AgOpenGPS and RTKbase

P. Linna[*] and H. Haapala

Jamk University of Applied Sciences, Jamk Institute of Bioeconomy, Tuumalantie 17, FI 43130 Tarvaala, Finland
[*]Correspondence: petri.linna@jamk.fi

Abstract. Precision agriculture increasingly relies on Real-Time Kinematic (RTK) services to perform highly accurate tasks in the field. Robotics are gradually entering farming, demanding precise and reliable correction signals. However, before widespread adoption of autonomous field robots becomes a reality, automated tractors will remain in use for a significant period, becoming progressively more advanced.

The market is currently filled with various manufacturers offering aftermarket autosteering systems, which incrementally bring farmers closer to the functionality of fully autonomous field robots. This study explores open-source solutions for cost-effective autosteering systems and RTK base stations. The project involved retrofitting a single farmer's tractor with an autosteering system and establishing an RTK base station.

As the pilot progressed, word of the implementation spread, leading to the creation of a dedicated communication channel for interested farmers. This platform has facilitated knowledge sharing and further adoption. Information about the project also reached other regions, inspiring similar initiatives that have significantly increased the number of RTK base stations in just two years.

The results of this project demonstrate a strong demand for alternative solutions. Many farmers lack the financial resources to invest in expensive, proprietary systems or are unwilling to commit to recurring subscription fees. The goal remains the same, regardless of the implementation method, agriculture is moving steadily toward smarter, more precise practices and the eventual adoption of field robotics.

Key words: AgOpenGPS, autosteering, open source, precision agriculture, RTK2Go, RTKBase.

INTRODUCTION

Agriculture is becoming increasingly robotized. Cheng et al. (2023) discuss as many as fourteen different agricultural robots, illustrating that field robotics is just one part of the broader spectrum of agricultural automation. In this context, automating tractors serves as an ideal starting point, offering a valuable opportunity for skill development before the arrival of more advanced robotic systems. Many of these robots will also require precise navigation, making the construction of a local RTK base station a cost-effective and accurate solution.

The future of agriculture will involve multiple technological development sectors, such as robotics and artificial intelligence, but localization and navigation will remain core components among other advancements, as Kisliuk et al. (2023) discusses. This observation supports the idea that developing an autosteering system is not only beneficial for immediate use but also lays the groundwork for future data collection and data-driven decision-making processes.

The market is filled with commercial autosteering solutions, which signals the significant market potential recognized by industry players. However, open-source alternatives provide a cost-effective option, particularly for farmers who prefer a do-it-yourself approach or whose investment budgets are already allocated elsewhere.

Currently, agriculture in Finland is struggling with profitability, making efficiency improvements vital for sustainability. Autosteering systems can contribute to this by reducing overlap during fieldwork, minimizing unnecessary turns, and allowing farmers to focus more optimizing the work machine (Bora et al., 2012).

As automation increases and the variety of agricultural equipment expands, precise positioning will be crucial for new applications, such as drones. Agricultural drones are becoming as common as traditional machinery, used for tasks such as spraying crops, detecting invasive species, and generating NDVI maps (Lipping et al., 2020; Linna et al., 2024).

This paper focuses on a case study of a farmer-led implementation of a retrofitted open-source autosteering system AgopenGPS (AgOpenGPS, 2025a) and an RTKbase (RTKbase, 2025) base station, demonstrating the feasibility and broader implications of these solutions. The study highlights both the technical and practical challenges encountered throughout the process.

MATERIALS AND METHODS

Method

Case studies play an important role in the development of farming systems and agribusiness research, as highlighted by Klein & Knight (2005) and Vanclay et al. (1998). This research is an engineering case study that combines constructive research with empirical field experiments. The study investigates open-source solutions for implementing a retrofitted autosteering system and the necessary RTK base station for correction signals.

We hypothesize that open-source solutions can offer a competitive alternative to commercial systems, thereby enabling a transition to precision agriculture with lower investments. If this hypothesis holds true, more farms will be able to adopt precision agriculture. While some users may eventually shift to commercial solutions and new users will join the open-source community, the overall use of open-source technologies is expected to increase the adoption of precision farming practices on Finnish farms. This outcome aligns with the primary objective of the study.

This case study was conducted on a mid-sized farm in western Finland, using a CASE 7220 series tractor without factory-installed autosteering capabilities. The farmer had a strong interest in precision farming and aimed to develop the farm cost-effectively by investing personal time and effort into the process. The project was initiated independently and carried out during the 2022–2024 seasons, with support from peer

networks and publicly available open-source resources. The implementation included building an RTK base station and integrating an AgOpenGPS-based autosteering system tailored to the farm's specific operational needs. The case represents a realistic example of how individual farmers can adopt precision agriculture tools with limited budgets and technical resources.

Searching and analyzing material

The research began with collecting relevant material on the topic. The primary sources were the AgOpenGPS websites (AgOpenGPS, 2025a; 2025b; 2025c) and other related documentation. While some additional sources were consulted, their overall significance was minimal. One particularly valuable practical resource was YouTube videos, which provided examples of various implementations. RTKbase material was mainly from Github account (RTKbase, 2025).

One of the biggest challenges was the inconsistency of available information. The software of AgOpenGPS was in an intense development phase, leading to numerous conflicting instructions and implementations. As a result, forming a comprehensive understanding of the system took considerable time in the beginning. This can, on the other hand, be seen as typical of open-source solutions, where the implementation process has not been given the same level of attention as in commercial systems.

Orders and building

After defining the implementation plan, printed circuit boards (PCBs) were ordered from circuit board manufacturing (JLBPCB, 2025) factory. Their delivery took some weeks, after arrival, there were plenty of boards for testing. Ordering individual components proved to be a complex process. Since no single supplier carried all the necessary parts, they had to be sourced from multiple vendors.

Additionally, there were significant supply chain issues with the Raspberry Pi due to global production challenges, likely caused by COVID-related manufacturing disruptions. Eventually, all the required hardware was successfully acquired, although a few incorrect components were ordered, and in some cases, there was an excess of certain parts.

Once all components were available, the soldering of the circuit boards began.

Software tools

After assembling the circuit boards, the software configuration phase began. This phase also took a considerable amount of time, as multiple software components and settings had to be studied, some of which were dependent on specific hardware versions.

The required software included:

- u-Blox (u-Blox, 2025) – Used to install the software and configure settings for the GPS module
- Command Prompt (Terminal) – Used for installing RTKBase
- Raspberry Pi Imager (Raspberry Pi Foundation, 2025) – Used to install the operating system onto the Raspberry Pi's memory card
- Arduino IDE (Arduino, 2025) – Used to upload software and configure settings for the board

- RTKBase (RTKbase, 2025) – The software running on the RTK base station
- RTK2go Service (RTK2go, 2025) – Used for distributing the correction signal
- AgOpenGPS Software (AgOpenGPS, 2025a) – The autosteering program. Contained all the necessary files for the autosteering system and related hardware configurations.

RTKBase - RTK base station

With the circuit boards assembled and the necessary software and configurations in place, the actual construction process began with the simplest task, building the RTK base station. The required components included the simpleRTK2 GNSS board with the u-blox ZED-F9P GNSS module (ArduSimple, 2025), Raspberry Pi and GNSS antenna.

The installation was relatively straightforward. The components were connected, the memory card was inserted into the Raspberry Pi, and the device was powered on. A connection to the Raspberry Pi was then established using the command prompt, where the following commands were executed:

- wget https://raw.githubusercontent.com/Stefal/rtkbase/master/tools install.sh -O install.sh
- chmod +x install.sh
- sudo./install.sh --all release.

This command sequence installed the RTKBase software, which serves as the main base station program. The installation also created a web-based configuration page, allowing users to monitor the system status, including the number and signal strength of satellites. Additionally, the page provided settings for defining services such as the NTRIP service.

We used RTK2go (RTK2go, 2025), a free service for distributing correction signals, which allows anyone to publish their own RTK signal and access publicly available signals. The registration details for the RTK2go service were entered into the base station configuration.

Accurately determining the base station's location is essential for transmitting correct correction signals. The base station included a feature for logging GNSS signals over a specified period. In our case, signals were logged for 24 hours to ensure accuracy. The resulting RINEX file was uploaded to the Canadian Geodetic Survey's GSRS-PPP service (Natural Resources Canada, 2025), which calculated the precise coordinates of

Figure 1. The layout of the RTK base station.

the base station. These coordinates were then entered into the base station configuration. After this step, the NTRIP service was activated, and the base station began transmitting RTK correction signals to the RTK2go service, making them publicly available for use. The base station is ready for wall installation, as shown in Fig. 1

AgOpenGPS - Retrofitting Autosteering

After setting up the base station, the more challenging part began: connecting the autosteering components, installing the necessary software, configuring the system, and conducting field tests.

The key components included a steering angle sensor for measuring the wheel position, GNSS antenna and its circuit board, hydraulic control unit, Windows-based laptop, USB hub and main control module.

Installing the steering angle sensor proved structurally challenging, as it needed to measure the angle of the front wheel accurately. The sensor was mounted on the front axle to measure the angle of the left wheel joint. For protection, a 3D-printed housing was used as seen in Fig. 2.

Figure 2. The angle sensor with protective case.

The GNSS antenna was mounted on the edge of the tractor's roof, as close to the tractor's center as possible along both the length and width axes.

Several options were available for controlling the steering wheel, including using a roller motor on the wheel, a center motor on the wheel, an electronic orbital steering unit, or a custom hydraulic control system. We chose the hydraulic solution, due to its technical benefits and alignment with our implementation goals. This setup involved cutting the hydraulic hoses between the orbital valve and the wheels and inserting a custom-made hydraulic control unit (Fig. 3). The box received control signals from the AgOpenGPS control unit.

Nowadays, ready-made electronic hydraulic control units are also widely available (Fig. 4).

Figure 3. The hydraulic control unit in this research case.

After completing the hydraulic system, the most challenging tasks were building the main control box and GNSS box. For the GNSS box, the u-blox configuration utility was used to install the firmware and configure the necessary settings for the simpleRTK2 board. The firmware for the control box was installed on the Teensy board using the Arduino IDE. This process required installing the correct drivers for the Teensy and

ensuring the right software version was selected in the Arduino IDE. Initially, there were significant communication issues between the GNSS board and the control box due to an incorrect baud rate, which prevented data transmission. Once the configurations were completed, the devices were connected to the Windows laptop via a USB hub. At this stage, the software and hardware setup were fully operational (Fig. 5).

Next, tractor-specific configurations were completed in the field, such as adjusting the turning radius and calibrating the system to match the tractor's characteristics. After several steps and fine-tuning, the system was successfully operational.

During practical use, issues began to arise with the USB connection, which would occasionally disconnect unexpectedly. It was discovered that the COM connection via USB experienced small, intermittent disconnections for unknown reasons. Similar issues had been reported by other users of the same implementation model.

To address this, the control box was replaced with an all-in-one board solution that used Ethernet connectivity instead of USB. The new all-in-one board integrated the GNSS functionality, eliminating the need for a separate GNSS board. The ZED-F9P GNSS module and the position sensor were both directly integrated into the new board (Fig. 6). Although it required learning a new implementation, the installation was completed relatively quickly. The new setup proved more reliable and streamlined, making it a significant improvement over the initial configuration.

Figure 4. The commercial hydraulic control unit.

Figure 5. On the left is the 2nd version with All-in-One board including also GNSS. In the middle is the 1st version of board. On the right is the 1st GNSS board and hydraulic electronic control which is linked with Fig. 3 hydraulic control unit.

Figure 6. The All-In-One board.

RESULTS AND DISCUSSION

The project successfully resulted in a fully functional retrofitted autosteering system integrated with an RTK base station connected to the RTK2Go service. The system achieved centimeter-level accuracy, enabling precise field tasks.

As the pilot progressed, word of the implementation spread through dedicated communication channels, primarily among farmers interested in low-cost precision agriculture solutions. There is significant expansion of the RTK base station network, in 2023/2, approximately 23 base stations and by 2024/4 (Fig. 7), this number had increased to 60 stations and 2024/10 about 70. One farmer involved in the network began selling custom-built hardware kits, enabling broader access to autosteering solutions. The project's success inspired similar implementations in neighbouring regions, contributing to the wider adoption of open-source precision agriculture technologies.
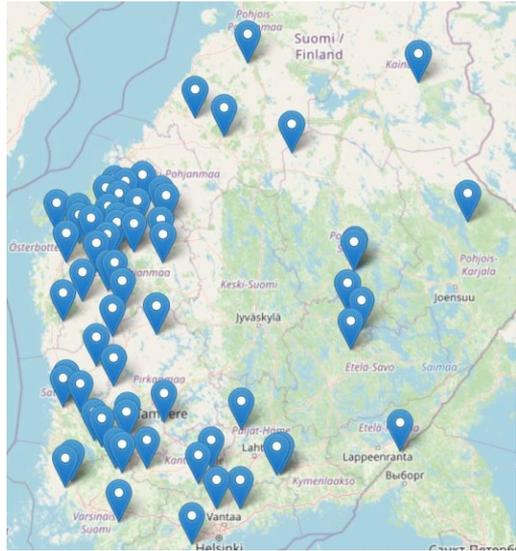
Figure 7. The RTK base stations in Finland in RTK2go-service at 2024/4.

Despite the challenges, the project demonstrated that open-source solutions are viable alternatives to commercial autosteering systems, especially for farmers seeking affordable and customizable options. The availability of almost ready-to-use kits has significantly lowered the barrier to entry over the past two years. As open-source solutions continue to evolve, they are expected to complement and even accelerate the adoption of commercial technologies, further promoting agricultural automation. Farmers purchasing tractors or harvesters have become more likely to install autosteering systems at the time of purchase due to the growing availability of open-source and hybrid options. Open-source implementations, when supported by knowledge-sharing networks, contribute to broader technological advancements in the agricultural sector.

There were some challenges. The initial USB connection instability due to COM port interruptions was a major hurdle. This was mitigated by switching to the Ethernet-based control unit, which streamlined communication and eliminated frequent disconnections. Incorrect baud rates initially caused communication problems, which were resolved through careful reconfiguration. The RTKBase system's limited reliability was attributed to external factors like power supply issues and Wi-Fi connectivity problems. Potential improvements include using a more robust power source and wired connections.

To approximate the effort required for setting up an RTK base station, the process can be divided into two components: technical installation and preliminary research. The preliminary phase, consisting of information gathering, hardware selection, and community engagement, can be significantly more time-consuming. The latter varies

considerably depending on the user's technical background and access to peer support. Therefore, in our analysis we focus on the concrete installation time, while acknowledging the challenge of quantifying the research phase.

Mounting the components into the enclosure typically takes 1 to 2 hours, or longer if the case lacks pre-drilled cable entry points or suitable mounting locations for boards. Installing the software on the Raspberry Pi takes approximately 30 minutes. Setting up the RTKbase application also takes around 30 minutes. Installing the base station in a suitable location may require drilling holes, building an antenna mount, or routing cables such as power lines. In this estimate, we assume that the antenna can be easily screwed into place and that both power and Ethernet are readily available. Under these conditions, physical installation can be completed in about 2 hours.

Following the hardware setup, a series of configuration steps are required: starting position logging about 10 minutes, using an online positioning service such as the Canadian Spatial Reference System Precise Point Positioning (Natural Resources Canada) service about 15 minutes, and importing the calculated coordinates to the base station about 15 minutes. Finally, setting up RTK information sharing, (e.g., registering the station on the RTK2Go service) entering service details into the base station configuration, and starting the correction data broadcast takes around 1 hour. All the above assumes an optimal setup. In practice, each phase may take significantly more time if any challenges arise.

The total estimated installation and configuration time for the RTK base station is approximately 6 to 7 hours under optimal conditions.

The total hardware cost for a self-built RTK base station ranges from approximately 380 € to 530 €, depending on the selected components. The GNSS receiver (SimpleRTK2b) and the computing unit (Raspberry Pi) constitute the majority of the cost. Although prices vary slightly depending on the supplier and specific models used. The listed estimate does not include labor or research time required to understand the setup process. The table below presents the prices of components.

As in the base station analysis, our focus for the AgOpenGPS-autosteering system is on the concrete installation and configuration steps. The time required for background research is difficult to estimate precisely, as it depends heavily on the desired implementation method and the specific tractor model and equipment used.

Table 1. RTK base station components

Component	Price
SimpleRTK2b (with u-blox F9P)	180
GNSS antenna	50–100
Raspberry Pi	100–200
Cables, box, and charger	50
	380–530 €

In our first case, we built a custom experimental hydraulic control unit between the orbital steering valve and the steering cylinder. Its installation took approximately 20 hours. Based on this, installing a ready-made hydraulic unit can be estimated to take around 10 hours. In another tractor, we implemented steering using a motor mounted on the steering wheel. This installation took approximately 5 hours, including 3D printing, welding, and mechanical attachment of parts. For steering wheel control, there are also ready-made solutions available, for example, steering wheel center hub motor kits, which can be installed very quickly, in about 1 hour.

The first autosteer PCB assembly required substantial manual effort. All components were hand-soldered, which took about 4 hours. The components had to be ordered from

different suppliers, and the selection process required considerable effort. In a later implementation, we used a so-called All-in-One board, which was significantly easier. When ordering the board, a parts list and installation instructions are also provided on the AgOpenGPS download site. It is essential to ensure that all components are available from the chosen vendor, to avoid manual soldering and rework. The result is a high-quality, factory-assembled PCB board. Placing the order takes about 1 hour, and no soldering or wiring is required after that.

Electrical wiring, including power supply, safety relays, and connections to the steering controller, adds approximately 2 hours. Installing and connecting the wheel angle sensor and GNSS antenna may take an additional 2 to 4 hours.

Software configuration, including installing AgOpenGPS on a PC, sensor calibration, and device communication setup, can take 2–4 hours under optimal conditions. Initial field calibration takes another 1 to 2 hours and includes practical testing of turning radii and steering performance.

All estimates assume optimal conditions and some prior familiarity with the hardware and software. In practice, any phase may take considerably longer if complications arise.

The total estimated installation and configuration time for the autosteering system is approximately 37 hours under optimal conditions, which is calculated our first case installation.

Table 2. AgOpenGPS components

Component	Price
Steering motor and mount	100
or hydraulic steering unit	600
or steering wheel hub	600
GNSS antenna	50–100
Autosteer PCB and connectors	300
u-blox F9P (micro)	200
Cables and enclosures	50
Wheel angle sensor	50–100 €
Windows tablet	300–1,000
	1,450–2,350 €

The hardware cost of an open- source autosteering system varies depending on the chosen implementation method and component availability. Based on our assessment, the total cost ranges from 1,450 € to 2,350 €. The Table 2 presents a typical breakdown.

CONCLUSIONS

This case study demonstrates that open-source solutions for autosteering and RTK base stations are viable, cost-effective alternatives to proprietary systems, offering farmers a pathway to adopting precision agriculture technologies without significant financial barriers. However, success with open-source implementations requires a willingness to experiment and access to a strong support network that can be relied on when challenges arise. While most problems encountered are minor, having someone to confirm or suggest a solution is often essential.

The goal of both open-source and commercial implementations is ultimately the same: to bring farmers closer to fully automated field robotics, where tasks become increasingly automated and optimized. Once farmers have experienced the benefits of systems such as autosteering and automated implement control, it becomes difficult to return to traditional methods. The adoption of such technologies represents a permanent shift in how farming is approached, driving the agricultural sector steadily toward greater efficiency and automation.

This study is a case-based investigation, limited to two self-built RTK base stations and a single implementation of autosteering. As such, the generalizability of the results is limited, particularly regarding the autosteering system, due to the diversity of tractors and the variety of possible implementation options. Additionally, the type of implements used may influence the chosen technical solutions. In contrast, the RTK base stations are significantly easier to replicate, as evidenced by the rapidly expanding RTK base station network in Finland.

Our next aim is to support farmers in adopting more precise and automated agricultural practices, while respecting their individual preferences. Some may be inclined to explore open-source solutions, while others prefer quick and easy commercial options. Ultimately, the goal is to shift the farmer's role from repetitive tasks toward data-driven decision-making.

The total hardware cost for a self-built RTK base station ranges between 380 € to 530 €, and the estimated installation and configuration time is approximately 6–7 hours. For the autosteering system based on AgOpenGPS, hardware costs vary from 1,450 € to 2,350 € depending on implementation (e.g., motor vs. hydraulic control), and installation time ranges from 7 to 13 hours. These figures include only the optimal technical installation, not the preliminary research or troubleshooting phases.

As a result of this study, setting up RTK base stations has become easier, as more guidance and documentation are now available. The threshold for building a personal base station may also have lowered as awareness of such setups has spread. In general, the installation of the base station was relatively simple to begin with. The software installation process has not changed significantly, nor has the required hardware, which has remained largely the same over the past few years. Unit costs have also remained fairly stable, as the core components have not undergone major changes. Therefore, the most significant change has been the growing viability of building a base station as an alternative to purchasing commercial correction signal services.

When compared to commercial alternatives, an RTK base station may not be needed at all, users can simply purchase an RTK subscription, typically costing around 600 € per year and usable anywhere in Finland. This option requires no installation time. It is sufficient to buy the license and configure the connection details into, for example, the tractor's autosteering device. An interesting emerging alternative is the Galileo High Accuracy Service (HAS), which eliminates the need for a local RTK base station altogether by providing correction data directly via satellite (Prol et al., 2024).

A wide range of commercial autosteering systems is available, with prices starting at approximately 5,000 € and extending significantly higher. The authors have not conducted commercial installations themselves, but according to product brochures and manufacturer claims, installation usually takes only a few hours at most.

Open-source solutions, while significantly cheaper, require intermediate-level proficiency in electronics, mechanical assembly, and software configuration. For example, the original PCB required hand-soldering, but newer All-in-One versions simplify the process considerably.

Despite the higher initial labor requirement, the open-source approach offers a viable alternative with substantial cost savings, especially for technically skilled users or those with peer support networks. In particular, the flexibility to tailor the solution to specific farm equipment and the absence of vendor lock-in are key benefits for early adopters.

REFERENCES

AgOpenGPS. 2025a. *Official GitHub repository*. [Online].
    Available at: https://github.com/AgOpenGPS-Official/AgOpenGPS [Accessed: 19 April 2025].

AgOpenGPS. 2025b. Documentation Wiki. [Online]. Available at: https://docs.agopengps.com/ [Accessed: 19 April 2025].

AgOpenGPS. 2025c. *User forum (Discourse platform)*. [Online]. Available at: https://discourse.agopengps.com/ [Accessed: 19 April 2025].

Arduino. 2025. Arduino Software (IDE). [Online].
    Available at: https://www.arduino.cc/en/software/ [Accessed: 19 April 2025].

ArduSimple. 2025. *SimpleRTK2b GNSS module*. [Online]. Available at:
    https://www.ardusimple.com/product/simplertk2b/ [Accessed: 30 January 2025].

Bora, G.C., Nowatzki, J.F. & Roberts, D.C. 2012. Energy savings by adopting precision agriculture in rural usa. *Energy, Sustainability and Society* **2**, 22. doi: 10.1186/2192-0567-2-22

Cheng, C., Fu, J., Su, H. & Ren, L. 2023. Recent Advancements in Agricultural Robots: Benefits and Challenges. *Machines* **11**(1), 48. doi: 10.3390/machines11010048

JLCPCB. 2025. *Printed circuit board manufacturing service*. [Online]. Available at: https://jlcpcb.com/ [Accessed: 15 April 2025].

Kisliuk, B., Krause, J.C., Meemken, H., Saborío Morales, J.C., Müller, H. & Hertzberg, J. 2023. AI in Current and Future Agriculture: An Introductory Overview. *KI - Künstliche Intelligenz* **37**, 117–132. doi: 10.1007/s13218-023-00826-5

Klein, K.K. & Knight, J.G. 2005. Using case studies as an approach for conducting agribusiness research. *International Food and Agribusiness Management Review* **8(**2), 111–126. https://doi.org/10.22004/ag.econ.8137

Linna, P., Halla, A. & Suomalainen, J. 2024. Concept for sharing drone data in agricultural data ecosystem. In: *2024 47th MIPRO ICT and Electronics Convention (MIPRO)*, 20–24 May 2024, Opatija, *Croatia. IEEE*, pp. 1900–1904. doi:10.1109/MIPRO60963.2024.10569579

Lipping, T., Linna, P. & Narra, N. (eds.) 2020. *New Developments and Environmental Applications of Drones*. Springer International Publishing. doi:10.1007/978-3-030-77860-6. Available at: https://link.springer.com/10.1007/978-3-030-77860-6.

Natural Resources Canada. 2025. *Canadian Geodetic Survey: CSRS-PPP service*. [Online]. Available at: https://webapp.csrs-scrs.nrcan-rncan.gc.ca/geod/tools-outils/ppp.php [Accessed: 30 January 2025].

Prol, F., Ferrer, C., Pazos, Á., Hernández-Pajares, M. & Juan, J.M. 2024. Enabling the Galileo high accuracy service with open-source software: Integration of HASlib and RTKLIB. *GPS Solutions* **28**, Article 17. https://doi.org/10.1007/s10291-024-01617-7

Raspberry Pi Foundation. 2025. Raspberry Pi OS and software. [Online]. Available at: https://www.raspberrypi.com/software/ [Accessed: 19 April 2025].

RTK2go. 2025. *Free NTRIP Caster service*. [Online]. Available at: http://rtk2go.com/ [Accessed: 30 January 2025].

RTKBase. 2025. *Base station software*. [Online]. Available at: https://github.com/Stefal/rtkbase [Accessed: 30 January 2025].

u-blox. (2025). Official website of u-blox. [Online]. Available at: https://www.u-blox.com/en [Accessed: 19 April 2025].

Vanclay, F., Mesiti, L. & Howden, P. 1998. The role of case studies in farming systems research. *Agricultural Systems* **57**(2), 259–272. https://doi.org/10.1016/S0308-521X(98)00009-9

Influence of fertilization on yield, nutritional and qualitative characteristics of potato tubers under different agro-climatic conditions in Armenia

A.O. Markosyan[1], M.H. Zadayan[2,*], S.K. Baghdasaryan[1], S.Z. Kroyan[3], S.A. Markosyan[4] and G.H. Gasparyan[1]

[1]Armenian National Agrarian University, Scientific Center of Soil Science, Agrochemistry and Melioration named after H. Petrosyan, 24 Admiral Isakov Ave, AM0004 Yerevan, Armenia
[2]Center for Agricultural Research and Certification, State Non-Commercial Organization of the Ministry of Economy of the Republic of Armenia, AM1139, v. Merdzavan, Armavir Marz, Yerevanyan highway 2nd deadlock, building 4, Armenia
[3]National University of Architecture and Construction of Armenia, Department of Engineering Geodesy, Teryan Str., 105, AM0009 Yerevan, Armenia
[4]Yerevan State University, Faculty of Biology, Department of Biology, 1 Alex Manoogian, AM0025 Yerevan, Armenia
*Correspondence: mhzadayan@gmail.com

Abstract. This study investigates the effects of mineral (N150P120K120, 'Control-Treatment 1') and organo-mineral fertilization (with 20 t ha^{-1} of farmyard manure (FYM), 'Treatment 2', and 40 t ha^{-1} of FYM, 'Treatment 3') on the yield, nutritional value, and quality of potato (*Solanum tuberosum L.*) tubers under different agro-climatic conditions in Armenia. Field trials were conducted in two contrasting regions: Dasht village (Ararat Plain, 850 m a.s.l.) with cultivated irrigated meadow-fulvous soils, and Vahan village (Gegharkunik Highlands, 2000 m a.s.l.) with mountain black soils (chernozems). The research measured tuber yield, dry matter, starch, vitamin C, and protein content over three years (2019–2021).

Application of T3 (NPK + 40 t ha^{-1} FYM) significantly improved all measured indicators. Yield increased by up to 28%, dry matter by 10.8%, starch by 12.0%, vitamin C by 13.4%, and protein content by 14.4%. Climate conditions also had a significant impact on tuber productivity and composition. In chernozems, compared to irrigated meadow-fulvous soils, yield increased by 15.1–21.6%, and the content of dry matter, starch, and vitamin C increased by 3.1–8.7%. However, protein content was higher in the irrigated meadow-fulvous soils by 3.4–5.2%.

These results demonstrate the importance of fertilization strategies tailored to specific agroecological zones and climatic trends, particularly in the context of climate change adaptation.

Key words: agro-climatic conditions, dry matter, farmyard manure, growth conditions, mineral and organic fertilization, potato, protein content, starch, tuber yield and quality, vitamin C.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most widely cultivated and nutritionally significant crops worldwide. It is a rich source of carbohydrates, vitamin C, potassium, and moderate amounts of high-quality protein, making it a key component of food security and human nutrition.

In Armenia, potato is cultivated across a wide range of agro-climatic zones, from lowland plains to high-altitude regions. Despite its importance, both yield and tuber quality often remain suboptimal, primarily due to inappropriate fertilization practices and the declining fertility of soils under intensive cropping systems (Hayrapetyan, 2000; FAO, 2010).

A growing body of research has confirmed that balanced mineral and organic fertilization is essential for improving both tuber yield and biochemical composition. The combined use of farmyard manure (FYM) and mineral fertilizers significantly enhances the accumulation of dry matter, starch, vitamin C, and protein in potato tubers (El-Sayed et al., 2015; Ahmed et al., 2019; Gelaye, 2023). Moreover, this integrated approach positively affects storability and marketability of tubers (Lombardo et al., 2013; Brazinskienė et al., 2014).

Global demand for potatoes is projected to increase further, driven by population growth and the need for affordable, nutrient-dense food sources (Lutaladio & Castaldi, 2009; Devaux et al., 2014; Jennings et al., 2020). At the same time, environmental sustainability and resource conservation are becoming increasingly urgent priorities for global agriculture (Ayupov et al., 2014).

Potato has high potential as a food crop, as it provides a nutritionally valuable yield per unit area within a relatively short growing season (< 120 days), compared to cereal crops such as maize (Hirpa et al., 2010). The nutritional value of potatoes depends on the balance of organic and mineral constituents necessary for human health. Depending on the variety, potato tubers contain 15–35% dry matter, of which 80–85% is starch and up to 3% protein (Danilchenko et al., 2008; Ayupov et al., 2014), along with up to 31 mg 100 g^{-1} (fresh weight) of vitamin C (Bista & Bhandari, 2019). Potatoes are also rich in vitamin B6, potassium, and various antioxidants that strengthen immunity (Camire et al., 2009; Pacier & Martirosyan, 2015; Beals, 2019; Mattoo et al., 2022; Xu et al., 2023).

Potato yield and quality are strongly influenced by variety, environmental conditions, and agronomic practices (Bártová et al., 2013; Lombardo et al., 2013; Brazinskienė et al., 2014; Novikova, 2021). Potatoes are highly sensitive to soil fertility, physical soil properties, and the availability and balance of macro- and micronutrients (El-Sayed et al., 2015; Ahmed et al., 2019; Awad et al., 2022; Mancer et al., 2024). Fertilization strategies must therefore be designed to ensure that vegetative growth is supported while promoting the formation of tubers with desirable qualitative and nutritional characteristics (White et al., 2007; Koch et al., 2020; Naumann et al., 2020).

Among mineral nutrients, nitrogen plays a particularly significant role in potato production, influencing vegetative growth, tuber yield, chemical composition, and quality (Hopkins et al., 2008; Sincik et al., 2008; Zelalem et al., 2009; Abreham, 2022;

Harraq, 2022). However, the positive effects of nitrogen occur only up to a certain threshold, beyond which excessive application fails to further increase yield and may even reduce tuber quality (Love et al., 2005; Olivier et al., 2006; Zebarth and Rosen, 2007; Eremeev et al., 2009). Overuse of nitrogen can also cause nitrate accumulation in tubers, posing serious health risks, including carcinogenic effects (Atafar, 2010; Chen et al., 2017; Alengebawy et al., 2021; Jovovic et al., 2021).

To address these challenges, researchers advocate combining mineral and organic fertilizers (Jhangiryan et al., 2024) to enhance soil physical, mechanical, biochemical, and fertility properties (Ahmed et al., 2019; Miskoska-Milevska et al., 2020; Kuht et al., 2023). Organic fertilizers improve soil water-holding capacity by approximately 17% and increase soil porosity by about 12% (Setiyo et al., 2021), creating conditions favorable for root and tuber development. Improved nutrient balance also promotes protein accumulation in potato tubers (Naghdi et al., 2022; Martirosyan et al., 2024).

Research and production data indicate that potato fertilization practices must be tailored to specific soil types and climatic conditions to achieve optimal productivity and quality (Sintsova & Lyskova, 2020; Tamrazov, 2021). Potatoes require extended daylight hours and high light intensity, as well as ample moisture, particularly during tuberization and flowering stages. They grow best in fertile, light-textured soils. The optimal temperature range for photosynthesis and tuber development is 20–25 °C (Jiménez et al., 2008; Andayani & Maryam, 2019; Setiyo et al., 2021). Physiological processes weaken at 26–29 °C and cease entirely above 33 °C (Sun et al., 2017; Wang et al., 2018; Obiero et al., 2020).

Soil temperature and moisture are critical environmental factors affecting tuber yield and quality. Potatoes grown at 85% relative humidity produce higher yields than those grown at 50% humidity (Setiyo et al., 2021). In high-altitude, cooler climates, tubers tend to accumulate more dry matter and vitamin C, whereas in warmer, drier regions, protein content is higher, largely due to increased nitrogen uptake and metabolism (FAO, 2010; Minasyan, 2015; Raymundo et al., 2018; Jennings et al., 2020; Jovovic et al., 2021).

Study objectives
This study aims to:

1. Evaluate the effects of mineral and organo-mineral fertilization under two contrasting agro-climatic and soil conditions - irrigated meadow-fulvous soils of the Armavir region (Dasht) and mountain chernozems of the Gegharkunik region (Vahan) - on the yield and nutritional quality of potatoes in Armenia.

2. Develop region-specific sustainable fertilization strategies to improve potato production.

3. Elucidate the relationships between growth conditions, yield, and tuber quality characteristics, thereby addressing a knowledge gap in scientific research on potato production in lowland versus highland agro-ecological zones.

MATERIALS AND METHODS

Research sites

Field trials were conducted in two villages. In Vahan village (Gegharkunik region, 2000 m a.s.l.), mountain carbonated chernozems predominate. These soils have a lumpy-granular structure and a loamy texture. Organic carbon content is quite high, reaching 3.5–4.7% in the upper horizons of uncultivated soils. However, they are deficient in mobile nutrients—nitrogen, weakly to moderately supplied with phosphorus, and weakly to moderately supplied with potassium. The Ca content in these soils is 39.0 mg eq per 100 g of soil, and the mg content is 9.3 mg eq per 100 g of soil. The soil pH ranges from 7.0 to 7.2 (Soils of the Armenian SSR, 1976).

According to the Köppen climate classification, the region has a temperate continental climate classified as Dfb (Köppen-Geiger, 2024). The average annual air temperature varies between 5.5–6.0 °C, with 16.5 °C during July–August. The sum of temperatures above 0 °C is 2,600 °C, and above 10 °C is 2,100 °C. The average annual precipitation ranges between 400–500 mm, with 280 mm falling during the warm months. Maximum precipitation occurs in late spring and early summer (Melkonyan et al., 2004).

Dasht village (Armavir region, 800 m a.s.l.) is characterized by hot, dry summers and irrigated meadow-fulvous soils. The relief of the Armavir region is flat. Irrigated meadow-fulvous soils are heavy clayey-granular in texture, with weak silty-granular structure and 3.7% carbonate content. The organic carbon content in the upper horizons varies between 0.9–1.2%. These soils are poorly supplied with mobile nitrogen, moderately to well supplied with phosphorus and potassium. The Ca content in these soils is 29.8 mg eq per 100 g of soil, and the mg content is 8.2 mg eq per 100 g of soil. The soil pH varies between 7.8 and 8.5 (Soils of the Armenian SSR, 1976).

According to the Köppen climate classification, the region has a semi-arid temperate continental climate classified as BSk (Köppen-Geiger, 2024). The climate is strictly arid and continental. The average annual air temperature reaches 11.8 °C, with absolute minimum temperatures of -27 to -30 °C and maximum temperatures of +39 to +40 °C. The sum of temperatures above 0 °C is 4,500–4,800 °C, and above 10 °C is 4,000–4,200 °C. The average annual precipitation varies between 250–280 mm, with maximum precipitation occurring in May (Melkonyan et al., 2004).

Soil sampling and analysis

To assess the agrochemical properties of soils, soil samples were collected before fertilization and again at flowering from two pits per experimental field at a depth of 0–30 cm. Soil pH was determined by the electropotentiometric method, organic carbon content by the Tyurin method, available nitrogen by the Tyurin-Kononova method, P_2O_5 by the Machigin method, and K_2O by the Maslova method (Arinushkina, 1970; Faithfull, 2002).

During the flowering stage, soil and air temperatures, and relative air humidity (in both interplant and interrow spaces) were measured. Soil moisture was determined from soil samples taken at 30 cm depth. Soil surface temperature at 15 cm depth was measured with a Savinov thermometer, and air temperature and humidity were measured with an

MB-4M aspiration psychrometer. Measurements were performed three times daily at 8:00, 13:00, and 19:00. Soil moisture was determined by drying samples in an oven until reaching a constant weight.

Experimental design

Field experiments were conducted from 2019 to 2021 using a randomized complete block design (RCBD) (Bush, 2023) with three replicates. The plot size was 50 m² (Matevosyan & Gyulkhasyan, 2000). Tubers (50–80 g) were planted at a spacing of 70×30 cm. The test variety was *Impala* (*Solanum tuberosum* L. cv. Impala), an early maturing, high-yielding Dutch variety that produces large (80–160 g), oval-shaped tubers with smooth yellowish skin and light yellow flesh. Fertilization treatments were:

1. T1 (Control): NPK (N150P120K120) mineral only.
2. T2: NPK + 20 t ha⁻¹ of well-rotted farmyard manure (FYM).
3. T3: NPK + 40 t ha⁻¹ FYM.

The inorganic fertilizers used were ammonium nitrate (N, 34% active ingredient), triple superphosphate (P, 50% active ingredient), and potassium chloride (K, 60% active ingredient).

Planting in Dasht village was performed in the third week of March and in Vahan village in the third week of May, using pre-germinated tubers. During the research years, tuber harvesting was carried out after the widespread natural death of the tops: in Dasht village in the last week of June, and in Vahan village in the third week of September.

Crop Management

Soil cultivation and plant care followed standard procedures for the respective climatic conditions. Irrigation was applied only in Dasht. During the growing season, insect pests and late blight were monitored, but no systemic pesticide applications were necessary; only standard cultural practices (hilling, irrigation, crop rotation) were used. In autumn, before deep plowing, the experimental plots were fertilized with mature FYM and the full amount of potassium and two-thirds of phosphorus fertilizers. During spring planting, the remaining phosphorus and two-thirds of the nitrogen were applied. The final nitrogen application was provided during the first loosening as top dressing.

Measurements and data collection

Phenological observations included the onset (10%) and completion (75%) of tuber germination, stolon formation, flowering, and tuber maturity. Growth and development parameters were measured at flowering on 25 plants per treatment, including plant height, number of stems, number of leaves, leaf area, weight, number of stolons, tuber formation efficiency, and tuber number.

Yields were determined by harvesting and weighing individual plots. Yield was recorded from each treatment, per replicate of 25 plants. Tuber samples were collected from each treatment to determine dry matter, starch, vitamin C, and protein content.

Dry matter content was determined by oven-drying at 105 °C, starch content by the specific gravity method (Arinushkina, 1970), total protein by the Kjeldahl method (N × 6.25), and vitamin C by iodometric titration (Dioha, 2011). Leaf area was measured using Easy Leaf Area software.

Statistical analysis

Data analysis was performed using R (v4.5.0). The following libraries were used:

- *tidyverse* (including dplyr) for data manipulation and summarization,
- *dplyr* for grouping and summarizing data,
- *agricolae* for Least Significant Difference (*LSD*) tests after *ANOVA*,
- *knitr* and *kableExtra* for professional table generation,
- *cor.test* (from base R) for Pearson's correlation analysis,
- *ggpubr* for visualizing correlation results.

Significance was tested using two-way *ANOVA*, and treatment means were compared using *LSD* at $p < 0.05$. Results are reported as means ± standard error (SE).

RESULTS AND DISCUSSION

Agro-climatic and soil conditions of the experimental sites

The two areas differed significantly in climate and soil characteristics. Dasht (Ararat Valley) is characterized by hot summers (> 30 °C) and low precipitation, while the high-mountainous Vahan remains cool (< 25 °C) with abundant precipitation, conditions that promote larger tuber size and greater starch accumulation. Monthly average temperature and precipitation data during the growing seasons (2019–2021) are summarized in Fig. 1, based on the National Hydrometeorological Service database (2021). Meteorological comparisons show that precipitation in Vahan during the growing season is 2.8 times greater than in Dasht (321 mm total). Conversely, average monthly temperatures in Vahan are about 9 °C lower, averaging 12.9 °C.
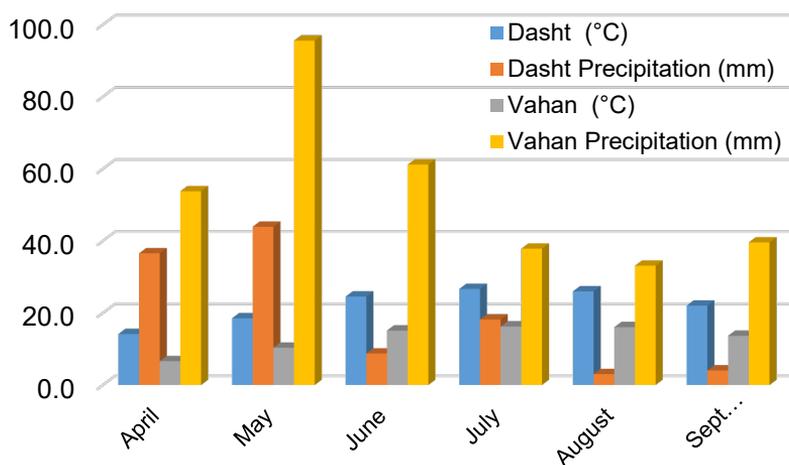
Figure 1. Monthly precipitation (mm) and average air temperature (°C) in Dasht and Vahan villages during the 2019–2021 vegetation seasons.

Using the Köppen-Geiger climate classification, Dasht is classified as BSk (semi-arid steppe), while Vahan is classified as Dfb (humid continental, warm summer) (Figs 2, 3) (Köppen-Geiger, 2024).
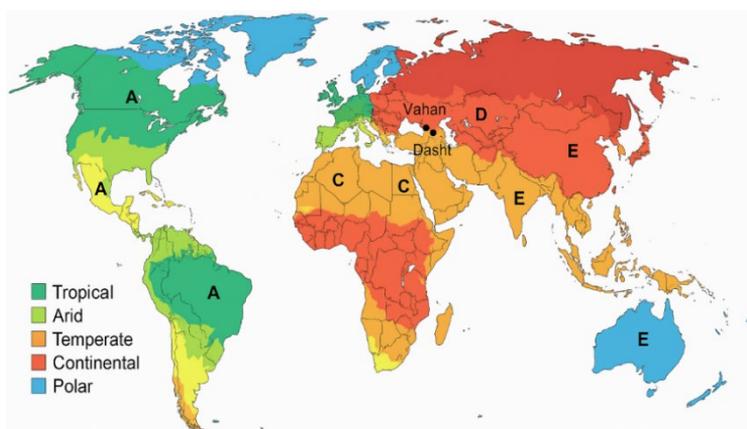
Figure 2. Climatic classification of the study areas according to the Köppen-Geiger system (world map). Source: Beck et al., *Scientific Data* 5:180214 (2018).
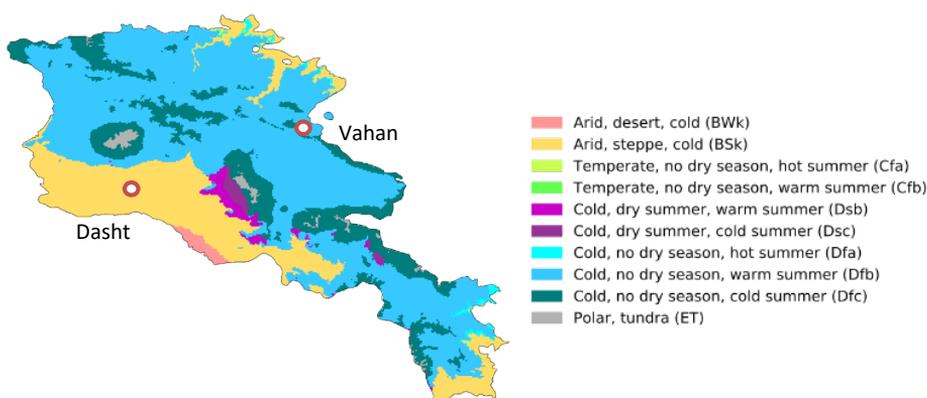
Figure 3. Climatic classification of the study areas according to the Köppen-Geiger system (Armenia map). Source: Beck et al., *Scientific Data* 5:180214 (2018).

Table 1 shows that the soils differed notably before planting and during flowering. In Dasht (irrigated meadow-fulvous soils), humus content was 2.15–2.25%, mobile nitrogen 3.58–3.98 mg 100 g^{-1} soil, phosphorus 2.22–2.33 mg 100 g^{-1} soil, potassium 38.25–38.61 mg 100 g^{-1} soil, and pH 8.0–8.2. In contrast, Vahan's mountain chernozems had humus contents of 4.39–4.53%, mobile nitrogen 5.75–5.91 mg 100 g^{-1}, phosphorus 4.8–4.9 mg 100 g^{-1}, potassium 15.5–20.9 mg 100 g^{-1}, and pH 7.0–7.2. Fertilization, particularly with combined organo-mineral inputs, significantly improved soil nutritional properties, especially under T3 treatment (NPK + 40 t ha^{-1} FYM).

Climatic differences had a direct impact on potato growth, particularly during flowering and tuber bulking. In Vahan, higher precipitation and moderate temperatures contributed to increased assimilate transport into tubers, while in Dasht, water stress limited their accumulation. Similar results have been reported by Rykaczewska (2015), Singh et al. (2019), Djaman et al. (2021), Gouerou et al. (2025), Molmann and Johansen (2025).

Table 1. Agrochemical properties of soils in Dasht and Vahan experimental sites before planting and at the flowering stage (average of 2019–2021)

Experimental site, village	Fertilization	Before fertilization					During flowering				
		Organic carbon content (%)	pH	Mobile nutrients, mg in 100g of soil			Organic carbon content (%)	pH	Mobile nutrients, mg in 100g of soil		
				N	P_2O_5	K_2O			N	P_2O_5	K_2O
Dasht	Control (Treatment 1)	1.3	8	3.85	2.33	38.61	1.6	8	5.12	4.08	40.01
	Treatment 2	1.25	8.1	3.58	2.22	38.39	1.88	8.1	7.58	6.12	42.09
	Treatment 3	1.28	8.2	3.98	2.25	38.25	2.12	8.3	8.98	7.95	43.25
Vahan	Control (Treatment 1)	2.55	7	5.75	4.9	15.5	2.64	7	7.85	6.54	23.8
	Treatment 2	2.58	7.1	5.84	4.84	18.8	3.2	7.2	9.78	8.89	25.5
	Treatment 3	2.63	7.2	5.91	4.8	20.9	3.74	7.3	11.25	12.54	28.8

Soil temperature dynamics

According to Table 2, fertilization influenced soil surface temperatures. In Dasht, during mornings (8:00), the control treatment registered soil temperatures 0.7–1.1 °C lower compared to T3, while at midday (13:00) and evening (19:00), the control registered 0.8–2.4 °C higher temperatures. The increase in soil temperature under organic fertilization is attributed to the darker color and organic matter content, which improve soil thermal properties and enhance biological activity (Konen et al., 2003; Kumar et al., 2020). Similar differences were observed between inter-row and inter-plant areas, as plant canopy coverage reduces soil exposure.

Table 2. Effect of fertilization on soil surface temperature (°C) during flowering stage

Experimental site, village	Fertilization	8 [00]		13 [00]		19 [00]	
		Interplant	Interline	Interplant	Interline	Interplant	Interline
Dasht	Control (Treatment 1)	15.3	15.0	21.8	23.4	19.2	19.0
	Treatment 2	15.8	15.4	20.1	22.6	19.0	19.1
	Treatment 3	16.4	15.7	19.4	22.0	18.5	18.3
Vahan	Control (Treatment 1)	12.8	12.3	17.2	18.8	15.9	15.2
	Treatment 2	13.1	13.2	17.0	18.1	16.3	15.5
	Treatment 3	13.4	13.4	16.5	17.5	16.5	16.0

Comparable patterns were observed at a soil depth of 15 cm (Table 3). In both Dasht and Vahan, soil temperatures under treatment T3 were consistently more stable compared to the control. These results align with previous findings indicating that organic amendments buffer soil thermal fluctuations (Lal, 2020; Kumar et al., 2021).

In Dasht village, at 15 cm depth during morning measurements (8:00), the control treatment showed temperatures 0.4–1.0 °C lower in interplant spaces and 0.4–0.9 °C lower in interline spaces compared to treatment T3 (NPK + 40 t ha^{-1} FYM). At 13:00, the control plots were 0.5–1.0 °C higher in interplant spaces and 0.7–1.2 °C higher in interline spaces relative to T3. In the evening (19:00), differences were 0.4–1.6 °C and 0.3–0.8 °C, respectively.

Table 3. Effect of fertilization on soil temperature at a depth of 15 cm (°C) during the flowering stage

Experimental site, village	Fertilization	8 00		13 00		19 00	
		Interplant	Interline	Interplant	Interline	Interplant	Interline
Dasht	Control (Treatment 1)	16.5	16.9	18.5	19.7	18.4	17.0
	Treatment 2	16.9	17.3	18.0	19.0	18.0	16.7
	Treatment 3	17.5	17.8	17.5	18.5	16.8	16.2
Vahan	Control (Treatment 1)	14.0	13.7	16.5	17.9	16.0	16.8
	Treatment 2	14.3	14.1	16.3	17.2	15.8	16.5
	Treatment 3	14.5	14.2	16.1	17.0	15.8	16.0

A similar pattern was observed in Vahan village. At 8:00, soil temperatures under the control were 0.3–0.6 °C and 0.9–1.1 °C lower in interplant and interline spaces, respectively. By 13:00, they were 0.2–0.7 °C and 0.7–1.3 °C higher, and by 19:00, 0.4–0.6 °C and 0.3–0.8 °C higher compared to treatment T3.

Additionally, climatic differences between the two locations caused significant variations independent of fertilization. In Dasht, the soil surface temperature in potato rows, interplant, and interline spaces was 2.5–4.6 °C higher than in Vahan village.

Observations indicate that the combined application of mineral and organic fertilizers had a pronounced effect on both soil moisture content and relative air humidity within potato crops. This effect is primarily linked to the increase in soil organic matter, which enhances soil structure and water-holding capacity by improving its physical properties (Ozlu et al., 2019; Lal, 2020; Kumar et al., 2021; Acar et al., 2025).

The role of relative air humidity is also crucial for potato growth and development. High relative humidity levels have been shown to support optimal physiological processes in potato plants (Setiyo et al., 2021). Therefore, in the present study, humidity was measured both between plants (interplant spaces) and between rows (interline spaces) to capture microclimatic variations.

In Dasht village, under control conditions (mineral fertilization only, N150P120K120), the relative air humidity at 8:00 was 55% in both interplant and interline spaces. By mid-afternoon (13:00), humidity dropped to 45% and 48%, respectively, before rising again to approximately 53% by 19:00.

Under T3 treatment (N150P120K120 + 40 t ha^{-1} FYM), relative humidity increased by 9–13% in the morning and 7–10% in the afternoon compared with the control, demonstrating the positive effect of FYM on microclimate regulation.

In Vahan village, a similar diurnal pattern was recorded. However, the overall relative humidity values were higher than in Dasht, with T3 treatment exceeding the control by approximately 8–9% throughout the day.

In addition to atmospheric humidity, soil moisture content showed a clear improvement under organo-mineral fertilization. Across both study sites, T3 treatment resulted in an average increase of 3.8% compared to the control, confirming the water-retention benefits of organic amendments.

It is noteworthy that the relative air humidity in Dasht was consistently 20–23% lower than in Vahan under similar fertilization conditions, reflecting the warmer and

drier microclimate of the Ararat Valley compared to the cooler, more humid highlands of Chambarak (Table 4).

Table 4. The effect of soil and climatic conditions and fertilization on changes in relative air and soil humidity (flowering stage, %)

Experimental site, village	Fertilization	8 00		13 00		19 00		13 00
		Interplant	Interline	Interplant	Interline	Interplant	Interline	Soil moisture at a depth of 30 cm, %
Dasht	Control (Treatment 1)	55	55	48	45	53	53	22.5
	Treatment 2	62	64	50	52	57	56	24.7
	Treatment 3	68	65	60	55	62	60	25.7
Vahan	Control (Treatment 1)	75	75	70	68	70	70	18
	Treatment 2	82	83	73	75	75	72	19.8
	Treatment 3	84	84	78	76	78	76	21.8

The results of the study demonstrated that variations in plant growth conditions, influenced by both fertilization and agro-climatic factors, had a direct effect on the growth, development, and yield formation of potato plants, as well as on the qualitative characteristics of the tubers.

As shown in Table 5, soil and climatic conditions, together with fertilization, significantly affected the transition between potato phenological phases and the overall duration of the growing season.

Table 5. Duration of potato phenophase transition depending on fertilization and climatic conditions

Experimental site, village	Fertilization	Duration of development stages, days			
		Planting to germination	Germination to cocooning	Cocooning to flowering	From flowering to natural death of the tops
Dasht	Control (Treatment 1)	21	23	15	33
	Treatment 2	21	24	18	36
	Treatment 3	20	23	19	38
Vahan	Control (Treatment 1)	26	26	16	33
	Treatment 2	27	27	17	35
	Treatment 3	27	28	19	38

According to field observations, in Dasht village, the period from planting to emergence lasted 20–21 days, whereas in Vahan village, where the climate is cooler, this period extended to 26–27 days.

In Dasht, the time span from emergence to natural senescence of the potato tops was 71 days under the control treatment and 80 days under the high fertilization treatment (T3: N150P120K120 + 40 t ha^{-1} FYM).

Similarly, in Vahan, the duration was 75 days for the control and 85 days under T3.

These results indicate that high fertilization rates prolonged the vegetation period by 9–10 days, irrespective of the location or prevailing agro-climatic conditions. The extended vegetation period reflects improved nutrient availability and more favorable physiological conditions for plant development, ultimately contributing to higher yields and better tuber quality (Table 5).

The combined application of mineral and organic fertilizers created a more favorable environment for the growth and development of both the aboveground and belowground organs of potato plants.

In Vahan village, intensive fertilization (T3: N150P120K120 + 40 t ha^{-1} FYM) resulted in notable improvements compared to the control. Specifically, stem height increased by 14.0 cm, the number of stems by 2.6, the number of leaves by 21, leaf biomass by 123.7 g, and leaf area by 1,894.3 cm².

	Control (Treatment 1)	Treatment 2	Treatment 3	Control (Treatment 1)	Treatment 2	Treatment 3
		Dasht			Vahan	
Plant height, cm	40	47	55	50	57	65
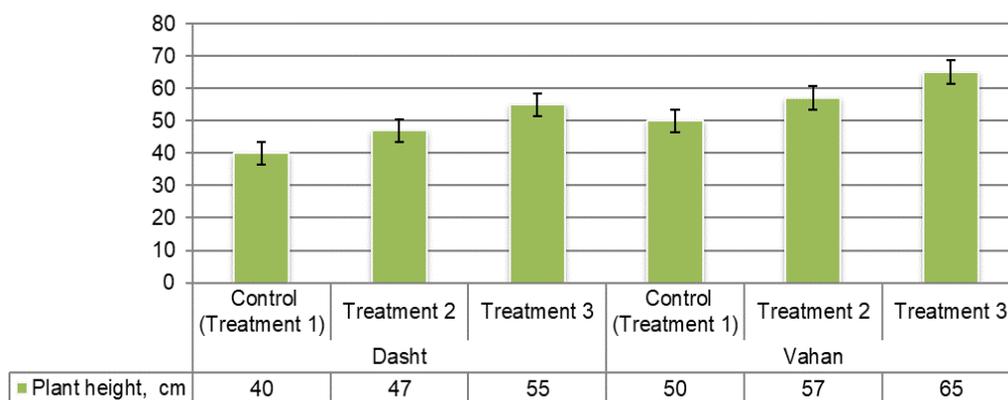
Figure 4. The height of potato plants depending on fertilization and climatic conditions during the flowering stage. $LSD_{0.5} = 3.27$.

In Dasht village, the values for these parameters were slightly higher, with respective increases of 14.3 cm, 2.3 stems, 19 leaves, 95.0 g of leaf biomass, and 2009.1 cm² of leaf area (Fig. 4, Table 6).

Table 6. Growth and development of aboveground organs of potato depending on fertilization and climatic conditions (during flowering stage)

Experimental site, village	Fertilization	One bush			
		Stems per plant, pcs.	Leaves per plant, pcs.	Leaf weight, g	Leaf area, cm^2
Dasht	Control (Treatment 1)	4.7	79	245.3	5,086.1
	Treatment 2	6	90.3	296.7	5,993.4
	Treatment 3	7	98	340.3	7,095.2
Vahan	Control (Treatment 1)	5.7	81	340.3	6,753.6
	Treatment 2	7.3	87.7	414.3	7,314.7
	Treatment 3	8.3	102	464	8,647.9
LSD_{05}		0.78	3.35	4.97	55.65

These results clearly demonstrate that higher levels of organo-mineral fertilization promote more vigorous vegetative growth by improving nutrient availability and enhancing soil moisture retention. The increase in leaf area and biomass is especially important, as it directly contributes to higher photosynthetic capacity, ultimately supporting tuber bulking and yield formation.

Soil and climatic conditions also had a significant effect on the formation of the aboveground biomass of potato plants. Under comparable fertilization treatments, potato plants grown in Vahan village were, on average, 10 cm taller, with 1.0–1.3 more stems and 2–4 additional leaves per plant compared to those grown in Dasht village. These additional leaves contributed to a higher total biomass, weighing 95.0–123.7 g, and covered 1,552.7–1,667.5 cm² more leaf surface area than plants from Dasht.

The influence of fertilization was also clearly evident in the development of the underground organs of potato plants. In Vahan, under T3 treatment (N150P120K120 + 40 t ha^{-1} FYM), plants exhibited markedly vigorous growth, producing 5.6 more stolons and 4.0 more tubers per bush compared to the control. Furthermore, stolon efficiency was 2.1% higher than in the control plots.

In Dasht village, the response to T3 was slightly lower but still substantial, with increases of 3.6 stolons, 3.5 tubers per bush, and a 2.0% improvement in stolon efficiency relative to the control (Table 7).

Table 7. Growth and development of underground organs of potato depending on fertilization and climatic conditions during the flowering stage

Experimental site, village	Fertilization	Quantity pcs., bush		Stolon efficiency, %
		Stolon	Tuber	
Dasht	Control (Treatment 1)	27.9	14.8	53
	Treatment 2	28.6	16.2	56.6
	Treatment 3	31.5	18.3	58
Vahan	Control (Treatment 1)	28.1	16.5	58.7
	Treatment 2	30.4	18.3	59.5
	Treatment 3	33.7	20.5	60.8
$LSD_{0.5}$		2.28	2.01	

Notes:
- Values represent the mean of three replications ± standard deviation (SD)
- T1 – Control (mineral fertilizer only, N150P120K120)
- T2 – N150P120K120 + 20 t ha^{-1} FYM (farmyard manure)
- T3 – N150P120K120 + 40 t ha^{-1} FYM
- *Stolon efficiency* was calculated as the ratio of stolon number to tuber number per plant, expressed as a percentage (%)
- Differences between treatments were evaluated using $LSD_{0.5}$ test; values followed by different letters in the same column are significantly different ($p < 0.05$).

The combined application of mineral and organic fertilizers had a significant positive effect on potato yield. In Vahan village, yield under the high fertilization treatment (T3: N150P120K120 + 40 t ha^{-1} FYM) increased by 139.1 c ha^{-1} compared to the control. In Dasht village, the corresponding yield increase was 90.5 c ha^{-1}.

Soil and climatic conditions also exerted a substantial influence on yield performance. Across all treatments, Vahan consistently outperformed Dasht by 66–115 c ha^{-1}, which can be attributed to higher rainfall, moderate temperatures, and favorable moisture conditions in the highland area, compared with the hotter and drier conditions of the Ararat Valley (Figs 1 and 4).

The combined application of mineral and organic fertilizers had a positive effect on the qualitative composition of potato tubers, particularly by increasing the content of dry matter, starch, vitamin C, and proteins.

In Vahan village, under control conditions (T1: mineral fertilizer only), the tubers contained 21.3% dry matter, 13.3% starch, and 21.3% vitamin C. Under T2 and T3 treatments, where both mineral and organic fertilizers were applied, these indicators increased by 0.9–1.7%, 1.1–1.6%, and 1.5–2.6%, respectively, compared with the control.

Similarly, in Dasht village, the content of dry matter increased by 0.8–1.8%, starch by 1.0–1.4%, and vitamin C by 1.3–2.3% compared to the control treatment. These improvements suggest that organo-mineral fertilization enhances nutrient accumulation in tubers by improving soil nutrient availability and plant physiological processes (Table 8).

Table 8. The effect of fertilization on the qualitative characteristics of potato tubers under different climatic conditions

Experimental site, village	Fertilization	Dry matter, %	Starch, %	Vitamin C, mg per 100g	Protein, g per 100g
Dasht	Control (Treatment 1)	20.5	12.3	20.5	2.01
	Treatment 2	21.3	13.3	21.8	2.14
	Treatment 3	22.3	13.7	22.8	2.3
Vahan	Control (Treatment 1)	21.3	13.3	21.3	1.98
	Treatment 2	22.2	14.4	22.8	2.08
	Treatment 3	23	14.9	23.9	2.16
$LSD_{0.5}$		0.90	0.92	1.22	0.12

Notes:
- T1 – Control (mineral fertilizer only, N150P120K120)
- T2 – N150P120K120 + 20 t ha^{-1} FYM (farmyard manure)
- T3 – N150P120K120 + 40 t ha^{-1} FYM
- Values represent the mean of three replications ± standard deviation (SD)
- Differences between treatments were determined using the $LSD_{0.5}$ test; values followed by different letters in the same column indicate significant differences ($p < 0.05$).

Protein content

Protein content was less influenced by fertilization and was primarily determined by climatic conditions. In the relatively warmer and drier conditions of Dasht village, the protein content in potato tubers was higher compared to the cooler and more humid mountainous conditions of Vahan.

Regardless of the fertilization treatment, protein levels in Dasht fluctuated between 2.01–2.30%, whereas in Vahan they ranged from 1.99–2.16%. This suggests that higher temperatures and lower relative humidity promote increased nitrogen mineralization and protein synthesis in potato tubers.

Yield performance

Tuber yield was significantly affected by both fertilization and location. In both study sites, the T3 treatment (N150P120K120 + 40 t ha⁻¹ FYM) produced the highest yields, reaching 639.6 c ha⁻¹ in Vahan and 525.7 c ha⁻¹ in Dasht (Fig. 5).

	Dasht			Vahan		
	Control (Treatment 1)	Treatment 2	Treatment 3	Control (Treatment 1)	Treatment 2	Treatment 3
Yield, c ha⁻¹	434.5	455.5	525.7	500.2	550.4	639.6
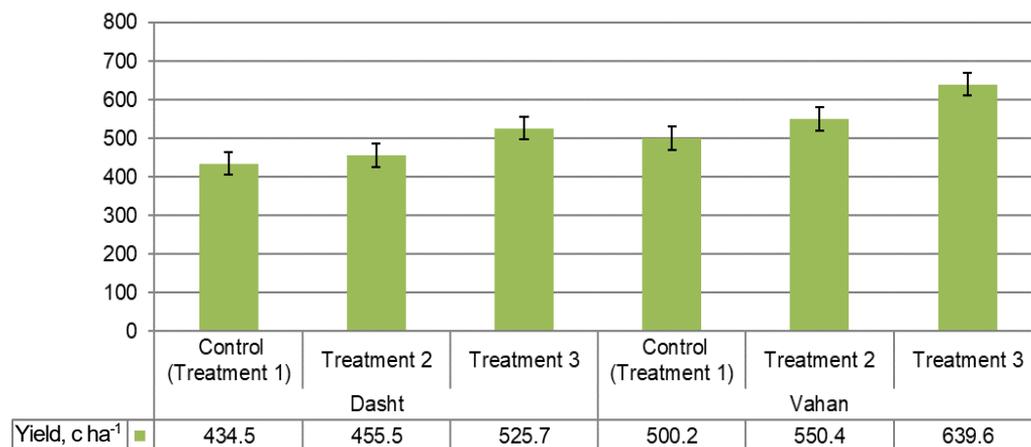
Figure 5. The effect of fertilization on potato yield under different climatic conditions. $LSD_{0.5} = 63.63$.

Notes:

- T1 – Control (mineral fertilizer only, N150P120K120)
- T2 – N150P120K120 + 20 t ha⁻¹ FYM (farmyard manure)
- T3 – N150P120K120 + 40 t ha⁻¹ FYM
- Values represent the mean of three replications ± standard deviation (SD)
- Bars marked with different letters indicate significant differences between treatments according to *LSD* test at $p < 0.05$
- Yield is expressed in centners per hectare (c ha⁻¹).

Overall, yields in Vahan exceeded those in Dasht by 15.1–21.6%, reflecting the higher soil fertility, greater rainfall, and moderate temperatures of the highland region. However, despite lower yields, protein content was consistently higher in Dasht-grown tubers, which can be attributed to enhanced nitrogen mineralization under warmer climatic conditions.

Quality parameters of tubers

The impact of fertilization on tuber quality traits is presented in Table 8. Key observations include:

- Dry matter content was highest in Vahan under T3, reaching 23.0%
- Starch content was also highest in Vahan under T3 (14.9%)
- Vitamin C content peaked at 23.9 mg 100 g⁻¹ in Vahan under T3
- Protein content was higher in Dasht-grown tubers (2.16%, T3), linked to higher temperatures that favor protein biosynthesis.

These findings are consistent with earlier studies reporting that the integration of organic and mineral fertilizers improves both yield and quality parameters of potato tubers under different agro-climatic conditions (Lombardo et al., 2013; Brazinskienė et al., 2014; Gelaye, 2023).

Comparative climatic influence on nutritional indices

The combined effects of fertilization and environmental conditions were evident across nutritional traits.

- The Vahan site favored carbohydrate and vitamin accumulation, as indicated by higher dry matter, starch, and vitamin C levels
- The Dasht site, with its warmer and drier climate, was more conducive to protein synthesis in tubers.

These results underscore the importance of adapting fertilization strategies to local agro-climatic conditions to enhance specific nutritional quality traits in potato production.

Correlation analysis

Pearson's correlation analysis revealed strong positive relationships between yield and several quality parameters (Table 8):

- Tuber yield and dry matter: $r = 0.91$
- Tuber yield and starch content: $r = 0.87$
- Tuber yield and vitamin C content: $r = 0.82$
- Moderate correlation with protein content: $r = 0.68$.

These correlations were computed using R statistical software (*cor.test* function) and visualized with the ggpubr package.

The results are consistent with those reported by Brazinskienė et al. (2014), Gelaye (2023), and Lombardo et al. (2013), reinforcing the conclusion that integrated fertilization strategies have a positive and interrelated effect on both potato yield and tuber quality under diverse environmental conditions.

CONCLUSION

The results of this study clearly demonstrate that both the yield and nutritional quality of potato tubers are significantly influenced by fertilization strategies and agro-climatic conditions.

The combined application of mineral fertilizers (N150P120K120) with 40 t ha^{-1} farmyard manure (T3 treatment) produced the highest yields and superior quality parameters, including increased dry matter, starch, vitamin C, and protein content.

Among the two experimental sites, the mountain chernozem soils of Vahan (Gegharkunik region) provided more favorable conditions for the accumulation of dry matter, starch, and vitamin C, owing to cooler temperatures and higher relative humidity.

In contrast, the warmer and drier conditions of Dasht (Armavir region) promoted higher protein synthesis in potato tubers.

These findings highlight the importance of adapting fertilization regimes to local environmental conditions in order to achieve optimal crop performance. Furthermore, the study revealed strong positive correlations between tuber yield and quality parameters such as dry matter, starch, and vitamin C content, confirming the interconnected nature of productivity and nutritional value.

Given the challenges posed by global warming and increasing climate variability, site-specific fertilization strategies are essential for:

- Enhancing sustainable potato production
- Improving nutrient use efficiency
- And developing potato cultivars with superior nutritional and functional properties.

Practical recommendations

Based on the findings, the following practical recommendations can be made for farmers, agronomists, and decision-makers:

1. Adopt integrated fertilization: Combining mineral fertilizers with farmyard manure ($40\,t\,ha^{-1}$) ensures optimal nutrient balance, leading to higher yields and improved tuber quality.

2. Adjust fertilization to local conditions:

✓ In highland regions like Vahan, prioritize practices that support carbohydrate and vitamin accumulation, such as balanced mineral nutrition and moisture conservation.

✓ In lowland regions like Dasht, focus on nitrogen management to control protein accumulation and prevent excessive vegetative growth.

3. Implement climate-smart practices: Given the predicted effects of climate change, techniques such as mulching, drip irrigation, and crop rotation should be integrated to mitigate soil moisture loss and maintain stable yields.

4. Use soil and climate monitoring data to guide fertilization schedules and ensure efficient nutrient use, reducing environmental impact and production costs.

REFERENCES

Abreham, K., Guja, U., Mekuria, T. & Tsegaye, H. 2022. Response of potato (*Solanum tuberosum* L.) to the combined application of organic and inorganic fertilizers at Chena district, Southwestern Ethiopia. *Int. J. Agric Res Innov Technol*. **12**(1), 18–22. doi: 10.3329/ijarit.v12i1.61026

Acar, M., Wahab, T.S., Karaca, Ö. K., Işık, M., Sariyev, A. & Exposing, İ.O. 2025. How Long-Term Organic and Inorganic Fertilizers Affect the Physical Characteristics of Soil. *Journal of Soil Science and Plant Nutrition* **25**, 5187–5201. doi: 10.1007/s42729-025-02457-1

Ahmed, F., Mondal, M.M.A. & Akter, Md.B. 2019. Organic fertilizers effect on potato (*Solanum tuberosum* L.) tuber production in sandy loam soil. *Int J Plant Soil Sci*. **29**(3), 1–11. doi: 10.9734/ijpss/2019/v29i330146

Alengebawy, A., Abdelkhalek, S.T., Qureshi, S.R. & Wang, M.-Q. 2021. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics*, **9**, 42. doi: 10.3390/toxics9030042

Andayani, U. & Maryam, S. 2019. The portrait of reading habit and interest of Tangerang City's community. *Adv Soc Sci Educ Humanit Res*. **302**, 219–221. doi: 10.2991/icclas-18.2019.56

Arinushkina, E.V. 1970. *Guide to Chemical Analysis of Soils*. Moscow, 488 pp. (in Russian).

Atafar, Z., Mesdaghinia, A., Nouri, J., Homaee, M., Yunesian, M., Ahmadimoghaddam, M. & Mahvi, A.H. 2010. Effect of fertilizer application on soil heavy metal concentration. *Environ Monit Assess* **160**, 83–89. doi: 10.1007/s10661-008-0659-x

Awad, M.M., Ali, A., Hegab, S. & El Gawad, A.M.A. 2022. Organic fertilization affects growth and yield of potato (Cara cv) plants grown on sandy clay loam. *Commun Soil Sci Plant Anal*. **53**(6), 688–698. doi: 10.1080/00103624.2022.2028808

Ayupov, Y., Apushev, A., Zamalieva, F.F. & Gabdulov, M. 2014. The effect of planting density on the crop yield, structure and quality of middle-early potato variety in West Kazakhstan. *Life Sci. J*. **11**(8), 545–548. http://www.lifesciencesite.com

Bártová, V., Diviš, J., Bárta, J., Brabcová, A. & Švajnerová, M. 2013. Variation of nitrogenous components in potato (*Solanum tuberosum* L.) tubers produced under organic and conventional crop management. *Eur. J. Agron* **49**, 20–31. doi: 10.1016/j.eja.2013.02.009

Beals, K.A. 2019. Potatoes, nutrition and health. *Am. J. Potato Res*. **96**, 102–110. doi: 10.1007/s12230-018-09705-4

Beck, H.E., Zimmermann, N.E., McVicar, T.R., Vergopolan, N., Berg, A. & Wood, E.F. 2018. Present and future Koppen-Geiger climate classification maps at 1-km resolution. *Scientific Data* **5**, 180214, doi: https://doi.org/10.1038/sdata.2018.214

Bista, B. & Bhandari, D. 2019. Potassium fertilization in potato. *Int. J. Appl. Sci. Biotechnol*. **7**(2), 153–160. doi: 10.3126/ijasbt.v7i2.24636

Brazinskienė, V., Asakaviciute, R., Miezeliene, A., Alencikiene, G., Ivanauskas, L., Jakstas, V., Viskelis, P. & Razukas, A. 2014. Effect of farming systems on yield, quality parameters and sensory properties of conventionally and organically grown potato tubers. *Food Chem* **145**, 903–909. https://doi.org/10.1016/j.foodchem.2013.09.011

Bush, J.R., Baisley, J., Harding, S.V. & Alfa, M.J. 2023. Consumption of Solnul™ resistant potato starch produces a prebiotic effect in a randomized, placebo-controlled clinical trial. *Nutrients* **15**, 1582. doi: 10.3390/nu15071582

Camire, M.E., Kubow, S. & Donnelly, D.J. 2009. Potatoes and human health. *Crit. Rev. Food Sci. Nutr*. **49**, 823–840. doi: 10.1080/10408390903041996

Chen, J., Wu, H., Qian, H. & Gao, Y. 2017. Assessing nitrate and fluoride contaminants in drinking water and their health risk of rural residents living in a semiarid region of Northwest China. *Expo Health* **9**, 183–195. doi: 10.1007/s12403-016-0231-9

Danilchenko, H., Pranaitiene, R., Tarasieviciene, Z. & Venskutoniene, E. 2008. The effect of inhibitors on the amino acid content in stored potato tubers. *Zesz Probl Post Nauk Rol*. **530**, 301–316.

Devaux, A., Kromann, P. & Ortiz, O. 2014. Potatoes for sustainable global food security. *Potato Res*. **57**(3–4), 185–199. doi: 10.1007/s11540-014-9265-1

Dioha, I.J., Olugbemi, O., Onuegbu, T.U. & Shahru, Z. 2011. Determination of ascorbic acid content of some tropical fruits by iodometric titration. *Int. J. Biol. Chem Sci*. **5**(5), 2180–84.

Djaman, K., Irmak, S., Koudahe, K. & Allen, S., 2021. Irrigation management in potato (*Solanum tuberosum* L.) production: a review. *Sustainability* **13**. https://doi.org/ 10.3390/su13031504

El-Sayed, S.F., Hassan, H.A. & El-Mogy, M.M. 2015. Impact of bio- and organic fertilizers on potato yield, quality and tuber weight loss after harvest. *Potato Res*. **58**(1), 67–81. doi: 10.1007/s11540-014-9272-2

Eremeev, V., Keres, I., Tein, B., Selge, A. & Luik, A. 2009. Effect of different production systems on yield and quality of potato. *Agron Res*. **7**(1), 245–250.

Faithfull, N.T. 2002. *Methods in Agricultural Chemical Analysis: A Practical Handbook*. CABI Publishing, Wallingford, 266 pp.

FAO. 2010. Climate Smart Agriculture: Policies, Practices and Financing for Food Security, Adaptation and Mitigation. Rome: Food and Agriculture Organization, 41 pp. Available online: https://www.fao.org/4/i1881e/i1881e00.pdf

Gelaye, Y. 2023. Effect of combined application of organic manure and nitrogen fertilizer rates on yield and yield components of potato: A review. *Cogent Food & Agriculture* **9**. doi: 10.1080/23311932.2023.2217603

Gouerou, M., Visse-Mansiaux, M., Brostaux, Deleu, Y.C., Val, F. Leport, L. & Dupuis, B. 2025. Effect of climate change on potato yield and starch content. *Field Crops Research* **330**, 109951. https://doi.org/10.1016/j.fcr.2025.109951

Harraq, A., Sadiki, K., Bourioug, M. & Bouabid, R. 2022. Organic fertilizers mineralization and their effect on the potato 'Solanum tuberosum' performance in organic farming. *Journal of the Saudi Society of Agricultural Sciences* **21**, 255–266. doi: 10.1016/j.jssas.2021.09.003

Hirpa, A., Meuwissen, M.P.M., Tesfaye, A.J.M., Lommen, W., Lansink, A.O., Tsegaye, A. & Struik, P.C. 2010. Analysis of seed potato systems in Ethiopia. *American Journal of Potato Research* **87**, 537–552.

Hopkins, B.G., Rosen, C.J., Shiffler, A.K. & Taysom, T.W. 2008. Enhanced efficiency fertilizers for improved nutrient management: Potato (Solanum tuberosum L.). *Crop Management*. https://doi.org/10.1094/CM-2008-0317-01-RV

Jennings, S.A., Koehler, A.K., Nicklin, K.J., Deva, C., Sait, S.M. & Challinor, A.J. 2020. Global potato yields increase under climate change with adaptation and CO_2 fertilisation. *Frontiers in Sustainable Food Systems* **4**, 519324. https://doi.org/10.3389/fsufs.2020.519324

Jhangiryan, T., Hunanyan, S., Markosyan, A., Yeritsyan, S., Eloyan, A., Barseghyan, M. & Gasparyan, G. 2024. Assessing the effect of joint application of mineral fertilizers and biohumus on potato yield quality indicators. *Functional Food Science* **4**(12), 508–520. https://doi.org/10.31989/ffs.v4i12.1528

Jiménez, L.I., González, A.Z., López, J.M., Martín, M.A.R.Q. & Ibarra, M.D.L.R. 2008. Photosynthesis, soil temperature and yield of cucumber as affected by colored plastic mulch. *Acta Agriculturae Scandinavica, Section B - Soil & Plant Science* **58**(4), 372–378. doi: 10.1080/09064710801920297

Jovovic, Z., Dolijanovic, Z., Spalevic, V., Dudic, B., Przulj, N., Velimirovic, A. & Popovic, V. 2021. Effects of liming and nutrient management on yield and other parameters of potato productivity on acid soils in Montenegro. *Agronomy* **11**, 980. doi: 10.3390/agronomy11050980

Koch, M., Naumann, M., Pawelzik, E., Gransee, A. & Thiel, H. 2020. The importance of nutrient management for potato production. Part I: Plant nutrition and yield. *Potato Research* **63**, 97–119. https://doi.org/10.1007/s11540-019-09431-2

Konen, M.E., Burras, C.L. & Sandor, J.A. 2003. Organic Carbon, Texture, and Quantitative Color Measurement Relationships for Cultivated Soils in North Central Iowa. *Soil Science Society of America Journal* **67**(6), doi: 10.2136/sssaj2003.1823

Köppen-Geiger. 2024. Classification Descriptions. Appendix C. Available online: https://open.oregonstate.education/permaculturedesign/back-matter/koppen-geiger-classification-descriptions/ (accessed on April 2024), L. & Luik, A. 2023. Changes in the content of soil organic carbon and total nitrogen in the organic and conventional cropping systems. *Agronomy Research* **21**(2), 814–823. https://doi.org/10.15159/AR.23.041

Kumar, M, Singh, S.K. & Patra, A. 2021. Effect of different nutrient sources on yield and biochemical properties of soil under rice-wheat cropping sequence in middle gangetic alluvial plain. *J Plant Nutr* **44**, 2310–2330. doi: 10.1080/01904167.2021.1899206

Kumar, Y.R., Kaushal, S., Kaur, G. & Gulati, D. 2020. Effect of soil organic matter on physical properties of soil. *Just agriculture* **1**(2), 25–30. www.justagriculture.in

Love, S.L., Stark, J.C. & Salaiz, T. 2005. Response of four potato cultivars to rate and timing of nitrogen fertilizer. *American Journal of Potato Research* **82**, 21–31. doi: 10.1007/BF02894916

Lutaladio, N. & Castaldi, L. 2009. Potato: The hidden treasure. *Journal of Food Composition and Analysis* **22**, 491–493. https://doi.org/10.1016/j.jfca.2009.05.002

Mancer, H., Rouahna, H., Souici, S., Otmani, Z., Bettiche, F., Menasria, H., Rechachi, M.Z., Chaib, W. & Mostephaoui, T. 2024. Effects of mineral and organic fertilization on potato production in sandy soil in arid region. *Scientific African* **23**, e02112. doi: 10.1016/j.sciaf.2024.e02112

Martirosyan, H., Galstyan, M., Aloyan, T., Gasparyan, N., Terteryan, K., Sahakyan, N. & Avagyan, G. 2024. The impact of mineral and organic fertilizers on potato yield quantitative, qualitative indicators, and functional value. *Functional Food Science* **4**(8), 309–324. https://doi.org/10.31989/ffs.v4i8.1400

Matevosyan, A.A. & Gyulkhasyan, M.A. 2000. *Plant Cultivation*. Yerevan: Astghik, 57 pp. (in Armenian).

Mattoo, A.K., Dwivedi, S.L., Dutt, S., Singh, B., Garg, M. & Ortiz, R. 2022. Anthocyanin-rich vegetables for human consumption—Focus on potato, sweet potato and tomato. *International Journal of Molecular Sciences* **23**, 2634. doi: 10.3390/ijms23052634

Melkonyan, K.G., Ghazaryan, H.Gh. & Manukyan, R.R. 2004. *Current ecological state of agricultural soils, level of land use, improvement of the management system and ways to increase the effective use of lands in the Republic of Armenia*. Yerevan, 53 pp. (in Armenian).

Miskoska-Milevska, E., Dimovska, D., Popovski, Z. & Iljovski, I. 2020. Influence of the fertilizers Slavol and Biohumus on potato leaf area and stomatal density. *Acta Agriculturae Serbica* **25**, 13–17. https://doi.org/10.5937/AASer2049013M

Molmann, J.A. B. & Johansen, T.J. 2025. Infuence of Growth Temperature on Development and Yield in a Medium Late and a Late Scandinavian Cultivar of Potato. *Potato Research.* https://doi.org/10.1007/s11540-025-09854-0

Naghdi, A.A., Piri, S., Khaligi, A. & Moradi, P. 2022. Enhancing the qualitative and quantitative traits of potato by biological, organic, and chemical fertilizers. *Journal of the Saudi Society of Agricultural Sciences* **21**, 87–92. https://doi.org/10.1016/j.jssas.2021.06.008

Naumann, M., Koch, M., Thiel, H., Gransee, A. & Pawelzik, E. 2020. The importance of nutrient management for potato production. Part II: Plant nutrition and tuber quality. *Potato Research* **63**, 121–137. https://doi.org/10.1007/s11540-019-09430-3

Novikova, L.Yu., Chalaya, N.A., Sitnikov, M.N., Gorlova, L.M., Kiru, S.D. & Rogozina, E.V. 2021. Dynamics of tuber weight in early potato varieties in the contrasting weather conditions of the Northwestern Russia. *Agronomy Research* **19**(1), 185–198. doi: 10.15159/ar.20.241

Lal, R. 2020. Soil organic matter and water retention. *Agron J.* **112**, 3265–3277. doi: 10.1002/agj2.20282

Lombardo, S., Lo Monaco, A., Pandino, G., Parisi, B. & Mauromicale, G. 2013. *The phenology, yield and tuber composition of 'early' crop potatoes: a comparison between organic and conventional cultivation systems. Renewable Agriculture and Food Systems* **28**(1), 50–58. doi: 10.1017/S1742170511000640

Hayrapetyan, E.M. 2000. *Soil Science.* Astghik Publishing, Yerevan, 456 pp. (in Armenian).

Obiero, C.O., Bell, R.W. & Milroy, S.P. 2020. Photosynthetic and respiratory response of potato leaves of different ages during and after an episode of high temperature. *Journal of Agronomy and Crop Science* **206**(3), 352–362. doi: 10.1111/jac.12391

Olivier, M., Goffart, J.P. & Ledent, J.F. 2006. Threshold value for chlorophyll meter as decision tool for nitrogen management of potato. *Agronomy Journal* **98**, 496–506. doi:10.2134/agronj2005.0108

Ozlu, E., Kumar, S. & Arriaga, F.J. 2019. Responses of long-term cattle manure on soil physical and hydraulic properties under a corn-soybean rotation at two locations in Eastern South Dakota. *Soil Sci Soc Am J* **83**, 1459–1467. https://doi.org/10.2136/sssaj2019.03.0077

Pacier, C.M. & Martirosyan, D. 2015. Vitamin C: Optimal dosages, supplementation and use in disease prevention. *Functional Foods in Health and Disease* **5**, 89. doi: 10.31989/ffhd.v5i3.174

Raymundo, R., Asseng, S., Robertson, R., Petsakos, A., Hoogenboom, G. & Quiroz, R. 2018. Climate change impact on global potato production. *European Journal of Agronomy* **100**, 87–98. https://doi.org/10.1016/j.eja.2017.11.008

Rykaczewska, K. 2015 The Effect of High Temperature Occurring in Subsequent Stages of Plant Development on Potato Yield and Tuber Physiological Defects. *American journal of potato Research* **92**(3). doi: 10.1007/s12230-015-9436-x

Setiyo, Y., Gunam, I.B.W., Harsojuwono, B.A. & Yuliadhi, K.A. 2021. Analysis of the impacts of technological implementation on the productivity and quality of seed potato tubers. *Journal of Applied Horticulture* **23**(2), 200–205. doi:0.37855/jah.2021.v23i02.37

Sincik, M., Turan, Z.M. & Goksoy, A.T. 2008. Response of potato (Solanum tuberosum L.) to green manure cover crops and nitrogen fertilization rates. *American Journal of Potato Research* **85**, 150–158. doi: https://doi.org/10.1007/s12230-008-9011-9

Singh, B., Kukreja, S. & Goutam, U. 2019. Impact of heatstress on potato (Solanum tuberosum L.): present scenario and future opportunities*, The Journal of Horticultural Science and Biotechnology*. doi: 10.1080/14620316.2019.1700173

Sintsova, N.F. & Lyskova, I.V. 2020. The study of the source material of potatoes under conditions of Kirov region. *Agrarnaya Nauka Evro-Severo-Vostoka = Agricultural Science Euro-North-East* **21**(6), 697–705. https://doi.org/10.30766/2072-9081.2020.21.6.697-705 (in Russian).

Soils of the Armenian SSR 1976. / Ed. R.A. Edilyan, G.P. Petrosyan, N.N. Rozov. – Yerevan: Hayastan, 383 pp.

Sun, Y., Geng, Q., Du, Y., Yang, X. & Zhai, H. 2017. Induction of cyclic electron flow around photosystem I during heat stress in grape leaves. *Plant Science* **256**, 65–71. doi:10.1016/j.plantsci.2016.12.004

Tamrazov, T.H. 2021. The influence of drought on the change in the area of the assimilation surface of the genotypes of durum and bread wheat, which differ from the ripening period. *Agrarian Science* **350**(6), 37–41. doi:10.32634/0869-8155-2021-350-6-37-41 (in Russian).

Wang, Q.-L., Chen, J.-H., He, N.-Y. & Guo, F.-Q. 2018. Metabolic reprogramming in chloroplasts under heat stress in plants. *International Journal of Molecular Sciences* **19**(3), 849. doi:10.3390/ijms19030849

White, P.J., Wheatley, R.E., Hammond, J.P. & Zhang, K. 2007. Minerals, soils and roots. In: Vreugdenhil, D. (Ed.), *Potato Biology and Biotechnology: Advances and Perspectives*. Elsevier, Amsterdam, pp. 739–752. doi:10.1016/B978-044451018-1/50076-2

Xu, J., Li, Y., Kaur, L., Singh, J. & Zeng, F. 2023. Functional food based on potato. *Foods* **12**, 2145. https://doi.org/10.3390/foods12112145

Zebarth, B.J. & Rosen, C.J. 2007. Research perspective on nitrogen BMP development for potato. *American Journal of Potato Research* **84**, 3–18. doi:10.1007/BF02986294

Zelalem, A., Tekalign, T. & Nigussie, D. 2009. Response of potato (*Solanum tuberosum* L.) to different rates of nitrogen and phosphorus fertilization on vertisols at Debre Berhan, in the central highlands of Ethiopia. *African Journal of Plant Science* **3**, 16–24.

Milk productivity, reproductive performance, hematological and clinical indicators of holstein, brown swiss, simmental, and jersey breeds of cows under Armenian agro-climatic conditions

L.M. Minasyan[1], H.R. Vardanyan[1], Zh.T. Chitchyan[2], M.H. Zadayan[3,*], J.T. Simonyan[4], N.A. Shahazizyan[1], M.S. Mirzoyan[1] and L.L Simonyan[1]

[1]Scientific Centre for Risk Assessment and Analysis in Food Safety Area of the Ministry of Economy of the Republic of Armenia, 107/2 Masis Highway, Shengavit, AM0071 Yerevan, Republic of Armenia
[2]Armenian National Agrarian University, Yerevan, Teryan 74, AM0009 Yerevan, Republic of Armenia
[3]Center for Agricultural Research and Certification, State Non-Commercial Organization at the Ministry of Economy, Yerevanyan highway 2nd block, building 4, AM1139 Merdzavan, Republic of Armenia
[4]Ministry of Economy of the Republic of Armenia, Mher Mkrtchyan 5, AM0010 Yerevan, Republic of Armenia
*Correspondence**: mhzadayan@gmail.com

Abstract. The research was conducted on the farms of 'Himnatavush' Foundation and 'Agroholding Armenia' LLC in the Tavush and Lori regions of the Republic of Armenia. This study aims to fill a research gap regarding the adaptability of elite dairy breeds in the South Caucasus under variable agro-climatic conditions. The scientific and practical goal of the work is to conduct, for the first time, a comparative study of the milk productivity, reproductive capacity, and hematological and clinical indicators of Holstein, Brown Swiss (Schwyz), Simmental, and Jersey cattle breeds imported into the Republic of Armenia from various European countries in recent years, as well as cows born and raised locally (local reproduction). The study is being carried out in two farms located in different agro-climatic zones of the Republic. At the farms of 'Agroholding Armenia' LLC and the 'Himnatavush' Foundation, the age at first calving of both imported full-grown cows of Holstein, Brown Swiss, Simmental, and Jersey breeds and their locally born and raised first-calf heifers corresponded to breed-specific norms. The service period for cows of all breeds exceeded the desirable range (80–90 days), ranging from 93.8 to 115.6 days. However, the reproductive capacity coefficient ranged between 92.9–97.3, which is considered a good indicator. Hematological and clinical parameters of all cows remained within physiological norms. Jersey cows exhibited slightly better adaptation markers. The study confirms the successful acclimatization of these breeds to Armenia's diverse agro-climatic zones and supports the continued use of these high-yielding breeds for sustainable dairy development.

Key words: adaptation traits, milk productivity, age at first calving, reproductive traits, breed comparison, physiological indicators.

INTRODUCTION

In Armenia, cattle breeding is the leading branch of animal husbandry. More than 95% of the milk and over 60% of the meat produced in the country come from cattle farming.

Primarily, the animals raised in the republic belong to the Caucasian Grey breed, which is used for dairy and meat production and accounts for over 90% of the total cattle population. However, the milk yield of this breed is not particularly high; in the best farms, it ranges from 3,000 to 3,500 kilograms per lactation.

In 2022, the average annual milk yield per cow in the country was 2,467 kilograms (Minasyan et al., 2024).

It should be noted that the low milk productivity of cows in the Republic is caused not only by the breeds raised but also by a number of other factors. For example, Armenia is a relatively small country (with a total area of 29.8 thousand km²), of which 2,041.4 thousand hectares, or 68.6% of the total area, are agricultural lands. These include 441.6 thousand hectares (21.6%) of arable land, 1,049.2 thousand hectares (51.4%) of pastures, and 121.3 thousand hectares (5.9%) of hayfields.

In fact, the feed base for cattle breeding remains weak. The productivity of hayfields and pastures is low, the potential for field-based feed production is limited, and arable lands are mainly used for cultivating other crops. As a result, the amount of feed available for livestock is insufficient, especially during the stall-feeding (winter) period.

In addition, cattle breeding in the country is still practiced extensively. Selective breeding programs and pedigree work are not properly implemented, artificial insemination of cows does not cover a large portion of the herd, and housing conditions require improvement, among other issues.

Considering the necessity of further developing cattle breeding in the republic, especially to increase the average milk yield per cow, several projects have been implemented in recent years aimed at expanding milk production. As part of these initiatives, heifers of various European breeds - mainly Holstein, Swiss (Schwyz), Simmental, and Jersey - have been imported.

Our studies have shown that both the imported heifers of these breeds and their locally born and raised offspring (local reproduction) demonstrate quite high milk productivity under the conditions of our republic. According to our data (Minasyan et al., 2023a), under the conditions of the 'Depi Agro' farm in Armavir Region, imported Holstein cows from Germany achieved an average first-lactation milk yield of 5,827 kilograms, with milk fat and protein contents of 3.82% and 3.27%, respectively.

According to the results of our studies (Muradyan et al., 2022), at the 'Agroholding Armenia' LLC farm, locally reproduced Brown Swiss breed cows had a 305-day lactation milk yield of 8,450 kilograms, with milk fat and protein contents of 4.0% and 3.12%, respectively.

Based on the above, we emphasize the importance of continuing research on Holstein, Swiss, Simmental, and Jersey cattle breeds - not only regarding their milk productivity but also their reproductive capacities and hematological clinical indicators - and presenting conclusions about their adaptability to the climatic conditions of our republic.

In modern cattle breeding, reproductive performance is recognized as a key economic trait, alongside breed improvement and technological advancements aimed at enhancing

productivity. Effective herd reproductive management significantly influences milk yield, animal longevity, utilization intensity, and overall production profitability.

Reproduction, as a fundamental biological function, ensures the continuity and survival of species. Suboptimal reproductive performance in cows leads to substantial economic losses for farmers. A shortage of calves contributes to herd size reduction, decreased milk and meat production, and limited opportunities for herd renewal and genetic improvement. Additionally, maintaining infertile animals imposes unnecessary costs related to feeding, care, and veterinary interventions.

According to Kozlo (1984), a 10% infertility rate in cows (with an average annual milk yield of 3,000 kg) can result in a 5% reduction in milk output and a 10% decline in calf production annually. Moreover, over 5% more feed units are required to produce every 100 kg of milk under such conditions.

Thus, optimizing reproductive function is essential for improving herd productivity, advancing breed development, and enhancing the economic sustainability of livestock systems.

Furthermore, reproductive efficiency is closely linked to an animal's adaptability to environmental conditions. In this context, clinical and hematological indicators serve as critical tools for evaluating animals' acclimatization and adaptation processes. These indicators provide insights into animals' physiological responses and their suitability for specific rearing environments.

Understanding the variability of these parameters under different ecological conditions is particularly important for assessing health status, metabolic function, and productive potential during the acclimatization period.

Accurate interpretation of adaptability traits requires reference to species-specific physiological norms. For cattle, normal ranges are as follows (Kudryavtsev & Kudryavtseva, 1974; Eidrigievich & Raevskaya, 1978):

- Pulse rate: 40–80 beats per minute
- Respiration rate: 17–30 breaths per minute
- Body temperature: 37.5–39.5 °C
- Erythrocyte count: 5.5–8 million mm^{-3}
- Leukocyte count: 5.0–9.4 thousand mm^{-3}
- Hemoglobin: 9–12 g %$^{-1}$
- Alkaline reserve: 410–540 mg %$^{-1}$
- Total serum protein: 6–8 g %$^{-1}$.

Among clinical indicators, body temperature, pulse rate, and respiratory rate are considered primary markers of physiological condition. Literature indicates that body temperature is generally stable, except in cases of illness, and is not significantly influenced by age, gestation, or lactation. In contrast, pulse and especially respiration rates are more sensitive to age-related changes. For instance, from birth to maturity, pulse rate decreases by approximately 18%, and respiration rate by 35%.

The effect of age at first calving on milk productivity and reproductive qualities of Holstein cows has been studied in the USA by Meyer et al. (2004) and in China by Mao et al. (2010).

Environmental factors also play a crucial role in shaping animals' physiological and morphological traits during growth and development, especially by affecting blood composition. Hematological parameters are commonly used to assess animal health,

body condition, and productivity potential, given their close correlation with metabolic and physiological status.

In a large-scale study conducted in Southern China on 786 clinically healthy Holstein cows, hematological values were determined. The results varied significantly with age, number of calvings, and lactation stage (Chen et al., 2022).

In Armenia, clinical and hematological characteristics have been previously investigated in local breeds. The Caucasian Grey cattle have been studied by Hovhanisyan (2001) and Simonyan (2004), while research on the second planned Black Pied breed was conducted by Chitchyan (1984), Abovyan (1994), and Minasyan (2013), among others.

Although Holstein and Jersey breeds are widely studied in Western Europe and North America, data from the South Caucasus - especially under diverse climatic zones - are scarce. Thus, this study aims to fill a key research gap regarding the adaptability of elite dairy breeds in the South Caucasus under variable agro-climatic conditions.

In various regions of the Russian Federation, the reproductive abilities of Grey Swiss cows have been studied by Solovyova et al. (2016), Salakhov (2017), and Skoptsova & Popova (2018).

Good reproductive abilities of Holstein and Jersey cows have also been reported in the USA by Worman et al. (2009).

Kostomakhin et al. (2021) studied the reproductive abilities of Holstein cows in Kurgan Oblast, Russian Federation.

According to Khamiruev (2009), in the 'Baikalskoye' farm in the Republic of Buryatia, Russian Federation, the service period of Austrian-bred Simmental cows was 92.4 days, the dry period was 65.9 days, and the inter-calving period was 418.2 days.

According to Svyazhenina (2020), in the Tyumen region of the Russian Federation, the age at first calving for Simmental cows was 25.9–27.6 months, and the service period lasted 119–130 days.

The scientific and practical goal of this work is to conduct, for the first time, a comparative study of the milk productivity, reproductive capacity, and hematological and clinical indicators of Holstein, Brown Swiss (Schwyz), Simmental, and Jersey cattle breeds imported into the Republic of Armenia from various European countries in recent years, as well as of cows born and raised locally (local reproduction). The study is being carried out on two farms located in different agro-climatic zones of the Republic.

Based on the data obtained, it will be possible to present scientifically grounded conclusions regarding the adaptability of these breeds to the climatic conditions of Armenia and to make recommendations on the future importation and breeding of these animals in the country.

Action Plan
- Selection of Holstein, Brown Swiss (Schwyz), Simmental, and Jersey cows and formation of experimental groups on two farms.
- Organization and implementation of research as outlined by the methodologies.
- Regular visits to the farms and on-site supervision of ongoing work.
- Collection, recording, and processing of data obtained from the research.

MATERIALS AND METHODS

Study Design and Location

The Republic of Armenia is a landlocked country in the South Caucasus, situated at the intersection of Eastern Europe and Western Asia. Its total surface area is approximately 29,743 km². Armenia's terrain is predominantly mountainous, with an average elevation of ~1,800 meters above sea level. The landscape features volcanic plateaus, high mountain ranges, and deep river valleys. The continental highland climate is characterized by hot, dry summers and cold, snowy winters, with significant regional variation. Annual precipitation ranges from less than 250 mm in arid zones to more than 1,000 mm in forested highlands.

Our research was conducted in the Tavush and Lori regions of the Republic of Armenia. Tavush, one of Armenia's ten administrative regions, is located in the northeastern part of the country. It covers 2,704 km² and is characterized by forested mountains, narrow valleys, and a relatively high proportion of biologically productive land. Tavush experiences a mildly humid climate influenced by prevailing westerly winds and orographic precipitation. Average annual precipitation ranges from 600 to 900 mm, with relatively warm, humid summers and moderate winters, particularly in lower altitudes. These conditions favor perennial grasses, forage crops, and natural pasturelands.

Lori, located immediately west of Tavush, spans approximately 3,799 km² and is topographically diverse, comprising uplands, river valleys, and subalpine zones. Its climate is temperate to moderately humid, with annual precipitation between 600 and 1,000 mm, and slightly cooler temperatures than Tavush due to elevation and exposure. Winter temperatures are lower, especially in higher altitudes, while summers remain relatively cool and moist.

The northeastern regions of Armenia, particularly Tavush and Lori, possess climatic and ecological conditions favorable for cattle farming. Key factors include:

- **Abundant precipitation and humidity:** Moderate to high rainfall supports lush pasturelands and diverse vegetation, enabling extended grazing seasons and reducing dependence on supplementary feed, which increases the cost-efficiency of extensive cattle rearing systems.

- **Cool summer temperatures:** Lower summer heat stress in highland regions like Lori contributes to higher milk yields and improved reproductive performance in cattle. Heat stress, a limiting factor in many semi-arid regions, is minimized here, especially for dairy breeds.

- **Availability of natural water resources:** Numerous rivers and springs in both regions provide reliable water supplies essential for livestock health and forage cultivation.

- **Longer vegetation periods:** Lower altitudes of Tavush allow multi-cut hay production and the growth of high-quality fodder crops such as clover, alfalfa, and mixed grasses.

- **Forest-pasture ecosystems:** Forest-steppe landscapes support agro-silvopastoral systems, enabling farmers to combine free-range grazing with woodland shelter, which improves animal welfare and productivity, especially in winter.

This study was conducted during 2022–2023 at two large commercial dairy farms in Armenia: 'Agroholding Armenia' LLC in Lori Province and the 'Himnatavush' Foundation in Tavush Province.

The farms housed fully grown Holstein, Brown Swiss, Simmental, and Jersey cows imported from various European countries, as well as locally born and raised first-lactation heifers derived from the same breeds. Each breed group included 10 clinically healthy cows. During the research period, the number of cows at the 'Agroholding Armenia' LLC farm ranged from 180 to 190, while at the 'Himnatavush' Foundation farm it was 80 to 90.

Both farms kept cows year-round in typical modern loose-box housing systems. Required zoo-hygienic standards were monitored and maintained in the barns. Cows were fed and milked twice daily, with mechanized milking performed in dedicated parlors equipped with appropriate milking units.

Loose-box housing was organized by grouping cows in separate barn sections, with individual boxes (resting/sleeping areas) prepared for each animal. Boxes were separated by metal bars and measured approximately 190–210 cm in length, 100–120 cm in width, and 100–110 cm in height (side bars). Boxes were elevated 15–20 cm above the barn floor, which was made of concrete or wooden planks and mostly covered with rubber mats. Bedding materials included wood shavings, peat, or chopped straw. Cows were fed in feeding troughs.

Milk yield was determined over a 305-day lactation period using test milking. Fat and protein content were measured using an 'Ekomilk' milk analyzer, and live weight was determined by weighing. The milk yield coefficient (milk yield to live weight ratio) was calculated based on these data.

Reproductive Performance Indicators

The following reproductive traits were evaluated using on-farm management records:

- Age at first calving
- Service period (interval from calving to successful conception)
- Calving interval (CI)
- Dry period (non-lactating period before calving)
- Reproductive capacity coefficient (RCC).

RCC was calculated according to Marmaryan (2001) using the formula:

$$\text{RCC} = \left(\frac{365}{\text{CI}}\right) \cdot 100$$

where 365 is the number of days in a year and CI is the calving interval in days.

Hematological Parameters

Measured hematological indicators included:

- Erythrocyte count (million mm^{-3})
- Leukocyte count (thousand mm^{-3})
- Hemoglobin concentration (g, %)
- Total serum protein (g, %)
- Alkaline reserve (mg, %).

Blood samples were collected early in the morning from the jugular vein prior to feeding. Analyses were conducted at the 'Neolab' diagnostic laboratory (Yerevan), following standard veterinary hematology protocols.

Clinical Indicators

Clinical indicators were recorded seasonally (spring, summer, autumn, winter) to assess physiological adaptation:

- Body temperature, measured rectally with a digital veterinary thermometer
- Respiration rate, observed by thoracic movements per minute
- Pulse rate, measured at the coccygeal artery using a stethoscope.

These indicators reflect baseline metabolic activity and animals' physiological responses to seasonal climatic variations.

The data obtained from the studies were subjected to biometric processing (mean ± standard error, standard deviation, coefficient of variation) using the computer software 'Biostat'.

RESULTS AND DISCUSSION

Milk Productivity

Milk productivity was studied in cows of all breeds at two farms, focusing on first and third or higher lactations, including 305-day milk yield, milk fat and protein content, and live weight. Results are presented in Table 1.

As shown in Table 1, cows of all breeds demonstrated fairly high milk productivity. Generally, milk yield increased with age. An exception was observed among Brown Swiss and Simmental cows at 'Agroholding Armenia' LLC, where first-calf cows significantly outperformed mature cows in milk yield. Notably, first-calf Brown Swiss cows reached a record-high milk productivity of 8,523 kg, exceeding Holstein and Simmental counterparts by 223 kg (2.7%) and 610 kg (7.7%), respectively.

Among mature cows of these three breeds, Holsteins had the highest milk yield (8,887 kg), surpassing Brown Swiss and Simmental cows by 1,114 kg (14.3%) and 2,691 kg (43.4%), respectively. Brown Swiss cows in their third and higher lactations outperformed mature Simmental cows by 1,577 kg (25.4%).

The high milk productivity of locally raised first-lactation cows of these breeds is mainly attributed to the use of high-quality bulls, excellent feeding and care practices, and favorable rearing conditions during growth and development.

Milk fat content was relatively high, ranging from 3.9% to 4.33%. First-lactation Simmental cows had a fat content of 4.07%, 0.17% higher than Holsteins of the same lactation. Among mature cows, Holsteins exhibited the highest fat content (4.33%), while Brown Swiss cows showed relatively higher milk protein content.

Live weight increased predictably with age (Table 1). Simmental cows had the highest live weights (576 and 726 kg), significantly surpassing Holstein and Brown Swiss counterparts. However, Simmentals had lower milk yield coefficients. First-lactation cows exhibited higher milk yield coefficients (13.7–15.8) compared to mature cows.

Table 1. Milk productivity, milk composition, live weight, and milk yield coefficient of Holstein, Brown Swiss, Simmental, and Jersey cows by lactation and farm

Breed	Lactation	Biometric parameters	Milk yield in 305 days, kg	Milk fat content, %	Milk protein content, %	Milk fat, kg	Milk protein, kg	Live weight, kg	Milk yield coefficient
Milk productivity indicators of different cow breeds in the farms of 'Agroholding Armenia' LLC and 'Himnatavush' Foundation (n = 10)
'Agroholding Armenia' LLC									
Holstein	I	M±m	8,300 ± 566	3.9 ± 0.05	3.04 ± 0.02	324 ± 21.7	252 ± 16.2	553 ± 19.7	15.0 ± 1.0
		C_v	21.6	3.9	2.4	21.2	20.4	11.3	20.8
	III and higher	M±m	8,887 ± 445	4.33 ± 0.13	3.04 ± 0.02	385 ± 16.3	270 ± 13.1	683 ± 12.4	13.0 ± 0.7
		C_v	15.8	9.7	2.6	13.5	15.3	5.8	18.1
Swiss	I	M±m	8,523 ± 530	3.9 ± 0.05	3.11 ± 0.04	332 ± 22.3	265 ± 18.0	540 ± 19.0	15.8 ± 0.9
		C_v	19.7	3.8	3.6	21.2	21.5	11.1	18.5
	III and higher	M±m	7,773 ± 338	4.3 ± 0.04	3.22 ± 0.05	334 ± 15.6	250 ± 10.8	660 ± 10.0	11.8 ± 0.5
		C_v	13.7	3.1	4.7	15.0	14.1	4.8	14.8
Simmental	I	M±m	7,913 ± 419	4.07 ± 0.09	3.14 ± 0.03	322 ± 16.2	248 ± 14.2	576 ± 16.5	13.7 ± 0.9
		C_v	16.7	6.8	3.0	15.9	18.0	9.1	21.6
	III and higher	M±m	6,196 ± 565	4.23 ± 0.08	3.1 ± 0.02	262 ± 19.5	192 ± 16.7	726 ± 9.0	8.5 ± 0.8
		C_v	28.8	5.8	2.4	21.7	27.7	3.9	29.2
'Himnatavush' Foundation									
Jersey	I	M±m	3,345 ± 147	5.82 ± 0.13	3.9 ± 0.05	195 ± 7.4	130 ± 6.2	440 ± 14.2	7.6 ± 0.3
		C_v	13.9	7.2	4.0	12.1	15.1	10.2	10.7
	III and higher	M±m	4,040 ± 182	5.4 ± 0.13	3.82 ± 0.04	218 ± 13.6	154 ± 7.2	485 ± 12.8	8.3 ± 0.5
		C_v	14.2	7.7	3.4	19.6	14.6	8.3	17.5
Simmental	I	M±m	3,670 ± 205	4.0 ± 0.06	3.18 ± 0.02	147 ± 7.2	117 ± 5.9	560 ± 10.5	6.6 ± 0.3
		C_v	17.7	4.9	2.5	15.7	16.1	5.9	14.8
	III and higher	M±m	5,115 ± 243	3.95 ± 0.05	3.15 ± 0.03	202 ± 8.7	161 ± 6.4	655 ± 11.4	7.8 ± 0.4
		C_v	15.1	3.7	2.7	13.7	12.7	5.5	14.7

At the 'Himnatavush' Foundation farm, Jersey and Simmental cows showed fairly high milk productivity increasing with age. Simmentals outperformed Jerseys in milk yield for both lactation groups but lagged in milk fat and protein content. Jerseys, known for their high milk fat globally, had fat contents of 5.82% and 5.4% for first and third or higher lactations, respectively.

Simmental cows at this farm had higher live weights (560 and 655 kg) than Jerseys but lower milk yield coefficients.

In summary, Holstein, Brown Swiss, Simmental, and Jersey cows - both imported and locally born, first-calf and mature - showed milk productivity typical for their breeds and quite high under Armenian conditions.

Similar positive results for these breeds have been reported in other countries:

• At 'Agroholding Armenia' LLC, milk yields for local reproduction Holstein, Swiss, and Simmental cows were 9,040, 8,450, and 9,313 kg, respectively (Minasyan et al., 2023b)

• At 'Himnatavush' Foundation, first lactation Jersey cows produced 2,532 kg milk with 5.79% fat, and third/higher lactations produced 3,132 kg with 5.28% fat (Chitchyan, 2017)

• In Kazakhstan, imported Holstein cows at 'Almati' farm yielded 7,598 kg (Muratova, 2021)

• In Russia, first-lactation Holsteins at 'Agrofirma Pakhma' farm produced 8,615 kg (Tyapugin et al., 2021); in Moscow's 'Avdeyevskoye' farm, first and third lactations yielded 7,297 and 8,773 kg respectively (Lepekhina et al., 2022)

• Simmental cows in Russia's Tyumen region produced 6,903 kg (first lactation) and 7,360 kg (mature) (Svyazhenina, 2020).

These data demonstrate consistently high milk productivity of the studied breeds under favorable feeding and management conditions.

Reproductive Performance

The comparative assessment of reproductive traits among Holstein, Brown Swiss, Simmental, and Jersey cows - both imported and locally born - indicated satisfactory adaptation and reproductive efficiency under Armenian agro-climatic conditions.

Data presented in Table 2 show that the age at first calving among locally bred first-lactation cows ranged from 24.6 to 28.0 months. Jersey cows exhibited the earliest calving (24.6–25.0 months), characteristic of early-maturing breeds. In contrast, Holstein, Brown Swiss, and Simmental cows calved at 27.0–28.0 months, consistent with medium-maturing breeds.

The service period exceeded the recommended 80–90 days in all groups. Locally bred cows showed shorter service periods (105.7 days on average) than imported ones (113.8 days), suggesting improved reproductive efficiency. The calving interval (CI) ranged from 374.4 to 393.3 days, and the reproductive capacity coefficient (RCC) varied between 92.9 and 97.3. Jersey cows had the highest RCC values (96.6–97.3), reflecting superior reproductive performance.

Dry periods ranged from 61.4 to 85.2 days - above the standard 45–60 days - but remained within acceptable physiological limits.

These findings align with prior studies conducted in Armenia (Minasyan, 2010; Navasardyan, 2015) and other regions (Abdullayev et al., 2021; Muratova, 2021; Kanev et al., 2024), which describe similar reproductive traits in continental climates.

Table 2. Reproductive performance indicators (mean ± SD) in imported and locally bred cows by breed and farm

Reproductive Performance Indicators of Different Breeds of Cows in the Farms of 'Agroholding Armenia' LLC and 'Himnatavush' Foundation (*n* = 10)

Breed	Lactation	Biometric parameters	Age at first calving (months)	Service period duration (days)	Gestation period duration (days)	Calving interval duration (days)	Dry period duration (days)	Reproductive capacity coefficient (RCC)
'Agroholding Armenia' LLC								
Holstein	I	M±m	27.3 ± 1.23	103.4 ± 4.12	277.3 ± 1.3	380.8 ± 5.53	61.4 ± 3.47	95.8 ± 0.92
		C_v	14.3	12.6	1.5	2.9	17.9	3.0
	III and higher	M±m	28.6 ± 1.16	113.0 ± 3.83	275.4 ± 1.2	388.5 ± 7.96	61.9 ± 4.87	94.0 ± 0.96
		C_v	12.8	10.7	1.4	3.2	24.9	3.2
Swiss	I	M±m	27.7 ± 0.81	106.5 ± 4.26	275.2 ± 1.1	381.8 ± 4.6	64.7 ± 4.7	95.7 ± 1.17
		C_v	9.2	12.7	1.23	3.84	23.1	3.85
	III and higher	M±m	30.6 ± 0.86	112.8 ± 4.1	274.2 ± 0.92	387.0 ± 4.35	68.7 ± 4.35	94.4 ± 1.1
		C_v	8.9	11.5	1.1	3.6	20.0	3.7
Simmental	I	M±m	27.3 ± 0.78	107.0 ± 4.12	278.5 ± 1.23	385.6 ± 4.2	69.0 ± 4.55	94.8 ± 1.0
		C_v	9.0	12.2	1.4	3.45	20.9	3.4
	III and higher	M±m	29.0 ± 1.24	115.6 ± 3.83	277.0 ± 1.3	393.3 ± 4.5	70.7 ± 4.5	92.9 ± 1.1
		C_v	13.5	10.5	1.5	3.6	20.1	3.7
'Himnatavush' Foundation								
Jersey	I	M±m	25.0 ± 0.66	97.5 ± 5.0	280.3 ± 0.8	378.0 ± 5.3	81.3 ± 4.7	96.6 ± 1.3
		C_v	8.4	16.0	0.9	4.4	18.2	4.3
	III and higher	M±m	24.6 ± 1.0	93.8 ± 7.5	281.6 ± 1.2	374.4 ± 7.2	85.2 ± 5.4	97.3 ± 1.9
		C_v	12.8	25.3	1.3	6.1	19.9	6.1
Simmental	I	M±m	27.0 ± 0.8	102.0 ± 3.85	279.1 ± 1.2	381.0 ± 4.2	65.0 ± 2.7	95.9 ± 1.0
		C_v	9.8	11.9	1.3	3.5	13.2	3.5
	III and higher	M±m	28.0 ± 0.63	105.7 ± 4.1	278.8 ± 1.2	384.5 ± 4.1	71.0 ± 2.8	95.0 ± 1.0
		C_v	7.1	12.4	1.3	3.4	12.5	3.3

Note: CI – calving interval; RCC – reproductive capacity coefficient.

Table 3. Hematological parameters in different cattle breeds (mean ± SD) measured during the study

Breed	Biometric parameters	Erythrocyte count, mln/1mm³	Leukocyte count, thous./1mm³	Hemoglobin, g%	Total protein, %	Blood alkaline reserve, mg%
Hematological Indicators of Mature Cows of Different Breeds in the Farms of 'Agroholding Armenia' LLC and 'Himnatavush' Foundation ($n = 10$)						
'Agroholding Armenia' LLC						
Holstein	M±m	7.1 ± 0.10	10.6 ± 0.48	11.0 ± 0.35	6.3 ± 0.11	424.0 ± 9.4
	C_v	4.4	14.3	10.1	5.7	7.0
Swiss	M±m	6.7 ± 0.14	8.8 ± 0.54	11.8 ± 0.37	6.4 ± 0.11	430.5 ± 7.0
	C_v	6.9	19.3	9.9	5.4	5.2
Simmental	M±m	7.2 ± 0.18	9.4 ± 0.52	12.0 ± 0.14	6.3 ± 0.12	433.1 ± 9.3
	C_v	7.8	17.4	3.6	5.9	6.8
'Himnatavush' Foundation						
Jersey	M±m	5.2 ± 0.18	7.5 ± 0.3	9.5 ± 0.2	6.3 ± 0.08	430.2 ± 9.5
	C_v	10.9	12.6	6.5	4.3	7.0
Simmental	M±m	7.1 ± 0.23	9.2 ± 0.6	11.6 ± 0.18	6.1 ± 0.1	424.1 ± 7.9
	C_v	10.2	20.3	4.9	4.7	5.9

Note: All values are within physiological ranges. Sample size n = 10 per group.

Table 4. Seasonal variation in clinical indicators in cows of different breeds

Clinical Indicators of Holstein, Swiss, and Simmental Breed Cows from the 'Agroholding Armenia' LLC Farm and Jersey and Simmental Breed Cows from the 'Himnatavush' Foundation Farm ($n = 10$)

Breed	Lactation	Spring			Summer			Autumn			Winter		
		Body temperature, °C	Respiratory rate, per min	Pulse rate, per min	Body temperature, °C	Respiratory rate, per min	Pulse rate, per min	Body temperature, °C	Respiratory rate, per min	Pulse rate, per min	Body temperature, °C	Respiratory rate, per min	Pulse rate, per min
'Agroholding Armenia' LLC													
Holstein	I	38.2	34.3	72.2	38.3	34.8	72.8	38.2	34.5	72.3	38.2	34.7	71.7
	III and higher	37.6	31.8	66.1	37.7	33.1	69.0	37.7	32.0	66.2	37.7	33.1	67.6
Swiss	I	38.3	34.4	73.1	38.4	34.8	73.8	38.3	34.3	73.3	38.2	34.2	73.4
	III and higher	37.8	33.4	72.0	37.8	33.8	72.7	37.8	33.4	71.8	37.7	33.3	71.0
Simmen-tal	I	38.8	32.7	68.8	38.8	33.6	69.6	38.6	33.1	69.0	38.5	32.5	69.0
	III and higher	38.1	33.0	70.0	38.0	33.5	71.1	38.0	32.9	70.2	38	32.6	70.4
'Himnatavush' Foundation													
Jersey	I	38.7	33.0	64.0	38.7	33.8	65.4	38.8	32.2	64.9	38.0	32.5	64.4
	III and higher	38.8	32.2	65.0	38.7	33.6	65.7	38.6	32.4	65.0	38.3	33.0	64.8
Simmen-tal	I	38.7	32.0	69.0	38.6	33.0	70.0	38.6	32.6	69.1	38.5	32.3	68.5
	III and higher	38.2	33.1	71.0	38.2	33.8	71.7	38.3	33.3	70.5	38.0	33.0	70.2

Note: Measurements recorded in spring, summer, autumn, and winter. Units: temperature (°C), respiration (breaths min^{-1}), pulse (beats min^{-1}).

Hematological Indicators

Table 3 shows hematological profiles of study cows. All values fell within physiological norms. At 'Agroholding Armenia' LLC, Holstein and Simmental cows had slightly higher erythrocyte and leukocyte counts than Brown Swiss cows, while hemoglobin, total protein, and alkaline reserve were stable across breeds.

At 'Himnatavush' Foundation, Jersey cows exhibited higher total serum protein and alkaline reserve levels compared to Simmental cows, suggesting enhanced metabolic activity and heat adaptability. Simmental cows showed elevated erythrocyte and hemoglobin levels, possibly reflecting robust oxygen transport capacity.

These results align with previous breed-specific hematological findings reported by Gaidukova (2011), Shevkhuzhev et al. (2014) and Chitchyan (2017).

Clinical Indicators

Seasonal clinical monitoring (Table 4, Figs 1 and 2) showed body temperatures within the normal physiological range (37.6–38.8 °C). Respiratory and pulse rates varied slightly by season, ranging from 31.8 to 34.8 breaths per minute and 64.0 to 73.8 beats per minute, respectively.

Slight increases during summer were most pronounced in Jersey cows, likely due to higher metabolic activity and environmental sensitivity.

These values are consistent with species-specific norms described by Kudryavtsev & Kudryavtseva (1974), confirming the animals' health and good adaptation to local climatic variations.
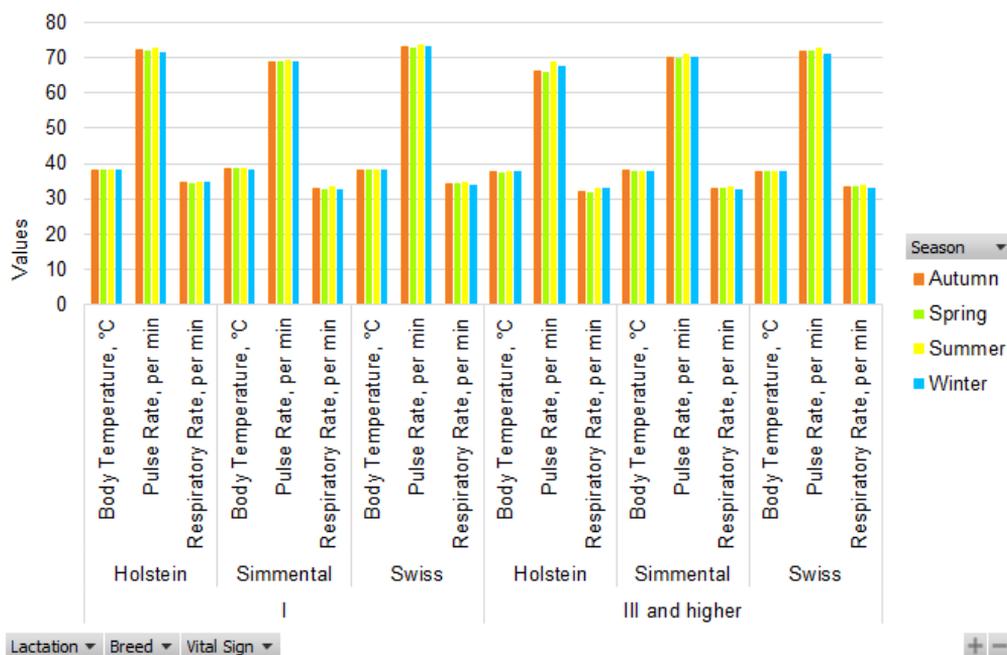
Figure 1. Clinical Indicators of Holstein, Swiss, and Simmental Breed Cows from the 'Agroholding Armenia' LLC Farm.
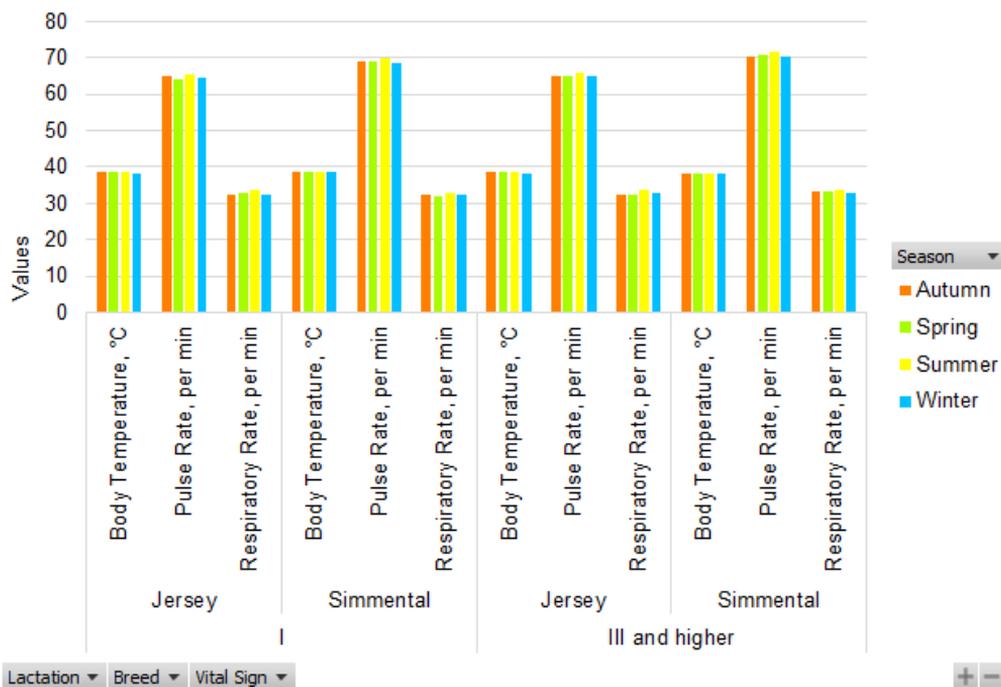
Figure 2. Clinical Indicators of Jersey and Simmental Breed Cows from the 'Himnatavush' Foundation Farm.

CONCLUSIONS

The findings of this study demonstrate that Holstein, Brown Swiss, Simmental, and Jersey cows – both imported and locally bred – exhibit satisfactory milk productivity, reproductive performance, and physiological adaptation under the diverse agro-climatic conditions of Armenia.

At the 'Agroholding Armenia' LLC farm, first-calf Brown Swiss cows produced 8,523 kg of milk, exceeding their Holstein and Simmental counterparts by 223 kg (2.7%) and 610 kg (7.7%), respectively. Among mature cows of these breeds, Holsteins had the highest milk yield (8,887 kg), surpassing Brown Swiss and Simmental cows by 1,114 kg (14.3%) and 2,691 kg (43.4%), respectively. Brown Swiss cows also outperformed Simmentals by 1,577 kg (25.4%).

These three breeds showed relatively high milk fat content, ranging between 3.9% and 4.33%. Live weights increased proportionally with age, with Simmental cows having the highest values (576 kg and 726 kg), significantly surpassing Holstein and Brown Swiss counterparts.

At the 'Himnatavush' Foundation farm, milk yields of Simmental cows were 3,670 kg and 5,115 kg for first and third or higher lactations, respectively; Jersey cows produced 3,345 kg and 4,040 kg. Jersey cows demonstrated characteristically high milk fat content – 5.82% and 5.4%, respectively.

Although service and dry periods slightly exceeded standard recommendations, reproductive efficiency indicators such as the reproductive capacity coefficient (92.8–97.2) remained within acceptable thresholds. Locally bred cows outperformed imported ones in several reproductive traits, indicating successful genetic adaptation and effective herd management.

Hematological indicators showed no significant differences among mature cows of all breeds at both farms, with all values within physiological norms. Clinical parameters including body temperature (37.6–38.8°C), respiration rate (31.8–34.8 breaths min^{-1}), and pulse rate (64.0–73.8 beats min^{-1}) were stable and within normal ranges throughout the study.

Hematological and clinical parameters confirm the absence of pathological stress. Jersey cows exhibited slightly better reproductive efficiency and protein metabolism indicators, while Simmental cows showed enhanced erythrocyte and hemoglobin profiles.

These results support the continued importation and selective breeding of Holstein, Brown Swiss, Simmental, and Jersey cattle in Armenia. Their adaptability, reproductive viability, and physiological stability suggest strong potential for improving the sustainability, productivity, and genetic advancement of the national dairy sector.

Further longitudinal studies under varying feeding regimes are recommended to fully exploit the genetic potential of these breeds in Armenia's evolving dairy industry.

REFERENCES

Abdullayev, K.Sh., Saniyazova, A.Zh. & Sultanbayeva, G.K. 2021. Selection status in the dairy cattle industry in southern Kazakhstan. In *Collection of Scientific Works of the Kazakh National Agrarian Research University*, Almaty, pp. 71–75.

Abovyan, Yu.H. 1994. *Influence of genotypic characteristics and paratypical factors on the development and manifestation of natural resistance in cattle*. Doctoral dissertation in biological sciences, Yerevan, 40 pp.

Chen, H., Yu, B., Liu, C., Cheng, L., Yu, J., Hu, X. & Xiang, M. 2022. Hematology Reference Intervals for Holstein Cows in Southern China: A Study of 786 Subjects. *Vet Sci.* **9**(10), 565.

Chitchyan, Zh.T. 1984. *Economic and biological traits of imported Black Pied cattle in Armenia's mountainous zones*. Abstract of thesis for Candidate of Agricultural Sciences. 06.02.04, Krasnodar, 23 pp.

Chitchyan, Zh.T. 2017. Biological and productive traits of imported Jersey cows in the northeast of Armenia. PhD dissertation, Yerevan, 23 pp.

Eidrigievich, E.V. & Raevskaya, V.V. 1978. *Conformation of Farm Animals*. Moscow: Kolos, 255 pp.

Gaidukova, E.V. 2011. Correlation between milk productivity and reproductive qualities of cows depending on breed in breeding farms in Kaluga region. *Zootechny* **12**, 8–10.

Hovhanisyan, A.O. 2001. Economic-biological characteristics of the Caucasian Brown cattle breed and the development and application of new selection parameters for breed improvement in the Republic of Armenia. Abstract of doctoral dissertation in agricultural sciences. 06.02.01, Yerevan, 37 pp.

Kanev, P.N., Gorelik, O.V. & Kharlap, S.Yu. 2024. Association of productive traits in Holstein dairy cattle. *Agrarian Science* **3**, 92–97.

Khamiruev, T.N. 2009. Simmental cattle of Austrian selection under the conditions of the Baikalskoye enterprise, Republic of Buryatia. In *Agrarian Science for Agricultural Production in Kazakhstan, Siberia, and Mongolia. Proceedings of the 12th International Scientific-Practical Conference (Shymkent, April 16–17, 2009)*, Vol. II, Almaty, pp. 64–67.

Kostomakhin, N.M., Voronova, O.A. & Gabedova, M.A. 2021. Milk productivity and reproductive capacity of cows with different Holstein blood levels. *Bulletin of Kurgan State Agricultural Academy* **3**(39), 43–50.

Kozlo, N.E. 1984. *Animal Reproduction*. Moscow, 224 pp.

Kudryavtsev, A.A. & Kudryavtseva, L.A. 1974. Clinical Hematology of Animals. Moscow: Kolos, 339 pp.

Lepekhina, T.V., Bakay, F.R. & Avdeeva, E.V. 2022. Milk productivity of Holstein cows in the Moscow region. *Zootechny* **5**, 5–7.

Mao, V.G., Vang, Z.P. & Jhi, X.K. 2010. Effect of the age of first mating on reproductive performance of Chinese Holsteins. *Chinese Journal of Animal Science* **46**, 52–54.

Marmaryan, Yu.G. 2001. *Fundamentals of Animal Husbandry*, Yerevan, 433 pp.

Meyer, M.J., Everett, R.W. & Van Amburgh, M.E. 2004. Reduced age at first calving: Effects on lifetime production, longevity, and profitability. In Stevenson, J.S. (ed.): *Dairy Day 2004*, Kansas State University, Tempe, AZ, pp. 42–52.

Minasyan, L.M. 2010. Reproductive abilities of Black Pied cows in the mountainous zones of the Republic of Armenia. *AgroScience*, 9–10, 409–412.

Minasyan, L.M. 2013. *The Black Pied cattle breed and ways to improve its efficiency in the Republic of Armenia*. Abstract of doctoral dissertation in agricultural sciences. 06.02.01, Yerevan, 45 pp.

Minasyan, L.M., Chitchyan, T.Zh. & Chitchyan, Zh.T. 2023a. Milk Productivity of Holstein Cows Imported from Germany Under the Conditions of the 'Smart' Barn at 'Depi Agro' Farm in the Armavir Region of Armenia. *Agroscience and Technology of the Armenian National Agrarian University* **3**, 273–277.

Minasyan, L.M., Simonyan, A.Kh., Chitchyan, T.Zh. & Chitchyan, Zh.T. 2023b. Results of a comparative study of milk productivity indicators in Holstein, Brown Swiss, and Simmental cows of local reproduction under the conditions of Armenia. *Effective Animal Husbandry* **1**(183), Krasnodar, 74–75.

Minasyan, L.M., Markosyan, T.H., Vardanyan, H.R., Simonyan, L.L., Shahazizyan, N.A. & Simonyan, A.Kh. 2024. *Bulletin on the Number of Farm Animals and Livestock Production in the Republic of Armenia*. Author's Edition, Yerevan, 45 pp.

Muradyan, A.M., Solovyeva, O.I., Minasyan, L.M., Chitchyan, Zh.T. & Ruzanova, N.G. 2022. Changes in the Milk Productivity Dynamics of Locally Bred Brown Swiss Cows in Armenia. *Agricultural Science* **365**(12), Moscow, 41–42.

Muratova, M.A. 2021. Specifics of milk productivity in Holstein cows under southeastern Kazakhstan conditions. *Collection of Scientific Works of the Kazakh National Agrarian Research University*, Almaty, pp. 109–114.

Navasardyan, D.S. 2015. *Biological and economic characteristics of Holstein, Brown Swiss, and Simmental cows*. PhD abstract, Yerevan, 24 pp.

Salakhov, F.D. 2017. *Comparative characterization of economically valuable and adaptive traits in Holstein and Brown Swiss cows under industrial milk production technology.* Dissertation for Candidate of Agricultural Sciences. Ufa, 133 pp.

Shevkhuzhev, A.F., Smarkuev, D.R. & Meremshaova, E.A. 2014. Morphological and biochemical blood indicators in Simmental cows. *Dairy and Meat Cattle Breeding* **4**, 29–32.

Simonyan, Kh.M. 2004. *Cattle breeds raised in Armenia and methods for their improvement.* Yerevan, 558 pp.

Skoptsova, T.I. & Popova, S.A. 2018. Milk productivity of first-calf Brown Swiss cows depending on their lineage. *Proceedings of Velikolukskaya State Agricultural Academy* **3**, Velikiye Luki, 28–32.

Solovyova, O.I., Ruzanova, N.G., Khramtsev, A.V., Levinkova, V.P. & Listrapenkova, V.I. 2016. Productive and reproductive qualities of Brown Swiss and Sychevka breed cows depending on mating type. *Dairy and Meat Cattle Breeding* **3**, 38–40.

Svyazhenina, M.A. 2020. Simmental cattle of the Tyumen region. *Proceedings of the Omsk State Agrarian University* **4**(84), 283–286.

Tyapugin, S.E., Meshcherov, R.K. & Dunin, M.I. 2021. Breeding characteristics of Holstein cattle from Northern European countries. *Dairy and Beef Cattle Breeding* **6**, 2–4.

Worman, H.D., Wright, J.R. & Hubbard, S.M. 2009. Reproductive status of Holstein and Jersey cows in the United States. *Journal of Dairy Science* **92**, 3517–3528.

Modeling the process of management of water-salt and food regime of salt soils of marginal land using waste water

Zh. Mustafayev[1,*], S. Umirzakov[2], Zh. Baimanov[2], K. Akylbayev[3] and K. Budikova[3]

[1]Department of Landscape Study and Problems of Nature Management, Institute of Geography and Water Security Science Committee, Seifullin av. 458/1, KZ050000 Almaty, Kazakhstan
[2]Kazakh Scientific Research Rice Growing named after, I, Zhakhaev, Abaya Str. 25b, KZ120000 Kyzylorda, Kazakhstan
[3]Korkyt-Ata Kyzylorda State University, 29A Ayteke Bi Str., KZ120000 Kyzylorda, Kazakhstan
[*]Correspondence: z-mustafa@rambler.ru

Abstract. Following the development and further improvement of the ecosystem approach to the development of saline marginal lands, a theoretical justification has been prepared for the conceptual model of the technology for the management of the salinity and nutritional regime of marginal lands, where the development of such land takes place in three symmetrical and parallel-sequential actions in time at annual intervals on the basis of using biologinization of agriculture, programming productivity of agricultural crops with desalinization of the saline soil to a certain acceptable level subject to leaching limit and chemical reclamation, taking into account the environmental management requirements and classification of the saline soil and salt tolerance of agricultural crops. In addition, the distinctive peculiarities of the proposed technology are the possibility to implement the entire complex of environment-forming functions of the natural system, including the assessment of energy resources of the soil and vegetation cover (various agro-ecological categories), productivity of agricultural land using the reference yield methods, water consumption deficit of agricultural land and leaching limits, ensuring the gradual achievement of the safe level of salinity, using the biological characteristics of salt-tolerant crops (halophytes) and chemical reclamation, based on application of gypsum to the topsoil, owing to a balanced supply of mineral fertilizers, and based on their rationing, taking into account planning the yield of cultivated crops. To illustrate the capability of the presented models, a two-year field experiment was conducted on saline lands using wastewater in the Kyzylorda region of the Republic of Kazakhstan and the results showed that supplementing the information with data from actual measurement results will provide even greater adaptability of the model. Based on the experimental research conducted to explore the possibility of managing the water-salt and nutrient regimes of soils in marginal lands using wastewater and salt-tolerant crops, and developed through mathematical models of soil water, salt, and nutrient dynamics along with a control technology algorithm for these natural-technogenic processes, it was demonstrated that the theoretical justification and the proposed conceptual model of the software and hardware system for managing the soil water-salt regime under wastewater irrigation comply with

environmental protection requirements and align with the ecological, economic, and social aspects of the sustainability triad.

The materials and methods of this manuscript, except for the experimental part of the study were presented as a preprint in Research Square (Mustafayev et al., 2024).

Key words: saline soil, marginal land, development technology, salinity management, leaching, gypsum application, irrigation, halophytes.

INTRODUCTION

The strategy for the development of agricultural land reclamation in the countries of the Eurasian region is based on the maximum possible crop yield through the creation of comfortable conditions for plants as objects of reclamation, and has been ensured over the last 50 years through the transformation of agricultural land irrigation standards from biological to reclamation and from reclamation to reclamation-leaching, in quantitative terms exceeding 1.5–2.0 times the evaporation capacity of the natural system. In this context, in areas of insufficient moisture, to drain excess water from the root layer of soil as a prevention of secondary salinization of irrigated land and adjacent territories, and not the causes, while relative to irrigation systems, collector-drainage (drainage) systems were designed, violating the basic principles of agricultural land reclamation '…improvement of the hydrothermal regime, acceleration of the biological and deceleration of the geological cycle of water and chemicals…'. As a result, almost all natural processes were dramatically disrupted: the hydrological regime of permanent and temporary watercourses of river systems changed; geochemical flows have increased many times due to the involvement of huge masses of salt, previously 'buried' by nature, in the active geological cycle; within the irrigated land and adjacent territories, soil, biological, hydrogeological and ecological processes have changed, as a result, saline and degraded marginal lands have developed, requiring functional-component and structural reconstruction for their reclamation and normalization of their ecological properties.

Purpose of the study is a scientific and methodological justification of the innovative approach and technology for the management of salinization of marginal lands, providing reclamation and normalization of the productivity of natural and hydroagrolandscapes for agricultural use.

To properly understand the goals and objectives of managing salinization of marginal agricultural land, it is necessary to define a system of values and the object of influence, namely, such values are man and his environment, and the object of influence is soil as the main component of the landscape biosphere as a whole and as the key instrument and object of labor in agricultural production.

In order to address the above-mentioned challenges and ensure sustainable productivity of saline soils in marginal lands, we developed a conceptual model for the integrated management of the water-salt and nutrient regimes using wastewater irrigation and gypsum application. This model is based on a combination of chemical and biological reclamation techniques, crop programming, and agroecological assessment tools. The methodological details of this approach are presented in the following section.

There is a large number of works devoted to the development of degraded agricultural land using chemical and biochemical reclamation and leaching in combination with irrigation, among which special mention should be given to works of Kireycheva et al. (2014), associated with the development of a fragment of the classifier of methods for the restoration of the fertility of degraded land; and works of Kaziyev et al. (2021), which presented the created multicomponent tiered agrophytocenoses during vegetative reclamation of desert pastures; works of Bilquees et al. (2003), which present results of seawater irrigation using local halophytes in the desert zone of Pakistan, and the possibility of creating a profitable oil-yielding industry; works of Abass Abdu et al. (2023), which present results of the study on the development of saline land using common soil parameters, and the soil fertility/nutrient/index approach, were employed to estimate the soil quality; while in the works of Shamsutdinov et al. (2009), the concept of biological reclamation during the development of degraded land in desert zones has been formulated. Unsustainable pressure on natural resources threatens the very ecosystem of services on which the global food system depends (Howe et al., 2014), and land degradation is a complicating factor in achieving not only sustainable development but also in increasing the productivity of land resources (Vlek et al., 2017), Mustafayev & Umirzakov (2013) presented the principle of an environmentally safe technology for wastewater utilization in specialized agro-landscapes.

At the same time, the strategy for reclamation of marginal lands and technologies for their restoration should be based on the morphological, physicochemical, genetic and biochemical properties of halophytes (Busby et al., 2017), ensuring their successful growth in saline areas in various climatic conditions and under different degrees of pressure on agricultural soils (Woldeyohannis, 2024), which is a promising environmentally friendly technology for saline lands and based on them, biosalt agriculture can be created (Rahman et al., 2023), using sea and poor-quality water for irrigation, thereby reducing dependence on dwindling freshwater resources (Alkharabsheh et al., 2021).

The works of Mitchell (1968), Kolb (1971), Petrov (1975), Eckholm & Brown (1977), Bresler et al. (1982), Darkoh (1989), Kharin (1992), Chhabra (1996), Ferrero (1996), Gilani (1997), Takamura (1997), Zonn & Kust (1999), Gabunshchina (2001), Petrov (2001), Kust (2002), Kruzhilin & Chasovskikh (2004), Mukhtar & Mustafa (2004), Kulik et al., (2007), Kizyaev (2009), Borodychev et al. (2011), Keller (1923), deal with the problems of prevention of desertification processes and development of degraded soil on the principles of ecological balance of hydro-reclamation, forest-reclamation, and agro-reclamation impacts.

The method of assessing the water-salt balance of irrigated lands has been developed in a broad aspect, and among them it is necessary to note the works of Averyanov (1978), who is the founder of the joint regulation of water, air, thermal, nutrient and salt regimes of the soil, as well as the works of Aidarov, Golovanov & Nikolsky (1990), where the principles of optimizing the meliorative regimes of irrigated and drained agricultural lands are presented, Rex (1995) – system studies of meliorative processes and systems, Borodychev, Dedova, Sozanov and Lytov (2016) – modeling the process of managing the water-salt regime of soils under irrigation conditions, Mustafayev & Abdeshev (2023) – modeling soil salinization and desalinization.

One of the key directions of the technology for the development of saline land is the direction developed in the works of Dedova (2012), associated with the development of a conceptual model for the increase of natural resource potential through complex reclamation and Mustafayev et al. (2015) associated with the formation of a new conceptual approach to the ecosystem substantiation of methods for the development of saline land, based on two symmetrical and parallel-sequential actions in time at annual intervals with desalinization of the saline soil to a certain acceptable level subject to leaching limit, taking into account the environmental management requirements and classification of the saline soil and salt tolerance of agricultural crops (Dedova, 2012; Mustafayev et al., 2015; Mustafayev et al., 2017a; Mustafayev et al., 2024a).

Based on the analysis of the methodological approach for assessing the anthropogenic impact on saline marginal lands within the framework of one of the laws of the materialistic dialectic of 'negation of negation', which allows us to improve the natural science understanding of modern ecological and meliorative mechanisms for modeling the water, salt and food regimes of the soil and to study their mutual functioning, under conditions of natural climatic moisture and taking into account meliorative measures (irrigation), including their environmental admissibility, provides a methodological basis for the creation of mathematical tools that ensure comprehensive regulation and management of this natural and man-made process.

MATERIALS AND METHODS

Data analysis. The methodological basis for managing the saline and nutritional regime of saline soils of marginal lands using urban wastewater has become the principles of environmental management, structural modeling of technological processes using biologinization of agriculture, ensuring the activation of natural mechanisms for increasing soil fertility, programming the productivity of agricultural crops, based on the development of a complex of interrelated agro technical processes within which it is possible to obtain a calculated yield with a known probability and regulation of the hydrochemical regime of the soil based on the laws of the evolutionary process of the natural system, ensuring environmentally safe desalinization of the soil-forming rock from water-soluble salts by dissolving and carrying them into groundwater when the hydrological regime of the soil changes.

As a basis for the development of the algorithm of the management technology for water-salt and nutritional regimes of the soil on marginal lands, a mathematical model of the integrated fertility index – Pegov & Khomyakov's (1991) 'soil index', consisting of three independent blocks, namely: humus reserves (S_G), mineral reserves (S_{NPK}) and hydrolytic acidity (S_{Hg}), which has the following expression:

$$S = S_G + S_{NPK} + S_{Hg} = \left[6.4 \cdot \left(G_{hh} + 0.20 \cdot C_{fh}\right)/600\right] + \\ + 8.5 \cdot \sqrt[3]{N \cdot P \cdot K \cdot \delta} + 5.1 \cdot exp\left[-\left(H_g - 1\right)/4\right], \quad (1)$$

where S_G – indicator of the humus impact on the generalized soil index; S_{NPK} – indicator of minerals elements impact (nitrogen, phosphorus and potassium) on the generalized soil index; S_{Hg} – indicator of hydrolytic acidity impact on the generalized soil index; G_{hh}–content of humate humus, t ha^{-1} or g cm^{-3}; G_{fh} – content of fulvic humus, t ha^{-1} or g cm^{-3}; 600.0 – maximum possible humus content in reference (chernozem) soil, t ha^{-1};

$N = N_i/N_{ont}$; $P = P_2O_{5i}/P_2O_{5\,ont}$; $K = K_2O_i/K_2O_{ont}$ – relative content of phosphorus and potassium in the soil of agricultural land; N_i, P_2O_5, K_2O_i – content of nitrogen, phosphorus and potassium in the soil of agricultural land, kg ha^{-1}; N_{ont}, $P_2O_{5\,ont}$, K_2O_{ont} – optimal content of nitrogen, phosphorus and potassium in the soil of agricultural land, kg ha^{-1}; $N = N_i/N_{ont}$, $P_2O_5 = P_2O_{5i}/P_2O_{5\,ont}$, $K_2O = K_2O_i/K_2O_{ont}$ – relative content of phosphorus and potassium in the soil of agricultural land; δ – mineral fertilizer digestion coefficient; H_g – hydrolytic acidity, mg-eq 100 g^{-1} of soil.

Based on the structural analysis of the mathematical model of the integrated fertility index – 'soil index', components of the maximum possible value of the generalized soil index were determined:

– under the conditions of a sufficiently high humus content in the soil, that is, when $(G_{hh} + 0.20 \cdot C_{fh})/600 = 1$, soil index in terms of humus content will be equal to:

$$S_G = 6.4 \cdot (G_{hh} + 0.20 \cdot C_{fh})/600 = 6, \qquad (2)$$

– in the absence of a deficiency of mineral reserves in the soil, that is, nitrogen $N = N_i/N_{ont} = 1$, phosphorus $P = P_2O_{5i}/P_2O_{5\,ont} = 1$ potassium $K = K_2O_i/K_2O_{ont} = 1$ and when the coefficient of mineral fertilizer digestion by plants (δ) reaches one, then the soil index in terms of mineral reserves will be equal to:

$$S_{NPK} = 8.5 \cdot \sqrt[3]{N \cdot P \cdot K \cdot \delta} = 8.5 \cdot \sqrt[3]{1 \cdot 1 \cdot 1 \cdot 1} = 8.5; \qquad (3)$$

– with neutral soil acidity, that is $H_g = 1$, the soil index in the presence of neutral acidity will be equal to:

$$S_{Hg} = 5.1 \cdot exp\left[-\frac{(H_g - 1)}{4}\right] = 5.1 \cdot exp\left[-\frac{(1 - 1)}{4}\right] = 5, \qquad (4)$$

Based on the results of forecast calculations, components of the soil index in terms of humus content (S_G), mineral reserves (S_{NPK}) and hydrolytic soil acidity (S_{Hg}), it is possible to identify the generalized soil index (S): $S = S_G + S_{NPK} + S_{Hg} = 6.4 + 8.5 + 5.1 = 20.0$.

In accordance with the obtained results, weight coefficients of the vegetation productivity (k_i) characterizing the impact of the humus reserve (S_G), mineral reserves (S_{NPK}) and hydrolytic acidity (S_{Hg}) on the soil fertility have been determined (of agricultural crops (Mustafayev et al., 2024b):

$$\begin{aligned} k_{Gi} &= S_G/S = 6.4/20.0 = 0.320; \\ k_{NPKi} &= S_{NPK}/S = 8.5/20.0 = 0.425; \\ k_{Hgi} &= S_{Hg}/S = 5.1/20.0 = 0.255; \\ k_i = k_{Gi} + k_{NPKi} + k_{Hgi} &= 0.320 + 0.425 + 0.255 = 1.00, \end{aligned} \qquad (5)$$

where k_i – weight coefficients of the vegetation productivity characterizing the impact of the humus reserve (S_G), mineral reserves (S_{NPK}) and hydrolytic acidity (S_{Hg}) on the soil fertility.

Consequently, the basic block in the implementation of the technology for the management of salinization of marginal agricultural land is the mathematical model of the integrated fertility index - 'soil index', which is produced on the basis of a comprehensive study of all components of nature, based on a systematic approach in comparing the requirements for soil and vegetation cover of agricultural land and their adaptive capabilities with the actual state of natural and technogenic landscapes and the prospect of regulating its properties.

To develop the algorithm of the management technology for salinization of marginal land, it is necessary to create a database of information and analytical materials characterizing their natural and technogenic state:

    – climatic indicators, including average monthly temperature (t_i, °C), relative air humidity (α_i, %), annual precipitation (O_{ci}, mm);

    – biological index of agricultural crops, including the coefficient of solar energy utilization and removal of mineral reserves from the soil and wastewater by the agricultural crop yield:

    – soil index, including the content of mineral reserves, humate and fulvic humus in the top soil and hydrolytic acidity of the soil;

    – hydrochemical index, including the content of chemical elements in the composition of surface and wastewater.

Thus, the process of biological reclamation of saline marginal lands is based on the quantitative regulation of soil enrichment with organic and mineral fertilizers, aimed at preserving and restoring the natural balance of soil components, while recognizing the importance of the 'water-soil-plant' triad. This integration of nutrient management and salinity control implies a comprehensive approach based on the combined application of fertilizers and soil amendments with irrigation water, allowing for fractional delivery of substances in required quantities and at appropriate stages of the growing season, thereby ensuring precise control over the nutrient and salt regimes of the soil.

RESULTS AND DISCUSSION

Based on the development and further improvement of the ecosystem method of development of saline land, technologies for management of the salinity of marginal land have been developed, where the development of saline land takes place in three symmetrical and parallel-sequential actions in time at annual intervals with desalinization of the saline soil to a certain acceptable level subject to leaching limit and chemical reclamation, taking into account the environmental management requirements and classification of the saline soil and salt tolerance of agricultural crops from very highly saline to highly saline, from highly saline to moderately saline, from moderately saline to slightly saline and from slightly saline to non-saline with the subsequent cultivation of corresponding salt-tolerant crops: very tolerant-tolerant-moderately tolerant-moderately sensitive-sensitive.

To address the problems of sustainable management of water-salt and nutrition regimes of the saline soil of marginal land, it is necessary to study the formation of their energy resources and moisture supply, which are a function of the average long-term values of annual precipitation and evaporation from the soil and vegetation surface, reflecting the ratio of heat and moisture characteristic of a given geographical area.

The energy resources of agricultural landscapes are a function of average monthly temperature (t_i, °C) and relative air humidity (α_i, %), expressed by the average annual sum of air temperature above 10 °C ($\sum t_{ci} > 10$ °C), total evaporation from the water surface (E_{oci}) and radiation balance of the daytime surface of the soil and vegetation cover (R_i) (Mustafayev et al., 2024a):

    – sum of monthly air temperature ($\sum t_i$): $\sum t_{mi} = t_i \cdot N_i$, where N_i – number of days per month;

– average annual sum of air temperature above 10 °C ($\sum t_{ci} > 10$ °C): $\sum t_{ci} > 10$ °C $= \sum_{i=1}^{n} t_m$, where n – number of months during the period of air temperature above10 °C;

– monthly evaporation from the water surface, which is determined using Ivanov's formula (1978): $E_{mi} = 0.0018 \cdot (t_i + 25)^2 \cdot (100 - \alpha_i)$, mm;

– total evaporation from the water surface (E_{tmi}): $E_{tmi} = \sum_{i=1}^{n} E_{mi}$, where n- number of months during the period of air temperatures above10 °C;

– natural moisture coefficient (C_{mi}): $C_{mi} = O_{ci}/E_{tmi}$;

– radiation balance (photosynthetic active radiation – PAR) of the daytime surface of the soil and vegetation cover (R_i) is determined using Nikolskiy & Shabanov's empirical formula (1986):

$$R_i = 13.39 + 0.0079 \cdot \sum t_{ci} > 10 \text{ °C, kcal cm}^{-2}$$
$$\text{or} \tag{6}$$
$$R_i = 4.1868 \cdot [13.39 + 0.0079 \cdot \sum t_{ci} > 10 \text{ °C], kJ cm}^{-2},$$

when using the radiation balance of the daytime surface of the soil and vegetation cover (R_i) it becomes necessary to bring their unit of measurement to kcal ha^{-1} or kJ ha^{-1} in further forecast calculations, that is, if 1 ha $= 10^7$ cm^2, then: $R_i = [13.39 + 0.0079 \cdot \sum t_{ci} > 10 \text{ °C}] \cdot 10^8$, kcal ha^{-1} or $R_i = 4.1868 \cdot [13.39 + 0.0079 \cdot \sum t_{ci} > 10 \text{ °C}] \cdot 10^8$, kJ ha^{-1}.

To determine the value of various agroecological categories of agricultural land productivity, we use Tooming's (1984) reference yield method, which is a logical outcome of the principle of maximum productivity.

The potential yield of agricultural land (PY_i) in ideal conditions is determined using Nichiporovich's formula (Nichiporovich et al., 1961):

$$PY_i = (R_i \cdot 10^8 \cdot K)/(10^2 \cdot q \cdot 10^3 \cdot 10^2) \tag{7}$$

where R_i – amount of photosynthetic active radiation (PAR) during the vegetation period of agricultural crops in a given geographical area, billion kcal ha^{-1}; K – planned PAR utilization factor, %; q – amount of energy released when burning 1 kg of dry matter (calorific value of agricultural crop yield), equal to $q = 4 \cdot 10^3$, kcal; 10^2 – conversion from kg to centner; 10^2 – 100% to convert PAR utilization factor to a relative value.

Actual-possible productivity (APP_i, c ha) of agricultural land, subject to limitation of the moisture of soil and vegetation cover by agrometeorological conditions, can be determined using the following formula (Mustafayev et al., 2017b):

$$APP = PY \cdot FW \tag{8}$$

where FW – function of the impact of moisture conditions on crop productivity (moisture coefficient), dimensionless:

$$FW = 1 - \left[1 - \left(E_i/E_{opt}\right)\right]^2 \tag{9}$$

where E_i – total water consumption by agricultural land; E_{opt} – optimal total water consumption by agricultural land.

The expected actual-possible productivity ($EAPP_i$, c ha) of agricultural land on the saline soil of marginal land is limited by the degree of soil salinity:

$$EAPP = APP \cdot exp\left[-k \cdot \left(S_S/S_{per} - 1\right)^b\right] = APP \cdot K_s \tag{10}$$

where S_s – salt content in the soil; S_{per} – maximum permissible level of soil salinity, providing the maximum possible productivity of landscapes used for agricultural land; k – parameter characterizing plant response to toxic salts; b – parameter characterizing

the type of soil salinization; $K_s = exp\left[-k \cdot \left(S_s/S_{per} - 1\right)^b\right]$ – coefficient of reduction in agricultural yield due to soil salinization of marginal land (non-saline soil $K_s = 0$; highly saline soil $K_s = 0.25$; moderately saline soil $K_s = 0.75$; slightly saline soil $K_s = 0.80$; non-saline soil $K_s = 1.0$).

By-products of agricultural crops are determined using the formula:

$$C_p = EAPP_0 \cdot \alpha_p = C_b \cdot \alpha_p \tag{11}$$

where $EAPP_0 = C_p$ – main products of agricultural crops, c ha; C_b – byproducts of agricultural crops, c ha; α_p – coefficient characterizing the ratio of the main products of agricultural crops to by-products.

Total water consumption of agricultural land is formed under the influence of the annual radiation balance of the soil and vegetation surface (R_i) and due to the heat spent on evaporation, that is, the result of multiplying the latent heat of vaporization (L) and determined using Budyko's formula (Budyko, 1956):

$$ET_{ci} = 4.1868 \cdot R_i \cdot L^{-1} \tag{12}$$

where L – latent heat of vaporization (kJ cm^{-2} per year per 1 mm of water layer), equal to 2.5 kJ cm^{-3}.

In addition, the maximum permissible water consumption deficit (irrigation rate) of agricultural land is determined taking into account geo-ecological restrictions that have regard to the hydrothermal regime of irrigated land according to the following water balance equation:

$$\Delta E_i = O_{pi} = [4.1868 \cdot (R_i/\overline{R}_i) \cdot L] - O_c \tag{13}$$

where $\overline{R}_i$ – 'radiation dryness index' or hydrothermal coefficient: $\overline{R}_i = R_i/L \cdot AP_i$; AP_{ci} – annual precipitation, mm; O_{pi} – irrigation rate of agricultural land, mm; ΔE_i – water consumption deficit (irrigation rate) of agricultural land, mm.

The ongoing natural changes in global and, consequently, regional climate are manifested through variations in air temperature, which is a function of the evaporative capacity of the natural system and atmospheric precipitation. These parameters characterize the natural moisture conditions of the soil and vegetation cover in both spatial and temporal dimensions.

A retrospective analysis and assessment of spatiotemporal changes in climatic characteristics (air temperature and relative humidity, atmospheric precipitation) are carried out using a hydrometeorological observation database and the trend method. In this context, the value of a climatic variable at a given time is referred to as the level of the climate time series, and the pattern of its evolution over time is described as the trend.

Long-term climatological research shows that air temperature (t_i) and relative humidity (a_i), which describe the evaporative capacity of the natural system (E_o), along with atmospheric precipitation (AP_i), which characterizes the natural moisture of soil and vegetation cover as a dynamic-stochastic process, can be represented by a linear trend equation:

$$t_i = a \cdot T_i + b_i;\ a_i = a \cdot T_i + b_i;\ AP_i = a \cdot T_i + b_i \tag{14}$$

where α is the regression coefficient, which shows how much the studied indicator (t_i, a_i, AP_i) depends on the influencing factor (T_i), characterizing the increase or decrease in the value of the time series; b is the zero regression coefficient, which shows the minimum value of the studied indicator, in the absence of the influence of the explanatory factor ($T_i = 0$); T_i is the period number or the ordinal number of the year in

the forecasting period or an independent variable.

In Eq. (14), the first terms express the random part of the climate indicators, the last terms express the deterministic part of this process, which is characterized by a tendency that is a function of time, which can serve as a basis for long-term forecasting (for several years ahead).

Based on the equation of the linear trend of the studied (t_i, a_i, AP_i) indicator, it is possible to develop a mathematical model of the absolute growth, growth rate and growth coefficient of the studied indicators, which have a sufficiently high physical and mathematical meaning, based on the law of nature:

– the absolute increase in the studied indicator (AI_i), which shows the quantitative value of any studied indicator (t_{ei}, a_{ei}, AP_i) at the end of the period under consideration (T_{ei}) to its initial value $(t_{bi}, a_{bi}, O_{cbi})$ at the beginning of the period under consideration (T_{bi}), where, $T_{bi} = 1 = const$ and is determined by the formula: $AI_i = \alpha \cdot (T_{ei} - 1)$;

– the growth rate of the studied indicator (RI_i) shows the relative value of the absolute growth of the studied indicator (t_i, a_i, AP_i) over the period under consideration, and is calculated using the formula: $RI_i = [\alpha \cdot (T_{ei} - 1)]/T_{ei}$;

– the growth coefficient of the studied indicator (GR_i) is the ratio of the quantitative value of any studied indicator (t_{ei}, a_{ei}, AP_i) at the end of the period under consideration (T_{ei}) to its initial value (t_{bi}, a_{bi}, AP_i) at the beginning of the period under consideration (T_{bi}) and is represented by the following formula: $GR_i = (\alpha \cdot EPUR_i + b)/(\alpha + b)$

When agricultural marginal land is irrigated by wastewater, chemical elements enter the soil layer along with irrigation water, including nitrogen, phosphorus and potassium, enriching agricultural crops with nutrients (Mustafayev & Kemelbekov, 1987):

$$NO_n = n_c \cdot O_{pi}/1{,}000; \quad PO_{ph} = ph_c \cdot O_{pi}/1{,}000; \quad KO_{pi} = p_c \cdot O_{pi}/1{,}000, \qquad (15)$$

where n_c, ph_c, p_c – content of nitrogen, phosphorus and potassium in wastewater, mg L^{-1}; NO_n, PO_{ph}, KO_p – expected amount of nitrogen, phosphorus and potassium entering the wastewater into the soil layer of irrigated land, kg ha^{-1}; 1,000 – conversion factor from mg L^{-1} to kg ha^{-1}.

The amount of mineral nutrients in the topsoil (0–30 cm) at the beginning of the vegetation period of agricultural crops per 1 ha is determined using the formula:

$$NS_i = 10 \cdot M \cdot n_s = 10 \cdot S \cdot h \cdot d_v \cdot n_s \cdot 10^6 \cdot 10^{-6};$$
$$PS_i = 10 \cdot M \cdot p_s = 10 \cdot S \cdot h \cdot d_v \cdot ph_s \cdot 10^6 \cdot 10^{-6}; \qquad (16)$$
$$PS_i = 10 \cdot M \cdot p_s = 10 \cdot S \cdot h \cdot d_v \cdot p_s \cdot 10^6 \cdot 10^{-6}$$

where M – topsoil mass of agricultural land: $M = S \cdot h \cdot d_v \cdot 1{,}000$, kg ha^{-1}; 1,000 – conversion factor of dm^3 to m^3; S – area 1 ha = 100·100 m^2; h – thickness of the topsoil of agricultural land, m; d_v – soil density, kg dm^{-3}; n_s, ph_s, p_s – content of available forms of nitrogen, phosphorus and potassium in the topsoil, mg 100 per g of soil; NS_i, PS_i, KS_i – reserve of nitrogen, phosphorus and potassium in the topsoil, kg ha^{-1}; 10^6 – conversion factor from mg to kg, mg kg^{-1}.

Maximum possible mineral reserves in the soil together with wastewater inflow during the vegetation period of agricultural crops cultivated on marginal land is determined using the formula:

$$MPRN_i = NS_i + NO_{pi}; \quad MPRP_i = PS_i + PO_{pi}; \quad MPRK_i = KS_i + KO_{pi} \qquad (17)$$

where $MPRN_i, MPRP_i, MPRK_i$ – maximum possible reserves of nitrogen, phosphorus and potassium in the topsoil, kg ha^{-1}.

Use of the maximum possible mineral reserves from the soil by the main and by-products (yield) of agricultural crops is determined as follows:

$$NU_i = MPRN_i \cdot \alpha_{Nmp} + MPRN_i \cdot \alpha_{Nuc};$$
$$PhU_i = MPRP_i \cdot \alpha_{Phmp} + MPRP_i \cdot \alpha_{Phuc}; \qquad (18)$$
$$PU_i = MPRK_i \cdot \alpha_{Pmp} + MPRK_i \cdot \alpha_{Puc},$$

where α_{Nmp}, α_{Phmp}, α_{Pmp} – coefficient of utilization of nitrogen, phosphorus and potassium by the main products of agricultural crops from the topsoil; α_{Nuc}, α_{Phuc}, α_{Puc} – coefficient of utilization of nitrogen, phosphorus and potassium by agricultural by-products from the topsoil; NU_i, PhU_i, PU_i – expected utilization of nitrogen, phosphorus and potassium from the topsoil by the main and by-products (yield) of agricultural crops, kg ha^{-1}.

Total (economic) removal of mineral reserves by the main (B_o) and byproducts (B_c) of agricultural crops is calculated using the formula:

$$PRT_i = B_o \cdot C_{No}/100 + B_c \cdot C_{Nc}/100$$
$$RPB_i = B_o \cdot C_{Pho}/100 + B_c \cdot C_{Phc}/100 \qquad (19)$$
$$RPU_i = B_o \cdot C_{Po}/100 + B_C \cdot C_{Pc}/100$$

where C_{No}, C_{Pho}, C_{Po} – content of nitrogen, phosphorus and potassium in the main products of agricultural crops, %; C_{Nn}, C_{Phc}, C_{Pc} – content of nitrogen, phosphorus and potassium in by-products of agricultural crops, %; PRT_i, RPB_i, RPU_i – possible removal by the main and by-products (yield) of agricultural crops from the topsoil, kg ha^{-1}.

The deficiency of mineral reserves to provide the expected yield of agricultural crops is determined using the expression:

$$\Delta N_i = PRT_i - NB_i \Delta Ph_i = RPB_i - PB_i \Delta P_i = RPU_i - KB_i \qquad (20)$$

where ΔN_i, ΔPh_i, ΔP_i – possible removal by the main and by-products (yield) of agricultural crops from the topsoil, kg ha^{-1}.

The rates of mineral fertilization of agricultural land are determined taking into account the content of active ingredients using the formula:

$$\Delta NF_i = \Delta N_i \cdot 100/\alpha_{No}; \Delta PhF_i = \Delta P_i \cdot 100/\alpha_{Pho}; \Delta PF_i = \Delta P_i \cdot 100/\alpha_{Po}, \qquad (21)$$

where C_{No}, C_{Pho}, C_{Po} – content of nitrogen, phosphorus and potassium active ingredients in mineral fertilizers, %; ΔNF_i, ΔPhF_i, ΔPF_i – rates of application of nitrogen, phosphorus and potassium in agricultural land, kg ha^{-1}.

For the integrated management of water-salt and food regimes of saline soils of marginal lands, based on the materialistic theory of scientific knowledge and the theoretical-logical concept of the direction and intensity of the cycle within the framework of the law of conservation of matter, equations of water, salt and food balance of the soil layer have been developed:

$$\Delta WR_i = (AP_i + O_{pwi} + O_{pwsi} + FR_i + ME_{sgwi}) - (E_{tmi} + ME_{gswi});$$
$$\Delta GS_{rsi} = (G_{pwi} + G_{wsi} + G_{api} + SI_{fi} + SF_i) - (RS_{hi} + GS_{rdi} + GS_{sli}); \qquad (22)$$
$$\Delta NR_{si} = (NW_{ofi} + NW_{mfi} + NW_{wwi} + NI_{sli}) - RN_{wci},$$

ΔWR_i – change in soil water reserves over the period under consideration, m^3 ha^{-1}; AP_i – total precipitation, m^3 ha^{-1}; O_{pwi} – rate of water supply by river water, m^3 ha^{-1}; O_{pwsi} – rate of water supply by wastewater, m^3 ha^{-1}; ME_{sgwi} – moisture exchange between soil and groundwater, m^3 ha^{-1}; E_{tmi} – total evaporation (evaporability), m^3 ha^{-1}; ME_{gswi} – moisture exchange between groundwater and soil water (DD_i – drainage flow), m^3 ha^{-1}; FR_i – leaching rate, m^3 ha^{-1}; ΔGS_{rsi} – change in salt reserves in the soil over the

considered period of time, t ha⁻¹; G_{pwi} – amount of salts supplied with river water, t ha⁻¹; G_{wsi} – amount of salts supplied with wastewater, t ha⁻¹; G_{api} – amount of salts supplied with atmospheric precipitation, t ha⁻¹; SI_{fi} – amount of salts supplied with fertilizers and chemicals, t ha⁻¹; SF_i – the amount of salts supplied by flushing water, t ha⁻¹; RS_{hi} – salt removal with the crop, t ha⁻¹; GS_{rdi} – the amount of salts removed by drainage, t ha⁻¹; GS_{sli} – the amount of salts in the soil layers, t ha⁻¹; ΔNR_{si} – the change in the reserves of nutrients in the soil over the period under consideration, kg ha⁻¹; NW_{ofi} – application of nutrients with organic fertilizers, kg ha⁻¹; NW_{mfi} – application of nutrients with mineral fertilizers, kg ha⁻¹; NW_{wwi} – application of nutrients with wastewater, kg ha⁻¹; NI_{sli} – amount of nutrients in soil layers, t ha⁻¹; RN_{wci} – removal of nutrients with the crop, kg ha⁻¹.

The equations of water, salt and food balance of the soil layer, reflecting the essence of the teachings of Dokuchaev (1949) and Kostyakov (1951) on the genesis and melioration of soils as a special natural body, allow us to quantitatively assess the direction of cycles, where the most important thing is to establish the relationship between them, since the formation of saline soils of marginal lands is the result of the combined action of climatic, environmental, soil and biological factors.

Increase of the productivity of saline soil of marginal land is possible through the use of special technologies that neutralize acidity and create favorable conditions in the soil for the growth and development of plants.

Theoretical basis of the agrochemical method of increasing the fertility of alkali soil is the restoration of the absorbing soil complex with calcium and the displacement of exchangeable sodium and magnesium. When gypsum is added to the soil, calcium passing into the colloidal complex of alkaline soil causes the coagulation of soil colloids and displaces sodium. Agrophysical, chemical and biological properties of the soil are improved, the displaced sodium (Na) forms a hydrolytically neutral, highly soluble salt with the sulfate anion (SO_4).

The interaction of gypsum with soil is possible according to the following pattern (2021):

$$Na_2CO_3 + CaSO_4 \rightarrow CaCO_3 + Na_2SO_4, \tag{23}$$
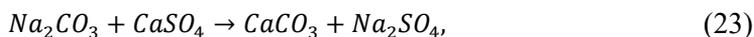
Thus, the absorbed sodium ion in the maximum permissible concentration (MPC) is replaced by a calcium ion. The reaction products are harmless in small quantities, however, when application of gypsum to the topsoil containing more than 20% Na in MPC, excess sodium sulfate is formed and it should be leached beyond the root layer after the end of the vegetation period of agricultural land.

It is recommended to calculate the optimal rate of gypsum (ameliorant), taking into account its utilization rate, using the formula (Mustafayev &Abdeshev, 2023):

$$G = [4.3 \cdot 10 \cdot (a \cdot b \cdot d_v \cdot h)]/c \tag{24}$$

where G – gypsum dose, t ha⁻¹; a – calcium concentration in a saturated gypsum solution, mg L⁻¹; b – volume of the saturated gypsum solution corresponding to the first coagulation threshold on the dispersion curve; c – soil lot, g; d_v – soil density, g cm⁻³; h – depth of reclaimed layer, cm; 10 – conversion factor, t ha⁻¹; 4.3 – gypsum conversion factor:

$$4.3 = (M \cdot CASO_4 \cdot 2H_2O)/M \cdot Ca \tag{25}$$

where M – molecular weight of $CASO_4 \cdot 2H_2O$; $M \cdot Ca$ – molecular weight of Ca.

In this case, the dose of gypsum should be agronomically optimal and knowingly safe for the agrocenosis, where the calculation is performed using the formula (Mustafayev & Abdeshev, 2023):

$$S_a = [(MPC - C_c) \cdot M \cdot 10^3]/C_i = [(MPC - C_c) \cdot d_v \cdot h \cdot 10^3]/C_i, \qquad (26)$$

where S_a – amount of substance that can be applied, t ha^{-1}; MPC – maximum permissible concentration of an element, mg kg^{-1}; C_i, C_c – content of the element in the ameliorant and in the soil, mg kg^{-1}; M – mass of 1 hectare of topsoil, t ha^{-1}; d_v – soil density, g cm^{-3}; h – topsoil thickness, cm.

Under modern conditions, successful implementation of the technology for salinity management on marginal land is possible only with continuous monitoring and timely forecasting of the soil water-salt regime using calculation and analytical methods. For the gradual desalinization of saline soil of marginal land, an effective technique is leaching - the process of step-by-step desalination of the top soil by removal of water-soluble salt into the lower horizons under the influence of small irrigation rates during the vegetation period, where the expected amount of leached salt from the soil layer is determined based on the level of manmade load of the natural system in the annual interval. The value of the required total leaching rate (net) for a meter layer of the soil is calculated according to Mustafayev's method (Mustafayev, 1986), using the formula:

$$N = 1000 \cdot (\alpha/\beta) \cdot lg(S_H/S_K), \quad N_n = V_t \cdot t \text{ and } t_d = (N - N_p)/K_f, \qquad (27)$$

where α – salt release coefficient; S_H and S_k – salt content in the soil at the beginning and end of leaching, %; 1000 – conversion factor from mm to m; K_f – filtration coefficient, m hour^{-1}; N – leaching rate, m^3 ha^{-1}; N_p – leaching rate in pressure mode of water absorption into the soil; $N_n = (N - N_p)$ – leaching rate in non-pressure mode of water absorption into the soil; t_d – duration of additional flushing leaching in non-pressure mode of water absorption into the soil, hour; V_t – average rate of water absorption into the soil over a given period of time, m hour^{-1}; n – number of absorption zones; t – duration of leaching; β – acceleration coefficient of the salt release, which depends on the speed of the infiltration flow:

$$\beta = 2.02 \cdot exp(-9.57 \cdot V_t^b), \qquad (28)$$

here V_t^b – speed of the infiltration flow, m hour^{-1} (Mustafayev, 1986).

The salt content in the soil layer after leaching can be determined using the formula:

$$S_k = S_n \cdot exp[-(\beta/\alpha) \cdot N_n], \qquad (29)$$

The amount of salt removed from the soil layer of marginal land in the annual interval is determined based on the salt balance equation, formed as a result of three symmetrical and parallel-sequential actions of leaching, gypsum application and cultivation of salt-tolerant crops:

$$\Delta S_n + \Delta S_k = S_H - S_k, \qquad (30)$$

where ΔS_n – amount of salt removed from the soil layer of marginal land in the annual interval during the leaching process in the vegetation period, t ha^{-1}; ΔS_k – amount of salt removed from the soil layer of marginal land in the annual interval of a salt-tolerant crop, t ha^{-1}.

Thus, the developed algorithm for the technology of management of marginal land salinity based on three symmetrical and parallel-sequential actions, including irrigation-leaching, gypsum application and cultivation of salt-tolerant crops, methodological support, using various models to determine the energy resources of soil and vegetation

cover, water consumption deficit and various agroecological productivity of agricultural land, focused on forecast calculations to determine the necessary elements of mineral nutrition and gypsum application, allow the formation of optimal water-salt and nutrition regimes.

Results of experimental studies

Experimental plot. The experimental site is located within the city of Kyzylorda, in the Tasboget area (44° 51′ N, 65° 31′ E), Kyzylorda region, in the southwest of the Republic of Kazakhstan (Fig. 1), near agricultural fields irrigation. In accordance with the requirement to create environmentally friendly meliorated agricultural landscapes using wastewater, the experimental site consists of two independent sites.
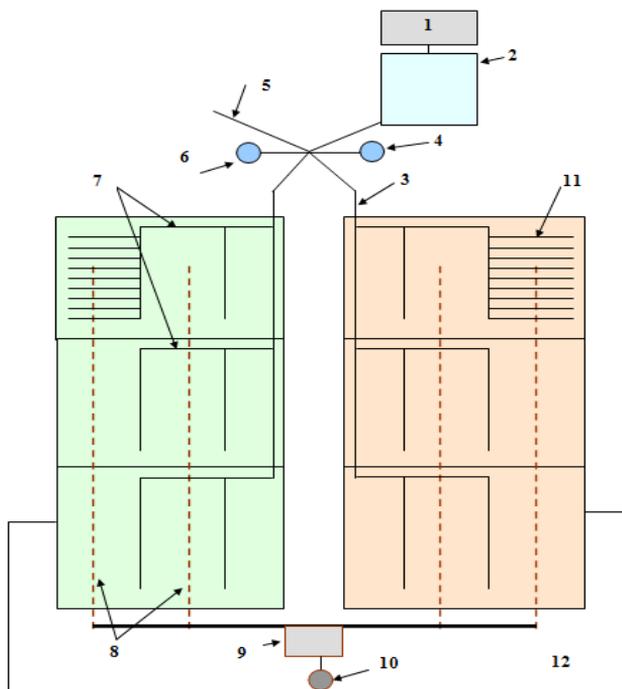
Figure 1. Layout of the experimental site: 1 – treatment facilities; 2 – storage pond; 3 – distribution network for wastewater supply; 4 – distribution network for wastewater supply; 5 – section of distribution channels; 6 – temporary sprinklers; 7 – drainage network; 8 – collector network; 9 – collecting well; 10 – hydraulic structure with a gate; 11 – gate valve; 12 – experimental sites; 13 – buffer zone for non-vegetation disposal of wastewater.

The first site is located within the agricultural fields irrigation, where, using river water, the processes of preserving and restoring the natural balance of soil components will be studied, recognizing the importance of the aspect of the triad 'water-soil-plants', and the second site is located outside of agricultural fields irrigation, where the process 'impact-change-consequences' on saline marginal lands will be studied using wastewater, for maximum utilization of wastewater based on the evaporative capacity of the natural system (Fig. 1).

Climatic conditions of the experimental site. Kyzylorda region is located to the east of the Aral Sea, in the lower reaches of the Syr Darya River, mainly within the Turan Lowland (elevation 50–200 m), The climate of the city of Kyzylorda is sharply continental with hot, dry, long summers. The tables below show the climate indicators: average monthly temperature (t_i, °C), average monthly relative humidity (α_i, %), monthly precipitation (AP_i, mm) and energy indicators for the sum of air temperatures above 10 °C ($\sum t_{ci}$) > 10 °C, radiation balance of the daytime surface (R_i), total evaporation from the water surface (E_{tmi}) and total water consumption of agricultural lands (ET_{ci}).

To analyze and evaluate the features of the development of climate processes in the experimental site and to determine the energy resources of the natural system, meteorological indicators of the Kyzylorda weather station for 2015–2024 and their fragments for 2020–2024 were used and are presented in Tables 1 and 2.

Table 1. Climate indicators of the experimental site for 2020–2024

Years	Months												Year-old
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Air temperature, °C													
2020	-2.5	1.3	7.0	15.6	23.5	27.2	29.6	26.2	17.9	10.0	-1.4	-11.0	12.0
2021	-8.7	-2.3	3.0	15.7	25.6	28.7	30.4	28.5	19.3	8.6	0.7	-0.3	12.4
2022	-2.1	-0.1	4.5	18.3	22.6	29.4	29.4	26.2	21.4	10.7	3.0	-8.3	12.9
2023	-5.8	-1.2	9.5	15.8	22.8	28.2	31.0	26.8	18.7	12.9	7.8	-1.9	13.7
2024	-4.1	-2.8	5.4	17.6	20.6	29.3	28.0	26.7	18.4	11.5	3.5	-4.0	12.5
Relative air humidity, %													
2020	81	76	51	50	40	28	32	36	41	46	68	78	52.3
2021	72	73	68	43	29	28	32	31	37	50	64	84	50.9
2022	86	79	69	49	42	29	34	34	34	49	73	75	54.4
2023	66	76	57	43	32	28	29	37	48	59	69	70	51.2
2024	80	71	70	44	45	32	42	40	38	60	76	78	56.3
Atmospheric precipitation, mm													
2020	17.8	27.9	0.4	28.5	24.9	3.7	4.2	0.5	0.0	0.4	11.4	1.9	121.6
2021	12.6	19.6	38.3	11.1	0.0	0.9	0.8	0.0	2.7	0.6	13.8	18.9	119.3
2022	18.4	5.9	6.4	12.4	4.3	0.0	2.3	0.5	0.0	5.3	25.5	7.8	88.8
2023	21.1	20.8	15.8	9.7	5.4	4.2	0.9	3.0	0.9	22.1	8.9	7.0	119.8
2024	17.9	4.4	54.1	3.0	42.9	2.4	5.7	3.2	0.7	13.2	17.0	13.0	177.5

Table 2. Energy indicators of the pilot site for 2020–2024

Years	Energy indicators					
	Sum air temperatures ($\sum t_{ci} > 10$ °C)	Radiation balance (R_i), kJ cm^{-2}	Evaporation (E_{tmi}), mm	Evaporation (ET_{ci}), mm	Natural humidification coefficient (C_{mi})	'Dryness Index' ($\overline{R}_i$)
2020	4,279.3	197.6	1,617.9	790.4	0.074	6.5
2021	4,530.5	205.9	1,824.6	823.6	0.064	6.9
2022	4,497.2	204.8	1,705.6	819.2	0.052	9.2
2023	4,379.6	200.9	1,701.1	803.7	0.070	6.7
2024	4,293.3	198.1	1,541.8	792.3	0.115	4.5

Analysis of the dynamics of long-term fluctuations in climatic and energy indicators of the Kyzylorda meteorological station for 2015–2024 using the trend method in the Microsoft program Excel produces linear trend equations that show:

– $t_i = 0.0934 \cdot T_i + 11.915$, with a determination index (R^2) of 0.1445, where the trend in the change in average annual air temperatures (t_i, °C) for the period under review is positive, observed from 10.89 to 12.90 °C, the absolute increase is – '+' 0.841 °C, the growth rate is '+' 0.0840 °C and the growth coefficient is 1.070;

– $a_i = -0.3273 \cdot T_i + 55.940$, with the determination index (R^2) of 0.1726, where the trend of change in the average annual relative air humidity (α_i, %) for the period under review is negative, observed from 50.90 to 58.20 °C, the absolute increase is – 2.946%, the growth rate is – 0.295% and the growth coefficient is 0.947;

– $-AP_i = -7.6721 \cdot T_i + 189.610$, with the determination index (R^2) of 0.2876, where the trend of change in annual atmospheric precipitation (AP_i, mm) for the period under review is negative, observed from 88.80 to 233.90 mm, the absolute increase is – 69.049 mm, the growth rate is – 6.095 mm and the growth coefficient is 0.620;

– $\sum t_{ci} > 10\,°C = 17.379 \cdot T_i + 4{,}202.2$, with the determination index (R^2) of 0.1605, where the tendency of change in the sum of air temperatures above 10 °C ($\sum t_{ci} > 10\,°C$), for the period under review, positive, observed from 4,023.8 to 4,497.0 °C, absolute growth is – '+' 156.411 °C, growth rate is – '+' 15.641 °C and growth coefficient is 1.037;

– $R_i = 0.5752 \cdot T_i + 195.070$, with the determination index (R^2) of 0.1604, where the trend of change in the radiation balance of the daytime surface (R_i), for the period under review is positive, observed from 189.120 to 204.810 kJ cm^{-2}, the absolute increase is – '+' 5.177 kJ cm^{-2}, the growth rate is '+' 0.518 kJ cm^{-2} and the growth coefficient is 1.026;

– $E_{tmi} = 10.894 \cdot T_i + 1{,}572.1$, with the determination index (R^2) of 0.1217, where the trend of change in total evaporation from the water surface (E_{tmi}) for the period under review is negative, observed from 1,488.6 to 1,824.6 mm, the absolute increase is – '+' 98.046 mm, the growth rate is '+' 9.804 mm and the growth coefficient is 1.062.

Thus, the analysis of the dynamics of climate and energy processes at the Kyzylorda weather station showed that they are characterized not only by a stochastic component, but also by a deterministic component in the form of multidirectional (positive and negative) trends, which are a function in time. Summarizing the above results, we can conclude that the climate indicators characterizing energy resources are increasing, and the indicator of natural moisture of the soil and vegetation cover is decreasing, which contributes to an increase in the water demand of agricultural lands.

Soil conditions of the experimental area. The conducted studies of chemical composition of saline soils of Tasboget experimental area show predominance of such cations as calcium, magnesium, sodium, potassium and anions of chlorine, sulphate-anions, hydrocarbonates (Table 3).

Soils of the experimental area at the depth of 0–20 cm and 20–40 cm by mechanical composition belong to sandy loam with slightly and moderately saline, chloride-sulphate type of salinization, Soils from 40 to 100 cm are medium loam with very strongly saline, sulphate type of salinization. Hydrogen index by depth of soil layer varies from 8.10 to 7.40, which refer to alkaline environment.

Table 3. Chemical and physico-chemical composition of soils

Name of indicators, unit of measurement	Depth, cm				
	0–20	20–40	40–60	60–80	80–100
Nitrogen (N), mg kg^{-1}	2.8	2.8	4.2	2.8	2.8
Mass fraction P_2O_5 in soil, mg kg^{-1}	7.2	9.2	14.8	16	13.6
Mass fraction K_2O in soil, mg kg^{-1}	20	14	26	26	26
Humus, %	1.21	1.519	2.64	2.865	1.32
Dense residue, %	0.389	0.501	1.908	1.961	1.821
Hydrogencarbonate (HCO_2), %	0.018	0.012	0.018	0.018	0.012
Chlorine (Cl), %	0.099	0.089	0.099	0.053	0.043
Chlorine (Cl), mg-eq per 100 g of soil	2.8	2.5	2.8	1.5	1.2
Sulphate (SO_2), %	0.173	0.254	1.08	1.210	1.210
Sodium (Na),%	0.012	0.005	0.012	0.012	0.012
Calcium (Ca),%	0.05	0.12	0.495	0.485	0.37
Magnesium (Mg), %	0.037	0.021	0.204	0.183	0.174
Hydrogen index (pH)	8.1	7.9	7.4	7.7	7.6
Salinisation type	Chloride-sulphate		Sulphate		
Degree of salinisation	Lightly saline	Medium saline	Very highly saline		
Mechanical composition	sandy loam	sandy loam	medium sandy loam		

Wastewater and surface water quality indicators. The total estimated volume of wastewater generation for the city of Kyzylorda is 31.1 thousand m³ per day or 11,351.5 thousand m³ per year. The capacity of the modular biological treatment plant in Tasboget settlement is up to 6,400 m³ per day, In the node of wastewater treatment for irrigation of agricultural land is provided for their treatment using activated carbon, Qualitative composition of wastewater of Kyzylorda city in filtration fields and wastewater treatment unit is given in Table 4.

Table 4. Qualitative indicators of wastewater before and after treatment with activated carbon

Name of indicators, unit of measurement	Wastewater quality indicators		Ground water	River wate
	before cleaning	after cleaning		
Colour, degree	600.0	510.0	-	-
Hydrogen index (pH)	8.05	7.65	7.50	7.20
Total hardness, mg-eq dm^{-3}	8.10	7.80	-	-
Alkalinity, mmol dm^{-3}	11.20	10.60	-	-
Ammonium (by nitrogen) (NH_4), mg dm^{-3}	20.90	19.90	-	0.020
Calcium (Ca), mg dm^{-3}	60.0	6.00	0.210	1.340
Magnesium (Mg), mg dm^{-3}	62.4	32.40	0.099	42.20
Total iron (Fe), mg dm^{-3}	2.3	1.40	-	-
Copper (Cu), mg dm^{-3}	0.9	0.60	-	-
Aluminium (Al), mg dm^3	48.7	32.30	-	-
Sulphates (SO_4), mg dm^3	0.27	0.20	0.436	382.4
Chlorides (Cl), mg dm^3	45.0	10.80	0.150	124.0
Nitrates (NH_2), mg dm^{-3}	0.33	0.31	-	0.008
Nitrates (NH_3), mg dm^{-3}	0.146	0.143	-	0.120
Carbonates (Ca), mg dm^{-3}	48.0	36.0	-	
Hydrocarbonates (HCO_3), mg dm^{-3}	524.6	512.4	0.866	1.340
Total mineralization (dry residue), g L^{-1}	2.04	1.61	2.625	

According to the quality composition of wastewater after the use of activated carbon and groundwater is suitable only for irrigation of special salt-tolerant fodder crops. In this regard, four levels of preparation of irrigation water were used in the study: wastewater after the use of activated carbon; mixing of wastewater after the use of activated carbon with groundwater at 1:3; groundwater (clean) water; river water.

Selection of salt-tolerant crops for cultivation on saline lands using mineralised wastewater and groundwater. For the experimental study, taking into account the soil and climatic factors of the experimental plot, more salt-tolerant forage crops were selected: barley, oats, alfalfa, sweet sorghum, mogar. African millet, corn, as well as from woody plantations: ash and poplar. Using the programmed yield theory, the productivity categories of some agricultural crops were scientifically substantiated taking into account interrelated climatic and agrotechnical factors (Tables 5 and 6),

Table 5. Projected productivity of agricultural crops by Kyzylorda region

Indicators	Agricultural crops, c ha			
	Barley	Oats	Sugar sorghum	Alfalfa
Pphotosynthetic active radiation (PAR) (R_i), kJ cm^{-2}	115.4	115.4	153.6	201.5
Planned PAR efficiency, %	1.0	1.0	2.0	1.5
Caloric value of crop biomass, (kJ kg^{-1})	18,506.0	18,422.0	16,328.0	21,771.4
Potential productivity (Pp_i), kg ha^{-1}	62.4	62.6	366.0	138.8
Climate indicator (FW)	1.0	1.0	1.0	1.0
Really-possible productivity (RPp_i) c ha	62.4	62.6	366.0	138.8
Soil reclamation indicator (K_s)	0.75	0.75	0.75	0.75
Expected productivity (EP_i), c ha	46.8	47.0	274.5	104.1
By-productivity coefficient (α_p)	0.83	0.83	0.67	1.00
By-productivity (U_p), c ha	38.8	39.0	183.9	104.1

The dependence of yield on salinity type and nutrient content is taken into account when calculating the level of expected productivity by introducing reduction coefficients for different soil availability of these elements into the calculation.

Table 6. Dose of mineral fertilizers for programmed harvest of alfalfa for hay according to nutrient removal

Indicators	Doses ofmineral fertilisers		
	N	P_2O_5	K_2O
Productivity of agricultural crops, c ha	104.1		
Nutrient removal from the soil by one centner of yield, kg	2.6	0.65	1.50
Total nutrient removal by crop, kg ha^{-1}	270.7	67.7	156.2
Soil nutrient content, mg per 100 g	2.8	7.2	20.0
Nutrients contained in the arable horizon (20 cm), kg ha^{-1}	39.0	266.4	740.0
Nutrient uptake rate from soil	0.53	0.14	0.16
Amount of nutrients received by plants from the soil	20.7	37.3	118.4
Nutrient content in wastewater, mg L^{-1}	16.0	6.0	17.0
Irrigation rate, m^3 ha^{-1}	8,100.0		
Amount of nutrients delivered with wastewater, kg ha^{-1}	129.6	48.6	137.7
Amount of nutrients received by plants from wastewater, kg ha^{-1}	32.4	12.2	34.4
Dose of mineral fertilizers to be applied to the soil, kg ha^{-1}	217.6	18.2	3.4

In order to obtain a given programmed yield, it is necessary to apply optimal rates of fertilisers to the crop, taking into account its biological characteristics, whose participation in obtaining a given productivity sometimes reaches 60–70%, as well as meeting the needs of plants in nutrients while maintaining and further improving the effective soil fertility and ensuring environmental protection.

Formation of the water-salt regime of the soil under conditions of irrigation with waste and river waters. For irrigated agriculture, in conditions of optimal water supply for sowing agricultural land, the irrigation regime for agricultural crops is determined based on ensuring water demand, taking into account the climatic features of the year, based on the equation of the water balance of the soil layer of irrigated lands (Table 7).

Table 7. Water balance of the soil layer of the experimental plot of irrigated lands with wastewater and river waters during the cultivation of alfalfa

| Years | Water balance indicators of the soil layer of the experimental plot | | | | | | | ΔWR_i, m³ ha⁻¹ |
| | Reception area | | | | Expenditure part | | | |
	O_{ci}, m³ ha⁻¹	A_{pi}, m³ ha⁻¹	FR_i, m³ ha⁻¹	Sum, m³ ha⁻¹	E_{oi}, m³ ha⁻¹	DD_i, m³ ha⁻¹	Amount, m³ ha⁻¹	
Lucerne fields irrigated with wastewater								
2020	1,216.0	13,022.0	4,000.0	18,238.0	14,561.1	3,366.9	17,928.0	310.0
2021	1,193.0	14,863.0	4,000.0	20,056.0	16,421.4	3,314.6	19,736.0	320.0
2022	888.0	14,121.0	4,000.0	19,009.0	15,350.4	3,318.6	18,669.0	340.0
2023	1,198.0	13,772.0	4,000.0	18,970.0	15,309.9	3,305.1	18,615.0	355.0
2024	1,775.0	11,793.0	4,000.0	17,568.0	13,876.2	3,359.8	17,236.0	332.0
Lucerne fields irrigated using river water								
2020	1,216.0	6,688.0	4,000.0	11,904.0	7,904.0	3,748.0	11,652.0	252.0
2021	1,193.0	7,043.0	4,000.0	12,236.0	8,236.0	3,735.0	11,971.0	265.0
2022	888.0	7,304.0	4,000.0	12,192.0	8,192.0	3,765.0	11,957.0	235.0
2023	1,198.0	6,839.0	4,000.0	12,037.0	8,037.0	3,785.0	11,822.0	215.0
2024	1,775.0	6,148.0	4,000.0	11,923.0	7,923.0	3,768.0	11,691.0	232.0

An examination of the water balance structure of an irrigated area where irrigation is carried out using wastewater leads to the conclusion that its incoming part is determined mainly by the irrigation rate (73–75%) supplied during the growing season, and partly by the leaching rate (18–20%) supplied at the end and beginning of the growing season to moisten the soil and remove salts from the active soil layer. At the same time, the outgoing part of the water balance of irrigated areas is the evaporation of moisture from the surface of the soil and vegetation cover (75–77%), and partly from drainage runoff (23–25%) formed during irrigation and leaching of salts from the active soil layer.

Calculation of the water balance of the irrigated area, where irrigation is carried out using river water, based on the totality of its incoming and outgoing components showed that the incoming part consists of the irrigation rate (60–65%) supplied during the growing season and the leaching rate (30–35%) supplied during the non-growing season to moisten the soil and remove salts from the active soil layer. At the same time, the outgoing part of the water balance of irrigated areas consists of moisture evaporation

from the surface of the soil and vegetation cover (65–70%) and drainage runoff (25–30%) formed during irrigation and leaching of salts from the active soil layer.

The methodological basis for studying the formation of the salt regime of the soil of an irrigated area using waste and river water was the information support for the water balance equation of the experimental site using calculation methods (Table 8).

Table 8. Forecasting the salt regime of the soil of the experimental site with irrigation with wastewater and groundwater (using alfalfa as an example)

Years	Water balance indicators of the soil layer of the experimental plot							ΔGS_{rsi}, t ha^{-1}
	Reception area				Expenditure part			
	G_{opi}, t ha^{-1}	G_{api}, t ha^{-1}	SF_i, t ha^{-1}	Sum, t ha^{-1}	RS_{hi}, t ha^{-1}	GS_{rdi_i}, t ha^{-1}	amount, t ha^{-1}	
Lucerne fields irrigated with wastewater								
2020	22.89	0.61	5.40	28.90	0.31	8.14	8.45	20.45
2021	25.85	0.60	5.32	31.77	0.30	7.88	8.18	23.59
2022	23.89	0.44	5.48	29.81	0.30	7.81	8.11	21.70
2023	26.20	0.60	5.76	32.56	0.30	9.04	9.34	23.22
2024	26.26	0.89	5.84	32.99	0.30	10.47	10.77	22.22
Lucerne fields irrigated using river water								
2020	11.07	0.61	5.40	17.08	0.31	7.62	7.93	9.15
2021	11.53	0.60	5.32	17.45	0.32	7.38	7.70	9.75
2022	11.47	0.44	5.48	17.39	0.33	7.68	8.01	9.38
2023	11.25	0.60	5.76	17.61	0.33	8.16	8.49	9.12
2024	11.09	0.89	5.84	17.82	0.34	8.28	8.62	9.20

To ensure increased productivity of agricultural lands on saline lands with relatively low natural moisture, one of the effective methods is the moisture-charging-leaching technology, aimed at: firstly, moistening the soil layer before sowing agricultural crops, and secondly, desalination of the upper soil layer (0–40 cm), the accumulated process of irrigation of the growing season, due to the removal of water-soluble salts to the lower horizons under the influence of large irrigation rates.

Analysis and assessment of the salt regime of the soil of irrigated areas using wastewater and river water showed that the soil and climatic region taken for the study is characterized not only by high mineralization of wastewater, but also river water. Under such conditions, in irrigated areas using wastewater, salts are constantly accumulating in the active soil layer, and where river water is used in irrigated areas, due to high mineralization, their desalting effect of the active soil layer is very low. Based on the above, we can conclude that the expected amount of precipitation does not provide natural washing of the active soil layer, and the available surface and wastewater, due to sufficiently high mineralization, do not have sufficient capacity for desalination of soil prone to secondary salinization. Under these conditions, widespread use of biological reclamation technology of saline marginal lands using salt-resistant crops can ensure effective productivity of agricultural land.

Assessment of soil fertility based on agrochemical indicators under conditions of irrigation with wastewater and river water. The assessment of the main ecological functions of soils is based on the reserves and composition of humus (humic and fulvic humus), reserves of the main elements of mineral nutrition (nitrogen, phosphorus,

potassium) and acid-base indicators (pH and hydrolytic acidity), and as an integral indicator for assessing soil fertility, one can use the 'soil index' [30], which varies from 0 to 20 units (0–4.00 – very low; 0.41–8.00 – low; 8.01–12.00 – average; 12.01–16.00 – high; 16.01–20.00 – very high).

To analyze and evaluate the agrochemical fertility index of agricultural lands irrigated using waste and river water, an information and analytical research base has been created for the 'dryness index' ($\overline{R}_i$), soil hydrolytic acidity (H_g), fertilizer assimilation coefficient by plants (δ_i), humus reserves (G_i), relative content of nitrogen (N_i/N_{max}), phosphorus (P_i/P_{max}) and potassium (K_i/K_{max}), the ratio of humates to fulvates (G_{hh}/G_{fh}), based on experimental data obtained in pilot production sites in the area of the village of Tasboget in the Kyzylorda region of the Republic of Kazakhstan (Table 9).

Table 9. Information and analytical base for assessing the 'soil index' in irrigated areas of the pilot production site in the area of the village of Tasboget, Kyzylorda region, Republic of Kazakhstan

Years	'Dryness Index' ($\overline{R}_i$)	Humus reserves, %	Ratio of humates to fulvates (G_{hh}/G_{fh})	Nutrient availability as a percentage of maximum value			δ_i	H_g, mg-eq/ 100 g^{-1} of soil
				N_i/N_{max}	P_i/P_{max}	K_i/K_{max}		
Lucerne fields irrigated with wastewater								
2020	6.5	1.41	1.683	0.221	0.099	0.433	0.98	8.10
2021	6.9	1.45	1.610	0.236	0.103	0.444	1.19	7.90
2022	9.2	1.48	1.493	0.246	0.106	0.452	1.93	7.40
2023	6.7	1.52	1.598	0.260	0.111	0.462	1.45	7.70
2024	4.5	1.61	1.639	0.292	0.120	0.485	1.59	7.60
Lucerne fields irrigated using river water								
2020	6.5	1.640	1.966	0.302	0.123	0.493	2.06	7.33
2021	6.9	1.645	1.911	0.304	0.124	0.494	2.34	7.20
2022	9.2	1.654	1.718	0.307	0.125	0.496	2.79	7.02
2023	6.7	1.659	1.845	0.308	0.126	0.498	2.39	7.18
2024	4.5	1.662	1.807	0.309	0.126	0.498	2.23	7.25

Based on the information and analytical base of the study (Table 9), the assessment of the integral fertility indicator – the 'soil index' of the experimental production site was carried out using an automated spreadsheet based on Microsoft Excel, which is shown in Table 10.

An assessment of the soil fertility indicator – the 'soil index' in irrigated areas of pilot production sites in the area of the village of Tasboget in the Kyzylorda region of the Republic of Kazakhstan based on the main agrochemical indicators (humus reserves, reserves of mineral nutrients and hydrolytic acidity) for the period 2020–2024 showed:

– in alfalfa fields where irrigation was carried out using wastewater, the indicator of the influence of humus on the generalized soil index (S_G) varies from 2.10 to 2.41 units, the indicator of the influence of mineral nutrition elements (nitrogen, phosphorus and potassium) on the generalized soil index (S_{NPK}) – from 1.79 to 2.55 units, the indicator of the influence of hydrolytic acidity on the generalized soil index (S_{Hg}) – from 0.88 to 1.05 units, and the 'soil index' – from 4.77 to 5.96 units and, in general, characterizes a low level of soil fertility;

– in alfalfa fields, where irrigation was carried out using river water, the indicator of the influence of humus on the generalized soil index (S_G) varies from 2.41 to 2.46 units, the indicator of the influence of mineral nutrition elements (nitrogen, phosphorus and potassium) on the generalized soil index (S_{NPK}) – from 2.85 to 3.19 units, the indicator of the influence of hydrolytic acidity on the generalized soil index (S_{Hg}) – from 1.07 to 1.15 units, and the 'soil index' – from 6.33 to 6.80 units and, in general, characterizes a low level of soil fertility.

Table 10. Integral indicators of soil fertility – 'soil index' in irrigated areas of the pilot production site in the area of the village of Tasboget, Kyzylorda region, Republic of Kazakhstan

Years	Soil index values			'Soil Index' (S_i)
	S_{Gi}	S_{NPKi}	S_{Hgi}	
Lucerne fields irrigated with wastewater				
2020	2.10	1.79	0.88	4.77
2021	2.17	1.99	0.93	5.09
2022	2.24	2.41	1.05	5.70
2023	2.28	2.28	0.97	5.53
2024	2.41	2.55	1.00	5.96
Lucerne fields where irrigation is carried out using river water				
2020	2.41	2.85	1.07	6.33
2021	2.42	2.99	1.10	6.51
2022	2.46	3.19	1.15	6.80
2023	2.45	3.05	1.11	6.61
2024	2.46	2.98	1.09	6.53

Thus, the conducted analysis of the state of agricultural lands based on agrochemical indicators using the 'soil index' showed that the reserve of humus and the main elements of mineral nutrition is quite low and requires a set of reclamation measures to increase soil fertility.

CONCLUSIONS

Based on the study, theoretical justification and a conceptual model was proposed for the technology of salinity management of marginal land to address problems of managing the soil water-salt and nutritional regimes, built on a mathematical model of the integrated fertility index – 'soil index', the distinctive peculiarities of which are as follows:

– possibility to implement the entire complex of functions of the technology of salinity management of marginal land, focused on increase of the 'soil index', based on humus reserves (S_G), mineral reserves (S_{NPK}) and hydrolytic acidity (S_{Hg}), using as an alternative to the calculation method using agroclimatic information to predict energy resources, water consumption deficits and various agroecological productivity of agricultural land;

– simultaneous use in combination of different estimated dependencies to assess the need of agricultural crops in nutrients, as well as chemical and biological reclamation and leaching of saline soil, providing the gradual achievement of environmentally safe level of salinization of the soil root layer and their use to adapt calculation models for

predicting water-salt and nutritional regimes, taking into account the natural characteristics of marginal land;

– the developed algorithm for the technology of salinity management of marginal land, including chemical reclamation and leaching, together with the cultivation of salt-tolerant crops serving as an instrument for salt removal from the root layer of the soil is not only limited to the desalinization effect, but also provides high-quality biological products of agricultural land, which create the prerequisites for meeting the needs of agriculture (feed base), food security and environmental sustainability of the region.

The presented analysis of the formation of soil fertility in agricultural lands allows us to determine the direction of the technology for managing salinization of marginal lands, allowing us to restore and increase soil fertility for agricultural use:

– the indicator of the influence of humus on the generalized soil index (S_G) varies from 2.10 to 2.46 units, where an increase in the humus reserve in the soil of marginal lands can be achieved by additional input of soil biomass in the form of by-products of the plant cover;

– the indicator of the influence of mineral nutrition elements (nitrogen, phosphorus and potassium) on the generalized soil index (S_{NPK}) varies from 1.79 to 3.19 units, an increase in the content of mineral nutrition elements in the soil can be achieved through an increase in humus reserves and a balanced supply of organic and mineral fertilizers, based on their standardization taking into account the planning of the yield of cultivated agricultural crops;

– the indicator of the influence of hydrolytic acidity on the generalized soil index (S_{Hg}) varies from 0.88 to 1.15 units, an increase in the value of the soil index for hydrolytic acidity can be achieved by carrying out chemical melioration to obtain a neutral soil reaction;

– the 'soil index' varies from 4.47 to 6.80 units and, in general, characterizes a low level of soil fertility; an increase can be achieved using a set of reclamation measures that can increase soil fertility.

REFERENCES

Abdu, A., Laekemariam, F., Gidago, G. & Getaneh, L. 2023. Explaining the Soil Quality Using Different Assessment Techniques. *Applied and Environmental Soil Science* **1**, 6699154. doi: 10.1155/2023/6699154

Aidarov, I.P., Golovanov, A.I. & Nikolsky, Yu. N. 1990. *Optimization of melioration regimes of irrigated and drained agricultural lands (recommendations)*. Moscow: Publ. Agropromizdat, 60 pp. (in Russian).

Alkharabsheh, H.M., Seleiman, M.F., Hewedy, O.A., Battaglia, M.L., Jalal, R.S., Alhammad, B.A. & Al-Doss, A. 2021. Field crop responses and management strategies to mitigate soil salinity in modern agriculture: A review. *Agronomy* **11**, 2299. doi: 10.3390/agronomy11112299

Averyanov, S.F. 1978. *Combating salinization of irrigated lands.* Moscow: Publ. Kolos, 288 pp. (in Russian).

Bilquees, G., Ajmal, M. & Khan. 2003. Saline agriculture: promises and prospects for future agriculture in degraded saline land. *Technology and Development in New Millennium*, 149–156.

Borodychev, V.V., Dedjva, E.B., Sazanov, M.A. & Lytov, M.N. 2016. Modeling the process of managing the water-salt regime of soils under irrigation conditions. *News of the Lower Volga Agro-University Complex: Science and Higher Professional Education* **2**(42), 26–33 (in Russian).

Borodychev, V., Levina, A., Dedova, E. & Ochirova, E. 2011. Ecological and energy efficiency of rice agrolandscapes of the Sarpinsk lowland. *Fertility* **2**, 21–23 (in Russian).

Bresler, E., McNeal, B. & Carter, D. 1982. *Saline and Sodic Soils: Principles, Dinamics, Modeling*. Springer-Verland, Berlin, Heidelberg, 296 pp.

Budyko, M. 1956. Thermal balance of the Earth's surface, Leningrad: *Gidrometeoizdat*, **3**, 256. (in Russian).

Busby, P., Soman, C., Wagner, M., Friesen, M., Kremer, J. & Bennett, A. 2017. Research priorities for harnessing plant microbiomes in sustainable agriculture. *PLoS Biol* **15**(3), e2001793. doi: 10.1371/journal.pbio.2001793

Chhabra, R. 1996. Soil salinity and water quality. New Delhi, 284 pp. doi: 10.1201/9780203739242

Darkoh, M. 1989. Desertification in Africa. *Journal of Eastern African Research & Development*, 1–50. https://www.jstor.org/stable/24325608

Dedova, E. 2012. *Increase of natural-resource potential of degraded agricultural lands of Kalmykia by means of complex reclamation*. PhD Thesis, All-Russian Research Institute of Hydraulic Engineering and Land Reclamation named after A. Kostyakov, 246 pp. (in Russian).

Dokuchaev, V.V. 1949. *Selected Works*. Edited by Academician B.B. Polynov. M., Publishing House of the USSR Academy of Sciences, 643 pp. (in Russian)

Eckholm, E. & Brown, L. 1977. The Hand of Man Spreading Desertification. *World Watch Paper* **13**, 26.

Ferrero, A. 1996. Prediction of Heteranthera reniformis competition with flooded rice using day–degrees. *Weed Res*. **36**, 197–201. doi: 10.1111/j.1365-3180.1996.tb01815.x

Gabunshchina, E.B. 2001. Concept of management of arid ecosystems of the Russian Caspian Sea. *Elista* **39**.

Gilani, A. 1997. Soil Degradation and Desertification in Arab Countries. *Journal of Water and Agriculture* **17**, 28–55.

Howe, C., Suich, H., Vira, B. & Mace, G. 2014. Creating win-wins from trade-offs? Ecosystem services for human well-being: A metaanalysis of ecosystem service trade-offs and synergies in the real world. *Global Environmental Change* **28**, 263–275. doi: 0.1016/j.gloenvcha.2014.07.005

Ivanov, N. 1978. Humidification zones of the globe. Izvestiya USSR. *Series of geography and geophysics* **3**, 261–288 (in Russian).

Kaziyev, M., Ibragimov, K., Umakhanov, M. & Teymurov, S. 2021. *Restoration of degraded forage lands in the Western Caspian region*. Monograph. Federal State Budgetary Scientific Institution 'Federal Agricultural Scientific Centre of the Republic of Dagestan'. Makhachkala. Publ. Riso-Press, 206 pp. (in Russian).

Keller, B. 1923. Plant world of Russian steppes, semi-deserts and deserts. *Sketches of ecological and phytocenotic*. Works on the study of nature and economy of arid desert regions of Russia, Voronezh, Vol **1**, 183 pp. (in Russian).

Kharin, N. 1992. *Methodical guidelines for studying desertification* (on the example of Mongolia). Eds.: Kharin, N.; Babaev, A.; Kurbanmuradov, K., Institute of Deserts, 78 pp. (in Russian).

Kireycheva, L., Glazunova, I.V., Yashin, V. & Nguyen, S. 2014. Classifier of methods and technical solutions for restoring the fertility of degraded lands. *Fertility* **6**(81), 30–34 (in Russian).

Kizyaev, B. 2009. *System of rice farming of the Republic of Kalmykia*. Elista Publishing House 'Dzhangar'. 167 pp.

Kolb, A. 1971. *East Asia, China, Japan, Korea, Vietnam*: *Geography of a cultural region*. Stanford University Press. 268 pp. (in Russian).

Kostyakov, A.N. 1951. *Fundamentals of Land Reclamation*. Selkhozgiz, 350 pp. (in Russian).

Kulik, K.N., Gabunschina,, I. & Kruzhilin,, E. 2007. Desertification and complex reclamation of agrolandscapes of arid zone. Volgograd: *Publ.: VNIALMI* **1**, 85. (in Russian).

Kruzhilin, I. & Chasovskikh, V. 2004. *The concept of agricultural land reclamation development in Russia*. Under general ed, by G,A, Romanenko, Moscow, pp. 43. (in Russian).

Kust, G. 2002. Desertification, droughts and soil degradation. *Soil degradation and protection*. Moscow, 551–600 (in Russian).

Mitchell, C. 1968–1981. Soil degradation mapping from Landsat imagery in North Africa and the Middle East. FAO/UNEP. *Agrarian radicalism in China*, *Harward University Press, Cambridge*, 296 pp.

Mukhtar, A. & Mustafa. 2004. Traditional knowledge and modern technology for sustainable agricultural development in the drylands of Sudan. *Assembler of scientists articles*, MAB UNESCO, Paris, 3543.

Mustafayev, Zh. 1986. Physical and mathematical modeling of the process of leaching of salts from soil. *Fertility of soils of Kazakhstan* **2**, 64–72 (in Russian).

Mustafayev, Zh. & Abdeshev, K. 2023. Modeling of Soilsalinization and Desalinizatiom Processes. Chisinau: *LAP LAMBERT Academic Publishing*, 185 (in Russian).

Mustafayev, Zh. & Kemelbekov, Sh. 1987. Methodology of calculation of irrigation norms for the use of manure runoff. *Herald of Agricultural Science of Kazakhsta,* **1**, 75–77, (in Russian).

Mustafayev, Zh., Kozykeeva, A., Kireicheva, L. & Zhusupova, L. 2015. Ecosystem justification of ways to develop saline soils. *Agroecology* **2**(4), 3–9 (in Russian).

Mustafayev, H., Kozykeeva, A., Karlykhanov, T. & Zhusupova, LK. 2017a. Method of development of saline lands. *Electronic Bulletin* **6**, 3 (in Russian).

Mustafayev, Zh., Kozykeeva, A. & Zhidekulova, G. 2017b. Model of crop productivity formation in hydro-agrolandscape systems. *International Technical and Economic Journal* **4**, 100–109 (in Russian).

Mustafayev, Zh., Medeu, A., Skorintseva, I., Bassova, T. & Aldazhanova, G. 2024b. Improvement of the Methodology for the Assessment of the Agro-Resource Potential of Agricultural Landscapes. *Sustainability* **1**, 419. doi: 10.3390/su16010419

Mustafayev, Zh., Skorintseva, I., Toletayev, A., Kuderin A. & Omarov, A. 2024a Assessment of soil resources of agricultural landscapes in Turkestan region of the Republic of Kazakhstan based on agrochemical indexes. *Open Geosciences* **16**(1). doi: 10.1515/geo-2022-0652

Mustafayev, Zh.S. & Umirzakov, S.I. 2013. Environmentally safe technologies for wastewater disposal in specialized agricultural landscapes. *Big Neo Service*, 248.

Mustafayev, Zh., Umirzakov, S. & Baimanov, Zh. 2024. Modeling the Process of Management of Water-Salt and Food Regime of Salt Soils of Marginal Land Using Waste Water, 16 April, *PREPRINT* (Version 1) available at Research Square. doi: 10.21203/rs.3.rs-4255939/v1

Nikolskiy, Yu. & Shabanov, V. 1986. Calculation of design yield depending on water regime of reclaimed lands. *Hydraulic engineering and melioration* **9**, 52–56 (in Russian).

Nichiporovich, A., Stroganova, L., Chmora, S. & Vlasova, N. 1961. Photosynthetic activity of plants in crops. Moscow. *Academy of Sciences of the USSR* **2**, 160 (in Russian).

Petrov, M. 1975. About borders of desert areas. *Problems of Desert Development* **2**, 3–10 (in Russian).

Petrov, V. 2001. Forest-melioration of arid territories. *Agroforestry science in the XX century*, Volgograd: Publ.: *VNIALMI*, 162–198 (in Russian).

Pegov, S. & Khomyakov, P. 1991. Modeling of ecological systems development. Leningrad: *Gidrometeoizdat* **2**, 224 (in Russian).

Rahman, K., Thaleth, M. & Shalpykov, K. 2023. Strategies for Remediation of Marginal Lands and Restoration Technology. *Biomedical Journal of Scientific & Technical Research* **48**(3), 39791–39794. doi: 10.26717/BJSTR.2023.48.007663

Rex, L.M. 1995. *Systematic studies of melioration processes and systems*. Moscow: Aslan Publishing House, 192 pp. (in Russian).

Shamsutdinov, Z., Kosolapov, V., Savchenko, I. & Shamsutdinov, N. 2009. Ecological restoration of pastures (based on new varieties of forage halophytes). *Russian Academy of Personnel Support of Agroindustrial Complex* **2**, 295 (in Russian).

Woldeyohannis, Y.S., 2024. Negative Effect of Soil Compaction and Investigation of Its Relation with Soil Physiochemical Properties in Mechanization Farming System. *Applied and Environmental Soil Science* **1**, 5654283. doi: 10.1155/2024/5654283

Takamura, H. 1997. Changes in the hydrological environment and land degradation in the Tarim Basin. *Proceedings of the International Symposium on Hydro-Environment in Asia*. November 5–7, 111–118.

Tooming, H. 1984. *Ecological principles of maximum crop productivity*. Leningrad: Gidrometeoizdat, 264 pp. (in Russian).

Vlek, P., Khamzina, A. & Tamene, L. 2017. Land degradation and the sustainable development goals: Threats and potential remedies. *CIAT Publication, International Center for Tropical Agriculture (CIAT)* **67** pp. https://hdl.handle.net/10568/81313

Zonn, I. & Kust, G. 1999. The problem of desertification in Russia: status, assessment, ways of solution. Desertification and soil degradation. *Proceedings of the International Scientific Conference*, Moscow: MSU Publishing House, pp. 52–65 (in Russian).

Susceptibility of Japanese plum and pluot cultivars to *Pseudomonas syringae*

I. Pánková[*], V. Krejzar and R. Krejzarová

Czech Agrifood Research Center, Department of Phytobacteriology, Drnovská 507, CZ16106 Praha, Czech Republic
[*]Correspondence: iveta.pankova@carc.cz

Abstract. The susceptibility of seven cultivars of Japanese plum (Black Star, Gauota, Golden Japan, Crimson Glo, Ozark Premier, Santa Rosa, Vanier) and three cultivars of pluot (Black Gigant®, Dapple Supreme pluot®, Flavorich pluot®) to *Pseudomonas syringae* pathovars *syringae* and *morsprunorum* was evaluated under temperate European climate conditions over a three-year period. The evaluation was carried out in a netted house. The susceptibility of the plants to the causal agents of blossom and terminal shoot infection was calculated on five occasions during the year using a formula summarising all the symptoms that appeared on the plants after artificial inoculation. Most cultivars were classified in BS class 2 as low susceptible to both pathovars of *Pseudomonas syringae*. The Japanese cultivar Santa Rosa was the only cultivar classified in class 4, as highly susceptible to *morsprunorum* pathovar. After terminal shoot inoculation, 60% of the Japanese plum and pluot cultivars were classified as very low susceptible in class 1 and 40% as low susceptible in class 2 to both pathogens tested. Apart from Santa Rosa, the remaining Japanese plums and all plum cultivars showed remarkable vigour and recovery after repeated artificial infection with economically important *Prunus* plant pathogens.

Key words: blossom inoculation, netted house, *Pseudomonas syringae* pv. *morsprunorum*, *Pseudomonas syringae* pv. *Syringae*.

INTRODUCTION

Plum is a drupe in the genus *Prunus* in the family Rosaceae. Commercially grown genotypes belong to the European plum group (mainly *Prunus domestica* L.) or the Asian plum group (*Prunus salicina* L.) and cherry plums (*Prunus cerasifera* Ehrh.) (Okie & Hancock, 2008). The attractive appearance and flavour of Japanese plum cultivars and pluots, complex *Prunus* hybrid with dominant parentage of plum (*Prunus salicina*) and apricot (*Prunus armeniaca* L.) having fruit resembling plum, are attracting increasing interest from growers. The added value of these cultivars is their nutritional value, particularly their high levels of health benefits anthocyanins (Rampáčková et al., 2021; Tomić et al., 2022). These cultivars are characterised by reduced resistance to cold hardiness and limited adaptability to different soil conditions (Dalbó et al., 2016; Milošević & Milošević, 2018). In temperate regions, climate change is increasingly

contributing to pronounced temperature fluctuations during dormancy and spring, which significantly affect the vigour of plum trees and increase their susceptibility to bacterial plant pathogens (Fadón et al., 2020; Seethapathy et al., 2022). Additionally, excessive fertilisation and pesticide application further sensitize plant tissues to pests (Husseini & Akköprü, 2020; Aprile et al., 2021; Tripathi et al., 2022).

In temperate orchards, significant economic losses are associated with bacterial infections caused by members of the *Pseudomonas syringae* (*Ps*) complex (Yang et al., 2023), notably *Ps* pv. *syringae* van Hall 1902 (*Pss*) and *Ps* pv. *morsprunorum* race 1 (Wormald) Young et al. (Lee et al., 2012) (*Psm*). These pathogens severely affect plum production across Central Europe, leading to symptoms such as blossom blast, shoot dieback, and stem cankers. Historical data indicate annual tree mortality rates reaching up to 30% in heavily infected orchards (Sundin, 2007). Recent reports have confirmed that losses of a similar magnitude continue to affect both plum and pluot cultivars in the region (Němcová & Buchtová, 2023).

The pathogens have led to widespread symptoms such as blossom blast, shoot dieback, and canker formation, especially during wet spring seasons. These pathogens contribute to widespread losses through blossom blast, shoot dieback, and canker formation, severely impacting orchard longevity and fruit yield (Konavko et al., 2014). Bacteria are naturally present on plants or plant debris in the agro-ecosystem and are transferred to susceptible plants by wind, rain, birds, insects and technology. The severity of *Ps* infection varies according to annual weather conditions. Outbreaks occur during rainy periods, prolonged high humidity and temperatures below 12 °C (Hunjan & Lore, 2020). Once suitable conditions are established, the initially unculturable *Ps* inoculum reaches a concentration capable of inducing infection within 24 h. Blossoms are most susceptible to *Ps* infection due to the amount of nutrients available (Chan et al., 2021). The changes in the pattern of precipitation along with the warmer weather have increased the incidence of *Ps* diseases in fruit trees in autumns (Roussos, 2024). Through various wounds and frost damage, *Ps* enters the vascular bundles and threatens tree vigour and production (Hulin et al., 2018). The course and severity of infection vary depending on the pathovar or race of the pathogen (Islam et al., 2024).

The susceptibility of Japanese plum and pluot cultivars grown in temperate climates to any bacterial pathogens is a topic that has yet to be extensively researched. The most common method for evaluating the susceptibility of plants to Ps bacteria is through inoculation of detached flowers, leaves, or shoots. This approach provides valuable insights into the interaction between the pathogen and the plant organ (Aleksandrova et al., 2023; Lienqueo et al., 2024). The assessment of fruit trees in a greenhouse setting by inoculation of blossoms or terminal shoots is a more compact approach, but the degree of agreement with the experience of agricultural practice is variable (Bophela et al., 2020). While cultivar susceptibility evaluations under different weather and soil conditions in field trials are less consistent, they are better transferable to practice, thereby reducing economic losses due to inappropriate variety selection (Carvalho et al., 2021; Burbank et al., 2023).

This study aimed to evaluate the susceptibility of blossom and terminal shoots to *Pss* and *Psm* in selected Japanese plum and pluot cultivars under netted house conditions simulating a temperate climate.

MATERIALS AND METHODS

Plant material

The level of susceptibility of 7 Japanese plum (Black Star, Gauota, Golden Japan, Crimson Glo, Ozark Premier, Santa Rosa, Vanier) and 3 pluot (Black Gigant®, Dapple Supreme pluot®, Flavorich pluot®) cultivars to *Pss* and *Psm* was evaluated for three consecutive growing seasons (2021–2023). All cultivars were grafted by chip-budding on GF 305 peach rootstocks. Twenty-four saplings of each Japanese plum and pluot cultivar were planted in the autumn in 40-litre containers, placed in two netted houses, and winterized. The potted trees were maintained under a uniform care regimen within the netted houses, which were designed to simulate field-like conditions. Trials were conducted at an altitude of 250 m a.s.l. in a major fruit-growing region of Central Europe. To mitigate environmental stress, trees were irrigated during summer to prevent drought and covered with coco mats in winter to protect against frost damage.

Bacterial strains and inoculum preparation

Suspensions of virulent *Pss* strains CPABB 138, CPABB 234 and CPABB 237 (Collection of Phytopathogenic and Agriculturally Beneficial Bacteria, Czech Republic) in sterile water at a concentration of 10^5 CFU mL^{-1} were mixed in equal ration and used for inoculation. Mixed suspension of *Psm* strains CPABB 23 and CPABB 24 was prepared and applied in the same way.

Inoculation of Japanese Plum and Pluot Cultivars

Potted trees of Japanese plum and pluot cultivars were inoculated at two distinct phenological stages following the BBCH scale (Meier et al., 1994). Five trees per cultivar were treated at blossom stage BBCH 61-65, when all flowers within a cluster were open. A single randomly selected flower cluster on each plant was sprayed with 1 mL of a mixed *Pss* suspension. In a separate set of five trees per cultivar, inoculation was performed at shoot stage BBCH 35-39, corresponding to shoots reaching 50–90% of their final length. On each plant, a randomly chosen shoot was cut below the first undeveloped leaf using scissors dipped in the same mixed *Pss* suspension.

In the same way, the same number of plants were inoculated with a mixed suspension of *Psm*. Four trees of each plum and pluot cultivar were used as negative controls. Two were sprayed with 1 mL of sterile water on one randomly selected blossom cluster, and in the other two, one randomly selected terminal shoot was cut below the first undeveloped leaf using scissors dipped in sterile water.

Evaluation of Cultivar Susceptibility

Blossom blight, shoot blight, and other symptoms of progressive pseudomonad infection affecting various plant organs were evaluated five times (at 1, 3, 6, 12, and 20 weeks after inoculation) during each growing season from 2021 to 2023.

For each plant (i), the following parameters were determined: the total number of infected plant organs (Yi), the percentage of necrotic blossoms in the inoculated cluster (NBi/TBi), the percentage of necrotic lesions on the inoculated terminal shoot (NSi/TSi), the percentage of leaves with necrotic lesions (Li), the proportion of damaged fruit (Fi), and the decline in overall tree vigour (Vi) in comparison to negative control. For each

evaluation date, the mean blossom susceptibility (*BSx*) and terminal shoot susceptibility (*TSx*) to *Pss* and *Psm* were calculated for each cultivar (*x*) using the formula:

$$BS_x\ (TS_x) = 1/5 \sum_{i=1}^{5} \left(\frac{NBi}{TBi} \cdot 100 + \frac{NSi}{TSi} \cdot 100 + L_i + V_i + F_i \right) / Y_i. \qquad (1)$$

The highest calculated values of BS_x and TS_x for each pathovar were than transformed into the international scale of susceptibility (Le Lezec et al., 1997): 1 – very low (0–20.0%); 2 – low (> 20.0–40.0%); 3 – moderate (> 40.0–60.0%); 4 – high (> 60.0–80.0%); 5 – very high (> 80.0–100%). Based on this scale, each Japanese plum and pluot cultivar was assigned a susceptibility class for blossom and terminal shoot infection by *Pss* and *Psm* for each year of evaluation.

The effects of place of infection (blossom, terminal shoot), evaluation term and year on the calculated *BS* and *TS* to *Pss* and *Psm* for Japanese plum and pluot cultivars were analysed using Fisher's Least Significant Difference (*LSD*) test in the R software version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). $P < 0.05$ was considered as the threshold for significance.

RESULTS AND DISCUSSION

For three consecutive growing seasons (2021–2023), a total of five calculated *BS* and *TS* values to the pathogens *Pss* and *Psm* for each plum and pluot cultivar tested are summarized in supplementary tables A–D (Susceptibility of Japanese plum and pluot cultivars to Pseudomonas syringae-Supplementary Tables.xlsx).

Blossom susceptibility
The findings revealed that, irrespective of the cultivar and *Ps* pathovar tested, *BS* values recorded at week 3 were lower than those calculated 7 days after blossom inoculation. From week 6 through week 20, a consistent trend was observed, characterized by a gradual increase in BS values, reflecting progressive symptom development as shown in Fig. 1, (A–C).

Figure 1. Symptoms of pseudomonas blight on Japanese plum and pluot cultivars observed during the 2022 growing season in a netted house, following artificial inoculation of randomly selected blossom clusters with a bacterial mixture of *Pseudomonas syringae* pv. *Morsprunorum*.
A – blossom blight on Japanese plum cultivar Black Star, observed during the first evaluation, one week after artificial inoculation; B – necrotic tips observed on pluot cultivar Black Gigant®, observed at the third evaluation term, 6 weeks after inoculation; C – necrotic leaf spots on Japanese plum cultivar Golden Japan, observed during the fourth evaluation, 12 weeks after inoculation.

A comparison of the annual *BS* classes of the tested cultivars, determined according to the highest *BS* values, revealed statistically significant differences ($P < 0.05$) in their susceptibility to *Pss* and *Psm* pathogens are summarized in supplementary tables E–F (Susceptibility of Japanese plum and pluot cultivars to Pseudomonas syringae-Supplementary Tables.xlsx) and Fig. 2. Most of the cultivars exhibited low susceptibility to blossom infection, categorized in class 2. Two exceptions were identified, Flavourish pluot® was classified into class 1 as being very low susceptible to *Pss* infection, and Santa Rosa was classified into class 4 as being highly susceptible to *Psm* infection. Six of the ten cultivars evaluated, namely Black Star, Crimson Glo, Santa Rosa and three pluot cultivars, were classified in the higher susceptibility category regarding infection by *Psm*. Two cultivars were assigned to the same susceptibility class for both pathogens. Only Vanier was assigned to a higher susceptibility class for *Pss*.

Figure 2. Comparison of the blossom susceptibility classes in Japanese plum and pluot cultivars to *Pseudomonas syringae* pv. *syringae* (*Pss*) and *Pseudomonas syringae* pv. *morsprunorum* (*Psm*) determined in 2021–2023 in a netted house.

Each bar represents the mean ± SD of the blossom susceptibility classes (1 – very low susceptibility; 2 – low susceptibility; 3 – moderate susceptibility; 4 – high susceptibility) to *Pseudomonas syringae* pv. *syringae* and *Pseudomonas syringae* pv. *morsprunorum*, evaluated in five trees of each cultivar over the period 2021–2023; * indicates a statistically significant difference at the 0.05 level according to Fisher's *LSD* test.

Terminal shoot susceptibility

Following the infection of the terminal shoots, the initial disease symptoms, manifesting as black water-soaked lesions progressing from the site of inoculation along the shoots (Fig. 3, A and B), were observed at week 3, regardless of the *Ps* pathovar used. As the infection progressed to other parts of the inoculated trees, *TS* values increased at varying rates throughout the remainder of the growing season, depending

on the specific plum or pluot cultivar. These data are summarized in supplementary tables C and D (Susceptibility of Japanese plum and pluot cultivars to Pseudomonas syringae-Supplementary Tables.xlsx).

A comparison of the annual *TS* classes of the tested cultivars revealed statistically significant differences ($P < 0.05$) in susceptibility to the pathogens *Pss* and *Psm* (supplementary tables E–F; Susceptibility of Japanese plum and pluot cultivars to Pseudomonas syringae-Supplementary Tables.xlsx) at the three cultivars tested (Fig. 4). Two of these, Black Star and Dapple Supreme pluot®, demonstrated heightened susceptibility to *Pss*. In contrast, the cultivar Santa Rosa was found to be the most susceptible cultivar to *Psm* infection on terminal shoots. Seven cultivars demonstrated comparable susceptibility to both pathogens. The plum cultivars Gauota, Ozark Premier, Vanier and the pluot cultivars Black Gigant® and Flavorich pluot® were classified as very low susceptibility in class 1
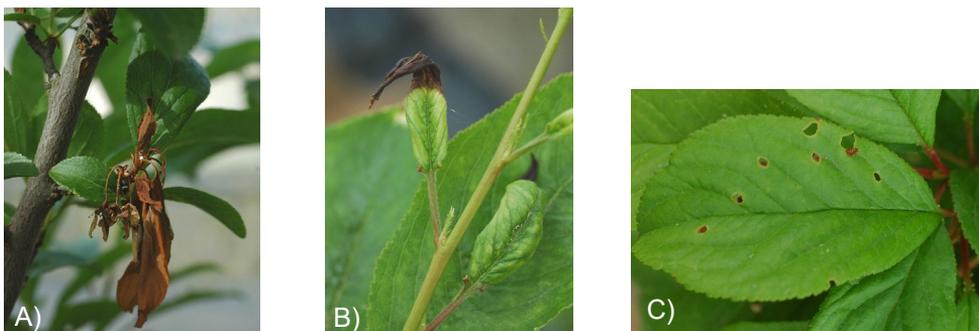

Figure 3. Symptoms of pseudomonas blight on Japanese plum and pluot cultivars observed during the 2022 growing season in a netted house, following artificial inoculation of randomly selected terminal shoots with a bacterial mixture of *Pseudomonas syringae* pv. *Morsprunorum*.

A – symptoms on Japanese plum cultivar Crimson Glo, observed during the fourth evaluation, 12 weeks after inoculation; B – necrotic terminal shoot on Japanese plum cultivar Santa Rosa, observed during the fifth evaluation, 20 weeks after inoculation.

throughout the study. The plum cultivars Golden Japan, Crimson Glo and the pluot cultivar Dapple Supreme® were equally susceptible to both pathogens and were placed in Class 1 in 2021 and Class 2 in 2022 and 2023.

Susceptibility to *Pseudomonas syringae*

Across all three growing seasons, the *BS* class ratings for *Ps* pathovars were consistently higher than *TS* class ratings. Plum and pluot cultivars were low susceptible to *Ps* blossom infection, typically falling into *BS* class 2. Regarding terminal shoot infection, 50% of the tested cultivars were classified as very low susceptible (class 1), while 40% were considered low susceptible (class 2). The only exception was the cultivar Santa Rosa, which was moderately susceptible to blossom infection (class 3) and low susceptible to terminal shoot infection (class 2). Over the years, greater variability was observed in BS class ratings for individual cultivars. Over the years, greater variability was observed in BS class ratings for individual cultivars. Statistical analysis using the *LSD* test ($P < 0.05$) revealed that the differences between *BS* and *TS* class ratings to *Ps* became more pronounced and statistically significant, particularly when comparing low and moderately susceptible Japanese plum and pluot cultivars (Fig. 5).
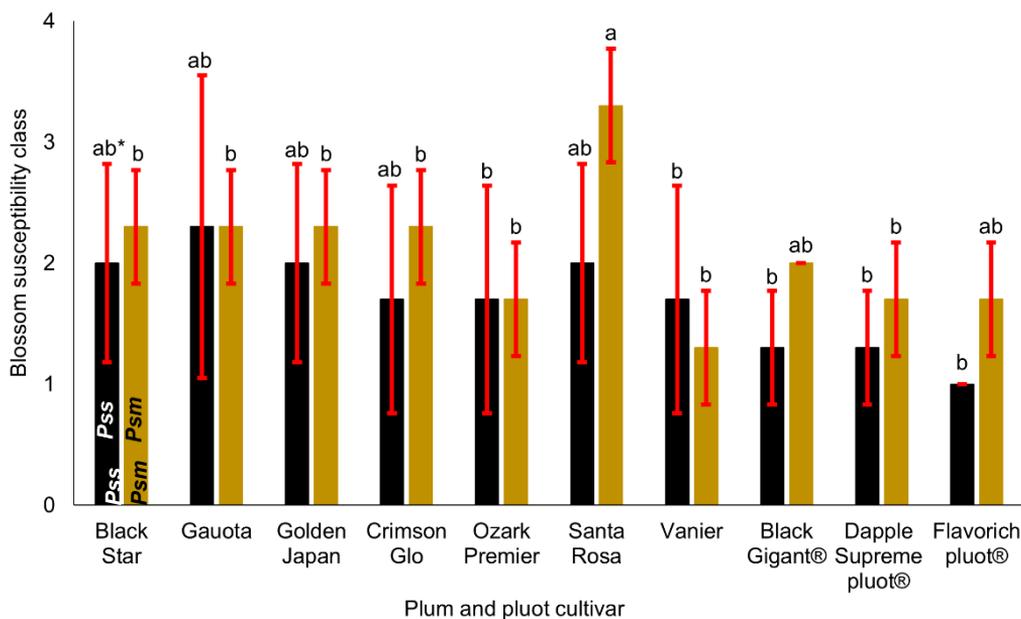
Figure 4. Comparison of the terminal shoot susceptibility classes in Japanese plum and pluot cultivars to *Pseudomonas syringae* pv. *syringae* (*Pss*) and *Pseudomonas syringae* pv. *morsprunorum* (*Psm*) determined in 2021–2023 in a netted house.

Each bar represents the mean ± SD of the terminal shoot classes (1 – very low susceptibility; 2 – low susceptibility; 3 – moderate susceptibility) to *Pseudomonas syringae* pv. *syringae* and *Pseudomonas syring*ae pv. *morsprunorum*, evaluated in five trees of each cultivar over the period 2021–2023; * indicates a statistically significant difference at the 0.05 level according to Fisher's *LSD* test.

Throughout the three-year study, both *Ps* pathovars exhibited a similar infection progression in inoculated plants during the growing season, inducing comparable symptoms such as desiccation and necrosis, despite belonging to distinct phylogroups within the *Ps* complex. Specifically, *Pss* belongs to PG2, while *Psm* is classified as PG3 for race 1 and PG1 for race 2 (Chen et al., 2022; He et al., 2023). *Pss* and *Psm* share key mechanisms of pathogenesis, notably the production of phytotoxins and deployment of the type III secretion system (T3SS), which delivers effector proteins into host cells to suppress plant immune responses (Hulin et al., 2018). This mechanism was evident during the inoculation of blossom clusters, which triggered a near-identical hypersensitive reaction across pathovars. Following this initial reaction, a secondary phase referred to as the 'symptom-free period' was observed. Consistent with previous findings (Yang et al., 2023), the duration of this phase and the subsequent timing and severity of disease symptoms varied depending on the susceptibility of the plum or pluot cultivar, the inoculation method, the specific pathovar involved, and the prevailing weather conditions during each evaluation period. To assess the implications of pathogenic mechanisms for orchard management, alongside the progression of *Pss* and *Psm* infections from the inoculation site to other plant organs, the vigour of inoculated trees was also evaluated in comparison to the negative control. These vigour assessments were incorporated into the mean *BS* and *TS* values calculated for each evaluation period.
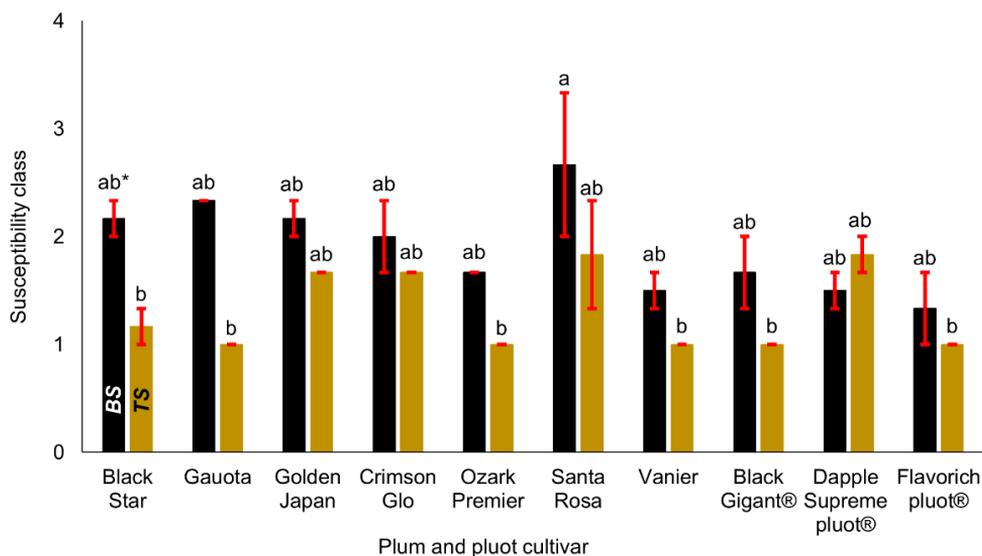
Figure 5. Comparison of the blossom (*BS*) and terminal shoot susceptibility (*TS*) classes in Japanese plum and pluot cultivars to *Pseudomonas syringae* determined in 2021–2023 in a netted house.

Each bar represents the mean ± SD of the susceptibility classes (1 – very low susceptibility; 2 – low susceptibility; 3 – moderate susceptibility; 4 – high susceptibility) to *Pseudomonas syringae*, evaluated in ten trees of each cultivar over the period 2021–2023; * indicates a statistically significant difference at the 0.05 level according to Fisher's *LSD* test.

Following blossom infection with *Pss*, a 'symptom-free period' persisted until the third evaluation date in week 6 for the low susceptible cultivars Golden Japan, Black Star, and Santa Rosa, when necrotic spots were observed on leaves and shoots near the inoculation site. In moderately susceptible plum varieties Black Star, Golden Japan, Santa Rosa, as well as in the almost moderately susceptible plum variety Gauota, the infection had spread to shoots near the inoculation site by the same evaluation date, which manifested itself in their gradual wilting. Following the application of the *Psm* inoculum to the blossom clusters, the interval between the onset of the hypersensitive reaction and the subsequent appearance of symptoms on the shoots was reduced. In the low susceptible plum cultivars Black Star, Gauota, Golden Japan, Crimson Glo and Santa Rosa, water-soaked lesions manifested immediately on the shoots below the cluster of inoculated blossoms. Subsequently, they appeared on the leaves from week 6 onwards, gradually drying up and the necrotic tissue falling off. In the moderately susceptible plum cultivar Santa Rosa, from week 12 onwards, the entire branches exhibited sudden desiccation, and the experimental plants demonstrated heightened susceptibility to water stress. The divergent post-hypersensitivity strategies exhibited by *Pss* and *Psm* in plant tissues, manifesting as differential rates of spread and intensity of infection symptoms on plant organs, are attributable to the presence of distinct effectors specific to individual pathovars (Hulin et al., 2018; Ruinelli et al., 2019; Santos et al., 2024). In accordance with the findings of several studies (Xin et al., 2018; Ruinelli et al., 2019; Chai et al., 2023), which highlight the critical role of high humidity in sustaining elevated epiphytic populations of *Ps* on plant surfaces and enhancing

bacterial survival and infectivity in aerosols, the frequency and severity of *Pss* and *Psm* infection symptoms increased in 2022 due to springtime precipitation. Across most of the evaluated cultivars, a statistically significant correlation was observed between *BS* values and the year of evaluation, regardless of the pathovar involved.

The physiological response of plum and pluot trees to terminal shoot infection was slower than their response to blossom infection. This difference in response speed and intensity can be explained by the fact that blossoms, being more succulent and nutrient-rich, offer a high-moisture environment with elevated metabolic activity, which promotes rapid bacterial multiplication. (O'Malley & Anderson, 2021). Two-way ANOVA revealed that, for both *Ps* pathovars, there were no statistically significant differences in the *TS* classification of individual plum and pluot cultivars across the 2021 to 2023 growing seasons. The weaker response to varying seasonal conditions can be attributed to differences in tissue physiology compared to blossoms. Actively growing terminal shoots are highly susceptible to *Ps* infection, particularly under conditions of high humidity and bacterial concentrations exceeding 10^5 CFU/ shoot (Hulin et al., 2018; Mustafa et al., 2021; Broniarek-Niemiec et al., 2023). However, the gradual maturation of shoots, accompanied by increasing lignification and structural integrity, contributes to a more robust defense mechanism that slows the spread of *Ps* pathogens. Mature tissues often have lower metabolic activity and fewer nutrient resources, making them less favourable for bacterial proliferation (Xin et al., 2018; Huang et al., 2022. The differing mechanisms of infection dissemination, regardless of *Ps* pathovar resulted in statistically significant discrepancies between blossom susceptibility class and terminal shoot susceptibility class in *LDS* tests across Japanese plum and pluot cultivars.

The first clearly visible symptoms of infection, including shoot tip desiccation and the development of small necrotic lesions on leaves and terminal shoots, were observed on Santa Rosa trees six weeks after inoculation with *Psm* bacteria. This cultivar was classified as low susceptible (*TS* class 2), or as moderately susceptible (*TS* class 3) in 2023. Santa Rosa was the only cultivar that showed a clearly observable progression of infection throughout the 20-week evaluation period. By week 20, premature yellowing, leaf drop, and complete desiccation of the inoculated terminal shoots were recorded in its trees. Plum and plum cultivars Golden Japan, Crimson Glo and Dapple Supreme pluot® were predominantly classified in *TS* class 2, regardless of the *Ps* pathovar or year of evaluation. These cultivars consistently exhibited visible symptoms of infection, including dark necrotic spots on the inoculated terminal shoots and, later, on surrounding shoots, typically observed in week 12 or 20. Disease progression in these cultivars was slow and often subtle, remaining nearly unnoticed throughout the growing season. Such mild disease symptoms were detectable primarily due to the extended 20-week evaluation period and could easily go unnoticed under typical commercial orchard conditions. The delayed onset of symptoms, sometimes appearing up to 20 weeks after inoculation, suggests that although certain cultivars may initially appear resistant to severe disease, they gradually express susceptibility if environmental conditions continue to support pathogen proliferation (Popović et al., 2021; Jin et al., 2023). The extended evaluation period allowed for observations during late summer in a temperate climate, when decreasing temperatures created more favourable conditions for the multiplication and spread of *Ps* pathogens. The increasing concentration of *Ps* inoculum toward the end of the growing season may enhance its survival in subcortical tissues

during dormancy and increase the risk of disease outbreaks in susceptible cultivars during the spring (Kostick et al., 2019).

This comprehensive three-year study, conducted under netted house conditions that closely simulated field environments, evaluated the vigour and susceptibility of promising Japanese plum and pluot cultivars to major bacterial pathogens in temperate climates (Kostick et al., 2019; Cui et al., 2021). These findings are essential for developing effective orchard management strategies to mitigate bacterial infections in stone fruit production.

CONCLUSIONS

- The plum and pluot cultivars tested showed higher susceptibility to blossom infection compared to terminal shoot infection and were more susceptible to bacterial strains of *Pseudomonas syringae* pv. *morsprunorum* than to *Pseudomonas syringae* pv. *syringae*.
- Notably, the plum cultivars Vanier and Ozark Premier, along with the pluot cultivar Flavorich pluot[®], exhibited very low susceptibility to *Pseudomonas syringae* infection.
- Fruit cultivars resistant to *Pseudomonas syringae* pathogens are crucial for maintaining orchard health in temperate regions, due to the significant financial losses caused by the premature death of infected trees.

REFERENCES

Aleksandrova, D., Nesheva, M. & Dimitrova, N. 2023. Reaction of plum cultivars and rootstocks to bacterial blight (*Pseudomonas* sp.). Scientific Papers. Series B. *Horticulture* **67**(1), 17–22.

Aprile, F., Heredia-Ponce, Z., Cazorla, F.M., de Vicente, A. & Gutiérrez-Barranquero, J.A. 2021. A large Tn 7–like transposon confers hyperresistance to copper in *Pseudomonas syringae* pv. *syringae*. *Applied and Environmental Microbiology* **87**(5), e02528-20. doi: 10.1128/AEM.02528-20

Bophela, K.N., Petersen, Y., Bull, C.T. & Coutinho, T.A. 2020. Identification of *Pseudomonas* isolates associated with bacterial canker of stone fruit trees in the Western Cape, South Africa. *Plant disease* **104**(3), 882–892. https://doi.org/10.1094/PDIS-05-19-1102-RE

Broniarek-Niemiec, A., Børve, J. & Puławska, J. 2023. Control of bacterial canker in stone fruit trees by chemical and biological products. *Agronomy* **13**(4), 1166. doi: 10.3390/agronomy13041166

Burbank, L., Sisterson, M.S., Wei, W., Ortega, B., Luna, N. & Naegele, R. 2023. High growing season temperatures limit winter recovery of grapevines from *Xylella fastidiosa* infection – implications for epidemiology in hot climates. *Plant Disease* **107**(12), 3858–3867. https://doi.org/10.1094/PDIS-03-23-0492-RE

Carvalho, L.C., Gonçalves, E.F., Marques da Silva, J. & Costa, J.M. 2021. Potential phenotyping methodologies to assess inter-and intravarietal variability and to select grapevine genotypes tolerant to abiotic stress. *Frontiers in Plant Science* **12**, 718202. doi: 10.3389/fpls.2021.718202

Chai, A., Yuan, L., Li, X., Li, L., Shi, Y., Xie, X. & Li, B. 2023. Effect of temperature and humidity on dynamics and transmission of *Pseudomonas a*mygdali pv. *lachrymans* aeroso ls. *Frontiers in Plant Science* **14**, 1087496. https://doi.org/10.3389/fpls.2023.1087496

Chan, C., Liao, Y.Y. & Chiou, T.J. 2021. The impact of phosphorus on plant immunity. *Plant and Cell Physiology* **62**(4), 582–589. https://doi.org/10.1093/pcp/pcaa168

Chen, H., Chen, J., Zhao, Y., Liu, F. & Fu, Z.Q. 2022. *Pseudomonas syringae* pathovars. *Trends in Microbiology* **30**(9), 912–913. https://doi.org/10.1016/j.tim.2022.03.002

Cui, Z., Huntley, R.B., Zeng, Q. & Steven, B. 2021. Temporal and spatial dynamics in the apple flower microbiome in the presence of the phytopathogen *Erwinia amylovora*. *ISME Journal* **15**(1), 318–329. https://doi.org/10.1038/s41396-020-00784-y

Dalbó, M.A., Bruna, E.D., Nodari, R.O. & Saifert, L. 2016. Plum selections with total resistance to leaf scald (*Xylella fastidiosa*). *Acta Horticulture* **1127**, 61–64. doi: 10.17660/ActaHortic.2016.1127.11

Fadón, E., Herrera, S., Guerrero, B.I., Guerra, M.E. & Rodrigo, J. 2020. Chilling and Heat Requirements of Temperate Stone Fruit Trees (*Prunus* sp.). *Agronomy* **10**(3), 409. https://doi.org/10.3390/agronomy10030409

He, L.L., Wang, X., Rothenberg, D.O.N., Xu, X., Wang, H.H., Deng, X. & Cui, Z.N. 2023. A novel strategy to control *Pseudomonas syringae* through inhibition of type III secretion system. *Pesticide Biochemistry and Physiology* **194**, 105471. doi: 10.1016/j.pestbp.2023.105471

Huang, J., Yao, C., Sun, Y., Ji, Q. & Deng, X. 2022. Virulence-related regulatory network of *Pseudomonas syringae*. *Computational and Structural Biotechnology Journal* **20**, 6259–6270. https://doi.org/10.1016/j.csbj.2022.11.011

Hulin, M.T., Mansfield, J.W., Brain, P., Xu, X., Jackson, R.W. & Harrison, R.J. 2018. Characterization of the pathogenicity of strains of *Pseudomonas syringae* towards cherry and plum. *Plant Pathology* **67**(5), 1177–1193. https://doi.org/10.1111/ppa.12834

Hunjan, M.S. & Lore, J.S. 2020. Climate Change: Impact on Plant Pathogens, Diseases, and Their Management. pp. 85–100. In: *Crop Protection Under Changing Climate*. Jabran, K.S., Florentine, B.S. Chauhan eds. Springer, Cham, Switzerland, 204 pp. https://doi.org/10.1007/978-3-030-46111-9_4

Husseini, A. & Akköprü, A. 2020. The possible mechanisms of copper resistance in the pathogen *Pseudomonas syringae* pathovars in stone fruit trees. *Phytoparasitica* **48**(5), 705–718. https://doi.org/10.1007/s12600-020-00828-1

Islam, T., Haque, M.A., Barai, H.R., Istiaq, A. & Kim, J.J. 2024. Antibiotic Resistance in Plant Pathogenic Bacteria: Recent Data and Environmental Impact of Unchecked Use and the Potential of Biocontrol Agents as an Eco-Friendly Alternative. *Plants* **13**(8), 1135. https://doi.org/10.3390/plants13081135

Jin, Y., Zhang, W., Cong, S., Zhuang, Q.G., Gu, Y.L., Ma, Y.N. & Wei, H.L. 2023. *Pseudomonas syringae* Type III Secretion Protein HrpP Manipulates Plant Immunity to Promote Infection. *Microbiology Spectrum* **11**(3), e05148-22. doi: 10.1128/spectrum.05148-22

Konavko, D., Moročko-Bičevska, I. & Bankina, B. 2014. *Pseudomonas syringae* as important pathogen of fruit trees with emphasis on plum and cherry. *Research for Rural Development* **1**, 19–25.

Kostick, S.A., Norelli, J.L. & Evans, K.M. 2019. Novel metrics to classify fire blight resistance of 94 apple cultivars. *Plant Pathology* **68**(5), 985–996. https://doi.org/10.1111/ppa.13012

Lee, Y.S., Koh, H.S., Sohn, S.H., Koh, Y.J. & Jung, J.S. 2012. Genetic diversity among *Pseudomonas syringae* pv. *morsprunorum* isolates from *Prunus mume* in Korea and Japan by comparative sequence analysis of 16S rRNA gene. *The Plant Pathology Journal* **28**(3), 295–298. https://doi.org/10.5423/PPJ.NT.02.2012.0032

Le Lezec, M., Lecomte, P., Laurens, F. & Michelesi, J.C. 1997. Sensibilite varietale au feu bacterien. *L'arboriculture Fruitiere* **503**, 57–62.

Lienqueo, I., Villar, L., Beltrán, F., Correa, F., Sagredo, B., Guajardo, V. & Almada, R. 2024. Molecular, phenotypic and histological analysis reveals a multi-tiered immune response and callose deposition in stone fruit rootstocks (*Prunus* spp.) against *Pseudomonas syringae* pv. *syringae* (*Pss*) infection. *Scientia Horticulturae* **324**, 112588. https://doi.org/10.1016/j.scienta.2023.112588

Meier, U., Graf, H., Hack, H., Hess, M., Kennel, W., Klose, R., ... & Boom, T.V.D. 1994. Phenological growth stages of pome fruits (*Malus domestica* Borkh. and *Pyrus communis* L.), stone fruits (*Prunus* species), currants (*Ribes* species) and strawberry (*Fragaria× ananassa* Duch.). *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **46**(7), 141–153.

Milošević, T. & Milošević, N. 2018. Fruits 3: Plum (*Prunus* spp.) breeding. pp. 165–215. In: *Advances in Plant Breeding Strategies.* (Al-Khayri, J.M., Jain, S.M., Johnson, D.V. eds.) Springer, Cham, Switzerland, 990 pp. https://doi.org/10.1007/978-3-319-91944-7

Mustafa, M.H., Bassi, D., Corre, M.N., Lino, L.O., Signoret, V., Quilot-Turion, B. & Cirilli, M. 2021. Phenotyping brown rot susceptibility in stone fruit. A literature review with emphasis on peach. *Horticulturae* **7**(5), 115. https://doi.org/10.3390/horticulturae7050115

Němcová, V. & Buchtová, I. 2023. *Situational and outlook report – Fruit 2023*. Ministry of Agriculture of the Czech Republic, pp. 99.

Okie, W.R. & Hancock, J.F. 2008. Plums. In: *Temperate Fruit Crop Breeding.* (Hancock, J.F., ed.). New York, NY:Springer, pp. 337–358.

O'Malley, M.R. & Anderson, J.C. 2021. Regulation of the *Pseudomonas syringae* Type III Secretion System by Host Environment Signals. *Microorganisms* **9**(6), 1227. doi: 10.3390/microorganisms9061227

Popović, T., Menković, J., Prokić, A., Zlatković, N. & Obradović, A. 2021. Isolation and characterization of *Pseudomonas syringae* isolates affecting stone fruits and almond in Montenegro. *Journal of Plant Diseases and Protection* **128**(2), 391–405. https://doi.org/10.1007/s41348-020-00417-8

Rampáčková, E., Göttingerová, M., Kiss, T., Ondrášek, I., Venuta, R., Wolf, J. &. Ercisli, S. 2021. CIELAB analysis and quantitative correlation of total anthocyanin content in European and Asian plums. *European Journal of Horticultural Science* **86**(5), 453–460. https://doi.org/10.17660/eJHS.2021/86.5.1

Roussos, P.A. 2024. Climate Change Challenges in Temperate and Sub-Tropical Fruit Tree Cultivation. *Encyclopedia* **4**(1), 558–582. https://doi.org/10.3390/encyclopedia4010036

Ruinelli, M., Blom, J., Smits, T.H.M. & Pothier, J.F. 2019. Comparative genomics and pathogenicity potential of members of the *Pseudomonas syringae* species complex on Prunus spp. *BMC genomics* **20**(1), 172. https://doi.org/10.1186/s12864-019-5555-y

Santos, M.G., Nunes, da Silva, M., Vasconcelos, M.W. & Carvalho, S.M. 2024. Scientific and technological advances in the development of sustainable disease management tools: a case study on kiwifruit bacterial canker. *Frontiers in Plant Science* **14**, 1306420. https://doi.org/10.3389/fpls.2023.1306420

Seethapathy, P., Gothandaraman, R., Gurudevan, T. & Malik, I.A. 2022. Diseases, Pests, and Disorders, pp. 133–176. In: *Handbook of Plum Fruit.* eds. Gull, A., Nayik, G.A., Wani, S.M., Nanda, V. CRC Press, Boca Raton, Florida, USA 364 pp. doi: 10.1201/9781003205449

Sundin, G. 2007. *Pseudomonas syringae* Diseases of Fruit Trees: Progress Toward Understanding and Control. *Plant Disease* **91**(1), 4–17. doi: 10.1094/PD-91-0004

Tomić, J., Glišić, I., Milošević, N., Štampar, F., Mikulič-Petkovšek, M. & Jakopič, J. 2022. Determination of fruit chemical contents of two plum cultivars grafted on four rootstocks. *Journal of Food Composition and Analysis* **105**, 103944. doi: 10.1016/j.jfca.2021.103944

Tripathi, R., Tewari, R., Singh, K.P., Keswani, C., Minkina, T., Srivastava, A.K. & Sansinenea, E. 2022. Plant mineral nutrition and disease resistance: A significant linkage for sustainable crop protection. *Frontiers in Plant Science* **13**, 883970. doi: 10.3389/fpls.2022.883970

Xin, X.F., Kvitko, B. & He, S.Y. 2018. *Pseudomonas syringae*: what it takes to be a pathogen. Nature reviews. Microbiology 16(5), 316–328. doi: 10.1038/nrmicro.2018.17

Yang, P., Zhao, L., Gao, Y.G. & Xia, Y. 2023. Detection, diagnosis, and preventive management of the bacterial plant pathogen *Pseudomonas syringae*. *Plants* **12**(9), 1765. doi: 10.3390/plants12091765

The starting material is the basis of heterotic wheat breeding

A. Rakov[1,*], Y. Dmytrenko[1], R. Spriazhka[1] and Y. Zaika[1,2]

[1]National University of Life and Environmental Sciences of Ukraine, 15 Heroyiv Oborony Str., UA03041 Kyiv, Ukraine
[2]The V.M. Remeslo Myronivka Institute of Wheat of National Academy of Agrarian Sciences of Ukraine, 68 Tsentralna Str., village Central, Myronivskyi district, UA08853 Kyiv region, Ukraine
*Correspondence: a.rakov@nubip.edu.ua

Abstract. This study addresses key aspects of developing initial breeding material for heterosis breeding in bread winter wheat (*Triticum aestivum L.*). In the context of climate change and threats to food security, particularly in Ukraine, the creation of new high-yielding hybrids resistant to biotic and abiotic stresses is of paramount importance. The research involved a comprehensive assessment of 78 winter wheat varieties from the Ukrainian National Gene Bank. Field trials were conducted during 2022–2024 at the Agronomic Research Station (Forest-Steppe zone of Ukraine) using a randomized complete block design with three replications.

The study focused on morphological and physiological traits influencing cross-pollination efficiency, including anther extrusion (VAEX-method scored on a 1–9 scale), plant height (cm), flowering synchrony (days from January 1 to heading), spike characteristics, grain yield (t ha^{-1}), and resistance to pathogens (septoria, powdery mildew; % leaf area affected) and winter hardiness (1–9 score).

Cluster analysis revealed three distinct groups with specific breeding potential. Group 1 (Achim, Mescal, Yuvileyna Patona) demonstrated the highest spike productivity (61.2–63.6 grains/spike; grain mass 2.8–3.4 g) and yield (6.1–6.2 t ha^{-1}). Female components (Group 2: Altigo, Soborna, Taira) exhibited short plant height (71.4–78.2 cm) and early heading time (140.3–144.0 days). Male components (Group 3: Zoreslava, Metelytsya Kharkivska, Urbanus, Liryka Bilotserkivska, and Kyivska 17, etc.) were characterized by tall plant height (80.3–89.8 cm) and high anther extrusion (6.3–7.5 score).

A topcross mating design is proposed for further evaluation of combining ability and heterosis effects. The results provide a foundation for the development of hybrid wheat breeding programs under Ukrainian conditions, with an emphasis on parental lines with complementary flowering biology and agronomic performance.

Key words: bread wheat, hybrid breeding, heterosis, parental selection, anther extrusion, *Triticum aestivum* L.

INTRODUCTION

Wheat (*Triticum aestivum L.)* is the most important cereal crop for global food security, supplying carbohydrates to nearly 40% of the world's population. In the context

of climate change and increasing biotic and abiotic stresses, the development of high-yielding and resilient hybrids has become a strategic goal of modern breeding programs (Zemoida et al., 2019; Kovalyshyna et al., 2020a; Polishchuk et al., 2024).

Therefore, obtaining consistently high wheat grain yields by selectively increasing the yield potential of varieties and hybrids is extremely relevant for agricultural production in Ukraine. To create high-yielding hybrids of bread winter wheat, it is necessary to have homozygous lines that meet the basic requirements of breeders - increased individual productivity, high donor properties, genetic protection from bio- and abiotic factors, increased biochemical indicators and baking properties, adaptability to soil and climatic conditions, and different ecological, geographical and genetic origins as breeding starting material (Domaratsky et al., 2018; Kyrylenko et al., 2021).

Hybrids have several significant advantages over varieties due to heterosis which is an effect that occurs as a result of crossing two genetically promising parental forms (Longin et al., 2014; Gupta et al., 2019). The main manifestation of heterosis is increased yield and stability of productivity, especially under adverse growing conditions (Whitford et al., 2013; Longin et al., 2014; Ferreira et al., 2024).

In addition to increased yield, hybrids demonstrate significantly better agronomic characteristics compared to traditional varieties. They have improved grain quality Gupta et al., 2019, drought tolerance, more efficient use of nutrients, and are characterized by a more developed root system. In addition, hybrids exhibit higher resistance to biotic and abiotic stress factors, which makes them more adaptable to climate change and adverse environmental conditions (Singh et al., 2010; Prysiazhniuk et al., 2023).

To successfully create wheat hybrids, both male and female parent plants must have morphological and biological characteristics that facilitate effective cross-pollination. However, the requirements for male and female flower characteristics differ significantly (Betul et al., 2022).

Flowering in spikelets occurs gradually, and the entire flowering period lasts from one to three days, depending on the genotype and environmental conditions (Whitford et al., 2013). One of the key factors is the presence of open flowers in the spike, which provides maximum exposure of the reproductive organs. Ideal characteristics for this are large lodicules and floral scale, as well as widely spaced flowers on long spikes (Murai et al., 2002). One of the important morphological features is the long, well-developed stigmas, which completely emerge from the flower and remain receptive to pollen for a long time. This structure allows the flowers to open fully, creating optimal conditions for pollen to reach the stigmas. In addition, according to literature data the extended period of receptiveness of the stigmas of more than 5 days contributes to increased pollination efficiency even under conditions of low pollen concentrations in the air (Longin et al., 2012; Whitford et al., 2013).

Plant height is important from a heterosis perspective, as it was the first trait on which dominance was observed and described in first-generation hybrids in wheat as early as 1919 (Singh et al., 2010). To successfully maintain cmS-sterile female parents, it is important to identify semi-dwarf lines that combine optimal height and high pollination ability (Garst et al., 2023).

Important characteristics of male plants that facilitate cross-pollination and efficient seed production include the ability to actively disperse pollen using long, well-extruded anthers that contain large amounts of viable pollen with high fertility

duration (Whitford et al., 2013; Longin et al., 2014; Langer et al., 2014; Hanafi et al., 2022). Studies have shown that this trait has a medium-high heritability (0.62–0.87), indicating the possibility of its improvement through selection (Langer et al., 2014; Boeven et al., 2016; Sade et al., 2022). High pollination productivity increases the likelihood of successful fertilization of female plants even under difficult weather conditions. Another key factor in hybrid wheat breeding is the synchronization of male and female flowering to maximize pollination (Whitford et al., 2013; Longin et al., 2014; Langer et al., 2014; Hanafi et al., 2022). The ideal time for female plants to flower is 1–2 days earlier than male plants, coinciding with the peak of pollen release (Garst et al., 2023. The anthers remain receptive to pollen for 4–13 days, and a prolonged pollination period promotes more efficient seed formation. One way to prolong pollination in male plants is to stimulate tillering by adjusting the crop density (Schmidt et al., 2024).

Therefore, a detailed understanding of wheat flowering mechanisms is critical for successful hybrid seed production. This knowledge allows for effective breeding strategies development, aimed at improving the hybrid's yield and stability.

Despite the potential of heterosis, commercial success of hybrid wheat in Ukraine and Europe remains limited due to insufficient adaptation of available hybrids to local environments. The efficiency of hybrid breeding strongly depends on the selection of parental components with complementary flowering biology, pollination traits, and agronomic performance. (Basnet et al., 2022).

MATERIALS AND METHODS

Germplasm collection. The research material consisted of 78 varieties of bread winter wheat obtained from the National Center for Plant Genetic Resources of Ukraine, The Institute of Plant Physiology and Genetics of the NAAS of Ukraine, the V.M. Remeslo Myronivka Institute of Wheat of the NAAS of Ukraine, the National Scientific Center 'Institute of Agriculture of the National Academy of Sciences of Ukraine', the Institute of Irrigated Farming NAAS, the Yuriev Plant Production Institute NAAS of Ukraine, the Plant Breeding and Genetics Institute - National Center of Seed and Cultivar Investigation, the Bilotserkivska Experimental Breeding Station of the Institute of Bioenergy Crops and Sugar Beet of the NAAS of Ukraine, and other breeding centers.

Experimental design. The purpose of this study was to comprehensively evaluate 78 varieties of bread winter wheat and to select promising parental forms for hybrid breeding programs in Ukraine. The evaluation of the breeding material was carried out during 2022–2024 in the experimental fields of the Department of Genetics, Breeding and Seed Production named after Professor M.O. Zelensky in the Separate Unit of the National University of Life and Environmental Sciences of Ukraine 'Agronomic Research Station'. The agricultural technology of the experiments corresponded to the generally accepted technology of growing bread winter wheat in the Forest-Steppe of Ukraine and was aimed at optimizing the growth and development of plants. The experiment in the collection nursery was established on randomized single-row plots using a manual seeder, with three replications. Each plot was 1.2 m in length with a row spacing of 20 cm, and the sowing density was 1.5 million seeds per hectare. All other agronomic practices were applied according to the standard regional recommendations.

Morphological data recording. Determination of plant height, winter hardiness, productive tillering coefficient, and weight of a thousand seeds was carried out by the Methodology for conducting the examination of plant varieties of the cereal and legume groups for suitability for distribution in Ukraine, approved by the Ukrainian Institute for the Examination of Plant Varieties (UIEPV) (Korzun, 2016). Earing calculated as the difference between the date of full earing of the plot and January 1 of the calendar year.

In the study, the International Classifier of the genus *Triticum L.* (1987) was used to determine the group of varieties by plant height, according to which wheat varieties are divided into dwarfs of the first group (< 36 cm), dwarfs of the second group (36–50 cm), short-growing of the first group (51–65 cm), short-growing of the second group (66–80 cm), medium-growing of the first group (81–95 cm), medium-growing of the second group (96–110 cm), tall-growing of the first group (111–125 cm), tall-growing of the second group (126–140 cm), and extremely tall-growing (> 140 cm) (Dorofeev et al., 1987; Samoilyk et al., 2024).

The anther extrusion was assessed according to the score of visual anther extrusion - VAEX method (Langer et al., 2014). Three days after the Zadoks 61, the number of anthers that protruded beyond the flowers was measured in five randomly selected plants from each plot or row, with the subsequent calculation of the proportion of such spikelets. The upper and lower two spikelets were not taken into account (Langer et al., 2014; Betul et al., 2022). The obtained results are presented in the form of a 9–point scale, according to which at 9 points, almost all anthers are extruded from the flowers of the spikelet (Whitford et al., 2013).

After the plants reached full maturity, 20 typical plants were selected from each recording plot for biometric analysis, which included determining the spike length, the number of spikes, the number of grains in the spike, and the mass of grains per spike.

Disease scoring. The assessment of the intensity of the lesion was carried out visually, analyzing 10 plants, and the degree of damage to the flag and subflagellar leaves was assessed using scales (Trybel et al., 2010) and the integral resistance scale (Petrenkova et al., 2018; Kovalyshyna et al., 2020b), according to which the lesion is expressed in relative percentages of the leaf area covered with pathogen pustules.

Statistical analysis. Statistical calculation of the results of field and laboratory studies was performed using the methods of variational statistics and analysis of variance (Dospekhov, 1985) using the Microsoft Excel 2016 application package. Cluster analysis and dendrogram creation were performed using the Statistica 13 program.

RESULTS AND DISCUSSION

The main obstacle to the transition from varieties to hybrid breeding of bread winter wheat is the method of pollination. *Triticum aestivum L.* is a self-pollinating species, but it can cross-pollinate at a low frequency. While the frequency of cross-pollination is usually from a few tenths of a percent to several percent (Waines & Hegde, 2003), in some years in certain genotypes it can increase to 10% and above (Kozub et al., 2017). One of the main features of a genotype that promotes cross-pollination is its ability to extrude anthers to ensure sufficient levels of cross-pollination in this self-pollinating crop (Sade et al., 2022). Anther extrusion is an important trait in hybrid wheat breeding programs that can be quickly determined in the field. If a genotype exhibits a high

percentage of anther extrusion, it is an excellent male parent for hybrid seed production. The selection of male parents with high pollination ability made it possible to create the first economically viable commercial wheat hybrids (Gupta et al., 2019).

Anther extrusion and heading synchrony. A study of plants of 78 varieties of bread winter wheat (Fig. 1) based on the anther extrusion from the flowers of the spike revealed 1 variety in which the anthers almost do not emerge from the flower scales (score 3) – the variety Perlyna Lisostepu. Most genotypes (26 varieties) threw out anthers at the level of 7 points, the maximum score was set in 4 varieties - Pam`yati Girka, Spivanka Poliska, Efektna, and Emil. Thus, according to the research results, it was established that in modern bread winter wheat varieties, included in the State Register of Varieties Suitable for Distribution in Ukraine, the anthers emerge from the flower scales at the level of 3–9 points, which is promising for heterosis breeding.
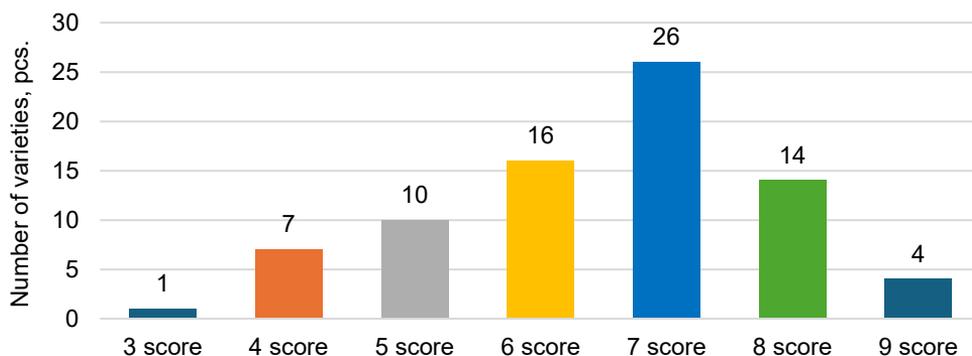
Figure 1. Distribution of anther extrusion among winter wheat varieties.

Based on the results of anther extrusion and morpho-biological traits, 11 varieties were identified as promising parental components for heterosis bread winter wheat breeding (Fig. 2).
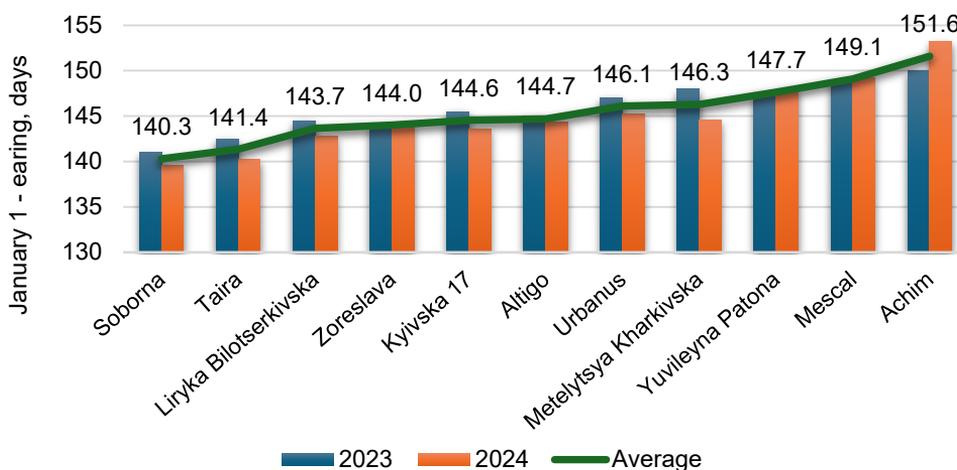
Figure 2. Days from January 1 to heading for different genotypes.

Heading time (days from January 1 to heading) ranged from 140.3 days (Soborna) to 151.6 days (Achim). Early-heading genotypes (Soborna, Taira, Liryka Bilotserkivska) contrasted with late- heading ones (Achim, Mescal, Yuvileyna Patona), providing an opportunity to design parental combinations with optimal synchrony between pollen release and stigma receptivity.

Significant variability was observed in the extent of anther extrusion among the evaluated genotypes. The highest average scores (7.4–7.8 score) were recorded in Altigo, Soborna, Taira, Metelytsya Kharkivska, Urbanus, and Kyivska 17 (Fig. 3), confirming their potential as pollen donors. In contrast, Achim and Mescal demonstrated relatively low extrusion (≤ 5.8 score) (Table 1), indicating limited cross-pollination capacity (Betul et al., 2022).

Plant height. Plant height ranged from 71.4 cm (Altigo) to 89.8 cm (Liryka Bilotserkivska) (Table 2). According to the international wheat classifier, short-statured genotypes (Altigo, Soborna, Taira) represent suitable candidates for maternal forms, while taller genotypes (Kyivska 17, Liryka Bilotserkivska, Metelytsya Kharkivska) meet the requirements for effective pollen dispersal as male forms.

Yield components. Elements of the yield structure are morphological characteristics of the plant that directly shape the yield level. The most important of them include the length of the spike, the number of spikelets in the spike, the number of grains per spike, the weight of grains per spike, and the weight of 1,000 seeds. Each of these indicators plays a specific role in shaping the harvest.

Figure 3. Anther extrusion in representative genotypes (2023–2024).

Table 1. Anther extrusion of winter wheat varieties

Varieties	Anther extrusion, score		
	2023	2024	Average
Altigo	8.0	6.8	7.4
Zoreslava	6.0	6.6	6.3
Metelytsya Kharkivska	6.0	7.8	6.9
Soborna	8.0	7.6	7.8
Taira	8.0	7.2	7.6
Achim	5.5	6.0	5.8
Mescal	5.0	5.0	5.0
Urbanus	7.0	6.6	6.8
Kyivska 17	7.5	7.4	7.5
Liryka Bilotserkivska	5.5	7.0	6.3
Yuvileyna Patona	6.0	6.5	6.3

Spike length is one of the first visual indicators that can be used to assess the potential productivity of the spike part of the plant. Typically, the longer the spike, the more spikelets and, consequently, grains it can contain. However, it is important to understand that length does not always directly correlate with productivity, as grain filling also matters (Gupta et al., 2019; Basnet et al., 2022).

Table 2. Plant height of winter wheat varieties

Varieties	2023		2024		x,	min,	max,	R,
	x ± S x, cm	V, %	x ± S x, cm	V, %	cm	cm	cm	cm
Altigo	71.0 ± 6.2	8.7	71.8 ± 5.7	7.9	71.4	65.0	78.6	13.6
Zoreslava	81.0 ± 3.0	3.7	83.0 ± 4.1	4.9	82.0	77.1	88.9	11.8
Metelytsya Kharkivska	82.0 ± 6.8	8.3	89.0 ± 4.6	5.2	85.5	76.1	94.4	18.3
Soborna	70.0 ± 5.3	7.6	85.6 ± 4.4	5.1	77.8	64.3	91.5	27.2
Taira	71.0 ± 4.8	6.8	85.4 ± 3.0	3.5	78.2	66.7	91.9	25.2
Achim	78.0 ± 3.4	4.4	76.6 ± 3.0	3.9	77.3	71.7	84.3	12.6
Mescal	74.0 ± 4.8	6.5	82.0 ± 5.9	7.2	78.0	67.4	87.6	20.2
Urbanus	73.0 ± 5.1	7.0	87.6 ± 6.3	7.2	80.3	69.1	90.9	21.8
Kyivska 17	82.0 ± 4.7	5.7	93.2 ± 5.0	5.4	87.6	75.3	96.7	21.4
Liryka Bilotserkivska	85.0 ± 6.7	7.9	94.6 ± 4.4	4.7	89.8	78.9	99.9	21.0
Yuvileyna Patona	87.0 ± 4.9	5.6	89.4 ± 6.8	7.6	88.2	83.9	94.9	11.0
x	77.6	6.6	85.3	5.7	81.5	72.3	90.9	18.6
LSD_{05}	4.1		4.0					

The spikelets number in a spike is an important characteristic, as each spikelet can contain several flowers capable of developing grains. The greater the number of spikelets, the higher the potential for grain formation. This indicator depends on both the genetic characteristics of the variety and the growing conditions.

The number of grains per spike indicates the overall level of spike productivity. This indicator depends on the number of spikelets and the number of grains in each of them. Accordingly, the more grains are formed, the higher the potential yield from the plant. At the same time, it is important to consider not only the number, but also the quality and weight of these grains (Kyrylenko et al., 2021; Garst et al., 2023).

The grain mass per spike is an indicator that combines the number and size of grains. It is one of the key parameters that allows you to assess the efficiency of grain filling processes. If the mass is high, this indicates good growing conditions, moisture, and nutrition, as well as a high ability of the variety to form full-fledged grain (Demydov et al., 2024).

The weight of a thousand seeds is used as a standard for assessing grain size and quality. It depends on the genetic characteristics of the variety, the intensity of grain filling, growing conditions, and fertilizers. A higher the weight of a thousand seeds is often a sign of good filling, a high content of reserve substances (Kyrylenko et al., 2021; Basnet et al., 2022; Avagyan & Martirosyan, 2024).

All of the listed elements not only form yield, but also reflect the interaction of the variety with the environment. Therefore, when assessing the yield potential, it is important to comprehensively consider all these indicators (Schmidt et al., 2024).

According to the analysis of spike length (Fig. 4), the varieties Achim, Mescal, Yuvileyna Patona, and Metelytsya Kharkivska exhibited the longest spikes, measuring 10.3 cm, 10.8 cm, 9.8 cm, and 9.7 cm, respectively. On the other hand, the varieties with the shortest spike (8.1 cm) were Altigo, Soborna, and Taira. Other varieties had spike lengths from 8.8 cm in the Liryka Bilotserkivska variety to 9.3 cm in the Urbanus variety.
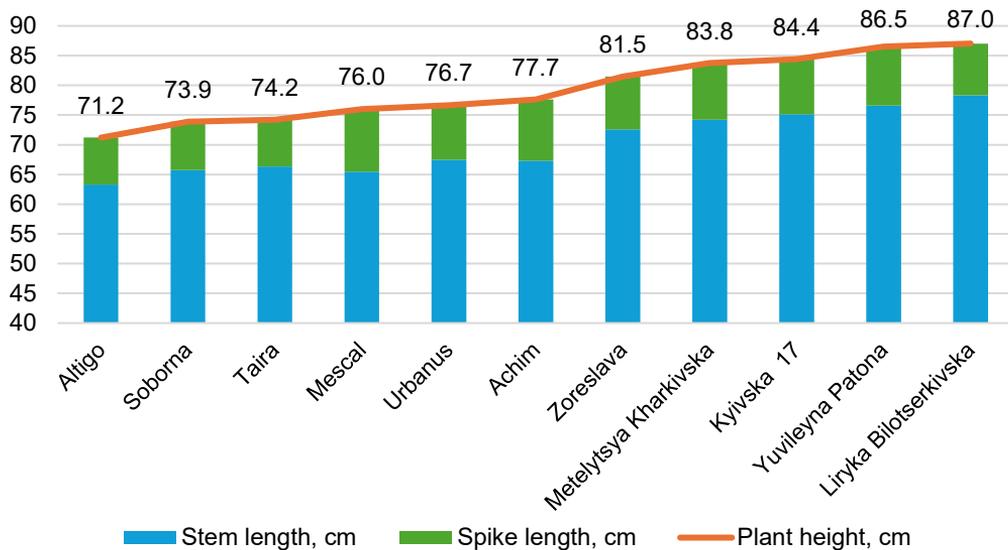
Figure 4. Stem and spike length of selected winter wheat varieties.

The highest spike density was observed in the Yuvileyna Patona variety - 20.6 (Table 3), which significantly exceeds other varieties. Close to it in value are Metelytsya Kharkivska and Mescal, which had 18.8 and 19.2, respectively. The Achim, Altigo, Zoreslava, and Urbanus varieties demonstrated average values within 15.3–18.0, which indicates an optimal combination of spike compactness and potential grain number.

Table 3. Spike density and number of spikelets per spike of tested varieties

Varieties	Spike density			Spikelet number, pcs		
	2023	2024	x	2023	2024	x
	x ± S x	x ± S x		x ± S x	x ± S x	
Altigo	14.5 ± 1.3	16.1 ± 1.7	15.3	15.9 ± 1.2	17.3 ± 1.6	16.6
Zoreslava	15.9 ± 1.6	15.5 ± 1.6	15.7	17.1 ± 1.5	16.6 ± 1.5	16.9
Metelytsya Kharkivska	17.9 ± 1.7	19.6 ± 1.2	18.8	19.0 ± 1.6	20.6 ± 1.1	19.8
Soborna	14.8 ± 1.6	14.9 ± 1.6	14.9	16.1 ± 1.4	16.2 ± 1.5	16.2
Taira	14.2 ± 1.4	14.6 ± 1.6	14.4	15.5 ± 1.3	15.9 ± 1.4	15.7
Achim	18.5 ± 1.7	17.4 ± 1.9	18.0	19.5 ± 1.6	18.4 ± 1.8	19.0
Mescal	18.1 ± 1.7	20.2 ± 2.1	19.2	19.1 ± 1.6	21.1 ± 2.1	20.1
Urbanus	16.2 ± 1.8	14.4 ± 2.0	15.3	17.3 ± 1.7	15.4 ± 1.8	16.4
Kyivska 17	14.5 ± 1.4	12.8 ± 1.5	13.7	15.6 ± 1.3	13.9 ± 1.4	14.8
Liryka Bilotserkivska	13.9 ± 1.5	14.7 ± 1.6	14.3	15.1 ± 1.4	15.8 ± 1.5	15.5
Yuvileyna Patona	20.2 ± 2.3	21.0 ± 1.3	20.6	21.2 ± 2.2	22.1 ± 1.3	21.7
X	16.2	16.5	16.4	17.4	17.6	17.5
LSD_{05}	1.5	1.5		1.4	1.4	

The highest average number of spikelets in the spike was observed in the varieties Metelytsya Kharkivska (19.8 pcs), Mescal (20.1 pcs), and Yuvileyna Patona (21.6 pcs). The lowest number was in Kyivska 17 (14.8 pcs), Liryka Bilotserkivska (15.5 pcs), and Taira (15.7 pcs). In other varieties, the indicator ranged from 16.2 pcs in Soborna to 18.9 pcs in the Achim variety.

Clear differences were recorded for spike productivity. The highest number of grains per spike was observed in Mescal (62.0 pcs), Achim (61.2 pcs), and Yuvileyna Patona (63.6 pcs) (Table 4). Grain mass per spike was also highest in these varieties (2.8–3.4 g). By contrast, Altigo and Soborna produced fewer grains (40–42 pcs) with lower grain mass (< 2.2 g).

Table 4. Grain number per spike and spike weight of winter wheat varieties

Varieties	Number of grains per spike, pcs.			Weight of grains per spike, g		
	2023	2024	x	2023	2024	x
	x ± S x	x ± S x		x ± S x	x ± S x	
Altigo	38.3 ± 5.9	45.4 ± 10.2	41.9	2.2 ± 0.4	2.1 ± 0.6	2.2
Zoreslava	46.6 ± 10.0	45.0 ± 11.0	45.8	2.3 ± 0.6	2.2 ± 0.7	2.3
Metelytsya Kharkivska	51.2 ± 8.4	54.6 ± 7.7	52.9	2.5 ± 0.5	2.6 ± 0.5	2.6
Soborna	43.5 ± 8.8	37.6 ± 7.9	40.6	2.0 ± 0.4	1.3 ± 0.4	1.7
Taira	44.0 ± 8.8	47.9 ± 11.4	46.0	2.2 ± 0.5	2.3 ± 0.6	2.3
Achim	71.3 ± 11.2	51.1 ± 13.0	61.2	3.7 ± 0.7	1.9 ± 0.8	2.8
Mescal	64.3 ± 9.7	59.7 ± 10.7	62.0	3.3 ± 0.7	2.7 ± 0.7	3.0
Urbanus	46.6 ± 8.1	47.6 ± 11.1	47.1	2.7 ± 0.5	2.6 ± 0.6	2.7
Kyivska 17	49.9 ± 7.3	44.7 ± 8.3	47.3	2.9 ± 0.5	2.5 ± 0.6	2.7
Liryka Bilotserkivska	54.1 ± 8.3	52.0 ± 8.3	53.1	2.9 ± 0.4	2.5 ± 0.7	2.7
Yuvileyna Patona	65.9 ± 13.5	61.2 ± 7.0	63.6	3.7 ± 0.8	3.1 ± 0.6	3.4
X	52.3	49.7	51.0	2.8	2.3	2.6
LSD_{05}	8.4	8.9		0.5	0.6	

The thousand-kernel weight ranged from 40.9 g (Soborna) to 57.1 g (Kyivska 17) (Table 5). Genotypes Urbanus, Kyivska 17, and Yuvileyna Patona showed a favorable combination of large kernels and high grain unit (> 820 g L^{-1}), indicating good grain quality.

Table 5. Weight of a thousand seeds and grain unit of selected genotypes

Varieties	Weight of a thousand seeds, g					Grain unit, g L^{-1}		
	2023		2024		x	2023	2024	x
	x ± S x	V, %	x ± S x	V, %				
Altigo	57.4 ± 2.6	4.5	46.0 ± 2.6	5.7	51.7	761.3	734.0	747.6
Zoreslava	49.4 ± 2.4	4.9	49.6 ± 2.8	5.6	49.5	767.0	780.0	773.5
Metelytsya Kharkivska	48.8 ± 2.2	4.5	48.3 ± 2.6	5.4	48.6	746.9	788.0	767.4
Soborna	46.0 ± 2.4	5.2	35.7 ± 2.5	7.0	40.9	779.5	816.0	797.8
Taira	50.0 ± 2.9	5.8	47.6 ± 1.9	4.0	48.8	793.0	843.0	818.0
Achim	51.3 ± 2.8	5.5	36.7 ± 2.2	6.0	44.0	742.1	780.0	761.0
Mescal	52.9 ± 2.4	4.5	32.1 ± 1.6	5.0	42.5	725.8	780.0	752.9
Urbanus	57.9 ± 1.7	2.9	54.3 ± 1.4	2.6	56.1	779.5	819.0	799.3
Kyivska 17	58.1 ± 2.1	3.6	56.1 ± 1.0	1.8	57.1	846.7	809.0	827.9
Liryka Bilotserkivska	53.6 ± 1.0	1.9	48.1 ± 3.0	6.2	50.9	784.3	807.0	795.7
Yuvileyna Patona	56.1 ± 2.7	4.8	50.4 ± 2.0	4.0	53.3	737.3	788.0	762.6
X	52.9	4.4	45.9	4.8	49.4	873.9	897.3	782.2
LSD_{05}	1.7		1.8			7.8	7.9	

s

Disease resistance and winter hardiness. Resistance to septoria and powdery mildew varied significantly (Table 6). Achim, Yuvileyna Patona, and Altigo demonstrated the lowest infection levels (< 13%), whereas Urbanus and Taira showed the highest susceptibility (> 28%). Winter hardiness scores ranged from 7.8 scores (Achim) to 8.9 scores (Taira, Kyivska 17), confirming high adaptation to cold stress across the collection.

Table 6. Disease resistance and winter hardiness of winter wheat varieties

Varieties	Septoria leaf spot, %			Powdery mildew, %			Winter hardiness, score		
	2023	2024	x	2023	2024	x	2023	2024	x
Altigo	5.0	24.0	14.5	5.0	16.8	10.9	9.0	8.4	8.7
Zoreslava	20.0	20.4	20.2	15.0	18.0	16.5	9.0	8.6	8.8
Metelytsya Kharkivska	20.0	21.6	20.8	10.0	30.0	20.0	8.5	8.6	8.6
Soborna	10.0	21.6	15.8	20.0	24.0	22.0	9.0	8.6	8.8
Taira	30.0	27.6	28.8	10.0	19.2	14.6	9.0	8.8	8.9
Achim	3.0	15.6	9.3	3.0	13.5	8.3	7.0	8.6	7.8
Mescal	5.0	20.4	12.7	3.0	18.0	10.5	9.0	8.4	8.7
Urbanus	35.0	33.6	34.3	5.0	24.0	14.5	9.0	8.2	8.6
Kyivska 17	5.0	18.0	11.5	8.5	34.8	21.7	9.0	8.8	8.9
Liryka Bilotserkivska	12.5	24.0	18.3	5.0	16.8	10.9	9.0	8.4	8.7
Yuvileyna Patona	3.0	21.6	12.3	3.0	15.0	9.0	8.0	8.0	8.0

Productive tillering and grain yield. The productive tillering coefficient varied from 2.1 (Soborna) to 3.3 (Metelytsya Kharkivska). The highest average yields over two years were recorded for Urbanus (6.7 t ha⁻¹), Achim (6.2 t ha⁻¹), Metelytsya Kharkivska (6.1 t ha⁻¹), and Yuvileyna Patona (6.1 t ha⁻¹). Lower yields were observed in Altigo and Soborna ($\leq$ 5.0 t ha⁻¹).

Table 7. Productive tillering coefficient and grain yield

Varieties	Productivity tillering coefficient			Yield, t ha⁻¹		
	2023	2024	x	2023	2024	x
	x ± S x	x ± S x		x ± S x	x ± S x	
Altigo	1.6 ± 0.4	3.3 ± 0.4	2.5	4.6 ± 0.3	4.6 ± 0.4	4.6
Zoreslava	1.7 ± 0.1	4.4 ± 0.2	3.1	5.2 ± 0.4	6.5 ± 0.2	5.9
Metelytsya Kharkivska	2.2 ± 0.1	4.4 ± 0.4	3.3	5.2 ± 0.4	6.9 ± 0.3	6.1
Soborna	2.1 ± 0.1	2.1 ± 0.4	2.1	3.6 ± 0.2	6.3 ± 0.2	5.0
Taira	1.7 ± 0.4	3.7 ± 0.4	2.7	3.8 ± 0.4	6.1 ± 0.4	5.0
Achim	1.8 ± 0.3	2.8 ± 0.1	2.3	5.0 ± 0.2	7.3 ± 0.4	6.2
Mescal	1.2 ± 0.2	3.4 ± 0.1	2.3	4.2 ± 0.4	6.8 ± 0.4	5.5
Urbanus	2.1 ± 0.2	3.4 ± 0.3	2.8	4.7 ± 0.2	8.6 ± 0.4	6.7
Kyivska 17	1.8 ± 0.4	3.3 ± 0.2	2.6	3.5 ± 0.2	7.9 ± 0.3	5.7
Liryka Bilotserkivska	2.4 ± 0.2	2.7 ± 0.3	2.6	5.0 ± 0.4	7.0 ± 0.2	6.0
Yuvileyna Patona	2.2 ± 0.4	3.4 ± 0.3	2.8	3.7 ± 0.2	8.4 ± 0.4	6.1
X	1.9	3.4	2.6	4.4	6.9	5.7
LSD_{05}	0.2	0.2		0.3	0.2	

Cluster analysis. Cluster analysis grouped the 11 most promising varieties into three distinct clusters (Fig. 5):

Group 1: Achim, Mescal, Yuvileyna Patona – high spike productivity and yield, but moderate anther extrusion.

Group 2: Altigo, Soborna, Taira – early maturity, short plant height, high anther extrusion → recommended as maternal forms.

Group 3: Zoreslava, Metelytsya Kharkivska, Urbanus, Liryka Bilotserkivska, Kyivska 17 – tall plants with high anther extrusion → suitable as male forms.

These results confirm the existence of complementary parental pools for hybrid breeding under Ukrainian growing conditions.
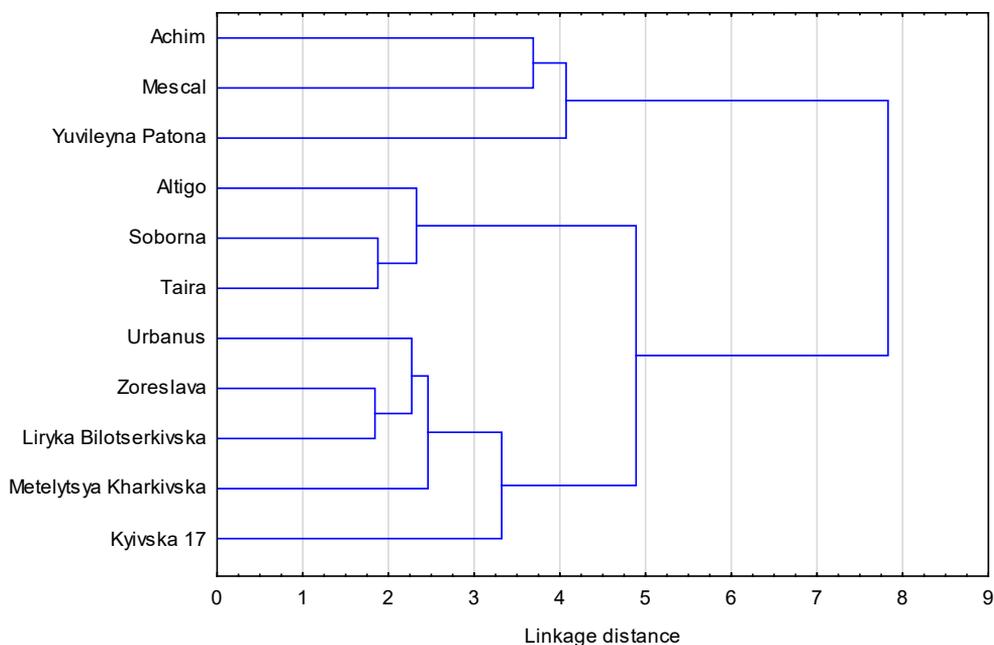
Figure 5. Cluster analysis of wheat varieties based on agronomic traits.

DISCUSSION

The success of hybrid wheat breeding largely depends on the identification of parental components with complementary flowering biology, pollination efficiency, and agronomic performance. In this study, the evaluation of 78 winter wheat varieties revealed substantial genetic diversity for traits directly influencing cross-pollination, yield formation, and stress resistance.

Anther extrusion proved to be one of the most critical traits for selecting male parents. High values recorded in varieties such as Soborna, Taira, Kyivska 17, and Altigo confirm their potential for hybridization schemes. Similar results were reported by Langer et al. (2014) and Sade et al. (2022), who emphasized that strong anther extrusion

is associated with enhanced pollen dispersal and improved seed set. However, some high-yielding varieties (e.g., Achim, Mescal) displayed lower extrusion levels, which may limit their efficiency as pollen donors but does not exclude them from use as female parents.

Flowering synchrony is another key determinant of hybrid seed production. In our study, early-heading varieties (Soborna, Taira, Liryka Bilotserkivska) contrasted with late-heading ones (Achim, Mescal, Yuvileyna Patona). Such diversity is advantageous for designing hybridization schemes, as it allows the combination of early-flowering females with slightly later male forms, thereby maximizing overlap between pollen release and stigma receptivity (Garst et al., 2023; Schmidt et al., 2024).

Plant height differences further support the division into potential maternal and paternal components. Shorter genotypes (Altigo, Taira, Soborna) are suitable for use as cmS-sterile female parents, as previously highlighted in hybrid wheat programs (Whitford et al., 2013). Conversely, taller varieties (Liryka Bilotserkivska, Kyivska 17, Metelytsya Kharkivska) provide an advantage as pollen donors due to improved pollen dispersal.

Yield-related traits also revealed clear contrasts. High spike productivity and grain weight were typical for Yuvileyna Patona, Mescal, and Achim, confirming their value as productivity donors. Previous studies demonstrated that the combination of high grain number with efficient grain filling is essential for achieving heterotic effects (Gupta et al., 2019; Basnet et al., 2022). At the same time, Altigo and Soborna displayed lower yield components, but their favorable flowering biology justifies their role as maternal forms.

Biotic and abiotic stress resistance is crucial under Ukrainian conditions, where climate instability and pathogen pressure have increased. The identification of Achim, Yuvileyna Patona, and Altigo as genotypes combining resistance to both septoria and powdery mildew provides valuable sources of tolerance for future hybrids. In contrast, Urbanus and Taira demonstrated higher susceptibility, suggesting a need for cautious use in breeding programs.

Finally, cluster analysis allowed the classification of genotypes into three distinct groups with clear functional differentiation into potential male and female components. Such clustering confirms the existence of complementary parental pools, which is consistent with findings from hybrid wheat breeding programs in Europe and Asia (Longin et al., 2014; Hanafi et al., 2022).

Overall, this study highlights the importance of integrating morphological, physiological, and resistance traits when selecting parental components. The identified varieties not only represent valuable breeding resources but also provide a practical basis for launching topcross experiments to evaluate combining ability and heterosis effects under Ukrainian growing conditions.

CONCLUSIONS

Significant genetic diversity was revealed among 78 winter wheat varieties for traits important in hybrid breeding, including anther extrusion, flowering synchrony, plant height, and yield structure.

Varieties Achim, Mescal, and Yuvileyna Patona combined high spike productivity and yield potential, making them suitable as paternal forms.

Varieties Altigo, Soborna, and Taira were characterized by early maturity, short plant height, and high anther extrusion, which are favorable traits for maternal components.

Varieties Zoreslava, Metelytsya Kharkivska, Urbanus, Liryka Bilotserkivska, and Kyivska 17 demonstrated high anther extrusion and tall stature, confirming their potential as male components.

Cluster analysis grouped the collection into three clusters, clearly separating maternal and paternal candidates for hybrid breeding.

The identified varieties form a basis for further evaluation of combining ability and heterosis expression in topcrosses under Ukrainian growing conditions.

REFERENCES

Avagyan, G.V. & Martirosyan, H.S. 2024. Fusarium head blight in winter wheat: development peculiarities and protective strategies. *Agronomy Research* **22**(1), 52–71. doi: 10.15159/AR.24.031

Basnet, R.B., Dreisigacker, S., Arun, K.J., Mottaleb, K.A., Vishwakarma, M.K., Bhati, P., ... & Rosyara, U. 2022. Status and Prospects of Hybrid Wheat: A Brief Update. In R.B. Basnet, S. Dreisigacker, K.J. Arun, K.A. Mottaleb, M.K. Vishwakarma, P. Bhati, ... U. Rosyara, New Horizons in Wheat and Barley Research. 637–679. Singapore: *Springer*. doi: 10.1007/978-981-16-4449-8_24

Betul, S., Amir, I.M., Nithya, S., Rudd, J.C. & Liu, S. February 2022. Assessment of floral characteristics for hybrid wheat (Triticum aestivum L.) production in Texas. *Agrosystems, Geosciences & Environment* **5**(1). doi: 10.1002/agg2.20228

Boeven, P.G., Longin, C.H., Leiser, W.L., Kollers, S., Ebmeyer, E. & Würschum, T. 2016. Genetic Architecture of Male Floral Traits Required for Hybrid Wheat Breeding. *Theor. Appl. Genet* **129**, 2343–2357.

Demydov, O.A., Kyrylenko, V.V., Murashko, L.A., Humenyuk, O.V., Suddenko, Y.M., Mukha, T.I., ... & Mazurenko, B.O. 2024. Breeding and genetic screening of F1 hybrids of soft winter wheat (Triticum aestivum L.) by manifestation of resistance to Fusarium graminearum Schwabe. *Agronomy Research* **22**(1), 96–109. doi: 10.15159/AR.24.013

Domaratsky, Ye.O., Bazaliy, V.V., Boyko, M.O. & Pichura, V.I. 2018. *Agrobiological Justification of Cereal Cultivation in the Steppe Zone under Climatic Changes*. Kherson: OLDI-PLUS, 334 pp. (in Ukrainian).

Dorofeev, V.F., Udachin, R.A. & Semenova, L.V. 1987. *Wheats of the world*. Leningrad: Agropromizdat, p. 560 pp. (in Russian).

Dospekhov, B.A. 1985. *Methodology of field experiment* (with the basics of statistical processing) (B.A. Dospekhov, Ed.). Moscow: Agropromizdat, 351 pp. (in Russian).

Ferreira, S.B., Gomes, B.H., Dias, P.S., Hamawaki, C.L., Hamawaki, R.L. & Nogueira, A.O. 2024. Diallel and generation analysis in F2 soybean populations. *Agronomy Research* **22**(S3), 1421–1433. doi: 10.15159/AR.24.100

Garst, N., Belamkar, V., Easterly, A., Guttieri, M.J., Stoll, H., Ibrahim, A.M. & Baenziger, S.P. 2023. Evaluation of pollination traits important for hybrid wheat development in Great Plains germplasm. *Crop Science* **63**(3), 1169–1182. doi: 10.1002/csc2.20926

Gupta, P., Balyan, H., Gahlaut, V., Gautam, S., Pal, B., Basnet, B. & Joshi, A. 2019. Hybrid wheat: past, present and future. *Theoretical and Applied Genetics* **132**, 2283–2294. doi: 10.1007/s00122-019-03397-y

Hanafi, S.E., Cherkaoui, S., Kehel, Z., Sanchez-Garcia, M., Sarazin, J.-B., Baenziger, S. & Tadesse, W. 2022. Hybrid Seed Set in Relation with Male Floral Traits, Estimation of Heterosis and Combining Abilities for Yield and Its Components in Wheat (Triticum aestivum L.). *Plants* **11**(4), 508. doi: 10.3390/plants11040508

Korzun, D.Yu. 2016. *Methodology for conducting examination of plant varieties of cereal, groat, and legume groups for suitability for distribution in Ukraine*. (S.O. Tkachyk, Ed.). Vinnytsia: Ukrainian Institute for Plant Variety Examination, 82 pp. (in Ukrainian).

Kovalyshyna, H.M., Dmytrenko, Y.M., Butenko, A.O., Mukha, T.I., Makarchuk, O.S., Tonkha, O.L., ... Bakumenko, O.M. 2020. Screening of winter wheat varieties for leaf diseases resistance. *Ukrainian Journal of Ecology* **10**(5), 287–290. doi: 10.15421/2020_245

Kovalyshyna, H., Dmytrenko, Y., Tonkha, O., Makarchuk, O., Demydov, O., Humenyuk, O., ... & Mushtruk, M. 2020. Diversity of winter common wheat varieties for resistance to leaf rust created in the V. M. Remeslo Myronivka institute of wheat. *Potravinarstvo Slovak Journal of Food Sciences* **14**, 1001–1007. doi: 10.5219/1447

Kozub, N.O., Sozinov, I.O., Bidnyk, H.Ya., Demianova, N.O., Blyum, Ya.B. & Sozinov, O.O. 2017. Cross-pollination in Triticum aestivum L. and its wild relative Aegilops biuncialis Vis. *Factors of experimental evolution of organisms* **21**, 143–147.

Kyrylenko, V.V., Dubovyk, N.S., Humeniuk, O.V., Volohdina, H.B., Los, R.M. & Dubovyk, D.Y. 2021. *Winter bread wheat breeding by using wheat-rye translocations under environments of the Central Forest-Steppe*: monograph. Kyiv: Comprint, 221 pp. (in Ukrainian).

Langer, S.M., Longin, C.H. & Würschum, T. 2014. Phenotypic evaluation of floral and flowering traits with relevance for hybrid breeding in wheat (Triticum aestivum L.). *Plant Breeding* **133**(4), 433–441. doi: 10.1111/pbr.12192

Longin, C., Muhleisen, J., Maurer, H.P., Zhang, H., Gowda, M. & Reif, J.C. 2012. Hybrid breeding in autogamous cereals. *Theoretical and Applied Genetics* **125**, 1087–1096. doi: 10.1007/s00122-012-1967-7

Longin, F.H., Mi, X., Melchinger, A.E., Reif, J.C. & Würschum, T. 2014. Optimum allocation of test resources and comparison of breeding strategies for hybrid wheat. *Theoretical and Applied Genetics* **127**(10), 2117–2126. doi: 10.1007/s00122-014-2365-0

Murai, K., Takumi, S., Koga, H. & Ogihara, Y. 2002. Pistillody, homeotic transformation of stamens into pistil-like structures, caused by nuclear-cytoplasm interaction in wheat. *Plant J.* **29**, 169–181.

Petrenkova, V.P., Borovska, I.Yu. & Luchna, I.S. 2018. *Methodology for determining the resistance of field crops to biotic and abiotic factors*. Kharkiv: FOP Brovin O.V., 242 pp. (in Ukrainian).

Polishchuk, V., Konovalov, D. & Brovdi, A. 2024. Influence of weather conditions on winter wheat (Triticum aestivum L.) overwintering. *Agronomy Research* **22**(3), 1266–1274. doi: 10.15159/AR.24.061

Prysiazhniuk, L., Honcharov, Y., Melnyk, S. & Kliachenko, O. 2023. The selection of maize parent lines within marker assisted selection (MAS) by crtRB1-3'TE marker for Steppe zone of Ukraine. *Agronomy Research* **21**(S2), 551–559. doi: 10.15159/AR.23.015

Sade, B., Ibrahim, A.H., Subramanian, N., Rudd, J., Liu, S., Opena, G. & Baenziger, S. 2022. Assessment of floral characteristics for hybrid wheat (Triticum aestivum L.) production in Texas. *Agrosystems, Geosciences & Environment* **5**(1). doi: 10.1002/agg2.20228

Samoilyk, M., Lozinskyi, M., Yurchenko, A. & Ustinova, H. 2024. Variation of winter wheat plant height depending on ecotype and meteorological conditions. *Agrobiology* **1**, 213–221.

Schmidt, C., Hinterberger, V., Philipp, N., Reif, J.C. & Schnurbusch, T. October 2024. Hybrid grain production in wheat benefits from synchronized flowering and high female flower receptivity. *Journal of Experimental Botany* **76**(2). doi: 10.1093/jxb/erae430

Singh, S.K., Chatrath, R. & Misra, B. 2010. Perspective of hybrid wheat research: a review. *Indian J Agric Sci* **80**, 1013–1027.

Trybel, S.O., Hetman, M.V. & Stryhun, O.O. 2010. *Methodology for Assessing the Resistance of Wheat Varieties to Pests and Pathogens*. (S.O. Trybel, Ed.), Kyiv: Koloobih, p. 392 pp. (in Ukrainian).

Waines, J.G. & Hegde, S.G. 2003. Intraspecific gene flow in bread wheat as affected by reproductive biology and pollination ecology of wheat flowers. *Crop Sci.* **43**, 451–463.

Whitford, R., Fleury, D., Reif, J. & Garcia, M. 2013. Hybrid breeding in wheat: Technologies to improve hybrid wheat seed production. *Journal of Experimental Botany* **64**(18), 5411–5428. doi: 10.1093/jxb/ert333

Zemoida, V.L., Bashkirova, N.V., Zinchenko, L.A., Karpuk, L.M., Alyokhin, V.I. & Dmytrenko, Y.M. 2019. Autogamy of alfalfa (Medicago sativa L.) and it's usage in breeding. *Plant Cell Biotechnology and Molecular Biology* **20**(23–24), 1137–1142.

Assessment of cultivation efficiency of several cultivars of blue lucerne (*Medicago sativa* L.) in the conditions of Shirak region of Armenia

R. Sadoyan[1], N. Mkrtchyan[3], L. Suvaryan[3], L. Matevosyan[2], A. Avetisyan[1,2], H. Nersisyan[2], N. Bayramyan[1,2], M. Zadayan[2,4,*], A. Shirvanyan[5] and H. Martirosyan[6]

[1]Armenian State Pedagogical University after Kh. Abovyan, 17 Tigran Mets Ave., AM 0010 Yerevan, Armenia
[2]Scientific Center of Agriculture CJSC of the Ministry of Economy of the Republic of Armenia, Issi-Le Mulino 1 Str, AM1101 Ejmiatsin, Armavir region, Armenia
[3]Gyumri Breeding Station CJSC of the Ministry of Economy of the Republic of Armenia, Shirak Marz, Akhuryan village, Armenia
[4]Center for Agricultural Research and Certification, State Non-Commercial Organization of the Ministry of Economy of the Republic of Armenia, Yerevanyan highway 2nd deadlock, building 4, Armavir Marz, AM1139 v. Merdzavan, Armenia
[5]Scientific Center for Risks Assessment and Analysis in Food Safety Area CJCS of the Ministry of Economy of the Republic of Armenia, Masisi Str., 107/2 Building, AM0071 Yerevan, Armenia
[6]Scientific Center of Agrobiotechnology, branch Armenian National Agrarian University, Issi-Le Mulino 1 Str, AM1101, Ejmiatsin, Armavir region, Armenia
*Correspondence: mhzadayan@gmail.com

Abstract. Background: Lucerne (*Medicago sativa* L.) is a highly productive forage legume valued for its exceptional protein concentration, vitamin richness, digestible fiber, and bioactive compounds. One kilogram of lucerne green mass contains approximately 50 g of carotene, alongside calcium, phosphorus, and other essential nutrients, making it a cornerstone of sustainable livestock farming and a potential functional food source.

Objective: The study aimed to assess agro-biological traits, green mass and hay yield, seed productivity, and protein concentration of 29 introduced lucerne cultivars from the All-Russian Institute of Crop Production (VIR) compared with the traditional local cultivar 'Aparani' under the agro-climatic conditions of the Shirak region in Armenia.

Methods: Field experiments were conducted from 2019 to 2022 using a randomized design with four replications (plot size: 25 m²). Standard agronomic practices were applied. Protein concentration in seeds was determined by the Kjeldahl method, while dry matter and nitrogen values were analyzed at the Organic Agriculture Laboratory of ANAU. Statistical analysis was performed using ANOVA, *LSD*, and relative error (Ex, %).

Results: Considerable variation was observed among cultivars in yield and adaptability. The Canadian cultivars 'Admiral' and 'Adge', the French cultivar 'Derby', and the American cultivar 'Schild' demonstrated the highest productivity. 'Admiral' showed superior early regrowth, growth vigor, leafiness, and seed productivity (8.8 c ha^{-1}). Green mass yield reached 325.5 c ha^{-1} for 'Admiral' and 308.4 c ha^{-1} for 'Adge', while their seed protein concentrations were 16.3% and 15.6%, respectively, exceeding the local 'Aparani'.

Conclusion: The results indicate that 'Admiral' and 'Adge' are highly suitable for the Shirak region due to their high yields, adaptability, and nutritional value. Their adoption could enhance fodder self-sufficiency, improve livestock productivity, and expand the role of lucerne as a functional food crop rich in protein and bioactive compounds.

INTRODUCTION

Lucerne (*Medicago sativa* L.) is one of the most important perennial forage legumes worldwide, highly valued for its high protein content, carotene concentration, and digestible fiber. Its cultivation plays a vital role in sustainable livestock production, soil fertility improvement through nitrogen fixation, and biodiversity conservation (Gaweł, 2012; Tanchyk, 2021; Akhtar et al., 2023; Pourebrahimi Foumani et al., 2023). In Armenia, lucerne has traditionally been the dominant forage crop and continues to serve as a cornerstone of animal husbandry systems.

The Shirak region is characterized by cold winters, frequent frosts, and relatively short vegetation periods, which significantly influence the productivity, persistence, and adaptability of lucerne stands. Consequently, evaluating foreign and local cultivars under these specific conditions is of great importance for improving fodder security and increasing the efficiency of livestock production. Although more than 29 cultivars introduced from international breeding centers have been tested in Armenia, comprehensive comparative studies remain scarce (Doguzova, 2020; Harutyunyan et al., 2023; Martirosyan et al., 2023c).

Previous research has shown that the average productivity of forage crops in Armenia remains lower than that of leading European countries. This limitation is not only attributed to the genetic potential of cultivars but also to inadequate fertilization, limited use of modern agrotechnologies, irrigation constraints, and poor adaptation of some imported cultivars to local agro-climatic conditions. As a result, the selection and introduction of lucerne cultivars with proven adaptability and productivity under regional conditions have become a national priority (Harutyunyan, 2022; Matevosyan et al., 2023).

Given these challenges, the present study was designed to conduct a comparative evaluation of 29 lucerne cultivars obtained from the All-Russian Institute of Crop Production (VIR) against the traditional Armenian cultivar 'Aparani'. Special emphasis was placed on agro-biological traits, green mass and hay yield, seed productivity, and protein concentration under the agro-climatic conditions of Shirak. The findings are expected to provide both scientific and practical guidance for improving fodder self-sufficiency and for introducing highly productive lucerne cultivars with potential applications not only in animal feed but also as functional food resources.

MATERIALS AND METHODS

The field trials were conducted from 2019 to 2022 at the experimental fields of the Gyumri Breeding Station, Shirak region, Armenia (40°47′ N, 43°50′ E; 1,550 m a.s.l.). The region has a temperate continental climate with cold winters and moderately warm summers. The average annual air temperature is 6.8 °C, ranging from –12 °C in January to 25–27 °C in July. Annual precipitation averages 520–560 mm, mostly occurring in spring and early summer. The experimental soils are meadow-chernozems with pH 7.4, humus 3.2%, and moderate levels of available phosphorus and potassium.

Seeds of 29 lucerne cultivars obtained from the All-Russian Institute of Crop Production (VIR, Russia) were compared with the traditional Armenian cultivar 'Aparani'. Among them, the cultivars Admiral and Adge (Canada), Derby (France), and Schild (USA) were subjected to detailed analysis due to their superior performance during preliminary evaluations.

The experimental layout followed a randomized complete block design (RCBD) with four replications. Each plot measured 25 m², with 15 cm row spacing. Seeds were sown without cover crops during the first decade of May. Standard agronomic practices—including weed control, irrigation when necessary, and pest management - were applied uniformly across all plots.

The following measurements were taken:

- Green mass yield – determined as fresh biomass weight (c ha⁻¹) at each mowing
- Hay yield – calculated after air-drying subsamples to constant weight
- Seed yield – measured at full maturity and expressed in c ha⁻¹
- Leaf-to-stem ratio – determined from 10 randomly selected plants per plot, dried at 70 °C, and weighed.

Protein concentration in seeds was analyzed using the Kjeldahl method ($N \times 6.25$). Dry matter and nitrogen contents were determined at the Organic Agriculture Laboratory of the Armenian National Agrarian University (Yagodin, 1987; Dospekhov, 1985; Conova & & Samoilov, 2015; Shaboyan et al., 2024).

Data were statistically processed using one-way ANOVA, and mean comparisons were performed with the *LSD* test at $p \leq 0.05$. Relative error (Ex, %) was also calculated to assess data accuracy. Statistical calculations were carried out using Microsoft Excel 2019.

RESULTS AND DISCUSSION

Morphological Traits

The evaluation of 29 lucerne cultivars under the agro-climatic conditions of the Shirak region revealed considerable variation in morphological characteristics. Four cultivars - Admiral, Adge, Derby, and Schild - demonstrated superior performance in terms of green mass, hay yield, seed productivity, and agro-biological traits. These cultivars also showed strong winter hardiness and rapid spring regrowth, with Admiral and Adge (Canada) being particularly well adapted, followed by Derby (France) and Schild (USA).

Morphological traits are recognized as key indicators of lucerne productivity and nutritional quality, since leaves and inflorescences contain 1.5–1.8 times higher protein and digestible nutrients compared to stems (Kudeyarov, 2021; Solozhentseva, 2021;

Stødkilde, 2023). As shown in Table 1, the Admiral cultivar was characterized by large, slightly serrated leaves, while Adge displayed predominantly oval leaves. Inflorescence type and flower number also varied: the local cultivar Aparani produced compact clusters with up to 40 flowers per cluster, whereas imported cultivars generally formed looser clusters with 15–30 flowers. Pod morphology and 1,000-seed weight differed moderately between cultivars but remained within ranges reported for lucerne in other temperate regions (Shi, 2017; Ramos-Ulate, 2022).

Table 1. Morphological and morphological description of tested cultivars

Morphological trait	Aparani (local)	Derby (France)	Schild (USA)	Adge (Canada)	Admiral (Canada)
Leaf shape	Compound, medium-sized, oval leaf with long petiole	Compound, lanceolate, toothed margin	Compound, elliptic, with petioles	Compound, obovate, serrated at the ends	Compound, large leaves, weakly serrated
Inflorescence	Compact cluster with short petiole	Elongated cluster (~4 cm)	Compact bunch without petiole	Scattered cluster with a short peduncle	Compact, small cluster with few flowers
Number of flowers/cluster	Up to 40	Up to 25	Up to 28	Up to 30	Up to 15
Pod shape	Spring-shaped, up to 3 coils	Spring-shaped, 1–2 coils	Spring-shaped, 1.5–2 coils	Spring-shaped, with 2 coils	Spring-shaped, up to 3 coils
1,000 seed weight (g)	2.3	2.1	2.1	2.3	2.2

Overall, the morphological variation confirms that imported cultivars - particularly Admiral and Adge - possess structural advantages such as larger leaf size and more favorable leaf-stem ratios, which are directly linked to higher forage quality. These results suggest that morphological traits, especially leaf size and leaf-stem ratio, are reliable indicators of lucerne adaptability under the cold and relatively short growing season of Shirak. Cultivars combining high leafiness with compact inflorescences can therefore be recommended as promising candidates for both productivity and nutritional improvement.

Growth and Development Phases

All tested cultivars completed three mowing cycles per season under the agro-climatic conditions of the Shirak region (Table 2). Among them, the Canadian cultivar Admiral demonstrated the earliest spring regrowth (March 13), which was 5–9 days earlier than the local control Aparani and 2–4 days earlier than the cultivars Derby, Schild, and Adge. Early regrowth allowed Admiral to reach the bud formation and flowering stages more quickly, thereby contributing to shorter harvest cycles.

The duration of the first harvest ranged from 62 to 71 days, depending on the cultivar. Admiral required the shortest period (62 days), while Aparani showed the longest (71 days). Subsequent harvests were also shorter in Admiral and Adge compared with the control, confirming their better adaptability and faster turnover under short growing seasons.

The advantage of earlier regrowth and shortened harvest cycles lies in the opportunity to achieve higher annual biomass production, particularly in regions with relatively short vegetation periods such as Shirak. Lucerne cultivars with early spring growth and rapid regrowth capacity are more productive and better suited to cooler climates (Vasileva, 2012; Mielmann, 2013; Osipova, 2018; Perpetuo, 2021).

Overall, the results suggest that phenological traits - especially early spring regrowth and shorter inter-harvest periods - are reliable indicators of cultivar adaptability. Cultivars such as Admiral and Adge, which combine early development with high productivity, can be recommended as valuable options for maximizing forage yield in highland environments.

Table 2. Phenological stages and harvesting durations (days) of tested lucerne cultivars

Cultivar	Stage I Regrowth	Cocooning	Blooming	Stage II Regrowth	Cocooning	Blooming
Aparani local	18.03	20.05	28.05	04.06	27.07	03.08
Derby (France)	15.03	14.05	20.05	27.05	16.07	22.07
Schild (USA)	15.03	16.05	23.05	30.06	21.07	27.07
Adge (Canada)	14.03	10.05	17.06	23.06	13.07	20.07
Admiral (Canada)	13.03	29.05	14.05	21.05	07.07	13.07

Table 2 (continued)

Cultivar	Stage III Regrowth	Cocooning	Blooming	Harvest I (days)	Harvest II (days)	Harvest III (days)
Aparani local	11.08	01.10	08.10	71	67	64
Derby (France)	01.08	26.09	02.10	65	63	62
Schild (USA)	05.08	29.09	06.10	68	65	63
Adge (Canada)	29.07	19.09	26.09	64	62	60
Admiral (Canada)	23.07	14.09	20.09	62	59	58

Note: Stage I–III = first, second, and third mowing cycles; 'Cocooning' = bud formation; 'Blooming' = onset of flowering. Dates are presented as day. month (dd.mm). Harvest duration is the period from regrowth to mowing.

Growth Characteristics and Foliage

The assessment of growth characteristics across three harvests revealed significant cultivar-dependent variation in stem density, plant height, and foliage contribution (Table 3). The Canadian cultivar Admiral showed superior performance in all traits compared with both the local Aparani and the other introduced cultivars.

Stem number and density. During the first harvest, Admiral produced 18.5 stems per plant and 3,977 stems per m², exceeding Aparani by 5.8 stems per plant and approximately 1,300 stems per m². Although stem numbers decreased in subsequent harvests for all cultivars, Admiral and Adge consistently maintained higher averages. This trend agrees with findings by some scientists who reported that greater stem density is strongly associated with higher biomass accumulation in lucerne (Mantovi, 2015; Hoppen, 2021; Tadevosyan, 2023; Teixeira, 2023).

Plant height. Admiral also outperformed the other cultivars in height, reaching 70.5 cm in the first harvest, which was 12.4 cm taller than Aparani. The difference remained significant in subsequent harvests, confirming its superior growth vigor. Adge ranked second, while Schild was closest to the control. Since plant height is known to correlate positively with forage yield (ARMSTAT, 2023), these results further support the high biomass productivity of Admiral and Adge.

Leaf mass and foliage ratio. Foliage contribution, a key determinant of nutritive value, was highest in Admiral, with 38.8 g of leaf mass per 10 plants, accounting for 54% of total biomass. Adge followed with 53.3%, while Aparani reached only 45.2%. The higher leaf-stem ratio in Admiral and Adge reflects their superior forage quality, as lucerne leaves contain 1.5–1.8 times more protein than stems.

Statistical validation. ANOVA analysis confirmed statistically significant differences ($p < 0.05$) among cultivars for all measured parameters. The consistently superior performance of Admiral and Adge aligns with results from Canadian and European breeding programs (FAO, 2018; Abdullayev et al., 2021), demonstrating their strong adaptability to the agro-climatic conditions of Armenia.

Table 3. Growth characteristics and foliage mass of tested lucerne cultivars during three harvests (mean values ± SE, $n = 4$)

Cultivar	Stems per plant (pcs)	Stems per m² (pcs)	Plant height (cm)	Leaf mass (g per10 plants)	Stem mass (g per10 plants)	Leaf % of total mass
Aparani (local)	12.7 ± 0.4	2,679 ± 85	61.2 ± 1.5	32.8 ± 1.2	39.8 ± 1.3	45.2
Derby (France)	16.2 ± 0.5	3,450 ± 92	63.4 ± 1.6	34.9 ± 1.3	41.9 ± 1.4	45.5
Schild (USA)	15.4 ± 0.6	3,234 ± 88	62.1 ± 1.4	32.2 ± 1.1	42.6 ± 1.2	43.0
Adge (Canada)	17.9 ± 0.4	3,830 ± 95	67.8 ± 1.8	37.2 ± 1.4	45.8 ± 1.6	53.3
Admiral (Canada)	18.5 ± 0.5	3,977 ± 97	70.5 ± 1.7	38.8 ± 1.5	46.2 ± 1.5	54.0
LSD ($p < 0.05$)	0.8	110	2.0	1.9	2.1	–

Note: Values are means of four replicates. Statistical differences among cultivars were determined by one-way ANOVA followed by *LSD* test ($p < 0.05$).

(Values for stems per plant, stems per m², and plant height are presented across I / II / III mowings. Data represent mean values of four replicates. Statistical analysis was performed by one-way ANOVA, and means were compared using *LSD* test at $p < 0.05$).

In summary, Admiral and Adge not only ensured higher stem density and plant height but also maintained a higher proportion of leaf biomass, directly contributing to both forage yield and nutritive value. These traits make them particularly promising for large-scale cultivation in the Shirak region.

Yield Performance

The analysis of forage productivity across three mowing cycles revealed statistically significant differences among cultivars (Table 4).

Table 4. Yield and feed value of tested lucerne cultivars (c ha^{-1})

Cultivar	Green mass yield (c ha^{-1})	Hay yield (c ha^{-1})	Feed units (c ha^{-1})*
Aparani (local)	246.1 ± 5.8	80.3 ± 3.1	43.8 ± 1.7
Derby (France)	278.1 ± 6.2	93.2 ± 3.6	50.5 ± 1.9
Schild (USA)	266.4 ± 6.0	88.8 ± 3.5	48.4 ± 1.8
Adge (Canada)	308.4 ± 6.5	102.8 ± 3.9	56.1 ± 2.0
Admiral (Canada)	325.5 ± 6.8	108.5 ± 4.1	59.1 ± 2.1
LSD ($p < 0.05$)	7.9	4.6	2.5

Note: *Feed unit conversion: 100 kg of hay = 54.5 feed units.

Green mass yield. The local Aparani cultivar produced the lowest green mass yield (246.1 c ha^{-1}), while the Canadian cultivar Admiral achieved the highest productivity (325.5 c ha^{-1}), exceeding the control by 79.4 c ha^{-1}. Adge also demonstrated high productivity (308.4 c ha^{-1}), confirming its adaptability to Shirak conditions. Derby and Schild performed moderately, with yields of 278.1 and 266.4 c ha^{-1}, respectively. These findings are consistent with earlier reports that Canadian lucerne cultivars show superior yield stability under temperate continental climates (FAO, 2018).

Hay yield and feed unit conversion. Hay yield followed similar trends, ranging from 80.3 c ha^{-1} in Aparani to 108.5 c ha^{-1} in Admiral. The feed unit conversion efficiency was highest in Admiral (59.1 c ha^{-1}), which exceeded Aparani by 15.3 c ha^{-1}, while Adge also performed well (56.1 c ha^{-1}). The high feed unit values reflect both greater dry matter accumulation and more favorable leaf-stem ratios. These results agree with Eldeiry & Garcia (2008), who emphasized that lucerne foliage proportion is a decisive factor determining nutritive value.

Nutritional efficiency. On average, approximately 31–32% of green mass was converted into hay. Admiral showed the highest efficiency in the second harvest, where hay yield accounted for 36.9% of total biomass. Such efficiency is particularly important for ensuring stable forage reserves under Armenia's semi-arid climatic conditions.

Statistical validation. ANOVA analysis confirmed that differences among cultivars in green mass yield, hay yield, and feed unit production were statistically significant ($p < 0.05$). The results clearly demonstrate the superiority of Admiral and Adge over both the local Aparani and the other imported cultivars.

In summary, Admiral and Adge combined high green mass productivity, elevated hay yields, and improved feed unit efficiency, confirming their potential to substantially increase fodder production and support livestock sustainability in the Shirak region.

Chemical Composition

The analysis of fresh-mass chemical composition revealed clear cultivar effects on moisture, dry matter, nitrogen, and crude protein concentrations (Table 5). Admiral demonstrated the highest dry matter (25.91%) and crude protein content (16.3%), followed by Adge (25.09% DM; 15.6% CP). Derby and Schild showed intermediate values (≈24.0–23.3% DM; 15.6–15.0% CP), while the local cultivar Aparani exhibited the lowest dry matter (23.41%) and crude protein content (15.0%). The inverse relationship between moisture and dry matter was consistent across all cultivars.

Statistical validation. ANOVA confirmed significant differences ($p < 0.05$) among cultivars for both dry matter and crude protein concentration. Pairwise *LSD* tests ranked Admiral > Adge > Derby ≈ Schild ≈ Aparani for CP. Although the absolute differences were modest - for instance, Admiral exceeded Aparani by 1.3 percentage points – the field-scale impact is meaningful. Considering Admiral's hay yield of 108.5 c ha⁻¹ (10,850 kg ha⁻¹), this protein advantage corresponds to approximately 141 kg more crude protein per hectare.

Link to morphology and forage quality. The higher crude protein concentrations in Admiral and Adge are consistent with their greater leaf mass and higher leaf-stem ratios (Table 3), reflecting the well-established gradient of higher protein content in leaves compared with stems (Eldeiry & Garcia, 2008). Reported crude protein values for lucerne at early bloom in temperate regions typically range from 15–20% (Goncharov, 1985; Guo, 2020), aligning closely with the results obtained under Shirak's agro-climatic conditions.

In summary, the chemical composition results confirm that Admiral and Adge are nutritionally superior cultivars, combining higher dry matter accumulation with enhanced protein concentration. These traits further increase their value not only for forage production but also for improving the overall efficiency of livestock feeding systems in the region.

Table 5. Chemical composition of green mass of studied cultivars (%)

Cultivar	Moisture (%)	Dry matter (%)	Nitrogen (%)	Crude protein (%)
Aparani local	76.59	23.41	2.4	15.0
Derbi (France)	75.93	24.07	2.5	15.6
Schild (USA)	76.73	23.27	2.4	15.0
Adge (Canada)	74.91	25.09	2.5	15.6
Admiral (Canada)	74.09	25.91	2.6	16.3

Note: Crude protein calculated as N × 6.25; The *Admiral* cultivar demonstrated the highest dry matter and protein content among all studied cultivars.

Seed Productivity

Seed yield was another critical performance indicator, and the results revealed substantial and statistically significant variation among cultivars (Table 6). Across the three-year testing period (2019–2021), the Canadian cultivars Admiral and Adge consistently achieved the highest yields, averaging 8.8 and 8.5 c ha⁻¹, respectively. These values markedly exceeded those of the local Aparani cultivar (5.9 c ha⁻¹), as well as Derby (6.8 c ha⁻¹) and Schild (6.3 c ha⁻¹). ANOVA confirmed that these differences were significant ($p < 0.05$).

The consistent performance of Admiral and Adge highlights their strong adaptation to the agro-climatic conditions of Shirak. Their superior reproductive capacity ensures a reliable seed supply, which is essential both for maintaining

Table 6. Average lucerne seed yield in the 1st year of use (2019–2021), c ha⁻¹

Cultivar	2019	2020	2021	3-year average
Aparani (local)	6.1	6.3	5.4	5.9
Derby (France)	7.0	6.8	6.6	6.8
Schild (USA)	6.4	6.6	5.5	6.3
Adge (Canada)	8.3	8.5	8.8	8.5
Admiral (Canada)	8.8	8.6	9.0	8.8

long-term lucerne cultivation and for supporting the expansion of forage-based production systems. Similar trends have been reported in international studies, where the introduction of high-performing cultivars resulted in stable yield increases in forage legumes (Bani Khalaf et al., 2021; Martirosyan, 2023a). There are scientific works that also emphasize that genetic potential, combined with agro-ecological adaptation, plays a decisive role in lucerne seed productivity (Burceva, 2020; Argenti, 2021; Beslemes, 2023).

The results of Armenian research once again confirm that cultivar selection is a key factor for achieving stable lucerne seed production under contrasting climatic conditions. The consistent superiority of the Canadian cultivars in Shirak confirms their potential as high-performing, well-adapted genotypes (Matevosyan, 2020; Martirosyan, 2023b).

In conclusion, Admiral and Adge not only outperform the local Aparani and other imported cultivars in forage yield and protein content but also ensure significantly higher and more stable seed productivity. Their integration into Shirak's forage production systems could substantially strengthen the regional fodder base, enhance livestock sector sustainability, and contribute to the long-term resilience of Armenia's forage cultivation.

CONCLUSION

This study evaluated the agro-biological performance of 29 lucerne (*Medicago sativa* L.) cultivars from diverse geographic origins under the soil and climatic conditions of the Shirak region of Armenia. Among them, the Canadian cultivars Admiral and Adge consistently demonstrated superior performance in terms of green mass, hay and seed yield, protein content, and adaptability to local environmental conditions.

Based on these results, Admiral and Adge are recommended for wider integration into Armenia's forage production systems. Their high productivity, early regrowth, short vegetation period, and tolerance to harsh winters make them suitable for intensive crop rotations and climate-resilient fodder production. The adoption of these cultivars can significantly strengthen the regional fodder base, improve feed quality, and contribute to the sustainability of livestock farming in Armenia.

In addition to their agronomic advantages, lucerne cultivars - particularly Admiral and Adge – exhibit nutritional properties relevant to functional food and nutraceutical applications. Their content of essential amino acids, vitamins, and bioactive phytochemicals highlights their potential for use in dietary supplements and health-promoting food products. Lucerne sprouts and protein concentrates could thus serve as valuable ingredients contributing to nutritional security.

Nevertheless, this research was conducted in a single region under specific climatic conditions. Further multi-year and multi-location trials are required to assess the long-term stability and wider adaptability of the selected cultivars. Complementary biochemical studies, including amino acid profiling and phytochemical characterization, are also recommended to substantiate their functional food potential.

REFERENCES

Akhtar, S., Farooqui, A., Younis, K., Mani, A. & Singh, H. 2023. Plant proteins for sustainable food production: 'Serving present to secure the future'. *International Journal of Food Science and Technology*. https://doi.org/10.1111/ijfs.16807

Argenti, G., Parrini, S., Staglianò, N. & Bozzi, R. 2021. Evolution of production and Forage quality in sown meadows of a mountain area inside Parmesan cheese consortium. *Agronomy Research* **19**(2), 344–356. https://doi.org/10.15159/AR.21.061

ARMSTAT. 2023. *Statistical Bulletin*, *2022*. Yerevan: National Statistical Service. https://armstat.am/file/article/29_gt_2022.pdf

Bani Khalaf, Y., Aldahadha, A., Samarah, N., Migdadi, O. & Musallam, I. 2021. Effect of zero tillage and different weeding methods on grain yield of durum wheat in semi-arid regions. *Agronomy Research* **19**(1), 13–27. https://doi.org/10.15159/AR.20.236

Beslemes, D.F., Tigka, E. & Kakabouki, L. 2023. Lentil crop rotation and green manuring effects on soil structural stability and corn yield in different soils in Central Greece. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca*. Horticulture, **80(**1), 7–13. https://doi.org/10.15835/buasvmcn-hort:2022.0039

Burceva, N. 2020. Agrotechnical methods of alfalfa cultivation for seeds under irrigation conditions in the Lower Volga region. *Agrarian Bulletin* **201**(10), 8–15. https://doi.org/10.32417/1997-4868-2020-201-10-8-15

Conova, A.M. & Samoilov, L.N. 2015. Removal of nutrients cultural and weeds in the crop rotation. *Agrochemistry* **5**, 46–53 (in Russian).

Doguzova, N.N. 2020. Seed productivity of various alfalfa cultivars for the foothill zone of the North Caucasus. *Agrarian Science* **342**(10), 64–67. https://doi.org/10.32634/0869-8155-2020-342-10-64-67

Dospekhov, B.A. 1985. *Methodology of Field Practice*. Moscow: Agropromizdat, pp. 207–248 (in Russian).

Eldeiry, A.A. & Garcia, Luis A. 2008. Detecting Soil Salinity in Alfalfa Fields using Spatial Modeling and Remote Sensing. *Soil & Water Management & Conservation* **72**(1), 201–211. https://doi.org/10.2136/sssaj2007.0013

FAO. 2018. https://www.fao.org/news/archive/news-by-date/2018/ru/

Gaweł, E. 2012. Chemical composition of lucerne leaf extract (EFL) and its applications as a phytobiotic in human nutrition. *Acta Scientiarum Polonorum – Technologia Alimentaria* **11**(3), 303–310. https://pubmed.ncbi.nlm.nih.gov/22744951/

Goncharov, P.L. & Lubenetc, P.A. 1985. Biological Aspects of Alfalfa Cultivating. Novosibirsk: *Nauka*, 256 pp. (in Russian).

Guo, Z., Wan, S., Hua, K., Yin, Y., Chu, H., Wang, D. & Guo, X. 2020. Fertilization regime has a greater effect on soil microbial community structure than crop rotation and growth stage in an agroecosystem. *Applied Soil Ecology* **149**, Article 103510. doi: 10.1016/j.apsoil.2020.103510

Harutyunyan, S.S., Ghazaryan, H.R., Ghukasyan, A.G., Osipova, R.H. & Mkrtchyan, A.T. 2023. Production removal of the main nutrient elements from winter wheat and barley crops in the conditions of the Ararat Valley of Armenia. *Agronomy Research* **21**(1), 78–86. https://doi.org/10.15159/AR.23.007

Harutyunyan, S.S., Matevosyan, L.G., Ghukasyan, A.G. & Galstyan, M.H. 2022. The system of soil protection and general balance of main nutrient elements in perennial plantations of semi-desert natural soil zone of Armenia. *Agronomy Research* **20**(3), 575–587. https://doi.org/10.15159/AR.22.039

Hoppen, S.M., Neres, M. & Moot, D.J. 2021. Factors related to productivity and persistence of lucerne (Medicago sativa) genotypes with different fall dormancy levels: a review. *Research, Society and Development* **11**(1), e11711124473. doi: 10.33448/rsd-v11i1.24473

Kudeyarov, V.N. 2021. Nitrous oxide emission factor from Russian arable soils at the fertilizers application. *Agrochemistry* **11**, 3–15 (in Russian).

Mantovi, P., Dal Pra, A., Pacchioli, M.T. & Ligabue, M. 2015. Forage production and use in the dairy farming systems of Northern Italy. *Grassland Science in Europe* **20**, 67–77. https://doi.org/10.5555/20153417857

Martirosyan, D.M. & Stratton, S. 2023a. Advancing functional food regulation. *Bioactive Compounds in Health and Disease* **6**(7), 166–171. https://doi.org/10.31989/bchd.v6i7.1178

Martirosyan, G., Sarikyan, K., Adjemyan, G., Pahlevanyan, A., Kirakosyan, G., Zadayan, M. & Avagyan, A. 2023b. Impact of green technology on content of bioactive components in eggplant. *Bioactive Compounds in Health and Disease* **6**(12), 351–363. https://doi.org/10.31989/bchd.v6i12.1261

Martirosyan, H.S., Sadoyan, R.R. & Avetisyan, A.S. 2023c. Ways of obtaining organic products from several bread crops (barley, emmer, and wheat). *Austrian Journal of Technical and Natural Sciences* **5–6**/2023. https://doi.org/10.29013/AJT-23-5-6-3-9

Matevosyan, L.G., Barbaryan, A.A. & Avetisyan, S.G. 2020. The Comparative Efficiency in the Cultivation of New Groundnut Varieties in the Piedmont Zones of Armenia. *Agriscience and Technology* **3**(71), 56–59 (in Armenian).

Matevosyan, L.G., Barbaryan, A.A., Ghukasyan, A.G., Ghazaryan, R.G., Alikhanyan, N.A. & Shaboyan, G.G. 2023. Organization of seed breeding activities for leguminous crops and introduction of new cultivars in conditions of piedmont and mountainous zones of Armenia. *Ecological and Biological Well-Being of Flora and Fauna (Part 1),* EBWFF 2023-International Scientific Conference, **420**, 1–6. doi: 10.1051/e3sconf/202342001022

Mielmann, A. 2013. The utilisation of lucerne. *British Food Journal* **115**(4), 590–600. https://doi.org/10.1108/00070701311317865

Osipova, V.V. 2018. *Scientific substantiation of alfalfa cultivation technology (Medicago L.) in adaptive agriculture of the Republic of Sakha (Yakutia)*. Doctoral dissertation, Russian State Agrarian University-Moscow Agricultural Academy named after K.A. Timiryazev. Retrieved from https://www.dissercat.com/content/nauchnoe-obosnovanie-tekhnologii-vozdelyvaniya-lyutserny-medicago-l-v-adaptivnom-zemledelii

Perpetuo, Á.V., Juan Antonio, E.D. & Joel, V.R. 2021. Productive performance of alfalfa (Medicago sativa L.) at different age of resprout in the spring season. *Agro Productividad* **13**(12). https://doi.org/10.32854/agrop.v13i12.1898

Pourebrahimi Foumani, M., Savoy, H., Atotey, N. & Yin, X. 2023. Effect of potassium application rate and timing on alfalfa yield and potassium concentration and removal in Tennessee. *Agronomy Research* **21**(1), 183–192. https://doi.org/10.15159/AR.23.014

Ramos-Ulate, C.M., Pérez-Álvarez, S., Guerrero-Morales, S. & Palacios-Monarrez, A. 2022. Biofertilization and nanotechnology in alfalfa (Medicago sativa L.) as alternatives for a sustainable crop. *Characterization and Application of Nanomaterials* **5**(2), Article 1769. https://doi.org/10.24294/can.v5i2.1769

Shi, S., Nan, L. & Smith, K.F. 2017. The current status, problems, and prospects of alfalfa (Medicago sativa L.) breeding in China. *Agronomy* **7**(1), 1–11. https://doi.org/10.3390/agronomy7010001

Shaboyan, G., Matevosyan, L., Sarikyan, K. & Martirosyan, G. 2024. Biochemical analyses in the ICARDA collection of unique dried materials of lentils. *Bioactive Compounds in Health and Disease* **7**(2), 131–140. https://doi.org/10.31989/bchd.v7i2.1291

Solozhentseva, L.F., Piskovatsky, Y.M. & Lomov, M.V. 2021. Alfalfa breeding to increase productivity, disease resistance. IOP Conference Series: *Earth and Environmental Science,* **901**(1), Article 012012. https://doi.org/10.1088/1755-1315/901/1/012012

Stødkilde, L. 2023. The composition and nutritional quality of biorefined lucerne protein depend on the precipitation method. *Science of Food and Agriculture*. https://doi.org/10.1002/jsfa.13226

Tadevosyan, L., Martirosyan, G., Tsereteli, I., Vardanian, I., Zadayan, M. & Avagyan, A. 2023. Dynamics of bioactive substances accumulation during cauliflower maturation as a way to ensure crop functional properties. *Functional Foods in Health and Disease* **13**(11), 584–594. https://doi.org/10.31989/ffhd.v13i11.119719

Tanchyk, S., Litvinov, D., Butenko, A., Litvinova, O., Pavlov, O., Babenko, A., Shpyrka, N., Onychko, V., Masyk, I. & Onychko, T. 2021. Fixed nitrogen in agriculture and its role in agrocenoses. *Agronomy Research* **19**(2), 601–611. https://doi.org/10.15159/AR.21.086

Teixeira, E., Guo, J., Liu, J., Cichota, R., Brown, H., Sood, A., Yang, X., Hannaway, D. & Moot, D. 2023. Assessing land suitability and spatial variability in lucerne yields across New Zealand. *European Journal of Agronomy* **148**, Article 126853. https://doi.org/10.1016/j.eja.2023.126853

Vasileva, V. 2012. Nitrogen content in yield of dry aboveground and root mass of forage lucerne (Medicago sativa L.) after mineral nitrogen fertilization and water deficiency stress. *Agronomy Research* **10**(1–2), 351–356.

Yagodin, B.A. 1987. Workshop on Agricultural Chemistry. Moscow: Agropromizdat, 12 pp. (in Russian)

Effect of zeolite-amended sandy soils on growth & yield of Copenhagen cabbage

O.A. Sindesi[1,*], M.N. Lewu[2], B. Ncube[3], A.R. Mulidzi[2] & F.B. Lewu[1]

[1]Lecturer & Researcher, Department of Agriculture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Private Bag X8, Wellington 7654, South Africa
[2]Senior Researcher, Soil & Water Science Programme, Agricultural Research Council Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa
[3]Lecturer & Senior Researcher, Centre for Water & Sanitation Research, Faculty of Engineering & the Built Environment, Cape Peninsula University of Technology, Bellville 7535, Cape Town, South Africa
*Correspondence: olwethusindesi@gmail.com

Abstract. A greenhouse pot experiment was conducted at the Agricultural Research Council, Infruitec-Nietvoorbij, Stellenbosch, South Africa, to assess the effect of zeolite on the fresh head yield & growth characteristics of cabbage cv. Copenhagen. Zeolite was applied at 0:10, 1:9, 2:8, & 3:7 zeolite to sandy soil (w/w). Cabbage growth parameters, plant height, & number of loose leaves showed significant improvements ($p < 0.05$) under zeolite-amended treatments in the second growing season. Maximum chlorophyll content index (CCI) values ranged from 70.03 to 78.04 in the first season & 52.37 to 61.59 in the second growing season. While leaf area showed no significant differences ($p > 0.05$) in the first growing season. Additionally, marketable cabbage head traits (head diameter, head circumference, & fresh weight) exhibited no significant differences ($p > 0.05$) among treatments in the first season. Still, significant improvements were observed in the second season. Zeolite applications at 30% reduced cabbage yields by 15.12% (without loose outer leaves) & by 11.64% (with outer leaves) compared to a 20% zeolite application. Furthermore, the findings highlight a practical implication: a 20% zeolite amendment appears to be the optimal level for improving cabbage yield without the negative effects observed at higher application rates. Overall, this study revealed that zeolite could enhance certain cabbage growth parameters & yield, particularly in the second season. This indicates that zeolite might require a fallowing period within the soil to fully benefit plant growth. Additionally, the results also indicate that zeolite soil amendment may have a limit to its beneficial effects. To support broader adoption, the study recommends applying zeolite at 20% prior to planting, integrated with conventional fertilisers, and guided by soil testing in follow-up seasons.

Key words: agronomic characteristics, growth rate, soil amendment, fresh market vegetables, *Brassica oleracea* var. *capitata* L.

INTRODUCTION

Vegetables are a cost-effective source of essential micronutrients & vitamins that support healthy diets (Sindesi et al., 2023). Among them, cabbage (*Brassica oleracea* var. *capitata* L.) is valued for its nutritional & medicinal properties, contributing to both food security & health promotion (Šamec et al., 2017). During the COVID-19 pandemic, cabbage was notably consumed in fermented forms as a potential mitigation strategy in regions such as Eastern Asia, Central Europe, & the Balkans Naicker et al., 2021; Bousquet et al., 2021; Sooriyaarachchi et al., 2022). It is also recognised as one of the top twenty important food crops globally (FAO, 1988; Gelaye, 2024). In Africa, cabbage & other leafy vegetables are predominantly grown by smallholder farmers who face constraints due to socio-economic, institutional, resource & environmental factors (Mdoda et al., 2022). These farmers often lack access to arable land & irrigation water, which limits their ability to produce food for the growing population (Chikozho et al., 2020). These limitations are further amplified by soil deterioration mainly caused by monocropping & the over-application of inorganic fertilisers (Lin et al., 2019; Tao et al., 2024).

To ensure increased crop production & productivity to meet future food demands, it is important to identify & research innovations that may reduce the constraints faced by small-scale & smallholder food producers (Gordillo & Jeronimo, 2017). The importance of cabbage as a food source & its contribution to food security & sovereignty (Mganga, & Sanga, 2024) support this claim. Cabbage is, however, a heavy nutrient feeder & poor soil conditions, such as soil fertility decline & climate-related soil moisture losses, limit its productivity among resource-poor farmers (Manyevere et al., 2014; Gelaye, 2024). Organic soil conditioners such as crop residues & manures have been used to reduce this limitation & increase crop productivity. However, they have a short life in soils as they are easily decomposed (Sindesi et al., 2023). Fallowing was also used to reclaim degraded & nutrient-depleted soils, however, the reduction of arable lands, particularly in African countries, prohibits its wide use (Tryphone & Thomas, 2023). Zeolites have shown potential as stable soil conditioners, with a longer soil life, in improving soil physicochemical properties, such as nutrient retention, nutrient availability & soil moisture holding capacity (Gondek et al., 2023; Hassan et al., 2024; Zheng et al., 2024).

Zeolites are microporous aluminosilicate minerals with high cation exchange capacity (CEC), water absorption & retention properties, & a strong affinity towards ammonium (NH_4^+) & potassium (K^+) ions (Liu et al., 2022). Zeolite has been used to improve crop growth & yield by improving soil conditions that affect crop growth & yield (Noori et al., 2006; Cairo et al., 2017; Jakkula & Wani, 2018; Sindesi et al., 2021; Hassan et al., 2024). The study by Sindesi et al. (2023) demonstrates that crops may have plant-specific growth & yield responses to zeolite application. Understanding the growth changes promoted by applying soil conditioners such as zeolite is vital for researchers before advocating for its use by farmers to maximise vegetable yields (Go et al., 2022). However, limited research exists on the response of specific vegetable crops, such as cabbage, to varying levels of zeolite under controlled conditions, especially across different growing seasons. However, limited research exists on the response of specific vegetable crops, such as cabbage, to varying levels of zeolite under controlled conditions, especially across different growing seasons. This study addresses that gap by

evaluating the effect of different zeolite application levels on growth parameters & fresh head yield of cabbage cv. Copenhagen across two growing seasons. In this study, zeolite was applied specifically to enhance the nutrient and water-holding capacity of sandy soil and to assess its effect on cabbage growth and marketable yield over two growing seasons. Cabbage growth has been measured using agronomic & morphophysiological characteristics such as leaf area, plant height, vegetation index, chlorophyll content, cabbage head size & others (Adzić et al., 2012; Jun et al., 2015). These characteristics are affected by environmental factors & soil conditions such as temperature, soil fertility & moisture (Borges et al., 2018). As such, it was expected that zeolite soil conditioning would positively influence cabbage fresh yield & growth parameters.

MATERIALS AND METHODS

Research Design & Site

The greenhouse pot experiment was conducted at the Agricultural Research Council, Infruitec-Nietvoorbij, Stellenbosch, South Africa (33.914476° S & 18.861322° E). Six-week-old cabbage (*Brassica oleracea var. capitata* L cv Copenhagen) seedlings were transplanted & grown in zeolite-amended sandy soil over 2 seasons, 2017–18 & 2018–19. The 2018 season commenced in late autumn & ended in late spring, while the 2019 season spanned from early autumn to early spring. The treatments were ratios of zeolite to sandy soil on weight-to-weight ratios of 0:10, 1:9, 2:8 & 3:7 zeolite to sandy soil (w/w). The baseline sandy soil had a $pH_{(KCl)}$ of 5.4, which is below the recommended range for optimal cabbage growth. It contained 0.89% organic carbon, 47.00 mg kg^{-1} of available phosphorus (P), 32.76 mg kg^{-1} of nitrate-nitrogen (NO_3-N), & 7.1 mg kg^{-1} of ammonium-nitrogen (NH_4-N) (Sindesi et al., 2024). Micromineral concentrations were 362.6 mg kg^{-1} for iron (Fe), 6.2 mg kg^{-1} for zinc (Zn), 24.2 mg kg^{-1} for manganese (Mn), & 0.4 mg kg^{-1} for copper (Cu) (Sindesi et al., 2022). The used zeolite was of clinoptilolite mineralogy, composed of 64.30% silicate (SiO_2) & 12.70% aluminium oxide (Al_2O_2); other minor minerals of the zeolite are shown in Table 1. The $pH_{(H2O)}$ of the zeolite was 9, & the cation

Table 1. Mineral composition of the used zeolite

Chemical analysis	(%)
TiO_3	0.1
MgO	1.3
Na_2O	2.3
Fe_2O_4	1.3
CaO	1.2
K_2O	1.7

exchange capacity was 16 mg kg^{-1}. Treatments were arranged in a randomised complete block design with six replications. Cabbage was harvested 133 days after transplanting; the management practices carried out during the growth of the cabbage were reported by Sindesi et al. (2021) & Sindesi et al. (2023).

Before transplanting, basal fertilisation was carried out using urea (46% N) & single superphosphate (20% P) at rates of 1.17 g pot^{-1} & 3.00 g pot^{-1}, respectively. Potassium chloride (50% K) was applied at 1.92 g/pot. A supplemental side-dressing of urea (1.11 g pot^{-1}) was applied in two equal split applications at 3 & 6 weeks after transplanting. Cabbage seedlings were transplanted at a rate of one seedling per 30 cm diameter & depth plastic pot. Weeds were controlled manually. Pest management included the application of Makhro Cyper® (cypermethrin, 200 g L^{-1}) at 1 mL per 10 L of water during the first growing season. In the second season, Avi Gard

Mercaptothion® (organophosphate, 500 g L^{-1}) was applied at 15 mL per 10 L of water, due to apparent pest resistance to cypermethrin. All treatments were irrigated to pot capacity (PC) before planting. Soil moisture was monitored throughout the experiment using the gravimetric method & periodic pot weighing. During the study, soil water content was maintained between 50% & 70% of PC. The volume of irrigation applied varied based on fluctuations observed in the gravimetric measurements to ensure consistent moisture levels across treatments. Seasonal irrigation results are published in Sindesi et al. (2023). Furthermore, Growth conditions within the greenhouse, including temperature, humidity, & light, were not actively controlled & were dependent on the external environmental conditions. The greenhouse was covered only with polythene plastic, without any additional climate control systems.

Data Collection

Data on plant height, the number of loose leaves per plant, leaf width & leaf length of cabbage were collected on a continuous basis from three weeks after transplanting until the 19th week after transplanting. Data on the head diameter & circumference were only measured on the day of harvest.

Number of loose leaves

Loose leaves were counted from the stage of pre-cupping till head maturity. All the leaves that started to fold in & were attached to the cabbage head were no longer counted, while only those that were dissociated from the head were counted.

Plant height

Plant height was measured with tape, observing the length between the soil surface & the highest leaf tip.

Leaf area

For leaf area, the leaf width (maximum value perpendicular to the midrib) & the leaf length (maximum value along the midrib) were measured. The leaf area was estimated using the formula of an oval shape, using the leaf length & leaf width as r_1 & r_2 in the formula, leaf area = pi x r_1/2 x r_2/2.

Leaf Chlorophyll content index

Data for leaf chlorophyll content index (CCI) was taken at the top edge of the biggest leaf on each plant, using a chlorophyll content meter CCM-200 plus manufactured by Opti-Sciences, United States of America.

Fresh yield

This study considered two types of cabbage fresh yield i) cabbage heads with loose outer leaves & ii) cabbage heads without loose outer leaves. Both the yield weights were taken on the day of harvest (133 days after transplanting).

Statistical analysis

The maximum values obtained for cabbage growth parameters, throughout the study (CCI, loose number of leaves, plant height & leaf area) were established. Data were then analysed using Statistical Analysis System (SAS) software (version 9.4, SAS

Institute Inc., Cary, NC, USA, 2000) for Analysis of Variance (ANOVA). Seasonal homogeneity of variance was tested with Levene's test, after which the results of both seasons were merged & studied in a single overall ANOVA. The Shapiro-Wilk test was carried out to test for deviation from normality & insignificant interactions. Fisher's least significant difference was calculated at the 5% level to compare treatment means. For all tests, a probability level of 5% was considered significant.

RESULTS AND DISCUSSION

Cabbage growth characteristics

The growth of vegetables, such as cabbage, typically follows a dynamic pattern of initial slow growth, followed by rapid growth, & then slow growth as plants reach maturity. It can be divided into four stages: the seedling stage, the growth acceleration stage (transplant & cupping), head formation, & the maturity stage (Go et al., 2022). According to Ogedegbe & Law-Ogbomo (2013), plant growth is regulated by physical input, while growth patterns & development are governed by input utilisation. In the growth acceleration stage of cabbage, there is little development until the cabbage starts to form the head. This stage mainly increases the number of cabbage leaves, plant height & leaf area (Stranderg & White, 1979). These are important agronomic attributes in cabbage production (Hossain et al., 2015). The faster the maximum values are reached for these growth parameters, the quicker the head is formed, often resulting in better yields during the season.

In this study, the maximum leaf chlorophyll content index (CCI) & leaf area (Fig. 1) were both higher ($p < 0.05$) in the first growing season compared to the second season in all the treatments. With values ranging from 70.03 to 78.04 for CCI in the first growing season & 52.37 to 61.59 in the second growing season. This may be due to favourable growing conditions in the first growing season (mostly spring) for these two cabbage growth parameters (Karungi et al., 2010). The maximum values obtained for leaf CCI were not significantly different ($p > 0.05$) across treatments in the first growing season. However, the values showed a tendency to increase with increasing zeolite application, with the zeolite 20% treatment (CCI =78.04) being the limit to the tendency.

The leaf CCI values obtained for cabbage grown on zeolite-amended soils were generally higher than those grown on the non-amended control treatment in both seasons. The CCI results were consistent with the findings of Saeed et al. (2019), who found that zeolite treatment improved the photosynthetic rate, transpiration rate, stomatal conductance & chlorophyll content-SPAD compared to the control. This observation can be attributed to zeolite's ability to adsorb heavy metals & potentially toxic elements, reducing their availability in the growth medium, thereby reducing uptake & promoting proper plant health (Naveed et al., 2020).

The results further showed a decrease ($p < 0.05$) in the maximum leaf area in the first growing season as zeolite application increased. Contrary to the first season, the leaf area tended to increase with the increase in zeolite application in the second growing season. The observed trends for leaf area may be attributed to zeolites' affinity towards NH_4^+, which has been reported to be responsible for lowering plant growth (Jakkula & Wani, 2018). Zeolite's high affinity for NH_4^+-N allows it to selectively adsorb & retain NH_4^+ ions within its porous structure (Zheng et al., 2024). This can temporarily reduce the availability of NH_4^+-N for plant uptake & nitrification, potentially leading to a

short-term delay in some plant growth parameters. Potentially accounting for the first season's decreased maximum leaf areas. Jarosz et al. (2022) & Liu et al. (2023) suggest that the duration of this effect can vary depending on factors such as the type of zeolite, soil conditions, & environmental factors. Additionally, the gradual increase in maximum leaf area within the second season can be due to nutrients released (from zeolite pores) by zeolite over time, thereby providing a more sustained supply to the plants in the second year (Szatanik-Kloc et al., 2021).
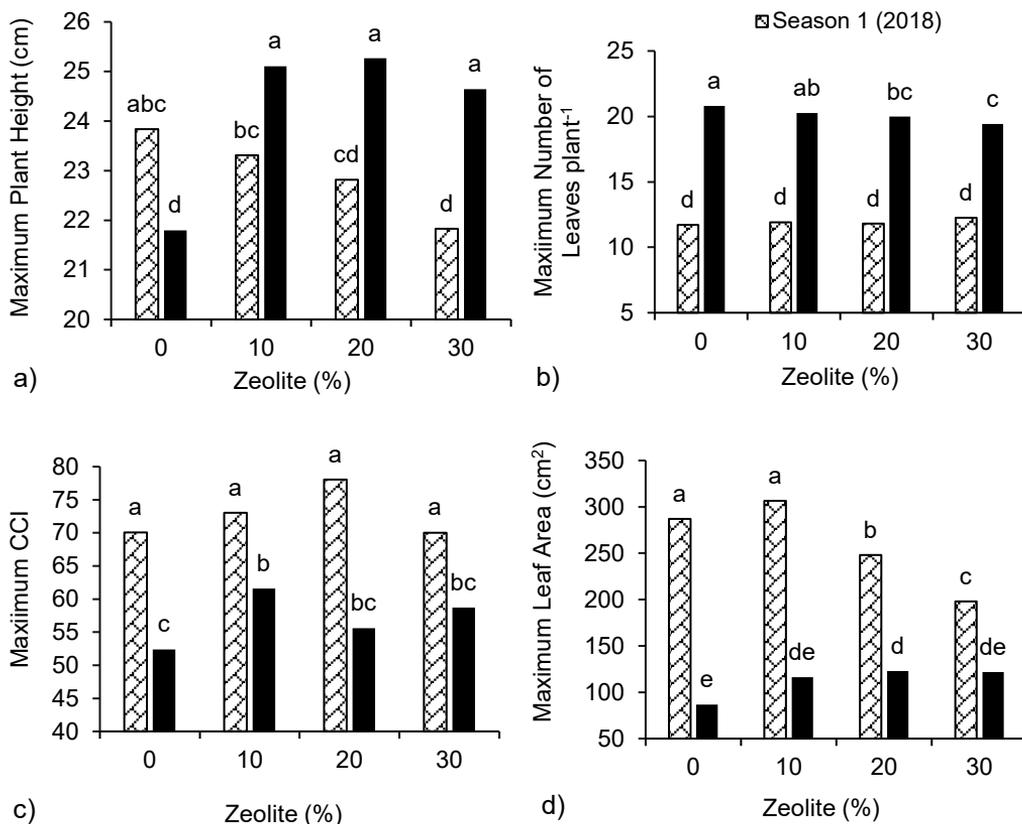
Figure 1. Response of cabbage maximum agronomic characteristics to zeolite application. Panels (a), (b), (c), and (d) represent different growth parameters. Bars sharing the same letter are not significantly different at $p > 0.05$ according to Fisher's Least Significant Difference (*LSD*) test.

For maximum plant height, when the two seasons were compared, the second growing season of cabbage showed improved plant growth in zeolite-amended soils. The plant height of the non-amended treatment reduced from the first growing season to the second season, from 23.84 to 21.79 cm, respectively. This reduction can be attributed to soil degradation & nutrient reduction in the non-amended soil in the second growing season. Belete & Yadete (2023) highlighted that monocropping, together with the removal of crop residues, depletes certain nutrients from the field & encourages soil degradation & reduced crop growth. This can be seen in the plant height result obtained from the non-amended treatment. The study by Sindesi et al. (2023) further reiterated

this, where the soil nitrate (NO_3-N), NH_4-N, phosphorus (P), soil $pH_{(KCL)}$ & Total K levels became reduced ($p < 0.05$) at the end of the second growing season of cabbage. More importantly, the two nitrogen (N) forms (NO_3-N, NH_4-N) are known to play a vital role in the biochemical & physiological functions of a plant in relation to the physical growth of plants such as plant height (Leghari et al., 2016).

On the other hand, the results obtained for plant height on the zeolite amended treatments show the potential of zeolite to slow-release nutrients to plant root zones (Al-Busaidi et al., 2008; Szatanik-Kloc et al., 2021; Sindesi et al., 2023). The results further highlighted the possibility of zeolite requiring time to fully activate within the soil before the full benefit is realised. Additionally, the seasonal order observed in results obtained for the maximum number of loose outer leaves & plant height was in contrast with the observed trends in leaf CCI & leaf area. The second season results tended to have larger values for plant height & number of leaves compared to the first growing season. This may suggest that different seasons influence various plant agronomic characteristics differently. Hatfield & Prueger (2015) found that warm temperatures increased the rate of phenological development, but they found no effect on leaf area or vegetative biomass when compared to normal temperatures.

Moreover, in this study, the relationship between leaf CCI, number of leaves & plant height showed that CCI does not present a holistic approach to monitoring plant growth & health, as suggested by Hidayah & Putri (2019). The results from the second growing season further revealed that cabbage tended to favour the production of several smaller but longer leaves than broader leaves, thereby limiting the biochemical processes (photosynthesis) of each leaf. The higher number of loose outer leaves & taller plants in the second season was consistent with the findings of Červenski et al. (2018), who reported that late planting of cabbage results in large above-ground biomass. This assertion was also confirmed by the cabbage dry matter yields reported by Sindesi et al. (2023). These results on the agronomic characteristics of cabbage also show that zeolite may have a different influence on various plant growth parameters.

Responses of marketable cabbage head traits to zeolite application

The size of the cabbage head is an important attribute considered by the fresh vegetable market. The head size may be rated by head weight or by head dimensions (Kołota & Chohura, 2015). The results obtained for cabbage head diameter & circumference in this study are shown in Fig. 2. In the first growing season, there were no significant differences in the cabbage head diameter & circumference ($p > 0.05$) across treatments. However, in the second growing season, all cabbages grown on the zeolite-amended treatments had significantly larger ($p < 0.05$) head sizes compared to those grown in the non-amended treatments. This may be due to a temporal restriction in nutrient availability caused by zeolites' adsorption of nutrients such as NH_4^+-N, phosphorus (P) & potassium (K). This restriction phenomenon was also observed by Doni et al. (2024), who found an increase in total K & a decrease in available K in vineyard soils amended with zeolite. The authors linked this with zeolites' strong affinity for K^+, which encouraged the cation to be held tightly in the structure of zeolite, thereby increasing its retention while reducing its availability in the soil solution. This reduced availability can also be true with NH_4^+-N. Another possibility for the observed non-significant influence on cabbage head size may be that the cabbages had reverted to gene-regulated sizes (Sun et al., 2021) due to the ineffectiveness of zeolite during this

period & potentially a non-optimal growing season, as most of the growth in the first growing season was in autumn.
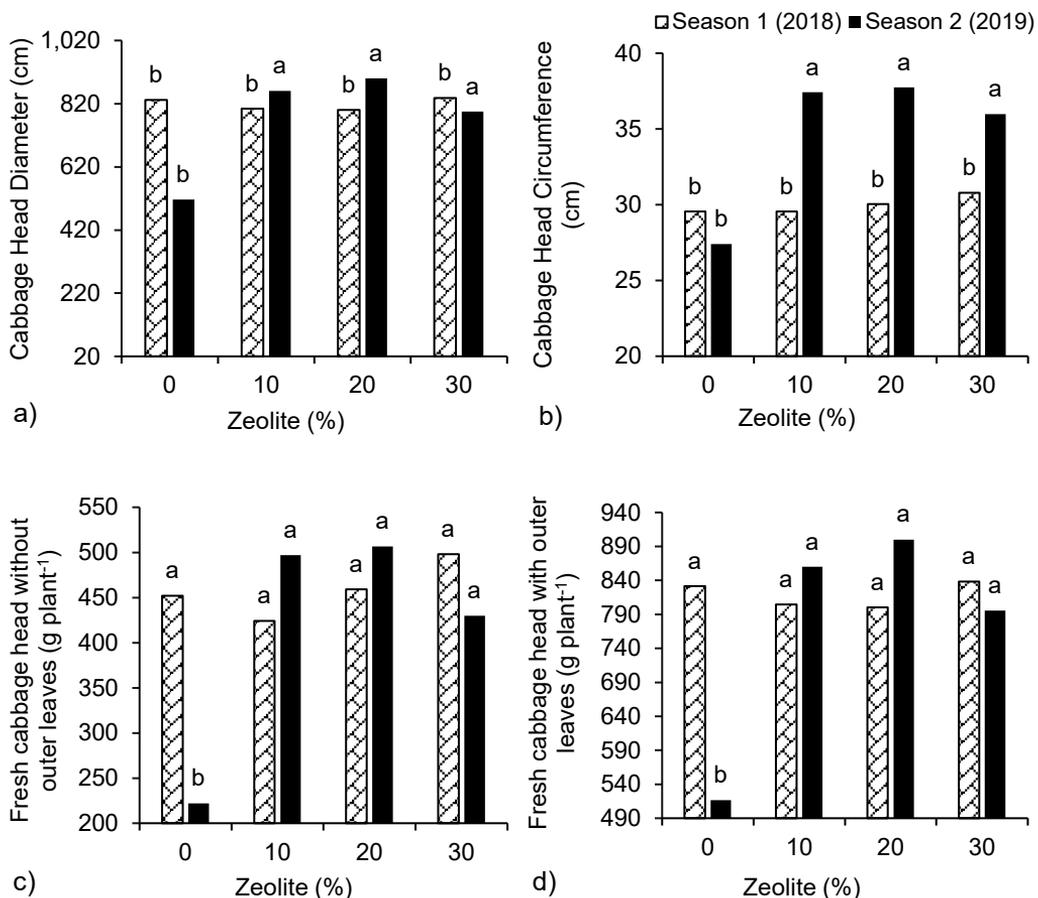
Figure 2. Effect of zeolite on cabbage marketable traits. Panels (a), (b), (c), and (d) represent different marketable traits of cabbage. Bars sharing the same letter are not significantly different at $p > 0.05$ according to Fisher's Least Significant Difference (*LSD*) test.

The head diameters obtained in this study were less than the diameters (12.79 to 14.52 cm) obtained by Tabor et al. (2022). Consequently, the head circumferences were also less than the circumferences ranging between 32.80 to 50.00 cm obtained by Ogedegbe & Ogbomo (2013). The difference in the diameter may be attributed to the differences in environment, plant available water & temperatures. The experiment by Tabor et al. (2022) was conducted in open-field conditions, while the current study was conducted in a greenhouse with no internal environmental manipulation. Hence, there might have been tendencies of heat extremes within the greenhouse. Environmental factors such as soil moisture, temperature and light intensity have been noted to affect crop production (Hazrati et al., 2017). Likewise, the variation between the head circumferences may be due to the fertilisation programme implemented. This study utilised less N fertiliser compared to the study by Ogedegbe & Ogbomo (2013). Gelaye

et al. (2024) note that cabbage is a highly demanding vegetable crop for major plant nutrients, including N. Nitrogen increases plant meristematic cells, which support continuous growth & development by containing active stem cells that divide to produce all the tissues & organs of the plant (Mira et al., 2018). In essence, if a plant is supplied with an optimum amount of nitrogen, there is a tendency to increase leaf cell number & cell size, with an overall increase in leaf production, hence, cabbage is a leafy vegetable.

However, the influence of zeolite on cabbage head circumference & diameter was more obvious in the second growing season. This further reiterated the ability of zeolite to initially adsorb nutrients into its structure, using its high cation exchange capacity properties, thereby reducing their solubility for a period before slowly releasing them into the plant root zone (Li et al., 2013). The results can also be an indication of a long-lasting improvement of soil physicochemical properties by zeolites (Szatanik-Kloc et al., 2021), as cabbage head sizes (diameter & circumference) significantly improved compared to the non-amended treatment in the second growing season, while those of the non-amended treatments reduced from their initial values. On the other hand, for cabbage head yields (with & without the loose outer leaves), there were generally no significant differences ($p > 0.05$) among the treatments and seasons, except for significantly lower weights of cabbage heads grown on the non-amended treatments in the second season. The comparable yields for the non-amended treatment with the zeolite treatments in the first growing season show that the used soil was in good condition to facilitate cabbage production. Additionally, the significantly reduced ($p < 0.05$) yields in the second growing season may be due to the potential decline of soil quality (Karungi et al., 2010), especially in the control treatment. Details of the soil fertility decline were reported by Sindesi et al. (2023).

The observed higher yields (Season 2) from cabbage grown on the zeolite-amended (10 & 20% zeolite) soils agree with the findings of Mahmoodabadi et al. (2009) & Li et al. (2013), who reported on the effects of zeolite on the yields of soybean & kale, respectively. The increase in yields in Season 2 due to zeolite application may be attributed to the high CEC of zeolites. High CEC reduces N losses & heavy metal toxicity, while the porous structure of zeolite increases water & nutrient holding capacity within the root zone, leading to improved crop yields (Ikhajiagbe et al., 2019). Chatzistathis et al. (2021) also found increased root biomass for chestnuts grown on zeolite-treated soils. The increase in root biomass from the study by Chatzistathis et al. (2021) may explain the increases in total biomass, as it is responsible for water & nutrient uptake. The slight decrease observed in cabbage yields from the 20% & 30% zeolite application may be an indication of a negative impact from an over-application of zeolite. The fresh yield of cabbage without loose outer leaves was reduced ($p > 0.05$) by an average of 76.59 g plant^{-1} from 20% zeolite application treatment to 30% zeolite application. This reduction accounted for a 15.12% reduction, while the reduction of the average weight of cabbage heads with outer leaves was only reduced by 11.64% between these treatments. Zeolites have selectivity for major essential nutrients, including NH_4^+, phosphate (PO_4^{2-}), NO_3^-, K^+ & sulphate (SO_4^{2-}), in their unique porous structure. This is beneficial as it reduces nutrient leaching (Mondal et al., 2021). However, when the zeolite is applied in large quantities, this adsorption of nutrients by zeolite may encourage much stronger bonds with the nutrients, which are much larger than the forces used by plants during nutrient assimilation, leading to less nutrient use by plants.

CONCLUSION

Cabbage *cv* Copenhagen responded positively to zeolite application in terms of growth & fresh yield, especially in the second growing season. The study demonstrated that zeolite requires time before it can be fully activated after soil incorporation. These results also demonstrate that zeolite can be used to improve some of the cabbage growth indicators, but not all of them. The results from this study also show that there is a possibility of zeolite over-application within cropping systems. From this study, there is a need to conduct further research on zeolite as a soil conditioner, especially under field conditions. More importantly, long-term field experiments that will determine whether zeolite will provide lasting improvements in the soil's physicochemical properties & plant growth should be explored. Additionally, studies should aim to develop practical application guidelines, including optimal rates, timing, & integration with existing fertilisation regimes, to facilitate the adoption of zeolite use by smallholder & commercial vegetable producers. As demonstrated by this study, smallholder and commercial vegetable producers are encouraged to apply zeolite prior to planting, at an optimum rate of 20% zeolite to sandy soil. Furthermore, zeolite should be applied alongside or just before conventional fertilisers to help synchronise nutrient release with crop demand. In follow-up seasons, nutrient applications should be guided by soil laboratory analyses to avoid over-fertilisation and ensure sustained soil health.

REFERENCES

Adzić, S., Pavlović, S., Jokanovic, M.B., Cvikić, D., Pavlović, N., Zdravković, J. & Prodanovic, S. 2012. Correlation of important agronomic characteristics and yield of medium late genotypes of head cabbage. *Acta Horticulturae* **960**, 159–164. doi: 10.17660/ActaHortic.2012.960.22

Al-Busaidi, A., Yamamoto, T., Inoue, M., Eneji, A.E., Mori, Y. & Irshad, M. 2008. Effects of zeolite on soil nutrients and growth of barley following irrigation with saline water. *Journal of Plant Nutrition* **31**(7), 1159–1173. doi: 10.1080/01904160802134434

Belete, T. & Yadete, E. 2023. Effect of Mono Cropping on Soil Health and Fertility Management for Sustainable Agriculture Practices: A Review. *Journal of Plant Science* **11**, 192–197. doi: 10.11648/j.jps.20231106.13

Borges, C.V., Seabra Junior, S., Ponce, F.S. & Lima, G.P.P. 2018. Agronomic factors influencing Brassica productivity and phytochemical quality. In: M. A. El-Eswari (Eds.), Brassica germplasm –Characterization, breeding and utilization London, UK: *IntechOpen*, pp. 7–74. doi: 10.5772/intechopen.74732

Bousquet, J., Anto, J.M., Czarlewski, W., Haahtela, T., Fonseca, S.C., Iaccarino, G., Blain, H., Vidal, A., Sheikh, A., Akdis, C.A. & Zuberbier, T. 2021. Cabbage and fermented vegetables: from death rate heterogeneity in countries to candidates for mitigation strategies of severe COVID-19. *Allergy* **76**(3), 735–750. doi: 10.1111/all.14549

Cairo, P.C., de Armas, J.M., Artiles, P.T., Martin, B.D., Carrazana, R.J. & Lopez, O.R. 2017. Effects of zeolite and organic fertilizers on soil quality and yield of sugarcane. *Australian Journal of Crop Science* **11**(6), 733–738. doi: 10.21475/ajcs.17.11.06.p501

Červenski, J., Danojević, D., Medić-Pap, S. & Savić, A. 2018. Late cabbage planting density. *Selekcija i semenarstvo* **24**(2), 26–31. doi: 10.5937/SelSem1802026C

Chatzistathis, T., Papaioannou, E., Giannakoula, A. & Papadakis, I.E. 2021. Zeolite and Vermiculite as Inorganic Soil Amendments Modify Shoot-Root Allocation, Mineral Nutrition, Photosystem II Activity and Gas Exchange Parameters of Chestnut (Castanea sativa Mill) Plants. *Agronomy* **11**(1), 109. doi: 10.3390/agronomy11010109

Chikozho, C., Managa, R. & Dabata, T. 2020. Ensuring access to water for food production by emerging farmers in South Africa: What are the missing ingredients?. *Water South Africa* **46**(2), 225–233. doi: 10.17159/wsa/2020.v46.i2.8237

Doni, S., Masciandaro, G., Macci, C., Manzi, D., Mattii, G.B., Cataldo, E., Gispert, M., Vannucchi, F. & Peruzzi, E. 2024. Zeolite and Winery Waste as Innovative By-Product for Vineyard Soil Management. *Environments* **11**(2), 29. doi: 10.3390/environments11020029

Food and Agriculture Organisation. 1988. Traditional Food Plant. Food and Agricultural Organization of the United Nations, Rome, Italy.

Gelaye, Y. 2024. A Systematic Review on Effects of Nitrogen Fertilizer Levels on Cabbage (*Brassica oleracea* var. *capitata* L.) Production in Ethiopia. *The Scientific World Journal* **2024**(1), 6086730. doi: 0.1155/2024/6086730

Go, S.H., Lee, D.H., Na, S.I. & Park, J.H. 2022. Analysis of Growth Characteristics of Kimchi Cabbage Using Drone-Based Cabbage Surface Model Image. *Agriculture* **12**(2), 216. doi: 10.3390/agriculture12020216

Gondek, K., Mierzwa-Hersztek, M. & Jarosz, R. 2023. Effect of willow biochar and fly ash-derived zeolite in immobilizing heavy metals and promoting enzymatic activity in a contaminated sandy soil. *Catena* **232**, 107429. doi: 10.1016/j.catena.2023.107429

Gordillo, G. & Jeronimo, O.M. 2017. Food Security and Sovereignty (Base document for discussion). Food and Agriculture Organisation, Rome.

Hassan, M.U., Shah, S.T., Basit, A., Hikal, W.M., Khan, M.A., Khan, W., Tkachenko, K.G., Brini, F. & Said-Al Ahl, H.A. 2024. Improving Wheat Yield with Zeolite and Tillage Practices under Rain-Fed Conditions. *Land* **13**(8), 1248. doi: 10.3390/land13081248

Hatfield, J.L. & Prueger, J.H. 2015. Temperature extremes: Effect on plant growth and development. *Weather and climate extremes* **10**, 4–10. doi: 10.1016/j.wace.2015.08.001

Hazrati, S., Tahmasebi-Sarvestani, Z., Mokhtassi-Bidgoli, A., Modarres-Sanavy, S.A.M., Mohammadi, H. & Nicola, S. 2017. Effects of zeolite and water stress on growth, yield and chemical compositions of Aloe vera L. *Agricultural Water Management* **181**, 66–72. doi: 10.1016/j.agwat.2016.11.026

Hidayah, F. & Putri, R.E. 2019. Study Relationship of Rice Chlorophyll Content Index (CCI) Value with Rice Prediction Yield Production on Rice Cultivation in West Sumatera. In *IOP Conference Series: Earth and Environmental Science* **327**(1), 012009. doi: 10.1088/1755-1315/327/1/012009

Hossain, M.F., Farhana, T., Raihan, M.Z., Hasan, M.S., Mia, M.M. & Rahman, M.M. 2015. Effect of different fertilizaion practices on the growth and yield of cabbage. *Asian Journal of Medical and Biological Research* **1**(2), 182–186. doi: https://doi.org/10.3329/ajmbr.v1i2.25610

Ikhajiagbe, B., Saheed, M.I. & Okeme, O.J. 2019. Effects of changes in soil cation exchange capacity on the reclamation of lead by Eleusine indica (L.) Gaertn. Fudma. *Journal of Sciences* **3**(4), 176–183.

Jakkula, V.S. & Wani, S.P. 2018. Zeolites: Potential soil amendments for improving nutrient and water use efficiency and agriculture productivity. *Scientific Reviews & Chemical Communications* **8**(1), 1–15.

Jarosz, R., Szerement, J., Gondek, K. & Mierzwa-Hersztek, M. 2022. The use of zeolites as an addition to fertilisers–A review. *Catena* **213**, 106125. doi: 10.1016/j.catena.2022.106125

Jun, T., Dongming, L., Zezhou, L., Limei, Y., Zhiyuan, F., Yumei, L., Mu, Z., Yangyong, Z., Honghao, L., Dengxia, Y.I. & Peitian, S. 2015. Preliminary study of the characteristics of several glossy cabbage (Brassica oleracea var. capitata L.) mutants. *Horticultural Plant Journal* **1**(2), 93–100. doi: 10.16420/j.issn.2095-9885.2015-0009

Karungi, J., Kyamanywa, S. & Ekbom, B. 2010. Organic soil fertility amendments and tritrophic relationships on cabbage in Uganda: Experiences from on-station and on-farm trials. *African Journal of Agricultural Research* **5**(21), 2862–2867.

Kołota, E. & Chohura, P. 2015. Control of head size and nutritional value of cabbage by plant population and nitrogen fertilization. *Acta Scientiarum Polonorum Hortorum Cultus* **14**(2), 75–85.

Leghari, S.J., Wahocho, N.A., Laghari, G.M., HafeezLaghari, A., MustafaBhabhan, G., HussainTalpur, K., Bhutto, T.A., Wahocho, S.A. & Lashari, A.A. 2016. Role of nitrogen for plant growth and development: A review. *Advances in Environmental Biology* **10**(9), 209–219.

Li, J., Wee, C. & Sohn, B. 2013. Effect of ammonium-and potassium-loaded zeolite on kale (Brassica alboglabra) growth and soil property. *American Journal of Plant Sciences* **4**(10), 1976. doi: 10.4236/ajps.2013.410245

Lin, W., Lin, M., Zhou, H., Wu, H., Li, Z. & Lin, W. 2019. The effects of chemical and organic fertilizer usage on rhizosphere soil in tea orchards. *Public Library of Science One* **14**(5), e0217018. doi: 10.1371/journal.pone.0217018

Liu, P., Zhang, A., Liu, Y., Liu, Z., Liu, X., Yang, L. & Yang, Z. 2022. Adsorption mechanism of high-concentration ammonium by Chinese natural zeolite with experimental optimization and theoretical computation. *Water* **14**(15), 2413. doi: 10.3390/w14152413

Liu, D., Huang, Z., Liu, D., Yang, Y., Ding, Y., Luo, Z., Xia, D., Xiao, H., Liu, L., Zhao, B. & Li, M. 2023. Synergistic effect of zeolite and biochar on geotechnical and fertility properties of vegetation concrete prepared by sandy soil. *Construction and Building Materials* **392**, 132029. doi: 10.1016/j.conbuildmat.2023.132029

Mahmoodabadi, R., Ronaghi, A.M., Khayyat, M. & Hadarbadi, G. 2009. Effects of zeolite and cadmium on growth and chemical composition of soybean (Glycine max L.). *Tropical and Subtropical Agroecosystems* **10**(3), 515–521.

Manyevere, A., Muchaonyerwa, P., Laker, M. & Mnkeni, P.N.S. 2014. Farmers' perspectives with regard to arable crop production and deagrarianisation: an analysis of Nkonkobe Municipality, South Africa. *Journal of Agriculture and Rural Development in the Tropics and Subtropics* **115**(1), 41–53.

Mdoda, L., Obi, A., Ncoyini-Manciya, Z., Christian, M. & Mayekiso, A. 2022. Assessment of profit efficiency for spinach production under small-scale irrigated agriculture in the Eastern Cape Province, South Africa. *Sustainability* **14**(5), 2991. doi: 10.3390/su14052991

Mganga, N.D. & Sanga, R.E. 2024. The efficacy of selected local pesticides in prevention of leaf damage and improvement of yield in Brassica rapa subsp. pekinensis L. (Chinese cabbage). *International Journal of Engineering, Science and Technology* **16**(2), 11–20. doi: 10.4314/ijest.v16i2.2

Mira, M.M., Huang, S., Hill, R.D. & Stasolla, C. 2018. Protection of root apex meristem during stress responses. *Plant Signalling & Behaviour* **13**(2), e1428517. doi: 10.1080/15592324.2018.1428517

Mondal, M., Biswas, B., Garai, S., Sarkar, S., Banerjee, H., Brahmachari, K., Bandyopadhyay, P.K., Maitra, S., Brestic, M., Skalicky, M. & Ondrisik, P. 2021. Zeolites enhance soil health, crop productivity and environmental safety. *Agronomy* **11**(3), 448. doi: 10.3390/agronomy11030448

Naicker, A., Palmer, K., Makanjana, O. & Nzama, P.F. 2021. The Impact of the COVID-19 Pandemic on Food Consumption Habits, Food Purchasing Behaviours, and Food Security Status among South Africans. *African Journal of Inter/Multidisciplinary Studies* **3**(1), 131–143. doi: 10.51415/ajims.v3i1.915

Naveed, M., Bukhari, S.S., Mustafa, A., Ditta, A., Alamri, S., El-Esawi, M.A., Rafique, M., Ashraf, S. & Siddiqui, M.H. 2020. Mitigation of nickel toxicity and growth promotion in sesame through the application of a bacterial endophyte and zeolite in nickel-contaminated soil. International *Journal of Environmental Research and Public Health* **17**(23), 8859. doi: 10.3390/ijerph17238859

Noori, M., Zendehdel, M. & Ahmadi, A. 2006. Using natural zeolite for the improvement of soil salinity and crop yield. *Toxicological & Environmental Chemistry* **88**(1), 77–84. doi: 10.1080/02772240500457928

Ogedegbe, S.A. & Law-Ogbomo, K.E. 2013. Growth and yield of cabbage (*Brassica oleracea* L.) as influenced by poultry, manure and NPK application. *Nigerian Journal of Agriculture, Food and Environment* **9**(4), 19–24.

Saeed, Z., Naveed, M., Imran, M., Bashir, M.A., Sattar, A., Mustafa, A., Hussain, A. & Xu, M. 2019. Combined use of Enterobacter sp. MN17 and zeolite reverts the adverse effects of cadmium on growth, physiology and antioxidant activity of *Brassica napus*. *Public Library of Science One* **14**(3), e0213016. doi: 10.1371/journal.pone.0213016

Šamec, D., Pavlović, I. & Salopek-Sondi, B. 2017. White cabbage (*Brassica oleracea* var. *capitata* f. alba): botanical, phytochemical and pharmacological overview. *Phytochemistry reviews* **16**(1), 117–135. doi: 10.1007/s11101-016-9454-4

Sindesi, O.A., Lewu, M.N., Ncube, B., Meyer, A., Mulidzi, A.R. & Lewu, F.B., 2024. Effect of zeolite application on soil enzyme activity of potted sandy soil cultivated with Swiss chard and cabbage. *Eurasian Journal of Soil Science* **13**(4), 284–293. doi: 10.18393/ejss.1496891

Sindesi, O.A., Lewu, M.N., Ncube, B., Mulidzi, R. & Lewu, F.B. 2021. Mineral Composition of Potted Cabbage (Brassica oleracea Var. capitata L.) Grown in Zeolite Amended Sandy Soil. *Agriculture (Pol'nohospodárstvo)* **67**(3), 103–112. doi: 10.2478/agri-2021-0010

Sindesi, O.A., Ncube, B., Lewu, M.N., Mulidzi, A.R. & Lewu, F.B. 2023. Cabbage and Swiss chard yield, irrigation requirement and soil chemical responses in zeolite-amended sandy soil. *Asian Journal of Agriculture and Biology* **1**(1), 202111387. doi: 10.35495/ajab.2021.11.387

Sindesi, O.A., Ncube, B., Lewu, M.N., Mulidzi, A.R. & Lewu, F.B. 2022. Micromineral Content of Swiss Chard (*Beta vulgaris* L. var. *cicla*) Leaves Grown on Zeolite-Amended Sandy Soil. *Indonesian Journal of Agricultural Research* **5**(03), 179–188. doi: 10.32734/injar.v5i03.10038

Sooriyaarachchi, P., Francis, T.V. & Jayawardena, R. 2022. Fruit and vegetable consumption during the COVID-19 lockdown in Sri Lanka: an online survey. *Nutrire.* **47**(2), 12. doi: 10.1186/s41110-022-00161-z

Stranderg, O. & White, J. 1979. Estimating Fresh Market Cabbage Maturity Dates. *Prococeedings Florida State Horticultural Society* **92**, 96–99.

Sun, X., Gao, Y., Lu, Y., Zhang, X., Luo, S., Li, X., Liu, M., Feng, D., Gu, A., Chen, X. & Xuan, S. 2021. Genetic analysis of the 'head top shape' quality trait of Chinese cabbage and its association with rosette leaf variation. *Horticulture Research* **8**, 106. doi: 10.1038/s41438-021-00541-y

Szatanik-Kloc, A., Szerement, J., Adamczuk, A. & Józefaciuk, G. 2021. Effect of low zeolite doses on plants and soil physicochemical properties. *Materials* **14**(10), 2,617. doi: 10.3390/ma14102617

Tabor, G., Atinafu, G., Gebretensay, F., Fikre, D., Asfaw, Y. & Taddese, F. 2022. Effects of spacing on yield and head characteristics of cabbage (Brassica oleracea var. capitata L.) in 2 agro-ecologies of Ethiopia. *African Journal of Agricultural Research* **18**(5), 322–329. doi: 10.5897/ajar2022.15993

Tryphone, G. & Thomas, S.P. 2023. Evaluating limitations of Agroecological practices and stakeholders' response: a case of Uluguru Mountains landscape in Morogoro municipality, Tanzania. *East African Journal of Science, Technology and Innovation* **4**. doi: 10.37425/eajsti.v4i3.676

Tao, D., Delgado-Baquerizo, M., Zhou, G., Revillini, D., He, Q., Swanson, C.S. & Gao, Y. 2024. Maize-alfalfa intercropping alleviates the dependence of multiple ecosystem services on nonrenewable fertilization. *Agriculture, Ecosystems & Environment* **373**, 109141. doi: 10.1016/j.agee.2024.109141

Zheng, J., Luo, X., Wang, R., Yu, H., Xia, G., Elbeltagi, A. & Chi, D. 2024. Zeolite application coupled with film mulched drip irrigation enhances crop yield with less N2O emissions in peanut field. *Soil and Tillage Research* **241**, 106130. doi: 10.1016/j.still.2024.106130

Assessing the yield potential of soybean maturity groups in different Ukrainian climatic zoness

V. Yatsenko[1], N. Yatsenko[2], N. Komisarenko[3],
V. Kravchenko[1], L. Vyshnevska[1], Z. Kovtunyuk[2], A. Lozinska[4] and S. Usyk[4]

[1]Uman National University, Faculty of Agronomy, Department of Crop Production, Instytutska Street, 1, UA20301 Uman, Ukraine
[2]Uman National University, Faculty of Horticulture, Ecology and Plants Protection, Department of Vegetable Growing, Instytutska Street, 1, UA20301 Uman, Ukraine
[3]Uman National University, Faculty of Management, Department of Ukrainian and Foreign Languages, Instytutska Street, 1, UA20301 Uman, Ukraine
[4]Uman National University, Faculty of Agronomy, Department of Agronomy, Instytutska Street, 1, UA20301 Uman, Ukraine
*Correspondence: slaviksklavin16@gmail.com

Abstract. In the context of global climate change, increasing demands for food security, and the need to expand sources of plant-based protein, soybean is gaining particular importance as a highly productive and valuable agricultural crop. Purpose. The study aimed to evaluate the yield potential of soybean cultivars from different maturity groups under various agro-climatic conditions of Ukraine by analysing their adaptability, productivity, and stability. The objective was to justify the selection of maturity groups best suited for specific regions to ensure sustainable soybean production. Methods. Field experiments were conducted in 2023–2024 across three agro-climatic zones: Odesa (Steppe), Cherkasy (Forest-Steppe), and Zhytomyr (Polissia). A total of 26 early- and mid-maturing soybean cultivars of Ukrainian and foreign origin were evaluated. Adaptive variability was assessed using standard statistical methods. Results. Among early-maturing cultivars, Taverna, Eri, and Calgary showed superior individual productivity, surpassing the standard by 9–13% in seed weight per plant and reaching yields up to 3.15 t ha^{-1} in Polissia. These cultivars demonstrated high plasticity and stability across environments. Among mid-maturing cultivars, ES Visitor and ES Collector delivered consistently high yields across all zones, exceeding the standard by 0.09–0.26 t ha^{-1}. Alicia also showed high productivity in the Forest-Steppe and Polissia, making it suitable for regions with moderate moisture. The highest average yield for early-maturing cultivars was recorded in Polissia (2.50 t ha^{-1}), and for mid-maturing ones - in the Forest-Steppe (2.68 t ha^{-1}). Regardless of the zone, Taverna, Eri, Calgary, ES Visitor, and ES Collector demonstrated stable and high productivity. Conclusions. The findings provide a basis for optimising cultivar selection and soybean production technologies, tailored to regional climatic conditions and challenges posed by climate change.

Key words: adaptability, cultivar, grain weight, soybean, yield.

INTRODUCTION

Soybean holds a leading position among oilseed crops worldwide, accounting for up to 44% of global oilseed production and approximately 35% of the total cultivated area under annual and perennial oil crops (Glauben & Svanidze, 2023). Due to its high protein content, soybean is a primary source of plant-based protein for human nutrition, livestock feed, and the processing industry (Voora et al., 2024). Global soybean production is largely concentrated in the United States, Brazil, Argentina, and China, which together account for up to 90% of the total output. At the same time, the growing demand for soybean in the feed and vegetable oil sectors opens up opportunities for further expansion of its cultivation.

The importance of soybean is increasing in the context of climate change, as the crop is highly sensitive to photoperiod and temperature regimes (Li et al., 2020; Abdala et al., 2025). High-temperature stress and droughts, particularly during the flowering (BBCH 60-69) and pod-filling (BBCH 70-79) stages, are increasingly causing significant yield reductions, as confirmed by studies conducted in the Forest-Steppe and Steppe zones of Ukraine (Liu et al., 2019; Biliavska et al., 2021).

Despite the high genetic potential of modern soybean varieties, only 50–60% of this potential is realised under production conditions (Shook et al., 2021). This is largely due to the use of standard cultivation technologies that often fail to account for varietal characteristics and regional environmental conditions. Addressing this issue requires the implementation of region-specific agronomic practices, scientifically justified crop rotation strategies, and the adaptation of technologies to evolving climate conditions (Tkachuk et al., 2022; Zabarna & Chereshniuk, 2024).

In Ukraine, interest in soybean cultivation is growing, as evidenced by an increase in the number of registered varieties from 125 to 279 over the past decade (Rybalchenko, 2022). However, the effective utilisation of this genetic resource requires a deeper understanding of varietal adaptability to local environmental conditions.

The need for this research stems from the necessity to develop scientifically grounded recommendations for the selection of suitable soybean varieties tailored to specific agro-climatic zones of Ukraine, aiming to enhance production efficiency, yield stability, and food security.

The scientific novelty of the study lies in the assessment of the yield potential of soybean varieties from different maturity groups under various soil and climatic conditions in Ukraine, considering their adaptability, ecological plasticity, and resistance to abiotic stress factors. The use of ecological data in analysing variety trials allows for a more accurate interpretation of genotype-environment interactions and enhances the ability to predict varietal performance under changing environmental conditions.

The aim of the research was to determine the yield potential of soybean cultivars of different maturity groups under the agro-climatic conditions of Ukraine by analysing their adaptability, productivity, and stability, with a subsequent justification for the feasibility of cultivating specific maturity groups in particular natural and climatic regions to ensure sustainable soybean production.

MATERIALS AND METHODS

To realise the yield potential of the cultivar, it is necessary to optimally interact with abiotic environmental factors (temperature, humidity, photoperiod), the dynamics of which during the growing season is complex and unpredictable. Humans have no direct control over these factors, and the possibility of partial regulation (in particular, through irrigation, mulching, covering, etc.) is accompanied by an increase in material costs. In this regard, an urgent task is to select and introduce adaptive, stress-resistant soybean cultivars capable of creating high efficiency of cultivation technology in different agroclimatic zones of Ukraine.

The study was conducted in three climatic zones of Ukraine - Steppe (Odesa region), Forest-Steppe (Cherkasy region) and Polissya (Zhytomyr region). The weather conditions during the study period differed significantly both by year and by climate zone. The distribution of precipitation was uneven throughout the study period (Table 1).

Table 1. Climatic conditions of the growing season of soybean plants (according to weather stations in Odesa, Uman and Zhytomyr)

Month	Steppe (Odesa)		Forest-steppe (Uman)		Polissya (Zhytomyr)	
	2023	2024	2023	2024	2023	2024
Precipitation, mm						
April	116.0	70.0	129.6	56.2	84	39.0
May	7.0	35.0	42.4	4.8	0.1	73.0
June	32.0	77.0	15.8	56.5	59.6	81.0
July	48.0	18.0	92.5	17.9	67.8	65.0
August	15.0	20.0	12.0	17.7	22.0	28.0
Σ	218.0	220.0	292.3	153.1	233.5	286.0
Air temperature, °C						
April	10.3	15.1	8.8	13.0	8.7	7.5
May	16.4	16.2	15.4	15.3	15.1	14.3
June	21.0	24.0	19.6	21.2	18.9	20.3
July	24.0	25.6	21.3	24.3	20.8	20.1
August	25.0	26.7	22.9	23.1	22.8	21.0
$\bar{X}, t\,°C$	19.3	21.5	17.6	19.4	17.3	16.6

Thus, although there was sufficient precipitation at the time of soybean sowing, the amount became inadequate after emergence and during the period of intensive growth in May. Rising temperatures further reduced productive moisture reserves, a trend that persisted across all zones until the end of the growing season, except in Polissya in 2024, where precipitation was relatively evenly distributed, ultimately impacting yield formation.

Soil conditions: Steppe - the soil of the experimental field is a low-humus heavy loamy chernozem with an average humus content of up to 2%. The thickness of the humus layer is 65–67 cm, and the humus horizon itself is 35–38 cm. The terrain of the area is predominantly flat with a gentle slope toward the Southwest.

In the Forest-Steppe zone, the soils are podzolized, low-humus, heavy loamy chernozems formed on carbonate loess, with a humus content of up to 2%, deep carbonates (115–120 cm), and a slightly acidic soil reaction.

Table 2. Origin of the studied soybean varieties

Ripeness group	Ripeness group	Cultivar	Originator	Yield, t ha^{-1}
Early maturing	Ukraine	Rhapsody st	Institute of Oilseeds of the National Academy of Agrarian Sciences of Ukraine	1.5–3.0
		Pallada	Institute of Feed and Agriculture of Podillia of the National Academy of Agrarian Sciences of Ukraine	1.5–3.5
		Perepilochka	National Scientific Center 'Institute of Agriculture of the NAAS of Ukraine'	1.6–2.5
		Taverna	Breeding and Genetic Institute - National Center for Seed Science and Variety Research	2.5–4.0
		Fortress	V.Ya. Yuryev Institute of Plant Growing of the National Academy of Agrarian Sciences of Ukraine	2.0–3.0
	Austria	Adelphia	SAATBAU PROBSTDORFER	3.8–5.2
		Adessa	SAATBAU PROBSTDORFER	1.5–3.5
	France	ES DECOR	Euralis Semences	1.8–3.0
		RGT SAKUZA	RAGT	1.6–3.4
	Canada	Eri	Semences Prograin INC.	1.8–3.2
		Calgary	Cerela Inc.	2.0–3.7
		Nunavik	Cerela Inc.	2.0–3.5
Mid-season	Ukraine	Titan st	Breeding and Genetic Institute - National Center for Seed Science and Variety Research, Institute of Feed and Agriculture of Podillia of the National Academy of Agrarian Sciences of Ukraine	2.0–3.3
		Ingus	LLC 'Institute of Organic Agriculture'	1.5–3.2
		Turizas	LLC 'Institute of Organic Agriculture'	1.5–2.5
	Austria	Acardia	Probstdorfer Zaatzucht Gez.m.b.H. and CoKG	2.0–3.5
		Alicia	SAATBAU PROBSTDORFER	4.0–4.5
	Canada	Dara	North American Plant Genetics	2.0–2.8
		Tersia	Semences Prograin INC.	2.5–3.3
		Neptune	Sevita Genetics	1.8–2.6
	France	ES VISITOR	Euralis Semences	2.0–3.5
		ES COLLECTOR	Euralis Semences	2.0–3.4
		ES COMPOSER	Euralis Semences	2.0–3.2
	Poland	Vitalina	Private enterprise 'Scientific breeding' and seed company 'Soybean Age'	2.0–2.5
		Zeus	Private enterprise 'Scientific breeding' and seed company 'Soybean Age'	2.0–2.5
		Carmelita	Monich Ruslan Vasylovych	2.0–3.2

In the Polissya region, the soils are sod-podzolized with a well-developed humus horizon, a humus content of up to 8%, and a neutral pH environment.

Sowing dates: Steppe – April 15–20; Forest-Steppe – April 28–May 5; Polissya - May 10–15. The sowing density was 450 thousand germinating seeds in all growing areas. The cultivation technology was typical and generally accepted for each zone.

In the experiment, new cultivars of cultivated soybeans of early maturing and mid-season groups recommended for the Steppe, Forest-Steppe and Polissya of Ukraine (Table 2).

The standard are the Rhapsody and Titan cultivars, which are the most tested in Ukraine (Methodology for testing cereal, groat, and legume crops for suitability to distribution in Ukraine, 2016). The area of the plot is 250 m^2, repeated four times. During the biometric measurements and formation of crop structure indicators, the generally accepted methods DSTU 4138-2002 (2004) and DSTU 4234:2003 (2004) were used; Methodical requirements in the field of seed production regarding the preservation of varietal and sowing qualities of soybean seeds (2023).

Proteins, oil content were determined by using standard methods described in the procedures of the American Organization of Analytical Chemists (International Organization of International, AOAC International) (Horwitz & Latimer, 2023). The crude oil (fat) was determined using a Soxhlet apparatus (Behr R 106 S, Germany) with petroleum ether, according to the AOAC 920.85 methodology (Horwitz & Latimer, 2023; Yatsenko et al., 2023).

Statistical processing of the results. A large number of methods are used to assess adaptability. Most of them are based on the method of regression analysis, the mathematical model of which for determining the stability and plasticity of cultivars was calculated according to Eberhart and Russell, and is also based on the principles of combining and transforming the effects of the environment and the interaction of the genotype with growing conditions.

$$bi = \Sigma \, (Xij \cdot Ij) \, / \, \Sigma \, Ij \qquad (1)$$

bi – the regression coefficient of the trait for the i-th cultivar under improving or deteriorating environmental conditions; Xij – the value of the trait for the i-th cultivar under the j-th set of conditions; Ij – the index of the j-th conditions, defined as the difference between the mean value of the trait for all cultivars under those conditions and the overall mean value of the trait across all trials.

The coefficient of linear regression of yield of a cultivar shows its reaction to changes in growing conditions. The higher the value of the coefficient ($bi > 1$), the better the response of the cultivar. In the case of $bi < 1$, the cultivar reacts weakly to changes in environmental conditions. Under the condition that $bi = 1$, there is a complete correspondence of the change in the yield of the cultivar in accordance with the change in growing conditions. The lower the stability coefficient, the more stable the cultivar (Eberhart & Russell, 1966).

The general homeostaticity of cultivars (H$_{om}$) was calculated according to the formula (Khangildin, 1984).

$$H_{om} = \frac{\overline{X^2}}{\sigma}, \qquad (2)$$

where $\overline{x}$ – arithmetic average by grade; σ – generalized root mean square deviation.

Breeding value of the cultivar:

$$(S_c) = \overline{X} \cdot \frac{\overline{X}_{lim}}{\overline{X}_{opt}}, \tag{3}$$

where $\overline{X}$ – arithmetic average by grade; $\overline{X}_{lim}$ – limited arithmetic mean; $\overline{X}_{opt}$ – optimal arithmetic mean.

Coefficient of multiplicity (CM). To avoid the linear artifact of the regression coefficient, - the coefficient of multiplicity, which allows comparing the variability of the trait. The higher the numerical value of this coefficient, the stronger the sign changes (Dragavtsev, 1984):

$$CM = \frac{\overline{X}i + bi \cdot yi}{Xi}, \tag{4}$$

where $\overline{X}i$ – average value of the studied characteristic in the i cultivar; bi – linear regression coefficient of i cultivar; yi –average value for all averages for all grades yi for each j point of the experiment.

The average index of ecological plasticity is calculated

$$IEP = \frac{\left(\frac{YC_1}{AYC_1} + \frac{YC_2}{AYC_2} + \cdots + \frac{YC_n}{AYC_n}\right)}{n}, \tag{5}$$

where YC_1, YC_2, YC_n value of trait (yield) in the cultivar in different years of trials; AYC_1, AYC_2, AYC_n – average value of quality of the cultivars in each of variants of the experiment (Gryaznov, 2019).

The annual adaptability coefficient (CA). To determine the adaptive capacity, the coefficient of adaptability of the cultivar (CA) was used.

The absolute average coefficient of adaptability (CAA) is calculated for the cultivar according to the formula (Zhivotkov et al., 1994):

$$CAA = \frac{(XiA) \cdot 100 \cdot Xm)}{100}, \tag{6}$$

where XiA – average yield of the cultivar over years of testing; Xm – multi-year average cultivar yield.

Stress resistance and compensatory ability of cultivars were determined by Rossielle & Hemblin (1981):

$$SR = Y_{min} - Y_{max}, \tag{7}$$

$$CA = \frac{Y_{min} + Y_{max}}{2}, \tag{8}$$

where Y_{min} and Y_{max} – minimum and maximum value of the cultivar characteristic.

The coefficient of variation is a relative value used to characterize the dispersion (variability) of a feature. It is the ratio of SD mean square deviation to the arithmetic mean, expressed as a percentage:

$$CV = \frac{SD}{\overline{X}}, \tag{9}$$

Coefficient of variation on the following ratio scale:

CV < 10% – variation is weak; CV 11–25% – variation is average; CV > 25% – variation is significant.

In the experiments, phenotypic, genotypic and ecological variability of cultivars was determined according to the following formulas (Burton et al., 1953; Shing, et al., 1993):

Genetic variance:

$$\sigma_G^2 = \frac{CM_p - CM_e}{r},\qquad(10)$$

Environmental variance:

$$\sigma_A^2 = CM_e,\qquad(11)$$

Phenotypic variance:

$$\sigma_F^2 = \sigma_G^2 + \sigma_A^2,\qquad(12)$$

Coefficient of genotypic variation (CVG):

$$\frac{\sqrt{\sigma_G^2}}{\overline{X}} \cdot 100,\qquad(13)$$

Coefficient of phenotypic variation (CVP):

$$\frac{\sqrt{\sigma_P^2}}{\overline{X}} \cdot 100,\qquad(14)$$

Coefficient of ecological variation (CVE):

$$\frac{\sqrt{\sigma_E^2}}{\overline{X}} \cdot 100,\qquad(15)$$

where CM_p – generalized root mean square value of the population trait; CM_e – generalized root mean square error, r – number of repetitions.

The results were statistically processed using the arithmetic mean (x) and standard deviation (SD) calculated using Microsoft Excel 2019. Correlations were calculated using Statistica 12 software. The Chaddock scale was used to qualitatively assess the correlation coefficients.

RESULTS

Experimental studies have shown that in terms of plant height, cultivars of the early-ripening group had an average variation (12%), and mid-ripening cultivars had a weak variation (9%). However, the difference in absolute values between the maturity groups was significant. Among the studied cultivars of the early ripening group, only the Pallada cultivar was found to be significantly taller than the standard (by 13 cm) and the Calgary cultivar (+4 cm to st), while all other cultivars were haracterized by a lower plant height of 2–19 cm. The absolute number of cultivars belongs to the medium-sized group, except for the Pallada cultivar, which is tall (Table 3).

Plant height. Mid-maturing cultivars had a higher average plant height (82 cm) compared to early-maturing ones (77 cm). This difference, supported by lower variability (CV = 9% vs. 12%), indicates greater morphological stability within the mid-maturing group. The tallest plants were observed in Pallada (97 cm), Neptune, Zeus, and Vitalina (93–94 cm), suggesting a genetic advantage in vegetative growth for these cultivars.

Total number of nodes. The number of nodes, which reflects morphophysiological activity, averaged 16 in mid-maturing cultivars and only 13 in early-maturing ones. This result is attributed to the longer vegetative period of mid-maturing genotypes and

indicates greater generative organ biomass. The highest node count (18) was recorded in Neptune, Vitalina, and Zeus.

Table 3. Individual productivity of soybean cultivars of different maturity groups, pcs/plant. (BBCH 99), 2023–2024

Cultivar	Height of plants, cm	Total number of nodes, pcs/plant	Height of attachment of lower bean, cm	Number of pods, pcs/plant	Number of grains, pcs/plant
Early ripening					
Rhapsody st	84	14	12	42	44
Pallada	97	17	14	38	40
Perepilochka	69	12	10	29	26
Taverna	65	11	10	53	54
Fortress	69	12	10	33	30
Adelphia	76	13	11	39	36
Adessa	65	11	10	39	35
ES DÉCOR	75	13	11	40	36
RGT SAKUZA	78	14	12	39	36
Eri	79	14	12	49	67
Calgary	87	15	13	46	63
Nunavik	82	14	12	36	39
$\bar{X}$	77	13	12	40	42
SD	9.05	1.6	1.38	6.43	12.17
CV, %	12	12	12	16	29
Mid ripening					
Titan st	71	14	10	42	57
Ingus	76	15	10	33	30
Turizas	79	15	11	33	30
Acardia	74	14	10	38	47
Alicia	86	16	12	46	53
Dara	75	15	10	34	31
Tersia	78	15	11	38	39
Neptune	94	18	13	35	32
ES VISITOR	82	16	11	47	64
ES COLLECTOR	82	16	11	47	64
ES COMPOSER	79	16	11	36	33
Vitalina	93	18	13	32	29
Zeus	94	18	13	35	32
Carmelita	80	16	11	43	47
$\bar{X}$	82	16	11	38	42
SD	7.28	1.3	1	5.24	12.75
CV, %	9	8	9	14	30

Height of the first pod attachment. This trait is critical for efficient mechanised harvesting. Both maturity groups showed average first pod attachment at 11–12 cm; however, Pallada, Neptune, Vitalina, and Zeus had the highest values (13–14 cm), confirming their technological advantage in terms of harvestability.

Number of pods per plant. Early-maturing cultivars showed a broader range of pod numbers - 29 to 53 (CV = 16%), compared to 32 to 47 in mid-maturing cultivars (CV = 14%). Taverna had the highest pod count (53), while ES VISITOR and ES COLLECTOR led the mid-maturing group with 47 pods each. These results demonstrate strong genetic potential for forming numerous generative structures.

Number of seeds per plant. This productivity-defining trait was highest in early-maturing cultivars Eri (147), Calgary (139), and Taverna (120), significantly exceeding the group average (93 seeds). Similarly, mid-maturing ES VISITOR (141) and ES COLLECTOR (140) demonstrated high reproductive potential. In contrast, Perepilochka, Ingus, Turizas, and Vitalina produced the fewest seeds (57–66), far below average, indicating the need for further assessment before widespread adoption.

The analysis confirms that Taverna, Eri, and Calgary are the most productive early-maturing cultivars, combining high pod and seed numbers with favourable pod attachment height. These characteristics suggest strong adaptability, making them well-suited to areas with shorter growing seasons, such as Polissia and the northern Forest-Steppe.

In the mid-maturing group, ES VISITOR, ES COLLECTOR, and Alicia were the most productive, offering high generative output and compatibility with mechanised harvesting. These cultivars are recommended for the Forest-Steppe and northern Steppe zones, where moisture levels are sufficient to support their yield potential.

Cultivars with low pod and seed numbers (Perepilochka, Ingus, Turizas, Vitalina) may still possess breeding value for traits such as stability or specific morphotypes but require further evaluation.

In conclusion, the individual productivity potential of soybean is determined by both genetic factors and the cultivar's adaptive response to environmental conditions. The integration of these factors is essential for optimising cultivar selection strategies under the pressures of climate change.

Among the early-maturing cultivars, the average seed weight per plant ranged from 6.34 to 9.42 g, with the lowest value recorded for Perepilochka, and the highest for Taverna (9.42 g), Eri (9.39 g), and Calgary (9.05 g). The overall trend indicates a relatively high level of variability in productivity within this group, which is likely attributed both to genetic differences among the cultivars and to their heightened sensitivity to fluctuations in agro-climatic conditions.

In the group of mid-maturing cultivars, the seed weight per plant varied from 6.39 to 8.69 g, with the lowest productivity observed in Vitalina, Ingus, Turizas, and Zeus, while the highest values were recorded for ES VISITOR (8.69 g), Titan st (8.44 g), ES COLLECTOR (8.41 g), and Alicia (8.33 g). Compared to the early-maturing group, this subgroup showed a narrower amplitude of variation, indicating a relatively higher level of trait stability and genetic uniformity.

Based on the distribution of seed weight per plant, three conditional categories were identified:

- Highly productive cultivars (> 8.3 g): Taverna, Eri, Calgary, ES VISITOR, ES COLLECTOR, Alicia, Titan st, and Rhapsody.
- Moderately productive cultivars (7.4–8.3 g): Pallada, Carmelita, Acardia, Tersia, RGT SAKUZA, Nunavik, Adelphia, Adessa, and ES COMPOSER.
- Low-productive cultivars (< 7.4 g): Fortress, Perepilochka, Vitalina, Ingus, Turizas, Zeus, Dara, and Neptune.

It is evident that the leading cultivars across both maturity groups include foreign selections (ES VISITOR, ES COLLECTOR, Alicia), as well as several Ukrainian or adapted cultivars (Taverna, Eri, Calgary) that consistently demonstrate high individual productivity under diverse agroecological conditions (see Fig. 1).

	$\bar{X}$	SD	CV, %	LSD_{05}
Early ripening	7.9	0.93	12	0.67
Mid ripening	7.5	0.78	10	0.4
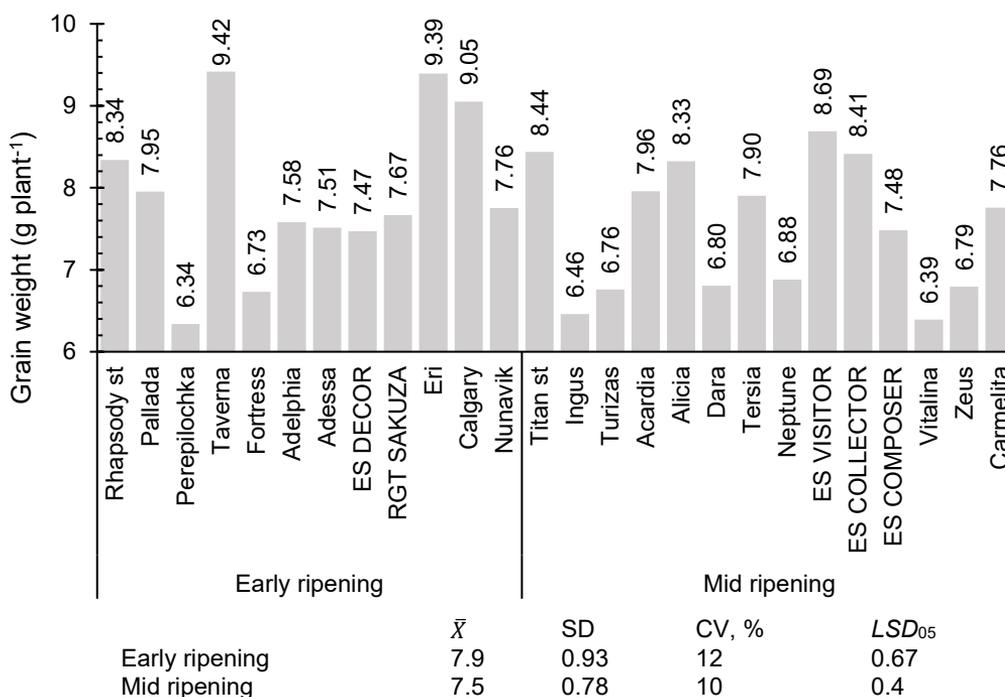
Figure 1. Seed weight per plant of soybean cultivars of different maturity groups.

Only one foreign selection cultivar of the mid-ripening group was found, characterised by a slightly higher grain weight per plant – 'ES VISITOR' -8.7 g (+0.26 g or 3%). All other studied cultivars had lower productivity by 1–24%. The variation of this indicator was average (12%) in the early maturing group and weak (10%) in the mid-ripening group of cultivars.

Thousand Seed Weight. Early-maturing cultivars exhibited a significantly narrower range of thousand seed weight - from 139 to 158 g. The lowest values were recorded for Taverna (139 g), Eri (140 g), and Calgary (142 g), whereas the highest

values were observed in Perepilochka and Adessa (both 158 g). It is worth noting that the seed weight within this maturity group generally corresponds to the medium- and large-seeded types, which is suitable for mechanised harvesting and ensures high seed vigour.

Mid-maturing cultivars were considerably heavier in terms of thousand seed weight - ranging from 151 to 195 g, which indicates a higher biomass potential and improved realisation of the generative function due to a longer vegetation period. Vitalina and Ingus reached the highest value of 195 g, exceeding the corresponding figures in the early-maturing group by 23–56 g.

Seed Bulk Density. The seed bulk density (test weight) reflects not only the thousand seed weight but also the internal structure and compactness of the seed.

In early-maturing cultivars, seed bulk density ranged from 557 to 633 g L^{-1}. The lowest values were found in Taverna (557 g L^{-1}), Eri (560 g L^{-1}), and Calgary (569 g L^{-1}), which correlates with their lower thousand seed weight. The highest values were recorded for Adessa (633 g L^{-1}) and Perepilochka (631 g L^{-1}).

In mid-maturing cultivars, higher bulk density values were observed - ranging from 603 to 781 g L^{-1} indicating denser and more fully developed seeds. The absolute leaders in this trait were Vitalina (781 g L^{-1}), Ingus (780 g L^{-1}), Zeus, and Dara (763 g L^{-1}), reflecting the high commercial quality of their seeds, which is of considerable importance for seed production and food processing.

The mid-maturing group clearly outperformed the early-maturing one in both thousand seed weight and seed bulk density. This advantage is attributable to a longer vegetation period, more efficient utilisation of photosynthetic potential, and greater accumulation of assimilates. Despite lower values, early-maturing cultivars demonstrated greater uniformity and technological stability, making them well-suited for areas with a shorter growing season.

The analysis revealed that the highest values of thousand seed weight and bulk density were produced by mid-maturing cultivars such as Vitalina, Ingus, Dara, Zeus, and Turizas. Due to their large seed size and high density, they can be recommended as benchmark genotypes for seed production and export. Cultivars ES COMPOSER and Tersia also showed high performance in these traits, effectively combining yield potential with seed quality.

Among the early-maturing cultivars, Adessa, Perepilochka, ES DECOR, and Nunavik deserve special attention, as they delivered relatively high thousand seed weight values (157–158 g) and bulk density above 626 g L^{-1}, making them valuable sources of technological traits for regions with a shorter warm season.

In conclusion, thousand seed weight and seed bulk density should be regarded not only as indicators of seed quality but also as markers of a cultivar's adaptive strategy, influencing its efficiency across different climatic zones. The combination of large seed size and high bulk density is of particular importance for improving varietal composition in terms of productivity, stability, and commercial seed quality (Fig. 2).

Figure 2. Weight of 1,000 grains (g) and natural content (g L^{-1}) of soybean cultivars of different maturity groups.
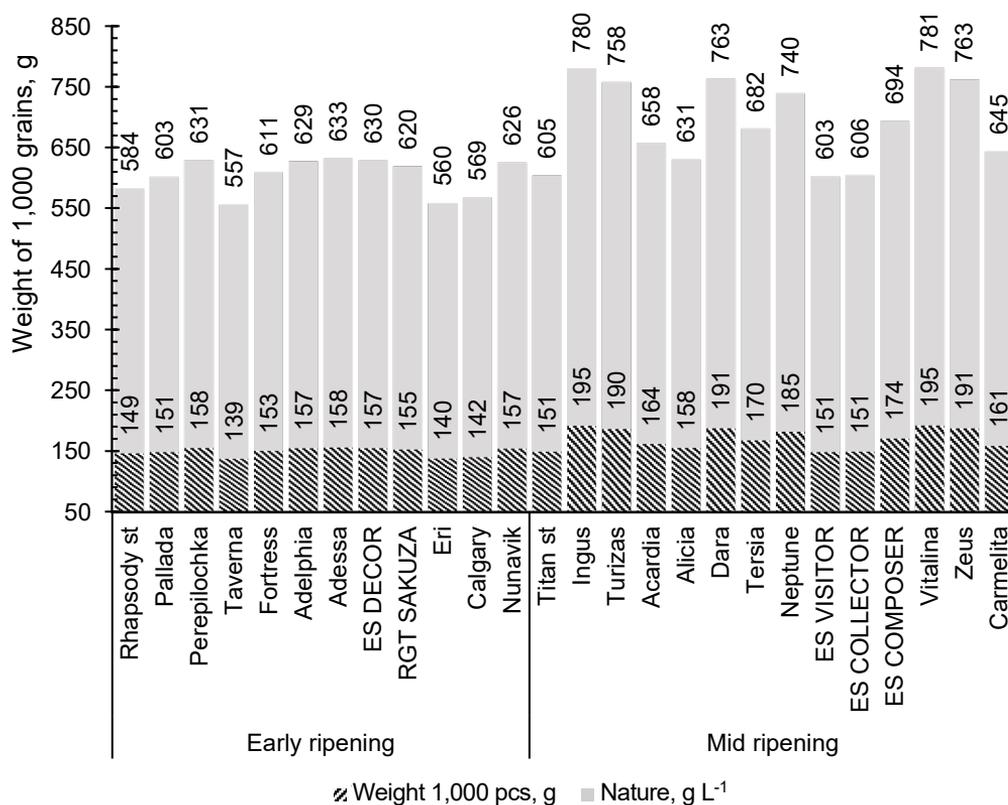
		$\bar{X}$	SD	CV, %	LSD_{05}
Early ripening	Weight 1,000 pcs	151	6.89	5	9.06
	Nature	604	28.05	5	36.24
Mid ripening	Weight 1,000 pcs	173	16.70	10	10.38
	Nature	693	66.81	10	41.58

The data obtained showed that the highest yield of early ripe cultivars can be obtained in Polissya - 2.50 t ha^{-1}, which is 4% higher than in the Forest-Steppe (2.41 t ha^{-1}) and Steppe (1.69 t ha^{-1}) by 48% or 0.81 t ha^{-1}. To ensure high soybean yields, a ratio of coefficients of genetic (CVG) and environmental (CVE) variation close to one or greater than one is required. In our studies, the environmental variation was greater than the genetic variation in each climate zone, indicating a strong dependence of the crop on growing conditions. The results in Table 4 show that the greatest compliance of growing conditions with crop requirements was found in the Forest-Steppe - CVG/CVE = 0.89. In the Steppe, where crops had a better supply of the sum of positive and effective temperatures and a lower supply of moisture, CVG/CVE was 0.80. In Polissia, where moisture supply was sufficient, temperature conditions were less

favourable, and yields were higher, CVG/CVE = 0.70, indicating that the biological potential of soybean cultivars in Polissia was not fully realised (Table 4).

Table 4. Yield of early-ripening cultivars of cultivated soybeans in different climatic zones of Ukraine

Cultivar	Steppe			Forest-steppe			Polissya		
	2023	2024	$\bar{X}$	2023	2024	$\bar{X}$	2023	2024	$\bar{X}$
Rhapsody st	1.78	1.38	1.58	2.77	1.98	2.38	3.03	3.20	3.12
Pallada	1.80	1.40	1.60	2.88	2.00	2.44	2.55	2.20	2.38
Perepilochka	1.74	1.24	1.49	1.90	1.31	1.61	2.12	1.62	1.87
Taverna	2.57	2.20	2.39	2.99	2.55	2.77	3.00	3.01	3.01
Fortress	1.98	1.46	1.72	2.29	1.55	1.92	1.85	1.70	1.78
Adelphia	1.66	1.33	1.50	2.95	2.10	2.53	2.28	2.40	2.34
Adessa	1.59	1.28	1.44	3.00	2.00	2.50	2.24	2.55	2.40
ES DECOR	1.67	1.35	1.51	2.77	2.00	2.39	2.35	2.64	2.50
RGT SAKUZA	1.71	1.38	1.55	2.91	2.06	2.49	2.35	2.40	2.38
Eri	2.40	1.79	2.10	3.14	2.51	2.83	3.00	2.92	2.96
Calgary	2.20	1.81	2.01	3.15	2.20	2.68	2.88	2.84	2.86
Nunavik	1.60	1.30	1.45	3.00	1.77	2.39	2.45	2.33	2.39
LSD_{05}	0.15	0.13	0.29	0.19	0.16	0.33	0.20	0.17	0.41
$\bar{X}$	1.89	1.49	1.69	2.81	2.00	2.41	2.51	2.48	2.50
SD	0.31	0.28	0.29	0.31	0.28	0.33	0.37	0.47	0.41
CV, %	17	19	17	12	17	14	15	19	16
CVG, %			17			20			12
CVE, %			21			22			17
CVP, %			27			29			21
CVG/CVE			0.80			0.89			0.70

The data analysis showed that regardless of the climatic zone of cultivation, high-yielding cultivars were Taverna (2.20–3.01 t ha^{-1} by zone), Eri (1.79–3.14 t ha^{-1}) and Calgary (1.81–3.15 t ha^{-1}).

Research has shown that the Perepilochka, Fortress and Nunavik cultivars were the least productive, with yields 12–30% lower than the standard. The cultivars Pallada, RGT SAKUZA, Adelphia, Adessa and ES DECOR were characterised by a yield lower than the standard by 9–10%, and the cultivars Taverna, Eri and Calgary were higher by 7–15%.

The genetic and statistical analysis of yields showed that the cultivars Perepilochka, Taverna, and Fortress were the most stable ($\sigma^2 d = 0.53$–0.56). The study found that the same cultivars had plasticity $bi > 1$ and stability $\sigma^2 d > 0$, meaning they would be productive under favourable growing conditions. Other cultivars had $bi < 1$ and $\sigma^2 d > 0$, indicating their ability to produce better results under favourable conditions, but unstable yields.

The cultivars Rhapsody st, Pallada, Adelphia, Adessa, ES DECOR, RGT SAKUZA, Eri, Calgary, and Nunavik can be classified as intensive in terms of plasticity (bi), while the others are plastic. The cultivars differed significantly in homeostaticity from 2.31 to 6.25, which confirms the stability or vice versa - the plasticity of a particular cultivar. The cultivars Taverna and Eri were distinguished by high breeding value and

compensatory ability. The Taverna, Eri, and Calgary cultivars were characterised by a higher coefficient of adaptability compared to the standard, where AAC was greater than 1 (Table 5).

Table 5. Generalised parameters of adaptive and productive potential of early-ripening cultivars of cultivated soybean in different climatic zones of Ukraine (2023–2024)

Cultivar	$\bar{X}$	$\sigma^2 d$	bi	Hom	Sc	CM	IEP	SR	CA	AAC
Rhapsody st	2.36	0.82	1.39	4.69	2.16	2.29	1.06	-1.82	2.29	1.07
Pallada	2.14	0.70	1.06	3.86	1.96	2.09	0.97	-1.48	2.14	0.97
Perepilochka	1.66	0.56	0.52	2.31	1.52	1.69	0.76	-0.88	1.68	0.75
Taverna	2.72	0.55	0.66	6.25	2.49	1.53	1.26	-0.81	2.61	1.24
Fortress	1.81	0.53	0.44	2.75	1.66	1.54	0.84	-0.83	1.88	0.82
Adelphia	2.12	0.72	1.13	3.79	1.94	2.17	0.96	-1.62	2.14	0.96
Adessa	2.11	0.76	1.25	3.76	1.93	2.30	0.95	-1.72	2.14	0.96
ES DECOR	2.13	0.71	1.12	3.83	1.95	2.15	0.96	-1.42	2.06	0.97
RGT SAKUZA	2.14	0.70	1.09	3.85	1.96	2.13	0.97	-1.53	2.15	0.97
Eri	2.63	0.68	1.01	5.83	2.41	1.85	1.20	-1.35	2.47	1.19
Calgary	2.51	0.69	1.06	5.33	2.30	1.92	1.15	-1.34	2.48	1.14
Nunavik	2.36	0.76	1.27	3.64	1.90	2.35	0.93	-1.70	2.15	0.94
LSD_{05}	0.13									
$\bar{X}$	2.20									
SD	0.30									
CV, %	14									
CVG, %	25									
CVE, %	26									
CVP, %	36									
CVG/CVE	0.97									

Note: $\sigma^2 d$ – stability; bi – coefficient of linear regression; Hom – homeostaticity; Sc – breeding value; cm – coefficient of multiplicity; IEP – index of ecological plasticity; SR – stress resistance; CA – compensatory ability; AAC – absolute average coefficient of adaptability.

Table 6 shows that the CVG/CVE ratio is 0.97, which confirms that the environmental conditions of the Steppe, Forest-Steppe and Polissya fully meet the requirements of the crop for the formation of a stable and high yield.

The study of the yield dynamics of cultivated soybean cultivars in the Steppe showed that only the Alicia cultivar was equal to the standard – the Titan cultivar with a yield of 1.63 t ha^{-1}. Two cultivars were significantly higher yielding than the standard - ES VISITOR (1.89 t ha^{-1}) and ES COLLECTOR (1.83 t ha^{-1}), which are 0.26 and 0.21 t ha^{-1} higher, respectively. All other tested cultivars in the Steppe zone had yields below the standard by 0.06–0.33 t ha^{-1}.

In the Forest-Steppe, the general trend was the same as in the Steppe, but the difference between cultivars was more significant. For example, the cultivars Alicia, ES VISITOR and ES COLLECTOR had yields that were slightly higher than or equal to the standard -2.63–2.68 t ha^{-1}. All other tested cultivars produced significantly lower yields - by 0.26–0.73 t ha^{-1}.

In Polissya, the general trend was the same: the Alicia cultivar significantly outperformed the standard at 2.96 t ha^{-1} (+0.28 t ha^{-1}), while the ES VISITOR and ES COLLECTOR cultivars were slightly better at 0.12 and 0.09 t ha^{-1}. The cultivar Carmelita was characterised by a slightly lower yield - 2.62 t ha^{-1} (-0.06 t ha^{-1}) and all other studied cultivars - significantly - 0.16–0.63 t ha^{-1} (Table 6).

Table 6. Yield of mid-season cultivars of cultivated soybeans in different climatic zones of Ukraine

Cultivar	Steppe			Forest-steppe			Polissya		
	2023	2024	$\bar{X}$	2023	2024	$\bar{X}$	2023	2024	$\bar{X}$
Titan st	1.83	1.42	1.63	3.07	2.20	2.64	2.70	2.66	2.68
Ingus	1.52	1.30	1.41	2.30	1.55	1.93	2.00	2.10	2.05
Turizas	1.57	1.20	1.39	2.40	1.60	2.00	2.12	2.20	2.16
Acardia	1.50	1.20	1.35	3.02	2.10	2.56	2.65	2.40	2.53
Alicia	1.85	1.40	1.63	2.89	2.40	2.65	2.76	3.16	2.96
Dara	1.59	1.27	1.43	2.44	1.63	2.04	2.10	2.14	2.12
Tersia	1.72	1.33	1.53	2.86	1.89	2.38	2.54	2.40	2.47
Neptune	1.59	1.30	1.45	2.41	1.55	1.98	2.20	2.44	2.32
ES VISITOR	1.97	1.80	1.89	2.96	2.40	2.68	2.90	2.70	2.80
ES COLLECTOR	1.85	1.81	1.83	2.93	2.33	2.63	2.80	2.75	2.78
ES COMPOSER	1.70	1.31	1.51	2.84	1.80	2.32	2.20	2.30	2.25
Vitalina	1.50	1.10	1.30	2.26	1.55	1.91	2.00	2.20	2.10
Zeus	1.56	1.12	1.34	2.46	1.80	2.13	2.10	2.37	2.24
Carmelita	1.74	1.40	1.57	2.94	2.20	2.57	2.31	2.92	2.62
LSD_{05}	0.12	0.08	0.17	0.22	0.15	0.29	0.16	0.22	0.29
$\bar{X}$	1.68	1.37	1.53	2.70	1.92	2.31	2.41	2.45	2.43
SD	0.15	0.21	0.17	0.29	0.32	0.29	0.31	0.30	0.29
CV, %	9	15	11	11	17	13	13	12	12
CVG, %			14			19			9
CVE, %			16			22			13
CVP, %			21			29			16
CVG/CVE			0.86			0.89			0.71

The analysis of the average grain yields of cultivated soybean cultivars showed that the yields in the Forest-Steppe (2.31 t ha^{-1}) and Polissya (2.43 t ha^{-1}) differed only slightly, within 5%. Whereas the productivity of cultivars in the Forest-Steppe and Polissya exceeded that in the Steppe (1.53 t ha^{-1}) by 51 and 59%, or 0.78 and 0.90 t ha^{-1}, respectively. At the same time, the analysis of compliance of growing conditions with crop requirements in the Polissya zone was the lowest -CVG/CVE = 0.71, and in the Steppe and Forest-Steppe, 0.86 and 0.89, respectively. In other words, the conditions of the Forest-Steppe and Steppe best meet the requirements of the crop for the formation of a stable and high yield.

Statistical analysis of the yield parameters showed that Vitalina, Inguz, Turizas, and Dara were the most stable ($\sigma^2 d = 0.60–0.65$) but low-yielding cultivars. The cultivars ES VISITOR and ES COLLECTOR proved to be consistently high-yielding, regardless of the growing zone. The Alicia cultivar, which was characterised by high yields in the Forest-Steppe and Polissya, was found to be unstable. The research showed that the cultivars Titan, Arcadia, Alicia, Tersia, ES COMPOSITOR and Carmelita were

characterised by a ratio of plasticity bi > 1 and stability σ²d > 0, i.e. they will be productive under favourable growing conditions. Other cultivars had bi < 1 and σ²d > 0, indicating their ability to produce better results under favourable conditions, but unstable yields (Table 7).

Table 7. Generalised parameters of adaptive and productive potential of mid-season cultivars of cultivated soybean in different climatic zones of Ukraine (2023–2024)

Cultivar	$\bar{X}$	σ²d	bi	Hom	Sc	CM	IEP	SR	CA	AAC
Titan st	2.31	0.75	1.19	4.05	1.67	2.07	1.10	-1.65	2.25	1.11
Ingus	1.80	0.60	0.75	2.44	1.29	1.88	0.87	-1.00	1.80	0.86
Turizas	1.85	0.65	0.89	2.58	1.33	2.00	0.88	-1.20	1.80	0.88
Acardia	2.15	0.80	1.32	3.48	1.55	2.28	1.01	-1.82	2.11	1.03
Alicia	2.41	0.78	1.24	4.39	1.74	2.07	1.15	-1.76	2.28	1.15
Dara	1.86	0.63	0.84	2.62	1.34	1.94	0.89	-1.17	1.86	0.89
Tersia	2.12	0.72	1.10	3.41	1.53	2.09	1.01	-1.53	2.10	1.02
Neptune	1.92	0.67	0.93	2.77	1.38	2.01	0.92	-1.14	1.87	0.92
ES VISITOR	2.46	0.67	0.92	4.56	1.77	1.78	1.19	-1.16	2.38	1.18
ES COLLECTOR	2.41	0.67	0.94	4.40	1.74	1.81	1.17	-1.12	2.37	1.15
ES COMPOSER	2.03	0.70	1.01	3.10	1.46	2.04	0.97	-1.53	2.08	0.97
Vitalina	1.77	0.65	0.88	2.36	1.27	2.04	0.84	-1.16	1.68	0.85
Zeus	1.90	0.68	0.98	2.73	1.37	2.08	0.91	-1.34	1.79	0.91
Carmelita	2.25	0.75	1.14	3.83	1.62	2.06	1.07	-1.54	2.17	1.08
LSD_{05}	0.14									
$\bar{X}$	2.09									
SD	0.25									
CV, %	12									
CVG, %	26									
CVE, %	26									
CVP, %	37									
CVG/CVE	0.98									

Note: σ²d – stability; bi – coefficient of linear regression; Hom – homeostaticity; Sc – breeding value; cm – coefficient of multiplicity; IEP – index of ecological plasticity; SR – stress resistance; CA – compensatory ability; AAC – absolute average coefficient of adaptability.

The cultivars differed significantly in homeostaticity from 2.36 to 4.56, which confirms the stability or vice versa - the plasticity of a particular cultivar. 'Titan', Alicia, ES VISITOR, ES COLLECTOR and Carmelita cultivars were distinguished by high breeding value (Sc). The high-yielding cultivars Alicia, ES VISITOR and ES COLLECTOR were characterised by a higher adaptability coefficient compared to the standard, where the AAC was more than 1, although the standard cultivars 'Titan' and 'Carmelita' also had AAC above 1. Table 5 shows that the CVG/CVA ratio is 0.98, which confirms that the environmental conditions of the Steppe, Forest-Steppe and Polissya fully meet the requirements of the crop for a stable and high yield.

As a result of statistical calculations, a strong inverse correlation was found on the Chaddock scale between soybean yield and air temperature during the growing season: for early-ripening cultivars, $r = -0.7120$ and and $r = -0.8031$ for mid-ripening cultivars. A noticeable relationship was found between yield and precipitation: in early-ripening cultivars, $r = 0.5077$ and – $r = 0.5597$ in mid-ripening cultivars (data not shown).

The content of crude protein and oil in soybean seeds is among the key biochemical indicators that determine its intended use - either as a source of plant protein for the food and feed industries or as an oil-rich raw material. Analysis of the variation in these parameters among cultivars of different origins and maturity groups, grown in three agro-climatic zones of Ukraine (Steppe, Forest-Steppe, and Polissia), allows identification of the most promising cultivars for specific utilization purposes (Table 8).

According to the results, the protein content in early-maturing soybean cultivars ranged from 34.32% to 41.46%. The highest average protein content was recorded in Canadian cultivars Nunavik (41.46%), Eri (40.42%), and Calgary (40.23%), indicating the high genetic potential of these lines for protein productivity. Ukrainian cultivars (Titan st, Turizas, Ingus) also showed worthy results, with mean protein contents ranging from 36.61% to 38.56%, which is acceptable for food and feed applications.

The variation analysis revealed that the lowest standard deviation in protein content was observed under Forest-Steppe conditions (SD = 1.91; CV = 5%), indicating high stability of trait expression in this agro-climatic zone. In contrast, the Steppe region showed the highest average value (X = 39%) due to the presence of high-protein cultivars, albeit with slightly higher variability (CV = 6%).

The average oil content in early-maturing cultivars ranged from 17.33% to 24.07%, while in medium-maturing cultivars it ranged from 18.58% to 24.01%. The highest oil content was observed in the Ukrainian cultivars Pallada (24.07%) and Fortress (24.00%), as well as in the Austrian cultivar

Table 8. Protein content in soybean grain of different maturity groups in different climatic zones of Ukraine (2023–2024)

Cultivar	Protein content, %			
	Steppe	Forest-steppe	Polissya	$\bar{X}$
Early ripening				
Rhapsody st	37.25	37.27	36.00	36.84
Pallada	36.81	35.51	34.95	35.76
Perepilochka	36.79	36.81	36.98	36.86
Taverna	37.57	36.99	36.33	36.97
Fortress	36.41	35.65	35.09	35.71
Adelphia	41.20	40.34	39.70	40.41
Adessa	37.53	36.90	36.32	36.91
ES DECOR	39.47	38.65	38.04	38.72
RGT SAKUZA	41.20	40.34	39.70	40.41
Eri	42.18	39.57	39.52	40.42
Calgary	41.04	40.04	39.61	40.23
Nunavik	42.16	41.28	40.96	41.46
X	39	38	38	38
SD	2.19	1.91	1.98	2.01
CV, %	6	5	5	5
Mid ripening				
Titan st	39.47	38.86	37.35	38.56
Ingus	36.58	37.38	35.89	36.61
Turizas	37.00	37.76	36.26	37.01
Acardia	36.29	34.68	32.01	34.32
Alicia	41.61	39.62	37.31	39.52
Dara	36.28	36.87	35.40	36.18
Tersia	34.46	35.02	33.63	34.37
Neptune	40.20	40.85	39.23	40.10
ES VISITOR	38.14	36.10	33.19	35.81
ES COLLECTOR	37.19	36.65	33.72	35.85
ES COMPOSER	38.29	38.91	37.36	38.19
Vitalina	36.66	37.26	35.78	36.56
Zeus	38.38	39.01	37.46	38.28
Carmelita	38.95	39.29	37.73	38.66
X	38	38	36	37
SD	1.79	1.73	2.00	1.81
CV, %	5	5	6	5

Akardia (24.01%). Conversely, Canadian cultivars such as Nunavik (17.33%) and Eri (18.01%) had significantly lower oil content, which is typical for protein-oriented genotypes.

The Polissia region exhibited the lowest variability in oil content (CV = 6%) with fairly high average values, making it suitable for the cultivation of oilseed-type cultivars with stable traits. In contrast, the Steppe region demonstrated the highest variability (CV = 14%), necessitating a careful selection of cultivars based on their adaptability to stress conditions (Table 9).

A clearly defined negative correlation between protein and oil content (data not shown) was established, which reflects the competitive allocation of resources within the seed. Therefore, cultivars with elevated protein levels (particularly Canadian ones) tend to exhibit lower oil content. This trade-off should be considered when defining the technological purpose of cultivar use.

Table 9. Oil content in soybean grain of different maturity groups in different climatic zones of Ukraine (2023–2024)

Cultivar	Oil content, %			
	Steppe	Forest-steppe	Polissya	$\bar{X}$
Early ripening				
Rhapsody st	23.81	22.81	21.19	22.60
Pallada	24.78	24.66	22.78	24.07
Perepilochka	24.76	23.13	20.35	22.74
Taverna	22.70	23.25	21.63	22.52
Fortress	24.92	24.31	22.76	24.00
Adelphia	19.00	18.70	18.06	18.58
Adessa	23.50	23.78	21.45	22.91
ES DECOR	19.89	20.09	18.86	19.61
RGT SAKUZA	18.40	18.14	17.66	18.07
Eri	17.55	18.39	18.11	18.01
Calgary	19.15	18.29	17.67	18.37
Nunavik	17.08	17.16	17.77	17.33
X	21	21	20	21
SD	2.92	2.71	1.96	2.50
CV, %	14	13	10	12
Mid ripening				
Titan st	20.28	21.07	21.05	20.80
Ingus	24.05	22.12	21.13	22.43
Turizas	22.61	21.56	21.73	21.96
Acardia	23.80	24.05	24.18	24.01
Alicia	18.90	18.36	20.85	19.37
Dara	23.80	22.73	22.91	23.15
Tersia	23.75	23.77	23.57	23.70
Neptune	19.95	18.50	20.31	19.59
ES VISITOR	21.82	23.52	23.84	23.06
ES COLLECTOR	22.22	22.60	23.46	22.76
ES COMPOSER	21.82	21.56	21.48	21.62
Vitalina	23.01	22.34	22.52	22.62
Zeus	21.82	21.56	21.08	21.48
Carmelita	21.32	20.58	20.60	20.83
X	22	22	22	22
SD	1.52	1.66	1.28	1.44
CV, %	7	8	6	7

DISCUSSION

The results of the conducted research fully confirm the key statements outlined in the introduction, particularly regarding the importance of varietal composition and agroecological adaptation of soybean cultivars to specific soil and climatic growing conditions. As noted in the introduction (Glauben & Svanidze, 2023, modern soybean production is characterised by a high degree of intensification and requires new cultivars capable of ensuring stable yields under changing climatic conditions. In this context, the observed variability in morphobiometric traits and yield structure elements between early- and mid-maturing soybean cultivars indicates significant differences in responses to environmental factors, which is fully consistent with the findings of Voora et al. (2024) and Zabarna & Chereshniuk (2024).

The research confirmed the conclusions of Liu et al. (2019) and Abdala et al. (2025) regarding soybean sensitivity to abiotic stresses, particularly high temperatures during the flowering–pod-filling phase. The identified strong negative correlation between air temperature during the growing season and yield ($r = -0.7120$ for early-maturing and $r = -0.8031$ for mid-maturing cultivars) confirms the critical impact of temperature stress on the formation of generative organs. Simultaneously, the established positive correlation between yield and precipitation ($r = 0.5077–0.5597$) aligns with the findings of Tkachuk et al. (2022), highlighting the need to adapt technologies to new moisture and temperature regimes.

The observed wide variation in plant height, number of nodes, pods and seeds, as well as thousand-seed weight and bulk density, indicates substantial genetic differences among the cultivars, which, according to Melnyk et al. (2020), points to differing levels of ecological plasticity. For instance, the cultivars Taverna, Eri, and Calgary demonstrated high individual productivity and adaptability across all climatic zones, confirming the hypothesis regarding their broad ecological suitability and supporting their recommendation for the Polissia, Forest-Steppe, and even Steppe zones.

The presence of significant inter-varietal differences in homeostatic index, breeding value, and compensatory ability makes it possible to identify cultivars with high breeding potential. Thus, the cultivars Taverna, Eri, Calgary, Alicia, ES VISITOR and ES COLLECTOR not only exceeded the standard in terms of yield but also had a high adaptability index, indicating their suitability for wide geographical testing, as noted by Hrabovskyi et al. (2024). At the same time, the observed trend of decreasing yields in the Steppe zone due to rising temperatures and water shortages confirms the need for the development of cultivars specialised for extreme conditions, as emphasised in the introduction.

Of particular note is the difference in yield potential realisation between zones: although the highest average yields were recorded in the Polissia zone, the the ratio of genetic and environmental variation (CVG/CVE) = 0.70 indicates lower efficiency of genetic potential realisation compared to the Forest-Steppe (0.89) and Steppe (0.80). This is entirely consistent with the statement regarding soybean's high dependence on growing conditions and the need to improve varietal agronomy (Shook et al., 2021).

Overall, the obtained results confirm the importance of long-term variety trials across different agroclimatic conditions using multifactorial analysis. They also demonstrate that breeding efforts aimed at increasing varietal adaptability should be based not only on yield potential but also on a comprehensive assessment of homeostatic stability, performance consistency, and responsiveness to changing climatic factors. Such approaches will enable the development of a scientifically grounded varietal policy in the context of agro-landscape transformations and climate instability, as required by modern agricultural science.

The results of this study are consistent with international research, emphasising the importance of taking into account climatic factors, cultivar adaptability, and the use of models to predict yields, which demonstrates the relevance of the study in the context of global climate change and the need to adapt agricultural practices.

CONCLUSIONS

Thus, based on the state results of experimental studies on agrobiological evaluation of soybean cultivars of different maturity groups, it can be stated that in different climatic zones (Steppe, Forest-Steppe and Polissya) of Ukraine, the structure of soybean grain yields largely depended on the biological characteristics of the cultivar and maturity group. The obtained results confirm considerable diversity in protein and oil content among soybean cultivars, which is driven by both genetic characteristics and environmental growing conditions. A differentiated approach to cultivar selection based on their intended processing direction enables effective adaptation of agronomic technologies to specific goals while maintaining production stability and profitability.

Recommendations for Soybean Cultivar Use
- **For protein processing and feed production**, it is advisable to grow cultivars with high protein content:
  - ✓ Nunavik, Eri, Calgary (Canada),
  - ✓ Titan st, Karmelita, Zeus (Ukraine – medium-maturing group).
- **For the oil-processing industry**, cultivars with oil content above 23% are recommended:
  - ✓ Pallada, Fortress, Ingus (Ukraine),
  - ✓ Akardia, Tersia (Austria).
- **For the Polissia and Forest-Steppe zones**, preference should be given to cultivars ensuring trait stability due to lower coefficients of variation (e.g., ES COMPOSER, Turizas, Dara).
- **For the Steppe region**, cultivars with enhanced tolerance to environmental fluctuations should be used – those demonstrating good plasticity and high potential for either protein (Calgary, Nunavik) or oil content (Pallada, Dara).

Prospects for further research include a detailed analysis of the influence of environmental factors on the formation of qualifying grain parameters in terms of sowing suitability and biochemical complex, and the widespread introduction of selected cultivars in the technological direction.

REFERENCES

Abdala, L., Santiago, T., Alejo, R., Schwalbert, R., Correndo, A. & Martin, N. 2025. Yield environment changes the ranking of soybean genotypes. *Field Crops Research* **321**, 109661. https://doi.org/10.1016/j.fcr.2024.109661

Biliavska, L.H., Biliavskyi, Yu.V., Diyanova, A.O. & Myrnyi, M.V. 2021. Soybean in Steppe and Forest-Steppe: Soybean cultivars for the Steppe and Forest-Steppe of Ukraine. *Scientific Progress & Innovations* **1**, 135–140. doi: 10.31210/visnyk2021.01.16 (in Ukrainian).

Burton, G.W. & De Vane, R.W. 1953. Estimating heritability in tall fescue (Festuca arundinacea) from replicated clonal material. *Agronomy Journal* **45**, 478–481. http://dx.doi.org/10.2134/agronj1953.00021962004500100005x

Dragavtsev, V.A., Tsilke, V.A. & Reiter, B.G. 1984. *Genetics of traits of spring wheat productivity in Western Siberia*. Novosibirsk: Nauka, 229 pp. (in Russian).

DSTU 4138-2002. 2004. Seeds of agricultural crops. Methods for determining quality. [Standard]. Effective from January 1, 2004 (in Ukrainian).

DSTU 4234:2003. 2004. Cereal crops. Determination of bulk density, so-called hectolitre weight. Part 2. Routine method (ISO 7971-2:1995, MOD). [Standard]. Effective from October 1, 2004 (in Ukrainian).

Eberhart, S.A. & Russell, W.A. 1966. Stability parameters for comparing cultivars. *Crop Science* **6**(1), 36–40.

Glauben, T. & Svanidze, M. 2023. FAO & Welthungerhilfe: *Global agricultural trade: Robust safety net to reduce the risk of hunger*. Welthungerhilfe. https://www.welthungerhilfe.org/global-food-journal/rubrics/agricultural-food-policy/global-agricultural-trade-as-safety-net-against-hunger

Gryaznov, A.A. 2019. *Barley naked. Monograph*. Chelyabinsk: South Ural State Agrarian University, 384 pp. Retrieved 27.08.2025, from https://j.twirpx.link/file/3364179 (in Russian).

Horwitz, W. & Latimer, G. 2023. *Official methods of analysis of AOAC International* (22nd ed.). AOAC International. https://doi.org/10.1093/9780197610145.002.001

Hrabovskyi, M.B., Fedoruk, Y.V., Hrabovska, T.O., Lozinskyi, M.V. & Kozak, L.A. 2024. Yield of soybean cultivars under conventional and organic cultivation technologies. *Agronom*. Retrieved April 10, 2025, from https://www.agronom.com.ua/urozhajnist-sortiv-soyi-za-tradytsijnoyi-ta-organichnoyi-tehnologij-vyroshhuvannya

Khangildin, V.V. 1984. Problems of selection for homeostasis and questions of the theory of the selection process in plants. *Breeding, Seed Production and Varietal Agricultural Technology in Bashkiria* **1**, 102–123 (in Russian).

Li, J., Nadeem, M., Chen, L., Wang, M., Wan, M. & Qiu, L. 2020. Differential proteomic analysis of soybean anthers by ITRAQ under high-temperature stress. *Journal of Proteomics* **229**, 103968.

Liu, Y., Li, J., Zhu, L., Jones, A., Rose, R. & Song, Y. 2019. Heat stress in legume reproduction: Effects, causes and future prospects. *Frontiers in Plant Science* **10**, 938.

Melnyk, A.V., Romanko, Y.O. & Romanko, A.Y. 2020. Adaptive potential and stress resistance of modern soybean cultivars. *Tavriya Scientific Bulletin* **113**, pp. 85–91. https://doi.org/10.32851/2226-0099.2020.113.12 (in Ukrainian).

Methodical requirements in the field of seed production regarding the preservation of varietal and sowing qualities of soybean seeds (2023). Retrieved April 10, 2025, from https://zakon.rada.gov.ua/laws/show/z0266-23#Text (in Ukrainian).

Methodology for testing cereal, groat, and legume crops for suitability for distribution in Ukraine. 2016. Kyiv. Retrieved February 5, 2025, from https://sops.gov.ua/uploads/page/5a5f4147d3595.pdf. (in Ukrainian).

Rossielle, A.A. & Hemblin, J. 1981. Theoretical aspects of selection for yield in stress and non-stress environments. *Crop Science* **21**(6), 27–29.

Rybalchenko, A.M. 2022. Features of formation of varietal resources and soybean yield in Ukraine. *Scientific Progress & Innovations* **3**, 18–25. doi: 10.31210/visnyk2022.03.02 (in Ukrainian).

Shing, M., Ceccarelli, S. & Hambling, J. (1993). Estimation of heritability from varietal trials data. *Theoretical and Applied Genetics* **86**, 437–441.

Shook, J., Gangopadhyay, T., Wu, L., Ganapathysubramanian, B. & Sarkar, S. 2021. Crop yield prediction integrating genotype and weather variables using deep learning. *PLOS ONE* **16**(6), e0252402. https://doi.org/10.1371/journal.pone.0252402

Tkachuk, O.P., Didur, I.M. & Pantsyreva, H.V. 2022. Ecological assessment of medium- and late-maturing soybean cultivars. *Agriculture and Forestry* **1**(24), 5–15. https://doi.org/10.37128/2707-5826-2022-1-1 (in Ukrainian).

Voora, V., Bermudez, S., Le, H., Larrea, C. & Luna, E. 2024. IISD Soybean Report: *Global market report: Soybean prices and sustainability*. International Institute for Sustainable Development. https://www.iisd.org/system/files/2024-02/2024-global-market-report-soybean.pdf.

Yatsenko, V., Yatsenko, N., Karpenko, V., Poltoretskyi, S., Lazariev, O., Kravchenko, V., Chynchyk, O., Vyshnevska, L., Tretiakova, S. & Kozyrsky, D. 2023. Agrobiological assessment of productivity and nitrogen fixation of vegetable soybean (edamame) in the conditions of Forest-Steppe of Ukraine. *Agronomy Research* **21**(2), 1006–1026, https://doi.org/10.15159/AR.23.097

Zabarna, T.A. & Chereshniuk, V.V. 2024) Agroecological aspects of soybean (Glycine max L.) cultivation in Ukraine. *Agroecological Journal* **1**, 108–116. https://doi.org/10.33730/2077-4893.1.2024.299945

Zhivotkov, L.A., Morozova, Z.A. & Sekatueva, L.I. 1994. Methodology for identifying the potential productivity and adaptability of cultivars and breeding forms of winter wheat in terms of yield. *Selection and Seed Production* **2**, 3–6 (in Ukrainian).

INSTRUCTIONS TO AUTHORS

Papers must be in English (British spelling). Authors are strongly urged to have their manuscripts reviewed linguistically prior to submission. Contributions should be sent electronically. Papers are considered by referees before acceptance. The manuscript should follow the instructions below.

Structure: Title, Authors (initials & surname; an asterisk indicates the corresponding author), Authors' affiliation with postal address (each on a separate line) and e-mail of the corresponding author, Abstract (up to 250 words), Key words (not repeating words in the title), Introduction, Materials and methods, Results and discussion, Conclusions, Acknowledgements (optional), References.

Layout, page size and font
- Use preferably the latest version of **Microsoft Word**, doc., docx. format.
- Set page size to **ISO B5 (17.6×25 cm)**, all **margins at 2 cm**. All text, tables, and figures must fit within the text margins.
- Use single line spacing and **justify the text**. Do not use page numbering. Use **indent 0.8 cm** (do not use tab or spaces instead).
- Use font Times New Roman, point size for the title of article **14 (Bold)**, author's names 12, core text 11; Abstract, Key words, Acknowledgements, References, tables, and figure captions 10.
- Use *italics* for Latin biological names, mathematical variables and statistical terms.
- Use single ('…') instead of double quotation marks ("…").

Tables
- All tables must be referred to in the text (Table 1; Tables 1, 3; Tables 2–3).
- Use font Times New Roman, regular, 10 pt. Insert tables by Word's 'Insert' menu.
- Do not use vertical lines as dividers; only horizontal lines (1/2 pt) are allowed. Primary column and row headings should start with an initial capital.

Figures
- All figures must be referred to in the text (Fig. 1; Fig. 1 A; Figs 1, 3; Figs 1–3). Use only black and white or greyscale for figures. Avoid 3D charts, background shading, gridlines and excessive symbols. Use font **Arial, 10 pt** within the figures. Make sure that thickness of the lines is greater than 0.3 pt.
- Do not put caption in the frame of the figure.
- The preferred graphic format is Excel object; for diagrams and charts EPS; for half-tones please use TIFF. MS Office files are also acceptable. Please include these files in your submission.
- Check and double-check spelling in figures and graphs. Proof-readers may not be able to change mistakes in a different program.

References
- **Within the text**

In case of two authors, use '&', if more than two authors, provide first author 'et al.':
Smith & Jones (2019); (Smith & Jones, 2019);
Brown et al. (2020); (Brown et al., 2020)

When referring to more than one publication, arrange them by following keys: 1. year of publication (ascending), 2. alphabetical order for the same year of publication:

(Smith & Jones, 2019; Brown et al., 2020; Adams, 2021; Smith, 2021)

- **For whole books**

Name(s) and initials of the author(s). Year of publication. *Title of the book* (*in italics*). Publisher, place of publication, number of pages.

Behera, K.B. & Varma, A. 2019. *Bioenergy for Sustainability and Security*. Springer International Publishing, Cham, pp. 1–377.

- **For articles in a journal**

Name(s) and initials of the author(s). Year of publication. Title of the article. *Abbreviated journal title* (*in italic*) volume (in bold), page numbers.

Titles of papers published in languages other than English, should be replaced by an English translation, with an explanatory note at the end, e.g., (in Russian, English abstr.).

Bulgakov, V., Adamchuk, V., Arak, M. & Olt, J. 2018. The theory of cleaning the crowns of standing beet roots with the use of elastic blades. *Agronomy Research* **16**(5), 1931–1949. doi: 10.15159/AR.18.213

Doddapaneni, T.R.K.C., Praveenkumar, R., Tolvanen, H., Rintala, J. & Konttinen, J. 2018. Techno-economic evaluation of integrating torrefaction with anaerobic digestion. *Applied Energy* **213**, 272–284. doi: 10.1016/j.apenergy.2018.01.045

- **For articles in collections:**

Name(s) and initials of the author(s). Year of publication. Title of the article. Name(s) and initials of the editor(s) (preceded by In:) *Title of the collection (in italics)*, publisher, place of publication, page numbers.

Yurtsev, B.A., Tolmachev, A.I. & Rebristaya, O.V. 2019. The floristic delimitation and subdivisions of the Arctic. In: Yurtsev, B.A. (ed.) *The Arctic Floristic Region*. Nauka, Leningrad, pp. 9–104 (in Russian).

- **For conference proceedings:**

Name(s) and initials of the author(s). Year of publication. Name(s) and initials of the editor(s) (preceded by In:) *Proceedings name (in italics)*, publisher, place of publishing, page numbers.

Ritchie, M.E. & Olff, H. 2020. Herbivore diversity and plant dynamics: compensatory and additive effects. In: Olff, H., Brown, V.K. & Drent R.H. (eds) *Herbivores between plants and predators. Proc. Int. Conf. The 38th Symposium of the British Ecological Society*, Blackwell Science, Oxford, UK, pp. 175–204.

Please note
- Use '.' (not ',') for decimal point: 0.6 ± 0.2; Use ',' for thousands – 1,230.4;
- Use '–' (not '-') and without space: pp. 27–36, 1998–2000, 4–6 min, 3–5 kg
- With spaces: 5 h, 5 kg, 5 m, 5 °C, C : D = 0.6 ± 0.2; $p < 0.001$
- Without space: 55°, 5% (not 55 °, 5 %)
- Use 'kg ha^{-1}' (not 'kg/ha');
- Use degree sign ' ° ' : 5 °C (not 5 ^{O}C).