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CONTENTS

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Germplasm diversity of sunflower volatile terpenoid profiles across vegetative and reproductive organs

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Abstract. Cultivated sunflower (*Helianthus annuus*) is the fourth most important oilseed crop globally and is known to have experienced multiple genetic bottlenecks during domestication and improvement. Homogenization of crop germplasm may limit breeding efforts to improve pest and pathogen resistance or optimize other biotic interactions like pollinator attraction. Such interactions are often strongly influenced by plant phytochemistry, especially volatile compounds like terpenoids. Here we use solid-phase microextraction gas chromatography mass spectrometry (SPME GC-MS) to evaluate volatile phytochemistry across leaves, involucral bracts, disc florets, and ray floret petals in a collection of twelve inbred lines selected to represent a cross-section of sunflower germplasm diversity. Results indicate considerable compositional diversity of volatiles among lines, though substantial reduction in total volatile abundance relative to wild *H. annuus*. From leaves and bracts to disc florets and petals, we observe a strong increase in the proportion of monoterpenoids relative to sesquiterpenoids accompanying the transition to reproductive structures, with consistently over 85% monoterpenoids in disc florets and petals. This pattern is driven by substantially higher production of monoterpenoids (especially alpha-pinene and sabinene) in reproductive structures. Sesquiterpenoid production is roughly similar across organs, and in leaves varies among lines from 21–55% of volatiles, dominated by cadinene-type sesquiterpenoids. This work suggests that the compositional diversity of volatile terpenoids within cultivated germplasm may be sufficient for many breeding applications, though for breeding increased volatile production the use of wild *H. annuus* and other wild *Helianthus* germplasm may be necessary.

Key words: SPME GC-MS, *Helianthus*, monoterpenoids, phytochemistry, sesquiterpenoids.

INTRODUCTION

Plant domestication is one of the most important events initiating human civilization (Childe, 1936). Although the Fertile Crescent (modern day regions of Iraq, Syria, Kurdistan, Lebanon, Iran, Turkey, etc.) is the oldest center of plant domestication and among the first and best-known cradles of civilization (Zeder, 2011; Haas et al., 2019), there are many other regions that have contributed crops to modern diets. Cultivated sunflower (*Helianthus annuus* L.) is one of the few crops that was domesticated in North America (Crites, 1993; Blackman et al., 2011). Native Americans as pioneer sunflower breeders developed the first sunflower landraces that had increased seed yield and oil content and were suitable for cultivation, descendants of which gave rise to diverse extant landraces like Hopi, Havasupai, Seneca, Mandan, Hidatsa, and Arikara, among many others (Heiser, 1954; Heiser et al., 1969; Seiler, 1984; Seiler, 1985; Seiler, 1992; Snow et al., 1998; Lentz et al., 2008; Park & Burke, 2020). These landraces became the foundational genetic material for all the other sunflower landraces, varieties, and breeding lines developed everywhere else (Blackman et al., 2011; Baute et al., 2015; Palmgren et al., 2015; Park & Burke, 2020). Today, modern sunflower lines are mostly short-statured and early-flowering, with specific oil profiles and decreased hull content (Heiser et al., 1969; Blackman, 2013; Baute et al., 2015). Sunflower seeds (achenes) can contain up to 55% oil by weight (Ismail & Arafat, 2014; Harun, 2019), as well as substantial protein content alongside phenolic compounds and essential oils (Ceccarini et al., 2004; Weisz et al., 2009; Zilic et al., 2010). Beyond nutritional value for human and animal consumption, a more recent application of sunflower oil and biomass is the production of biofuels (Jasinskas et al., 2008; Kolchinskij, 2008; Cedik et al., 2018). Given that global food security is under threat by climate change and land degradation, the use of edible oils for biofuel production has been questioned (Naylor et al., 2010; Ghosh et al., 2019), and technologies have been sought for the use of precursor-rich non-edible crop byproducts like sunflower stalks (Ziebell et al., 2013; Nargotra et al., 2018; Vital Brazil et al., 2019; Manmai et al., 2021.

There are two major market types of sunflowers: oilseed varieties, and confectionary (or non-oil) varieties. Generally, oilseed types have smaller seeds with thinner hulls and higher oil content, while confectionary types have larger seeds with thicker hulls and lower oil content (Heiser et al., 1969; Adeleke & Babalola, 2020). Oilseed sunflower is a profitable annual crop, and the fourth most important source of edible oil worldwide (FAO, 2019). Sunflower varieties also can be grouped based on their membership in major breeding pools (Korell et al., 1992), the most important of which are the HA (maintainer) and RHA (restorer) pools which have experienced major genetic divergence (Mandel et al., 2013; Badouin et al., 2017; Talukder et al., 2019). In addition, marker-based, phylogenetic, and genome-wide assessments have more fully described the impacts of founder events and genetic bottlenecks on the cultivated sunflower germplasm, indicating that wild sunflower accessions have around three times the number of alleles per microsatellite locus as elite inbred lines used in breeding (Tang & Knapp, 2003), and that cultivated sunflower as a whole has lost approximately one-third of allelic diversity present in wild *H. annuus* (Mandel et al., 2011). Further, modern cultivars contain about half the expected heterozygosity genome-wide as wild *H. annuus* accessions (Hübner et al., 2019), and cultivated sunflower exhibits around a 12-fold reduction in effective population size (Park & Burke, 2020). However, approximately 27% of the 61,205 genes in cultivated sunflower are variable across the 483 cultivated genotypes in the sunflower pangenome (Hübner et al., 2019), such that both cultivated and wild *H. annuus* are both viable sources for genetic variation for use in breeding. However, only a single cytoplasmic male sterility system and very few fertility-restoring alleles are used to create commercial hybrid seed for oilseed and confectionary production, resulting in much lower genetic variability on-farm (Seiler et al., 2017; Talukder et al., 2019).

Although domestication and improvement turned the grassland wildflower *Helianthus annuus* into a modern uniform high-yielding crop, cultivated sunflower is susceptible to numerous environmental stresses including diseases (rust, powdery mildew, downy mildew, charcoal rot, Verticillium, Phomopsis, Sclerotinia, and sunflower mosaic virus), insect pests (weevils, moths, and beetles), and abiotic stresses like drought and salinity (Seiler, 1984; Seiler, 1992; Palmgren et al., 2015; Seiler et al., 2017). A long-standing hypothesis in crop evolution posits that the process of domestication and improvement has favored selection for growth and yield at the expense of resistance to stress, particularly biotic stresses like herbivory (Becerra et al., 2009; Agrawal, 2011; Carmona et al., 2011; Whitehead et al., 2016). Across global agriculture, insect pests alone destroy around 32% of potential crop yield annually (Oerke, 2006). Stress resistance is a complex phenotype that is not linked consistently to specific chemical, physiological, or morphological traits across species (Whitehead et al., 2016). Several studies of the effects of domestication on insect interactions in sunflower have identified substantial increases in herbivore oviposition, feeding preference, survival and reproduction, and even reductions in beneficial parasitoid engagement in cultivated sunflower relative to wild *H. annuus* (Rogers et al., 1987; Chen & Welter, 2002, 2003, 2005, 2007; Michaud & Grant, 2009; Mayrose et al., 2011). Studies of phytochemistry have noted a domestication-associated reduction in the production of nonvolatile sesquiterpene lactones (Rowe et al., 2012; Prasifka et al., 2015), though no known comparisons to date have been published for volatile terpenoids. Leaves and flowers of wild and cultivated sunflowers are fragrant and rich in both volatile and nonvolatile terpenes (Marechal & Rigal, 1999; Ceccarini et al., 2004; Ukiya et al., 2007; Prasifka et al., 2015; Lawson et al., 2019). It is well documented that terpenoids mediate plant-environment interactions (Pichersky & Raguso, 2018; Liu et al., 2020; Zhou & Pichersky, 2020), and in sunflower are involved in a wide range of functions including repelling or killing herbivores (Rogers et al., 1987; Charlet et al., 2008; Gopfert et al., 2009; Rowe et al., 2012; Prasifka et al., 2015), inhibiting fungal pathogen growth (Mayrose et al., 2011; Lawson et al., 2019), allelopathic effects against competing plants (Macias et al., 2002), and even free radical scavenging (Liu et al., 2020). Beside these, volatile compounds in different species are responsible for many antimicrobial, antifungal, and antioxidant activities as well (Vasinauskiene et al., 2006; Lawson et al., 2019; Liu et al., 2020; Lukosiute et al., 2020). Volatile terpenoids have also been demonstrated to influence the quality of biofuel production (Mikulova et al., 2014; Pausas et al., 2016; Vitazek et al., 2018), and common monoterpenoids and sesquiterpenoids can form the basis of the production of specialty biofuels (Peralta Yahya et al., 2011; Joyce et al., 2012; Zhang et al., 2014; Yang et al., 2016; Mewalal et al., 2017).

Further work is needed to clarify the roles of specific volatile terpenoid metabolites in cultivated sunflower, as well as to describe the diversity of phytochemical profiles present in sunflower germplasm that can be leveraged to breed cultivars with desirable terpenoid-mediated phenotypes - whether that be repelling or inhibiting harmful pests or pathogens, attracting beneficial pollinators or parasitoids, or for industrial applications. The specific composition and relative ratios of terpenoids can be as important as their abundance, due to synergistic effects that occur when multiple compounds act together in a cocktail (Richards et al., 2016), or antagonistic effects such as those that occur due to preferential oxidation of major compounds by insect detoxification enzymes resulting in enhanced effects of minor compounds (Scalerandi et al., 2018). Sparse previous work performed in individual sunflower cultivars indicates that volatile profiles vary among organs and that monoterpenoids are dominant compounds (Ceccarini et al., 2004; Lawson et al., 2019). In wild *H. annuus*, geographic origin appears to drive large-scale variation in both abundance and proportional composition of profiles (Adams et al., 2017), such that genetic variation within the cultivated sunflower germplasm should be predicted to translate into parallel variation. Despite the value to breeding efforts, to date there has not been a comprehensive evaluation of volatile profiles across the cultivated sunflower germplasm. Here in this study, we performed analytical chemistry to describe volatile profile variation in four aerial organs - leaves, involucral bracts, disc florets, and ray floret petals - across twelve cultivated lines spanning breeding pools and market classes within the Sunflower Association Mapping (SAM) panel (Mandel et al., 2011; Mandel et al., 2013). The objectives of this study were to determine abundance and composition of volatile compounds and estimate overall quality and quantity of volatile compounds in cultivated sunflower. The results of this work inform the approach for broader targeted screening of the germplasm resources available for sunflower (Kane et al., 2013; Kantar et al., 2015; Seiler et al., 2017), germplasm selection for studies evaluating the consequences of volatile terpenoid variation on biotic interactions (e.g., Prasifka et al., 2015), and potential limits on the independence of phytochemistry among organs that may constrain the development of cultivars that optimize multiple functions like foliar pest resistance, floral pest resistance, and pollinator attraction.

MATERIALS AND METHODS

Germplasm selection

Twelve inbred lines were selected for evaluation in this study, the so-called 'Core 12' lines within the Sunflower Association Mapping (SAM) panel (Mandel et al., 2011; Mandel et al., 2013). The full panel contains 288 inbred lines of cultivated sunflower selected to capture approximately 87% of allelic diversity present within the sunflower germplasm repositories of the United States Department of Agriculture (USDA) National Plant Germplasm System and the French Institut National de la Recherche Agronomique (INRA), propagated by single-seed descent to remove residual heterozygosity (Mandel et al., 2011; Mandel et al., 2013). The 'Core 12' lines were selected by rarefaction to represent the most divergent genotypes, together containing just under half of the allelic diversity within the full SAM panel (pre Mandel et al., 2011), and includes three HA-Oil lines, two HA-NonOil lines, three RHA-Oil lines, one RHA-NonOil line, one INRA-HA line, and two open-pollinated varieties (Table S1). These twelve genotypes should reflect a cross-section of genetic diversity across all sunflower germplasm.

Plant growth

In 2019, the Core 12 lines were grown alongside the full 288-line SAM panel in a randomized complete block design across two agricultural high tunnels on the University of Central Florida campus in Orlando, FL, United States. The Core 12 lines were planted in mid-March, with six replicate plants of each line grown in each agricultural high tunnel, which served as statistical blocks, totaling a target of 12 replicates for each line. Seeds were planted directly into 18.6 liter pots filled with pine-bark-based potting soil. Each pot received four tablespoons (64 grams) of slow-release fertilizer (Osmocote Plus 15-9-12; Scotts, Marysville, OH, USA) to ensure non-limiting nutrient supply. Plants were watered daily to field capacity with automatic drip irrigation to ensure non-limiting water availability. Photoperiod, light, and temperature levels were ambient, with flowering of the Core 12 lines occurring during a two-week period in May based on genotype-derived variation in flowering phenology.

Sampling

At flowering (R5 stage; Schneiter & Miller, 1981), samples were taken of the four target aerial organs. The lamina of the most recently fully-expanded leaf (hereafter 'leaf') was cut with scissors down the midrib, with one side rolled and placed into a microcentrifuge tube. One or more involucral bracts on the back of the composite head (hereafter 'bract') were removed with scissors and placed into a microcentrifuge tube. Multiple ray floret petals (hereafter 'petal') were plucked from the circumference of the composite head with forceps and placed into a microcentrifuge tube. Several dozen newly open disc florets were removed from the center disc of the composite head with forceps and placed into a microcentrifuge tube. All organ samples were immediately snap-frozen in liquid nitrogen upon sampling, and kept in a -80 °C freezer until preparation for analysis. Scissors and forceps were cleaned with ethanol between samples to prevent cross-contamination. Only undamaged healthy structures were sampled, excluding any organs with visible wilting, damage, or necrosis, and excluding any replicate plants with substantial herbivory, pathogen infection, partially broken stems, or other visible factors that might influence phytochemistry. Within each genotype, between 2–9 samples were obtained for each organ type - typically more for leaves and bracts, and fewer for petals and disc florets given the narrower time window for sampling these more ephemeral organs (just a few days for each composite head) and our strict quality criteria. The total number of samples obtained was 240, or an average of $n = 5$ samples per organ per genotype.

Phytochemical analysis

In this experiment, solid-phase microextraction gas chromatography-mass spectrometry (SPME GC-MS) was performed using a single quadrupole GCMS-QP2020 (Shimadzu, Inc.) to identify volatile compounds in the samples. The leaf, bract, petal, and disc floret samples were ground with a mortar and pestle to a fine powder in liquid nitrogen, and 200 (± 20) mg of the tissue was put into 10 mL glass headspace vials with the total sample mass recorded. To start the phytochemical analysis, the vials were incubated at 75 °C for 15 minutes with agitation at 250 rpm. Then to extract volatiles from the headspace, a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVS/CAR/PDMS) SPME fiber was introduced to the vial and incubated at 75 °C with agitation at 250 rpm for 10 minutes. The SPME fiber was then desorbed for 3 minutes into the inlet of the GC-MS at 250 °C. The fiber between samples was conditioned for 10 minutes at 270 °C. Column flow was 1.91 mL min^{-1} with splitless injection using a purge flow of 3.0 mL min^{-1} after 3.5 minutes sampling time. Initial GC temperature was 35 °C, then increased to 80 °C at 10 °C min^{-1}, held for 5.5 minutes, then increased to 140 °C at 15 °C min^{-1}, held for 5.5 minutes, then increased to 220 °C at 20 °C min^{-1}, and held for 2 minutes. The MS source and interface temperatures were kept at 200 °C and 250 °C, respectively. The mass spectra of peaks were compared against the National Institutes of Standards and Technology standards database (Lemmon et al., 2017), and minimum similarity of 75% was used to select peaks identities as naming conventions. Potential mislabeling was avoided by manually processing the raw data using retention time and mass spectra similarity hits for each peak. For our purposes, the top NIST library hit for each peak was reported regardless of isomer identity (full differentiation of isomers can be difficult for many compounds with GC-MS); due to this our dataset contains multiple instances of some metabolites that might be isomers of the same compound. The peak area for each compound was divided by the sample mass placed into the headspace vial to generate mass-normalized peak area, our metric of compound abundance.

Statistical analysis

Additional summary statistics were calculated for each sample using mass-normalized peak areas. Sums of mass-normalized peak areas for compounds in particular focal classes were calculated to derive estimates of the abundance of total monoterpenoids, total sesquiterpenoids, total diterpenoids, total terpenoids (the sum of monoterpenes, sesquiterpenes, and diterpenes), total fatty acid derivatives, and an additional category of total 'other compounds' for all other miscellaneous non-terpenoid compounds (including various ketones, epoxides, benzaldehydes, alkanes, alkenes, and alcohols). By summing all mass-normalized peak areas, a semiquantitative relative estimate of the total volatile abundance in each sample was generated. The proportional contribution of each individual compound to the total volatile abundance was calculated by dividing the mass-normalized peak areas for each compound by the total volatile abundance in each sample, then expressed as a percentage. Likewise, the proportional contribution of monoterpenoids, sesquiterpenoids, diterpenoids, fatty-acid-derivatives, and other compounds were similarly calculated by dividing the total abundance of each class by the total volatile abundance in each sample.

The number of compounds detected and identified within each sample was also recorded as an estimate of volatile compound diversity. To improve focus on the dominant compounds in volatile profiles, 'major compounds' were identified in three ways. First, across the entire dataset 'major compounds' were identified as compounds that were both present in all organs of all genotypes and contributed on average > 1% of total volatile abundance across the 48 organ-by-genotype combinations. Within each organ, this process was again repeated to identify 'major compounds' that were present in all 12 genotypes and contributed on average > 1% of total volatile abundance across the 12 genotypes. Within each line, this process was again repeated to identify 'major compounds' within each line, identified as those compounds that were present in all four organ types and contributed on average > 1% of total volatile abundance across the four organs. Potential trait-trait associations within and between organs were investigated using pairwise Pearson correlations with the corr package (Makowski et al., 2020) in R version 1.4.1717 (R Core Team, 2022). Graphs were drawn using Microsoft Excel v. 2210.

RESULTS AND DISCUSSUION

Volatile compound diversity across organs and genotypes

Across the 240 samples analyzed, 196 unique compounds were detected and identified, of which 69.4% were terpenoids (33.7% monoterpenoids, 34.7% sesquiterpenoids, and 1% diterpenoids), 3.6% fatty acid derivatives, and 27% other compounds (Appendix 1). Among organs, leaves had the fewest unique compounds while disc florets and bracts had the most (Table 1). Across organs the proportion of identified compounds that were terpenoids (72.6–77.7%), fatty acid derivatives (0–4.8%), and other compounds (19.7–25.2%) were quite similar, though the proportional breakdown within the terpenoid class was more variable (Table 1). Within leaves, there were around twice as many sesquiterpenoid compounds identified as monoterpenoids, while the opposite pattern was observed in petals (Table 1). Both disc florets and bracts had roughly even proportions of both monoterpenoids and sesquiterpenoids (Table 1). Among the twelve genotypes, the total number of identified compounds varied from 71 to 107, with between 74.8–88.2% terpenoids, 0–3.7% fatty acid derivatives, and 11.7–21.6% other compounds (Table S2). Within the terpenoid class, the proportion of monoterpenoids and sesquiterpenoid compounds were roughly similar among genotypes with more of either subclass in different genotypes (Table S2).

Table 1. Total number of volatile compounds detected and identified via SPME-GC-MS in the four organ types assessed (pooling all twelve plant genotypes), as well as the proportional breakdown of compounds classified as terpenoids (divided into monoterpenoids, sesquiterpenoids, and diterpenoids), fatty acid derivatives, and other compounds

Organ	Number of compounds	% terpenoids	% monoterpenoids	% sesquiterpenoids	% diterpenoids	% fatty acid derivatives	% other compounds
Petals	83	73.5	49.4	24.1	0	4.8	21.6
Disc florets	95	72.6	38.9	32.6	1.0	2.1	25.2
Bracts	94	76.4	38.2	37.2	1.0	0	23.4
Leaves	72	77.7	25.9	50.6	1.2	2.4	19.7

Volatile compound abundance and profile composition

Across all genotype-by-organ combinations, mean total volatile abundance varied by nearly an order of magnitude, and the mean number of compounds detected ranged from 12.0 to 38.6 (Table 2, Fig. 1). Profiles were consistently terpenoid-dominated (87–99% of abundance), but with very large variation in the proportional abundance of monoterpenoids (38–98%) and sesquiterpenoids (0.5–55%) (Table 2). Regardless of organ or genotype, the diterpenoid and fatty acid derivative classes were minute fractions of total volatile abundance (< 1% in all cases, often undetected).

Considering organ-driven variation, petals contained on average the fewest detected compounds (16.8), while leaves contained the most (29.2) and disc florets and bracts were intermediate (Table S3). This cross-organ pattern holds within most of the twelve genotypes considered individually (Table 2). The mean number of compounds in each organ was unrelated to the total volatile abundance, which on average was highest in disc florets and under half as abundant in leaves, with bracts and petals intermediate (Table S3). On average, 93–98% of total volatile abundance was composed of terpenoids, but the balance of monoterpenoids and sesquiterpenoids varied widely among organs (Fig. 1, Table S3). In reproductive structures, total abundance was dominated by monoterpenoids (90–98% in petals, 85–94% in disc florets, 71–91% in bracts; Fig. 1, Table 2, Table S3). In leaves the balance between monoterpenoids and sesquiterpenoids was much more variable, with 38–69% monoterpenoids and 22–55% sesquiterpenoids among genotypes (Fig. 1, Table 2). Leaves have by far the highest proportion of sesquiterpenoids, as well as the highest proportion of non-terpenoid compounds (Table 2, Table S3).

Table 2. Volatile profiles for each organ within each plant genotype, as assessed by SPME-GC-MS. The average number of compounds detected and total volatile abundance (mass-normalized peak area) are reported, along with the proportional breakdown of mass-normalized peak area by compound class: terpenoids (divided into monoterpenoids, sesquiterpenoids, and diterpenoids), and other compounds. Values represent mean ± SE for each metric reported, calculated across all replicate samples. Fatty-acid derivatives are excluded as a category in this table, as only five organ-genotype combinations had detectable quantities. Entries with values representing detected compounds between 0–0.1% of volatile profile composition are rounded up to 0.1%, and percentages may not sum to 100% due to rounding

Core 12 genotype	Organ	Number of compounds	Total volatile abundance	% terpenoids	% monoterpenoids	% sesquiterpenoids	% diterpenoids	% other compounds
SAM 020	Petal	16.8 ± 1.0	32,193 ± 672	99.3 ± 0.3	95.8 ± 0.8	3.4 ± 0.8	0	0.6 ± 0.3
	Disc Floret	21.5 ± 1.6	56,381 ± 16,607	98.4 ± 0.3	89.0 ± 2.7	9.2 ± 2.5	0	1.7 ± 0.3
	Bract	27.0 ± 2.5	26,831 ± 2,120	95.8 ± 1.2	73.0 ± 1.4	22.7 ± 1.6	0.1 ± 0.1	4.1 ± 1.2
	Leaf	28.1 ± 3.2	19,797 ± 4,814	89.4 ± 4.9	40.6 ± 2.9	48.3 ± 3.9	0.4 ± 0.2	10.5 ± 4.9
SAM 022	Petal	18.2 ± 0.4	43,053 ± 18,789	97.6 ± 1.4	95.4 ± 1.3	2.2 ± 0.3	0	2.3 ± 1.4
	Disc Floret	24.7 ± 3.1	55,973 ± 25,032	96.9 ± 0.8	85.5 ± 6.0	11.3 ± 6.2	0	2.8 ± 0.9
	Bract	21.4 ± 2.4	36,174 ± 14,221	95.8 ± 0.9	79.5 ± 4.0	16.2 ± 4.1	0.1 ± 0.1	4.1 ± 0.9
	Leaf	25.1 ± 2.5	16,762 ± 2,645	91.9 ± 3.2	55.2 ± 5.7	36.1 ± 5.6	0.5 ± 0.1	8.0 ± 3.2
SAM 027	Petal	17.5 ± 1.3	32,568 ± 3,785	96.4 ± 1.5	90.6 ± 1.6	5.8 ± 0.5	0	3.3 ± 1.4
	Disc Floret	22.6 ± 2.0	40,946 ± 5,777	94.3 ± 1.4	87.8 ± 1.4	6.4 ± 0.4	0	5.6 ± 1.3
	Bract	26.2 ± 1.0	30,837 ± 3,672	97.5 ± 0.7	81.0 ± 1.5	16.5 ± 1.7	0	2.4 ± 0.7
	Leaf	26.8 ± 4.9	21,356 ± 8,474	95.0 ± 1.1	57.5 ± 4.1	37.1 ± 3.8	0.3 ± 0.1	4.9 ± 1.1
SAM 093	Petal	16.0 ± 1.8	20,579 ± 3,480	97.6 ± 1.0	92.1 ± 0.8	5.5 ± 0.5	0	2.3 ± 1.0
	Disc Floret	24.5 ± 2.8	43,219 ± 6,242	96.4 ± 1.2	91.4 ± 1.2	5.0 ± 0.8	0.1 ± 0.1	3.5 ± 1.2
	Bract	22.1 ± 2.7	20,855 ± 3,423	96.0 ± 1.3	85.0 ± 1.2	10.9 ± 0.6	0	3.9 ± 1.3
	Leaf	18.5 ± 2.6	7,779 ± 1,538	87.0 ± 4.8	55.3 ± 6.3	31.0 ± 5.2	0.6 ± 0.5	12.9 ± 4.8
SAM 094	Petal	17.0 ± 1.7	31,427 ± 5,742	98.0 ± 0.8	95.2 ± 1.0	2.7 ± 0.4	0	1.9 ± 0.8
	Disc Floret	23.7 ± 3.4	55,033 ± 14,133	98.0 ± 0.5	93.4 ± 0.4	4.5 ± 0.6	0	1.9 ± 0.5
	Bract	25.6 ± 1.7	23,948 ± 1,887	91.8 ± 3.3	73.8 ± 3.4	18.0 ± 1.7	0	8.1 ± 3.3
	Leaf	34.0 ± 2.8	36,717 ± 9,236	97.0 ± 0.7	58.6 ± 3.7	38.3 ± 3.3	0.1 ± 0.1	2.9 ± 0.7
SAM 176	Petal	12.0 ± 0.4	33,519 ± 8,194	99.1 ± 0.2	96.8 ± 0.4	2.3 ± 0.4	0	0.8 ± 0.2
	Disc Floret	24.2 ± 1.5	46,579 ± 2,097	98.6 ± 0.3	92.5 ± 1.0	6.0 ± 0.9	0.1 ± 0.1	1.3 ± 0.3
	Bract	21.2 ± 1.7	27,179 ± 7,499	96.1 ± 1.4	84.2 ± 2.3	11.8 ± 1.5	0	3.8 ± 1.4
	Leaf	23.1 ± 1.5	14,518 ± 1,384	94.1 ± 1.3	61.6 ± 5.2	31.7 ± 4.2	0.7 ± 0.2	5.8 ± 1.3

Table 2 (continued)

SAM 185	Petal	16.6 ± 4.0	21,906 ± 6,279	97.7 ± 1.7	92.8 ± 2.3	4.8 ± 2.0	0	2.2 ± 1.7
	Disc Floret	22.8 ± 2.3	30,926 ± 2,861	93.2 ± 3.9	86.3 ± 4.4	6.9 ± 0.6	0	6.7 ± 3.9
	Bract	25.7 ± 2.1	25,477 ± 3,319	98.3 ± 0.6	78.0 ± 1.5	20.3 ± 1.4	0	1.6 ± 0.6
	Leaf	29.5 ± 3.8	20,733 ± 4,974	94.1 ± 2.3	51.7 ± 3.4	41.9 ± 4.2	0.4 ± 0.2	5.8 ± 2.3
SAM 191	Petal	17.7 ± 1.6	35,969 ± 5,178	98.9 ± 0.4	95.0 ± 1.4	3.8 ± 1.3	0	1.0 ± 0.4
	Disc Floret	30.7 ± 1.4	53,286 ± 2,961	98.5 ± 0.2	89.8 ± 1.1	8.6 ± 0.9	0	1.4 ± 0.2
	Bract	22.0 ± 1.7	22,987 ± 2,157	93.2 ± 2.2	71.3 ± 4.8	21.8 ± 4.6	0	6.7 ± 2.2
	Leaf	30.5 ± 3.4	22,195 ± 4,838	94.1 ± 1.3	38.1 ± 6.1	55.4 ± 5.3	0.5 ± 0.2	5.8 ± 1.3
SAM 203	Petal	18.0 ± 0.9	43,979 ± 4,321	98.5 ± 0.3	97.9 ± 0.4	0.5 ± 0.1	0	1.4 ± 0.3
	Disc Floret	20.5 ± 2.1	50,213 ± 10,528	96.6 ± 1.0	94.0 ± 0.7	2.6 ± 0.7	0	3.3 ± 1.0
	Bract	17.3 ± 1.2	28,527 ± 13,367	96.9 ± 1.2	91.2 ± 2.1	5.3 ± 1.1	0.3 ± 0.2	3.0 ± 1.2
	Leaf	26.8 ± 2.2	18,996 ± 3,275	91.8 ± 2.4	62.5 ± 3.1	28.4 ± 1.5	0.8 ± 0.2	8.1 ± 2.4
SAM 237	Petal	14.3 ± 0.6	19,245 ± 1,703	99.1 ± 0.6	93.9 ± 1.3	5.1 ± 0.7	0	0.6 ± 0.6
	Disc Floret	24.6 ± 0.3	32,925 ± 3,077	94.8 ± 0.8	85.0 ± 0.1	9.7 ± 0.9	0	5.1 ± 0.8
	Bract	25.0 ± 1.5	22,806 ± 2,152	94.9 ± 2.3	75.3 ± 3.9	19.5 ± 2.2	0	5.0 ± 2.3
	Leaf	38.6 ± 2.6	28,727 ± 5,449	95.2 ± 0.1	44.7 ± 1.9	50.1 ± 2.0	0.3 ± 0.1	4.7 ± 0.1
SAM 240	Petal	18.3 ± 2.7	42,294 ± 7,998	97.8 ± 1.0	94.1 ± 2.9	3.7 ± 1.8	0	2.1 ± 1.0
	Disc Floret	26.5 ± 3.5	66,735 ± 9,890	98.4 ± 0.2	93.7 ± 1.1	4.7 ± 0.8	0	1.5 ± 0.2
	Bract	32.0 ± 2.0	59,356 ± 10,996	98.5 ± 0.2	84.4 ± 2.4	14.1 ± 2.6	0	1.4 ± 0.2
	Leaf	37.8 ± 1.3	46,592 ± 2,361	97.2 ± 0.5	59.0 ± 2.2	38.1 ± 2.3	0.1 ± 0.1	2.7 ± 0.5
SAM 262	Petal	19.5 ± 0.5	40,037 ± 3,575	96.3 ± 1.6	93.8 ± 2.1	2.4 ± 0.4	0	3.6 ± 1.6
	Disc Floret	25.0 ± 1.0	72,697 ± 5,727	97.0 ± 0.1	94.3 ± 0.1	2.7 ± 0.1	0	2.9 ± 0.1
	Bract	14.6 ± 1.4	15,832 ± 1,116	97.7 ± 0.9	88.9 ± 2.5	8.6 ± 1.4	0.2 ± 0.1	2.2 ± 0.9
	Leaf	31.6 ± 3.4	27,771 ± 4,980	91.1 ± 4.2	68.8 ± 5.9	21.9 ± 3.5	0.3 ± 0.1	8.6 ± 4.2

While organ-driven variation was large, several substantial genotype-driven patterns are evident. First, overall volatile abundance varies substantially among genotypes no matter which organ is considered (Fig. 1, Table 2). Genotype-level means averaged across organs show two-fold variation in total volatile abundance (Table S4), while in comparison across all 48 genotype-by-organ combinations the variation in total volatile abundance was over nine-fold (Table S5). Taking each organ individually, variation among genotypes in total volatile abundance was a bit over two-fold in petals and discs, over three-fold in bracts, and nearly six-fold in leaves (Table S6).

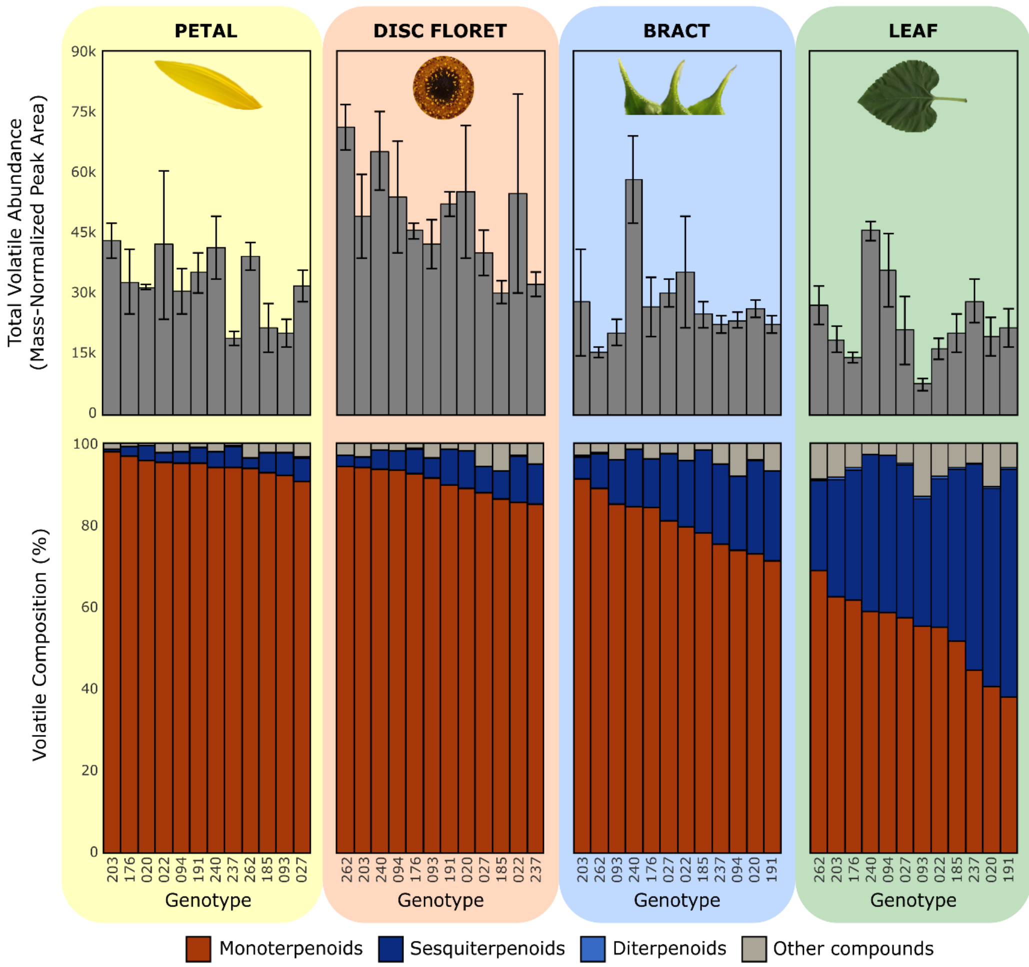

Figure 1. Total volatile abundance (top panels) and volatile profile composition (bottom panels) for the four focal organs (petal, disc floret, bract, and leaf) across the Core 12 genotypes (SAM 020, SAM 022, SAM 027, SAM 093, SAM 094, SAM 176, SAM 185, SAM 191, SAM 203, SAM 237, SAM 240, SAM 262). Total volatile abundance is expressed as the total mass-normalized peak area of all detected compounds averaged across replicate samples of a given organ within a genotype, and error bars represent standard error of the mean. Volatile profile composition is the relative proportion of total mass-normalized peak area comprised of monoterpenoids, sesquiterpenoids, diterpenoids, or other non-terpenoid compounds.

This variation among genotypes within each organ is similar in magnitude to the variation among organs within each genotype (Table S7). Second, genotypic variation in bract and leaf monoterpenoid-sesquiterpenoid balance is substantial (Fig. 1, Table 2), indicating that the relative composition of volatile profiles in these organs could be altered by targeted breeding efforts. Third, the proportional contribution of individual major compounds to the overall profile is highly variable.

Genetic variation in major compounds across sunflower organs

While 196 unique compounds were detected and identified in this study, only sabinene was present in every sample analyzed. Major compounds present in all organs of all genotypes were the monoterpenoids alpha-pinene, sabinene, gamma-terpinene, and o-cymene (Table S8). In the reproductive structures (petals, disc florets, and bracts), the monoterpenoids alpha-pinene and sabinene together comprised on average 60–72% of total volatile abundance, with the remaining portion of the profile up to > 85% made up of a combination the monoterpenoids beta-pinene, D-limonene, alpha-terpinene, gamma-terpinene, terpinene-4-ol, o-cymene, and bornyl acetate, along with the sesquiterpenoids beta-gurjunene, beta-cubebene, and beta-elemene, and the non-terpenoid methoxyphenyloxime and desmethoxyencecalin (Table 3). Variation in the composition of these compounds in each organ varied substantially among genotypes (Table 3). Alpha-pinene varied from 33–77% in petals, 35–62% in disc florets and 40–64% in bracts, while sabinene varied from 11–31% in petals, 9–24% in disc florets, and 4–12% in bracts (Table 3). The other major compounds varied at least 2-fold to as high as 50-fold among genotypes in each of these three organs (Table 3). Among genotypes, there was a strong negative correlation between the proportions of alpha-pinene and sabinene in both petals (R^2 = 0.81) and disc florets (R^2 = 0.65), though not in bracts where these two compounds are less dominant (Fig. S1).

In leaves, the most abundant compounds were the monoterpenoids D-limonene, sabinene, alpha-pinene, gamma-terpinene, and endo-borneol, the sesquiterpenoids beta-cubebene, alpha-cadinene, beta-cadinene, gamma-cadinene, alpha-muurolene, gamma-muurolene, and caryophyllene, and the non-terpenoids methoxyphenyloxime and 1,5,9,9-tetramethyl-Z,Z,Z-1,4,7-cycloundecatriene (Table 3). Among genotypes, D-limonene varied from 19–32%, beta-cubebene from 8–23%, sabinene from 6–14%, and alpha-pinene from 6–14% (Table 3). The other major compounds varied at least 2-fold and as high as 18-fold among genotypes in leaves (Table 3).

Diversity of volatile profiles compared with other cultivated and wild Helianthus

The results obtained here for a systematic cross-section of cultivated *Helianthus* germplasm are consistent with previous research on scattered varieties of cultivated sunflower. An assessment of essential oils derived from dried leaves and whole capitula of two varieties (‘Carlos’ and ‘Florom 350’) identified 51 and 49 compounds, respectively, of which 84–88% were terpenoids, with slightly more sesquiterpenoids than monoterpenoids (Ceccarini et al., 2004). Another assessment of essential oils derived from fresh leaves of two different cultivars (‘Mammoth’ and ‘Chianti’) identified 64 compounds, of which 95.1% were terpenoids with about twice as many sesquiterpenoids as monoterpenoids (Lawson et al., 2019). The most abundant compounds in these studies overlapped heavily with those identified here, including alpha-pinene, sabinene, limonene, bornyl acetate, terpinene-4-ol, beta-pinene, beta-gurjunene, and camphene. This indicates that a core set of terpenoid compounds in cultivated sunflower are present across most germplasm, and can be captured by assessing even a few accessions, but that there are many additional non-core compounds that are unlikely to be detected without screening far more genotypes.

Research conducted on wild *H. annuus*, the progenitor to cultivated sunflower, demonstrates a similar degree of qualitative phytochemical diversity to that observed in the Core 12 genotypes assessed here. Assessment of volatiles in snap-frozen tissues from greenhouse-grown plants of one accession of wild *H. annuus* from Konza Prairie, Kansas using identical analytical methods identified 79 compounds in leaves and 67 compounds in petals (17 shared between organs), only slightly less than observed in the Core 12 lines here (Table S10, Table S11; Bahmani et al., 2022). In both petals and leaves, terpenoids made up a higher proportion of identified compounds in wild *H. annuus* than in the Core 12 lines here (Table S10, Table S11). In petals, there were fewer monoterpenoid and non-terpenoid compounds and more sesquiterpenoid and diterpenoid compounds than observed in the Core 12 lines here (Table S10). In leaves, there were more monoterpenoid and diterpenoid compounds, and fewer sesquiterpenoids and diterpenoids than observed in the Core 12 lines here (Table S11). A broader assessment of essential oil extracts derived from air-dried leaves sampled in the field from 20 populations distributed across the native range of *H. annuus* identified 83 total compounds, 20 of which were shared across all populations, with a very similar average breakdown of compounds as those from Konza Prairie (Table S11; Adams et al., 2017; Bahmani et al., 2022). Considering total volatile abundance estimated from mass-normalized peak areas, wild *H. annuus* from Konza Prairie had over six-fold higher volatile abundance in both petals and leaves than observed on average in the Core 12 lines here (Table S12), though the proportional abundance was similar between wild and cultivated sunflower for petals (> 90% monoterpenoids in wild). In leaves, this proportional abundance was slightly shifted toward monoterpenoids in wild *H. annuus* from Konza Prairie (66% monoterpenoids, 31% sesquiterpenoids) relative to cultivated sunflower (Table S12). The average proportional abundance identified in leaves across the range of wild *H. annuus* was further shifted toward monoterpenoids (72% monoterpenoids, 15% sesquiterpenoids) (Table S13), suggesting that domestication and improvement have increased the relative abundance of volatile sesquiterpenoids in cultivated genotypes of *H. annuus*. However, given that total volatile production is far lower in cultivated sunflower than wild *H. annuus*, even accounting for these shifts total monoterpenoid and total sesquiterpenoid abundances are quantitatively on average 6-8 times lower in both petals and leaves (Table S3, Table S12). The abundance of nonvolatile sesquiterpene lactones has been previously demonstrated to be far higher in wild *H. annuus* accessions than in cultivated accessions (Prasifka et al., 2015), such that our findings here extend this pattern to volatile sesquiterpenoids and monoterpenoids as well.

A broader assessment of leaf and petal volatile profiles across 40 species of wild *Helianthus* using identical sampling and analytical methods identified approximately 500 compounds, with no single compound shared among petals of all species, and only four compounds shared among leaves of all species (Bahmani et al., 2022). Across the genus, total volatile abundance varied over 130-fold in leaves and 320-fold in petals, with the proportion of terpenoids varying from 9–99% of volatile abundance in petals and 29–99% in leaves (Bahmani et al., 2022). The balance of terpenoid subclasses varied from 2–92% monoterpenoids and 0–91% sesquiterpenoids among species in both petals and leaves (Bahmani et al., 2022).

Table 3. Proportional contribution (based on mass-normalized peak area) of major compounds identified across the Core 12 genotypes in each of the four organs, as assessed by SPME-GC-MS. Values represent the mean percentage for each listed compound, calculated across all replicate samples, and the grand mean across all genotypes. Percentages may not sum to 100% due to rounding

	Mean	SAM 020	SAM 022	SAM 027	SAM 093	SAM 094	SAM 176	SAM 185	SAM 191	SAM 203	SAM 237	SAM 240	SAM 262
Petal													
Alpha-Pinene	49.7	44.6	36.2	40	53.7	37.7	77.1	52.2	53.3	57.6	33.2	51.8	59.4
Sabinene	22.6	25.9	27.4	27.9	18.8	31.3	10.9	23.8	23.8	11.2	29.5	21.7	19.3
Gamma-Terpinene	5.1	6.1	6.0	5.6	4.5	6.5	2.5	4.5	4.8	4.1	8.0	5.4	2.9
Terpinen-4-ol	3.7	5.0	5.8	3.3	5.0	3.9	1.5	2.5	2.9	2.1	6.5	2.7	2.9
Beta-Gurjunene	2.6	2.2	1.8	5.2	4.5	2.5	1.6	3.5	1.4	0.1	4.8	1.5	1.8
Alpha-Terpinene	2.4	3.2	3.2	3.0	1.2	3.2	0.7	2.3	2.0	1.3	4.1	2.6	1.6
O-Cymene	2.1	2.1	2.5	2.1	2.4	2.2	1.0	1.9	2.3	2.3	3.7	1.7	1.4
Disc Floret													
Alpha-Pinene	52.6	45.1	51.7	48	54.3	40.6	62.1	55.4	62.2	60.6	34.7	57	59.9
Sabinene	16.3	22.5	12.2	15.3	15.1	25	13.8	15.2	10.7	9.0	24.1	13.8	19.2
D-Limonene	5.3	6.0	6.0	7.2	6.1	3.6	5.7	2.3	4.6	6.6	7.1	4.7	4.1
Gamma-Terpinene	4.1	5.6	3.2	4.6	3.6	7.4	3.6	3.2	3.1	2.5	5.9	4.0	3.0
Beta-Gurjunene	3.1	4.4	6.1	4.7	2.7	3.2	1.2	3.1	2.3	0.3	5.2	2.6	1.6
Terpinen-4-ol	2.1	2.2	1.4	2.8	1.7	2.6	1.9	2.0	1.5	1.6	3.2	1.6	2.2
Alpha-Terpinene	2.0	2.7	1.6	2.1	1.8	3.7	2.0	1.5	1.3	1.0	3.3	1.7	1.5
Bornyl-acetate	1.4	0.6	3.6	1.8	0.5	2.1	0.3	1.1	1.1	2.7	0.6	1.8	0.6
O-Cymene	1.4	1.5	1.2	1.4	1.3	1.7	1.2	1.5	1.3	1.5	2.0	1.2	0.7
Methoxyphenyloxime	1.2	0.7	1.8	1.9	1.3	0.5	0.4	2.7	0.6	0.7	1.3	0.4	1.6
Beta-Cubebene	1.1	1.7	1.5	0.5	0.7	0.4	1.2	1.3	2.2	0.5	1.5	0.4	0.7

Table 3 (continued)

Bract													
Alpha-Pinene	51.7	50.8	55.3	51	49.7	39.5	63.7	44.8	55.8	54.5	44.5	47.9	63
Sabinene	8.7	10.4	9.8	7.8	8.7	12.4	7.3	8.7	4.0	7.7	9.2	9.5	8.7
Beta-Gurjunene	8.0	10.7	8.8	11.5	7.0	10.5	4.0	11.3	10	0.8	9.9	6.0	5.8
D-Limonene	4.7	3.7	5.7	5.3	3.9	3.0	4.9	1.9	4.2	6.9	7.5	4.1	5.0
Bornyl acetate	3.7	0.6	1.8	3.4	6.6	4.9	0.6	6.6	1.1	5.2	3.5	6.6	3.1
Beta-Pinene	3.6	3.2	0.2	4.3	3.9	5.1	1.5	4.9	3.1	7.0	4.9	2.6	2.3
Beta-Cubebene	1.8	2.8	2.5	0.5	0.4	1.9	2.3	1.2	3.1	1.3	2.2	2.5	1.4
Beta-Elemene	1.4	1.8	1.2	0.8	1.2	1.3	0.9	1.4	2.5	1.5	1.6	1.3	1.2
Gamma-Terpinene	1.1	1.3	1.2	1.4	1.0	1.6	0.7	1.2	0.4	1.1	0.6	1.7	1.4
Desmethoxyencecalin	1.0	0.4	0.1	0.4	0.6	3.7	0.5	0.3	4.1	0.2	1.1	0.1	1.0
Leaf													
D-Limonene	23.6	20.8	29.5	23.1	23.8	21.3	32.4	19.0	19.2	23.8	19.3	23.6	27.1
Beta-Cubebene	13.8	15.4	14.6	12.2	8.1	13.4	10.4	13.3	22.5	13.4	17.2	15.8	9.0
Sabinene	10.5	9.1	9.8	11.3	12.8	13.6	11.3	13.7	6.0	8.3	8.3	6.9	14.4
Alpha-Pinene	9.2	6.3	8.2	6.5	10.1	8.1	12.4	11.7	6.5	10.8	7.0	9.3	13.6
Methoxyphenyloxime	4.1	8.0	5.9	3.1	8.6	0.8	2.6	3.3	3.1	5.4	1.9	0.8	5.8
Beta-Cadinene	3.7	5.0	3.4	4.0	3.9	3.4	3.4	4.6	5.2	2.6	4.0	3.7	1.9
Caryophyllene	3.1	4.2	2.7	2.7	3.4	2.8	3.1	3.3	4.3	2.1	4.1	2.8	1.8
Gamma-Terpinene	2.6	1.5	2.3	2.4	2.5	4.0	2.7	2.9	2.0	2.4	2.3	2.9	3.6
Gamma-Cadinene	2.5	3.0	2.5	2.8	2.6	2.3	2.4	2.8	3.5	1.7	2.5	2.4	1.3
Endo-Borneol	2.4	0.5	1.8	4.4	1.5	2.0	0.6	1.2	1.9	9.1	0.8	3.8	1.0
Gamma-Muurolene	1.5	2.1	1.6	1.6	1.4	1.5	1.4	1.7	1.9	1.5	1.6	1.4	0.7
*Cycloundecatriene	1.3	1.4	1.6	1.6	0.9	1.3	1.3	1.5	1.7	1.3	1.4	1.2	0.8
Alpha-Muurolene	1.3	1.9	1.1	1.1	1.2	1.5	1.2	1.2	1.8	1.2	1.5	1.4	0.7
Alpha-Cadinene	1.2	2.0	1.0	1.3	0.5	1.2	1.3	1.1	1.7	0.9	1.5	1.4	0.8

*Cycloundecatriene is an abbreviation of 1,5,9,9-tetramethyl-Z,Z,Z-1,4,7-Cycloundecatriene.

This dramatic variation in volatile abundance and composition indicates that the secondary and tertiary germplasm of sunflower is rich in quantitative phytochemical diversity that could be leveraged for cultivar improvement, far beyond that that exists in the primary *H. annuus* germplasm alone (Kantar et al., 2015).

Contributions of monoterpenoids and sesquiterpenoids to volatile profiles

Within and among organs, overall total volatile abundance was heavily influenced by the total production of monoterpenoids (Fig. 2). In reproductive structures, monoterpenoids dominated total volatile production, such that variation in total volatile abundance was not significantly correlated with the production of sesquiterpenoids or other compounds. In leaves, however, both total monoterpenoid and total sesquiterpenoid abundance contributed significantly to variation in total volatile production among genotypes, with R^2 values of 0.88 and 0.71 respectively (Fig. S2). In leaves, monoterpenoid and sesquiterpenoid production were weakly correlated ($R^2 = 0.39$), indicating that among genotypes increasing leaf volatile abundance is associated with a general increase in both major classes of terpenoids, but that monoterpenoid and sesquiterpenoid abundance do not move in lockstep.

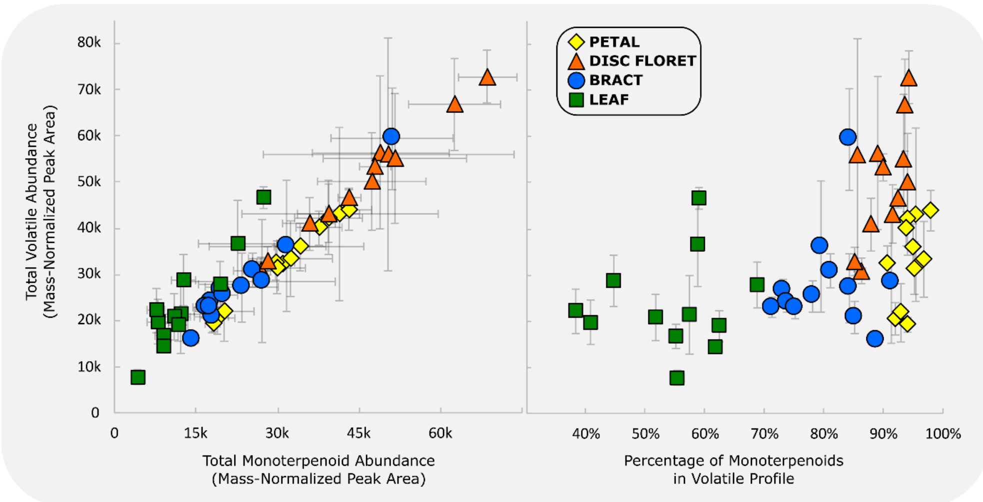

Figure 2. Contribution of total monoterpenoid abundance to total volatile abundance across organs and genotypes (left panel), and distribution of the organs and genotypes for total volatile abundance and proportion of monoterpenoids in the volatile profile (right panel). Points represent the mean across replicates of a single organ-genotype combination, with error bars representing the standard error of the mean.

Among genotypes, the proportion of the volatile profile comprised of monoterpenoids was highly correlated between leaves and bracts ($R^2 = 0.68$), as was the proportion of sesquiterpenoids ($R^2 = 0.80$), indicating that profile composition is not independent across these two organs and that genotype variation affects both organs simultaneously (Fig. S3). No significant correlations were observed between petal and disc floret proportions, or between proportions in these two organs and those in bracts or leaves, likely attributable to the low variation in sesquiterpenoid abundance in petals and disc florets.

Underlying secondary metabolism and applications in biotic interactions

The strong gradient in monoterpenoid-sesquiterpenoid balance observed between vegetative and reproductive structures across all Core 12 genotypes, as driven by variation in monoterpenoid production, is a major underlying driver of phytochemical differentiation among sunflower organs. Variation in this same monoterpenoid-sesquiterpenoid balance is observed within and among organs in wild *H. annuus* (Adams et al., 2017) as well as diverse *Helianthus* species (Bahmani et al., 2022), indicating that it is an inherent property of terpenoid secondary metabolism common to all sunflowers. High monoterpenoid production in reproductive structures is likely related to sunflower floral fragrance and its role in pollinator attraction, a trait that has to date received very little attention other than documenting variation among a few cultivars (Pham-Delegue et al., 1990; Bertoli et al., 2011). While the role of traits like floret morphology and nectar rewards in pollinator attraction have been systematically studied in cultivated sunflower using diverse germplasm (Mallinger & Prasifka, 2017; Portlas et al., 2018), the role of floral volatiles has been limited to valuable but narrow comparisons of few cultivars and honeybee choice and conditioning experiments with compounds derived therefrom (Pham-Delegue et al., 1986; Pham-Delegue et al., 1989; Pham-Delegue et al., 1990). The substantial quantitative variation observed among the Core 12 lines for disc floret volatile abundance, as well as relative composition of major compounds, indicates that the chemical signaling provided to pollinators is diverse among cultivated sunflower germplasm. Given this, broader screening of germplasm has the potential to identify particularly attractive floral fragrance profiles, either leveraging existing honeybee choice data or expanding this to wild bees given the critical pollinator services they provide to hybrid seed production where outcrossing is required, and potential to increase yield in oilseed production despite self-compatibility (Greenleaf & Kremen, 2006; Portlas et al., 2018). Optimization of cultivar floral fragrance would be highly facilitated by description of the genetic architecture of disc floret volatiles in sunflower (Dudareva & Pichersky, 2006; Pichersky & Dudareva, 2007).

The documentation provided here that cultivated sunflower exhibits substantially lower volatile abundance than wild *H. annuus* or other wild *Helianthus* may be an important factor related to the observation that cultivated sunflower is more susceptible to a wide range of pests and pathogens than wild *H. annuus* (Rogers et al., 1987; Chen & Welter, 2002; Charlet et al., 2008; Michaud & Grant 2009; Mayrose et al., 2011). While sesquiterpene lactones have been shown to contribute to resistance against head-feeding insects like the sunflower moth *Homeosoma electellum* (Rogers et al., 1987; Prasifka et al., 2015), few assessments of the anti-herbivore or anti-pathogen effects of volatile monoterpenoids or sesquiterpenoids have been conducted in sunflower. However, adjusting the relative ratios of five major sunflower monoterpenoids (alpha-pinene, beta-pinene, limonene, camphene, and bornyl acetate) substantially alters lure attractiveness to the red seed weevil *Smicronyx fulvus*, and substitution of sabinene reduces attraction further (Roseland et al., 1992). Ontogenetic variation in relative ratios of these same sunflower volatiles has also been demonstrated to alter attractiveness to the brown marmorated stink bug *Halyomorpha halys* (Wong et al., 2021). These examples indicate that at minimum, manipulation of volatile profiles is a potential route to developing cultivars that have reduced attractiveness to pests. The lack of tight phenotypic integration in volatile profiles among sunflower organs reported here suggests that volatile metabolism can be independently optimized in vegetative and reproductive structures.

Now that a substantial diversity of volatiles has been documented in a representative cross-section of cultivated sunflower germplasm, an obvious next step is to leverage the full Sunflower Association Mapping panel to identify the genetic architecture of volatile abundance and composition in sunflower. The genetic resources now exist to identify the genetic basis of this phytochemical variation (Badouin et al., 2017; Hübner et al., 2019), and permit optimization of sunflower volatile profiles through either genetic engineering or traditional breeding approaches. Furthermore, expanding investigation of the genetic basis of volatile production to wild *H. annuus* through use of the sunflower pangenome (Hübner et al., 2019) is a likely avenue for increasing volatile production beyond the range currently described in cultivated sunflower, whether for improvement of floral fragrance output, repelling pests, or other goals. Beyond this, leveraging the much broader qualitative and quantitative diversity available for volatiles in wild *Helianthus* is a yet another route if wild *H. annuus* diversity proves insufficient for a desired application (Kane et al., 2013; Kantar et al., 2015). Sunflower is already a more sustainable choice compared to many other crops, producing substantially lower greenhouse gas emissions than cereals or rapeseed both per hectare and per ton of yield (Debaeke et al., 2017), and the development of improved host plant resistance and improved pollinator-mediated seed set through phytochemical optimization of cultivars can further improve agricultural sustainability under a changing climate. In addition, the substantial genetic variation within sunflower in the composition of volatile terpenes deserves more attention in the context of terpenoid-based specialty biofuel production (Pausas et al., 2016; Mewalal et al., 2017).

CONCLUSION

Volatile terpenoids in cultivated sunflower mediate biotic interactions and are of value for cultivar improvement. To clarify the level of terpene diversity in cultivated sunflower, terpene profiles were evaluated across four vegetative and reproductive organs in twelve cultivated sunflower genotypes (known as 'Core 12') that capture about 50% allele diversity in a sunflower association mapping population. Results indicated a significant compositional diversity of volatiles among the studied lines, though substantial reduction in total volatile abundance relative to wild *H. annuus*. In the Core 12 genotypes, leaves produce a mixture of mono- and sesquiterpenoids, while reproductive organ composition is monoterpenoid dominated, although absolute sesquiterpenoid production is roughly similar across organs. Across the Core 12 genotypes, there is limited qualitative but substantial quantitative variation in volatile profiles, suggesting that for breeding increased volatile production the use of wild *H. annuus* and other wild *Helianthus* germplasm may be necessary.

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AUTHOR CONTRIBUTIONS. CMM and JAD designed the study. CMM and JAD led the greenhouse experiment and JAD led sampling of tissue. JAD and KB prepared samples for analysis. KB conducted GC-MS. MG and KB quantified metabolomic data from raw GC-MS output. KB and CMM performed data analysis and created figures. KB and CMM wrote the manuscript with input from MG and JAD.

DATA AVAILABILITY. Data used for this study are included in the supplement (Appendix 1) as well as the Dryad Digital Repository (Dryad: 10.5061/dryad.6wwpzgn31).

SUPPLEMENTAL MATERIAL: https://agronomy.emu.ee/index.php/category/running-issue/?aid=9386&sa=0#abstract-9379

Appendix1: https://agronomy.emu.ee/index.php/category/running-issue/?aid=9387&sa=0#abstract-9379

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SUPPLEMENTAL MATERIAL

Table S1. The 12 cultivated sunflower genotypes (inbred lines) for which volatile phytochemistry was assessed in this study.
Table S2. Total number of volatile compounds detected and identified via SPME-GC-MS in each genotype (pooling all four organ types assessed), as well as proportional breakdown of compounds.
Table S3. Volatile profiles as assessed by SPME-GC-MS in the four organ types assessed (averaged across the twelve plant genotypes).
Table S4. Volatile profiles as assessed by SPME-GC-MS in the twelve plant genotypes assessed (averaged across the four organ types).
Table S5. Fold-change variation in volatile compound profile metrics across the Core 12 genotypes and four organ types (48 genotype-by-organ combination means).
Table S6. Fold-change variation in volatile compound profile metrics across the Core 12 genotypes within the four organ types.
Table S7. Fold-change variation in volatile compound profile metrics across the four organs within each Core 12 genotype.
Table S8. The four most abundant compounds identified by SPME-GC-MS as a percentage of the overall volatile profile across the Core 12 genotypes and four organ types (48 genotype-by-organ combination means).
Table S9. The most abundant compounds identified by SPME-GC-MS as a percentage of the overall volatile profile in each of the Core 12 genotypes across the four organ types.
Table S10. Total number of volatile compounds detected and identified via SPME-GC-MS in *Helianthus* petals in recent studies, as well as the proportional breakdown of compounds.
Table S11. Total number of volatile compounds detected and identified via SPME-GC-MS in *Helianthus* leaves in recent studies, as well as the proportional breakdown of compounds.
Table S12. Volatile profiles as assessed by SPME-GC-MS in leaves and petals of one population of wild *Helianthus annuus* from Konza Prairie, Kansas (KON) by Bahmani et al. (2022).
Table S13. Volatile profiles as assessed by SPME-GC-MS in leaves of 20 wild populations of *Helianthus annuus* by Adams et al. (2017).
Table S14. Volatile profiles as assessed by SPME-GC-MS in leaves (n = 37) and petals (n = 24) of 40 species of wild *Helianthus* by Bahmani et al. (2022).
Figure S1. Negative correlations among the Core 12 genotypes between alpha-pinene and sabinene in petals and disc florets.
Figure S2. Positive correlations among the Core 12 genotypes between the total monoterpenoid abundance, total sesquiterpenoid abundance, and total volatile abundance in leaves.
Figure S3. Positive correlations among the Core 12 genotypes between the proportion of monoterpenoids and sesquiterpenoids in bracts and the proportion of monoterpenoids and sesquiterpenoids in leaves.

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Theoretical study of the conditions of the maximum allowable slipping of wheel tractors

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Abstract. The process of tractor wheels slipping is accompanied by two forms of ground deformation: displacement and shearing. From the point of view of preserving the structure of the soil environment, wheel slip should be limited to displacement only. The limit of soil strength [σ_o] at this deformation can be a standardizing parameter. In this article an analytical dependence is developed, which allows using the parameter [σ_o] to establish the maximum permissible level of slipping of wheeled tractors. Of the soil parameters, the specified dependence includes the coefficient of sliding friction between the particles of soil medium and the coefficient of wheel rolling resistance on a particular agrotechnical background. Theoretical studies have established that the greater the value of this coefficient, the smaller should be the maximum permissible value of tractor slipping with a more economical effect on the soil environment. Compared to a tractor with a nominal drawbar pull of 14 kN, the use of a heavier tractor with a drawbar pull of 30 kN is possible with higher values of maximum permissible towing. This result is due to the magnitude of the vertical load on the wheel of the heavier tractor, which gives it a greater friction force between the tire wheel and the ground and allows the same contacting traction force to be realized at a lower level of slipping. The results of mathematical modeling using the developed analytical relationships indicate that with increasing the value of the angle of placement (slope) of the tractor wheel to the longitudinal axis of symmetry, the maximum permissible level of its slipping should be less. This will help to reduce the value of deformation (longitudinal shift) of the soil by the tractor during its working movement. When using the ground shear strength limit [σ_o] y as a limiting parameter for increasing the pitch and height of the tractor tire, as well as the width of the tractor wheel tyre, the maximum permissible level of its towing is more than 15%, which is an undesirable fact.

Key words: rolling resistance coefficient, soil conditioner attachment, soil shear strength, tangential driving force, tractor attachment.

INTRODUCTION

Preservation and improvement of soil fertility has been and will always be one of the most urgent problems of agricultural production. Since soil structure and fertility play a decisive role in this, the full potential of agricultural science should be directed to their systematic improvement. First of all, this concerns the engineering direction, since the negative man-made impact on the soil is one of the determining factors in the decline of its fertility.

One of the most destructive processes of soil structure is its compaction and deformation due to the towing of heavy tractors (Ansorge & Godwin, 2007; Medvedev, 2010; Antille et al., 2015; Shafaei et al., 2021). In the first place wheeled tractors. It should be noted that the study of these processes has received considerable attention from the world scientific community (Farrakh et al., 2013; Chamen et al., 2015; Damanauskas & Janulevičius, 2015; Moitzi et al., 2016; Abrahám et al., 2017; Antille et al., 2019; Bulgakov et al., 2022). As a result, scientists and practitioners have developed a fairly informative system of recommendations to reduce the negative impact of towing the running gear systems of machine-tractor units on the soil structure.

However, it should be noted that these recommendations bypass such a problem as determining the maximum permissible level of slipping of tractor wheels. On the one hand, according to scientific data, it is known that a tractor as a part of a machine-tractor unit has high towing performance of the undercarriage system at the level of 20–22% (Macmillan, 2002; Bulgakov et al., 2020). On the other hand, it can be argued a priori about the unacceptability of such a man-made impact on the soil in terms of preserving its structure. At the same time, we note that our conscious use of the term 'a priori' is justified by the current absence of any restrictive-legislative documents concerning the permissible level of towing wheels of agricultural tractors.

The current document of similar purpose in Ukraine is DSTU 4521:2006 'Mobile agricultural machinery. Standard rates of impact on the soil by undercarriage'. It regulates the norms of maximum vertical pressure of agricultural machinery driving systems on the agricultural background $[Q_{max}]$ on its granulometric composition and moisture, as well as the terms of agricultural works in different soil and climatic zones.

The parameter $[Q_{max}]$ was used by us to establish the maximum permissible level of slipping δ_{max} of wheeled tractors, taking into account their deforming impact on the soil (Adamchuk et al., 2016; Battiato & Diserens, 2017; Nadykto et al., 2017; Bulgakov et al., 2020 and 2021). At the same time, the practical application of this parameter as a limiting parameter is rather problematic. Mainly due to the lack of methodological basis for the practical application of the requirements of the above DSTU. First of all, it concerns the practical determination of the specific pressure of the undercarriage system of wheeled power vehicles on the soil in kPa. The problem is that the value of such an indicator depends not only on the operating weight of the tractor, but also on the air pressure in the tires, their width and diameter, and most importantly on the hardness of the soil and its humidity. That is, in each case of using a particular tractor the specific pressure of its wheels on the ground will be different. Finally, everything is complicated by the problematic nature of measuring this indicator in the field in terms of determining the area of the bearing surface of the tractor wheels.

Given this, there is a need to find a compromise option for determining the maximum allowable slipping of wheeled undercarriage systems. This process is known to be accompanied by two forms of soil deformation: displacement and shearing. It is quite clear that in terms of preserving the structure of the soil environment wheels slipping should be limited only by displacement. The limit of soil strength at such a deformation can be a normative indicator. The nature of this parameter is quite clear, and the method of practical determination is widely known in scientific circles (Stefanow & Dudziński, 2020).

The aim of the study was determination of the maximum permissible slipping of wheeled tractors in the absence of shearing and manifestation of such a level of its shearing by the tyres, which does not exceed the specified strength limit.

THEORY AND MODELLING

As mentioned above, the process of slipping of wheel undercarriage systems is caused by displacement and shearing of soil by tyres of the soil in the direction opposite to the movement of the wheeled tractor. These deformations of the soil environment is its response to the action of the tangential force of wheel traction F_k (Fig. 1).

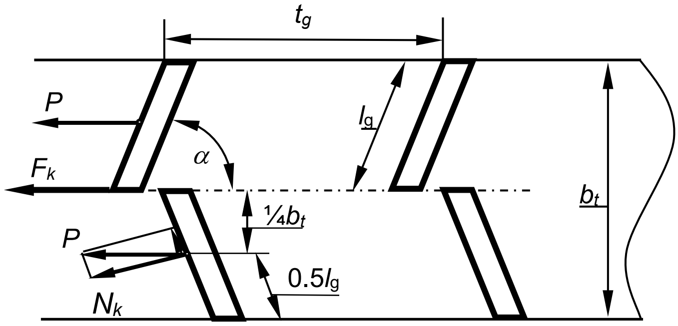

Figure 1. Scheme of the forces acting on the ground grips of the tyre of the tractor wheel (Nadykto et al., 2015).

In our opinion, the most complete connection between this force and the resulting shear and shear deformations of the soil is reproduced by the following analytical dependence:

$$F_k = \frac{f_{cl} \cdot k_\tau \cdot G}{\delta \cdot L} \left[\text{lnch} \frac{\delta L}{k_\tau} - f_{sup} \left(\frac{1}{\text{ch} \frac{\delta L}{k_\tau}} - 1 \right) \right] + 2\tau_{sh} \frac{h_g \cdot L}{t_g}, \quad (1)$$

where f_{cl} – coefficient of sliding friction between particles of the soil environment; k_τ – coefficient of uniaxial soil deformation, m; G – vertical load on the wheel, N; δ – wheel slip coefficient; L – the length of the contact area of the wheel with the supporting soil environment, m; f_{sup} – given coefficient of friction; τ_{sh} – soil shear strength, Pa; h_g, t_g – the height and step of the ground contact, respectively, m.

The force F_k can be represented by the sum of its two components P_k (Fig. 1). Each of them, in turn, has tangential and normal N_k components. The latter (i.e. the force N_k) carries out the displacement of the soil medium by the ground hitch, located on the wheel tire at an angle α.

We used dependence (1) in Bulgakov et al. (2021) and Nadykto et al. (2015) to determine the maximum permissible slip δ_{max} of a wheeled tractor. The limiting factor in this case was the above-mentioned normative parameter $[Q_{max}]$, which was used not in the vertical (as provided by DSTU 4521:2006), but in the horizontal direction of soil deformation by tire shoes, located at an angle α:

$$[Q_{max}] \leq \left\{\frac{f_{cl}G}{k_\delta}\left[\ln\text{ch}k_\delta - f_{sup}\left(\frac{1}{\text{ch}k_\delta} - 1\right)\right] + 2\tau_{sh}\frac{h_g L}{t_g}\right\}\frac{\sin^2\alpha}{\text{Integer}\left(\frac{L}{t_g}\right)b_t h_g}. \quad (2)$$

In equation (2), the values of the components will be as follows:

$$k_\delta = \frac{\delta \cdot L}{k_\tau}, \quad (3)$$

and

$$L = R_k \cdot \left(\text{arctag}\frac{f_k \cdot \sqrt{1 - f_k^2}}{0.5 - f_k^2} + 2f_k^2\right), \quad (4)$$

where R_k – rolling radius of the wheel, m; f_k – coefficient of rolling resistance.

The subject analysis of analytical dependence (2) allows us to establish two important points. According to the first term of the expression in curly brackets $2\tau_{sh}h_g L \cdot t_g^{-1}$ is the ground shear deformation, which, in principle, should be made impossible in determining the maximum allowable wheel slip, and therefore in the further analysis is not necessary to consider.

The second point is related to the parameter k_δ. The analysis of equations (3) and (4) for tractors with rated drawbar pull of 14, 30 and 50 kN reveals that the value of hyperbolic cosine k_δ differs from the one by 5–6%. Taking it into account with sufficient accuracy for practice, we can also take into account in expression (2) that:

$$\text{ch}k_\delta = \text{ch}\frac{\delta \cdot L}{k_\tau} = \frac{e^{\frac{\delta L}{k_\tau}} + e^{-\frac{\delta L}{k_\tau}}}{2} \cong 1, \quad (5)$$

and

$$f_{sup}\left(\frac{1}{\text{ch}\frac{\delta L}{k_\tau}} - 1\right) \cong 0. \quad (6)$$

Taking into account dependences (5) and (6) and replacing in expression (2) the parameter $[Q_{max}]$ by a new normalizing parameter $[\sigma_o]$, we obtain a new dependence for determining the maximum permissible slipping of a wheeled tractor, taking into account such limiting factor as the limit of strength of the ground in the presence of its displacement:

$$[\sigma_o] \leq \frac{f_{cl} \cdot k_\tau \cdot G}{\delta_{max} \cdot L} \cdot \ln\text{ch}\frac{\delta_{max} \cdot L}{k_\tau} \cdot \frac{\sin^2\alpha}{\text{Intger}\left(\frac{L}{t_g}\right) \cdot b_t \cdot h_g}. \quad (7)$$

If we take into account that earlier for k_τ the following analytical dependence was proposed (Nadykto et al., 2015)

$$k_\tau = 0.4 \cdot t_g. \quad (8)$$

Taking this into account, we finally have a new analytical expression for determining a new normalizing indicator $[\sigma_o]$:

$$[\sigma_o] \leq 0.4 \frac{f_{cl} \cdot t_g \cdot G}{\delta_{max} \cdot L} \cdot \text{lnch} \frac{2.5 \cdot \delta_{max} \cdot L}{t_g} \cdot \frac{\sin^2\alpha}{\text{Integer}\left(\frac{L}{t_g}\right) \cdot b_t \cdot h_g}. \quad (9)$$

The obtained equation (9) together with dependence (4) allows us to determine the influence of soil and tractor parameters on the maximum permissible level of slipping of its wheels under the action of the limiting factor in the form of the landslide strength limit $[\sigma_o]$.

MATERIALS AND METHODS

The research methods are based on the use of sections of the tractor traction dynamics theory considering the process of rolling of an elastic traction wheel on a deformed ground surface. The theoretical calculations of the obtained analytical regularities were performed in the MathCad 15.0 software environment. The initial data were the parameters of a typical soil medium and construction parameters of the most common tractors with nominal drawbar pull of 14 and 30 kN in this area.

To conduct theoretical research of dependence (9) taking into account expression (4) to determine the maximum allowable slipping of wheeled tractors, let us define the nomenclature of their nominal drawbar pull. At the present time, in spite of proposed by us project of new type of wheeled tractors for Ukraine, though formally, but still valid GOST 27021-86 (ST SEV 628-85). Of the ten traction classes declared in it, tractors with nominal drawbar pull of 14 and 30 kN, represented in this study by type models of MTZ-892 and HTZ-170 series (HTZ-17221), respectively, are most common almost all over the country (Table 1).

Table 1. Construction parameters of wheeled tractors

Nominal drawbar pull category of tractor series	Type size tires	Structural parameters of the tractor and wheel					
		G, N	t_g, m	h_g, m	b_t, m	R_k, m	α,°
1.4 (MTZ-892)	16.9R38	25,260	0.23	0.038	0.43	0.770	43
3 (HTZ-170)	23.1R26	30,900	0.23	0.045	0.59	0.715	47

Wheeled tractors with smaller nominal drawbar pull (2, 6 and 9 kN) are used much less as part of traction machine-tractor units. In the recent past, they were generally regarded as non-systematic and for them a trail of appropriate agricultural machines and tillage and other implements were not even developed.

Tractors with nominal drawbar pull of 50, 60 and 80 kN develop satisfactory traction-energy indicators, as a rule, on technological operations on basic tillage. Usually it is in such aggregate state, when its resistance to shear in horizontal direction is much higher than that of agrotechnical backgrounds characterized by rolling resistance coefficient of 0.12–0.18. Practice shows that on agrotechnical backgrounds with such a characteristic, the optimal loading of tractors with drawbar pull of 50–80 kN is quite problematic.

As for some parameters of the soil environment, for loamy soils: f_{cl} = 0.72–0.79; f_k = 0.12–0.18. Their uniaxial deformation strength σ_o varies within a wide range (Macmillan, 2002). Moreover, the better the soil structure, the lower the value $[\sigma_o]$. At the same time, it is possible to assert this only a priori, since there is practically no experimental data on the nature of the relationship between the structure of chernozems or other soils and their landslide strength. From the small amount of available information (Torikov et al., 2016), it follows that for soils with medium structure the value of the parameter $[\sigma_o]$ is at the level of 100 kPa. It is this value of this parameter that was used in the theoretical calculations.

RESULTS AND DISCUSSION

According to calculations of dependence (9), taking into account expression (4), the maximum permissible value of wheeled tractor slipping δ_{max} significantly depends on the state of agricultural background, on which the wheeled tractor is moving. The quantitative characteristic of the background in this case is the value of the rolling resistance coefficient f_k. As it turned out, the intensity (steepness) of the function change $\delta_{max} = f(f_k)$ for both compared power tools (MTZ-892 and HTZ-17021 tractors) is practically the same (Fig. 2).

That is, if the increase of f_k coefficient from 0.12 to 0.18 for the tractor HTZ-17221 requires the reduction of the maximum allowable slipping by 35% (in relative measure), then for the tractor MTZ-892 it is 37%. The resulting difference of 2% (in absolute measurement) can be considered insignificant.

There is a reason to claim that the regularity of δ_{max} decrease with increase of rolling resistance coefficient f_k of wheeled drivers is quite logical. After all, the greater is the value of f_k, the lower is the agricultural background density, the fluffier it is, and therefore more sensitive to the deforming effect in the form of displacement. Based on this we have the conclusion: the greater the coefficient of rolling resistance of the wheeled mover on a particular agricultural background, the lower should be the maximum permissible value of its towing.

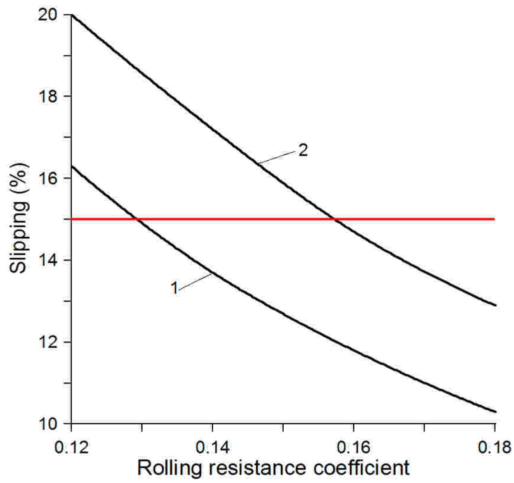

Figure 2. Dependence of the permissible slippage of the driver on the coefficient its rolling resistance: 1 – tractor MTZ-892; 2 – tractor HTZ-17221.

Analysis of comparison of calculation data for both tractors indicates that the use of tractor with nominal traction force of 30 kN (in this case - tractor HTZ-17221) is possible at high values of parameter δ_{max}. The reason of such result is caused by the value of vertical (or normal) load on the wheeled engine. Namely, it is by 23% higher in tractor HTZ-17221 than in tractor MTZ-892 (Table 1). Bigger value of G parameter in its turn causes bigger friction force between wheel tire and ground. And it allows one and the same contacting drawbar pull to be realized at lower level of wheel mover slipping.

Of the values included in expressions (4) and (9), a significant influence on the value of the maximum permissible slipping of wheeled power vehicle engines has the angle of position α of the tiller on the tire (Fig. 1). Fig. 3 shows the character of change of function $\delta_{max} = f(\alpha)$ at different values of rolling resistance coefficient f_k for wheeled power tool MTZ-892.

The results of mathematical modeling show that with increasing the value of the angle α the maximum permissible level of slipping of the wheel propeller should be less (curves 1 and 2, Fig. 3). This is explained by the location of the tire's hitch. As the value of α increases, it occupies a position close to the transverse with respect to the longitudinal axis of wheel symmetry. As a result, the tangential traction force component - force N_k, acting in the transverse (normal) direction to the side surface of the hitch (Fig. 1), increases. Because of this, the amount of shear deformation of the soil medium in the direction opposite to the tractor's movement increases.

As in the previous case, the intensity (steepness) of the function change $\delta_{max} = f(\alpha)$ for both variants of the coefficient value f_k is also almost the same (Fig. 3).

The cancellation is that with smaller values of f_k the value of the maximum permissible slipping of wheeled propellers may be greater. The result is quite logical, because at larger values of the coefficient of rolling resistance, the density of the soil environment, on which the tractor moves as part of a machine-tractor unit, is higher, its looseness is less, and the ability to resist shear is higher. Therefore, the greater the value of the coefficient f_k, the more sparing should be the impact on the agrotechnical background in the form of slipping of wheeled propellers of power vehicles. One of the technical options for solving this problem is the use of the latter with doubled or even tripled tires. Technological feasibility of such a technical solution is reflected quite substantially by Adamchuk et al. (2016).

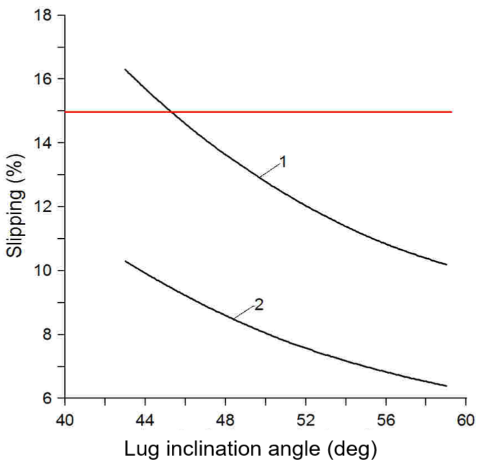

Figure 3. Dependence of the maximum permissible slipping of MTZ-892 tractor on the angle α of its tread on the tyre: 1) f_k= 0.12; 2) f_k= 0.18.

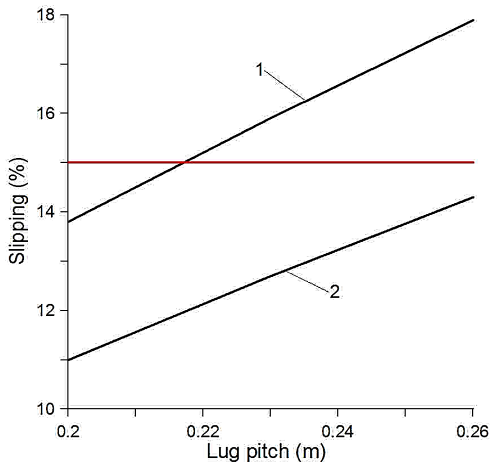

Figure 4. Dependence of the permissible skidding of the driver from the tread of the tyres: 1) tractor HTZ-17221; 2) tractor MTZ-892.

According to theoretical calculations we have that increasing the ground Clearance clearance pitch of the wheel propeller tyre allows us to increase the level of its permissible slipping. This level of tractor HTZ-17221 (curve 1, Fig. 4) is by 25% (in relative terms) higher than that of tractor MTZ-892 (curve 2).

The reason for this result is greater vertical load on the wheel of the tractor HTZ-17221. As already explained above, a larger value of parameter G causes greater friction force between the wheel tyre and the ground. And it gives an opportunity to realize more drawbar pull to the wheel at the same level of towing.

The height of the wheel hitch of both considered tractors qualitatively affects the value of maximum allowable slipping in the same way: the value of h_g max increases with increasing δ_{max} value (Fig. 5).

The logic behind this result is as follows. Increasing the height of the ground hook leads to a corresponding increase in the area of the side surface, which is located on the tire at an angle α and directly contacts the ground in the direction of the force N_k (Fig. 1). The increase in the specified area at the same value of force N_k causes a lower specific pressure of the trailing edge on the ground. This, in turn, leads to a decrease in the deformation of its displacement, which allows you to increase the maximum permissible value of towing the wheel.

As in the case with the pitch of the tractor tyre tread t_g, increasing its height h_g prefers increasing the value of δ_{max} to the heavier tractor (curve 1, Fig. 5) compared to the lighter tractor (curve 2, Fig. 5).

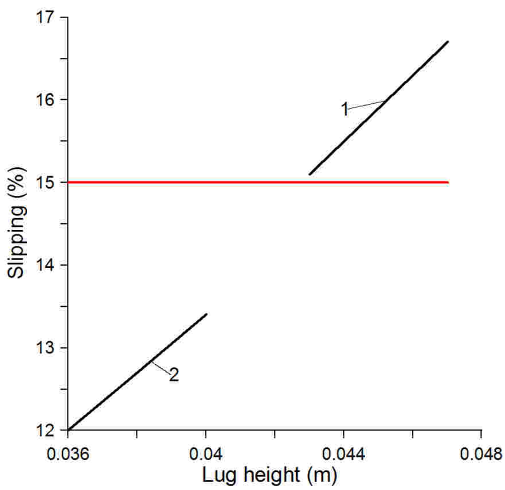

Figure 5. Dependence of the permissible slippage of the mover on the height of the tire ground hook: 1) tractor HTZ-17221; 2) tractor MTZ-892.

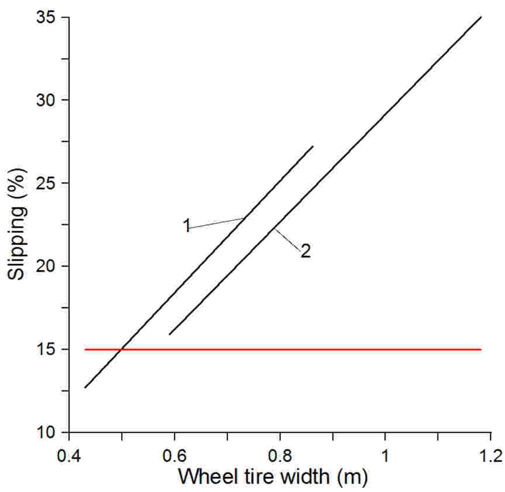

Figure 6. Dependence of allowable towing of mover on width of its tire: 1) tractor MTZ-892; 2) tractor HTZ-17221.

Now we will consider how the value of the maximum permissible wheel towing is affected by increasing the width of its tyre b_t. Increase of value of this parameter for both compared tractors twice causes corresponding increase of value of maximum permissible wheel towing (Fig. 6). This result is quite logical for the following reasons. The increase of the parameter b_t – is the corresponding increase in the length of the tyre tread l_g (Fig. 1), and therefore in the area of its supporting surface, directly in contact with the ground.

And the growth of bearing surface of the ground hook is the reduction of landslide value, which is the corresponding prerequisite for the increase of δ_{max} parameter. It should be noted that for the heavier tractor (in this case the HTZ-17221 tractor) the maximum permissible value of wheel slip for the reasons explained by us above is more.

Our preliminary studies have established that when using the normalization parameter $[Q_{max}]$ the maximum allowable value of towing of propulsion engines of wheeled power vehicles should not exceed 15%.

From the analysis of Fig. 2–6, where this limit is indicated by the red line, we see that when the shear strength of the soil $[\sigma_o]$ is used as a limiting parameter, the condition ($\delta_{max} \leq 15\%$) is not always satisfied. We will analyze this in more detail.

From Fig. 2 we see that the condition $\delta_{max} \leq 15\%$ almost completely corresponds to the tractor with a pulling force of 14 kN (tractor MTZ-892). For tractors with drawbar pull 30 kN (tractor HTZ-17221) this condition is true at their work on agricultural backgrounds with value of rolling resistance coefficient not less than 0.16 (curve 1, Fig. 2). It follows that on less loose (i.e., denser) backgrounds, the value of the maximum allowable slipping of these tractors can be increased up to 20%. But, as emphasized in (Nadykto et al., 2017), this should not be allowed in order to preserve the soil structure. That is, even when operating tractors such as HTZ-17221 on agricultural backgrounds with rolling resistance coefficient $f_k \leq 0.16$ the maximum permissible level of their slipping should not exceed 15%. It must be taken into account when assembling one or another machine-tractor unit on the basis of similar wheeled tractors.

Changing the angle of starters placement for both tractors under consideration within 43–59° practically does not contradict the requirement $\delta_{max} \leq 15\%$. Insignificant (by 1°) exceeding of this maximum allowable value of slipping takes place at α = 46° on the agricultural background with rolling resistance coefficient $f_k \leq 0.12$ (Fig. 3).

The values of pitch and height of ground hooks of MTZ-892 tractor wheels of 0.20 – 0.26 m (Fig. 4) and 0.036–0.40 m (Fig. 5) correspondingly fully meet the requirement $\delta_{max} \leq 15\%$. At the same time we have a different situation with the tractor HTZ-17221. Changing the height of its starters from 0.043 to 0.047 m allows you to set the maximum permissible value of wheel slip at the level of more than 15%, which, as has been repeatedly emphasized above, is unacceptable. As for the ground hooks pitch, only when their values are less than 0.22 m (curve 1, Fig. 4) the condition $\delta_{max} \leq 15\%$ is fulfilled. In practice, this means that the tyre tread of HTZ-17221 tractor type can be located in increments of more than 0.22 m, but the maximum permissible level of slipping of its wheels should not exceed 15%.

At width of the tire b_t > 0.5 m for tractor MTZ-892 and values of this parameter in the range of 0.59-1.18 m for tractor HTZ-17221, the considered condition of maximum permissible value of wheels slipping at a level no more than 15% is not satisfied. The use of doubled tires increases twice the area of the side surface of the tires, through which the ground is shifted. Therefore, as already emphasized above, a higher level of tractor wheel slip can theoretically be allowed. In practice, this cannot be done.

From the above analysis follows one logical question: why, when using the parameter $[\sigma_o]$ the maximum permissible level of tractor wheel slip δ_{max} can exceed the level of 15%, obtained when using as a limiting parameter $[Q_{max}]$? Such discrepancy of requirements on δ_{max} is caused, in our opinion, by the limited correct data on the value of σ_o parameter. Especially those that reflect the regularities of the dependence of the strength limit of the landslide on its structural-aggregate state. Obtaining such research information should be considered as one of the main research tasks of the nearest future.

CONCLUSIONS

The analytical dependence, which allows determining the maximum permissible level of towing of wheeled power tools, taking into account the limit of the soil medium strength for displacement, is developed.

Theoretical studies have established that the greater the coefficient of rolling resistance of a tractor wheel on a particular agricultural background, the less the maximum permissible value of its towing with a more economical impact on the soil environment.

In comparison with tractor with nominal drawbar pull 14 kN (tractor MTZ-892) the use of tractor with drawbar pull 30 kN (tractor HTZ-17221) is possible with higher values of maximum permissible towing. Such a result is due to the greater vertical load on the wheel of the tractor HTZ-17221. As a result, it provides him a greater friction force between the wheel tire and the ground and allows the same tangential traction force to realize at a lower level of slipping.

The results of mathematical modeling show that with increasing the angle of placement (inclination) of the hitch to the longitudinal axis of symmetry of the tractor wheel, the maximum permissible level of its slipping should be less. This will reduce the amount of deformation (longitudinal shear) of the soil by the tractor in the process of its working movement.

When using the limit of soil shear strength as a limiting parameter, increasing the values of the pitch and height of the tractor wheel and the width of the tractor wheel tire allows the maximum permissible level of its towing more than 15%, which is an undesirable fact.

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Potential impacts of chitosan on growth, yield, endogenous phytohormones, and antioxidants of wheat plant grown under sandy soil conditions

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Abstract. A field experiment was carried out in sandy soil, during two winter successive seasons to study the impacts of different concentrations of chitosan (50, 100 & 150 mg L^{-1}) on several growth parameters and biochemical changes as well as quantitative and qualitative grain yield. Foliar treatment of chitosan significantly increased the growth parameters concurrently with an increment in the photosynthetic pigments, total soluble sugar, proline, free amino acid total carbohydrates, antioxidant activities, phenol, flavonoids, and some minerals nutrition of wheat plant. Wheat plants treated with chitosan at different concentrations significantly increased different endogenous phytohormones auxins (IAA), abscisic acid (ABA), gibberellins (GAs), and cytokinins (Cyt), as compared with the untreated plants. Moreover, chitosan concentrations induced significantly increments in grains yield, nutritive values, carbohydrates %, proteins %, antioxidant compounds and macronutrients of the grain yield. Cultivation of wheat plants under sandy soil conditions and treated with foliar application of 100 mg^{-1} chitosan gave the higher values of the grain yield as well as the nutritional values contents.

Key words: chitosan, endogenous phytohormones, antioxidants, nutritional values, wheat, yield.

INTRODUCTION

In recent years, the forever-growing request for food worldwide, the ongoing climate change, the severe consumption of farmlands and the rising consideration of consumers to high-quality, secure and environmental-friendly food production collectively have encouraged the search for other biological methods that could encounter this request. Moreover, the changeable environmental conditions form a challenge to agriculture production, thus improving any agricultural system of the country is a necessity to encounter its people's requirements. Scientists paid their interest to compensate for this reduction by reclaiming and cultivating new sandy soils. So, due to the bad properties of sandy soils, the experiment was carried out in the reclaimed sandy soil in Egypt. The chosen district, is a portion of the Sahara Desert of Northern

Africa, and it is insecure due to the to the presence of different environmental stress conditions such as low water availability, high irradiances, temperature variations, and nutrient deficiency (Ramadan et al., 2020).

Thus, it is significant to increase wheat production by utilizing several planning, like extending wheat cultivation in sandy soil and conditions utilizing either high crop varieties or natural compounds as bio-stimulants and elicitors in agriculture to enhance its growth and yield in such type of soil. Elicitor is one of the important efficient strategies for promoting the plant defense mechanism. Moreover, elicitor is promoting transportation via a signal transduction system leading to alteration in the various aspects of cellular processes and finally enhancing the biosynthesis of secondary metabolites (Delaunois et al., 2014 and Suarez-Fernandez et al., 2020). Elicitorshave an important influence on various metabolic processes which promote plant growth and development through rising photosynthesis, endogenous bioregulator, nucleic acid,protein synthesis and ion uptake (Abbas, 2013). The discernment of sustainable crop production and rising agricultural yield with inexpensive inputs are the requirements for farmers. Accordingly, various strategies for the exogenous application of various natural compounds must be used to achieve this goal. One of those natural substances which could be provided safely to crops is the chitosan shell of shrimp extract. Chitosan is a natural compound and is commercially proceeded from seafood shells (Boonlertnirum et al., 2010). Chitosan is a low toxic and cheap substance beside being biodegradable and environmentally friendly with different applications in agriculture. Chitosan is a biopolymer, from the carbohydrate family primly created from a glucose ring and has a free amino group at carbon number - 2, however, the employment of chitosan increments with rising amino groups (Badawy & Rabea, 2011). Moreover, because of its cationic properties, chitosan submits a broad set of physicochemical and biological characteristics, inclusiveof both antimicrobial, and antioxidant characteristics (Aranaz et al., 2009). Chitosan is considered by individual characteristics, like bioactivity and biocompatibility (Dias et al., 2013). Chitosan application could increase the plant's yield (Mondal et al., 2012), decrease transpiration and induce a range of metabolic changes (Dzung et al., 2011). Also, the usage of chitosan improved key enzyme activities of nitrogen metabolism (nitrate reductase, protease, and glutamine synthetase) and increased the transmission of nitrogen in the workable leaves which increased plant growth and development. Chitosan stimulates plant hormones, lipid signaling and protection compounds in tomato root exudates, such asphenolic compounds (Suarez-Fernandez et al., 2020). Chitosan also propitiates the accumulation of auxin in the apex of plant roots (Lopez-Moya et al., 2017), and promotersmetabolic pathways (e.g., phenylpropanoid) participated in the biosynthesis of phenolic compounds (Singh et al., 2020).

Wheat (*Triticum aestivum* L) is the utmost substantial cereal crop grown in the world.At least one-third of the world's people consists of wheat as its major staple (Abdallah et al., 2015). The grains of wheat contain great amounts of proteins, carbohydrates, several minerals and vitamins.For these purposes, efforts must be directed toward the incrementwheat yield to fill the gap between production and consumption; so, the cultivated area could be increased by reclaiming new lands, supplying them with water from wells and devoting them to wheat production.

This study was conducted to investigate the performances of different doses of chitosan on grain yield and some biochemical aspects of wheat plants (Gimmeza 9) under sandy soil conditions. The specific objectives of the current study were to (i) Keep up the growing attention to high-quality, secure, and environmentally friendly grain production. (ii) Eliminate pollutants and use environmentally friendly materials by using the remaining shrimp shell by recycling action (iii) Utilizing chitosan as a natural compound (bio-stimulant) in agriculture toevaluate the effects of three different doses of foliar spray of chitosan on growth and grain yield of wheat (iv) Assess the effects of foliar spray of chitosan on the endogenous phytohormones, photosynthetic pigments and total antioxidant activities. induction of total carotenoids, lycopene, flavonoid and phenolic compounds and (vi) determine the effect of chitosan spray on the nutritional values of the yield grains.

MATERIALS AND METHODS

Materials

Wheat grains variety (Gimmeza 9) with 98 germination percentage by certified seed was obtained from Agricultural Research Center, Giza, Egypt.

Experiment location

The experimental farm of the National Research Centre was located in Nubaria region, Egypt, (30_86'67" N 31_16'67" E, with a mean altitude 21 m above sea level), two field trials were sown in two consecutive winter seasons in 2019/2020 & 2020/2021. The soil Farm is considered to be either an arid or semi-arid zone. The temperature at night was 8.7 to 16.23 °C, with an average of 11.8 and 10.12 to 16.54 with an average of 12.81, while daytime temperatures varied from 18.82 to 28.15 °C with an average of 22.52 °C and 17.05 to 28.91 °C with an average of 22.43 °C. During the years 2019–2020 and 2020–2021, respectively, the relative humidity ranged from 58.0 to 69.38% with an average of 64.4% and 58.22 to 70.9% with an average of 65.52%. According to Chapman & Pratt (1978), the soil at the experimental site was reclaimed sandy soil, and mechanical and chemical analyses are described in (Table 1).

Table 1. Analysis of the experimental soil's mechanics, chemistry, and nutrition

Sand		Silt	Clay	Soil texture
Course 2000-200µ	Fine 200-20µ	20-0µ	< 2µ	
47.44%	36.21%	12.88%	4.26%	Sandy

Chemical analysis:

pH 1:2.5	EC dSm^{-1}	$CaCo_3$	OM %	Soluble Cations meq/l				Soluble anions meq/l			
				Na^+	K^+	Mg^+	Ca^{++}	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
7.60	0.14	5.30	0.07	0.56	0.14	0.93	1.10	0.0	1.27	0.46	0.87

Nutritional analysis:

Available nutrients

Macro element ppm		Micro element ppm				
N	P	K^+	Zn^+	Fe^{++}	Mn^{++}	Cu^{++}
53.0	12.0	75.0	0.14	1.40	0.30	0.00

Experiment design

Four replications were used in the randomized complete block design of the experiment. In both seasons, wheat grains were sown on November 20th in rows that were each 3.5 meters long and spaced 20 cm apart on a 10.5 m^2 plot (3.0 m in width and

3.5 m in Length). When wheat was sown in sandy soil, agricultural operations were carried out as recommended, and the seeding rate was (140 kg ha^{-1}). The soil was amended with 360 kg ha^{-1} of calcium super-phosphate (15.5% P_2O_5) before sowing. Following plant emergence, ammonium nitrate 33.5% at a rate of 180 kg ha^{-1} was used as a nitrogen fertilizer, it was divided into five equal amounts before the first, second, third, fourth, and fifth irrigation water. Before the first and third irrigation water, two equal dosages of 120 kg ha^{-1} of potassium sulphate (48.52% K_2O) were applied. The new sprinkler irrigation system, which added water every five days, was used for irrigation. Chitosan at concentrations (0.0, 50, 100, & 150 mg L^{-1}) were applied twice to wheat plants in the tillering and elongation stage (about 4 to 5 leaves) at 45 & 60 days after sowing.

Irrigation water requirements:

Irrigation water requirements calculated using Penman Monteith equation and crop coefficient according to Allen et al. (1989). The average amount of irrigation water applied with sprinkler irrigation system was 5,950 m^3 ha^{-1} season^{-1} for both seasons of the experimental work (Table 2).

The amounts of irrigation water were calculated according to the following equation:

$$IWR = \left[\frac{ET_0 \times K_c \times K_r \times I}{E_a} + LR\right] \times 4.2 \quad (1)$$

where IWR = Irrigation water requirement m^3 ha^{-1}/ irrigation; ET_0 = Reference evaporation - transpiration (mm day^{-1}); K_c = Crop coefficient; K_r = Reduction factor (Keller & Karmeli, 1975); I = Irrigation interval, day; E_a = Irrigation efficiency, 90%; LR = Leaching requirement = 10% of the total water amount delivered to the treatment.

Preparation of Shrimp shells

In the current study, we described the low-cost chitosan's extraction from shrimp shells via chitin extraction, followed by alkaline deacetylation of chitin with a strong alkaline solution at various times. Chitin can be taken from a variety of sources and transformed to chitosan using variable levels of deacetylation and varying NaOH concentrations. The technique used to extract the chitosan was essentially the same as that used by Saleh et al. (2016).

Table 2. Water for growth stages of the wheat crop at Nubaria station during two successive winter seasons 2019/2020–2020/2021

Growth stages	No. of days	The applied water to the growth stages (m^3 ha^{-1})
Initial	20	326.04
Develop.	30	1,086.76
Mid	65	3,260.27
Late	40	1,141.08
Harvest	27	135.85
Total	182	5,950

Materials

Shells of shrimp was obtained from Suez Shrimp (Egypt). Sodium hydroxide (NaOH), hydrochloric acid (HCl), and acetic acid gotten from Aldrich, Egypt. They were then diluted to the concentration required for the methodology with distilled water.

Methods (Extraction of chitosan)

The extraction of chitosan can be carried out after removing the loose tissue from the shrimp shells. The shells were washed for 6 h. For chitin and chitosan productions drying, thoroughly with water to remove impurities in a hot-air oven at 90 °C and grinding to obtain a dry powder. The major procedure for obtaining chitosan is based on the alkaline deacetylation of chitin with a strongly alkaline solution at different periods of time.

Demineralization process

The demineralization was carried out by weighing 50 gm of powdered shrimp shells and using 4% HCl (1.3 N) at a ratio of 14 mL: 1 g (w/v) for 24 h at room temperature. The product was washed to neutrality under running tap water. Electricoven at 70 °C was used to dry the solid after it had been collected and rinsed with distilled water.

Deproteinization process

5% NaOH (1.25 N), 12 mL: 1 g (w/v), 90 °C, and 24 hours of deproteinization were performed. The deproteinized material was gathered and cleaned with distilled water.

Deacetylation

The product was deacetylated with 70% NaOH (17.5 N) with a ratio of 14 mL: 1 g (w/v) at room temperature for 75 h. with stirring. The solid was collected and washed with distilled water. The deacetylated product was then dried in an oven at 70 °C.

Plant sample

After 75 days from seeding, plant samples were taken in order to determine the growth characteristics (shoot height (cm), number of leaves per tiller, tiller fresh and dry weight per plant (g), and water content %). Photosynthetic pigments, endogenous phytohormones, organic solutes (proline, total free amino acids, and total soluble sugar), total carbohydrates, total proteins, flavonoids, total phenol contents, lipid peroxidation total antioxidant activities, and a few minerals (nitrogen, phosphorus, potassium, and calcium) were all measured in wheat leaves as part of the biochemical analysis.

The following characteristics were measured at harvest on randomly selected groups of 10 guarded tillers in every plot to estimate the following characteristics: plant height (cm), spikelets no/spike, 1,000-grains weight (g), grain yield/spike (g), straw yield, biological yield, and grain yield (ton ha^{-1}). In wheat grains, the percentages of carbohydrates, proteins, flavonoids, lycopene, and minerals (nitrogen, phosphorus, potassium, and calcium) were measured.

Water Content

Water content was determined according to (Jin et al., 2017). The fresh leaves of each sample were weighed and then dried at 80 °C for 72 hourswas record as dry weight. The leaf water content was calculated as the following:

Water content(%) = (Wf − Wd)/Wf × 100 Where, Wf, fresh weight and Wd, dry weight. Each sample was measured in biological triplicate.

Water-productivity (WP):

WP was determined by (Howell et al., 1990). The relationship between grain yield and irrigation water quantity is known as water productivity (WP). WP in (kg/mm ha^{-1}) was computed using the following formula:

Water productivity = wheat grain yield (kg ha^{-1}) / total utilized of irrigation water, m^3ha^{-1} /season.

Physiological and biochemical studies

Photosynthetic pigments: The method previously described (Li & Chen, 2015) was used to quantify the amounts of both chlorophyll a and b and carotenoids in fresh leaves using spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). In terms of g per 100 g of fresh weight, photosynthetic pigment levels were expressed.

Extraction, isolation, and identification of endogenous phytohormones: The methylation process was carried out in accordance with the previously stated approach (Urbanová et al., 2013), and the hormone extraction procedure was carried out in accordance with the previous method employed by Zhang et al. (2015). Hewlett Packard carried out the identification and quantification of auxins, gibberellins, and abscisic acid, gas-liquid chromatography (5890) with a flame ionization detector (Tarkowská et al., 2014). In accordance with Tarkowski et al. (2009) approach, cytokinin fractions were identified by HPLC isocratic UV analyzer.

Organic solute: The phenol sulphuric acid methodwas used to determine total soluble sugar (TSS) concentrations Mecozzi (2005). Proline (Pro) and free amino acids (FAA) were extracted using the technique outlined by Vartanian et al. (1992). Using Yemm et al. (1955) ninhydrin reagent technique for determination free amino acids. According to Bates et al. (1973) approach, proline was measured.

Total soluble protein (TSP) was appointed using the technique of(Maehreet al., 2018). Total carbohydrate was measured according to DuBois et al. (1956).

Antioxidant compounds: The total phenolic compound was measured using the spectrophotometer as previously determined (Siddiqui et al., 2017).With the use of the technique described by Dewanto et al. (2002) total flavonoids were estimated. Lycopene levels were measured using the method of Nagata & Yamashita (1992) approach.

Lipid peroxidation, through estimating the quantity of malondialdehyde (MDA) produced via the previously published thiobarbituric acid (TBA) reaction, lipid peroxidation was ascertained (Wang et al., 2013).

Antioxidant activity: in order to measure the antioxidant activity (DPPH radical scavenging) method of Liyana-Pathiranan & Shahidi (2005).

Mineral contents: The grains samples were powdered after being oven-dried at 70 °C for 72 hours. N, P, and K were measured. According to Paul et al. (2017) the Kjeldahl procedure was used to determine the nitrogen content of plant leaves. Paul et al. (2017) state that the spectrophotometer approach was used to estimate the phosphorus content. According to Paul et al. (2017) the potassium content of the plants was assessed using a flame photometer method.

Statistical analysis

Complete randomized block design statistical analysis was performed on the data. Given that the trend was consistent across two seasons, the homogeneity test using Bartlet's equation was used to combine the analyses of the two seasons. Using SAS software, the Duncan's multiple range test was measured to compare the means at $P < 0.05$ (SAS Institute Inc. 2002; Steel & Torrie, 1980). Correlation coefficient was calculated to determine the relationship between grain yield and each of the physiological and chemical traits.

RESULTS AND DISCUSSION

Growth parameters

The influence of various concentrations of chitosan (50,100 and 150 mg L^{-1}) on growth parameters of wheat plants are presented in (Table 3). The results revealed that, using chitosan as foliar treatment at different concentrations significantly increased plant height, leaves number / tillers, tiller fresh and dry weight and relative water contents as compared with untreated plants. While, the maximum significant ($P < 0.05$) increment was achieved in number of leaves/tiller, fresh and dry weight and relative water content were reported at 100 mg L^{-1} chitosan. The percentage of increases in response to 100 mg L^{-1} chitosan extended to 20% & 9% in fresh and dry weights of shoots as compared with the untreated plants.

Table 3. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1}) on morphological, criteria of wheat plant (Gimmeza 9) variety at 75 days from sowing, (mean of two seasons)

Chitosan (mg L^{-1})	Plant height (cm)	Number of leaves /tiller	Tiller Fresh wt. (gm)	Tiller Dry wt. (gm)	RWC %
control	70.0c	4.52d	7.12d	1.92c	72.97c
50	73.0b	6.11b	8.36b	2.05ab	75.45b
100	76.5a	6.60a	8.87a	2.13a	76.00a
150	78.87a	5.63c	7.82c	2.01bc	74.19bc

The obtained value came in line with Zayed et al. (2017) who stated that the application of chitosan improved the morphological characters of common beans. The beneficial effects of chitosan may be attributed to its role in various physiological processes. Sheikha & AL-Malki (2011) reported that chitosan improved bean growth parameters may be due to improving photosynthetic machinery. Moreover, Ke et al. (2001) stated that the application of carboxymethyl chitosanimproved enzyme activities of nitrogen metabolism which in turn enhanced photosynthesis and that followed byimproved plant growth.Chitosan increases plant growth might be because of an increment in the availability ofwater uptake and necessary nutrients led to adjusting cell osmotic pressure, cell division and elongation, increased protein biosynthesis and induction of the antioxidant defense system (Ma et al., 2014). Moreover, Chitosan also induces endogenous plant hormone synthesis (Uthairatanakij et al., 2007) or encourages closure of stomata, which decreases transpiration (Karimi et al., 2012).

Photosynthetic pigments

The effect of different concentrations of chitosan (50, 100 & 150 mg L^{-1}) on photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) of wheat plants are illustrated in (Fig. 1, A, B, C, D). Chitosan applications significantly ($P < 0.05$) enhanced photosynthetic pigments. Chitosan at 100 mg L^{-1} induced the highest values by 37.5%, 29.3%, 53.3% & 38.5% at chlorophyll a, chlorophyll b, carotenoid and total pigments respectively a contrast to the corresponding untreated plant.

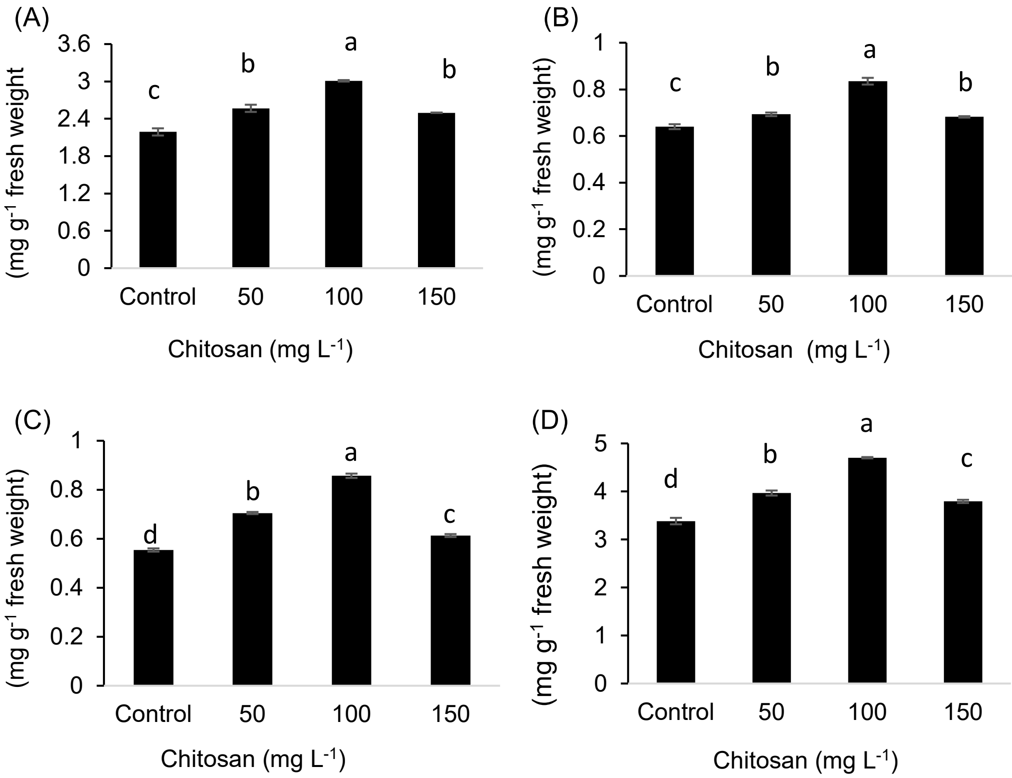


Figure 1. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1}) on photosynthetic pigments as (mg g^{-1} fresh wt.) (A) chlorophyll a (B) chlorophyll b (C) carotenoids (D) total chlorophyll on wheat plant (Gimmeza 9) variety at 75 days from sowing. The different letters (a–d) show statistical significance at $p < 0.05$; vertical bars indicate ± SE.

The significant increase in photosynthetic pigments of wheat plants due to chitosan treatment under sandy soil conditions (Fig. 1)might be due to improving cytokinin contents (Fig. 2, D) that encourage chlorophylls synthesis or preventing the reduction in the light-harvesting pigment protein complexes or raise the availability of amino compounds liberated from chitosan (Chibu & Shibayama, 2001). Chitosan could progress the plant defense mechanism via activating photochemistry and enzymes associated with photosynthesis (Faqir et al., 2021). It is obvious that chitosan enhances the photosynthesis performance and the accumulation of organic matter in wheat plants. This may be due to an increase in total carbohydrate contents (Fig. 3, E), N % and K % in plant leaves (Fig. 5, A, C) which assists in raising the numbering of chloroplasts per cell, so participating in the improved synthesis of chlorophyll. Farouk & Ramadan (2012) found that chitosan improved chlorophylls and total carbohydrates in cowpea (*Vigna unguiculata* L.) after foliar treatment with chitosan at 250 mg L^{-1}. Moreover, the application of chitosan induces carbon assimilation which leads to an increase in the photosynthetic pigment (Bistgani et al., 2017). The application of chitosan to coffee seedlings led to increase in chlorophyll contentswhich of attributed to enhance uptake of nutrients (Nguyen Van et al., 2013). Moreover, the application of chitosan-enhanced photosynthetic pigments in barley plants which may be because of the increments of N and Mg contents in the leaves which are the essential elements in the chemical composition of chlorophylls (Behboudi et al., 2018).

Endogenous phytohormones

Data in (Fig. 2, A, B, C, D) showed that, chitosan with different concentrations caused increases in IAA, ABA, GA, and Cyt as compared with untreated plants. The highest values of hormones were achieved with 100 mg L^{-1} chitosan treatment by 78%, 30%, 44%, and 73% in IAA, ABA, GA, and cyt respectively.

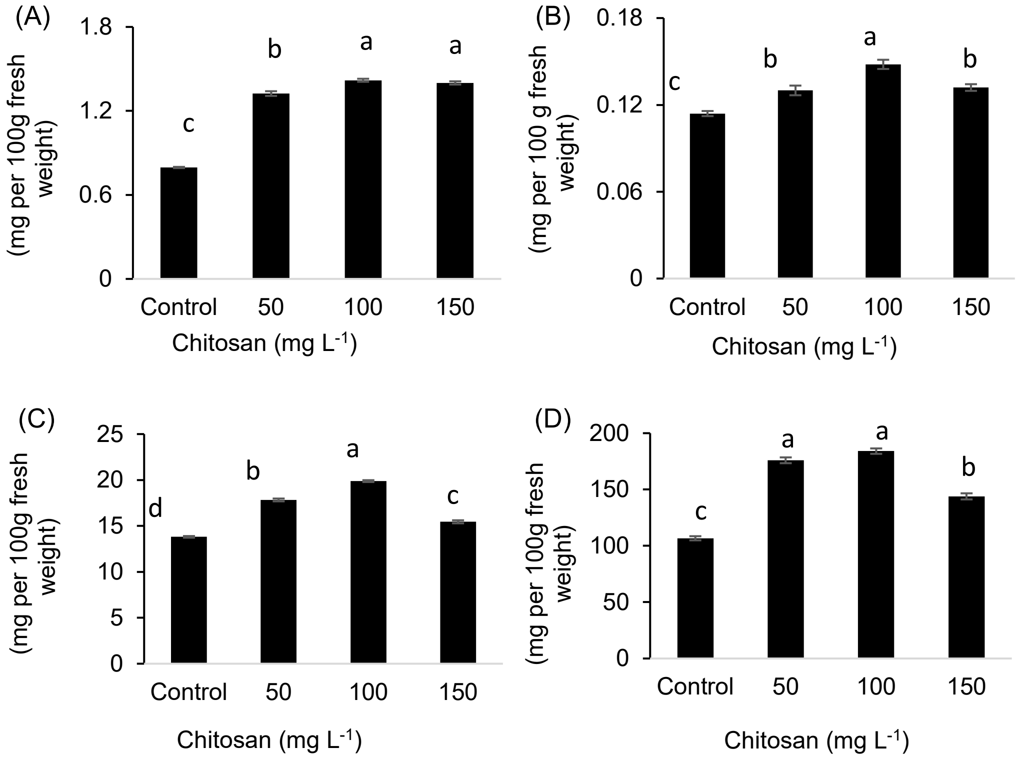


Figure 2. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1}) on endogenous phytohormones as (mg/100 g fresh wt.) (A) IAA, (B) ABA, (C) GA, and (D) Cytokinin on wheat plant (Gimmeza 9) variety at 75 days from sowing. The different letters (a–d) show statistical significance at $p < 0.05$; vertical bars indicate ± SE.

Iglesias et al. (2019) and Ma et al. (2019) reported that chitosan plays an important role in plant hormone production and systemic gained resistance. Chitosan might improve growth and development through signaling pathway associated to auxin biosynthesis through the tryptophan-independent pathway (Uthairatanakij et al., 2007). Similarly, Oligochitosan was able to increase IAA concentration, which improved the growth of tobacco plants (Guan et al., 2009). Moreover, chitosan induce the accumulation of IAA in the root apex of the Arabidopsis (Lopez-Moya et al., 2017). Muthukrishnan et al. (2019) recognized the enhancing role of chitosan on the IAA contents of the chickpea plants. These increments may be because of the promoted influence of chitosan on auxin-related gene expression, improved IAA biosynthesis and transport and decreased IAA oxidase activity (Li et al., 2019).

Moreover, chitosan elevated ABA activity, which plays an important role in the regulation of the stomatal aperture and decreased the rate of transpiration when the plant is subjected to stress condition (Lim et al., 2015). These results are in harmony with Iriti et al. (2009) who reported that the application of chitosan increased the ABA content in bean leaves. Srivastava et al. (2009) observed that chitosan treatment improved the plant under water deficit. The participation of ABA in this operation is also proposed through the notice that chitosan and ABA have approximate signaling components, similar to calcium and ROS. Uthairatanakij et al. (2007) stated that the treatment with chitosan increased the length of the stalks of the orchid *Dendrobium*. Chitosan might stimulate a signal to synthesize plant hormones like gibberellins.

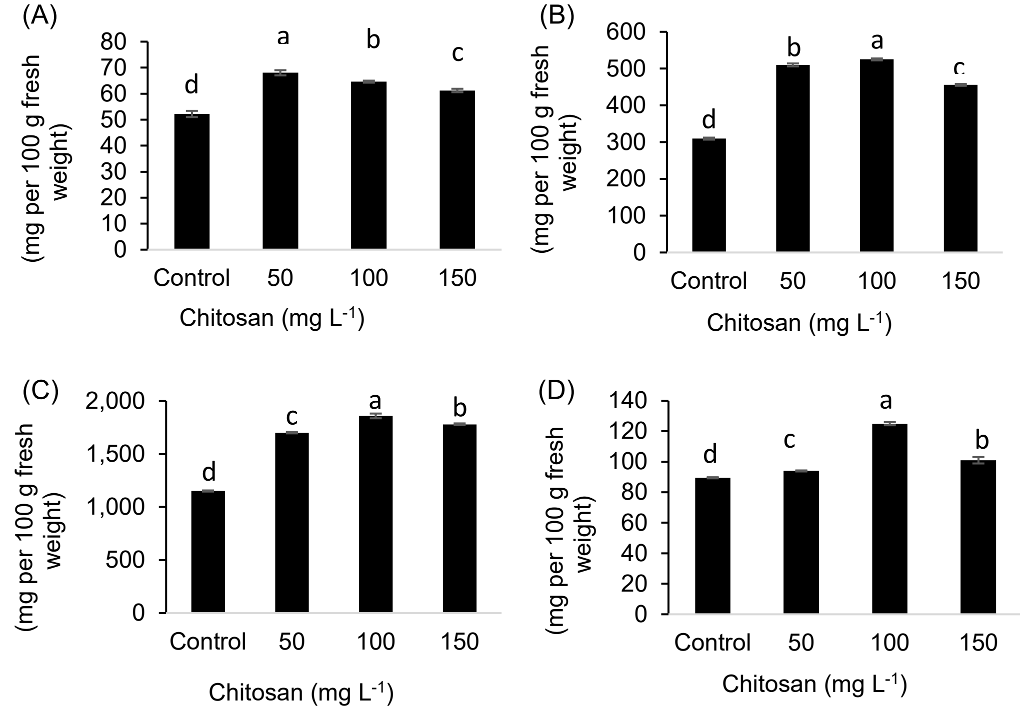
Organic solutes

The impact of chitosan (50, 100 & 150 mg L^{-1}) increased significantly the organic solutes [proline (Pro) free amino acids (FAA) total soluble sugars (TSS) and total soluble protein (TSP)] in wheat plants when compared with untreated plants (Fig. 3, A, B, C, D). The maximum increases in organic solutes were noted by using 100 mg L^{-1} chitosan except proline was increased at 50 mg L^{-1} chitosan.

Rising the osmolytes progress plant cells tolerance to the growth in sandy soil through enhancing osmotic pressure in the cytoplasm in addition to relative water contents is vital for plant growth. The present results are in harmony with their obtained by Geng et al. (2020) after chitosan application in Creeping bent-grass plants. Rabêlo, et al. (2019) reported that, chitosan induced an increment in the contents of soluble sugars, and soluble proteins during its function in enhancing the expression of enzymes that participated in glycolysis. In addition, It seems that chitosan improved the concentration of osmoprotectant compounds, whichplay a role in regulating plant osmosis and subsequently leading to better growth, yield and improved plant tolerance to environmental stress conditions (Li et al., 2017). Treatment with chitosan increased the accumulation of proline levels in the thyme plant (Bistgani et al., 2017). Chibu & Shiayama (2001) stated that these favorable influences the greater availability of amino compounds emitted from chitosan. Li et al. (2017 and Geng et al. (2020) reported that chitosan treatment promoted the production of metabolites and amino acids such as proline,γ-aminobutyric acid, aspartic acid, threonine, serine, isoleucine, valine, lysine, and phenylalanine on white clover under drought stress. Also, Hidangmayum et al. (2019) demonstrated that chitosan treatment promotes antioxidant enzymes via nitric oxide and hydrogen peroxide signaling pathways. It promotes the production of organic acids, sugars, amino acids and other metabolites that are essential for osmotic adjustment, stress signaling, and energy metabolism under stress. Abdallah et al. (2020a) found that the application of chitosan increased the protein content in the stressed wheat leaves.

Total carbohydrates

Data in (Fig. 3, E) showed that, chitosan with different concentrations increased significantly ($P < 0.05$) total carbohydrates. The highest values of carbohydrates were obtained at treatment with 100 mg L^{-1}chitosan. These results are in agreement with those obtained by Abdallah et al. (2020b) andstated that foliar treatment of chitosan, significantly enhanced growth parameters, photosynthetic pigments and carbohydrate constituents in sunflower plants. Leaf total carbohydrates were increased significantly in response to foliar chitosan application at 250 ppm on cowpea plants or 100 and 150 ppm on sour orange seedlings according to Farouk & Ramadan (2012) and Mohamed et al. (2018).



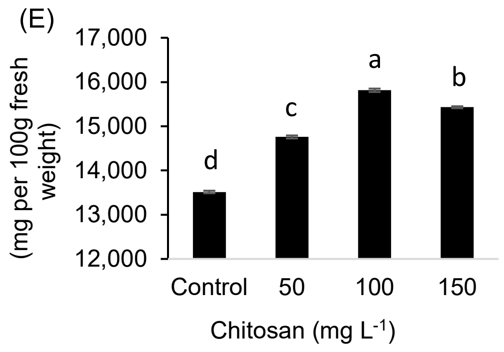


Figure 3. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1})on (A) proline, (B) total free amino acid, (C) total soluble sugars, (D) total soluble protein and (E) total carbohydrates as (mg 100 g^{-1} fresh wt) on wheat plant (Gimmeza 9) variety at 75 days from sowing. The different letters (a–d) show statistical significance at $p < 0.05$; vertical bars indicate ± SE.

Flavonoids and total phenol contents:

The influence of various concentrations of chitosan (50, 100 & 150 mg L^{-1}) on flavonoids and total phenol contents of wheat plant are shown in (Fig. 4, A & B). Chitosan significantly increased flavonoids and phenolic contents. The highest increment of flavonoids contents was achieved via foliar treatment with chitosan 150 mg L^{-1} and phenol contents in 100 mg L^{-1}.

Phenolics are important constitutive with scavenging capability because of their hydroxyl groups, which might participate straight to their antioxidant properties that trigger a chain of secondary metabolites molded by shikimic acid or malonic acid cycles like it has a cellular signaling function (Michalak, 2006). In this concern, Abdallah et al. (2020a) reported that chitosan application significantly raised phenolic compounds concomitantly with lipid peroxidation decrease (Fig. 4, D). Also, Hawrylak-Nowak et al. (2021) stated that, chitosan stimulated secondary metabolites as phenolic compounds by promoting specific genes that participate in the biosynthesis of secondary metabolites. Chitosan promotes metabolic pathways (e.g., phenylpropanoid) and may be involved in the signaling pathway for the biosynthesis of phenolics (Singh et al., 2020). Moreover, Chen et al. (2009) reported that chitosan treatment raised gene expression of phenylpropanoid and flavonoid biosynthesis in soybean sprouts.

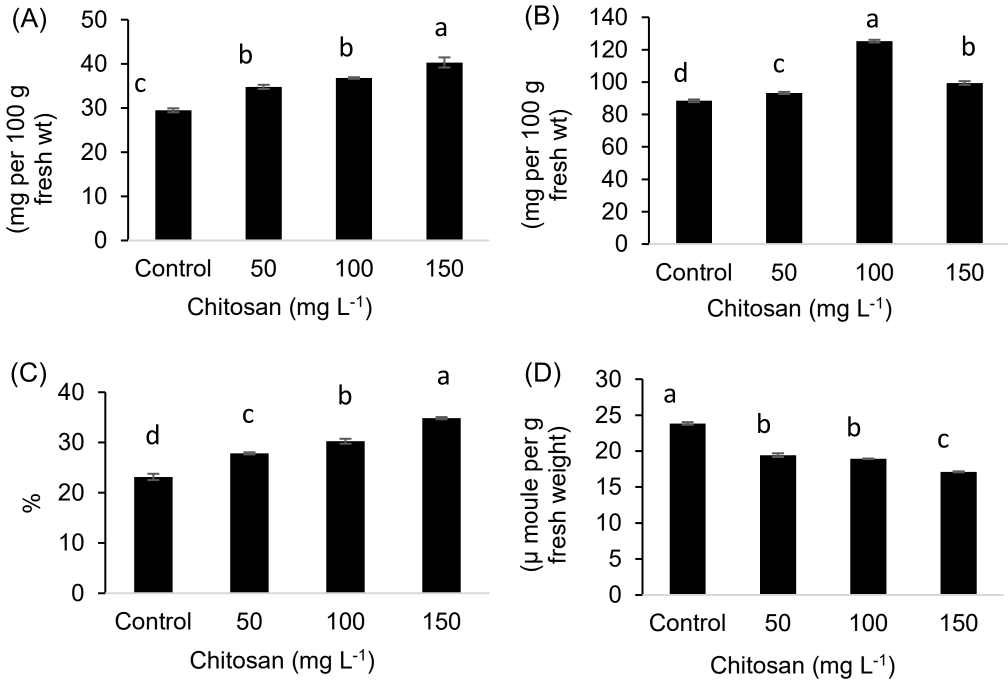


Figure 4. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1}) on flavonoids (A), total phenols (B) as (mg per 100 g fresh wt), total antioxidant activities (DPPH%) (C), and lipid peroxidation (µ mole per g fresh weight) (D) on wheat plant (Gimmeza 9) variety at 75 days from sowing. The different letters (a–d) show statistical significance at $p < 0.05$; vertical bars indicate ± SE.

Total antioxidant activityand Lipid peroxidation

Chitosan significantly increased total antioxidant activities with the increase of chitosan concentration (Fig. 4, C). The highest value of antioxidant activities was achieved by foliar treatment with chitosan (150 mg L^{-1}). Fig. 4, D illustrated that foliar spraying with chitosan on wheat plant grown in sandy soil reduced significantly lipid peroxidation in treated plants than the untreated. This noticed gradual decrease in MDA contents was reported with rising concentrations of chitosan-sprayed plants.

The current study indicated that chitosan treatment in wheat plants significantly reduced the malondialdehyde (MDA) content and increased antioxidant activities (Fig. 4, C, D). Xie et al. (2001) proposed that the antioxidant characteristic of chitosan are attributed mostly to its plentiful active hydroxyl and amino groups, that could interact with ROS to compose constant and comparatively nontoxic macromolecular radicals. Chitosan treatment showed an important role in the inhibition of malondialdehyde content which is one of the decomposition products of polyunsaturated fatty acids (PUFA) of bio-membranes (Seckin et al., 2010) where PUFA which are the major membrane lipid constituents liable to peroxidation and decay. Moreover, Al-Tawaha et al. (2018) and Geng et al. (2020) reported that the treatment of chitosan significantly reduced lipid peroxidation by enhancing the antioxidant activities, directing to inhibited membrane permeability.

The role of chitosan in inhibiting malondialdehyde contents may be due to that chitosan receptors are existing on the plasma membrane; however, through a signaling cascade, the chloroplast is the initial chitosan action organelle (Hadwiger, 2013). Charge-charge interactions among positively charged chitosan amine groups and negatively charged phospholipids stimulate a signal that will lead to the octadecanoid pathway activation; this metabolic pathway is straight correlated to reduced H_2O_2 forming (Almeida et al., 2020).

Minerals contents

Data in Fig. 5 (A, B, C, D) showed that, chitosan with different concentrations caused increases in percentage of nitrogen, phosphorus, potassium and calciumas compared with untreated ones. The highest levels of minerals were recorded with 100 mg L^{-1} chitosan application which caused an increase 47%, 11%, and 11% in N, K, and Ca respectively. Its worthy to mention that, 150 mg L^{-1} chitosan induced maximum increase of P by 54%.

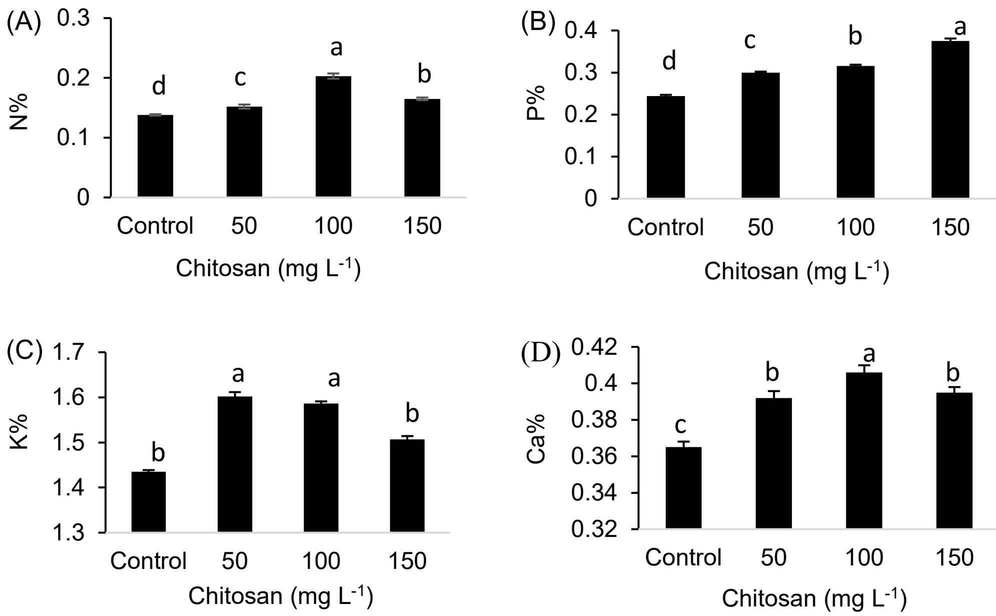


Figure 5. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1}) on minerals contents (Nitrogen % (A), phosphorus % (B), potassium % (C) and calcium % (D) as on wheat plant (Gimmeza 9) variety at 75 days from sowing. The different letters (a–d) show statistical significance at $p < 0.05$; vertical bars indicate ± SE.

Abdallah et al. (2020a) observed that chitosan application raisedN, P, K and Ca contents in the wheat plants grown in saline soil. Likewise, Farouk & Ramadan (2012) observed that chitosan significantly improved N and K contents in cowpea plants. The positive role ofchitosan in plant growth may be related to its influence in increasing nutrient uptake like N, P and K which play a substantial function in the biosynthesis and translocation of carbohydrates and promotes cell division, cell turgor, DNA and RNA (Farouk & Ramadan, 2012).

Changes in yield and yield components

The influences ofvarious concentration of chitosan (50, 100 & 150 mg L^{-1}) on yield parameters of wheat plants are shown in (Table 4) Chitosan significantly improved ($P < 0.05$) all yield parameters e.g. [shoot length, spike length, spike weight, number of spikelets /spike, grain yield/ spike (g), 1,000 grains weight (g), straw yield, biological yield and grain yield (ton ha^{-1})] as compared with the control. The results indicated that the treatment with chitosan at the rate of (100 mg L^{-1}) on wheat was more efficient in enhancing yield component properties except for shoot length (cm) which showed their increase with 150 mg L^{-1} treatment. Grains yield (ton ha^{-1}) increased significantly ($P < 0.05$) by 31% with 100 mg L^{-1} chitosan foliar spray.

Table 4. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1}) on yield component of wheatplant (Gimmeza 9) variety (combined analysis of two seasons)

Chitosan mg L^{-1}	Shoot length (cm)	Spike length (cm)	Spike wt. (g)	No. of spikelets /spike	Grain yield /spike(g)	1,000 grains weight (g)	Straw yield, ton ha^{-1}	Straw yield, ton ha^{-1}	Grain yield, ton ha^{-1}	WP, %
Control	85.4d	9.2d	2.74d	16.0d	2.247d	44.82d	5.195d	9.751d	4.505d	0.747d
50	86.5c	9.8c	3.36c	17.3c	2.96c	51.08c	6.501c	11.658c	5.153c	0.864c
100	89.3b	11.8a	4.09a	19.3a	3.283a	52.66a	7.058a	12.866a	5.832a	0.975a
150	92.4a	10.2b	3.58b	19.0b	2.993b	51.41b	6.68b	12.234b	5.550b	0.937b

Chitosan application led to the increment in wheat yield might be because of its influences in activating physiological processes, progressing vegetative growth (Table 3), photosynthetic pigments (Fig. 1), endogenous phytohormones content (Fig. 2) and mineral contents (Fig. 5) of treated wheat plants followed by active translocation of photo-assimilates from source to sink tissues. Moreover, foliar application of chitosan improved yield and grain quality as shown from the nutrient elements, protein and carbohydrate contents in the grains (Fig. 6). In this respect Abdallah et al. (2020b) showed that chitosan exerts a significant role in ameliorating growth, photosynthetic effectiveness and chlorophyll contents,improved yield & yield component and water productivity in sunflower plants.Chitosan is supposed to be a growth regulator and a signal molecule, as well as its function as a highly efficient biomolecule (Gornik et al., 2008). Moreover, The role of chitosan in improving the yield of the wheat plants may be due to increasing activities of key enzymes of nitrogen metabolism (nitrate reductase, glutamine synthetase, and protease) as well as meliorative translocation of nitrogen in the efficient leaves so raised plant growth and development (Sultana et al., 2017). Ghoname et al. (2010) found that chitosan foliar application in sweet pepper enhanced significantly the fruits quantity and quality. Also, seed soaking and foliar spraying with chitosan at various growth stages in wheat (*Triticum aestivum* L.) improved the yield components (Ma et al., 2014).

Water productivity (WP)

The present work showed that wheat plants treated with various concentrations of chitosan raised significantly water productivity in contrast with the untreated plants (Table 4).

Abdallah et al. (2020a) observed that foliar treatment with chitosan raised the water productivity of wheat. This increase may be due to the chitosan inhibited transpiration by stimulating the closure of stomata. The obtained results suggested that chitosan mightbe effective as anti-transparent which means keeping water to utilize in agriculture (Abdallah et al., 2020b). Geng et al. (2020) found that chitosan application promoted WP and carbohydrates in creeping bent-grass.

Nutritious value of the grains yield

Foliar spraying for chitosan increased ($P < 0.05$) significantly carbohydrate %, protein %, lycopene %, flavonoids %, of the grains wheat (Fig. 6, A, B, C, D). It seems from data that 100 mg L^{-1} foliar treatment was the most efficient to increase carbohydrates, lycopene, flavonoids, by 12.3%, 51.1%, and 77.3%respectively. Meantime, protein contentincreased by 19.9%resulted from grains of plants treated with 150 mg L^{-1} chitosan (Fig. 6, B).

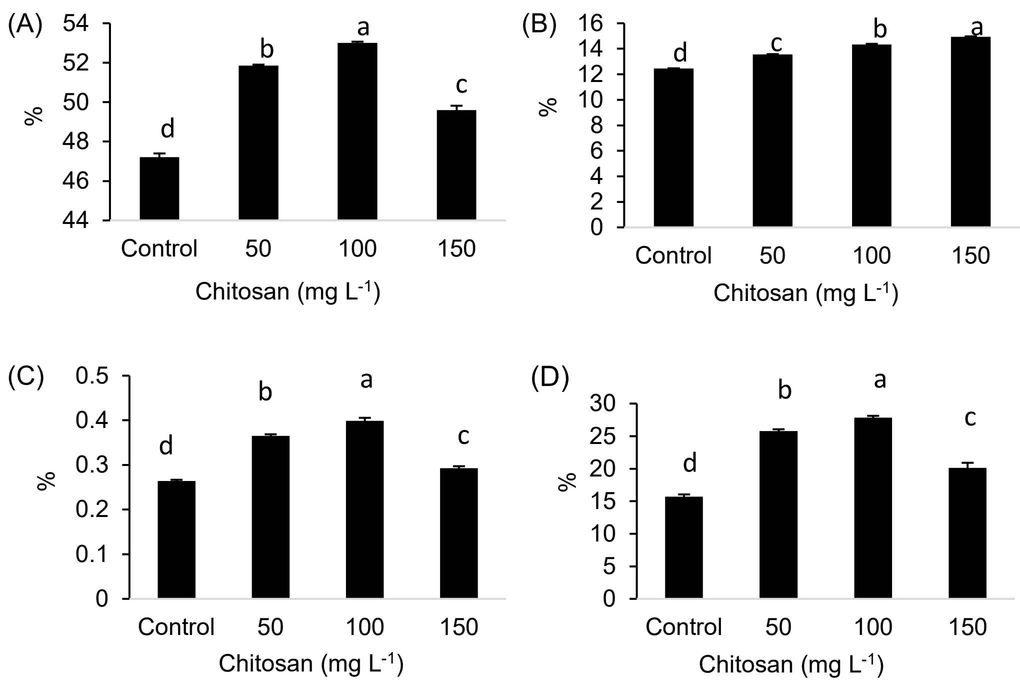


Figure 6. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1}) on Carbohydrate % (A), Protein %(B), Lycopene % (C),Flavonoids % (D)in grain yield on wheat plant (Gimmeza 9) variety (combined analysis of two seasons). The different letters (a–d) show statistical significance at $p < 0.05$; vertical bars indicate ± SE.

Foliar spraying of chitosan increased ($P < 0.05$) significantly carbohydrate % and protein % of the wheat grains (Fig. 6, A, B). Abdallah et al. (2020b), found that application of chitosan increased carbohydrates % and protein %in the yielded wheat grains.

Antioxidant compounds (lycopene and flavonoids) increased in wheat grains in response to various concentrations of chitosan (Fig. 6, C, D). Lycopene and flavonoids are considered plant secondary metabolites of phenolic nature which havingantioxidant properties, include cell signaling and communication. These results agree with that of Abdallah et al. (2020a) on wheat cultivars. Padayatty et al. (2003) recorded that, flavonoids in the human diet inhibit the danger of different cancers and avoid menopausal symptoms so the high contents of flavonoids have a much significant influence on human health.

Mineral contents of the grains yield:

Foliar spraying of chitosan increased ($P < 0.05$) significantly N%, P%, K% and Ca% of the wheat grains (Fig. 7, A, B, C, D). It seems from the data in (Fig. 7, A, C) chitosan that 150 mg L^{-1} gave the most efficient increment inN and K% by 20.6 and 32.2% respectively. Meantime, the increase in P% and Ca% was 28.6% and 27.0%, respectively resulted from grains of plants treated with 100 mg L^{-1} chitosan (Fig. 7, B, D).

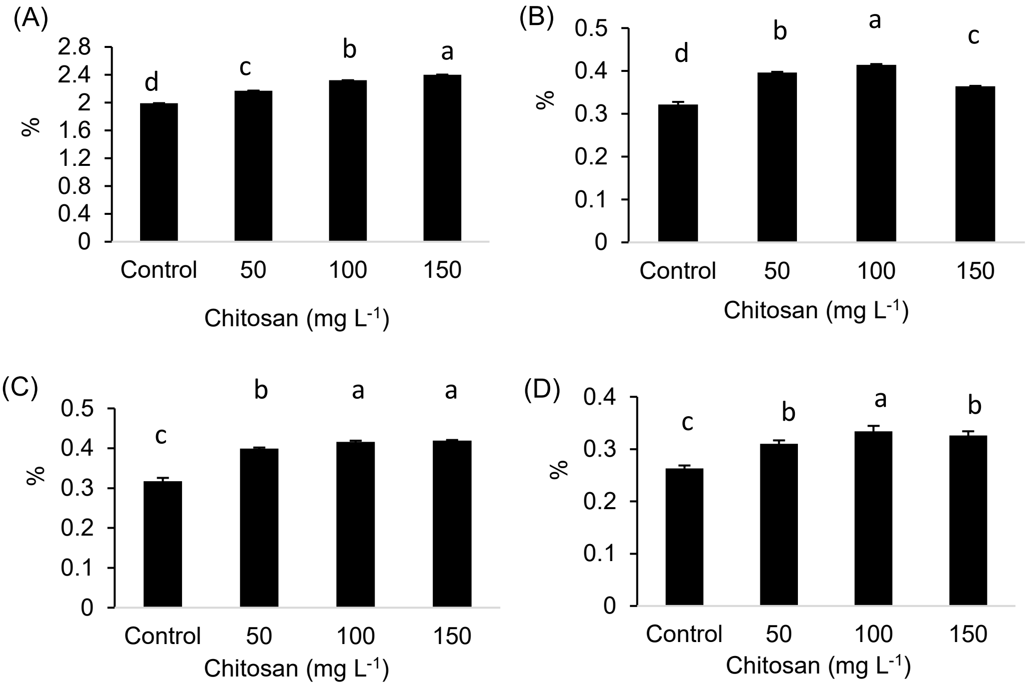


Figure 7. Effect of different concentrations of Chitosan (50, 100 and 150 mg L^{-1}) on Nitrogen % (A), Phosphorus % (B), Potassium % (C) and Calcium % (D) in grain yield on wheat plant (Gimmeza 9) variety (combined analysis of two seasons).The different letters (a–d) show statistical significance at $p < 0.05$; vertical bars indicate ± SE.

Foliar application of wheat plants grown in sandy soil with chitosan might work as fertilizer sources that enables the plant to overcoming the unfavorable conditions of thesoil such as deficiency in nutrients required for plant growth and development and consequently its yield and quality. Abdallah et al. (2020a) found that the application of chitosan increased the contents of N, P and K in wheat grains under water stress. Also, Farouk & Ramadan (2012) demonstrated that chitosan increased significantly N and K contents in cowpea plants. This may be due to the role of chitosan to stimulate plant growth, uptakeand transport of nutrients and photosynthesis efficiency (Guan et al., 2009).

Correlation Matrix

Pearson's correlation coefficients of grain yield among all deliberate characters of wheat plants growing in sandy soil conditions and three chitosan levels are presented in (Fig. 8). There was observed a potent correlation among grain yield and all of the related studied traits, i.e., shoot length, leaf No/plant, tiller fresh and dry wt, Chl a, Chl b, Car, total chl, IAA, ABA, GA, Cyt, FAA, TSS, total carbohydrates, protein, N%, P%, Ca%, flavonoids, total phenol, DPPH%, nutritional values in wheat grains (N%, P%, K%, Ca%, Carb%, Protein%, and flavonoids) which are highly positively associated with grain yield. However, RWC, proline andK% in wheat leaves and lycopene in nutritional value in grain yield are positive between grain yield.Moreover, there was a negative association among grain yield and lipid peroxidation.

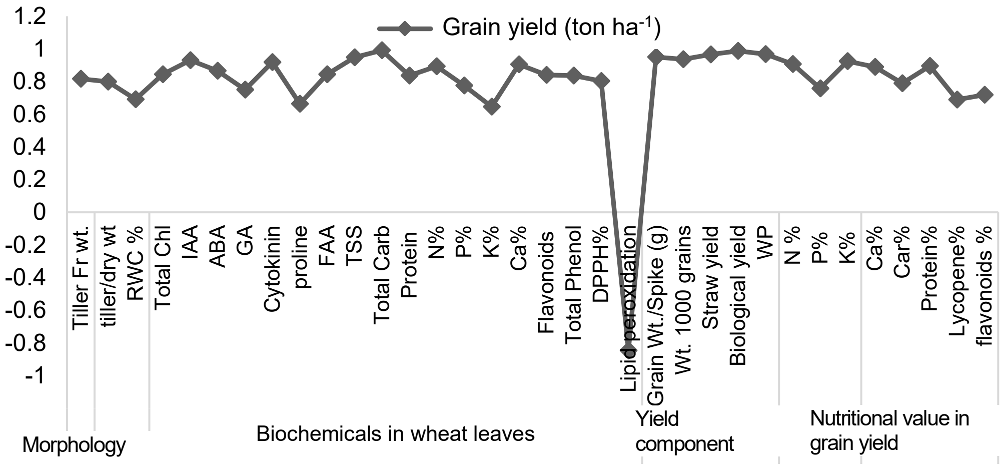


Figure 8. Pearson's correlation coefficients between all studied characters of wheat plants growing in three chitosan levels (50, 100 and 150 mg L^{-1}).

CONCLUSION

Chitosan is a low toxic and cheap substance that is biodegradable and environmentally friendly. Adding exogenous chitosan is an inexpensive and efficient measure which improved growth and yield of wheat plants. Chitosan significantly affects some metabolic processes; it enhances plant growth and development through enhancing photosynthesis, endogenous phytohormones, protein synthesis and ions uptake.

Chitosan-induced increase in osmoprotectants and secondary metabolites such as antioxidant compounds (lycopene, flavonoids and total phenol) and antioxidant activities played an essential role in contributing to enhanced growth, yield and water productivity in the wheat plants. Additionally, the antioxidants decrease the malondialdehyde levels in wheat plants. The application of chitosan increased significantly the carbohydrate, protein, flavonoids, N, P, K and Ca of the wheat grains. Chitosan at a concentration of 100 mg L^{-1}, was the most effective to be appliedas a foliar to wheat plants growing under sandy soil conditions. The perspective of sustainable crop production and rising agricultural yield with inexpensive contributions are farmers' requests for cultivating wheat plants under sandy soil conditions. This study illustrates the possible roles of chitosan in increasing the horizontal expansion of plant cultivation in the sandy soils. The results of the article can be implemented on a large scale in the new lands and the application of sustainable farming methods are safer to the environment. Thus using chitosan help to solve the problem of the bad properties of sandy soils and food gap. It can finally help in filling the food gap of grain crops in the third world.

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Combined effect of soil practices and chemical treatments on weeds growth, soil features, and yield performance in field wheat crop under Mediterranean climate

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Abstract. Soil management techniques influence the biological and physicochemical properties of the soil and lead to changes in soil quality and cover and thus on crop profitability and yield. In this study, the effects of short-term tillage and no-tillage methods combined with a chemical treatment using Glyphosate on weed abundance, selected soil physical properties and yield components were evaluated in durum wheat under Mediterranean climatic conditions. The no-tillage (NT) treatment resulted in higher weed community density during wheat cultivation and moisture consumption than the conventional tillage treatment. The tillage practice and the application of Glyphosate showed a very high efficiency on weeds. Furthermore, the results obtained showed a significant variation and effect of the treatments on the soil characteristics. The application of the no-tillage technique induced a small increase in soil moisture at the seed germination stage (25.6%), while at the last sampling a small increase was recorded in the CT treatment (9.5% for CT and 8.8% for NT). The results of the soil porosity, showed during the whole test period high values in the conventional technique (with or without herbicide application); but for the resistance to soil penetration, the results showed higher values in the no-till technique. Finally, the effect of the tillage system on crop yield was evaluated. In our study, the results showed that significant increases in the number of heads per m^2 (351.3 heads per m^2), the number of grains per head (45.8) and the weight of 1,000 grains (41.2 g) were obtained with the tillage treatment combined with glyphosate application. When comparing the two tillage methods, the highest values were always revealed with the tillage technique.

Key words: tillage system, no tillage system, herbicides, weeds, soil features, wheat.

INTRODUCTION

Soil management practices are a fundamental step to improve soil quality and crop yields. It is the essential way to ensure the sustainability of the agro-systems under the growing demand for food. In recent years, various studies have been realized to compare between these practices and to demonstrate their effect on biological, chemical and physical soil properties that, in turn, affect plant performance (Weber et al., 2017).

Conventional tillage practices are mechanical operations aimed at turning over the soil to create ideal conditions for seedling development and plant nutrient uptake (Garane et al., 2017). This practice is carried out using a chain of tillage tools that mainly consists of a plough, a cover crop and other tillage tools. Tillage often has a positive effect on soil moisture, bulk density, porosity, organic matter and microbiome abundance (Kaurin, et al., 2015; Niewiadomska, 2020). The tillage method regulates the sustainable use of crop soil resources, improves soil penetration, increases root absorption and development, and promotes crop growth and yield (Laurent et al., 2014).

Several studies compared different tillage techniques (conventional and conservation tillage) (Blevins et al., 2018; Hu et al., 2021), with the aim of getting the best from each technique.

Conventional practices based on tillage with turning are fundamental agro-technical operations in agriculture because of their influence on soil properties, the environment and agricultural production aimed at creating ideal conditions for seedling development and plant nutrient uptake. It is a technique that positively affects certain soil characteristics; soil loosening and leveling for seedbed preparation, as well as, soil fertilizer mixing and crop residue management (Busari et al., 2015). This technique helps in weed control, as well as, crop residue management and organic matter burial (Garane et al., 2017; Boko et al., 2020).

In addition, plowing can negatively influence soil bulk density, penetration resistance, increase soil compaction, the average weight diameter of aggregates and surface roughness (Carman, 1996). Mechanical properties, can also be affected by conventional tillage can, by disrupt soil structure becoming more vulnerable to wind, runoff, and general erosion. On cereal farming, there is a tendency to reduce tillage, motivated by the desire to reduce production and mechanization costs, to protect the soil from erosion or to promote carbon storage at the rate of soil mineralization by reducing the mineralization of organic matter (Roger-Estrade et al., 2011).

Conservation agriculture practices aimed at permanent soil cover followed by reduced tillage could be an innovation in farmers' strategies for sustainable agriculture (Kouelo et al., 2017). Conservation tillage (minimum tillage and direct seeding), is a method of reducing the use of implements to the point of allowing only one implement to minimize pressure on the soil and avoid compaction problems. No-till management, or direct seeding, is supposed to eliminate the undesirable consequences of conventional tillage, including soil degradation, and to improve several soil properties and water retention capacities; ploughed soil has a damaged pore network and so less water and mineral constituents. Furthermore, by inhibiting water infiltration, the crust increases the danger of erosion and runoff (Avramovic et al., 2022).

No-till has been shown to have a positive effect on increasing organic matter levels and structural stability (Moussadek, 2011). Many studies have confirmed that, compared to conventional tillage, conservation tillage can reduce soil erosion (Roger-Estrade et al., 2011). Other studies have also shown that no-till and reduced tillage methods, such as chisel tillage, reduced soil losses significantly compared to conventional mouldboard tillage. The increase in structural stability is directly proportional to the increase in stable organic matter content (Hu et al., 2021).

The absence of soil disturbance promotes denitrification processes (Labreuche et al., 2011). The main disadvantages of conservation agriculture concern weed management, in semi-arid zones, weeds such as brome that develop in the absence of deep tillage lead to increased control costs (Rouabhi et al., 2018). Weeds are plants that spread naturally without human intervention in natural habitats or natural seedlings and are undesirable herbaceous or woody plants at the site where they occur (Pipon, 2013). Competition between the latter and a given cereal constitutes a constraint to crop development through the competitive power of weeds (Morison et al., 2008).

According to Pipon (2013), the various socio-economic reasons plead today for an increased rationalization of weed control in order to avoid unnecessary or superfluous treatments as much as possible. Another approach to weed control could be practiced in the management of these bio-aggressors, an integrated control based on all mechanical, chemical and biological methods. These combined methods will give the best efficiency to keep the damage below the nuisance threshold (Néron, 2011). Indeed, many weed species are now resistant to herbicides, especially glyphosate (Heap, 2019 in Yash, 2020).

Given the importance of yield losses due to weeds, this study was designed to develop an environmentally adapted weed control system to minimize the use of plant protection products under two different tillage systems (conventional tillage and direct seeding).

MATERIALS AND METHODS

The experimentation was conducted in the National Higher School of Agronomy, ENSA; Algiers (36° 43’ N, 3°09’77’’ E) Fig. 1 under Mediterranean climate characterized with a wet winter between January & early May and a hot dry summer between May & October (average annual precipitation of approx. 63.6 mm and average annual temperature of 15.9 °C) Fig. 2. The soil analysis performed before experimentation were revealed rate of 25%, 26% and 16% of clays, fine loam and coarse loam respectively, pH7, K 2.6 mg per 100 g and N 0.07%.

Figure 1. Satellite image of the experimental station (Google map, 2021).

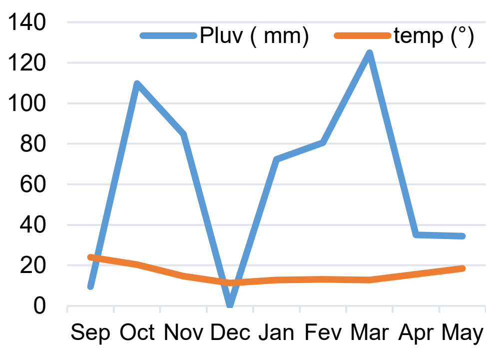

Figure 2. Umbrothermal graph for the 2015/2016 season.

Experimentation design

A 3744 m² (72 m × 52 m) plots were assigned for the treatments, divided into 12 micro-plots with 15 m long and 10 m wide (150 m²) Fig. 3. In this experiment, wheat (*Triticum durum L.*) '*Vitron*' was cultivated using the method of factorial block with two factors: tillage practice and chemical treatments. With 3 three replicates. Wheat was grown at a rate of 300 seeds m^{-2} in late November 2015, and was conducted using two tillage systems; conventional (CT) and no-till (NT), it is important to mention that the experimental plot is a fallow land. Each practice combined with or without glyphosate herbicide application respectively Fig. 3, tools used in soil preparation, a bisocs plough (25 cm deep) on 19 of November 2015, followed by one passage of a cover crop to crush large clumps, then using Roto-harrow to improve soil crumbling, on 22 November. No practice was carried out for NT method. For each practice, only nitrogen fertilizer was applied in the dose (100 kg ha^{-1}), in two terms in the form of 46% urea was applied, half at the early tillering stage and the other half at the ear 1cm stage for both techniques. Herbicides treatments were performed using Glyphosate© (3 L ha^{-1}) 10 days prior to crop planting was sprayed on 15 of November 2015. Moreover, sowing (CT and NT) 24 of November 2015 with two different seeders.

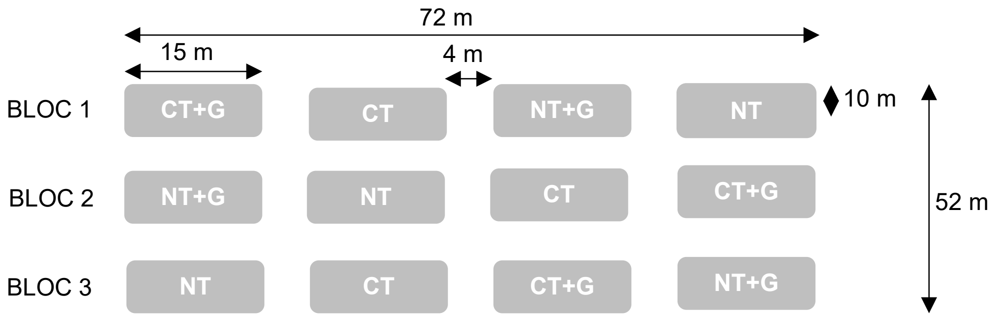

Figure 3. Experimental device of our study. NT: No tillage practice without glyphosate application; NT+G: No-tillage practice with application of glyphosate; CT: Conventional tillage method without glyphosate and CT+G: Conventional tillage method with application of glyphosate.

Soil sampling and physical analyses

Soil samplings were performed, after seeds germination, tillering stage, and at seed filling at a depth the 15 to 30 cm using a 5 cm diameter auger. Soil sampling was carried out, before soil preparation and, considered as control. The soil samples, were firstly weighed, then oven-dried at 105 °C for 24 h, and stored for analysis. Soil physical properties such as soil penetration resistance, humidity soil and bulk density were calculated from each sample. Soil penetration resistance was evaluated to a maximum, depth of 30 cm at every 10 cm depth interval using a manual cone penetrometer with 2 cm^2 surface area in the base. Porosity, soil humidity and bulk density were calculated using formula, (1) (2) and (3) respectively, a value of real density (Rd) is 2.49.

$$P\% = \left(1 - \frac{\mathrm{Bd}}{\mathrm{Rd}}\right) \times 100 \quad (1)$$

P – Total Porosity (%); Bd – Bulk density; Rd – Real density.

$$H\% = \frac{Ww - Dw}{Dw} \times 100 \quad (2)$$

H – Soil Humidity (%); Ww – Wet weight (g); Dw: Dry weight (g).

However, bulk density was performed by collecting soil cores between 0–10 cm, 11–20 cm, and 21–30 cm depth, using a metal core with known volume by placing the core in the middle of each soil level.

$$Bd = \frac{Dw}{V} \quad (3)$$

Bd – bulk density; Dw – dry weight; V – volume of soil sample (cm^3).

Weed community survey

The number and the abundance (number of individuals per species) of weed species in each treatment were determined using aquadrat (0.2 m^2) per plot: before seeds sowing, after seeds germination, at tillering stage, and at the seed filling. Sampling quadrats were located along a linear itinerary, every time at least 2.5 m away from the plot borders and at least 6 m away from other sampling site. Three measures of weed diversity were computed in each treatment.

Yield parameters

Wheat grain yield were recorded as described in standard procedures. Number of heads per m^2 was counted using a quadrat (1 m^2) randomly placed in the micro-plot. Three replicates were performed for each treatment. Moreover, number of grains per ear, was recorded directly after wheat harvest, ten heads were randomly taken from each of three sampling sites in each micro-plot. heads were manually threshed then counted. 1,000-grain weight was measured using a precision balance from three repetitions of each treatment. Finally, estimated grain yield was determined for each sampling site using the formula (4).

$$Estimated\ yield\ (qx\ per\ ha) = number\ of\ head\ per\ m^2 \times \text{number of grainper head} \times \frac{1{,}000\ grain\ weight}{10{,}000} \quad (4)$$

Statistical analysis

A one-factor analysis of variance (Anova 1) was used to compare, conventional tillage with herbicide treatment, conventional tillage with no herbicide treatment, no-till with herbicide treatment and no-till with no herbicide treatment on the weeds and during the three stages of wheat. Differences between means were tested using the post-hoc tuckey test at the 5% significance level. Correlations at the 5% threshold were performed between the number of heads per m^2, the number of grains per head, the weight of 1,000 grains and the theoretical yield. All analyses were performed using Excel and Statigraphic 19.

RESULTS AND DISCUSSION

Soil practices and chemical treatments affect weed growth

The results of our study showed that during wheat cultivation, tillage with or without glyphosate treatment were very effective on weed survival. After seed germination, no weeds appeared under CT+G and CT. However, weed density decreased from 127.4 plants per m^2 to 0 plants per m^2.The weed density value is low under NT+G

(25 plants per m^2) and high for NT (176.6 plants per m^2) Fig. 4, A. The ploughing predicted a lower weed density and variety than direct sowing (Pilipavicius et al., 2009; Alletru & Labreuche, 2019). During the germination stage, the analysis of variance (Anova test) showed a very highly significant difference ($F = 76.9$; $P < 0.001$) observed between the treatment on weed density. At the tillering stage in the CT (after 45 days of treatment) Fig. 4, C the effect of the tested technical itineraries on weed density shows that there were still 22.1 weeds plants per m^2 in the treated methods, the CT showed low weed growth whose achieved density was 10 plants per m^2 Fig. 4, B. Woźniak (2018) confirmed this result as well distinguished that tillage and soil turning significantly reduce weed growth.

For no-tillage, the highest weed density was obtained without glyphosate (NT) application, with 176.6 plants per m^2 (Fig. 4, A). According to Gruber & Claupein (2009), the no-till method increases the weed seed reserve in the superficial soil layers, which leads to an intense manifestation of weeds with the crop. Under (NT+G) a decrease in weed density observed (25 plants per m^2) Fig. 4, A. Similarly, Hayden et al. (2012) were recorded a reduction of 78% in weeds after turning the soil under a tillage practice.

These results were in accordance with previous studies (Abdellaoui et al., 2011; Gathala et al., 2011; Alarcón et al., 2018), in which higher weed degradation under the tillage method and high weed density with no-till practices were observed. On the contrary, Streit et al. (2002) found that weed density was lower in the no-till technique compared to a conventional method.

In addition, at tillering stage, NT showed the highest growth and weed level with a density of 226 plants per m^2 Fig. 4, B, At Seed filling stage, the highest weed level was always recorded with NT (354.4 plants per m^2). However, CT+G showed the lowest value with a density of

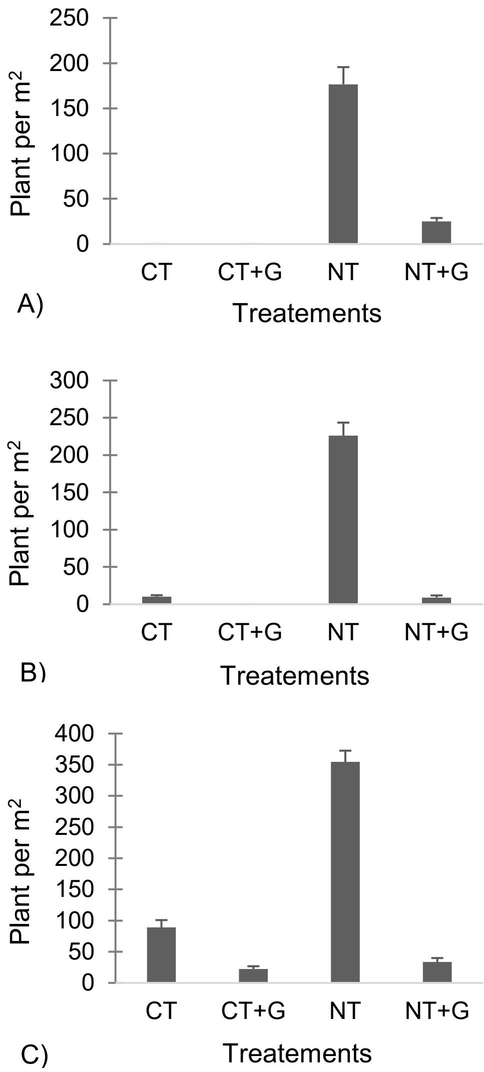

Figure 4. Weeds abundance under soil practices management and Glyphosate application under wheat crop cultivation, (A) after germination, (B) at the tillering stage and (C) the seed filling. Each value is the mean of ten replicates. Error bars represent ± standard deviation.

plants m^{-2} while CT and NT+G showed a density of 33.4 plants m^{-2} and 89 plants m^{-2} respectively. At the tillering stage, a very highly significant difference ($F = 152.3$; $P < 0.001$) in the effect of the four technical itineraries (CT, CT+G, NT, NT+G).

According to Petit et al. (2013), reducing dependence on herbicides is a current issue. However, the actual state of knowledge does not provide enough solutions or generic alternatives to manage the weed flora and thus minimize losses to yield. In deed, for the seed filling stage, statistical analysis recorded a very highly significant difference ($F = 182.6$; $P < 0.001$), for the effect of the technical itineraries tested on weed density. Weed density was low 22.1 plants per m^2 and 33.4 plants per m^2 for CT + G, NT + G respectively; average 89 plants per m^2 for CT, and high 354.40 plants per m^2 for NT Fig. 4, C.

Soil features under different tillage management and chemical treatments

Humidity

Water conservation and maintenance of soil fertility is one of the major challenges of soil management. The results of this research showed that soil humidity was higher in no-till (CT) practices. At the germination stage, soil tests revealed 25.6% and 23.4% humidity under NT and NT+G treatments respectively Fig. 5, A. Our results are in agreement with several studies that report that no-till improves soil humidity and water storage compared to conventional techniques (Abdellaoui et al., 2011).

Statistical analysis showed a significant effect of the effect of soil itineraries on moisture at the germination stage with ($F = 5.3$; $P < 0.001$). According to Guzha (2004) a significant influence of tillage practice on soil humidity, and water storage, especially in dry climates, and by its ability to maintain or increase the availability of organic matter and improve the physical properties of the soil. Ji et al. (2013) found that the water content of the soil under the no-till (NT) method at 30–40 cm depth of loam soil was significantly higher than tillage method (CT).

However, a proportion of 22.2% and 21.4% was obtained for CT and CT+G respectively Fig. 5, A. Then, the humidity content has been decreased for the whole treatments in the second samples with slightly high values for the conventional techniques. In the NT treatment, the humidity was reduced to 8.1% and NT+G to 7.1%. While the highest value 9.1% was obtained with CT+G (Fig. 5, B) tillering stage at the last sample, slight increases were recorded compared to the 2nd sampling, the highest rates were revealed in CT and NT with 9.4% and 8.8% respectively.

At seed filling stage, soil humidity is high in CT 9.4%; it is stable in NT and NT+G with similar values (8.8% and 8.4% respectively). The lowest value is observed in CT+G (7.8%). the reduction of humidity observed in the last two stages is due to the climatic conditions (since March, significant drop in rainfall). The anova analysis revealed that no significant effect for all technical itineraries considerate on humidity for the last both stages ($F = 0.9$; $P > 0.05$ tillering stage and $F = 1.9$; $P > 0.05$ seed filling stage).

Nouiri et al. (2004) conducted a comparative study of water retention and humidity in soil layers, between the no-tillage technique and that of tillage carried out in Tunisia. The results obtained are in agreement with Sadeghi & Bahrani (2009), which confirm that there was little difference of soil humidity under conventional tillage compared to no-till and that no-till provides a better humidity than conventional tillage. The higher soil humidity content under no-tillage would be the result of reduced evaporation from the low runoff due to the presence of crop residues on the surface and/or the higher water retention.

Porosity and penetrometry

Soil porosity was not influenced by plant development stages, but rather by soil practices (tillage and no tillage). Soil porosity throughout the season, the highest rate was revealed under the tillage method with or without herbicide application. At the germination stage, as reported in several studies, tillage significantly increases porosity; a value of 62.7% was obtained with CT and 59.3% with CT+G Fig. 6, A.

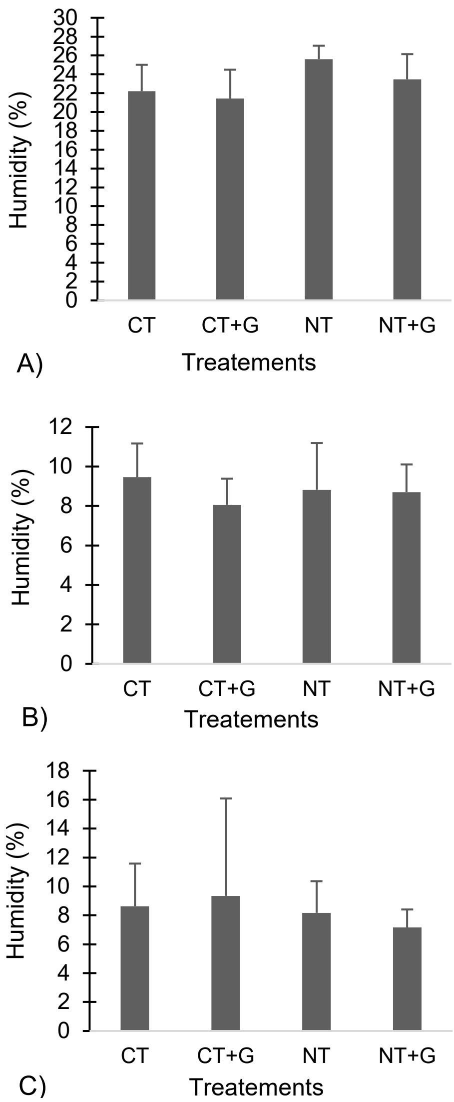

Figure 5. Effect of soil practices management and Glyphosate application on soil humidity under wheat crop cultivation, (A) after germination, (B) at the tillering stage and (C) the seed filling. Each value is the mean of ten replicates. Error bars represent ± standard deviation.

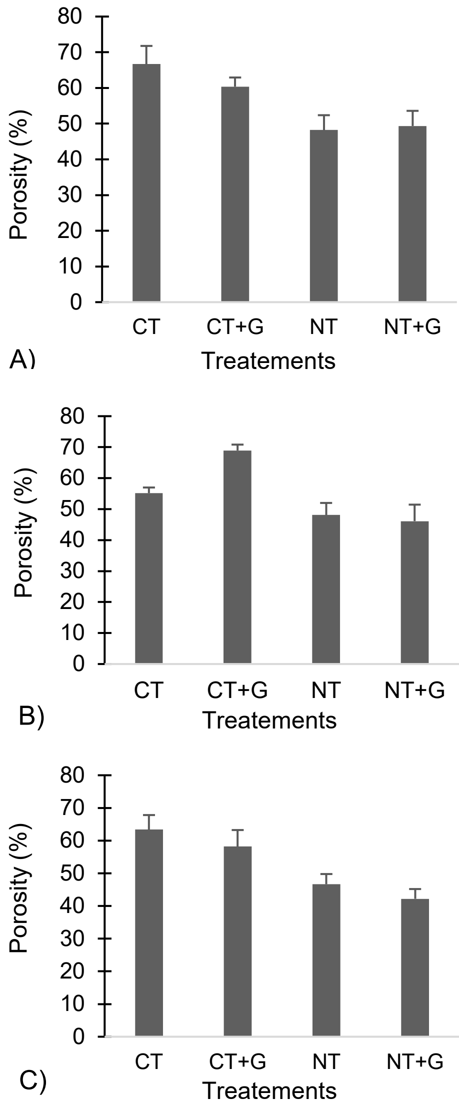

Figure 6. Soil practices management and Glyphosate application affected soil porosity under wheat crop cultivation, (A) after germination, (B) at the tillering stage and (C) the seed filling. Each value is the mean of ten replicates. Error bars represent ± standard deviation.

The results achieved by Lipiec et al. (2006) explained that porosity and pore size were highly influenced by the tillage method. In this study, the soil tillage conventional technique indicated the highest porosity and pore size especially in the surface layers of the soil. Moreover, at tillering stage, porosity increased by 68.9% with CT+G treatment and decreased for CT treatment (55.1%).

On the other hand, the porosity was around 48.1% and 46.1% with NT and NT+G respectively Fig. 6, B. At the end of cultivation, the analyses showed soil porosity of 63.3%, 58.1%, 46.6% and 42.1% with CT, CT+G, NT and NT+G respectively. A higher percentage of soil porosity under tillage than no tillage was obtained in the studies of Hill et al. (1985) and Kay & Vanden Bygaart (2002).

The grain filling stage shows high porosity values in the CT and CT+G techniques. An approaching value was found between germination and filling stage for the CT technique (about 60%), but these values drop in the NT and NT+G techniques (42% and 45.7% respectively) Fig. 6, C.

The Anova analysis shows a highly significant effect of the impact of the four techniques on porosity for all four stages of wheat with ($P < 0.0000$). Pastorelli et al. (2013) observed significantly an effect of the soil preparation ($p < 0.001$) on the total porosity in the first 10 centimeters.

Soil penetration resistance was the third trait studied. The results showed higher values in soils with no tillage practices. At germination, the NT treatment showed a penetration of 39.5 daN per cm^2, while the CT+G treatment showed the lowest penetration with 26.3 daN per cm^2 Fig. 7, A. At the second sample, the highest value was obtained with the NT+G treatment (32.4 daN per cm^2). The Anova-test following by tuckey-test indicated a significative difference between these four itineraries. Miyamoto et al. (2012) and Ji et al. (2013) found similar results in the previous study.

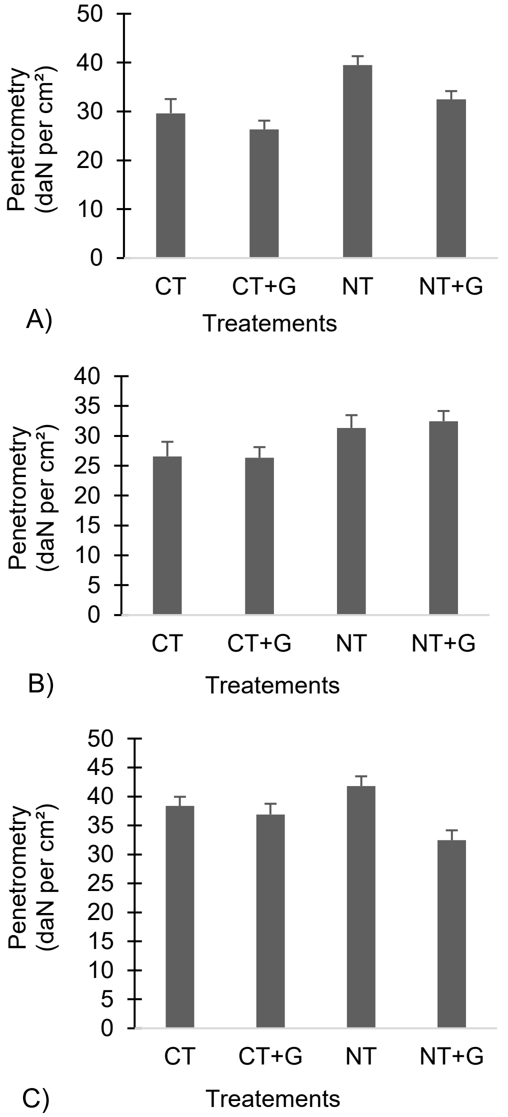

Figure 7. Effect of soil practices management and Glyphosate application on soil penetration resistance under wheat crop cultivation, (A) after germination, (B) at the tillering stage and (C) the seed filling. Each value is the mean of ten replicates. Error bars represent ± standard deviation.

At tillering stage, high values of resistance to penetration were observed in NT and NT+G treatment respectively 41.7 daN per cm^2 and 41.1 daN per cm^2 the CT+G and CT treatment showed the lowest penetration with 36.9 daN per cm^2 and 38.3 daN per cm^2 Fig. 7, A. The Anova-test and tuckey-test indicated significative difference between these four itineraries. Miyamoto et al. (2012) and Ji et al. (2013) and Das et al. (2014) showed similar results in a previous study, where tillage and soil depth, and soil penetration resistance significantly affected soil penetration resistance was significantly affected by tillage and soil depth. (Das et al., 2014) reported the same observation. Soil penetration resistance was increased after tillage (Alesso et al., 2019), followed by NT (31.3 daN per cm^2) and CT (26.5 daN per cm^2) treatment Fig. 7, B.

Finally, at Seed filling stage, the values of this parameter were significantly increased compared to the other two samples. A penetration of 41.7 daN per cm^2 and 38.3 daN per cm^2 was recorded in the NT and CT treatments, respectively Fig. 7, C.

However, after comparing between tillage systems Özgöz et al. (2007) reported higher soil penetration after no tillage application than a soil beforehand managed by conventional tillage. Soil compaction can cause unfavorable physico-chemical and biological properties of the soil that affect root growth and crop yield. The results of Dahou et al. (2018) have specified that tillage techniques have a direct influence on penetration resistance and root depth.

Weeds modulate yield growth parameters of Wheat

In this work, the soil practices and/or herbicide treatments significantly affected their various yield components as the number of heads per m^2, and the number of grains per heads and the weight of the 1,000-grains that assesse the yields capacity of wheat. The results showed that notable increases for the three yield components studied were obtained with the treatment tillage combined with glyphosate application. The effect of different treatments on number of heads per m^2 is shown in Fig. 8, A. The highest values 351.3heads per m^2 and 252 heads per m^2 were recorded with the treatments CT+G and CT respectively. Through the results of linear correlations, there are high positive correlations (the correlation coefficient r ranked between (0.7 and 0.9) and highly significant ($P < 0.01$) between all the yield parameters such as number of heads per m^{-2}, number of grains per head, weight of 1,000 grains and the theoretical yield.

These results are in agreement with those of Abdellaoui et al (2011), who show that conventional tillage techniques, when newly applied, especially in semi-arid lands, have the highest yields compared to the no-till method. The effect of tillage method on yield was significantly affected by the interaction between the tillage system, and the years of application, under the no-till method yields are lowest in the first years and are highest after five or six years (Alarcón et al., 2018).

The lowest value was obtained by NT (28.6 heads per m^2), which explains the effect of weeds and caused by the competition that weeds have on crops and primarily affects crop yields. This competition was related to space, light, water expressed as the difference between the yield with weeding and the yield without weeding. The anova test reported a very highly significant difference ($F = 50.7$; $P < 0.001$) in the number of heads per m^2 between the techniques tested.

According to our results, anova-test revealed a very highly significant difference ($F = 139.8$; $P < 0.001$). In effect, the CT+G treatment had a significant influence on the number of grains per head. The application of glyphosate increased this parameter up to an average of 45.8. Furthermore, CT and NT+G were showed a similar effect with an average of 40 grains per heads Fig. 8, B.

However, for the weight of 1,000 grains, only a slight increase was obtained with CT+G, which showed a value of 41.2 g, compared to the other treatments. On the other hand, the lowest weights, 25.2 g and 29.8 g, were recorded with CT and NT, respectively. However, NT+G increased the weight of 1,000 grains to an average of 33.5 g Fig. 8, C, while, Rieger et al. (2008) reported that at the maturity stage of wheat, shoot, and biomass was 2% higher in the no-tillage system compared to the tillage method, but grain yield was 3% lower in the no-tillage system compared to the tillage method. According to the statistical analysis the anova-test, there was a highly significant difference between the techniques itineraries and the 100-weights ($F = 8.6$; $P > 0.05$). Anova analysis noted that there was a very highly significant difference ($F = 42.6$; $P < 0.001$) for the yield parameter. The lowest estimated yield levels, (1.3 q ha^{-1}), was recorded in the treatment NT, CT and NT+G. The values were increased up to 18.4 q ha^{-1} à 31 q ha^{-1} respectively compared to NT, while the results showed a yield of 66.3 q ha^{-1} with the treatment CT+G Fig. 8, D. Ciha (1982) was observed an average grain yields and a 100-seed weight with no-tillage significantly greater than yields using tillage system. According to De Vita et al. (2007), there were no effects of NT system during the first 2 years in either wheat yield or quality under Mediterranean climate conditions.

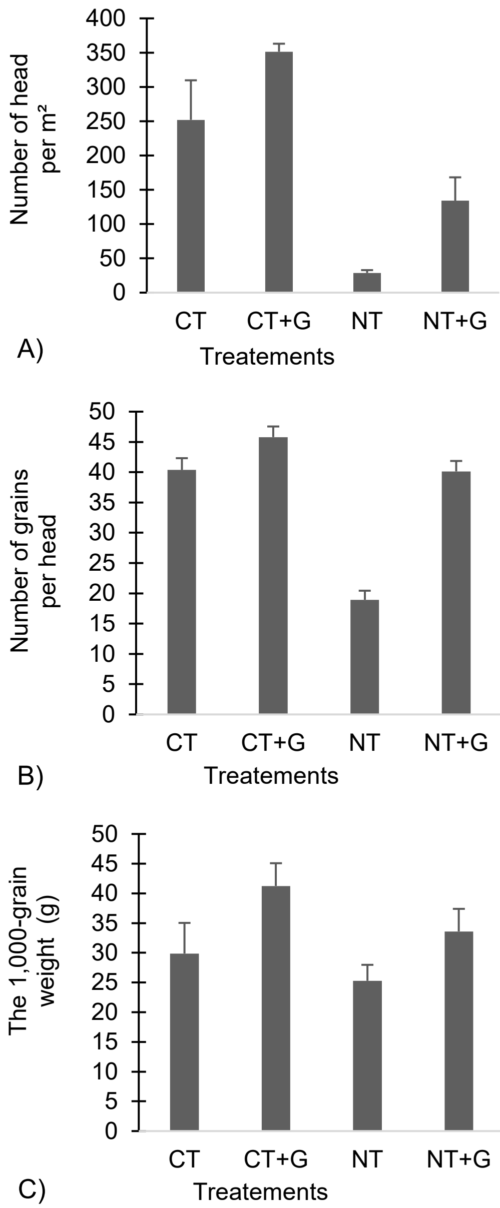

Figure 8. Effect of soil practices management and Glyphosate application on wheat yield components parameters, (A) Number of heads par m^2, (B) Number of grains per head and (C) The 1,000-grain weight. Each value is the mean of ten replicates. Error bars represent ± standard deviation.

Through this results, there are strong positive correlations (the correlation coefficient r located between (0.7 and 0.9) and highly significant ($P < 0.01$) between all the yield parameters such as; number of heads per m², number of grains per head, weight of 1,000 grains and the theoretical yield. Results showed a slight effect of weeds on yield Fig. 9. Alarcón et al. (2018) were mentioned that higher cereal yields were associated with a negative impact on weed diversity and richness. Increasing density of weeds affect directly crop yields (Pretty & Bharucha, 2014). Management, weeds and yield component relationship using the Scatterplot Matrix Fig. 9 demonstrate that weeds are strongly related to the NT and NT+G treatments. Whereas, the number of grains per head, the number of heads per m² and the 1,000-grain weight have a close relationship with CT and CT+G treatments.

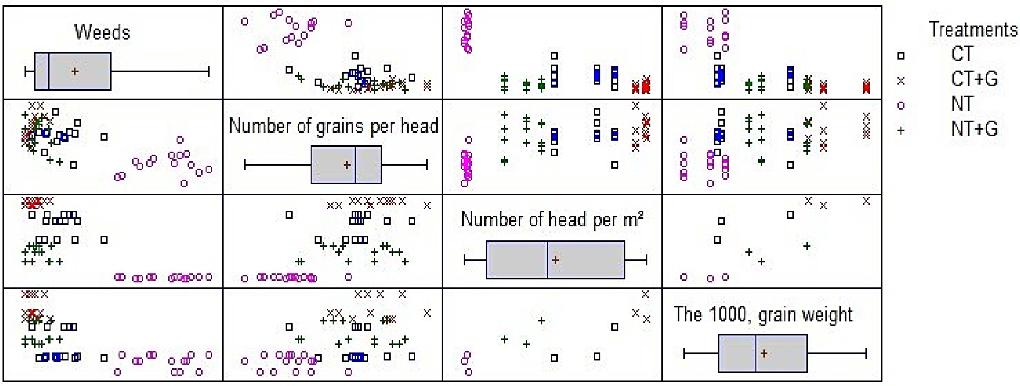

Figure 9. Scatterplot Matrix analysis of the correlations between weeds abundance and the three yield components parameters under soil practices management and Glyphosate application. Each value is the mean of ten replicates. Error bars represent ± standard deviation.

CONCLUSION

Soil management plays an important role in designing sustainable farming systems. Utilizing this approach requires more detailed study and careful experimental design than the traditional comparison between conventional and reduced tillage. A more precise analysis that takes into account soil characteristics and weed management Therefore, it is important to implement integrated weed management programs for weeds with evolutionary resistance to any weed control method. Tillage strategy as part of agricultural control is an interesting way to reduce the use of herbicides.

Our results focused on two main parts, the first one is the of influence agricultural practices on soil physical properties, soil humidity, porosity and penetrometry, and the second one is the influence of chemical and mechanical processes in weed infestation, and its consequences on the crop.

The humidity value is high in the NT and NT+G (of 25.6% and 23.4%) technique compared and to CT (22.2 and 21.4), as the plant cover frequently favors water conservation in the upper soil layer. Reduced evapotranspiration leads to lower humidity losses in the SD system; water penetrates the NT soil about three times faster than CT in ploughed soil (Chervet et al., 2016).

Nevertheless, soil porosity is better in CT than in NT tillering the soil significantly increases the porosity, a of 66.7% has been obtained with CT and 60.3% with CT+G; which is explained by the turning of the soil which favors its restructuring by rearranging the aggregates, contrary to NT where the soil is less disturbed. In general, in NT, weed control by herbicide was partial and reappearance was faster compared to CT where the seed stock was buried deep by the ploughing operation, which slowed down the germination of weed seeds.

The combination of mechanical tillage and chemical control (CT+G) allows the eradication of weeds with a significant reduction in the quantity of herbicide, this combination seems to be the most efficient to face one of the constraints of the yield decrease which are weeds and to preserve the environment. The results obtained confirm that yield is directly influenced by weed competition; this is less important in NT than in CT and more precisely in relation to the chemical treatment (yield is high in CT, NT+G and TC+G, while it is low in NT). Traditional tillage, with its deeper action on the soil, resulted in a high yield. The highest values 351.3 heads per m^2 and 252 heads per m^2 were recorded with CT+G and CT treatments respectively, while the lowest value was obtained by NT (28.6 heads per m^2), which explains the effect of weeds and competition on wheat yields.

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Production removal of the main nutrient elements from winter wheat and barley crops in the conditions of the Ararat Valley of Armenia

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Abstract. The aim of the research is to identify the extent of production removal of the main nutrient elements in the irrigated grain-growing lands of Armenia, to optimize the norms of organo-mineral fertilizers, to stabilize the yield of plants and prevent dehumification. The field experiments were carried out in the conditions of the Ararat Valley on winter wheat and barley in 2020–2022. The production removal of nitrogen by 5–7 t ha^{-1} grain of winter wheat and 9.6–13.3 t ha^{-1} straw varies between 155–247, P_2O_5: 60–88, K_2O: 134–197 kg ha^{-1}, and the amounts removed by barley grain 4.5–6.5 t ha^{-1} and straw 6.9–9.7t ha^{-1} were 122–194, 49–77 and 106–159 kg ha^{-1}, respectively. The amounts of nitrogen and potassium production removal from plant crops are about 2 times higher than the doses of applied fertilizers, and the amount of phosphorus is almost balanced by these doses.

Key words: winter wheat and barley, organo-mineral fertilizers, main nutrient elements, production removal.

INTRODUCTION

One of the main ways of increasing the yield of agricultural crops and stabilizing the soil fertility is the application of organo-mineral fertilizers, as the nutrient elements removed with commodity and non-commodity crops must somehow be returned to the soil. The amounts of nitrogen, phosphorus and potassium removal depend on the species and varietal characteristics of the plants, the level of the yield, soil and climatic conditions, the applied agricultural machinery, norms of fertilization and other factors. Global climate changes in various countries of the world, including Armenia, are becoming a crucial factor for ecological security, as well as for crop growth and yield. The studies conducted in 2009–2015 on different varieties of winter wheat in Latvia have shown that the effect of nitrogen fertilizers on grain yield and protein content is 3–35%, climatic conditions - 33–46%, and cultivation conditions - 34% (Skudra & Ruza, 2016; Linina & Ruza, 2018).

In Armenia, the sowing of grain crops regularly occupy120–200 thousand hectares of land. From this point of view, the Ararat valley is considered the most active agricultural region of Armenia and occupies 8.4% of the total area (29.8 thousand km^2). About 45 thousand hectares of grain (mainly winter wheat and barley) are cultivated in the area. The irrigation factor is problematic here. Regardless of the level of agricultural machinery, variety, fertilization, it is impossible to cultivate any plant without water in this semi-desert zone. The sum of active temperatures (over 10 °C) here reaches 4,000–4,300 °C annually, and the average temperature is 10.6 °C. The absolute minimum air temperature in winter reaches -30. -33 °C, the amount of precipitation is 200–260 mm, the evaporation capacity (annual deficit) is 600 mm, the humidity coefficient according to Shashko is 0.20–0.25.

The Scientific Center of Agriculture, with its production and experimental base, where the field experiments were carried out, is located in the central part of the Ararat valley, at an altitude of 853 m above sea level. During the years of research, the climatic and irrigation conditions of this zone were quite stable, although the influence of the greenhouse effect is also noticeable here. Moreover, because of the deepening deficit of nutrients, especially nitrogen, the yield of crops has been gradually reducing in the agricultural lands of the republic.

Almost everywhere in the world, insufficient amount of nitrogen is considered to be a limiting factor for good yield of the crops. The researches in this field have shown that the $N_{150}P_{50}$ combination noticeably increases the yield of winter wheat and barley and protein content in the grains (Hlisnikovsky et al., 2019).

The research conducted on grain crops shows that in different soil and climatic conditions, the amounts of production removal of the main nutrient elements by 1 t of grain and the corresponding mass of straw differ significantly. Thus, nitrogen removed by winter wheat varies between 26–37, P_2O_5: 8–13 and K_2O:16–27 kg, by winter rye 22–28, 8–10 and 24–26 kg, respectively, by barley N: 24–28, P_2O_5: 8–10, K_2O: 23–30 kg, by spring wheat N: 31–47, P_2O_5: 11–12, K_2O: 18–19 kg etc. (Peterburgsky, 1979; Babayan, 1980; Voitovich & Laboda, 2005; Shafran et al., 2008; Khachidze & Mamedov, 2009). Much lower data are reported by experiments in the USA in 1950-s. 11.7 kg of N, 2.1 kg of P_2O_5, 5.6 kg of K_2O are removed from wheat, 12.2; 1.9; 9.0 kg from barley. The release of K_2O in the commodity harvest of all crops was 3–4 times less than that of nitrogen, and by straw it exceeded the release of nitrogen by 1.5–3 times (Collings, 1960). The position of the slopes in the agrolandscape also has a certain effect on the root mass of winter wheat and the macro- and micro- elements accumulated in them. Root mass on northern slopes exceeded that of the plants growing on southern slopes, and the nutrient content was higher on southern slopes (Dubovik & Dubovik, 2015).

The studies conducted in Armenia show that 2.0–2.8 kg of N, 1.0–1.2 kg of P_2O_5, and 3.3–4.0 kg of K_2O are removed by 1 t of the tomato variety 'Lia' and corresponding vegetative mass, and in the case of the variety 'Sunrise' it was 3.3–3.8; 1.4–1.6 and 4.3–5.2 kg, respectively. 2.4 kg of N, 1.5–1.7 kg of P_2O_5 and 3.0–3.4 kg of K_2O are removed by the pepper variety 'Almond 55' (Harutyunyan, 2009; Harutyunyan & Miqaelyan, 2021). Monoculture farming in the cereal farms of Armenia has caused unprecedented soil fatigue. The inclusion of pea, lentil, soybean, korghan, alfalfa in the crop rotation of grain crops prevents not only soil fatigue, but also enriches the soil with biological nitrogen. It has been found out that chickpeas fixed 109–288 kg ha^{-1} of nitrogen, soybean 264–312 kg ha^{-1} and alfalfa 486 kg ha^{-1} over a year (Tanchyk et al., 2021).

Purpose of the research and problems

The purpose of the research is to study the reasons for the decline of soil fertility and plant yield in the cereal lands of Armenia, related to the disruption of crop rotation and the insufficiency of organo-mineral fertilizers in farms. The main issues arising from the goal are: 1. to identify the extent of production removal of the main nutrient elements in winter wheat and barley crops, 2. to adjust the doses of organic and mineral fertilizers, which will allow to stabilize the soil fertility, prevent dehumification and create a dynamic balance of nutrient elements in the crops.

The scientific novelty of the work is directly related to the main problem of agrochemistry, which is to ensure the balance of nutrients and humus in agrocenoses. From this point of view, calculations of the entrance and losses of main nutrient elements in irrigated and waterless grain phytocenoses of the republic have not been carried out during the last 45 years, while their regular implementation is considered a mandatory monitoring task in the control of soil fertility reproduction.

Materials and methods

The field experiments were carried out in 2020–2022 at the Ejmiatsin production experimental farm of Armenian Scientific Centre of Agriculture, on irrigated meadow gray soils (40°16' and 44°29'). The experiments were based on the varieties of Armenian red grain soft wheat 'Nairi 68' and barley 'Ara' with 4 repetitions (each repetition: 50 m^2). The schemes are given in tables, where the principles of the only difference and comparability are preserved. In field experiments, the norm of sowing wheat was 250 kg ha^{-1} (about 4.2–4.5 million grains), and that of barley - 200 kg ha^{-1} (about 4.0–4.5 million grains). Phosphorous-potassium fertilizers and 40% of nitrogen were introduced into the soil in the autumn under the plow, during the 3rd decade of October, and 60% of nitrogen was introduced in the spring as nutrition during the 1st and 3rd decades of April. The sowing was done in the 3rd decade of October. In field experiments, as well as in production sowing, 4 irrigation periods are performed (in the autumn, immediately after sowing, in the 2nd decade of April, in the 2nd decade of May and in the 1st decade June), with an irrigation norm of 900 m^3 ha^{-1}. Harvesting was performed in the 1st decade of July. Ammonia nitrate, granulated super-phosphate (P_2O_5: 19.5%) and potassium chloride (K_2O: 60%) were used as mineral fertilizers, semi-rotted cattle manure (N: 0.48; P_2O_5: 0.23; K_2O: 0.55%), as organic fertilizer, biohumus (N: 1.8–2.0; P_2O_5: 0.85–2.0; K_2O: 0.51–0.73% according to the certificate). In April, chemical control was carried out against two-stemmed weeds with the herbicide Grodil maxi at 0.11 l/ha, and against one-stemmed weeds with the doses 1 L ha^{-1} of Lastiktop.

The climatic conditions of the Ararat valley are represented by average data (Agroclimatic resources of Armenia edited by R.S. Mkrtchyan et al., 2011). Laboratory analyses of soils and plants were performed using the well-known methods in the agrochemistry laboratory of the Scientific Centre of Agriculture (Alexandrova & Naidonova, 1976; Yagodin, 1987). The mechanical composition of soils was determined by the classical pipette method and evaluated according to the classification scale of N.A. Kachynski (Soil science edited by J.S. Kaurichev, 1982). The pH of the water extract was determined by the potentiometric method, and the dry residue was not determined, as salinization is excluded in the studied and adjacent lands, since the groundwaters are at a depth of 7–10 m and do not pose any threat to crops. Humus was determined by I.V. Turin method, and total nitrogen by the Kjeldahl method. Easily hydrolyzable nitrogen in the soil was determined by the method of I.V. Tyurin and M.M. Kononova (Yagodin, 1987), and the mobile forms of phosphorus and potassium were determined by the Machigin method, which is based on the principle of their removal by a 1% $(NH_4)_2CO_3$ solution. Total nitrogen, P_2O_5 and K_2O in plant samples were determined by K. Ginzburg's wet ashing method, after which nitrogen was determined by Kjeldahl's micro-method. P_2O_5 was determined by photoelectrocalorimeter, and K_2O by flame photometer. The mathematical processing of the yield was done by the method of dispersion analysis (Dospekhov, 1985).

RESULTS AND DISCUSSION

The entire production and experimental plot of the Scientific Centre of Agriculture in Ejmiatsin is a homogeneous plane, where an area of about 5 hectares was selected for field experiments. At the beginning of October, 2019, in the central part of the plot one soil cut was made, from which, soil samples were taken according to genetic horizons, for laboratory analysis. Within that plot, field research was conducted in 2020–2022, where lentils and chickpeas were sown alternately in different parts of the experimental plot, followed by wheat and barley. Lentils and chickpeas were of background importance but nitrogen fixed by them was not calculated due to methodological difficulties. The irrigated meadow gray soils are characteristic of the semi-desert natural zone of the Ararat Valley and occupy 53 thousand hectares. The agrochemical characteristic of the soil is given in the first table.

From the data in the first table, it can be seen that the soil of the test plot is quite strong (A + B = 81 cm). Its mechanical composition is mainly medium and heavy loam, the physical clay (< 0.01 mm particles sum) in the horizon A is 35.09; in the horizon B_1 it is 59.92; in the horizon B_2 it is 33.4%, and in the horizon C it is considered to be light loam (22.6%). The roots of wheat and barley in these soils mainly spread in the 0–30 cm layer, and individual roots penetrate to a depth of 60–80 cm. The carbonate content in the topsoil is small, 1.1–1.5%, and the pH of the water extract is alkaline and varies between 7.6–8.3. The content of humus and total nitrogen is quite low, because the processes of mineralization of organic matter in these soils are quite intensive at high temperatures. The content of the available forms of the main nutrient elements is average. With optimum fertilization in these soils, all crops grow well and produce high yields.

The indices of grain and straw in the studied crops have been quite stable during the research years (Table 1), which is mainly explained by the stability of climatic conditions and agrotechnical measures. However, it should also be noted that under irrigation conditions a very strong competition arises between crops and weeds. Weed plants germinate and bloom intensively from early spring to late autumn, aggressively using up soil and fertilizer nutrients. In case of weak ecological and chemical control, an unprecedented drop in the yield of grain crops (up to 80%), as well as a decrease in the quality of the grain takes place. The degree of competition between crops and weeds in a flax crop rotation system can be represented in the following descending order: flax-potato-barley-winter wheat-winter rye (Conova & Samoilov, 2015).

Table 1. Physico-mechanical and agrochemical characteristics of test site soils

Genetic horizon and depth, cm	Hygroscopic humidity, %	< 0.01 mm sum of particles (phys. clay), %	The pH of the water draft	Humus, %	Total N, %	Mobile forms mg per 100 g in the soil		
						N	P_2O_5	K_2O
A 0–30	4.5	35.09	7.9	1.85	0.20	4.5	2.6	36
B_1 30–57	3.5	59.92	7.6	1.12	0.08	4.2	2.8	25
B_2 57–81	4.4	33.40	8.3	0.78	0.05	3.5	1.9	21
C 81–106	2.3	22.60	8.4	0.45	0.03	1.7	1.6	16

The data in Table 2 show that the 3-year average grain yield of wheat and barley is almost the same in all the options, while there are significant differences between the masses of the straw. The mass of wheat straw exceeds the mass of barley straw by about 30–40%, which can be explained by species and varietal characteristics. As for the effects of fertilizers, the yield of wheat grain in those options increased by 27.3–37.2 compared to the control; and the mass of straw - by 27.7–38.9%. In barley these indices were 36.1–42.5 and 33.8–41.0%, respectively, with the highest values recorded for $N_{120}P_{80}K_{90}$, as well as for biohumus in options. Relatively high results were also obtained in the $N_{120}K_{90}$ version, which is due to the strength of the plant stems, disease resistance, and full ripening of the grains. The surplus in wheat and barley yields in the fertilization options are reliable compared to the control in all years (LSD_{05} values).

Table 2. Effect of mineral and organic fertilizers on grain and straw yield of wheat and barley

Crop	Options	Grain harvest, t ha^{-1}				Straw, t ha^{-1}			
		2020	2021	2022	3 year average	2020	2021	2022	3 year average
Wheat	1. No Fertilization (Checker)	5.11	4.72	5.02	4.95	10.14	8.75	9.85	9.58
	2. $N_{120}P_{80}K_{90}$ kg ha^{-1}	7.29	6.37	6.72	6.79	13.96	12.35	13.63	13.31
	3. $N_{120}P_{80}$	6.58	6.16	6.26	6.33	13.19	11.92	11.26	12.46
	4. $N_{120}K_{90}$	6.76	6.47	6.53	6.59	13.53	11.68	12.82	12.68
	5. Biohumus-6 t ha^{-1}	6.57	6.21	6.48	6.42	13.34	11.47	12.41	12.41
	6. Manure-25 t ha^{-1}	6.43	6.13	6.35	6.30	13.04	11.36	12.29	12.23
	Sx, %	1.28	2.06	2.15	1.24				
	LSD$_{05}$, t	0.23	0.41	0.38	0.24				
Barley	1. No Fertilization (Checker)	4.54	4.52	4.65	4.57	6.82	6.86	6.89	6.86
	2. $N_{120}P_{80}K_{90}$ kg ha^{-1}	6.48	6.36	6.69	6.51	9.69	9.67	9.64	9.67
	3. $N_{120}P_{80}$	6.10	6.27	6.30	6.22	9.12	9.21	9.21	9.18
	4. $N_{120}K_{90}$	6.31	6.12	6.46	6.30	9.45	9.35	9.47	9.42
	5. Biohumus-6 t ha^{-1}	6.38	6.58	6.52	6.49	9.64	9.30	9.51	9.48
	6. Manure-25 t ha^{-1}	6.24	6.26	6.43	6.31	9.30	9.08	9.31	9.23
	Sx, %	2.36	3.29	0.83	0.87				
	LSD$_{05}$, t	0.43	0.56	0.18	0.17				

LSD – Lowest Significant Difference.

Removal of nitrogen, phosphorus and potassium from soil and fertilizers through crop yield and vegetative mass is considered to be the main factor in nutrient balance losses, while the content of nitrogen and ash elements in grains increases with favourable agrotechnical measures, such as optimal chemical treatment, targeted soil cultivation, active crop rotation and etc. In the case of the basic minimum soil cultivation and fertilization, with a yield of 3–4 t ha^{-1}, the nitrogen content in the grains of spring wheat reaches 2.61–2.82%, the removal of nitrogen makes 78–113 kg tha^{-1}. Nitrogen removal increases when chemicalization is intensified (Sineshchekov & Tkachenko, 2018).

In our field experiments, some increase in main nutrient elements was observed in wheat and barley grain and straw due to the use of organo-mineral fertilizers (Table 3). Thus, the total nitrogen content in the wheat grain of the test version was 2.00; in straw it was 0.58%, in the grain of the fertilization options it ranged from 2.16 ($N_{120}P_{80}$) to 2.42% ($N_{120}P_{80}K_{90}$), and in the straw it was 0.62–0.64%. The content of phosphorus in the grain and straw of the test version was 0.78 and 0.22%, respectively, and that of potassium - 0.68 and 1.05%. In the fertilization options these ash elements did not is almost indistinguishable from similar values in wheat. It shows that in flat-homogeneous soils the biological requirements of wheat and barley, as well as their selective and nutrient absorption properties are similar. It can be seen from Table 3 that the content of nitrogen in grains is about 3–4 times higher than that of straw, and the content of K_2O in straw is 1.5 times higher than in grain. As for phosphorus, its content in commodity plant crops is about 3.5–4 times more than in straw and about 3 times less than the amount of nitrogen contained in grain.

Table 3. The content of the main nutrient elements in the biomass of wheat and barley in relation to the application of organo-mineral fertilizers, % (average data for 2020–2021)

Crop	Options	Grain			Straw		
		Total N	P_2O_5	K_2O	Total N	P_2O_5	K_2O
Wheat	1. No Fertilization (Checker)	2.00	0.78	0.68	0.58	0.22	1.05
	2. $N_{120}P_{80}K_{90}$ kg ha^{-1}	2.42	0.84	0.71	0.62	0.24	1.12
	3. $N_{120}P_{80}$	2.16	0.83	0.70	0.64	0.24	1.08
	4. $N_{120}K_{90}$	2.29	0.86	0.74	0.63	0.22	1.14
	5. Biohumus-6 t ha^{-1}	2.32	0.81	0.69	0.63	0.23	1.10
	6. Manure-25 t ha^{-1}	2.22	0.82	0.69	0.64	0.22	1.11
Barley	1. No Fertilization (Checker)	1.82	0.76	0.72	0.57	0.21	1.07
	2. $N_{120}P_{80}K_{90}$ kg ha^{-1}	2.00	0.83	0.74	0.66	0.23	1.15
	3. $N_{120}P_{80}$	1.89	0.83	0.72	0.65	0.24	1.12
	4.$N_{120}K_{90}$	1.97	0.81	0.75	0.67	0.21	1.13
	5. Biohumus-6 t ha^{-1}	2.03	0.80	0.73	0.66	0.25	1.14
	6. Manure-25 t ha^{-1}	1.98	0.82	0.73	0.67	0.23	1.14

Based on the data in Tables 2 and 3, the production removal of the main nutrient elements related to the application of organomineral fertilizers in winter wheat and barley crops has been calculated (Table 4).

It can be seen from Table 4 that the removal of nitrogen by the grain of the studied plants is significantly higher than the amount of potassium, and the removal of potassium by straw exceeds the amount of nitrogen. The removal of phosphorus by grain is about 2 times higher than the amount removed by straw. As for the amounts of production removal of basic nutrients with the biomass of field-harvested wheat and barley, the amounts of nitrogen and potassium are about 2 times higher than the norms of applied fertilizers, and the amount of phosphorus removed is almost balanced with the norms of fertilizers. In the control versions of wheat and barley, nitrogen production removal was 154.6 and 122.3; P_2O_5-59.7 and 49.1; K_2O-134.3 and 106.3 kg ha^{-1}, respectively.

Table 4. Production removal of nitrogen, phosphorus and potassium from wheat and barley seeds, according to the average data of 2020–2022

Crop	Options	Through grain, kg ha^{-1}			Through straw, kg ha^{-1}			The amount of production removal, kg ha^{-1}			1 t by grain and corresponding straw mass*, kg		
		Total N	P_2O_5	K_2O	Total N	P_2O_5	K_2O	Total N	P_2O_5	K_2O	Total N	P_2O_5	K_2O
Wheat	1. No Fertilization (Checker)	99.0	38.6	33.7	55.6	21.1	100.6	154.6	59.7	134.3	31.3	12.1	27.2
	2. $N_{120}P_{80}K_{90}$ kg ha^{-1}	164.3	57.0	48.9	82.5	31.1	149.1	246.8	88.1	197.3	36.3	13.0	29.1
	3. $N_{120}P_{80}$	136.7	52.5	44.3	79.7	29.9	134.6	216.4	82.4	178.9	34.2	12.9	28.3
	4. $N_{120}K_{90}$	150.9	56.7	48.8	79.9	27.9	144.6	230.8	84.6	193.4	35.0	12.8	29.3
	5. Biohumus -6 tha^{-1}	148.9	52.0	44.3	78.2	28.5	136.5	227.1	80.5	180.8	35.4	12.5	28.1
	6.Manure -25 tha^{-1}	139.9	51.7	43.5	78.3	26.9	135.8	218.2	78.6	179.3	34.6	12.5	28.4
Barley	1. No Fertilization (Checker)	83.2	34.7	32.9	39.1	14.4	73.4	122.3	49.1	106.3	26.7	10.7	23.2
	2. $N_{120}P_{80}K_{90}$ kg ha^{-1}	130.2	54.0	48.2	63.8	22.2	111.2	194.0	76.2	159.4	29.8	11.8	24.5
	3. $N_{120}P_{80}$	117.5	51.6	44.8	59.7	22.0	102.8	177.3	73.6	147.6	28.5	11.8	23.8
	4. $N_{120}K_{90}$	124.1	51.0	47.3	63.1	19.8	106.4	187.2	70.8	153.7	29.7	11.3	24.4
	5. Biohumus-6 tha^{-1}	131.7	51.9	47.4	62.6	24.9	108.1	194.3	76.8	155.5	29.9	11.8	23.9
	6. Manure-25 tha^{-1}	124.9	51.7	46.1	61.8	21.2	105.2	186.7	72.9	151.3	29.6	11.6	23.9

*For example: In the control version of wheat, the average grain yield of 3 years was 4.95 t ha^{-1}, and the mass of straw was 9.58 t ha^{-1}. The mass of straw equivalent to 1 t of grain will be 9.58:4.95 = 1.94 t ha^{-1}.

In fertilization options, those indices in wheat crop ranged from N - 216.4–246.8; P_2O_5-80.5–88.1 to K_2O - 178.9–197.3 kg ha^{-1}, and in barley, respectively; in the range of 177.3–194.3; 70.8–76.8 and 147.6–159.4 kg ha^{-1}.

The amount of nutrients removed by 1 t of grain and corresponding straw mass in wheat crop varied between N: 31.3–36.3; P_2O_5: 12.1–13.0; K_2O: 27.2–29.3, and in the barley scheme in the range of 26.7–29.9; 10.7–11.8 and 23.2–24.5 kg ha^{-1}, respectively. Taking into account the loss of nutrients from fertilizers and their assimilation coefficients in the year of application (N: 50–60%; P_2O_5: 20–30%, K_2O: 60%), we can assume that the danger of a deepening deficit of basic nutrients is quite obvious. The reconsideration and optimization of doses of organo-mineral fertilizers in the Ararat valley and in the cultivated lands of the republic has become urgent.

CONCLUSION

1. The soils of the experimental fields belong to the type of irrigated meadow gray soils, which are quite strong (A + B = 81 cm), have an average content of available nutrient elements, are alkaline (pH: 7.6–8.4) and are favorable not only for cereals, but also for cultivation of all plant species.
2. The crop of winter wheat and barley grain in the Ararat valley provided a high-quality yield of 5–7 t ha^{-1} grain within the tested fertilization scheme. In the case of optimization of organo-mineral fertilizers, the yield of plants can reach 8–9 t ha^{-1}.
3. The amounts of nitrogen and potassium production removal from winter wheat and barley crops are about 2 times higher than the doses of applied fertilizers, and the amount of phosphorus is almost balanced with the doses of fertilizers, that is, the plants use more of the nitrogen and potassium in the soil.
4. The amount of nutrient elements removed by 1 t of grain and corresponding straw mass in the wheat sowing varied between N: 31–36; P_2O_5: 12–13, K_2O: 27–29, and in barley seeds in the range of - 27–30, 11–12 and 23–24 kg ha^{-1}, respectively.

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Effect of fertilizers on growth and productivity of saffron: a review

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Abstract. Saffron management involves a systematic approach to nutrient management. Controlling the amount, form, combinations and timing of nutrients delivered to plants is a pre-requisite for getting optimum yield and quality potential of saffron. Therefore, nutrient use efficiency and integrated nutrient management is a crucial tool for balanced fertilization and sustainable crop production. The impact and the need of organic and inorganic fertilization for saffron growth are discussed along with the possibilities of increasing qualitative and quantitative parameters by the integration of multiple fertilizers. The goal of this review is to give an overview of saffron nutrition management in order to maximize saffron growth and yield.

Key words: fertilization, saffron, stigma yield, nutrient management, organoleptic properties.

INTRODUCTION

Saffron (*Crocus sativus*) is a flowering plant of the genus Crocus in the family Iridaceae. It is one of the most valuable agricultural and medicinal plants that for centuries has been cultivated in Iran, India, and southern Europe (Menia et al., 2018). Saffron as the most expensive agricultural and medicinal plant has a unique status among industrial and export products, and important for producing employment opportunities (Golmohammadi, 2014) Saffron spice is made from the filaments that grow inside the flower. Saffron contains over 100 biologically active compounds, the most important of which are crocin, crocetin, picrocrocin, and safranal (Singletary, 2020).

A variety of factors affect saffron cultivation, including climate, crop density, irrigation, soil fertility, agricultural practices, and saffron type (Al Madini et al., 2019; Rezvani-Moghaddam, 2020). In addition, the main restrictions to saffron production are the fragmented and small land holdings in the nations where it is traditionally grown, low production and productivity levels, high labor costs, inconsistent climatic conditions, lack of scientific cultivation practices, and most importantly, to lack of awareness and interest on the appropriate choice and doses of manures and fertilizers among the saffron growers (Gresta et al., 2009). Thus, a balanced and timely supply of fertilizer is a pre-requisite for getting optimum yield and quality potential of saffron (Ghanbari & Khajoei-Nejad, 2022). Despite the low fertilizer requirement of saffron, studies confirmed that most changes of saffron flower yield depend on soil variables as affected by fertilizers and soil amendments (Temperini et al., 2009; Nehvi et al., 2010).

Soil fertility management is a proper strategy for increasing soil organic matter, strengthening microbial communities, preserving biodiversity, enhancing agricultural input efficiency, and eventually improving plant quantitative and qualitative yields (Koocheki et al., 2015; Rasouli et al., 2015). To attain this goal, the fertilization management program should be designed to increase nutrient use efficiency (Chen & Liao, 2017). Optimal nutrient delivery (Chen & Liao, 2017), controlled-release fertilizers (Vejan et al., 2021), integrated fertilization management (Alley & Vanlauwe, 2009), the use of organic nutrient resources, and beneficial symbiotic microorganisms with plant roots are all examples of methods that helps achieving nutrient use efficiency (Ghanbari & Khajoei-Nejad, 2021).

Chemical fertilizers are the most often used fertilizers in saffron fields, and their excess can jeopardize farmed soil and water quality, and reduce the yield of saffron (Dourandish et al., 2019). As a result, organic and biological fertilizers have attracted more interest for being more safe, low-cost, and have fewer negative environmental effects (Ebrahimi et al., 2022).

Organic fertilizers, such as soil amendments or surface mulches, and ecological fertility approaches, such as microbial inoculation and humic acid can increase agricultural productivity while preserving the environment (Shajari et al., 2022). Organic amendments have also been regarded as crucial means of improving plant nutritional status and soil properties, which can help to ensure long-term sustainability and positive economic returns in the fields (Chen et al., 2018). Organic fertilizers show compatibility with perennial crops, including saffron, and are natural and safe products that can be suitable for agricultural sustainability. The benefits of organic fertilizers on soil health, such as improved soil carbon sequestration, balanced pH, higher cation-anion retention, and micronutrient availability, have become increasingly apparent as compared to inorganic fertilizers, especially in the field of saffron cultivation (Singh et al., 2011).

Compost, animal manures, and vermicompost, as well as their integrated application, promote higher saffron growth, yield, and nutrient use efficiency, and improve soil properties and nutrient status (Cavagnaro, 2015; Koocheki & Seyyedi, 2015; Guo et al., 2016). It is proved that encapsulating fertilizers in nanoparticles increases nutrients uptake and reduces macro and micro nutrient deficiency (Chhipa, 2017; Shukla et al., 2019). Nanofertilizers have a greater capacity for absorption and are preferred over regular fertilizers (Solanki et al., 2015; Belal & El-Ramady, 2016; Khan & Rizvi, 2017). For a proper growth, plants require macronutrient in a fairly large proportion and micronutrients in relatively smaller amounts (Mandal, 2021).

The knowledge of the optimum saffron fertilization to improve yield and quality is lacking and requires comprehensive researches to reach a reliable long-term strategy to address the current issues. Considering the above facts, this review was undertaken to provide knowledge and better understanding of saffron fertilization requirement and to support the fertilizer management and development strategies based on available scientific findings. The reported information will help to predict the effective fertilizer requirement to improve saffron production.

Chemical fertilizers

Nitrogen (N). Nitrogen is one of the most important elements to increase saffron flowers and corm yield per plant and unit area. Nitrogen is known as a moving element and can be transferred from the aerial to t underground plant parts during growth, and especially at the end of each growing season (Koocheki & Seyyedi, 2015). Nitrogen fertilization increases nitrogen levels in saffron leaves, contributing in the formation of more chlorophyll molecules and higher photosynthetic activity, resulting in enhanced vegetative growth and biomass. Furthermore, the amount of material transported to underground organs and roots is determined by the photosynthetic level of saffron plant leaves, which increase the formation of daughter corms (Chaji et al., 2013). Nitrogen's ability to speed up plant growth resulted in larger flowers and heavier stigmas, but with lower percentages of safranal, crocin, and picrocrocin, reducing the stigma's flavor because the growth rate may be faster than the rate and amount of essential oil production by the plant, reducing its concentration in the stigma (Heydari et al., 2014).

Several studies discussed the effect of different nitrogen fertilizers on the growth and production of saffron. According to Kirmani et al. (2014), adding nitrogen at 90 kg ha^{-1} increases saffron yield by 57.57% and corm output by 79.62% over control. Omidi et al. (2009) conducted a study to determine the effects of nitrogen chemical and bio-fertilizer on quantitative yield and some quality components of saffron and found that using 150 kg ha^{-1} of urea or a mixture of 2.5 L ha^{-1} bio-fertilizer as Nitroxin (containing Azospirillum and Azotobacter) + 75 kg ha^{-1} urea resulted in the highest yield of stigma and style, and the highest crocine content. Also, adding 50 kg ha^{-1} of chemical nitrogen fertilizer to a biofertilizer containing plant growth promoting rhizobacteria, like Pseudomonas and Bacillus maximized saffron output and quality (Heydari et al., 2014).

Urea has shown to increase fresh and dry weight of corm and leaf, as well as the number of daughter corms (Chaji et al., 2013; Koocheki & Seyyedi, 2015). Further, the combined application of 10 t ha^{-1} vermicompost and 50 kg ha^{-1} urea nitrogen fertilizer significantly increased yields and enhanced soil characteristics. In fact, stigma dry yield, soil nitrogen content, soil organic matter, and available soil phosphorus increased by 42.6, 66.67, 68.39, and 43.75%, respectively, compared to the control (Feli et al., 2018).

Nitrogen fertilizer combined with manure is recommended for better saffron quality; compared to cow manure treatment, urea application increased safranal, crocin, and picrocrocin concentrations by 6%, 3% and 5%, respectively, while the former increased saffron yield by 20% in comparison to the latter (Abbasi & Sepaskhah, 2022).

Phosphorus (P). As the second most important element in plant nutrition, phosphorus plays a critical role in many physiological and biochemical activities in plants and its availability is one of the most effective variables in increasing saffron yield and quality (Fageria et al., 2013). Phosphorus promotes the synthesis of organic substance in leaves and their transport to the saffron corm, which aids in reproductive growth and boosts saffron output (Chaji et al., 2013; Koocheki et al., 2014). Studies have demonstrated that essential oils, such as phytolexins and safranal, are in dire need of phosphorus-containing compounds (Oftadeh et al., 2018).

The use of phosphorus fertilizer in various forms improved saffron corms' quality, vegetative characteristics, and increased stigma yield (Amirian & Kargar, 2016). In particular, it is shown that the use of 35 kg ha^{-1} of phosphorus increased the fresh weight of saffron corms and leaves, but decreased the number of corms (Chaji et al., 2013).

Also, the use of super phosphate (40 kg ha^{-1}) lead to early flowering (Amiri, 2008) and the application of Bio super phosphate increased leaf length (Alipoor Miandehi et al., 2014).

Potassium (K). Potassium is the second most important nutritional element for the growth and development of spice crops, and is frequently referred to as a quality component in crop production, as it enhances the usage of nitrogen and protein production, as well as size, weight, oil content, and color (Jiku et al., 2020; Fatematuzzohora & Karim, 2020). In plants like saffron, potassium is used as a flowering stimulator and is an important nutritional supply during the reproductive growth stage (Jabbari et al., 2017; Khayyat et al., 2018). Potassium fertilizer can also reduce the deleterious effects of salinity stress on saffron plant biomass formation in the roots and shoots, resulting in a higher yield in saline soil (Avarseji et al., 2013; Shayganfar et al., 2021). Foliar application of potassium at the rate of 3 L ha^{-1} increases leaf length, Leaf area index (LAI), stigma dry weight, crocin, picrocrocin and Safranal content (Akbarian et al., 2012). The application of 20 kg ha^{-1} of potassium in form of K_2SO_4 had the highest saffron dry stigma yield when compared to control and potassium in form of KCl (Zabihi & Feizi, 2014). The application of 200 kg ha^{-1} potassium sulfate led to increase in flower number and weight (5.5 and % 5.6 respectively), and stigma fresh and dry weights (by 10 and 8.5% respectively) (Akrami et al., 2015). Research on the use of 200 kg ha^{-1} of sulfur fertilizer and 100 kg ha^{-1} of potassium sulfate increased 90% the stigma dry weight (476 g ha^{-1}) in comparison to control (250 g ha^{-1}) (Basatpour et al., 2022).

NPK. Choosing the right NPK fertilizer ratio, form, and placement is able to supply all of the nutrients needed by the saffron simultaneously, which is more effective than applying independent nitrogen, phosphorus and potassium. Foliar spraying of different NPK fertilizers improved flowering rate, flower yield, and stigma yield of saffron due to increasing in production assimilates and their translocation to corm and below ground organs (Mollafilabi & Khorramdel, 2016) (Table 1).

Foliar application of NPK fertilizer; Phosamco™4 combined with cow manure increased the leaf appearance rate (by 6 leaves per day), flower emergence rate (by 9.5 flowers per day), maximum fresh weight of flower (by 42.5 g m^{-2}), and stigma dry weight (by 0.4 g m^{-2}) compared to control (Mollafilabi & Khorramdel, 2016). Application of NPK fertilizer (60-30-60 kg ha^{-1}) increased stigma dry weight by 146% compared to control (1.16 g m^{-2} and 0.47 g m^{-2} respectively) (Shahriary et al., 2018).

Flower fresh weight, stigma dry weight, and daughter corms number obtained from 5–10 g mother corms increased following foliar application of Dalfard 15® (a commercialized saffron fertilizer with 12% N from Urea and Nitrate sources, 8% P, 4% K and also Zn, Cu, Mg, Fe, chelates) by 16.44 g m^{-2}, 0.24 g m^{-2}, and 142% respectively compared to control (Khorramdel et al., 2015). In comparison to Nitroxin biofertilizer, Dalfard 15® was able to increase saffron flower number per unit area, flower dry weight, and stigma yield by nearly three fold (Koocheki et al., 2009), while it increased corm weight and number by 40 and 72% over the control, with comparable results obtained from fertilization using Manure 40 t ha^{-1} + vermiwash + effective microorganisms (Madahi et al., 2017).

Table 1. Effects of chemical fertilizers on growth and quality characteristics of saffron

Fertilizers	Dose of application	Soil type	DSY	FN	RCN	RCW	Source
Urea	150 kg ha^{-1}	Clay loam	+150%	-	-	NS	Omidi et al., 2009
	90 kg ha^{-1}	Clay loam	+49%	-	+90%	-	Kirmani et al., 2014
	45 kg ha^{-1}		+37%	-	+19%	-	
Urea foliar application	7 kg ha^{-1}	Sandy loam	+41%	+18%	+28%	+42%	Azizi et al., 2020
Triple superphosphate	35 kg ha^{-1}	Silt loam	-	-	-60%	+50%	Chaji et al., 2013
Super phosphate	40 kg ha^{-1}	Sandy Loam	+17%	-	-	-	Amiri, 2008
Ammonium phosphate	120 kg ha^{-1}	-	+40%	NS	-	NS	Amirian & Kargar, 2016
Potassium sulfate	200 kg ha^{-1}	Loam	NS	NS	-	-	Akrami, 2015
	100 kg ha^{-1}		NS	NS	-	-	
Foliar application of potassium	3 lit ha^{-1}	-	+15%	-	-	-	Akbarian, 2012
Potassium sulfate	20 kg ha^{-1} concentration	Loam	+134%	-	-	-	Zabihi & Feizi, 2014
Phosamco™4 foliar NPK	7 mg kg^{-1} concentration	Silt loam	+108%	+110%	-	+24%	Mollafilabi & Khorramdel, 2016
Dalfard 15® foliar spraying	15% concentration	Silt loam	+200%	+138%	+142%	108%	Khorramdel et al., 2015
FerTrix foliar fertilizer	2 L ha^{-1}	Sandy Loam	+34%	+61%	-	-	Emami et al., 2018

Values shown are percentage increase or decrease when compared to control; +: increased; -: decreased; NS: No significant difference according to the study; DSY: Dry stigma yield; FN: Flower number; RCN: Replacement corm number; RCW: Replacement corm weight.

Foliar application of FerTrix foliar fertilizer (NPK 20-20-20 fertilizer at 2 L ha^{-1}) combined with amino acids (0.5 L ha^{-1}) improved flower number by 85% and stigma yield by 46% compared to control (Emami et al., 2018). Integration of NPK fertilizer (50 kg ha^{-1} urea), triple superphosphate fertilizer (40 kg ha^{-1}), and potassium sulfate (50 kg ha^{-1}) with combined biofertilizer (A. Sp., *P. aeruginosus* and *B. subtilis*) increased stigma dry yield (by 57%), and contents of picocrocin (by 44%), safranal (62%), and crocin (by 47%) in comparison with control (Aalizadeh et al., 2018).

Humic acid

The beneficial impact of humic acid on plant growth are attributed to a variety of variables, including greater water and nutrient uptake, increased nutrient availability, better root system development, higher chlorophyll content, and improvements in the plant's enzyme activity (Barea et al., 2005; Sabzevari et al., 2010). Humic acid content was found to have a good influence on the quantity of saffron flowers and to improve saffron yield and quality (Osmani Roudi et al., 2015; Mollafilabi & Khorramdel, 2016).

In the study of Ahmadi et al. (2017), picrocin content was influenced in response to soil application of humic acid recording the highest value (40.60%) with 15 kg ha^{-1} humic acid applied. The highest safranal (20.1%) and crocin (55.57%) were obtained in plants treated with 10 kg ha^{-1} humic acid. Soil application of humic acid with 10 L ha^{-1} improved corm weight (by 7%) (Rivandi et al., 2016), and with100 kg per h, it improved the number of flowers (by 38%), flower yield (by 39%) and stigma dry yield (by 183%) compared to control (Koocheki et al., 2015). Moreover, Ahmadi et al. (2018) reported the highest stigma dry (0.23 g m^{-2}) yield in plants treated with 10 kg ha^{-1} humic acid and the highest total weight of corms (23.09 g per plant) with 5 kg ha^{-1} humic acid. In a more recent study conducted by Gerdakaneh et al. (2020), it was found that the use of 20 kg ha^{-1} of solid humic acid and 3 L ha^{-1} of foliar humic acid has increased flower number (by 42.72%) and yield of dry stigma (by 78.61%) compared to control, while combining 10 kg ha^{-1} solid humic acid and 2 L ha^{-1} foliar humic acid has enhanced picrocrocin content (by 4.9%), and the combination 10 kg ha^{-1} solid humic acid and 3 L ha^{-1} foliar humic acid was the most successful in increasing Safranal content (by 4.4%). Armak et al. (2021) also obtained higher stigma dry weight following the application of Super Humic treatment (improvement by 86.49% relative to control).

Vermicompost

Organic compost boosts soil fauna and microbial biomass, as well as enzyme activity, resulting in higher organic matter mineralization and pest and disease resistance, both of which are critical in organic farming (Erhart & Hartl, 2010). The final products of composting technologies like vermicomposting are nutrient-dense and ecofriendly, with a wide range of agricultural applications as soil conditioners (Usmani et al., 2017). On saffron, the use of vermicompost proved to be more effective compared to chemical fertilizers as well as other types of organic fertilizers; For instance, the application of vermicompost (10.2 t ha^{-1}) proved to be more effective than mineral fertilizer (N 225 kg ha^{-1} + P 129.08 kg ha^{-1}) in increasing the number, weight, N and P contents of medium and large daughter corms per plant (Seyyedi et al., 2018).

Gholami et al. (2017) reported that applying 10 t ha^{-1} vermicompost yielded more corms than applying 10 t ha^{-1} of cow manure, and from spraying 10 L ha^{-1} Humaster Saffron fertilizer, and that applying vermicompost buried under planting corm rows yielded more corms than spreading vermicompost. Besides, vermicompost application (10 t ha^{-1}) produced higher flower number and stigma yield than other fertilizer treatments (hen manure 15 t ha^{-1}, humic acid 2 kg ha^{-1}, and chemical fertilizer containing 200 kg ha^{-1} urea and 140 kg ha^{-1} P and K) (Rezaie et al., 2019).

Animal manure

Animal-waste-derived organic fertilizer is a mainstay for sustainable agriculture since it improves soil fertility, microbial abundance, disease prevention, and economic issues. Furthermore, waste to fertilizer conversion is a low-energy requiring process that promotes circular bio-economy (Bhunia et al., 2021). In the long run, manure's small and constant release of nutrients enhances soil texture and structure while also meeting plant nutritional needs, resulting in better saffron yields on farms (Koocheki & Teimouri, 2014). In general, livestock manures have been shown to have a greater impact than chemical fertilizers on saffron yield and yield components (Alipoor et al., 2015).

Cow manure. The usage of livestock manure improved many soil fertility parameters (organic carbon, soil K, Mg, Ca, N-NH and CEC) and is one of the main causes of superior saffron quality and yield (Amiri, 2008; Jami-Alahmadi et al., 2009; Mohammad et al., 2012). For instance, in the study of Yarami & Sepaskhah (2015), the application of cow manure in 60 t ha^{-1} improved soil fertility and thus increased saffron yield by about 23%. It also mitigated the effect of saline irrigation on saffron. Further, cow manure applied in lower dose of 40 t ha^{-1} increased flower and total fresh weight of corm by 15.78 and 37.44%, respectively (Osmani Roudi et al., 2015). In 30 t ha^{-1}, cow manure increased corms number and total corms weight per plant by 15 and 13%, respectively, compared with non-treated plants (Fallahi & Mahmoodi, 2018). According to Mollafilabi & Khorramdel (2016), the application of cow manure at a rate of 20 t ha^{-1} resulted in enhanced growth and yield of corm and flowers, and was able to double the stigma dry weight compered to control treatment.

When compared to chemical fertilizer (150 kg ha^{-1} urea + 75 kg ha^{-1} superphosphate), the use of 25 t ha^{-1} cow manure resulted in a greater improvement in total replacement corm yield, number, weight, and phosphorus content of replacement corms (Feizi et al., 2015; Koocheki et al., 2015a). The nitrogen (N) and phosphorus (P) use efficiency in saffron of composted cow manure treatment was found to be higher than that of chemical fertilizer (Koocheki & Seyyedi, 2015; Koocheki et al., 2015b). Moreover, cow manure applied in 20 t ha^{-1} induced higher fresh and dry stigma yield compared to foliar application of Delfard (NPK) in 7 kg ha^{-1} and floral Phosphorus in 2.5 kg ha^{-1} (Kianimanesh et al., 2021). Furthermore, improvements in stigma dry weight as a result of cow manure application at a rate of 40 t ha^{-1} reached 77.3, 71.7, and 58.9% increase respectively compared with non-treated plants, plants treated with humic acid, and plants subjected to Omic treatments (organic-mineral-based emulsion) (Ebrahimi et al., 2020).

The combination of cow manure (20 t ha^{-1}) and mycorrhizal fungus inoculation (*Glomus mosseae)* doubled flower number and yield, and enhanced organoleptic properties (picrocrocin and safranal contents) as well as total phenolic and total flavonoid contents in tepals (Ghanbari et al., 2019). The application of cow deep litter manure (20 t ha^{-1}) with combination of super phosphate at (40 kg ha^{-1}) and urea (50 kg ha^{-1}) resulted in highest yield (0.45 g m^{-2}) and maximum flower fresh weight (0.99 g), while, the lowest (0.24 g m^{-2}) and (0.50 g) with control, respectively (Amiri, 2009). Besides, combined effects of cow manure (20 t ha^{-1}) and urea (50 kg ha^{-1}) on saffron consisted of an optimization in flower fresh weight, stigma length, and yield (Mohammad et al., 2012).

Chicken manure. Aminifard & Gholizade (2018) investigated the effect of chicken manure application on saffron, and reported that a dose of 5 t ha^{-1} produced the maximum dry weight of stigma (increase by 0.32 g over the control). In drought conditions, the combined use of chicken manure and chemical fertilizer (ratio 3:1) improved saffron growth and yield, but the highest quality of saffron was obtained with a ratio (1:3); where chemical fertilizer share is higher (Aboueshaghi et al., 2022). Compared to a conventional chemical fertilizer (100 kg ha^{-1} urea + 80 kg ha^{-1} triple superphosphate), chicken manure applied in 5 t ha^{-1} provided significant improvement in many indicators, as follows: flowers number per m^2 (by 86.0), fresh flower weight (by 32.6 g m^{-2}), stigma dry weight (0.236 g m^{-2}), style dry weight (0.605 g m^{-2}), stigma harvest index (by 0.0071). In addition, chicken manure caused the greatest improvements in major studied characteristics of replacement corms (Shariatmadari et al., 2018).

Farmyard manure

Kirmani et al. (2014) found that applying farmyard manure at a rate of 90 kg ha^{-1} has increased saffron yield by 43.26% and corm output by 260.97% over the control. Over the course of three years, the application of Farmyard manure (FYM) at 350 kg ha^{-1} in combination with N:P:K at 30:20:15 kg ha^{-1} resulted in a maximum saffron yield with 91% improvement over control plots (Nehvi et al., 2010). The combination of 350 kg ha^{-1} FYM with Compound Liquid Fertilizer (12% N, 7% P2O5, K2O, Fe, Zn Chelates) at the rate 7 g per 1,000 m resulted in the maximum corm yield (1,047 g m^{-2}) (Nehvi et al., 2010).

Microbial inoculation

The use of biological fertilizers, which is one of the most successful management approaches for maintaining soil quality at the optimum level, is another novel method for providing the nutrients needed by the plant (Fallahi et al., 2009). Beneficial microorganisms have been used in agricultural activities for over 60 years, and helped enhancing plant resilience to different environmental challenges, such as drought, nutrients deficiency, and heavy metal toxicity (Wu et al., 2005).

In general, studies on the effect of microbial application on saffron have shown that rhizosphere bacteria act by stabilizing atmospheric nitrogen, increasing the availability of nutrients in the rhizosphere, increasing root contact and improving beneficial coexistence with host plants, and thus improving saffron growth and yield at different stages of the growing cycle (Rasool et al., 2021). Plant growth-promoting rhizobacteria (PGPR) release metabolites that directly stimulate growth, supporting the host plant by the ability to promote asymbiotic nitrogen fixation (Khan, 2005); produce phytohormones that promote plant growth (Marques et al., 2010); solubilize organic and inorganic phosphates (Alori et al., 2017) and has antagonistic activity against phytopathogenic microorganisms (Ren et al., 2020).

Azospirillum and Azotobacter. The bacteria in nitroxin biofertilizer (Azospirillum and Azotobacter) facilitated flower weight gain in saffron through an enhanced secretion of plant growth-regulating hormones, an improved development of saffron roots and aerial parts, a better nitrogen fixation and uptake by plant roots, and a more balanced uptake of essential nutrients and micronutrients required by the plant (Alipoor Miandehi et al., 2013).

In another study conducted by Pazoki et al. (2017), the same product (Nitroxin) increased vegetative traits and saffron dry yield (stigma + style weight) to 2.08 kg ha^{-1} compared to control (1.59 kg ha^{-1}) and significantly improved qualitative traits like Safranal, Picrocrocin, and Crocin. On the other hand, biofertilizer application in the form of viable strain of *Azotobacter* (5 kg ha^{-1}) has only positively influenced corm production, but its combined application with 90 kg of chemical nitrogen fertilizer has increased saffron yield (Kirmani et al., 2014). Besides, it was demonstrated that 0.2% Azotobacter-1 biofertilizer (containing *Azotobacter vinelandii*) is recommended to obtain higher production of saffron, and 1% Nitrokara (containing *Azorhizobium* *caulinodans*) to achieve higher content of active substances including antioxidants and total phenol (Parsa et al., 2018).

Pseudomonas putida and Pantoea agglomerans. One of the possible solutions needed to mitigate the problem of phosphorus deficiency is the use of phosphate-solubilizing bacteria to improve phosphorus uptake and reduce phosphorus fertilizer use. In this regard, *Pseudomonas putida* and *Pantoea agglomerans* are more efficient than chemical fertilizers at dissolving phosphorus from organic and inorganic substances (Aalizadeh et al., 2018). Farahani et al. (2014) found that using fertile phosphate biofertilizers with phosphorus-soluble bacteria helped to increase saffron yield by allowing organic chemicals and minerals to be absorbed more effectively. In addition, Parray et al. (2013) reported that the application of biofertilizers containing Pseudomonas bacteria encouraged the growth and enlargement of corms and increased stigma biomass.

The use of 100 g ha^{-1} of Phosphate Barvar2 (containing *Pseudomonas putida* strain P13 and *Pantoea agglomerans* strain P5) produced the highest saffron yield (improvement by 13.77%) compared to the application of 150 kg ha^{-1} of phosphorus as ammonium phosphate, and caused the highest picrocrocin content. In a similar study, 100 g ha^{-1} of Phosphate Barvar2 biofertilizer increased the corm number and flower dry weight (Bekhradiyaninasab et al., 2020). Further, a mixture of 50 g ha^{-1} of biophosphore (containing *Pseudomonas putida* strain P13 and *Pantoea agglomerans* strain P5) + 75 kg ha^{-1} chemical phosphorous as ammonium phosphate maximized safranal and crocin content (Naghdibadi et al., 2011). Additionally, the application of plant growth promoting bacteria (biofertilizer containing Pseudomonas and Bacillus) caused improvement in leaf length, leaf fresh and dry weight, chlorophyll b, total chlorophyll, carotenoids, zinc, and phosphorus concentrations (by 61.64%, 79.71%, 82.05%, 4.01%, 4.90%, 4.23%, 20.18% and 20.23% respectively compared to control) (Rasouli et al., 2013).

Mycorrhiza. Mycorrhizal colonization has been successfully implemented on saffron roots leading to significantly promoted mineral nutrient acquisition (Lone et al., 2016), as well as yield, quality, and volatile profile of saffron, particularly when integrated with fertilization (Ghanbari & Khajoei-Nejad, 2021).

For instance, the use of 10 g mycorrhiza (for every two corms with the same weight 7.5 ± 0.5 g) enhanced the stigmas dry yield and leaf dry weight by 46.21% and 137.5% respectively compared to control. Saffron roots were better colonized with mycorrhiza under organic nutritional treatments, as the treatment of 24 t ha^{-1} vermicompost and 10 g mycorrhiza fertilizer (for every two corms with the same weight 7.5 ± 0.5 g) increased saffron flower number in two consecutive years of study (Jami et al., 2020). Integration of arbuscular mycorrhizal (AM) fungus inoculation (*Funneliformis mosseae*) and different fertilizer types was reported to cause significant increases in leaf parameters, leaf nitrogen and phosphorus uptake, and leaf dry matter, yielding higher corm dry matter (Ghanbari & Khajoei-Nejad, 2022).

Integrated fertilizer application

The use of organic and inorganic fertilizers in the form of cow manure, vermicompost, Urea (nitrogen), diammonium phosphate (phosphorous) and Muriate of Potash (potassium) (in the ratio of 90 N:60 P_2O_5:50 K_2O kg ha^{-1}) in combination with 10 t ha^{-1} FYM and 0.5 t ha^{-1} vermicompost recoded maximum number of flowers plot associated with maximum saffron yield plot showing an increase in saffron yield to the extent of 154.86% and corm yield by 150% over control (Naseer et al., 2012). Also, the integration of 90 kg ha^{-1} N was combined with 30 t ha^{-1} FYM has optimized saffron yield, while the application of 90 kg ha^{-1} N coupled with 60 t ha^{-1} FYM has maximized N, P, and K content in leaves (Sofi et al., 2013). Seaweed extracts (2 L ha^{-1}) proved to be effective in improving many saffron indicators, like stigma dry weight, flowers number, corm and leaves dry weights (Azizi et al., 2020).

Nano-fertilizers

Nanofertilizers have had different effects on many physiological characteristics of saffron like antioxidant enzymes, reducing and non-reducing sugars, photosynthetic pigments, total phenol content and relative water content of leaf (Rostami et al., 2019). For instance, the use of different nanofertilizers (iron: Fe, boron: B, manganese: Mn, potassium: K, and zinc: Zn) increased leaf protein and relative water content (Rostami et al., 2017). Moreover, Amirnia et al. (2014) reported a positive effect of Fe, P, and K nanofertilizers on saffron flowering and production traits; flower number, stigma length, fresh and dry stigma weights, fresh and dry flower weights, and dry stigma yield. Hashemabadi et al. (2020) reported that the use of nanofertilizers increased both the quantitative and qualitative yield of saffron by making the best nutrients available (Table 2).

Table 2. Effects of Nano fertilizers on growth and quality characteristics of saffron

Fertilizers	Soil type	Dose of application	DSY	FN	RCN	RCW	Source
Nano Zn	Silt loam	6 g L^{-1} concentration	+25%	+40.5%	-	-	Rostami et al., 2019
Nano Chelated Fe	Silt loam	10 kg ha^{-1}	+133%	+93%	+102%	+219%	Baghai & Maleki Farahani, 2013
Nano TiO2	-	2,000 ppm concentration	+15%	-	52%	-	Nazari & Feizi, 2021
Nano silver	Loam	50 ppm concentration	+44%	-	-	-	Mahmoodi et al., 2021
Nano silicon	Sandy loam	1.5 ppt concentration	+17%	+11%	+7%	NS	Khoshpeyk et al., 2022
Nano NPK[1]	Loam	5 mg L^{-1} concentration	+70%	+71%	-	-	Hashemabadi et al., 2020

Values shown are percentage increase or decrease when compared to control; +: increased; -: decreased; NS: No significant difference according to the study; DSY: Dry stigma yield; FN: Flower number; RCN: Replacement corm number; RCW: Replacement corm weight; [1]Results shown for the treatment Nano NPK was on the planting date of 5th of September.

Foliar spraying of Fe is one of the quickest ways to meet the plants need for iron (Malhotra et al., 2020). According to Azarpour et al. (2013) foliar spraying of nano iron fertilizers with 2 g L^{-1} resulted in the highest amount of fresh flower yield (173.3 kg ha^{-1}). Furthermore, the application of 10 kg ha^{-1} Nano Chelated iron fertilizer improved many vegetative and productive indicators; including dried stigma yield, flower fresh weight, flowers number, leaves number, leaves length, main corm diameter, and corm total weight (Baghai & Maleki Farahani, 2013; Maleki Farahani & Aghighi Shahverdi, 2015). The latter experiments also revealed that nano-iron was more effective than common iron chelate fertilizer; where 5 kg ha^{-1} of the first had the same effects on most saffron traits as 10 kg ha^{-1} of the second. Salariyan et al. (2021) study shows that application of nano fertilizer of Fe caused a significant increase of dry weight of stigma.

Foliar application of nano Zinc Oxide (6 g L^{-1}) and conventional Zinc Oxide (9 g L^{-1}) had significant effects on the saffron yield and number of flowers, and morpho-physiological characteristics of saffron (Rostami et al., 2019). Foliar application of Zn improves leaf traits, dry matter production, and antioxidant enzymes that have a direct and indirect effect on improvement of saffron yield (Akbarian et al., 2012).

CONCLUSION

When compared to other fertilizers, foliar NPK fertilizers was able to increase the dry stigma yield by two and three-fold. In addition, the application of Nano chelated Fe at a rate of 10 kg ha^{-1} increased dry stigma yield by 133%, while cow manure at a rate of 20 t ha^{-1} increased stigma dry weight by 100%. It is also shown that the vermicompost at a rate of 10 t ha^{-1}, and 10 kg ha^{-1} humic acid were the best rates of application for improving the quantitative and qualitative yield of saffron, but the use of any of these organic fertilizers in combination with biofertilizers (*Azotobacter*, *Pseudomonas aeruginus*, and *Bacillus subtilis*) has a greater effect on increasing the quantitative and qualitative yield of saffron. The use of livestock manure and vermicompost together had a synergistic effect on saffron bacterial fertilizer. Even though foliar application of NPK fertilizers as well as the nano Fe could obtain a higher yield increase, it is recommended to use organic fertilizers for improving soil formation and structure and creating more stable agriculture system. In other words, organic fertilizers increase saffron growth, flowering, and quality properties by improving soil granulation, increasing water storage capacity, improving soil nutrient exchange capacity, and generally creating a suitable environment for bacterial growth and multiplication. In addition to having good impacts on most of the examined traits, the use of bio and organic fertilizers can enable the viability of saffron ecological production while reducing the use of chemical fertilizers, and can be considered a step toward sustainable agriculture.

Further testing of the reviewed fertilizers combined or alone in other doses and timing of application is required to understand the optimal nutritional management for the saffron crop. In addition, long-term use of organic and biological fertilizers should be investigated further to achieve sustainable and productive stage that is suitable and ideal alternative to chemical fertilizers.

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Application of environmentally safe chemical reclamation on an innovative basis in Russia

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Abstract. In the context of world food crisis, potato production and increasing its yield is an urgent topic of research. In agricultural production, one of the most basic directions of increasing production is the introduction of organic and mineral fertilizers. The purpose of the study is to develop agro-methods for the use of environmentally friendly biopolymers when applying mineral fertilizers for the effective cultivation of potato and to determine production efficiency. To end this purpose, two field experiments and one control variant without the use of biopolymers were used in our study to assess the effect of Nutricharge and Growgreen preparations on the yield of Gala potato variety. To obtain reliable experimental results, several of the following requirements were considered: representativeness, accuracy, and compliance with the principle of single difference. Experiment No. 1 - treatment of ammonium nitrate phosphate fertilizer with Nutricharge at the rate of 3 kg per 1 ha. As a result of the Nutricharge biopolymer application, the biological yield of potato increased by 83.5 c ha^{-1}. Experiment No. 2 - treatment of mineral fertilizers with Nutricharge and Growgreen, 2.5 L ha^{-1}. As a result of the biopolymers application, the biological yield of potato in this experiment exceeds the control data by 237.5 c ha^{-1}. At the same time, the yield in the second experiment turned out to be higher than the yield in the first experiment by 154 c ha^{-1}. An equally important task in conducting this study was to determine, with the help of economic assessment, the production efficiency of biopolymers application. As a result of economic analysis, we found out that the complex use of preparations allowed to reduce the cost of production of 1 ton of potato in the first experiment by 21%, in the second - by 7.7% compared with the control. Cost reduction and revenue increase in the second experiment allowed to make a profit from 1 ha of 298,020 rubles, and in the first - 221,440 rubles. The obtained research data give us reason to say that the use of biopolymers Nutricharge and Growgreen is effective and their scale should be spread throughout the entire region of the country.

Key words: potato, innovative mineral fertilizers, intensification, environmentally friendly chemical biopolymers Nutricharge and Growgreen, field experiment, potato yield and acreage, economic assessment.

INTRODUCTION

Potato (Solánum tuberósum) occupies the second place in food value after grain products. It is distinguished by its exceptional versatility in relation to its use as a product of agricultural production. The following qualities of this product should also be noted: high nutrient content and excellent taste qualities. Potato consumption is growing every year as a result of an increase in the world's population. In the conditions of the impending food crisis in the world, the increase in yields and the increase in the gross yield of potato is relevant (Kovalenko, 2019).

Recently, the government of the Moscow region has paid great attention to the development of the agricultural sector in this region. For the further development of the fruit and vegetable food sub-complex of this constitutional entity of the Russian Federation, a number of measures were taken to increase the gross yields of crop production in order to provide the population of Moscow and the Moscow Region with food products of domestic production, in accordance with medical consumption standards. It is especially important to note that the main emphasis was placed on the cultivation of such crops as: potatoes, carrots, white cabbage, table beets, which are most adapted to growing in this region according to the botanical characteristics of these crops and the combination of local natural and climatic conditions. In addition, the economic efficiency of their production is determined by the proximity to the sales market and its potential consumption volumes (Ibiev et al., 2021).

The use of biostimulators in combination with mineral fertilizers on all types of soils has an effective impact on economic fertility, and also improves the conditions for increasing soil microflora. The content of microflora (microorganisms) in well-loosened soil proves that it has a positive effect on the qualitative and quantitative composition of the soil, and this affects the increase in crop yields (Bulgakov et al., 2021).

When applying mineral fertilizers and other biological products, it is necessary to consider a number of technical and operational characteristics of machines and equipment, the efficiency of using these groups of fertilizers depends on this. In modern conditions, when almost all branches of the national economy are being converted to digital technologies, including agriculture, it is necessary to complete machines and equipment with digital installations, such as agrodrones, sensors, sensor dispensers and other digital resources that contribute to the normalized consumption of applied mineral fertilizers and biological products to provide the soil with the necessary plant nutrition elements in agriculture (Chernikova et al., 2021).

The most promising direction for increasing yields and increasing the efficiency of potato production is the use of innovative environmentally friendly mineral fertilizers - biodegradable polymers. Their main advantage is that they are capable of spontaneous destruction as a result of natural microbiological and chemical processes, without causing harm to the environment (Ushachev et al., 2017).

To successfully solve this problem in our scientific work, we conducted a study using innovative mineral fertilizers Nutricharge and Growgreen - biodegradable polymers and environmentally friendly chemicals for plant growth and development (Volkova, 2019).

Nutricharge and Growgreen are the new generation biopolymers for use in industrial crops, which increases the efficiency of expensive phosphorus-containing fertilizers, increasing the time that phosphorus remains in the state accessible to plants. Nutricharge and Growgreen have appeared on the Russian market recently, but, according to the opinion and assessments of leading experts engaged in this field of research, they show good results in the process of application in the cultivation of agricultural crops (Manzhina, 2017).

As mentioned above, in the abstract of the article, one of the most promising areas for increasing yields and improving the production efficiency of potato is the introduction of organic and mineral fertilizers. Usually, in a scientifically based farming system for potato cultivation, it is recommended to apply a complex mineral ammonium nitrate phosphate fertilizer ($NH_4H_2PO_4$+NH_4NO_3+ KCL), which contains three main components necessary to ensure the normal quality of plant life at different stages - nitrogen, phosphorus, and potassium (NPK).

In agricultural practice, it has long been widely known that such an important and rather expensive plant nutrition element as phosphorus, when it enters the soil in the form of mineral fertilizers, falls into a precipitate insoluble in water and becomes inaccessible to plants precisely because of the reaction with metals. According to various data, from 75 to 95% of this element is blocked in the soil solution in an insoluble form (Lipsky, 2014).

This leads to a lack of phosphorus, which in turn manifests in a delay in the growth and development of plants - small leaves are formed, flowering and fruit ripening are late. The lower leaves acquire a dark gray or dark green shade. Over time, they curl and die prematurely. The phosphorus binding reaction in the soil occurs quickly as soon as there is enough moisture and a soil solution is formed. Calcium cations present in alkaline soils, or aluminum and iron cations in acidic soils bind phosphorus, preventing plants from taking it up (Khromov, 2017).

Nutricharge is a new generation biopolymer for use in industrial crops. This is a macromolecule with a large negative charge, which takes on the maximum amount of calcium cations (or metal cations), as far as the capacity of cation exchange allows. For comparison: chernozem has a cation exchange capacity of 40 units, and Nutricharge molecule has 1,200 units. Therefore, a protective environment is formed around the phosphorus molecule, which works for at least 10 months. Nutricharge is gradually utilized in the soil by soil bacteria, does not accumulate in plants or in the soil ('ARTEL' LLC, 2017).

The advantage of this technology is that this water-soluble polymer is a slightly viscous liquid that is simply sprayed and applied to fertilizer granules in a similar way to seed dressing. This fertilizer has no shelf life. After treatment with polymer, the fertilizer is used on the farm in the same way as usual: as a basic fertilizer scattered for the main processing, cultivation or in a seeder during sowing, and as a top dressing with embedding in the soil. That is, the application technology does not change in any way (Khromov, 2017).

Another effective tool that the Artel is engaged in is complex liquid organomineral fertilizers of the Growgreen brand.

Growgreen is a biologically active fertilizer obtained during a unique patented production process, including mixing and preparation of ammonium sulfate with trace elements with the help of beneficial microbes. The fertilizer can be used for leaf feeding without the risk of burning the leaves. This provides excellent plasticity with respect to the type and frequency of fertilization ('ARTEL' LLC, 2017).

Both with leaf feeding and with tillage, Growgreen perfectly provides crops that have a high level of demand for these two nutrients. This provides an effective alternative to other products with more than twice high level of nitrogen.

Growgreen with a lower but more effective concentration of nutrients does not acidify the soil, in the same way as traditional ammonium sulfate. Growgreen is a biologically active product obtained as a result of a specially developed microbial production process.

Due to a specially developed microbial production process that repeats natural soil processes, transforming nutrients into a form 100% accessible to plants, Growgreen fertilizers are as effective as possible with small application rates.

The special 'stickiness' of Growgreen fertilizers allows to save nitrogen by 70% of the initial fertilizer level in the fertile soil layer after experiments on 'water outwash' on the lightest - sandy soils, where conventional fertilizers are washed out by 100% ('ARTEL' LLC, 2017).

Since the appearance of Nutricharge, since 2017, 220 production experiments have been laid on the Russian market in 17 regions, 45 farms, on 16 crops, including winter wheat, rapeseed, sunflower, potato, corn, sugar beet, soy, etc.

Gala potato variety was bred by breeders in 2013, high-yielding, unpretentious in care, resistant to diseases, it is classified as early-maturing. It ripens 75–80 days after planting, with proper cultivation, the crop yields from 12 to 20 medium-sized tubers from one bush.

In this regard, the purpose of the study was to determine, with the help of agricultural techniques and field experiments, the effectiveness of the use of Nutricharge and Growgreen biopolymers in the process of growing Gala potato, and also to determine the commercial feasibility of these actions in production.

MATERIALS AND METHODS

In 2020, in the spring-summer period, the Artel organization together with the scientific staff of the RSAU-MTAA and the specialists of the Lenin Collective Farm cooperative, which is located in the Ryazan region, laid two scientific field experiments to assess the effect of Nutricharge and Growgreen preparations on the yield of Gala potato, and also, for comparison with two field experiments, a control variant was used without application of biochemical polymers.

When setting up a field experience, the requirements and conditions of conducting a similar kind of research were considered: the soil topography, the configuration and size of the experiment area, the diversity of soil fertility, vegetation, and littering, the number and size of plots on the selected planting area, the shape of plots.

The size, number, and repetition of plots are of particular importance for the accuracy and reliability of the experiment. These requirements are determined by the chosen crop for the study, the botanical and biological features of the crop, and the method of potato planting are considered, in our study the size of plots in production conditions was 1 ha, the number of plots - 300 units, three land plots with an area of 300 hectares were chosen and selected for each experiment and control comparison option, the increase in repetition most significantly affects the accuracy of the experiment, the indicators chosen by us reduce the error of the experiment to 3%.

The shape of the plots was taken as an elongated rectangle - this is the most optimal form of efficient use of high-performance machinery and equipment. The GreenSeeker RT200 system, which is equipped with a modern Trimble FMX field computer, was used to conduct field experiment, for application and introduction of mineral fertilizers. A multifunctional device to which an autopilot or a thruster EZ-Steer, EZ-Pilot can be connected. With the help of GreenSeeker RT200, you can apply the planned fertilizer rate by GPS coordinates, 2 complexes of 6 sensors (for 2 tractors). The kit consists of 6 sensors with Trimble Recon PPC for variable speed fertilizer application. Such a system can be used with Trimble or John Deere GPS equipment (Sadykova et al., 2021).

Hereafter, let's look at the summary of the field experiment methodology:

During the field experiment in production conditions, three variants of the experiment were selected:

– control option, for comparison of experiment No. 1 and experiment No. 2, without the use of biopolymers Nutricharge and Growgreen. In the control variant, equality was observed in all the conditions that were given above, except for the studied one, known as the principle of single difference. The industrial technology of potato cultivation, scientifically-based potato farming systems by zones, the use of optimal norms of mineral fertilizers, in particular, such as: ammonium nitrate phosphate fertilizer ($NH_4H_2PO_4$+NH_4NO_3+KCL) - a complex fertilizer, which contains three main components necessary to ensure the normal quality of plant life at different stages of growth and development - nitrogen, phosphorus, and potassium (NPK), and ammonium sulfate ((NH_4) $2SO_4$, mainly contains 21% nitrogen and 24% sulfur. The planting area was 300 hectares, as mentioned above.

The dose of applying ammonium nitrate phosphate fertilizer for potato was 400 kg ha per 1 ha, considering the active substance nitrogen - 20%, phosphorus - 24%, and potassium - 30%. The application rate of ammonium sulfate is 300 kg ha.

– the control option of the field experiment for comparative analysis with experiments No. 1 and No. 2 was carried out without the addition and use of biochemical polymers, the remaining parameters of the field experiment methodology were preserved and left unchanged, which were listed above. The size of the plots is 1 ha, the number of plots is 300 units, the total area of Gala variety potato planting was 300 ha, the plot shape is an elongated rectangle. The control option was carried out using industrial potato growing technology, ammonium nitrate phosphate mineral fertilizers were used for planting potatoes 400 kg ha, ammonium sulfate - 300 kg ha of working solution for leaf feeding, the method of planting tubers is a bulk bed (Dutch method) at a distance of 50–60 cm from each other, the size of tubers is on average 70–80 g, the planting rate - 4.5 t.

– experiment No. 1 - treatment of complex ammonium nitrate phosphate mineral fertilizer with Nutricharge at the rate of 3 kg per 1 ha. The experiment technology, as in the control version: industrial technology of potato cultivation, mineral fertilizers: ammonium nitrate phosphate mineral fertilizer for planting potato 400 kg ha, ammonium sulfate - 300 kg ha of working solution for leaf feeding, the method of planting tubers is a bulk bed (Dutch method) at a distance of 50–60 cm from each other, the size of tubers on average 70–80 g, the planting rate is 4.5 t, but considering the processing of the complex ammonium nitrate phosphate mineral fertilizer with Nutricharge at the rate of 3 kg per 1 ha.

– experiment No. 2 - in addition to the Nutrichardge treatment, potato leaf fertilization was carried out in the phase of 5–7 leaves with micro-fertilizer Growgreen, 2.5 L ha^{-1} for a working solution of 300 L ha^{-1}. When conducting experiment No. 2, the conditions and requirements were considered, as in experiment No. 1, but in addition to the Nutrichardge treatment, potato leaf fertilization was carried out in the phase of 5–7 leaves with microfertilizer Grwgreen, 2.5 L ha^{-1} for a working solution of 300 L ha^{-1}.

RESULTS AND DISCUSSION

When conducting the experiment, it is necessary, first of all, to take into account indicators in physical units such as: gross harvest, biological and production yields, they are the basis for calculating the main results in our study. Biological yield is the potential yield based on selective sampling prior to harvest, when the crop is at its optimum ripeness. It is calculated according to the formula:

$$\text{y} = \frac{P \times A}{10{,}000}$$

where, P – number of plants per 1 hectare before harvest; A – mass of tubers from 1 bush, centner per hectare.

Production yield - the amount of products received after harvesting, according to the actual threshing, losses during harvesting and transportation to the place of its storage.

In the control option of the field experiment, the following production results were obtained:

– the gross yield amounted to 112,955 hundredweights from the potato planting area of 300 hectares;

– the production yield of potatoes was:

$$\text{Y}i_{sown\ area} = \frac{Bulk\ yield, \text{c}}{\text{S}^2_{\text{sowing}}} = \frac{112{,}955\ \text{c}}{300\ \text{ha}} = 376.56\ \text{c ha}^{-1}$$

– biological yield - 554.5 c ha^{-1}, production yield - 376.56 c ha^{-1}.

Production indicators during experiment No. 1 - treatment of complex ammonium nitrate phosphate mineral fertilizer with Nutricharge at the rate of 3 kg per 1 ha.

– biological yield - 638 c ha^{-1}, production yield - 418.31 c ha^{-1}, the difference was 83.5 c ha^{-1} and 41.75 c ha^{-1}, respectively.

After conducting experiment No. 2, we obtained the following results:

– biological yield - 792 c ha^{-1}, production yield - 495.31 c ha^{-1}. The difference is significant compared to both the control variant and experiment No. 1, the obtained biological yield in the second experiment exceeds the control data by 237.5 c ha^{-1}, and in experiment No. 1 - by 154 c ha^{-1}, the difference in production yield was 118.75 c ha^{-1} and 77 c ha^{-1}, respectively.

Further, for the purpose of economic assessment and identification of potato production growth reserves and the potential of the selected potato variety 'Gala', the actual costs of the grown potato crop were calculated.

Table 1 shows the actual costs of growing potato in 2020 by experiments.

Table 1. The actual costs of growing potato in the agricultural complex Collective Farm n.a. Lenin in 2020, rubles

Name	Total	per 1 c	per 1 ha	as a % of the total
Salary	12,300,026	102.07	38,438	18.45
Fuel and lubricants	1,926,109	15.98	6,019	2.89
Seeds	6,967,967	57.83	21,775	10.45
Mineral fertilizers	7,530,272	62.49	23,532	11.29
Means of protection	12,231,455	101.51	38,223	18.35
Costs of previous years	1,495,354	12.41	4,673	2.24
Tractor services	7,959,235	66.05	24,873	11.94
Motor transport	2,038,888	16.92	6,372	3.06
General expenses of production	4,321,268	35.86	13,504	6.48
Work of collective agricultural enterprise	2,583,760	21.44	8,074	3.88
Depreciation	5,255,964	43.62	16,425	7.88
Implementation costs	1,960,654	16.27	6,127	2.94
Other	102,746	0.85	321	0.15
TOTAL	62,653,598	53.31	208,355	100.00

The cost of production of 1 c of potato is:

$$C_{\text{potato}} = \frac{\sum \text{costs}}{\text{Bulk yield}} = \frac{62{,}653{,}598 \text{ rubles}}{112{,}955 \text{ c}} = 553.31 \text{ rubles c}^{-1}$$

The cost of production of 1 ton of potato, respectively, is 5,533.09 rubles per ton.

Let's calculate performance indicators separately for each experiment and compare them.

Results of experiment No. 1 with the treatment with Nutricharge fertilizers

At the first stage of the analysis, we will determine the indicators of the production efficiency of potato cultivation in physical units, which are the basis for calculating the cost indicators of potato production. However, indicators of production efficiency in physical units reflect only one side of the achieved result. To identify the full economic effect of the experiment, we will determine and calculate the cost indicators of the effectiveness of fertilizer application.

We will calculate the values of indicators that will allow to compare the effect of using fertilizers treated with Nutricharge according to experiment No. 1 (Table 2).

Processing of fertilizers with Nutricharge led to an increase in the biological yield of potato from 554.5 c ha^{-1} to 638 c ha^{-1}, which is equal to 15% in percentage terms.

Since the actual yield was not measured at the site, it was assumed that the increase in production yield was 50% of the increase in production yield, that is, 41.75 c ha^{-1} (the change in production yield to control is 11%). According to experiment, the production yield was determined as the sum of the increase in production yield and the yield of the sown area, i.e.:

$$Yi_{\text{prod.}} = \text{Increase } Yi_{\text{prod.}} + Yi_{\text{sown area}} = 41.75 \text{ c ha}^{-1} + 376.56 \text{ c ha}^{-1} = 418.31 \text{ c ha}^{-1}$$

The actual experiment costs per 1 ha increased by the cost of Nutricharge per 1 ha, i.e. by 5,250 rubles/ha or 2.5% to the control, i.e. the amount is relatively small. At the same time, the cost of production of 1 ton of potato in the experiment decreased to 5,106.36 rubles/ton, i.e. 7.71%.

The proceeds from the sale of potato from 1 ha at a price of 10,400 rubles per ton amounted to 391,625 rubles according to control, 435,045 rubles according to experiment. The profit calculated as the difference between revenue and actual costs, according to experiment, showed an increase of 38,170 rubles/ha, which amounted to 20.83%.

Table 2. Data from experiment No. 1 with the processing of ammonium nitrate phosphate fertilizers with Nutricharge for sowing potato in the agricultural complex Collective Farm n.a. Lenin in 2020

Indicators	Agricultural complex Collective Farm n.a. Lenin, Kasimovsky district	
	Potato	
	control	experiment
Applied on 1 ha on the experimental plot, kg	700	700
Actual costs per 1 ha, rubles	208,355	213,605
including: actual cost of fertilizers applied for the crop, including work in progress, rubles	23,532	23,532
Of these: cost of fertilizers per 1 ha in experiment, rubles	14,000	14,000
The cost of Nutricharge per 1 ha, rubles	0	5,250
Total: including the cost of fertilizers and Nutricharge per 1 ha in the experiment	14,000	19,250
Biological yield, c ha^{-1} (Gala variety) (Act)	554.5	638
The difference in the yield of experiments in comparison with the control, (+,-), c ha^{-1}		83.5
Change in biological yield in % to control, %		115,06
Production yield (adjusted in the experiment by 50% of the increase in biological yield)	376.56	418.31
Increase in production yield, c ha^{-1}		41.75
Change in production yield in % to control, %		111.09
Change in costs per 1 ha to the control variant, rubles		5,250
Change in costs per 1 ha to the control variant, in %		102.52
Cost of 1t of production yield, rubles	5,533.09	5,106.36
Change in the cost of 1 t to control, rubles		-426.73
Change in the cost of 1 t to control, %		-7.71
Actual selling price of potato, rubles/t	10,400	10,400
Estimated revenue from 1 ha, rubles	391,625	435,045
Profit from 1 ha, rubles	183,270	221,440
Increase in profit from 1 ha in comparison with control, %		20.83
Increase in profit from 1 ha in comparison with the control, rubles		38,170
Profitability, %	87.96	103.67
Change in profitability to control, % (+, -)		15.71

Profitability, calculated as the ratio of profit to actual costs, according to control was 87.96%, according to experiment - 103.67%, showing an increase of 15.71%.

We will calculate the indicators of economic efficiency, with the condition that the entire sown area of 300 hectares was sown with potato, and fertilizers were treated with Nutricharge.

Let's determine the volume of additional products that could be obtained by using the entire sown area, having previously transferred the increase in yield from c ha^{-1} to t ha^{-1}:

$$Add. products = Selling\ price \cdot S_{sowing}^{2} \cdot Increase\ Yi_{prod.} = 10{,}400 \text{ rubles} \cdot 300 \text{ ha} \cdot 4{,}175\ t\ ha^{-1} = 13{,}894{,}400 \text{ rubles}$$

We will calculate the additional costs of processing fertilizers for 300 hectares:

$$Add. \text{costs} = \text{Cost of Nitricharge per 1 ha, rubles} \cdot S_{sowing}^{2} = 5{,}250 \text{ rubles} \cdot 300 \text{ ha} = 1{,}680{,}000 \text{ rubles}$$

Additional costs to the total cost as a percentage will amount to 2.52%.

The cost of the potato harvest at the selling price of all products made will be:

$$V_{act} = Bulk\ yield, \text{t} \cdot Selling\ price\ 1 \text{ t} = 12{,}050 \text{ t} \cdot 10{,}400\ rubles = 125{,}320{,}000 \text{ rubles}$$

Let's determine the actual amount of profit as the difference between revenue and costs:

$$Pr = V - C = 125{,}320{,}000 \text{ rubles} - 62{,}653{,}598 \text{ rubles} = 58{,}646{,}302 \text{ rubles}$$

Let's determine the estimated cost of the crop when processing fertilizers with Nutricharge as the sum of the volume of additional products and the cost of the crop at the selling price, we get 13,894,400 rubles + 125,320,000 rubles = 139,214,400 rubles.

We will find a profit when using Nutricharge for the entire sown area of potato:

$$Pr = V - C = 139{,}214{,}400 \text{ rubles} - 62{,}653{,}598 \text{ rubles} - 1{,}680{,}000 \text{ rubles} = 70{,}860{,}702 \text{ rubles}$$

The increase in profit as a percentage of the original variant will be (70,860,702 rubles / 58,646,302 rubles) * 100 - 100 = 20.83%

The estimated additional profit will be equal to 13,894,400 rubles - 1,680,000 rubles = 12,214,400 rubles.

Results of experiment No. 2 with the treatment of fertilizers with Nutricharge and Growgreen

We will carry out the same calculation of indicators as in the previous paragraph. The results are presented in Table 3. Let's determine how the processing of fertilizers with Nutricharge and Growgreen will affect the economic results.

The difference in actual costs per 1 hectare of sown area between control and experiment amounted to 8,750 rubles, of which the cost of Nutricharge amounted to 5,250 rubles, the cost of Growgreen - 3,500 rubles. The change in costs in percentage terms increased by 4.2%.

According to the second experiment, the obtained biological yield exceeds the control data by 237.5 c ha^{-1}, increasing from 554.5 c ha^{-1} to 792 c ha^{-1} (42.83%). At the same time, the yield in the second experiment turned out to be higher than the yield in the first experiment by 154 c ha^{-1}, i.e. by 24.13%.

The conducted field experiment No. 2 and the analysis of the experimental data obtained, gives us reason to say that the use of Nutricharge and Growgreen biopolymers are effective and their scale needs to be increased and distributed throughout the Non-Chernozem zone of Russia.

The production yield in the second experiment was also adjusted by 50% of the biological yield increase, since no measurement was made on the site. The change in the production yield according to experiment in comparison with the control shows an increase of 118.75 c ha^{-1}, which is 31.54%.

Table 3. Data from experiment No. 2 with the processing of ammonium nitrate phosphate fertilizers with Nutricharge and Growgreen for sowing potato in the agricultural complex Collective Farm n.a. Lenin (Ryazan region) in 2020

Indicators	Agricultural complex Collective Farm n.a. Lenin Kasimovsky district	
	Potato	
	control	experiment
Applied on 1 ha on the experimental plot, kg	700	700
Actual costs per 1 ha, rubles	208,355	217,105
Including: Actual cost of fertilizers applied for the crop, including work in progress, rubles	23,532	23,532
Of these: The cost of fertilizers per 1 ha in experiment, rubles	14,000	14,000
The cost of Nutricharge per 1 ha, rubles	0	5,250
The cost of Growgreen per 1 ha, rubles	0	3,500
Total: including the cost of fertilizers, Nutricharge and Growgreen per 1 ha in the experiment	14,000	22,750
Biological yield, c ha^{-1} (Gala variety) (Act)	554.5	792
The difference in the yield of experiments in comparison with the control, (+,-), c ha^{-1}	-	237.5
Change in biological yield in % to control, %	-	142.83
Production yield (adjusted in the experiment by 50% of the increase in biological yield)	376.56	495.31
Increase in production yield, c ha^{-1}	-	118.75
Change in production yield in % to control, %	-	131.54
Change in costs per 1 ha to the control variant, rubles	-	8,750
Change in costs per 1 ha to the control variant, in %	-	104.20
Cost of 1t of production yield, rubles	5,533.09	4,383.20
Change in the cost of 1 t to control, rubles	-	-1,149.89
Change in the cost of 1 t to control, %	-	-20.78
Actual selling price of potato, rubles t^{-1}	10,400	10,400
Estimated revenue from 1 ha, rubles	391,625	515,125
Profit from 1 ha, rubles	183,270	298,020
Increase in profit from 1 ha in comparison with control, %	-	62.61
Increase in profit from 1 ha in comparison with the control, rubles	-	114,750
Profitability, %	87.96	137.27
Change in profitability to control, % (+, -)	-	49.31

The cost of production of 1 ton of potato using Nutricharge and Growgreen decreased by 1,149.89 rubles, which amounted to 20.78%.

With the potato selling price of 10,400 rubles per 1 ton, the estimated revenue from 1 ha for control amounted to 391,625 rubles, according to experiment - 515,125 rubles. The excess in revenue amounted to 123,500 rubles per 1 ha or 31.5%.

In terms of profit, the difference between control and experiment amounted to 114,750 rubles per 1 ha of area, or 62.61%.

The profitability of production showed an increase from 87.96% by control to 137.27% by experiment, i.e. the difference is 49.31%.

The use of Nutricharge and Growgreen drugs showed greater effectiveness in comparison with the use of Nutricharge alone.

For example, the complex use of drugs allowed to reduce the cost of production of 1 ton of potato: in the first experiment it amounted to 5,106.36 rubles, in the second - 4,383.20 rubles (change of 723.16 rubles). The revenue received from the sale of potato according to experiment No. 1 amounted to 435,045 rubles per 1 ha, whereas in experiment No. 2 - 515,125 rubles. The difference is 80,080 rubles per 1 ha. Cost reduction and revenue increase in the second experiment allowed to make a profit from 1 ha of 298,020 rubles, and in the first - 221,440 rubles, i.e. 34.5% more. The difference in profitability is significant: experiment No. 1 - 103.67%, experiment No. 2 - 137.27%, i.e. 33.6%.

We will calculate the economic efficiency indicators of the results of the use of Nutricharge and Growgreen for the entire potato planting area and compare them with the indicators from the first experiment (Table 4).

Table 4. Calculation of the expected results of the use of Nutricharge and Growgreen for the entire area of potato planting and comparison with experiment No. 1

Indicator	Experiment No. 1	Experiment No. 2	Change of indicators
The use of drugs for the entire area of potato planting would allow to obtain additional products, rubles	13,894,400	39,520,000	25,625,600
Additional costs for processing fertilizers for 320 ha (estimated calculation), rubles	1,680,000	2,800,000	1,120,000
As a % of total costs	2.52	4.20	1.7
Calculated cost of the crop when processing fertilizers with drugs, rubles	139,214,400	164,840, 000	25,625,600
Cost of the crop increase, rubles	13,894,400	39,520,000	25,625,600
Calculated net profit when using drugs for the entire planting area, potato, rubles	70,860,702	95,366,302	24,505,600
Net profit increase in % to the original variant	20.83	62.61	41.78
Calculated additional net profit, rubles	12,214,400	36,720,000	24,505,600

Higher yields and lower cost of production according to the second experiment allowed to obtain high indicators of economic efficiency. Thus, the cost of additional potato production according to experiment No. 2 exceeded the additional production volume according to experiment No. 1 by 25,625,600 rubles (almost 3 times). The volume of additional costs in the second experiment increased by 1,120,000 rubles in comparison with the first experiment, which is approximately 67%. Net profit is higher according to experiment No. 2 by 24,505,600 rubles in comparison with the first experiment. The increase in net profit in the second experiment is 41.78% more than in the experiment No. 1.

As is known in the scientific world, as a criterion of the truth and falsity of scientific knowledge, a scientific statement or phenomenon is determined by two positivism principles. The first is the principle of verification (verifiability) of a hypothesis, theory, and the second is the principle of falsification (refutation) of a hypothesis, theory. Everything must be confirmed by economic practice. Following this statement, we decided to check, by conducting scientific field experiments, how promising and effective the actions we are conducting are, as a result of the use of biochemical polymers Nutricharge and Growgreen to increase yields and production efficiency of potato crops in production conditions.

Treatment of ammonium nitrate phosphate fertilizer with Nutricharge during potato cultivation provides a significant improvement in production and economic indicators: potato yield increased from 554.5 c ha^{-1} to 638 c ha^{-1}, which is 15% in percentage terms. There was a decrease in cost by 1,149.89 rubles, which amounted to 20.78%, the excess in revenue was 123,500 rubles from 1 ha or 31.5%, in profit terms, the difference between control and experiment was 114,750 rubles from 1 ha of area or 62.61%. An even more tangible result is obtained with the combined use of processed fertilizers and leaf dressing Growgreen.

In modern conditions, the entire mechanism of agricultural production is aimed at ensuring the economic efficiency of production, often neglecting product quality and environmental safety. In their studies, agricultural scientists I. Ushachev, I. Trubilin, E. Ogloblin, and a number of other authors note that the most promising direction for solving this problem is the use of environmentally friendly mineral fertilizers (Ushachev et al., 2017). These statements cause certain discussions, disputes and discussions of the designated problem. The use of new generation biopolymers 'Nutricharge' and 'Growgreen' in the process of potato production confirms the correctness of this direction.

S. Khromov, R. Lipsky, S. Manzhina, and a number of other specialists involved in the study of the effect of mineral fertilizers on the soil environment, in their studies argue and prove that such an important and rather expensive element of plant nutrition as phosphorus, when it enters the soil in the form of mineral fertilizers, it precipitates into a water-insoluble precipitate and becomes inaccessible to plants. According to various sources, from 75 to 95% of this element is blocked in the soil solution in an insoluble form for the growth and development of crops, and the introduction of ammonium sulfate, a much-needed mineral fertilizer for plants, also causes problems in the digestibility and availability of such elements as nitrogen and sulfur. Processing of mineral fertilizers with biopolymers 'Nutricharge' and 'Growgreen' due to a specially developed microbial production process that repeats natural soil processes, transforms nutrients into a 100% available form for crops (Khromov, 2017; Lipsky, 2014; Manzhina, 2017; 'ARTEL' LLC, 2017). Thus, we can conclude that the treatment of mineral fertilizers with biopolymers 'Nutricharge' and 'Growgreen' increases the productivity of crops, which was proved in the course of our experiments No. 1 and No. 2.

The authors represented by V. Bulgakov, O. Adamchuk, S. Pascuzzi in their studies suggest that the use of biological products based on living beneficial microorganisms, in combination with mineral fertilizers, has a positive effect on the absorption and availability of such an important element of phosphorus for growth and development of plants, increase the beneficial micro flora in the soil (Bulgakov et al., 2021).

Also noteworthy are the studies by O. Chernikova, T. Seregina, Yu. Mozhaisky, S. Buryak, who argue that when using mineral fertilizers and other biological products, it is necessary to take into account a number of technical and operational characteristics of machines and equipment, their acquisition, paying attention to the possibility of using digital settings. These conditions will contribute to the normative-dosed consumption of the applied mineral fertilizers and biological products, to provide the soil environment with the necessary plant nutrients. (Chernikova et al., 2021).

After a comprehensive review and analysis of a scientific statement or phenomenon, everything must be confirmed by economic practice. Following this statement, we decided to check, by conducting scientific field experiments, how promising and effective is the use of biochemical polymers 'Nutricharge' and 'Growgreen' in terms of increasing yields and increasing the production efficiency of potato crops under production conditions.

The results obtained during the study proved that the scientific hypothesis put forward by us, the theory (the use of biochemical polymers Nutricharge and Growgreen for the effective cultivation of Gala potato variety) was true and correct. It has been proven by production and economic practice, by conducting a number of scientific field experiments. This is clearly evidenced by the obtained production and economic indicators of the Gala potato variety cultivation.

CONCLUSIONS

During the research, we determined that the use of biodegradable polymers to improve the absorption of minerals and fertilizers by plants is one of the promising areas. So far, in the agriculture of the Russian Federation, they have not yet been widely introduced and used in the production of agricultural products.

The use and implementation of biochemical polymers, as we see from application technology and the experiments carried out, will contribute to the production of environmentally friendly products, without negative environmental consequences, primarily on the soil and the environment. It is also necessary to note the economic component of the experiment, from economic calculations we see that the use of biochemical polymers will contribute to the growth and increase in crop production, and this affects the indicators of increasing the efficiency of potato production.

According to the obtained economic indicators, using the monographic method of economic research, it can be concluded that the profitability of potato production increases when fertilizers are processed with Nutrichage and Growgreen. If in the first case, the use of only Nutricharge made it possible to obtain an increase in profitability by 15.71%, then in the second case, the increase was already 49.31% compared to the control.

The next conclusion to be noted from our study is that Nutricharge and Growgreen appeared on the Russian market relatively recently - only 4 years; now, after receiving verified data on the effectiveness of their use, the question of promoting this product and introducing the use of a new technology for processing mineral fertilizers for other agricultural producers arises. As it was defined in the introduction, the main feature in agriculture when spreading innovations is that this innovation needs to be spread to hundreds and even thousands of producers

Based on the study, the following directions for the effective development of agribusiness can be outlined:

– distribution and implementation of this innovative tool among agricultural producers, considering the peculiarities of their agro-climatic conditions;

– prospects for the use of Nutricharge and Growgreen in the Non-Chernozem zone of Russia;

– the use of biochemical polymers, together with legumes in the crop rotation system, which will increase the production efficiency of other agricultural crops, and in particular such demanded crops as cereals (winter and spring wheat), grain products, which play a crucial role in the world grain market.

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Increased biogas production from lignocellulosic biomass by soaking in water

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Abstract. Due to its large production worldwide, lignocellulosic biomass represents a substrate with great potential to produce biogas. However, this type of biomass is characterized by a complex and solid structure, which is difficult to decompose by anaerobic microorganisms. Applying the correct pre-treatment method can increase its biodegradability. Lignocellulosic substrate was pre-treated by soaking in water for one day at room temperature to increase biogas production and monitoring of long-term operation of laboratory models of anaerobic reactors for anaerobic digestion of such pre-treated maize waste was employed. Monitoring results in two reactors, R1 with biogas produced from a substrate soaked in water for one day and R0 with the production of biogas from a substrate mixed with water just before dosing into the reactor, were compared showing positive effect of the pre-treatment method. This was expressed by higher values of biogas production and higher methane content in biogas from the substrate soaked in water for one day. The achieved specific biogas productions during four different phases of reactor operation in reactor R1 were in the range of 190–335 mL g^{-1} of VS (volatile solids) and 101–221 mL g^{-1} of VS in reactor R0. Methane content of biogas during reactor operation was 49.3–55.2% in reactor R1 and 42.5–45.5% in reactor R0. During long-term operation of another reactor, pre-treated maize waste was used as a co-substrate for maize silage, in the ratio of 1:1 based on VS of the substrates proving as a suitable co-substrate for maize silage, as the achieved average value of specific biogas production during reactor operation at OLR (organic loading rate) = 1.75 kg VS m^{-3} d^{-1} was 510 mL g^{-1} of VS and during first 67 days at OLR = 2 kg VS m^{-3} d^{-1} it was 454 mL g^{-1} of VS.

Key words: anaerobic digestion, bioenergy, maize waste, methane, pre-treatment.

INTRODUCTION

The use of lignocellulosic biomass as a substrate for biogas production is important for the development of sustainable energy sources due to its large worldwide production. Lignocellulosic biomass includes energy crops, agriculture and forest residues, sewage sludge, animal and food waste, municipal solid waste etc (Abraham et al., 2020). However, due to its complex structure formed mainly by cellulose, hemicellulose, and lignin, it is difficult to produce biogas (Martínez-Gutiérrez, 2018). Factors affecting the recalcitrance of this biomass to the action of anaerobic microorganisms include structural and compositional properties of lignocellulosic biomass, accessible surface area of cellulose, cellulose polymerization and crystallinity, as well as cross-linkages of hemicellulose and lignin (Xu et al., 2019; Yoo et al., 2020).

Application of a pre-treatment method is the key step in the disintegration of its main components and their subsequent conversion to bioenergy (Mankar et al., 2021). Studied pre-treatment methods include various biological, chemical, and physical methods. Biological pre-treatment methods include pre-treatment by fungi (Mustafa et al., 2016; Singh, 2021), bacterial systems (Xu et al., 2018; Shah et al., 2019), and enzymes (Wang et al., 2018a; Abraham et al., 2020).

Chemical pre-treatment methods, characterized by high efficiency, include the use of lime, acid, ionic liquid, steam explosion, sulfur dioxide explosion, ammonia fiber explosion and others (Norrrahim et al., 2021). Mancini et al. (2018) used alkaline pre-treatment of wheat straw with 1.6% (w/w) NaOH at 30 °C for 24 hours, cumulative biomethane production yield was enhanced by 15% compared to the untreated sample. In Sarto et al. (2019), the use of 5% H_2SO_4 for the pre-treatment of water hyacinth for 60 minutes increased biogas production by 132% compared to the untreated substrate. However, when choosing a suitable chemical method, possible high economic costs associated with the price of the chemical agent, and dangerous properties of some chemical substances such as toxicity, flammability or corrosivity have to be considered together with the production of inhibitors of anaerobic processes and toxic waste, the disposal of which can further increase the economic costs of the used pre-treatment method. In the work of Jankovičová et al. (2022a), significant increases in biogas production were achieved even when using chemical agents at lower concentrations, 0.5% NaOH and 0.5% H_2SO_4. Pre-treatment by 0.5% NaOH increased the biogas production by 159% for rapeseed straw, 240% for wheat straw and by 59% for maize waste. However, the use of reagents even at low concentrations increases the costs and as well as the salinity of the digestate.

Physical processes can salso be used to improve the biodegradability of lignocellulosic materials, including mechanical pre-treatment (Dahunsi, 2019), ultrasound (Wang et al., 2012), and microwave radiation (Kaur & Phutela, 2016). In Dell'Omo & La Froscia (2018), an average yield gain of 50% compared to the untreated material was achieved when the particle size of wheat straw was reduced from a fraction of 30 mm to 300 μm and to 1,200 μm. However, energy requirements and high costs make physical methods disadvantageous. The effect of other pre-treatment methods enhancing solubilization and anaerobic biodegradability of lignocellulosic biomass as wheat straw are described in Rahmani et al. (2022).

The goal is to find an effective pre-treatment method employing eco-friendly reagents, low energy consumption and cost-effective operation. Therefore, the aim of this study was to investigate the effect of simple pre-treatment methods, such as soaking the lignocellulosic biomass in water, on the biodegradability and biogas yield. Efficiency of the chosen pre-treatment method for biogas production was monitored during long-term operation of the reactors, providing good insight into the ongoing anaerobic degradation of the pre-treated lignocellulosic substrate. Since long-term monitoring of the process is applied, adaptation of the biomass to the dosed substrate is considered and various parameters of the digestate are monitored to indicate the inhibition of biogas production.

The studied pre-treated lignocellulosic material was one of the most common materials produced in agriculture, i.e. maize waste (also known as maize straw, corn stover). This material is a valuable source of energy because maize production constantly increases and the total mass of these residual parts of the plant (leaves, stalks, husk) is approximately 1.01 kg of dry matter per 1 kg of harvested maize grains (expressed in dry matter) (Mazurkiewicz et al., 2019). The use of such pre-treated maize waste as a co-substrate for maize silage was also investigated, whether the pre-treated maize waste can replace a significant part of the used maize silage in practice, since maize silage is still used as the main substrate for biogas plants in the European Union. However, this is not a long-term sustainable substrate (Hutňan, 2016).

MATERIALS AND METHODS

Substrate and inoculum

The used lignocellulosic substrate was maize waste, which contains various parts of the plant as stalks, leaves, and husks (except grains), as well as post-harvest residues. The plant was harvested at the stage of full physiological maturity and dry, i.e. with high dry matter content, from agricultural fields in Slovakia. It involved harvesting grain maize, and the residues of the plant (maize waste) was used as material in experiments. Furthermore, the material was stored at room temperature with no special drying in laboratory conditions.

Table 1. Characteristics of the used substrates

	TS (g g^{-1})	VS (g g^{-1})
Maize waste	0.94	0.86
Maize silage	0.41	0.39

The dry material was disintegrated into two different fractions. One of the fractions was ground with the particle size of 2 mm and the other was cut with the particle size of 1–3 cm. During the long-term operation of one reactor, maize waste was used as a co-substrate for maize silage, which is one of the most used substrates in biogas plants.

Table 2. Characteristics of the used inoculum

	TS (g L^{-1})	VS (g L^{-1})
Digestate from biogas plant	30.54	20.37
Sludge from wastewater treatment plant	15.32	9.50

During the reactor long-term operation, digestate from biogas plant in Hurbanovo (Slovakia) was used as inoculum. This biogas plant uses maize silage as a substrate. As an inoculum during the long-term operation of the reactors, where only pre-treated maize waste was processed, anaerobic sludge from the wastewater treatment plant Devínska Nová Ves (Slovakia) was used. Characteristics of the used substrates are given in Table 1 and those of the used inoculum are given in Table 2.

Degradation test

Lignocellulosic substrate, maize waste, was pre-treated using two different methods: boiling maize waste in water, and soaking maize waste in water at room temperature. The ratio of solid to liquid was 1 g of dry maize waste: 50 mL of water in both cases. Particle size of the used substrate was 1 cm. COD (chemical oxygen demand) of initial material was 1.108 g g^{-1}.

During pre-treatment by boiling the substrate in water (95 ± 5 °C), a sample of the liquid portion was taken every 15 minutes for 1 hour and its COD was determined. During pre-treatment by soaking the substrate in water (22 ± 2 °C), a sample of the liquid portion was taken at certain time intervals for 10 days to determine the value of COD of the liquid phase.

To determine the solubility of the material, the parameter degree of solubilization was used according to Penaud et al. (1999). The measurements were performed three times and the results show the average values of the degrees of solubilization obtained from these measurements. The degree of solubilization was calculated according to equation:

$$degree\ of\ solubilization = \frac{COD_{released}}{COD_{initial}} . 100\ (\%) \qquad (1)$$

where $COD_{released}$ is COD released from 1 g of maize waste in the liquid phase sample taken at certain time intervals during pre-treatment; $COD_{initial}$ is COD of 1 g of untreated maize waste.

Monitoring of long-term operation of model of anaerobic reactor

The device for monitoring long-term operation of the reactor consists of a closed reactor vessel with an opening for dosing the substrate and with a small opening with a tube connected to a device for measuring the produced biogas, a heating device, and a stirrer with adjustable speed.

Two reactors (R1, R0) for anaerobic digestion of pre-treated maize waste were performed under mesophilic conditions (37 °C) in semi-continuous reactors with a working volume of 1.4 L. As a daily dosed substrate to reactor R1, maize waste pre-treated by soaking in water for one day was used. And as daily dosed substrate to reactor R0, maize waste mixed with water just before dosing into the reactor was used.

Reactors were operated in four different phases, during which the organic loading rates (OLR) of the reactors and the particle size of used substrate changed. Characteristics of the operating phases of the reactors, i.e. the duration of the phases, changing OLR, and the particle size of the used substrate are shown in Table 3. Thus, in phases I and II, the ground fraction of the substrate was used and in phases III and IV, cut fraction of substrate was used.

Table 3. Characteristics of operating phases of reactors R1 and R0

Phase	Duration (days)	Particle size of substrate	organic loading rate (kg VS m^{-3} d^{-1})
I	0–31	2 mm	0.62
II	32–80	2 mm	1.24
III	81–145	1–3 cm	1.24
IV	146–189	1–3 cm	1.24

In the first three phases, the substrate was dosed to reactors R1 and R0 together with water in which it was soaked. In phase IV, only the substrate itself was dosed into reactor R1, i.e. without soaking water, to achieve a similar water content of maize waste as that of maize silage, so that the pre-treated maize waste could be used in practice as a suitable co-substrate for maize silage.

To evaluate the suitability of maize waste pre-treated by soaking in water for one day as a co-substrate for maize silage, long-term operation of the anaerobic reactor was monitored, where anaerobic digestion of pre-treated maize waste as a co-substrate for maize silage was performed under mesophilic conditions (37 °C) in a semi-continuous reactor with the working volume of 15 L. Particle size of the pre-treated maize waste was 1–3 cm and the substrate soaked in water for one day was dosed into the reactor without the soaking water. The reactor was started-up only by dosing maize silage and gradually increasing the OLR up to 1.75 kg VS m^{-3} d^{-1}, and it was operated at two different values of organic loading rate: 1.75 kg VS m^{-3} d^{-1} for 154 days and 2 kg VS m^{-3} d^{-1} for 84 days. These conditions bring the operation of the model closer to the OLR at real biogas plants. Maize waste pre-treated by soaking in water for one day was used as a co-substrate for maize silage in the ratio of 1:1 based on VS of the substrates.

During long-term operations of the anaerobic reactors, substrates were dosed daily and biogas production was monitored. Once a week, parameters of excess digestate such as pH, total solids (TS), and volatile solids (VS) were determined, together with parameters such as chemical oxygen demand (COD), concentration of ammonia nitrogen ($N\text{-}NH_4$), phosphate phosphorus ($P\text{-}PO_4$), and volatile fatty acids (VFA), which were determined from the filtered digestate sample. Methane content of biogas was determined also in each monitored phase.

Analytical methods

Parameters such as chemical oxygen demand (COD), ammonia nitrogen ($N\text{-}NH_4$), phosphate phosphorus ($P\text{-}PO_4$), volatile fatty acids (VFA), total solids (TS) and volatile solids (VS) were determined according to APHA, AWWA, WEF (2017); pH values were determined using pH meter Hach HQ11d. Biogas composition was measured by gas analyzer GA 2000 Plus (Geotechnical Instruments, UK).

RESULTS AND DISCUSSION

Degradation test

Regarding the economic difficulty of pre-treatment by boiling in water (heat consumption), the possibility of soaking in water at room temperature was tested. In the degradability test, both pre-treatment methods of lignocellulosic biomass were compared based on their degree of solubilization.

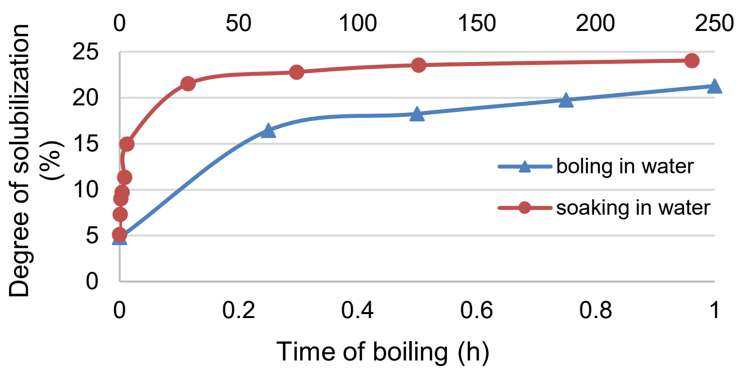

Figure 1. Course of the degree of solubilization during maize waste pre-treatment.

From the courses of the degree of solubilization during the pre-treatment shown in Fig. 1, a comparable course of the degree of solubilization in the selected methods is evident. It is obvious that degradability of the material increased with the increasing pre-treatment time for both chosen methods, as the values of the degree of solubilization increased with pre-treatment time of both methods.

The fastest increase in the solubilization degree in case of boiling in water was observed in the first 15 min, when the achieved value was 16.5%. After boiling maize waste in water for 1 hour, the degree of solubilization was 21.3%.

Of course, soaking maize waste in water at room temperature requires much longer pre-treatment time. The most significant solubilization of the material was observed in the first days of soaking. After approximately 1 day (29 hours) of soaking, the degree of solubilization in water was 21.5%. With longer time, the material decomposed gradually at much lower speed. After 10 days of soaking, the degree of solubilization was measured to be 24.1%.

Similar degrees of solubilization on carbon basis (approximately 25%) were achieved by Rogalinski et al. (2008), where the liquid hot water method (100 bar, 190 °C, 12 min) in combination with other methods was used for the pre-treatment of rye straw. However, the yield of unwanted degradation products, as HMF and furfural, increased with the treatment severity.

As soaking the material at room temperature for one day resulted in the same solubilization degree as when boiling the material for 1 hour, the method of soaking lignocellulosic biomass in water at room temperature was used in further experiments. This method does not have high energy demand and therefore it represents a simpler and more advantageous method in practice.

Long-term operation of anaerobic reactors for anaerobic digestion of pre-treated maize waste

The effect of the selected pre-treatment method, soaking maize waste in water for one day, was observed by monitoring biogas production in reactor R1 during long-term operation. To assess whether this chosen method has a positive effect on biogas production, the long-term operation of reactor R0 was also monitored, where the dosed maize waste was mixed with water just before its dosing into the reactor. Monitoring results are presented as cumulative biogas production in Figs 2–5, which are divided into individual graphs for each phase of operation for better representation of the differences in biogas production for reactors R1 and R0. Average value of cumulative biogas production, daily biogas production, specific biogas production and methane content of produced biogas are reported in Table 4.

Table 4. Results of long-term operation of reactors R1 and R0

Phase	Cumulative biogas production (L)		Daily biogas production (L d^{-1})		Specific biogas production (L g^{-1} VS)		Methane content (%)	
	R1	R0	R1	R0	R1	R0	R1	R0
I	6.73	2.81	0.166	0.086	0.192	0.101	49.3	42.5
II	41.29	19.21	0.597	0.308	0.335	0.173	49.3	42.5
III	61.24	23.07	0.515	0.261	0.296	0.150	55.2	43.7
IV	17.90	22.36	0.503	0.383	0.289	0.221	51.1	45.5

The initial phase of operation of the reactors (at OLR = 0.62 kg VS m^{-3} d^{-1}) lasted 32 days. During the first days, the inoculum in the reactors adapted to the used substrates, which can be seen in the almost zero biogas production in Fig. 2. It is clear from the previous results that the pre-treatment by soaking maize waste in water causes solubilization of the material and the release of the organic part of the solid material into the solution thus simplifying the availability of substances necessary for anaerobic digestion. A significantly positive effect was observed when using the substrate soaked in water for one day (R1) compared to the substrate mixed with water immediately before its dosing into the reactor (R0). Biogas production from the substrate soaked for one day was by about 140% higher compared to the substrate in reactor R0.

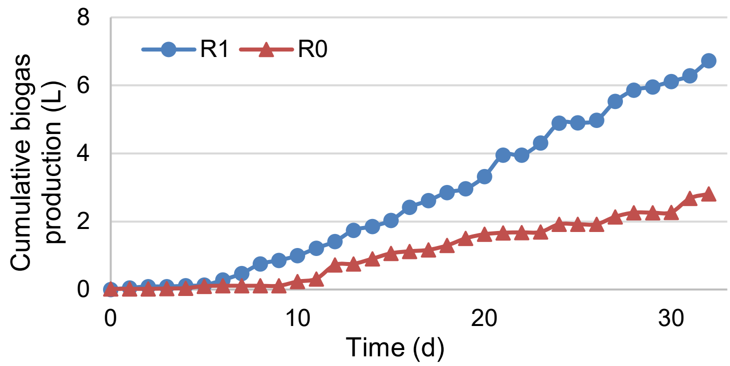

Figure 2. Cumulative biogas production during phase I of long-term operation of reactors R1 and R0.

After 32 days of operation, OLR in the reactors was doubled (1.24 kg VS m^{-3} d^{-1}). Pre-treatment conditions remained unchanged. Similar to the lower OLR, an almost 120% increase in biogas production was observed for the one-day-soaked substrate. When comparing the values of specific biogas production, 0.335 L g^{-1} VS for R1 and 0.173 L g^{-1} VS for R0, an increase of 94% was observed.

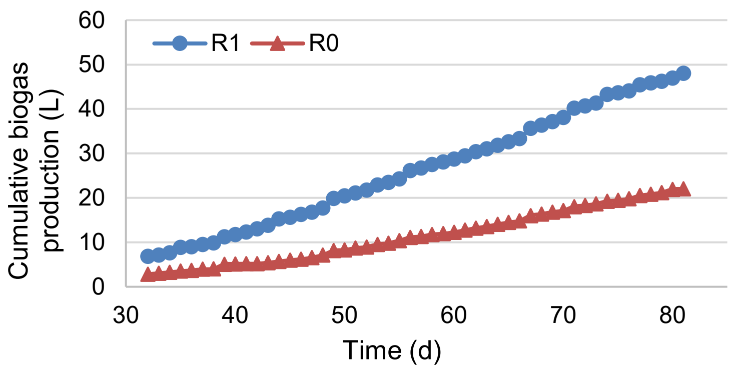

Figure 3. Cumulative biogas production during phase II of long-term operation of reactors R1 and R0.

Although the fraction with smaller particle size has increased accessible surface area and porosity of the particles, the process of mechanical disintegration of the material is energetically and economically demanding (Hernández-Beltrán et al., 2019). Due to the difficulty of maize waste pre-treatment by disintegration and to compare the effect of material disintegration on biogas production, the cut fraction of maize waste (1–3 cm) was used in phase III. A slight decrease in daily biogas production was observed compared to phase II. The use of a larger fraction of the lignocellulosic substrate decreased the value of specific biogas production in phase III by only 13% for R1 and by 15% for R0 compared to phase II, where a smaller fraction was used. However, the increase in specific biogas production in R1 (0.296 L g^{-1} VS) compared to R0 (0.150 L g^{-1} VS) is still quite high, up to 98%.

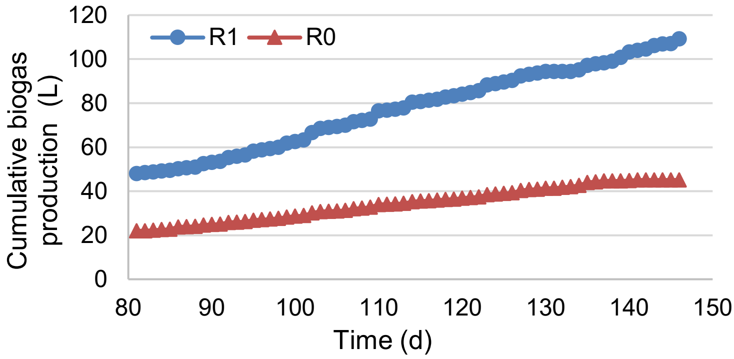

Figure 4. Cumulative biogas production during phase III of long-term operation of reactors R1 and R0.

In the last phase, when only the solid phase of the substrate soaked in water for one day was dosed into reactor R1, the water content of the substrate (80–85%) was comparable to that of maize silage (about 70%) (Şenol et al., 2020). The use of solid substrate without soaking water in phase IV was done to achieve water content of maize waste similar to that of maize silage to use pre-treated maize waste as a suitable co-substrate for maize silage. Thus, in phase IV, the closest approach to real conditions was used. Also, when dosing substrate with soaking water into the reactor, HRT (hydraulic retention time) decreased, which is a disadvantage. Even when processing maize silage at biogas plants, which has even higher TS than soaked maize waste without soaking water, no technological problems were observed. From the achieved value of SBP, 0.289 L g^{-1} VS, in phase IV for reactor R1, it is clear that part of well degradable organic matter available for biogas production was released into the soaking water; however, this amount is small. Also, in full scale conditions, soaking water would not be disposed of but reused for soaking another batch of substrate (with the necessary addition of fresh water). Organic substances released into the soaking water would therefore be present when soaking the following new batch of substrate the next days.

Gradual dosing of only the solid phase into reactor R1 had an inhibitory effect. The substrate was more difficult to decompose, which can also be seen in the qualitative parameters of digestate and liquid phase of digestate. Slowdown in the biogas production rate, as seen in Fig. 5, led to the shut-down of the operation of R1.

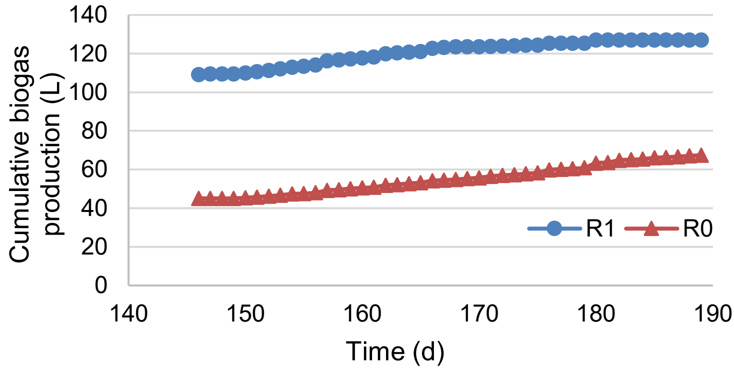

Figure 5. Cumulative biogas production during phase IV of long-term operation of reactors R1 and R0.

As it can be seen in Table 4, higher methane content of biogas was achieved in reactor R1 compared to reactor R0 in each phase of operation. The table shows average values of methane content during the phases; however, at the end of phase IV, the methane content in reactor R1 was only 45.2%.

Monitoring of digestate and liquid phase of digestate water parameters showed that the reactors were operated at a neutral pH value, i.e. at pH values of 6.7–7.5. Concentrations of N and P were sufficient for the COD:N:P ratio to be considered suitable for anaerobic digestion, COD:N:P = 500:7:1 (Gil et al., 2019). When maize waste is used as a mono-substrate for biogas production, deficiency of selected micronutrients cannot be excluded. As stated in the work of Lebuhn et al. (2014), synergistic detrimental effects on biocenosis cannot be excluded if several trace elements concomitantly approach limiting levels at anaerobic treatment of maize silage. However, as sludge from the municipal wastewater treatment plant was used here as inoculum in reactors R1 and R0, it is assumed that no micronutrient deficiency occurred during the experiments and no additional addition was done during the experiment. However, this can be a problem in long-term use in practice.

The change in operating parameters of the reactors such as OLR and the size of the substrate particles affect mainly the change of parameters such as COD (Fig. 6) and VFA (Fig. 7). The OLR increase in phase II led to an almost double increase in COD. In R1, the average value of COD in phase I was 540 mg L^{-1} and in phase II it increased to 1,139 mg L^{-1}. A similar increase in COD also occurred in R0, from 566 mg L^{-1} to 915 mg L^{-1}. VFA concentration varied during phases I and II in similar values for both reactors, i.e. between 100-200 mg L^{-1}. By changing the size of the substrate particles in phase III, the material decomposed more slowly and accumulated in the reactors, which was also reflected in the COD values, which increased by 80% in R1 and 110% in R0. Average VFA concentration after changing the size of the substrate particles also increased to 426 mg L^{-1} (R1) and 433 mg L^{-1} (R0), which represents an increase of 156% and 168%. During phase IV, concentrations of COD and VFA continued to increase but a more visible increase was observed in R1 when only the solid phase of the one-day-soaked substrate was used. Gradual densification of the reactor volume of R1 led to problems with operation and its subsequent termination.

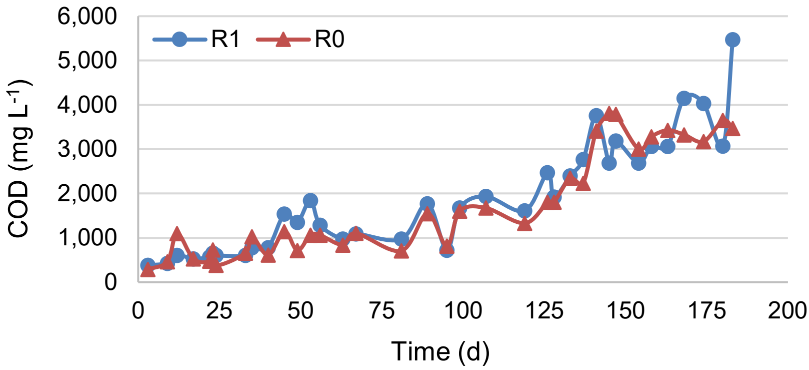

Figure 6. Variation of COD concentration during operation of reactors R1 and R0.

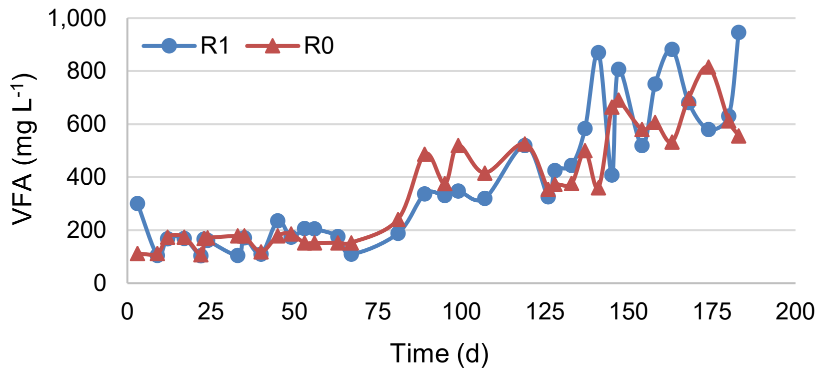

Figure 7. Variation of VFA concentration during operation of reactors R1 and R0.

Average values of digestate parameters as TS and VS did not change significantly during individual phases (Table 5). By increasing OLR and the particle size of the substrate, the substrate was more difficult to decompose and a part of the material remained undecomposed in the reactor, which was reflected in the change in the composition of the collected digestate, which contained more rigid undissolved parts. The solid undissolved material apparently settled at the bottom of the reactor, which also causes occasional jamming of the stirrer. Towards the end of phase IV in reactor R1, TS values reached up to 23–24 g L^{-1}.

Table 5. Average values of TS a VS of digestate during operation of reactors R1 and R0

Phase	TS (g L^{-1})		VS (g L^{-1})	
	R1	R0	R1	R0
I	14.1	12.7	9.1	8.3
II	17.4	15.2	12.3	10.1
III	16.4	18.2	10.9	12.1
IV	19.5	18.6	15.1	14.9

One of the reasons for substrate accumulation in the reactor was the technical limitation of the reactor model, where the only possible method of digestate removal from the reactor - from the reactor surface, contributed to the constant increase in the digestate density since the more liquid part of the digestate was removed.

Since in phase IV, only the solid part of the substrate was loaded to reactor R1 and the solid and liquid part was dosed to rector R0, more digestate was taken from reactor R0 than from R1. This increased OLR in R1. The undecomposed maize waste was also removed from R0, but this undecomposed material remained in R1, which significantly increased the retention time so that it could decompose more deeply. Thus, the collapse of R1 could be caused by a substantial extension of the retention time, when the accumulated substrate could begin to decompose. However, mainly the phases where hydrolysis and acidogenesis of anaerobic decomposition took place, which was manifested by an increase in VFA.

Positive effect of soaking lignocellulosic biomass in water at room temperature for one day was demonstrated as higher biogas production in reactor R1 in each phase of operation. Wang et al. (2018b) pre-treated rice straw hydrothermally at different temperatures (90 °C, 150 °C, 180 °C and 210 °C) achieving biogas yield increase of only 3% compared to untreated rice straw. Even a 30% decrease in biogas yield at 210 °C was observed as severe inhibitions appeared at higher pre-treatment temperatures.

Long-term operation of anaerobic reactor for anaerobic digestion of pre-treated maize waste as co-substrate for maize silage

Results of long-term operation of the reactor for anaerobic digestion of such pre-treated maize waste as co-substrate for maize silage are shown as the course of SBP (specific biogas production) (Fig. 8) during the operation of this reactor at two different values of OLR.

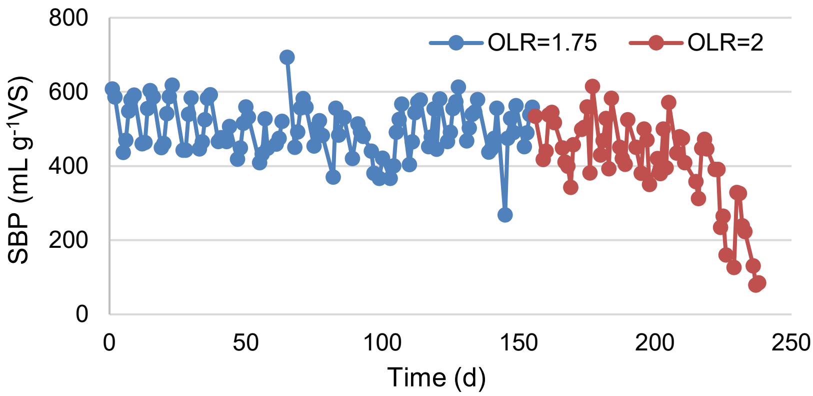

Figure 8. Specific biogas production during long-term operation of the reactor.

At OLR of 1.75 kg VS m^{-3} d^{-1}, anaerobic digestion of these co-substrates was characterized by stable operation. Average value of daily biogas production was 13.4 L d^{-1} and that of specific biogas production was 510 mL g^{-1} VS. Methane content of biogas was 54%. Specific biogas production has proven the suitability of using such pre-treated maize waste as co-substrate for maize silage. Specific biogas production during long-term operation of the reactor for mono-digestion of maize silage given in literature ranges from 479 mL g^{-1} VS (Jankovičová et al., 2022b) up to the maximum of 655 mL g^{-1} VS in Hutňan et al. (2010).

At OLR of 2 kg VS m^{-3} d^{-1}, the reactor operation was relatively stable for the first 67 days, then operational problems occurred, which further led to the termination of monitoring due to reactor overload, as it can be seen in Fig. 8. During the first 67 days, average value of daily biogas production was 13.6 L d^{-1} and that of specific biogas production was 454 mL g^{-1} VS. Methane content of biogas was also lower in comparison with that obtained at a lower OLR, namely 51%.

Performance of the anaerobic digestion process was also monitored via the changes in digestate liquid phase (filtered sample) parameters. At OLR of 1.75 kg VS m^{-3} d^{-1}, COD values ranged between 2,000–4,000 mg L^{-1}, N-NH_4 between 200–400 mg L^{-1}, and P-PO_4 was in the range of 20–50 mg L^{-1}. At OLR of 2 kg VS m^{-3} d^{-1}, COD values ranged between 3,000–5,000 mg L^{-1}, N-NH_4 was in the range of 300–600 mg L^{-1}, and P-PO_4 in the range of 25–70 mg L^{-1}. Therefore, N and P concentrations were sufficient for the COD:N:P ratio to be suitable for anaerobic digestion. Even in this case, micronutrients were not supplied since the digestate from the biogas plant, used as the inoculum in this reactor, supplies these micronutrients during the maize silage processing.

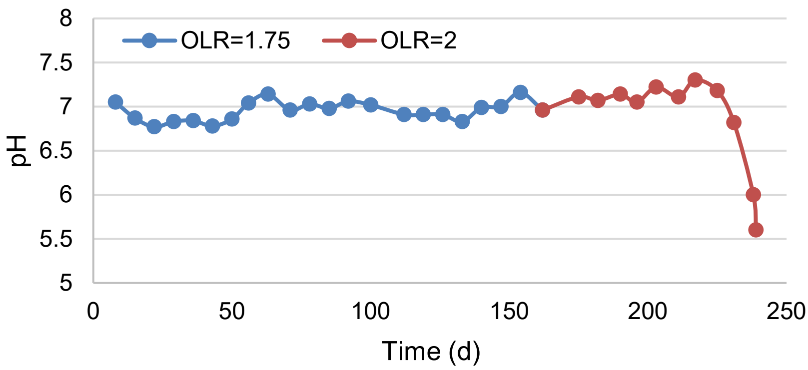

Figure 9. Variation of pH during long-term reactor operation.

For proper process operation, the optimum operating pH range for maximum methanogens growth is above 6.8 (Björnsson et al., 2000; Azizan et al., 2021). Fig. 9 shows pH changes during the reactor operation, while at OLR = 1.75 kg VS m^{-3} d^{-1} there were no significant changes in pH, at OLR = 2 kg VS m^{-3} d^{-1}, pH began to decrease from approximately the 220th day of operation - i.e. similar to the observed decrease in biogas production. The achieved low pH values, up to 5.6 when the reactor operation stopped, thus indicated reactor overload. Similarly as in Braz et al. (2019), pH values below 6 were observed after organic overload. Drastic pH drop was caused by the accumulation of VFAs, acidogenic and acetogenic bacteria, which reflects kinetics imbalance between acid production and consumption rate. Over-acidification/souring problem is the most common issue in the anaerobic digestion process (Alavi-Borazjani et al., 2020). Rapid accumulation of VFAs is shown in Fig. 10.

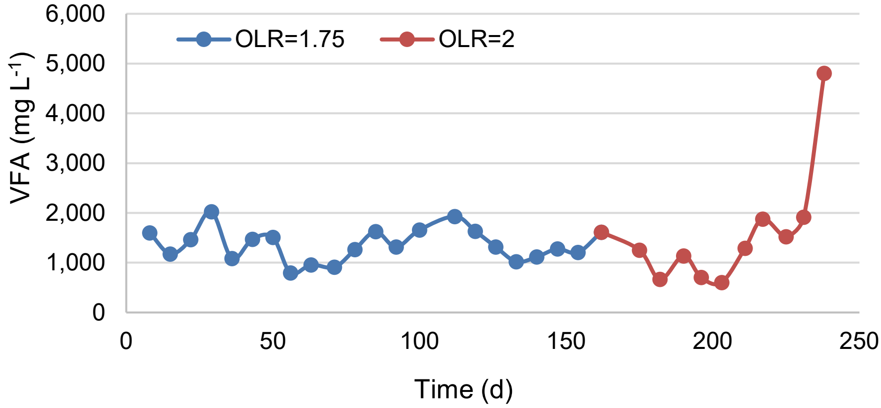

Figure 10. Variation of VFA concentration during long-term reactor operation.

Reactor overload can be caused by the accumulation of substrate at the bottom of the reactor. Accumulation of undecomposed material is also confirmed by the increase in TS and VS values of the digestate shown in Fig. 11.

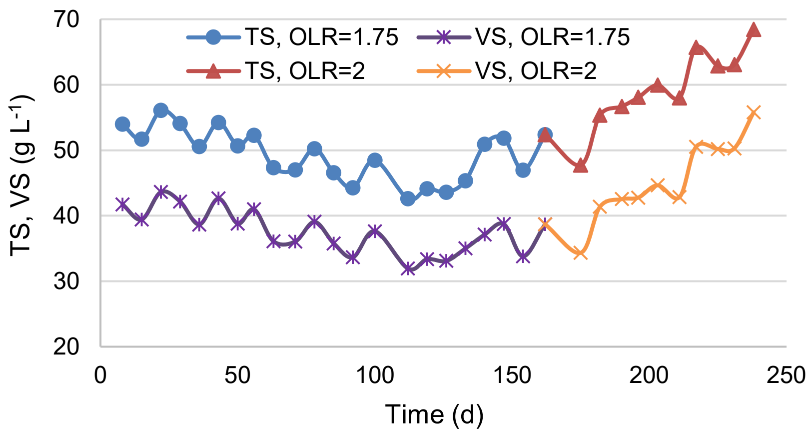

Figure 11. Variation of TS and VS concentration during long-term reactor operation.

CONCLUSIONS

The studied pre-treatment method, soaking lignocellulosic biomass at room temperature in water for one day, has been proven to have great potential in increasing biogas production from lignocellulosic materials such as maize waste. Higher values of cumulative biogas production, daily biogas production, specific biogas production, and higher methane content in biogas prove that pre-treatment of maize waste by soaking in water for one day has a positive effect on biogas production compared to maize waste mixed with water just before its dosing into the reactor. Based on the achieved average value of specific biogas production during the reactor operation at OLR of 1.75 kg VS m^{-3} d^{-1}, 510 mL g^{-1} VS, maize waste pre-treated by this method has been found to be suitable co-substrate for maize silage. Also during the first 67 days of reactor operation at OLR of 2 kg VS m^{-3} d^{-1}, sufficiently high average value of specific biogas production was achieved (454 mL g^{-1} VS), but at this higher OLR, technological problems associated with reactor overload occurred.

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Impact of some herbicides on the growth and the yield of common vetch (*Vicia sativa* L.)

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Abstract. The production and use of common vetch has great potential, but the lack of approved pesticides makes the success of cultivation difficult and unpredictable. The research was carried out on sandy soil at the Nyíregyháza Research Institute of the University of Debrecen in Hungary in April 2022. Five different herbicides, pendimethalin, metolachlor, flumioxazin, bentazon in doses 5 L ha^{-1}, 1.4 L ha^{-1}, 0.06 kg ha^{-1}, 2 L ha^{-1}, respectively, and imazamox in four different doses (0.6, 0.8, 1.0, 1.2 L ha^{-1}) were applied with the consideration of the ranges specified in the Hungarian legislation. The results showed that the highest NDVI (Normalized Difference Vegetation Index) values were obtained with flumioxazin compared to the other treatments. The maximum harvested seed yield (194.1 kg ha^{-1}) was obtained with the application of flumioxazin compared to the control treatment (132.5 kg ha^{-1}). Flumioxazin was also the best as it had the fewest weeds per plot and the lowest phytotoxicity score. Although pendimethalin approached the cleaned and harvested average seed yield of the control plots with 121.3 kg ha^{-1}, it did not feature prominently in the other indicators. With regard to plant trash after cleaning of vetch seed, the highest plant trash (179.4 kg ha^{-1}) was obtained with bentazon. Pendimethalin had the highest percentage ratio between seed yield ha^{-1} and plant trash ha^{-1} (61.8%), followed by flumioxazin (60.7%). The results also showed that there was a positive correlation between NDVI values and seed yield and a positive correlation between NDVI values and plant trash, while there was a negative correlation at the 0.01 level between NDVI values and phytotoxicity.

Key words: flumioxazin, NDVI values, phytotoxicity, weeds, pre-emergent, post-emergent.

INTRODUCTION

The historical background of vetch (*Vicia sp.*) goes back to ancient times. In the 1960s, common vetch (*Vicia sativa* L.) was grown on 75,000 hectares in Hungary. The growing area gradually decreased year by year, and in 2017, 25 hectares with a production amount of 12 tons were documented in Hungary, while in the world the cultivated area of vetch was 353,630 hectares, and the production amount was 711,203 tons in 2020 (FAO, 2022). According to FAO, more than half of total production of vetch species was harvested in Europe between the years 1994 and 2016 (Kutasy, 2019). Based on Hungarian experience, common vetch adapts well to field and crop year extremes. In terms of the pH value of the soil, it can be successfully grown between 4.5–8.2 (Molnár, 2019). The production area of vetch in Hungary correlated with the change in the livestock population, i.e. it decreased. On the positive side, several domestic sources state that it can be grown everywhere in our country. Optimal soils for cultivation are those with medium compaction and humic soils, as well as soils supplied with sufficient nutrients, which is the case in Hungary (Zsombik, 2019). The *Vicia sativa* is usable in many ways, like animal fodder or greening plants, it is one of the leguminous plants, that are able to increase the soil nitrogen content, as well as for human consumption in some part of the world, because it is a cheap substitute of lens (Islam et al., 2003). However, the lack of chemical control and the absence of authorized pesticides may discourage farmers from growing vetch. The active ingredient imazamox is currently allowed to use on Hungarian vetch (*Vicia pannonica* L.). Imazamox is an imidazolinone herbicide used to control weeds in legumes (Bukun et al., 2012). Common vetch is often grown with supporting plants, such as oats (*Avena sativa*), and in this case it is able to suppress weeds better, but in this case, the strong growth of oats may have a negative effect on the yield of the common vetch and other positive effects of the plant (nitrogen fixation) are not as dominant. The diversity, effects and its usability of the plant as well as its historical background, justify the development of plant protection technology for common vetch in Hungary. During our research, we used the experience found in the international literature as a basis for the selection of pesticides. When searching for herbicides, it was a difficulty that most of the literature considers vetch as a weed (Ahmad et al., 1984). Several studies referred to pesticides that were not or no longer on the market in Hungary. It is difficult to find post-emergence herbicides that are effective and do not damage the crop. In the case of common vetch, Balyan & Malik (1991) observed a decrease in dry matter and seed yield with the use of methabenzthiazuron and 2,4-D (2,4-dichlorophenoxyacetic acid). In addition to examining phytotoxicity to common vetch, an important factor was the effectiveness of the given active ingredients in inhibiting the weed species found in the plots. Pendimethalin was phytotoxic to common vetch in a three-year field trial in Spain. In two of the years studied, the active substance reduced the yield of vetch (Caballero et al., 1995). Vasilakoglou et al. (2013) examined the response of several leguminous plants to herbicides. S-metolachlor, pendimethalin and flumioxazin were applied pre–emergence, while imazamox was applied post-emergence in several doses. The results showed that pendimethalin, metolachlor and flumioxazin did not cause phytotoxic effects during the first year of the trial. Pendimethalin at a dose of 1.98 kg ha^{-1} and the active ingredient Metolachlor + terbuthylazine combination at both doses (0.94 + 0.56 and 1.25 + 0.75 kg ha^{-1}) were both effective against the *Chenopodium album* L. Imazamox at a dose of 0.03–0.04 kg ha^{-1} can also be used as an early post-emergence herbicide in common vetch (Vasilakoglou et al., 2013). Two doses of imazamox (0.014 and 0.028 kg 0.4 ha^{-1}) alone and two doses in combination with bentazon (0.11 kg) were applied. Imazamox treatments, including the combination of imazamox and bentazon (0.014 + 0.11 kg 0.4 ha^{-1}), caused significant phytotoxicity in common vetch. Seed yield and germination percentage were similar to the control, but the rate of phytotoxic symptoms ranged from 33–80% (Hinds-Cook et al., 2009). This research could be used to demonstrate that applied sciences are extremely important in life due to their numerous current and past applications (Abido & Zsombik, 2018).

MATERIAL AND METHODS

Study area and experimental design

Our research work was carried out in 2022 April on the sandy soil of the Research Institute of Nyíregyháza, IAREF, University of Debrecen in Hungary. The soil of the experiment is humic sandy soil. The soil pH of our experiment was 7.46. The soil plasticity according to Arany was 26, the amount of water soluble total salt (m/m) was less than 0.02%. The carbonated lime content (m/m) was 0.844%, and the organic carbon content (m/m) was 1.11%. The amount of phosphorus pentoxide was 440 mg kg^{-1}, potassium oxide 252 mg kg^{-1}. The forecrop of the experiment was maize. Common vetch (*Vicia sativa* L.) was sown at the beginning of April 2022 with a seed rate of 138 kg ha^{-1}. The size of each plot was 8.5 m^2. Five different herbicides were used, including imazamox at four different doses. Four replicates were used for each treatment. Herbicide doses were chosen based on application rates already allowed for other crops under Hungarian legislation. Where ranges were given for doses, the mean doses were used. The imazamox was applied in different doses. This ingredient is approved for several cultivars similar to common vetch, e.g. Hungarian vetch (*Vicia pannonica* L.). We have taken into consideration the recommended dose for this species. The common vetch is sensitive to herbicides and there has no herbicide approved in Hungary, yet, therefore we also used lower doses. Table 1 shows the active substances included in the experiment, their dosage and the time of application. The experiment was set up with a Randomized Complete Block Design (RCBD). The herbicides were applied pre-emergent and post–emergent. The pre-emergent and post-emergent treatments were carried out using a Stihl branded Sg 71 type back sprayer. The aim of our research was to investigate the effect of some herbicides on the productivity and weed conditions of common vetch (*Vicia sativa* L.). To monitor plant development, we used the BBCH scale based on UPOV (2012). During the breeding season, the meteorological data were recorded by a Micro Metos 2002 meteorological station.

MEASUREMENTS

Normalized Difference Vegetation Index (NDVI) data collection

Normalized differential vegetation index data were measured twelve times weekly from April 21 to July 7 using by Trimble GreenSeeker HCS-100 hand-held instrument. Two measurements were performed per plot each time. The obtained NDVI values are related to the relative chlorophyll content, the closer the value is to 1, the higher the chlorophyll content (Hatala, 2012). It is important to note that during the measurements, the instrument does not only measure the values of the indirect photosynthetic activity of common vetch (*Vicia sativa* L.), but also the weed values. NDVI values also reflect the phytotoxic effect caused by herbicides on plants.

Table 1. Common name, trade name, chemical name, time of application and rate of application of herbicides under study (Nyíregyháza, 2022)

Common name	Trade name	Chemical name	Time of application	Rate of application
Pendimethalin	Stomp 330 EC	N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine	pre-emergence	5.0 L ha^{-1}
Metolachlor	Dual Gold 960 EC	2-chloro-N-(2-ethyl-6-methylphenyl)-N-(1-methoxypropan-2-yl)	pre-emergence	1.4 L ha^{-1}
Flumioxazin	Pledge 50 WP	2-[7-fluoro-3,4-dihydro- 3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione	pre-emergence	0.06 kg ha^{-1}
Bentazon	Basagran 480 SL	1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide substituted by an isopropyl group at position 3	post- emergence	2 L ha^{-1}
Imazamox	Pulsar 40 SL	(2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5- oxo-1H-imidazol-2-yl]-5-(methoxymethl)-3- pyridinecarboxylic acid	post- emergence	0.6 L ha^{-1}
Imazamox	Pulsar 40 SL	(2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5- oxo-1H-imidazol-2-yl]-5-(methoxymethl)-3- pyridinecarboxylic acid	post- emergence	0.8 L ha^{-1}
Imazamox	Pulsar 40 SL	(2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5- oxo-1H-imidazol-2-yl]-5-(methoxymethl)-3- pyridinecarboxylic acid	post- emergence	1.0 L ha^{-1}
Imazamox	Pulsar 40 SL	(2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5- oxo-1H-imidazol-2-yl]-5-(methoxymethl)-3- pyridinecarboxylic acid	post- emergence	1.2 L ha^{-1}
Control	-	-	-	-

Phytotoxicity data based on the Dancza (2004) herbicide testing methodology

Phytotoxicity values were also recorded continuously in accordance with the herbicide methodology. The phytotoxicity measurements took place from May 3 to July 7 (9 times). Phytotoxicity was determined based on Dancza (2004) (Table 2), however, the statistical software could not properly handle the % values determined based on this, so for a successful test, an individual ratio had to be prepared.

Table 2. Phytotoxicity percentage and degree of damage according to (Dancza, 2004) (Nyíregyháza, 2022)

Phytotoxicity (%)	Scale values	Degree of damage
0	1	symptom free
1	2	very mild symptom
2	3	mild symptom
5	4	definite symptom
10	5	damaged
25	6	strong damage
50	7	serious damage
75	8	very serious damage
100	9	extinct

Weed measurements

In the herbicide trial, the 50×50 cm sampling frame was used with one replicate per plot, and weed species monitoring and tracking on the plot was also continuous. From the beginning of May to the beginning of June, we recorded the different weed species and their number per plot using a 50×50 cm sampling frame. Weed surveys were continued until the foliage closed (June 10). The following weeds were found: Annual ragweed (*Ambrosia artemisiifolia* L.), White goosefoot (*Chenopodium album* L.), Puruagrass, (*Bolboschoenus maritimus* L.), Common knotgrass (*Polygonum aviculare* L.), Wild radish (*Raphanus raphanistrum* L.), Redshank (*Persicaria maculosa* L.), Wild oat (*Avena fatua* L.), Field horsetail (*Equisetum arvense* L.), Hemp (*Cannabis sativa* L.), Redroot pigweet (*Amaranthus retroflexus* L.), Field pennycress (*Thlaspi arvense* L.), Bindweed (*Convolvulus arvensis* L.), Common chichkweed (*Stellaria media* L.), Giant sumpweed *(Iva xanthiifolia* L.), Scentless mayweed (*Matricaria inodora* L.), Shepherd's purse (*Capsella bursa-pastoris* L.). The number of plants for each weed species recorded during the observation times was added and then averaged in the MS Excel program.

Harvesting and post-harvest data collection

The vetch was desiccated with diquat-dibromid active ingredient on July 20 in order to facilitate the harvest, which took place on August 4. 120 days after sowing, all the plants in each plot (8.5m^{-2}) were harvested and air-dried, then threshed, standardizing the amount of the crop to 13% moisture content based on the measured amount. The harvested seeds were cleaned using a Kamas Westrup lab cleaning machine, the size and opening of the sieves used for cleaning were as follows: the upper sieve is 3.75 mm round, the lower sieve is 3.5 mm round. The grain cleaner fractionated grains of different sizes and waste with the help of sieves of different sizes and cleaning air. During the test, we determined the mass of clean grains and plant trash.

Statistical analysis

The basic data was recorded using the MS Excel program (NDVI and phytotoxicity values, degree of weed infestation, waste, seed weight). Descriptive statistical analysis, homogeneity analysis and one-factor analysis of variance were performed using IBM SPSS Statistics v22 statistical software. Homogeneity tests were performed using Duncan's test, and Pearson's correlation tests were performed on the obtained results.

RESULTS

Data from Micro Metos 2002 statistical station

he months of early March to late July, which have the greatest influence on the growing season, were the driest in the last 22 years in terms of rainfall (Fig. 1). Only 125 mm of rain fell during the growing season. In the last 22 years, apart from 2022, only three years had precipitation of less than 160 mm during this period.

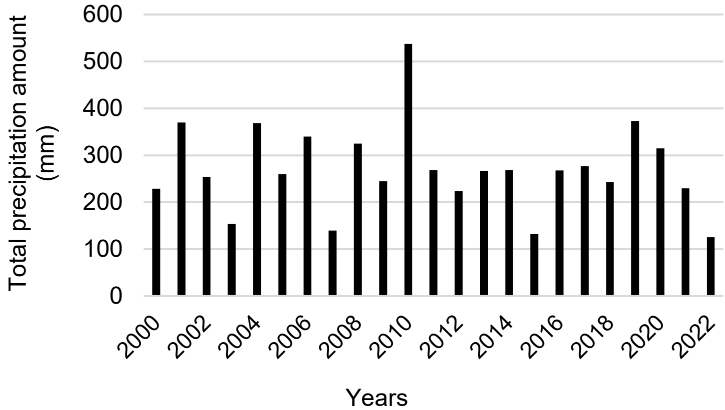

Figure 1. Total precipitation in mm over the last 22 years (Nyíregyháza, 2022).

In April, the amount of precipitation exceeded the average of the last 30 years, which provided suitable conditions for the emergence and early development of the common vetch (Fig. 2). However, there was only 3.9 mm of precipitation in May. In addition to the low amount of precipitation, the temperature values were higher than average. The extreme drought during the flowering phenophase negatively affected the success of generative development, however, it also harmed the development of weeds.

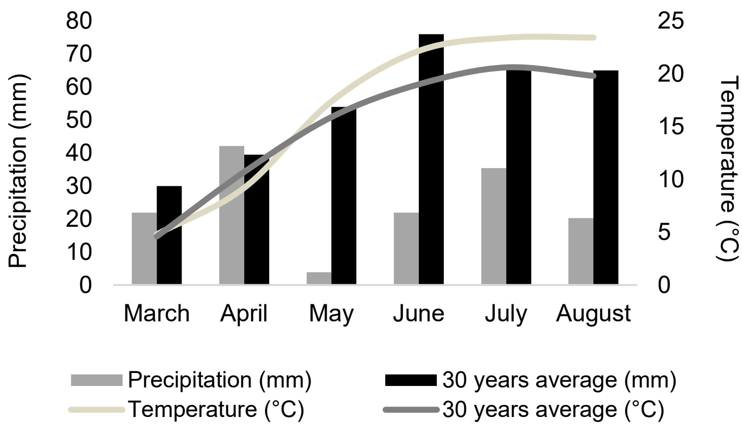

Figure 2. Average monthly precipitation and temperature datas during the growing season compared to the 30 years average (Nyíregyháza, 2022).

Measurement results of the Normalized Difference Vegetation Index (NDVI)

The first time was on April 21, (BBCH stage 10 - pair of scale leaves visible, the plots treated with pendimethalin showed the highest NDVI value (0.193), which was statistically different from the value measured when metolachlor was applied (0.176) as illustrated in Fig. 3.

The second time was on April 26, when the BBCH stage was 12 (2 leaves unfolded), the lowest NDVI value was shown by the metolachlor treatment (0.194). The value of the plots treated with pendimethalin was higher (0.231) than the value of the metolachlor treatment and they were statistically different from each other. Both treatments were statistically different from the value of the control plots (0.249). Fig. 4 illustrates the measured values.

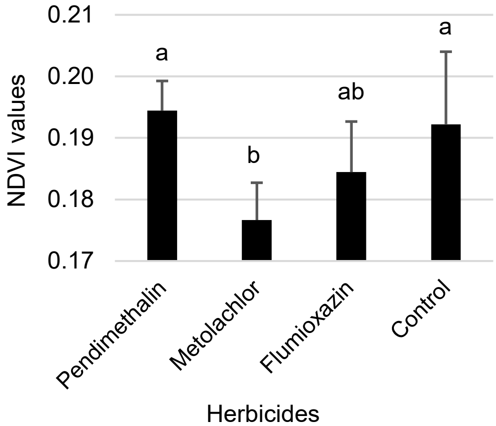

Figure 3. Evaluation of NDVI values in different common vetch herbicide treatments, when pair of scale leaves were visible.

Based on the NDVI values measured on May 3, when the BBCH stage was 19 (9 leaves unfolded), there was no significant difference between plots treated with flumioxazin and control plots, but control plots were statistically different from pendimethalin treated plots and metolachlor treated plots. The measured values are shown in Fig. 5.

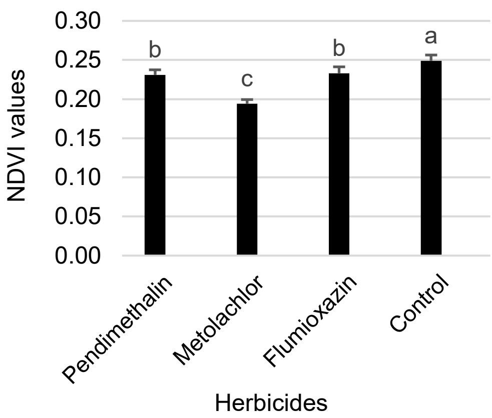

Figure 4. Evaluation of NDVI values in different common vetch herbicide treatments, when was 2 leaves unfolded.

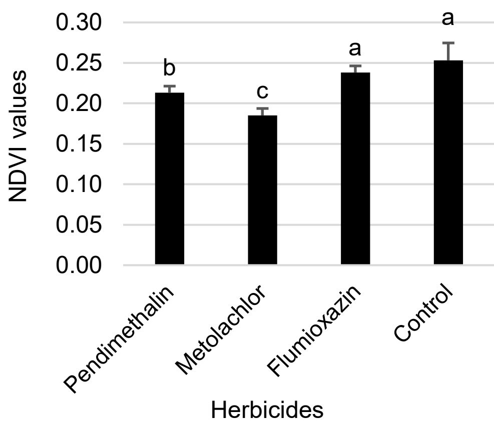

Figure 5. Evaluation of NDVI values in different common vetch herbicide treatments, when was 9 or more leaves unfolded.

Based on the NDVI values recorded on May 11, when the BBCH stage was 30 - beginning of stem elongation, the values of the plots treated with pendimethalin and metolachlor (0.265 and 0.260) treated plots were almost the same and statistically different from the control treatment (0.373). The values recorded for the other treatments are not outstanding here, as the treatment took place two days before, thus the post-emergence herbicides could not yet have exerted their effect here. The measured values are shown in Fig. 6.

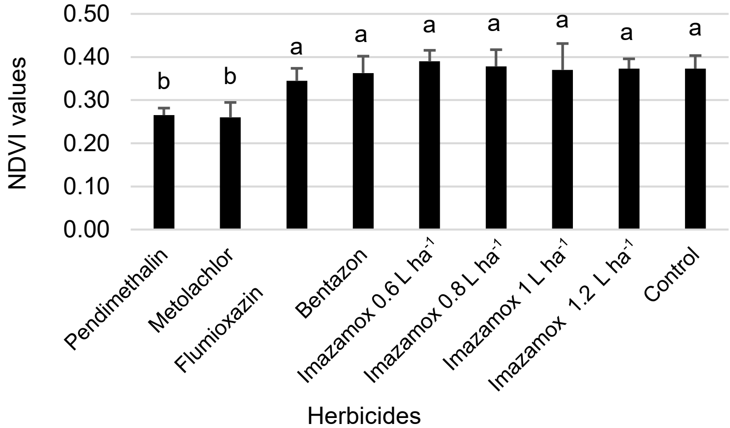

Figure 6. Evaluation of NDVI values in different common vetch herbicide treatments, when stem elongation started.

During the NDVI recording on May 18, when the BBCH stage was 33 - 3 visibly extended internodes, the plots treated with bentazon showed the lowest value (0.350), thus as a result of the post-emergence treatment, the normalized differential vegetation index value decreased compared to the value recorded at the previous recording time. However, the values of other treatments and the control increased. The highest value was measured in the control treatment (0.543) as shown in Fig. 7.

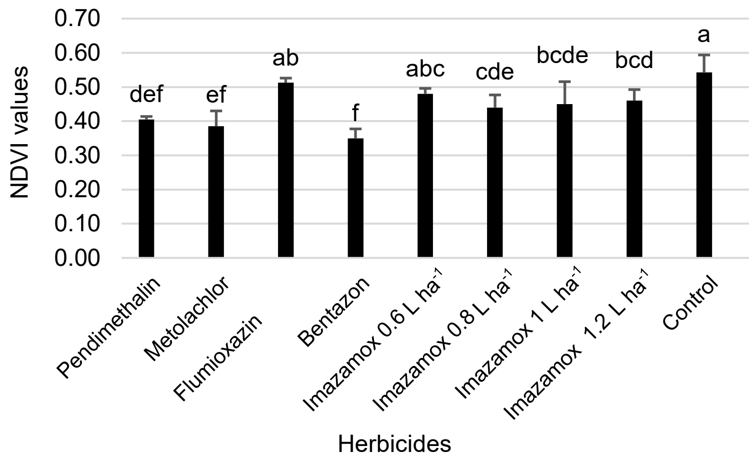

Figure 7. Evaluation of NDVI values in different common vetch herbicide treatments, when there were 3 visibly extended internodes.

The plots treated with flumioxazin showed the highest NDVI value (0.608) at data collection on May 25, when the BBCH stage was 39 - 9 visibly extended internodes (Fig. 8). It was statistically different from the metolachlor treatment, as well as from most post-emergence treatments (bentazon, imazamox 0.8 L ha^{-1}, imazamox 1 L ha^{-1}, imazamox 1.2 L ha^{-1}). The plots treated with bentazon showed the lowest value (0.378) and this treatment was not statistically different from the metolachlor and imazamox treatments.

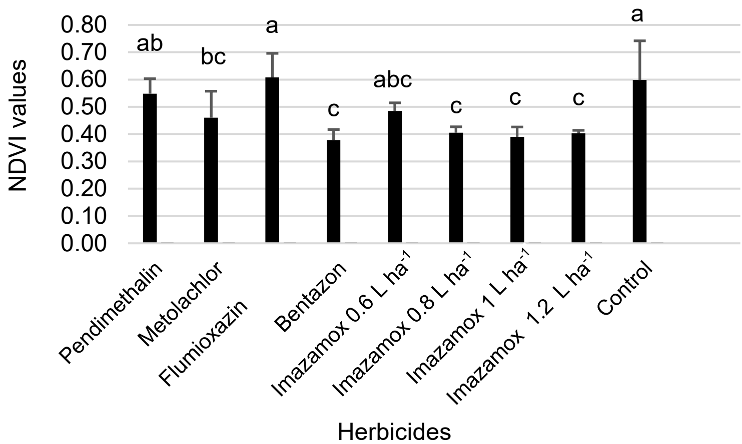

Figure 8. Evaluation of NDVI values in different common vetch herbicide treatments, when there were 9 or more visibly extended internodes.

Subsequently, the NDVI values changed to a large extent, significant differences were measured on May 30, when the BBCH stage was 51 - first flower buds visible outside leaves. The highest values were measured in the plots treated with flumioxazin (0.728) and in the control (0.678), which were statistically different from the values of the bentazon and imazamox 0.8 L ha^{-1}, 1 L ha^{-1}and 1.2 L ha^{-1} treatments. Weeds influenced the results in the control. Fig. 9 shows the results on May 30.

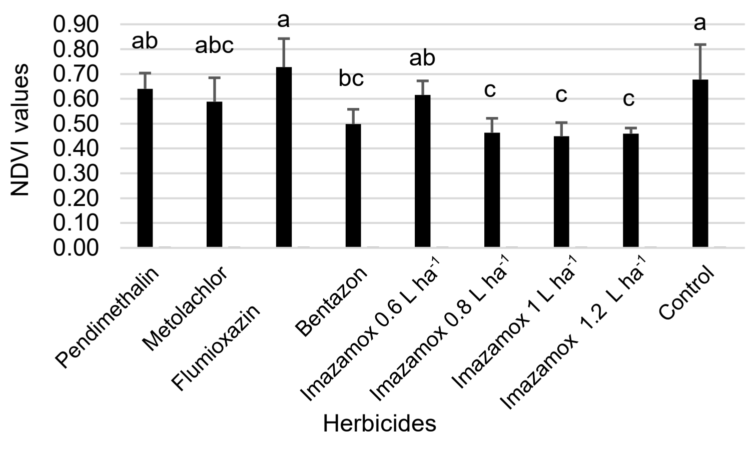

Figure 9. Evaluation of NDVI values in different common vetch herbicide treatments, when the first flower buds were visible outside leaves.

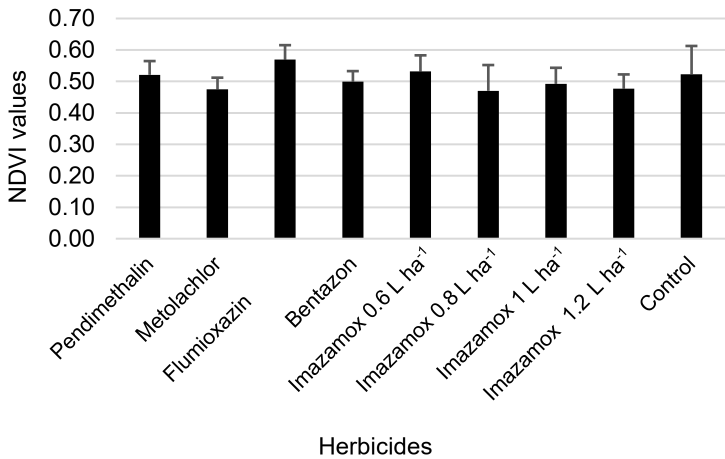

Figure 10. Evaluation of NDVI values in different common vetch herbicide treatments.

Based on the post hoc test of the NDVI data from the average of the twelve measurement times, the data show no statistical difference (Fig. 10). Regardless, it can be said that the plots treated with flumioxazin showed the highest value (0.570), while the plots treated with imazamox 0.8 L ha^{-1} yielded had the lowest value (0.470).

Phytotoxicity test results

Statistical analysis showed that seven out of nine test dates, were statistically different. In the initial period at the beginning of May, when the BBCH stage was 19 - 9 leaves unfolded, pendimethalin and metolachlor applied pre-emergent caused outstanding phytotoxicity values, as detailed in Fig. 11. In vetch, pendimethalin caused clear symptoms, the plant population was more deficient here than in the other treatments, and the treatment resulted in smaller plants. The phytotoxicity caused by pendimethalin and bentazon, as examples is illustrated in Fig. 12. On common vetch, metolachlor also resulted in deficient stands, leaf twisting, paleness, and dwarfed plants. However, plants regenerated over time.

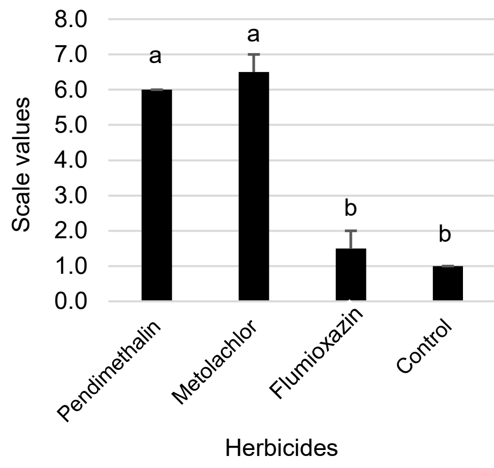

Figure 11. Evaluation of phytotoxicity values in common vetch herbicide treatments, when 9 or more leaves were unfolded.

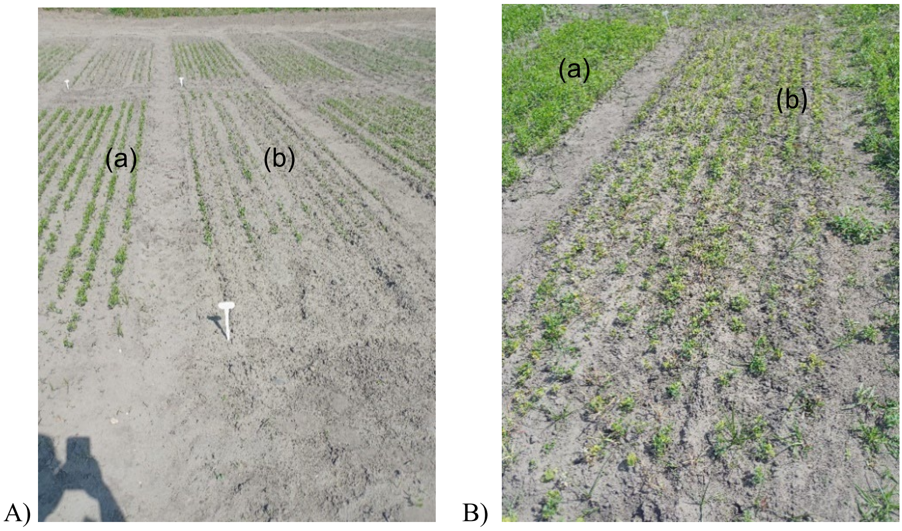

Figure 12. Phytotoxicity caused by pendhimethalin (A) and bentazon (B) on common vetch. (a) plots are untreated, while (b) plots are treated with the herbicides.

Based on data from the end of May to the beginning of June, when the BBCH stage was 39 - 9 visibly extended internodes, bentazon ingredient caused more severe pale yellow symptoms, while the symptoms seen with imazamox were milder. The plots treated with bentazon showed the highest phytotoxicity (6.25). This was followed by imazamox treated with amounts of 1 L ha^{-1}, 1.2 L $ha^{-1}$and 0.8 L ha^{-1}, and there was no significant difference between them (Fig. 13).

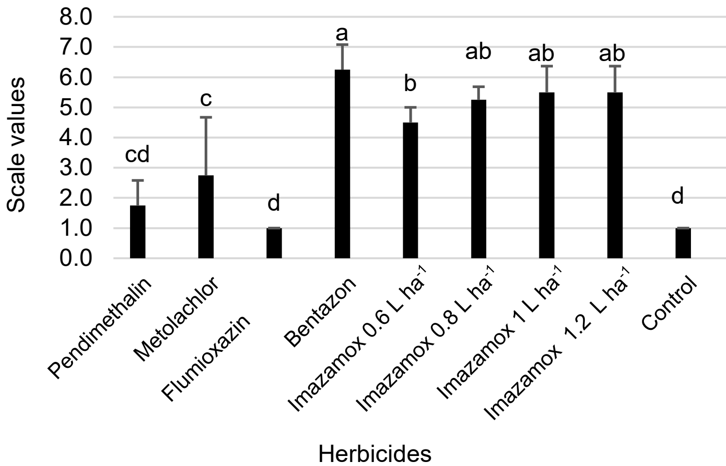

Figure 13. Evaluation of phytotoxicity values in common vetch herbicide treatments, when there were 9 or more visibly extended internodes.

Subsequently, when BBCH stage was 61 - beginning of flowering, the treatment with imazamox 1 L ha^{-1} showed the highest phytotoxicity (Fig. 14), which was statistically different from the phytotoxicity value of flumioxazin (1.75), which was the lowest.

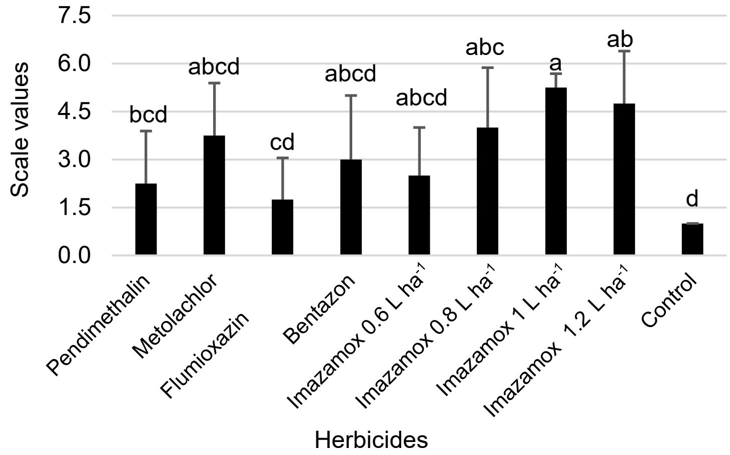

Figure 14. Evaluation of phytotoxicity values in common vetch herbicide treatments, upon the beginning of flowering.

Among the herbicides, the results of plots treated with flumioxazin showed the lowest phytotoxicity value (1.80). The highest phytotoxicity averages resulted from the imazamox 1 L ha^{-1} treatment (Fig. 15).

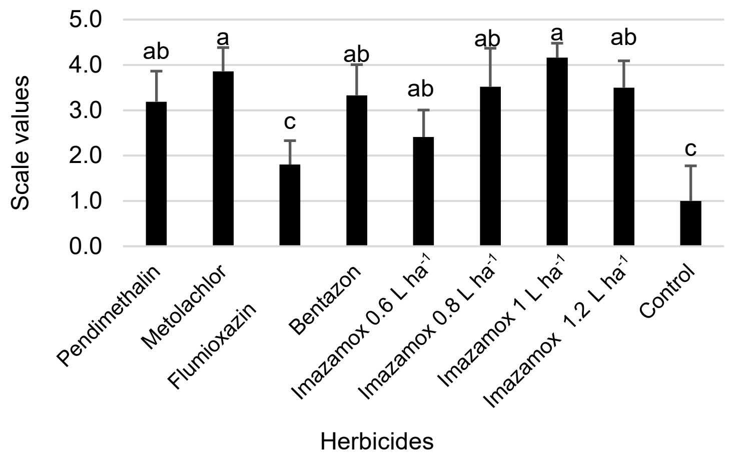

Figure 15. Evaluation of average phytotoxicity values in common vetch herbicide treatments.

Evaluation of the effect of different herbicide treatments on weed characters

We evaluated 16 different weed species occurring in the area. Based on samples collected on five occasions on the Post Hoc test, a significant difference was observed in the abundance of ragweed and white goosefoot. Based on the average of all wild grasses sampled on five occasions, with a value of 2.7 pieces m^{-2}, most of the wild grasses occurred in the control treatment, and the value of the plots treated with pendimethalin was only slightly different from this (2.6 pieces m^{-2}). These plots are significantly different from the other treatments, except for the imazamox 0.6 L ha^{-1} treatment (Fig. 16).

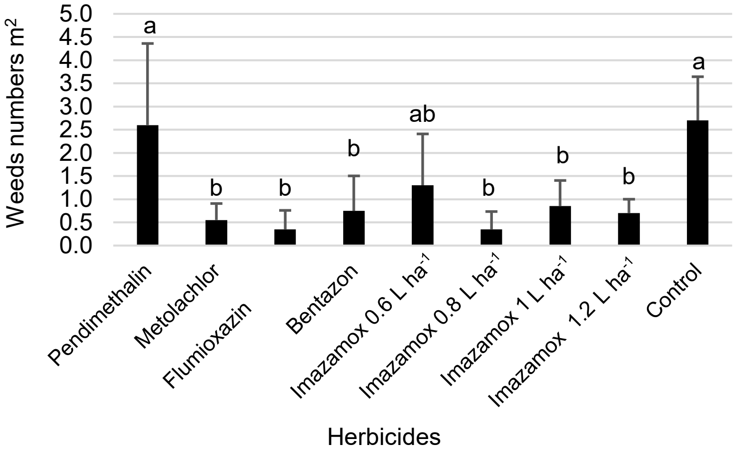

Figure 16. Effect of different herbicide treatments on the number of ragweed (*Ambrosia artemiisifolia* L.) in common vetch.

In the case of the added and then averaged white goosefoot, with a value of 3.75 pieces m^{-2}, the weed occurred in the highest number in the control plots (Fig. 17). Several weed species were present in large numbers, however, the statistical analysis did not show any significant difference between the treatments. Among the weed species occurring in larger numbers, the puruagrass, with a value of 2.5 pieces m^{-2} occurred in the highest amount in the plots treated with imazamox 1 L ha^{-1}, followed by the plots treated with pendimethalin with a value of 2.4 pieces m^{-2}. Redshank occurred in greater numbers with a value of 2.85 pcs m^{-2}, the largest amount occurred in the plots of 0.6 and 0.8 L ha^{-1}of imazamox, but it was second in the plots treated with flumioxazin with a value of only 0.5 pcs m^{-2} lowest, and it did not occur in the plots treated with pendimethalin.

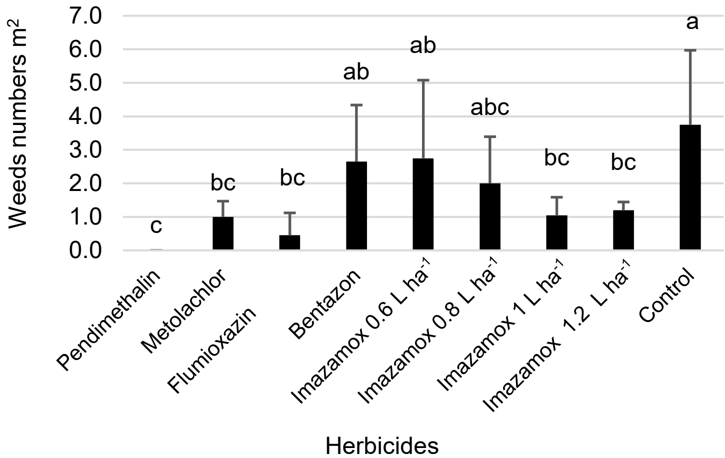

Figure 17. The effect of different herbicide treatments on the number of white goosefoot (*Chenopodium album* L.) in common vetch.

We analysed the total number of weeds at different weed recording times. Based on the data from the first recording on May 4, when the BBCH stage was 19 - 9 leaves unfolded, the metolachlor treatment showed the lowest value of weeds 3 pcs m^{-2}, which was not statistically different from the control value, which was 7.50 pcs m^{-2} as presented in Fig. 18.

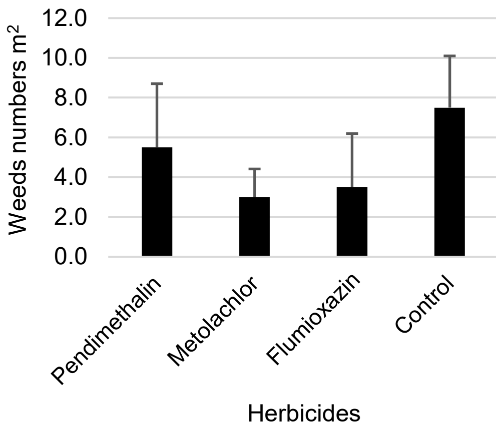

Figure 18. Total weed numbers as a result of herbicide treatments in the common vetch experiment at BBCH 19 phenological stage.

Fig. 19 shows the total number of weeds recorded on May 25, when the BBCH stage was 39, 9 visibly extended internodes. With a value of 12.50 pcs m^{-2}, the control plots had the highest number of weeds; this is statistically different only from the value of the plots treated with flumioxazin (4.75 pcs m^{-2}), which had the lowest number of weeds.

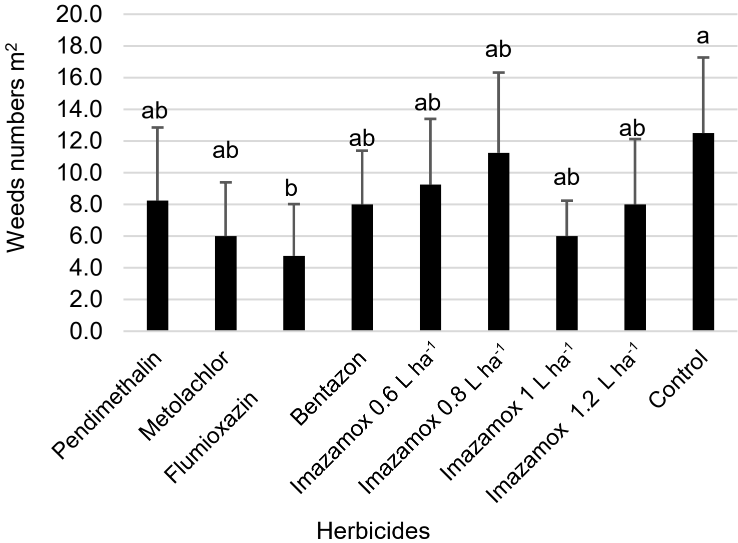

Figure 19. Total weed numbers as a result of the herbicide treatment in the common vetch experiment at BBCH 39 phenological stage.

Based on the data from the total number of weeds survey on June 2, when the BBCH stage was 51 - first flower buds visible outside leaves (Fig. 20), it can be seen that the plots treated with flumioxazin had the fewest number of weeds with a value of 0.50 pcs m^{-2}, followed the plot treated with pendimethalin, followed by the imazamox 1.2 L ha^{-1} treatment, while the most weeds were in the control plots with a value of 12 pcs m^{-2}.

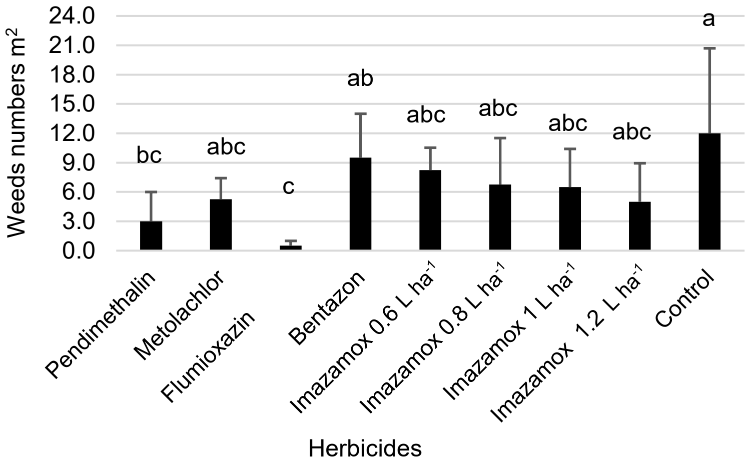

Figure 20. Total weed numbers as a result of the herbicide treatment of the common vetch experiment at BBCH 51 phenological stage.

The last weed collection took place on June 10, when the BBCH stage was 59 - first petals visible, flowers still closed. As expected, the control plots had the most weeds at this time (10.75 pcs m^{-2}). The treatment with 1 L ha^{-1} imazamox resulted in the lowest number of weeds (1.75 pcs m^{-2}), followed by the values of the plots treated with flumioxazin, then the values of the imazamox 1.2 L ha^{-1} treatment, which were not significantly different from each other (Fig. 21).

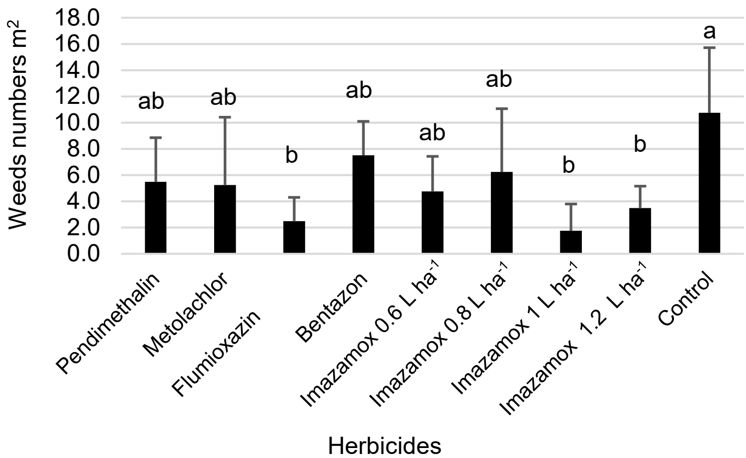

Figure 21. Total weed numbers as a result of the herbicide treatment in the common vetch experiment at BBCH 59 phenological stage.

During the statistical analyses, the average total number of weeds developed according to preliminary expectations, i.e. it was probable that most weeds would grow in the control plots. On average, the control plots had the most weeds (10.4 pcs m^{-2}). This was followed by 8.4 pcs m^{-2} of the plots treated with 0.6 L ha^{-1} imazamox. Flumioxazin was the best performing herbicide with an average of 2.9 pcs m^{-2} as shown in Fig. 22.

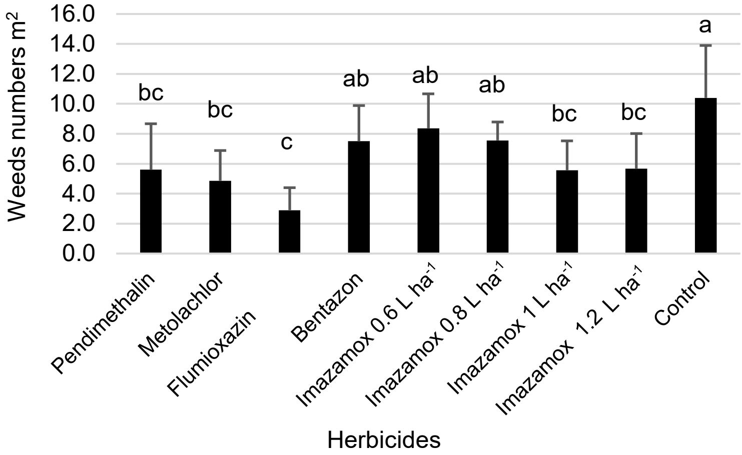

Figure 22. Total weed numbers as a result of the herbicide treatment in the common vetch experiment.

Effect of different herbicides on seed yield of common vetch

It is important to note that the year 2022 was particularly droughty, especially in the eastern parts of the country, thus the harvested crop amounts turned out to be low. In 2022, only 125.2 mm of rain fell between March and July. The lowest yield result was the application of the imazamox at a dose of 1 L ha^{-1} with an average of 34.5 kg ha^{-1}. All plots treated with imazamox had a harvested seed weight below 60 kg ha^{-1}. The imazamox treated plots were significantly different from the flumioxazin treated plots, but the imazamox treated plots and the flumioxazin treated plots were not statistically different from the other treatments and the control. The control plot produced a yield of 132.5 kg ha^{-1}, which was only exceeded by the average results of the flumioxazin treated plots with a yield of 194.1 kg ha^{-1} (Fig. 23).

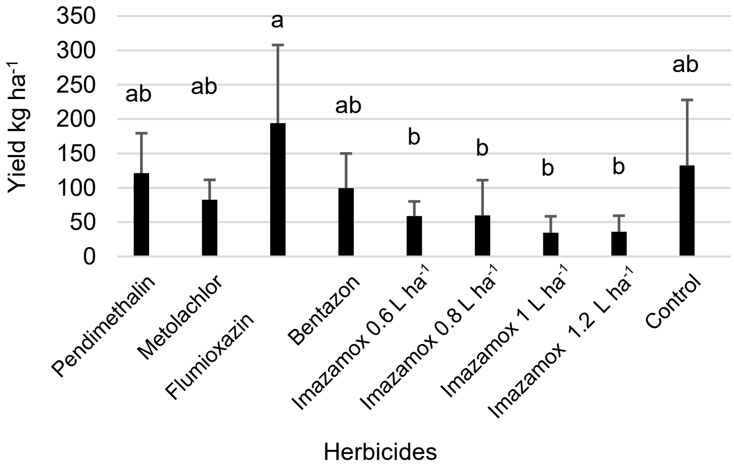

Figure 23. Effect of different herbicide treatments on common vetch yields.

Effect of different herbicide treatments on the amount of plant trash generated during cleaning seeds

Different levels of contamination were measured during the cleaning of common vetch seed lots. The highest contamination was found in the bentazon treated plots with an average of 179.4 kg ha^{-1}. We compared the % of seed yield with the % of plant waste, based on this, pendimethalin was the best with 61.8%, closely followed by flumioxazin (Fig. 24), here the % ratio of seed yield was 60.7%.

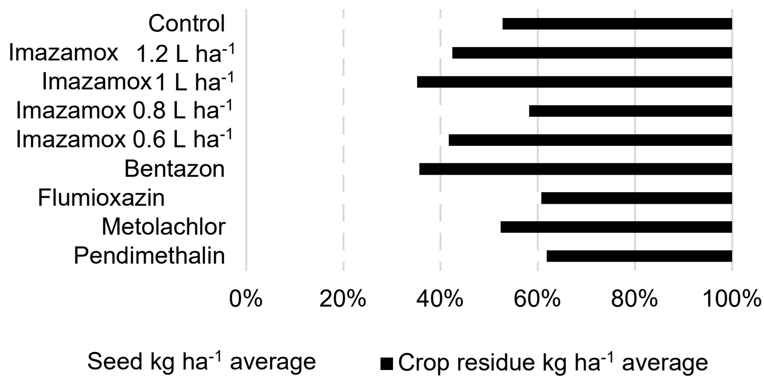

Figure 24. Ratio between quantities of seed and plant waste of common vetch experiment.

Table 3 shows the overall averages of all the parameters studied in the common vetch experiment. Based on the data, the herbicide with the best performance in the experiment was flumioxazin. This herbicide had the highest total average NDVI value (0.570).

Table 3. Averages of total NDVI, phytotox, weeds number, seed yield (kg ha^{-1}) and plant trash (%) as influenced by different herbicide treatments in the common vetch experiment (Nyíregyháza, 2022). Means for groups in homogeneous subsets are displayed. The superscripts indicate if a parameter is significantly different for the treatments

Treatments	NDVI value	Phytotox	Average of weeds m^2	Average seed kg ha^{-1}	Contamination (%)
Pendimethalin	0.521	3.19^{ab}	5.6^{bc}	121.3^{ab}	38.18
Metolachlor	0.475	3.86^{a}	4.9^{bc}	82.6^{ab}	47.62
Flumioxazin	0.570	1.81^{c}	2.9^{c}	194.1^{a}	39.27
Bentazon	0.499	3.33^{ab}	7.5^{ab}	99.4^{ab}	64.35
Imazamox 0.6 L ha^{-1}	0.532	2.42^{ab}	8.4^{ab}	59.0^{b}	58.29
Imazamox 0.8 L ha^{-1}	0.471	3.53^{ab}	7.6^{ab}	59.9^{b}	b41.79
Imazamox 1 L ha^{-1}	0.492	4.17^{a}	5.6^{bc}	34.5^{b}	64.87
Imazamox 1.2 L ha^{-1}	0.477	3.50^{ab}	5.7^{bc}	36.1^{b}	57.58
Control	0.523	1^{c}	10.4^{a}	132.5^{ab}	47.27
Total average	0.5	3.1	6.5	91.0	38.18

Favourable NDVI values were also measured for the imazamox 0.6 L ha^{-1} treatment, but the total average of all weeds pieces in the plots of this treatment was the second highest after the control treatment. The lowest phytotoxicity was observed in the flumioxazin treatment (1.81). In the total average of all weed numbers, the lowest value was for flumioxazin (2.9 pcs m^{-2}), while the highest value was obtained from the control plots (10.4 pcs m^{-2}). In addition, the highest seed yield was recorded in the flumioxazin treatment (194.1 kg ha^{-1}) followed by the control treatment (132.5 kg ha^{-1}).

For plant trash (kg ha^{-1}), the highest value was found in the plots treated with bentazon (179.4 kg ha^{-1}). Furthermore, the ratio between seed yield and plant trash as (%), based on this, the percentage of plant trash in the plots treated with imazamox 1 L ha^{-1} was the highest (64.87%), followed by the plots which treated with bentazon (64.35%) with no significant differences between them.

Pearson's correlation test

According to Pearson correlation test as shown in Table 4, there was a positive correlation between NDVI values and seed yield at 0.01 level with 0.557, which is a medium strong value, and a positive correlation between NDVI values and plant trash (kg ha^{-1}) at level at 0.01 level with 0.467, which is also a medium strong value. There was a negative correlation at the 0.01 level between mean NDVI values and mean phytotoxicity, at -0.745, which is considered a high strength correlation. There was a positive correlation between seed yield (kg ha^{-1}) and plant trash at the 0.05 level, with a medium correlation of 0.360. Seed yield (kg ha^{-1}) was negatively correlated with phytotoxicity at the 0.01 level, with a medium strength correlation of -0.518. Plant trash was negatively correlated with phytotoxicity at the level of 0.05 and had a medium strength correlation of -0.421.

Table 4. Pearson's correlation between average of NDVI, average seed yield (kg ha^{-1}) average of plant trash (kg ha^{-1}), average of phytotoxicity, average of all total weeds under herbicide treatments in common vetch experiment (Nyíregyháza, 2022)

Results	Average of NDVI	Average seed yield (kg ha^{-1})	Average of plant trash (kg ha^{-1})	Average of phytotox	Average of all total weeds
Average of NDVI	1	.557**	.467**	–.745**	.209
Average seed yield (kg ha^{-1})	.557**	1	.360*	–.518**	.141
Average of plant trash (kg ha^{-1})	.467**	.360*	1	–.421*	–.007
Average of phytotox	–.745**	–.518**	–.421*	1	–.206
Average of all total weeds	.209	.141	–.007	–.206	1

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

The results showed that the seed yield of pendimethalin treated plots was not statistically different from the control plots. Control treatment yielded 132.5 kg ha^{-1}, while the average seed yield of the pendimethalin treated plots was 121.3 kg ha^{-1}. Similarly, in the experiment of Caballero et al. (1995) who indicated that, pendimethalin reduced the yield of common vetch in two out of three years of the experiment. Initially, plants in the pendimethalin treated plots showed clear symptoms, more incomplete stands and smaller plants. Later field studies showed that the plants recovered from these symptoms. Although pendimethalin did not perform outstandingly in terms of weed control, white goosefoot was best controlled by this herbicide, which confirms the findings of Vasilakoglou et al. (2013) that the application of a higher dose of pendimethalin of 1.98 kg ha^{-1} provided the best control of white goosefoot.

The metolachlor treatment on common vetch initially resulted in leaf twisting, wilting, smaller plants and incomplete emergence, but the field survey on May 24, when the BBCH stage was 39 - 9 visibly extended internodes, showed that the plants had almost completely recovered. In terms of herbicidal effect, only flumioxazin performed better than metolachlor. This result is in good agreement with Vasilakoglou et al. (2013), who concluded that a higher herbicidal efficacy (48.8–50%) was recorded with the higher dose of metolachlor + terbutylazine (1.25 + 0.75 kg ha^{-1}), respectively.

Test results on 18th May, when the BBCH stage was 33 - 3 visibly extended internodes, showed that bentazon applied after emergence damaged common vetch. The average phytotoxicity results showed significantly higher values for bentazon as compared to the control treatment. This result contradicts the results reported by Insidecotton (2006) that Basagran at 2 L ha^{-1} did not cause phytotoxicity 3 and 6 weeks after application. In other studies, the application of bentazon resulted in the eradication of common vetch (Dumont & Serpeile, 1981). In a study carried out in 1995, the application of 1 kg ha^{-1} bentazon + 1.5% oil on common vetch leaves showed symptoms of scorch and thinned leaves with a reduced ability to regenerate, whereas in 1997 they were almost fully regenerated and in 1996 they were also damaged but then fully regenerated. In common vetch, the application of both doses of bentazon + oil (1 and 1.2 kg ha^{-1}) in 1995 slightly reduced yields (Americanos & Droushiotis, 1998). In our study, the yields of plots treated with bentazon were also lower than the control.

Application of imazamox resulted in milder symptoms in common vetch based on observations on May 18, when the BBCH stage was 33 - 3 visibly extended internodes. According to Vasilakoglou et al. (2013), imazamox at 0.03–0.04 kg ha^{-1} post-emergence was slightly phytotoxic to common vetch and resulted in 14–16% lower yield than the control treatment (no treatment). Hinds-Cook et al. (2009) found that imazamox treatments caused significant damage to common vetch, but germination percentage and seed yield were similar to those of the control. In a study by García-Garijo et al. (2014), imazamox accumulated mainly in common vetch, where concentrations were more than six times higher than those detected in beans (*Phaseolus vulgaris* L.). Herbicide use can negatively affect symbiosis and biological nitrogen fixation (Zaidi et al., 2005; Vieira et al., 2007). In our study, imazamox at a dose of 1 L ha^{-1} resulted in the highest (worst) average phytotoxicity value of 4.17. Based on the average seed yield per hectare, imazamox 1 L ha^{-1} had the lowest seed yield at 34.5 kg ha^{-1}, closely followed by imazamox 1.2 L ha^{-1} at 36.1 kg ha^{-1}.

CONCLUSION

Based on our findings in the present work, it can be said that flumioxazin herbicide performed best. The highest average NDVI value was observed for this herbicide (0.570), and the plant treated with flumioxazin was also the best performer in terms of seed yield per hectare with an average of 194.1 kg ha^{-1}. In addition, flumioxazin had the lowest average of phytotoxicity value (1.81) and also the lowest average number of weeds (2.9 pcs m^{-2}). Our results showed that the white goosefoot was well controlled by using pendimethalin at the recommended doses. In addition, metolachlor was second only to flumioxazin in terms of total weed number (4.9 pcs m^{-2}), but seed yield was low at 82.6 kg ha^{-1} and phytotoxicity (3.86) was second only to imazamox at 1 L ha^{-1}. All imazamox treatments seed yield per hectare below 60 kg ha^{-1}. Bentazon did not perform prominently either in any of the indicators. Thus, our research suggests that the higher doses of flumioxazin (0.08 and 0.1 kg ha^{-1}) may be worth investigating in the future.

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Effect of water deficit on maize seeds *(Zea mays* L.*)* during germination

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Abstract. Global climate changes cause the frequent recurrence of droughts, which reduce crop production more than any other environmental factor. This study was conducted to access the effect of water deficit on maize seeds *(Zea mays* L.*)* DKC 5143 hybrid during germination. The tasks were to assess the influence of different rank of osmotic stress on the maize lipid peroxidation (LPO), proline content, catalase and aminotransferases activities, and morphometric parameters during the early stages of maize seeds germination. The maize seeds were exposed to five levels of water availability which produced by PEG-1500 solutions (0, 20, 50, 100, 200 g L^{-1}). Seeds of maize were germinated on Petri dishes for 7 days under controlled parameters. Amounts of TBARS were increased in maize sprouted seeds by 1.9 times, coleoptiles by 1.4 times, and in roots - by 1.9 times under water deficit. Proline content increased by 9.2 times in coleoptiles and by 6.0 times in 7 days maize roots while PEG-1500 (200 g L^{-1}) treatment. An increasing of catalase (CAT), aminotransferases (ALT, AST) activities according to osmotic potential value was also observed. A remarkable development of maize oxidative reaction was associated with a significant reduction in emergence, wet weight and length of water-stressed plants. These results assume that the maize adaptive strategy to osmotic stress during germination was found in the activation of LPO and antioxidant components. The findings provide useful help for correcting the stress state of maize using osmotically active regulators.

Key words: aminotransferases, catalase, drought, germination, lipid peroxidation, maize, osmotic stress, proline.

INTRODUCTION

The water deficit is the most limiting factor of yield formation among the factors that cause plants stress. Drought periods repeated very frequently during the seeding period and have a negative impact on the plants during the vegetation period in the semi-arid and arid part of Ukraine and Europe, in general (Lipper et al., 2014; Vogel et al., 2019; Horváth et al., 2021). Disorders of water balance in the plant organism lead to changes in the photosynthesis intensity, carbohydrate and protein metabolism. Water stress promotes the accumulation of various toxic products, including activated oxygen metabolites (AOM), lipid peroxides, and oxidized proteins (Kolupaev et al., 2019).

It is known, that an antioxidant system plays a significant role while plants adapt to unfavorable environmental factors. The antioxidant system provides the tolerance of damaging environmental stresses which is correlated with an increased capacity to scavenge or detoxify ROS (Yang et al., 2021). Various enzymatic and nonenzymatic antioxidant components eliminate AOM, inhibit the development of the peroxidation process, and prevent DNA fragmentation (Masoumi et al., 2010). Antioxidative enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) play an important role against drought stress (Habibi & Hajiboland, 2011; Moharramnejad et al., 2019).

Protein metabolism of plants shows significant sensitivity in implementing adaptive reactions. When plant's leaves are under water stress, their protein content decreases. Besides that, the water deficit reduced the pool of total protein in C_3 plants more intensively than in C_4 plants (Ozturk et al., 2021).

Transferases take an important role in these processes participating not only in the synthesis of amino acids and proteins but also in maintaining energy metabolism. It is found that C_4 plants have high activity of aminotransferases (Schlüter et al., 2019). Alanine aminotransferase (ALT) (EC 2.6.1.2) catalyzes the transfer of amino between alanine and glutamate involving α-ketoglutarate and pyruvate. Aspartate aminotransferase (AST) (EC 2.6.1.1) catalyzes the transamination of glutamate and aspartate involving α-ketoglutarate and oxaloacetate. These enzymes are represented by several isoenzymes forms in plant cells and localized in the cytosol, plastids, mitochondria and peroxisomes (de la Torre et al., 2014). Aspartic and glutamic acids play an important role in plant adaptation to adverse factors as a reserve for the synthesis of new amino- and ketoacids. The role of aminotransferase reactions in the detoxification of xenobiotics, heavy metals, and pesticides (Singh et al., 2015) has enough investigated. The changes in aminotransferase activity under the influence of temperature, water, phytohormones, and nutrients were described (Kendziorek et al., 2012; Xue et al., 2021). However, the ontogenetic changes of aminotransferase activity of germinated maize under a water deficit have not been sufficiently investigated.

Water is an activation factor of biochemical and physiological processes that accompany seed soaking and germination. Plants are highly sensitive to even slight depression of water potential at seedling stages. Therefore, the physiologically normal level of free radical processes is disturbed and the synthesis of compatible osmolytes is increased in embryonic axes under osmotic stress conditions (Zivcak et al., 2016; Kolesnikov et al., 2019).

Maize (*Zea mays* L.) is one of the most important cereal crops in the world after wheat and rice. Normally, it needs 500–800 mm of water during its life cycle (80–110 days). Water availability and movement into the seeds are very important to promote germination, initial root growth, shoot elongation, and therefore at the establishment of a uniform stand. The germination starts with seed imbibition as a result of water uptake. This process occurs due to the distinct levels of osmotic potential between the dry seed and water in the substrate of germination. In general, the seed water content of cereal crops must reach at least 35 to 45% of seed dry mass to occur the germination process. The highly negative osmotic potential may affect the seeds' water uptake, making germination not possible. The most common responses of plants to the reduction of osmotic potential are a delay in initial germination and a reduction in the rate and total germination (Ashraf et al., 2016; Queiroz et al., 2019; Hunter et al., 2021). Under low moisture levels of the steppe, water deficit negatively influences different stages of growth and development of maize and grain crops, in general (Akıncı & Lösel, 2012; Golabadi et al., 2015; Mazhar et al., 2020). Although maize is a drought-resistant crop, it sprouts best when the upper soil layers are well-hydrated and heated (Bhusal et al., 2021; Khaeim et al., 2022).

There are numerous studies noted changes in the activity of the antioxidant system and metabolic components under drought conditions during seed germination, but the obtained results are different and sometimes contradictory. At present, the major focus of maize research is to improve abiotic stress tolerance characteristics. The study of maize resistance to water deficits during seedling stages is important for understanding the mechanism of the stress damage, for finding techniques that optimize metabolic processes, and for the selection of optimum moisture at sowing. As a result of this research, we will be able to develop agrotechnologies for growing maize hybrids in arid conditions without compromising the grain yield under stress conditions.

The present study was performed with a hypothesis that water deficit-induced change in pro-antioxidant system could affect yield and growth of maize plants grown under drought conditions. Therefore, the aim of present study was to evaluate the influence of different water stress levels on the maize lipid peroxidation, proline content, catalase, and aminotransferases activities and morphometric parameters during the early stages of maize seeds germination.

This study provides essential information regarding germination requirements, and it investigates tolerance to drought stress.

MATERIALS AND METHODS

Plant material, experimental procedures

The seeds of maize *(Zea mays* L.*)* DKC 5143 hybrid (produced by Monsanto Company) were used to study the effect of osmotic stress. DKC 5143 is a mid-late maize hybrid (FAO 430) with high plasticity and medium drought resistance. The maize seeds belong to the tooth-shaped type, weight of 1,000 grains is 330–370 g. Maize seeds were placed in growth chamber conditions with a temperature regime of 24 ± 2 °C in the dark. Three days after sowing, the germinated seeds were grown at 14 h of photoperiod, 60% relative humidity (RH), and 100 mmol m^{-2} s^{-1} of photosynthetic photon flux density (PPFD) provided by fluorescent lamps.

The design of the experiment included 5 groups. One group was used for control where maize seeds were germinated on water. The other four groups were used for water deficit treatments by exposure to PEG-1500 (polyethyleneglycole) at concentrations of 20, 50, 100, 200 g L^{-1}. Each concentration produces an osmotic potential (-0.5, -1.5, -3 and -6 bar) accordingly (Michel & Merrill, 1973). Seeds of the control and drought treatments were germinated according to the International Seed Testing Association (ISTA) protocol. For each treatment 250 seeds were placed on five 90 mm diameter Petri dishes (50 seeds on each dish). Two layers of filter papers were moistened with 5 mL of incubation medium (ISTA, 2014).

Dry seeds, endosperm of germinated seeds and embryonic axis (primary roots and coleoptile) were used for determination of biochemical parameters. The endosperm of germinated seeds was taken at 2, 4, 6, 12, 24 hours after sowing. The embryonic axis was taken on the 3rd and the 7th day after sowing. The seeds did not germinate in the group with 200 g L^{-1} PEG-1500 treatment, so the parameters were not determined in the coleoptiles and roots on the 3rd and the 7th day.

Measurements

Water uptake and growth measurement

The water uptake was measured by putting known weights seeds of maize in water or water solutions of PEG-1500 at different concentrations. After different intervals of time, the weight of the water absorbed by the seeds was ascertained and then weighed. The kinetics of water uptake was monitored in 2, 4, 6, 12, 24 hours from seeds sowing on Petry dishes. Seeds water content was calculated as the difference between the raw weight of soaked seeds (SRW) and their start dry weight (SDW) divided by the SDW according to the Eq. (1) and the result is presented as a percentage (Djébali, 2012).

$$\mathrm{WC} = \frac{\mathrm{SRW} - \mathrm{SDW}}{\mathrm{SDW}} \times 100\% \qquad (1)$$

The energy of seed's germination (ESG) was measured after 3 days of growing. The amount of normally germinated seeds was calculated when ESG accounting was conducted. Normally germinated maize kernels have a developed main germinal root, which was larger than half of the seed length and formed sprouts. This index was calculated using the formula (2):

$$\mathrm{ESG} = \frac{50 - n}{50} \times 100\% \qquad (2)$$

where, n – number of seeds, which didn't germinate for 72 hours, un.

The laboratory germination (LG), raw weights of roots and coleoptiles, and length of coleoptiles of maize were measured on the 7th day after sowing. The calculation of laboratory germination was made using the ratio (%) of the total number of seeds that were taken for germination to the number of not germinated seeds. The swollen, rotten, and abnormally germinated seeds are referred to as not germinated ones (ISTA, 2014).

Seedlings were separated into roots and coleoptiles on the 7th day after germination. The raw weights (g 100 units) of roots and coleoptiles were quantified using analytical scales. Coleoptile length (cm) was recorded as the distance from the scutellum to where leaf one had broken through the tip of the coleoptile (Hakizimana, 2000).

TBA-reactive substances (TBARS) determination. Plants samples were homogenized in LN with a mortar and pestle at the presence of 100 mmol tris-HCl buffer (pH 7.8) at ratio 1:9 (v/v) under temperature 0–4 °C. The tissue homogenate was centrifuged for 10 min (8000g) and the supernatant liquid was analyzed.

Plant homogenate with a volume of 1.0 mL was incubated in the boiled water bath with 3.0 mL of 0.5% thiobarbituric acid (TBA) on 20% trichloroacetic acid (TCA) solution for 30 min. After that, the reaction mixture was placed on ice and then centrifuged for 10 min at 5,000 g. The supernatant was used for photometric analyses. The absorbance of supernatant was read at 532 nm ('Unico UV-2800') subsequent to subtraction of non-specific absorption at 600 nm. The level of TBARS was measured according to malondialdehyde (MDA) concentration, which was calculated using its extinction coefficient 155 mM^{-1} cm^{-1} and expressed in $\mu M \times g^{-1}$ of raw tissue (Heath & Packer, 1968; Dhindsa et al., 1981).

Proline determination. Proline contents ($\mu g \times g^{-1}$) were measured using the rapid colorimetric method by Bates et al. (1973). Proline was extracted from 1.0 g of raw tissue by grinding in 10 mL of 3% (v/v) sulfosalicylic acid. The mixture was then centrifuged at 4,000 g for 10 min. In a test tube, 2 mL of the supernatant followed by 2 mL of freshly prepared acid-ninhydrin solution and 2 mL of acetic acid was placed. The tube was incubated in a boiled water bath for 1 hour and the reaction was terminated in an ice-bath. Then the reaction mixture was extracted with 4 mL of toluene and shook for 20 sec. After the separation, the toluene phase was collected into a test tube and its absorbance was read at 520 nm. Proline concentrations were determined from a standard curve prepared using analytical grade proline.

Catalase activity determination. Catalase activity (EC 1.11.1.6) was determined by measuring the rate of H_2O_2 conversion to O_2 at room temperature and the result was presented in mcatal$\times mg^{-1}$ protein (Goth, 1991). Plant homogenate with a volume of 0.1 mL, obtained as previously described, was added to 2.0 mL of 0.03% H_2O_2 and incubated for 5 min. The reaction was terminated by 1.0 mL of 4% ammonium molybdate solution. The absorbance of the colored complex was read at 410 nm. Catalase activity was calculated using its extinction coefficient 22,400 $mM^{-1} cm^{-1}$. The protein content in plant homogenate was measured by Lowry O.H. et al. (1951) with a help of Pholine reagent.

Alanine- and aspartate- aminotransferase activities determination. The activity of aminotranferase was determined by the colorimetrical method, with 2,4-dinitrophenyl-hydrazine in some modifications, the results being expressed in $\mu mol \times h^{-1} \times mg^{-1}$ of protein (Reitman & Frankel, 1957; Sato et al., 2016). The substrate (0.25 mL) was incubated with 0.05 mL of homogenate at 37 °C for 1 hour. For ALT (EC 2.6.1.2) determination, substrate contains: phosphate buffer 0.1 M, DL-a-alanine 0.2 M, 2-oxoglutarate 2 mM; and for AST (EC 2.6.1.1) determination substrate contains: phosphate buffer 0.1 M, L-aspartate 0.1 M, 2-oxoglutarate 2 mM. The reaction was terminated by adding 0.25 mL of 1mM 2,4-dinitrophenylhydrazine solution in 1M HCl. After 20 min, 2.5 mL of 0.4 M NaOH solution was poured and the mixture was incubated for 10 min again. The absorbance was read at 540 nm.

Statistical analysis

The data were analyzed using Analysis of Variance (ANOVA). All measurement represents the means and standard error (± SE) of five replicas. Statistically significant differences between means were compared at the 0.05 probability level by t-Student's test. Pearson's correlation test was conducted to determine the correlations between biochemical parameters (Edwards, 1976) and correlation coefficient values were determined using Excel.

RESULTS AND DISCUSSION

The water uptake of maize kernels during the soaking process was uneven. In the first hours, the kernels absorbed water vigorously, but as the kernels were saturated with water, the process slowed down. The required humidity was reached slowly in the last hours. Achieving the required humidity occurs after 24–30 hours. As known, the initial water uptake is carried out by seed shells that have a large number of capillaries, pores, and voids serving as a reservoir for the primary water accumulation. Then water penetrates the seminal membranes, the germ, and the aleurone layer and is bounded by proteins and carbohydrates strongly. Further movement of water is directed inside the endosperm (Agarry et al., 2014).

Maize seeds, germinated on water, are characterized by the fastest water absorption in compare with seeds germinated on PEG-1500 solutions during the first day (Fig. 1).

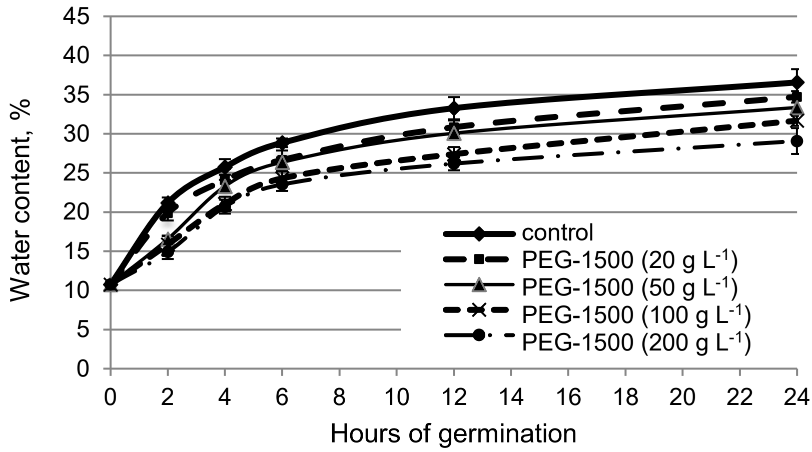


Figure 1. Changes in maize seeds water content during germination under different water deficit. Vertical line – standard error of mean.

Water absorption intensity was decreased while maize seeds germinated under water deficit conditions. Moreover, the reduction of seeds soaking directly depended on the degree of water potential depression in the substratum. A reduction in soaking was observed 2 hours after germination had begun. The most active inhibition of soaking process (up to 7.5%) was observed in seeds that were growing in the solution of PEG-1500 (200 g L^{-1}). The results indicate the formation of water deficit during maize seeds germination under the PEG solutions.

It is known that water absorption is the initiating factor of seed germination. The depression of medium water potential slows the physiological processes of reserve substances hydrolysis and enzymatic activity, which inhibits the activity of the embryonic axis ultimately (Khodarahmpour, 2011; Badr et al., 2020).

The next phase of germination is characterized by the seeds' transition to a saturated steady state water supply. The water content of maize kernels was increased by 25.9% in the control group by the end of the first day of germination. At the same time, the water content of maize kernels germinated on the PEG-1500 (200 g L^{-1}) increased only by 18.3% compared to the SDW. Certainly, differences in seeds water uptake while germination processes cause some peculiarities in metabolic pathways of germinated maize and formation of general resistance of plant (Liu et al., 2015).

The nature of ontogenetic peculiarities of lipid peroxidation (LPO) processes intensity is complex and depends on many factors: the availability of substrate, the quality of the substrates, germination conditions, etc. The LPO processes and antioxidant protection of plants depend on the strength and validity of adverse factors. The data presented in Table 1 show that TBARS content is increased in all of the studied treatments during the six hours after the maize seeds germination has started.

The TBARS content in the endosperm of seeds increased in 1.75–1.89 times during 6 h after sowing under the water deficit. It is necessary to mention, that TBARS level in the seeds` endosperm, which was incubated in water, reached its maximum (7.31 µmol MDA·g^{-1}) in 2 h after sowing and then the TBARS content reduced.

Table 1. TBA-reactive substances content in germinated seeds of maize under water deficit (µmol MDA·g^{-1}, mean ± *SE*)

Time, h	Treatments				
	H_2O	PEG-1500 20 g L^{-1}	PEG-1500 50 g L^{-1}	PEG-1500 100 g L^{-1}	PEG-1500 200 g L^{-1}
0	3.99 ± 0.04	3.89 ± 0.03	4.10 ± 0.06	4.06 ± 0.13	3.87 ± 0.04
2	7.31 ± 0.14	5.72 ± 0.43*	5.81 ± 0.35*	6.84 ± 0.10	4.85 ± 0.22*
4	5.05 ± 0.19	5.42 ± 0.04	5.68 ± 0.12	6.57 ± 0.15*	6.15 ± 0.20*
6	5.70 ± 0.18	7.00 ± 0.02*	7.26 ± 0.14*	7.54 ± 0.04*	7.37 ± 0.13*
12	2.83 ± 0.08	3.78 ± 0.03*	4.03 ± 0.03*	4.11 ± 0.04*	3.96 ± 0.06*
24	4.35 ± 0.17	4.55 ± 0.08	5.18 ± 0.22*	5.21 ± 0.07*	4.70 ± 0.10

* compare to control (H_2O treatment) ($P < 0.05$).

The intensity of peroxidation process was decreased in the maize seeds of all PEG-1500 treated groups from 6 h up to 24 hours after the germination starts. The TBARS content in seeds incubated on PEG solutions of different concentration was higher by 1.23–1.32 times compared to the control group after 6 h of germination. The maximum increase of TBARS content in the maize seeds (by 1.45 times) was recorded under 100 g L^{-1} PEG-1500 treatment in 12 h after sowing. The intensity of LPO in seeds was at the range of 4.35–5.21 µmol MDA g^{-1} under all examined treatments at the end of the first day of germination. Generally, it was noted, that TBARS level in the endosperm of seeds was found higher than the control ones during the first day of germination under PEG-1500 solutions.

The data illustrated in Fig. 2 indicate the significant increase of lipid peroxidation intensity in maize coleoptiles after 3 days exposure in water deficit conditions.

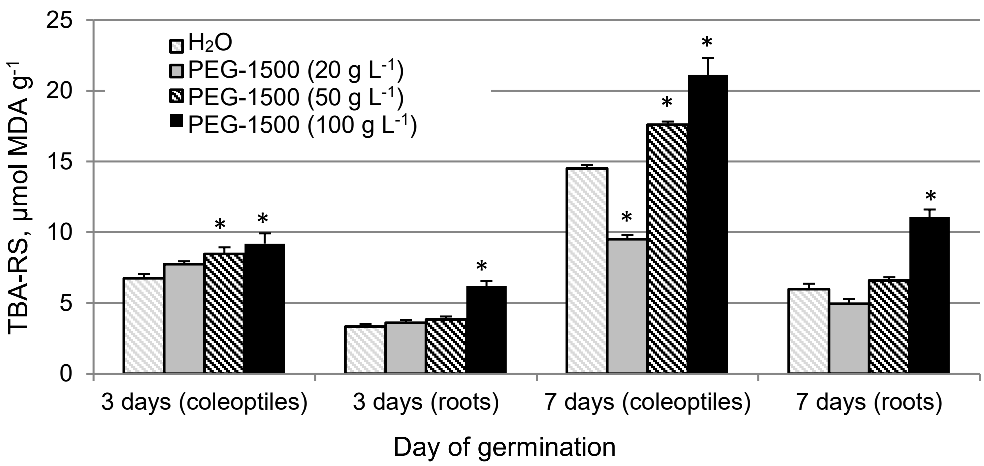


Figure 2. TBA – reactive substances content in coleoptiles and roots of maize under water deficit. Vertical line – standard error of mean; * compare to control (H_2O treatment) ($P < 0.05$).

The rising of TBARS content in seedlings tissues under water deficit gives evidence of a higher oxidative metabolism level in their cells compared with plants that germinated under normal water supply (Pyngrope et al., 2013; Anjum et al., 2017a). Moreover, the level of PEG-1500 (100 g L^{-1}) solution was found to be the most active, increasing TBARS content in maize coleoptile by 36.5% compared to the control. In addition, the TBARS content in 3 days maize roots was almost 2 times higher than in the one germinated on water under the influence of PEG-1500 (100 g L^{-1}).

The embryonic axes organs of 7-days seedlings were characterized by a high content of peroxidation products compared to the previous period of ontogenesis. TBARS level of maize coleoptiles and roots continued to increase significantly under the influence of PEG-1500 solution in concentrations of 50 and 100 g L^{-1}. Thus, TBARS content in coleoptiles and roots of 7-days maize seedlings increased by 46.2% and 84.8% respectively, under PEG-1500 (100 g L^{-1}) and compared with control seedlings. Any significant alteration in the intensity of LPO in 3 days embryonic axis maize organs were indicated under the influence of PEG-1500 (20 g L^{-1}). Although, the intensity of LPO in 7 days maize seedlings was even lower ($P \leq 0.05$) than control values under the light water deficit.

The nature of LPO processes intensification and functioning of antioxidant system depends to a large extent on the strength and duration of the unfavorable factor (Labudda, 2013; Killi et al., 2020).

It should be noted that the proline content in maize seeds decreased in the control variant by 28,6% during the first 4 hours of germination (Table 2).

Table 2. Proline content in germinated seeds of maize under osmotic stress (μg·g^{-1}, mean ± *SE*)

Time, h	Treatments				
	H_2O	PEG-1500 20 g L^{-1}	PEG-1500 50 g L^{-1}	PEG-1500 100 g L^{-1}	PEG-1500 200 g L^{-1}
0	18.89 ± 0.37	18.15 ± 0.35	19.45 ± 0.50	19.50 ± 0.85	18.94 ± 0.46
2	17.77 ± 0.41	17.70 ± 0.40	16.92 ± 0.62	16.50 ± 0.69	20.30 ± 0.81*
4	13.54 ± 0.35	13.54 ± 0.41	18.61 ± 0.56*	16.50 ± 0.63*	19.88 ± 0.75*
6	16.92 ± 0.55	16.90 ± 0.43	18.61 ± 0.68	21.15 ± 0.95*	21.57 ± 0.92*
12	19.88 ± 0.93	20.73 ± 1.05	22.00 ± 0.88	22.10 ± 1.12	22.20 ± 1.06*
24	21.15 ± 0.98	24.53 ± 0.95	24.75 ± 1.02*	24.11 ± 1.38	25.38 ± 1.11*

* compare to control (H_2O treatment) ($P < 0.05$).

However, the proline content in seeds varied slightly over the period under the influence of osmotic stress. Further, the proline content increased in the maize seeds of all groups up to the 24-hour germination period. Seeds were characterized by a consistently higher proline content compared to control seeds during the first germination day under water deficiency conditions. Thus, the proline content of maize seeds, which were treated with PEG solutions of different concentrations during the first day, exceeded the control values by 14–20% ($P \leq 0.05$).

The entry of water into the cells is the result of a concentration gradient. The accumulation of osmolytes precedes the phase of cell expansion in the germinal axis, when roots and seedlings are forming. The accumulation of osmolytes and proline, in particular, continues to support the water flow to the cell in order to keep the osmolality of the cell matrix (Naser et al., 2010; Anjum et al., 2017b). Therefore, the gradation of the osmotic stress influence was observed more evidently in the organs of the maize germinal axis.

The proline concentration increased by 1.44 and 1.19 times in coleoptiles and roots of 3-day maize germinated under PEG-1500 solution (20 g L^{-1}), respectively (Fig. 3).

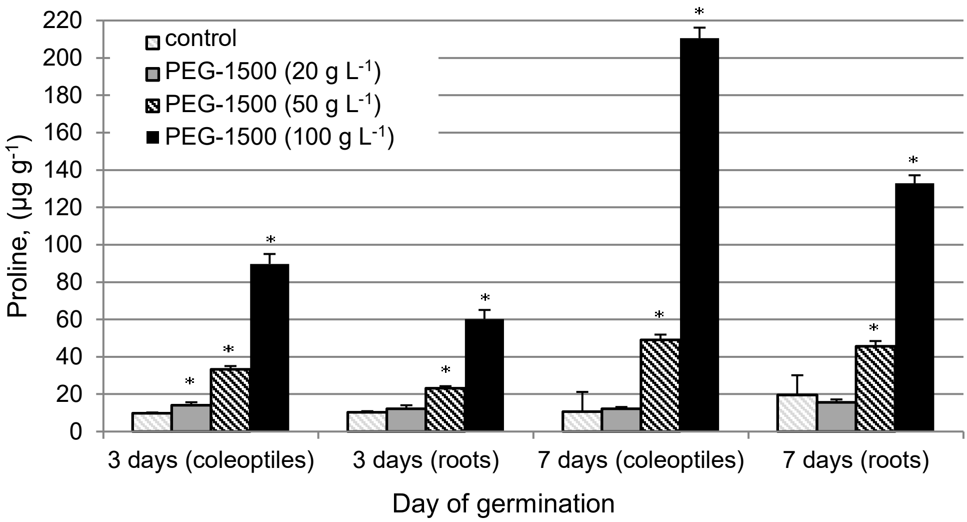


Figure 3. Proline content in coleoptiles and roots of maize under water deficit. Vertical line – standard error of mean; * compare to control (H_2O treatment) ($P < 0.05$).

Whereas, the proline content increased by 9.2 and 5.9 times respectively in the experimental tissues under the action of PEG-1500 (100 g L^{-1}) solution and compared with the control seeds. A similar trend was observed in 7-day seedlings of maize, when excessive activation and accumulation of proline in coleoptiles and roots were recorded, the content of which one was in 19.8 and 6.8 times higher than the concentration of proline in control seedlings, respectively.

A close direct correlation ($r = 0.86 \div 0.91$) between PEG-1500 concentration (osmotic potential) and tissue proline content was found out.

The accumulation of osmotic active substances is a universal adaptive response of plant organism to osmotic stress. Low molecular weight compounds and amino acids, in particular, provide the regulation of osmotic potential, the detoxification of free ammonia, and the normalization of energy metabolism under water deficiency conditions. Proline is referred to the so-called 'stress' amino acids, and the ability of plants to accumulate proline provides their osmotolerance (Tarighaleslami et al., 2012; Hosseinifard et al., 2022). The obtained data confirm the research on different varieties of maize, where it was shown that drought stress had a negative impact on chlorophyll and proline content. (Pawar et al., 2020).

In a series of papers (Raymond & Smirnoff, 2002; Verslues & Sharma, 2010), the synthesis of proline by the glutamate synthase pathway during the stress reaction was proved. It was found that there is a direct correlation between the content of proline and TBARS in germinating maize tissues. The correlation coefficient between these indexes is stronger in the maize roots ($r = 0.95$) than in the coleoptiles ($r = 0.70$) of 3- and

7-days seedlings. This fact probably indicates a higher adaptive capacity of the maize root system in the early stages of germination to osmotic stress.

Antioxidant system plays a key role in eliminating oxidative stress products. Catalase is one of the key enzymes involved in plant organism protection from free radical oxidation of biomolecules (Gomes & Garcia, 2013; Mafakheri et al., 2019).

The initial stage of maize germination is marked by enzymatic activity increased due to the hydro-stimulating initiation of protein complexes. It was observed an increase of CAT activity in the control group from 0.688 to 1.912 mcatal·mg^{-1} protein during the first 24 hours from seeds sowing (Table 3).

Table 3. Catalase activity in germinated seeds of maize under water deficit (mcatal·mg^{-1} protein, mean ± *SE*)

Time, h	Treatments				
	H_2O	PEG-1500 20 g L^{-1}	PEG-1500 50 g L^{-1}	PEG-1500 100 g L^{-1}	PEG-1500 200 g L^{-1}
0	0.688 ± 0.008	0.675 ± 0.010	0.693 ± 0.009	0.680 ± 0.011	0.626 ± 0.007
2	1.237 ± 0.019	1.396 ± 0.011*	1.412 ± 0.010*	1.291 ± 0.005	1.190 ± 0.005
4	1.047 ± 0.055	1.290 ± 0.027*	1.231 ± 0.020*	1.324 ± 0.006*	1.225 ± 0.026*
6	1.466 ± 0.023	1.524 ± 0.006	1.524 ± 0.013	1.754 ± 0.014*	1.818 ± 0.071*
12	1.224 ± 0.002	1.372 ± 0.012*	1.861 ± 0.021*	2.834 ± 0.037*	2.859 ± 0.079*
24	1.912 ± 0.019	1.756 ± 0.057	2.035 ± 0.059	2.236 ± 0.057*	2.578 ± 0.075*

* compare to control (H_2O treatment) ($P < 0.05$).

Changes in seeds CAT activity varied from 3.8 to 14.1% after 2 hours of incubation under the influence of PEG-1500 solutions compared with control. CAT activity in maize seeds of all PEG-1500 treated groups exceeded activity one in the control group after 4 hours from sowing (from 17 to 26%) and after 6 hours (from 4 to 24%). A significant increase of CAT activity in 1.20 and 1.24 times ($P < 0.05$) was proven only under the 100 and 200 g L^{-1} PEG-1500 treatment after 6 hours from seeds sowing, respectively. The maximum increase of CAT activity by 1.24, 2.34 and 1.35 times ($P < 0.05$) was recorded under PEG-1500 (200 g L^{-1}) treatment at 6, 12, and 24 hours from seeds sowing, respectively, and compared with control.

The increase in CAT activity is consistent with the increase of the TBARS content values in endosperm during the 12 hours of germination and can be considered as an adaptive response to the effect of osmotic stress.

PEG-1500 (20 g L^{-1}) solution caused a decrease in coleoptiles CAT activity of both 3- and 7-day seedlings of maize. A non-significant decrease of CAT activity in coleoptiles is associated with a decrease of LPO processes under the influence of PEG-1500 (20 g L^{-1}) solution, which does not create a pronounced effect of water deficit. Maize roots are more sensitive to water stress. An increase of CAT activity in 3- and 7-days maize roots was recorded even under PEG-1500 (20 g L^{-1}) solution. Miller et al. (2010) research believe that the change in CAT activity can be explained by the change in the content of hydrogen peroxide in coleoptiles. It was noted, that hydrogen peroxide accumulates under water stress in plant tissues (Kang et al., 2022). The activation of CAT in coleoptiles and maize roots, incubated with PEG-1500 solutions of higher concentrations, was noted. The highest stimulation of CAT activity was observed under the effect of PEG-1500 (100 g L^{-1}) solution. Thus, CAT activity in 3- and 7-days maize coleoptiles increased in 1.8 and 1.4 times ($P \leq 0.05$), respectively, under water deficit. A sharp leap of CAT activity in roots was registered when activity one increased in 13.7 and 12.3 times ($P \leq 0.05$) (Fig. 4).

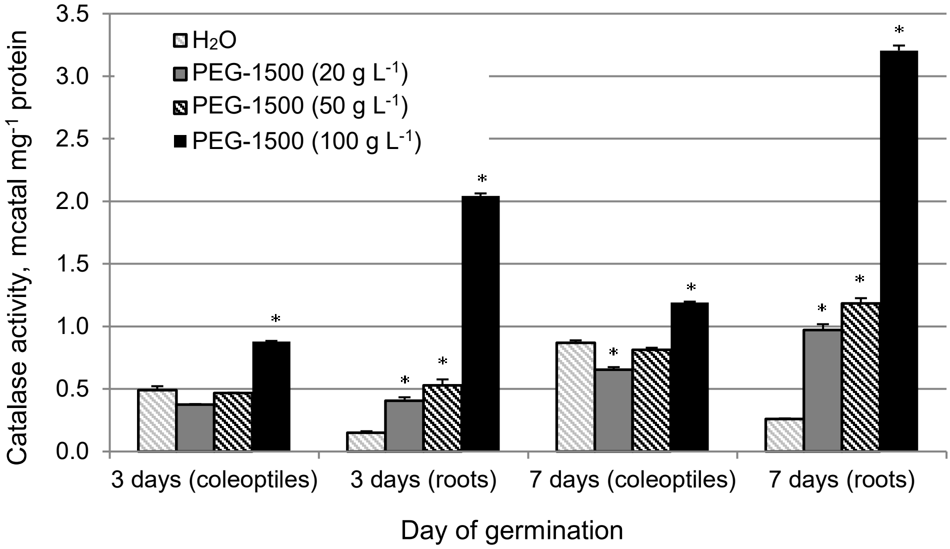


Figure 4. Catalase activity in coleoptiles and roots of maize under water deficit. Vertical line – standard error of mean; * compare to control (H_2O treatment) ($P < 0.05$).

Plants have the ability to avoid oxidative stress and may efficiently scavenge harmful ROS by up-regulating antioxidant enzymes, not only CAT but SOD, POX, and APX (Yang et al., 2021). Moharramnejad et al. (2019) reported a higher level of CAT in drought stressed maize seedling, which coordinates with SOD, POX activity. Numerous studies have reported that higher levels of antioxidants are associated with plant drought tolerance (Habibi & Hajiboland, 2011; Ashraf, 2016; Moharramnejad et al., 2019).

Probably, one of the dominant regulator of LPO processes is catalase or peroxidase in the root system, whereas in the aboveground part of plant, the regulation of peroxidation processes is carried out by low molecular weight antioxidants (Eriyamremu & Lolodi, 2010). Excessive activation of CAT in maize roots compared to coleoptiles allows keeping the TBARS content in roots at lower level than in coleoptiles under the condition of model water deficit. Similar activation of CAT was recorded in model experiments with water deficit of plants (Rafiee et al., 2011; Anjum et al., 2017a; Moharramnejad et al., 2019).

There is a high correlation between the content of peroxidation products and CAT activity in coleoptiles ($r = 0.85$) and roots ($r = 0.89$) of 3- and 7-days maize seedlings. The analysis of these dependences indicates that the root adaptation response was more active than the coleoptiles one under water deficiency.

The effect of any external factors is reflected in the protein metabolism in plants, which is associated with the ALT and AST enzymes. Alanine aminotransferase catalyzes the reversible reaction of the conversion of alanine and 2-oxoglutarate into pyruvate and glutamate. Aspartate aminotransferase catalyzes the reversible transfer of an α-amino group between aspartate and glutamate. The aminotransferase activity of maize seed endosperm increased during the first germination day. The seeds` ALT activity increased in 2.65 times and AST activity - in 1.58 times (Fig. 5).

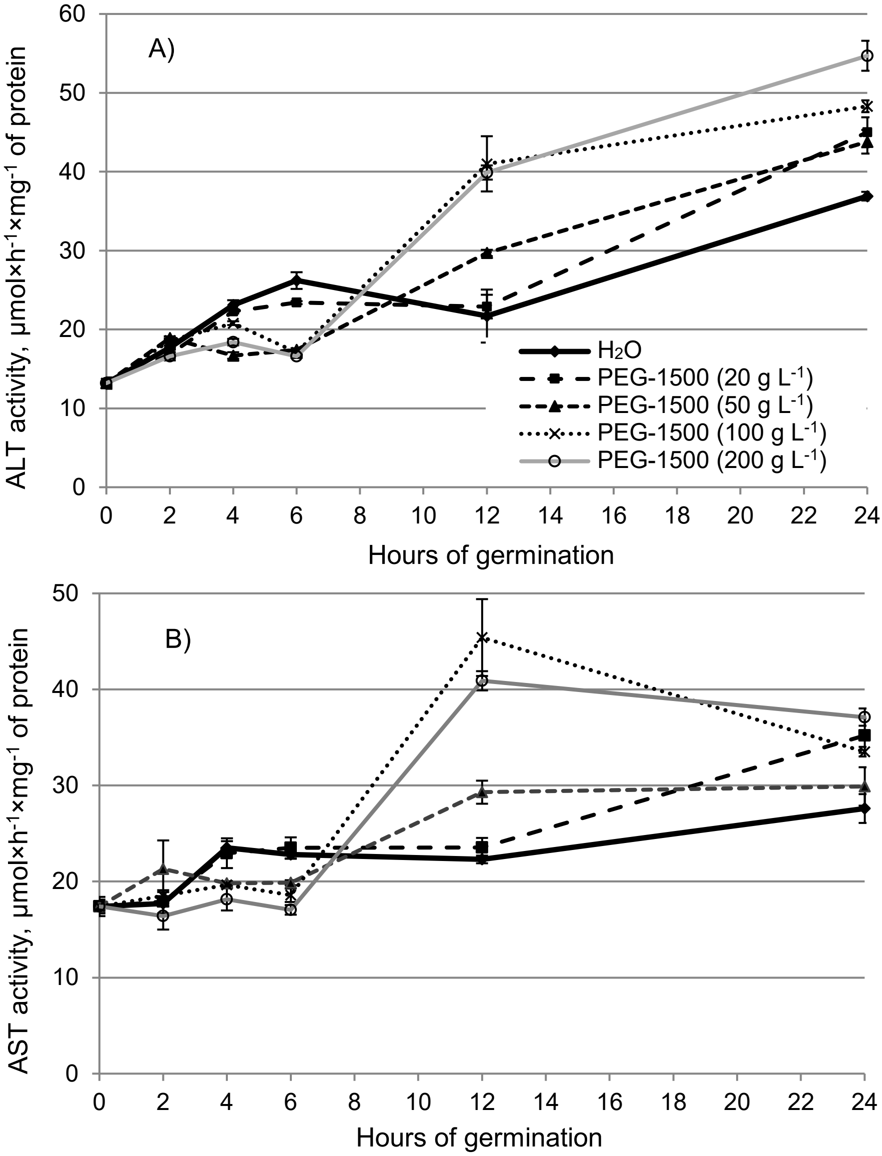
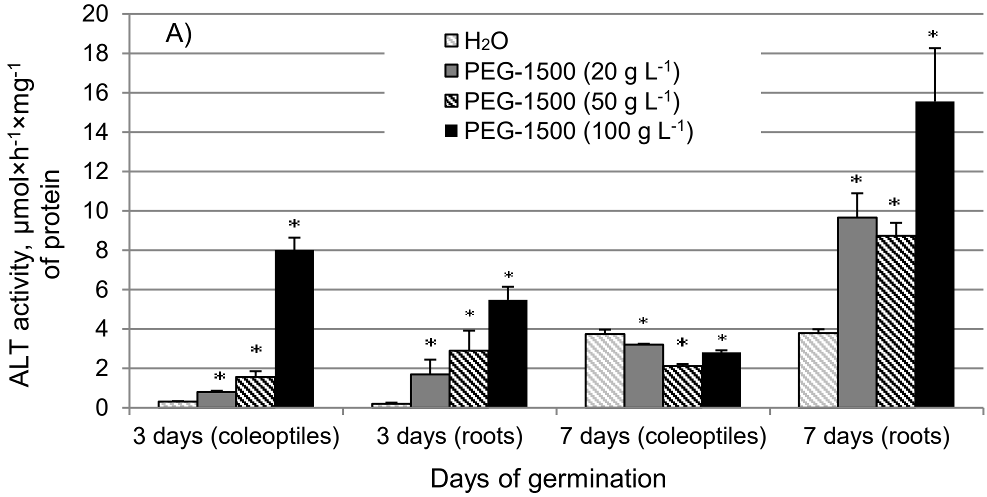


Figure 5. Alanine– (A) and aspartate– (B) aminotransferase activities in germinated seeds of maize under water deficit. Vertical line – standard error of mean.

The activity of aminotransferases under water deficiency was slightly lower than the control parameters during the phase of seed swelling. In the next phase of seed ringing, intensive division and stretching of germinal cells begins, accompanied by an increase in the aminotransferase activity of the seed endosperm under the condition of water deficiency. Moreover, the rise of ALT and AST activity of maize endosperm had a direct correlation with the osmotic potential of PEG-1500 solutions during the 12- and 24-hour seed germination periods. It was marked a close correlation ($r = 0.76 \div 0.98$) between AST and ALT activity of maize seed endosperm during the first day of germination.

The obtained data indicate that the exposure of seedlings caused a significant increase in ALT activity under conditions of model water deficit (Fig. 6).



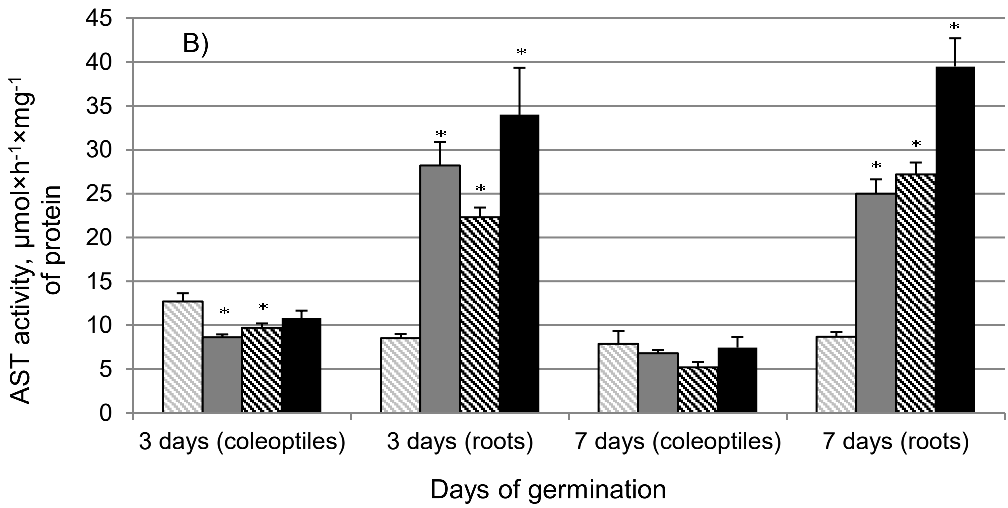


Figure 6. Alanine– (A) and aspartate– (B) aminotransferase activities in coleoptiles and roots of maize under water deficit. Vertical line – standard error of mean; * compare to control (H_2O treatment) ($P < 0.05$).

The most active stimulation of ALT activity in 3-day maize coleoptiles was observed in 25.8 times and roots - in 27.4 times when incubated in PEG-1500 (100 g L^{-1}) solution. It should be noted that the activity of ALT remains high in maize roots in 4.1

times, compared with control seedlings after 7 days seed germination under the water deficit, while some inhibition of the activity of this enzyme in the coleoptile was detected. Pyruvic acid, which is formed during the ALT reaction, is a necessary substrate for gluconeogenesis, that's why the activation of this enzyme contributes to the carbohydrate synthesis activation, which is required for further root growth. The accumulation of glutamate is a result of increased activity of transaminases, which allows to direct metabolic transformations towards the formation of proline, which together with carbohydrates is an important osmoprotector in water stress conditions (Kendziorek et al., 2012; Mostafa et al., 2021).

AST activity of 3- and 7-days maize roots incubated in PEG-1500 solutions exceeded its activity in the roots of control seedlings significantly. Thus, the activity of AST in the roots increased in 2.9–3.3 times even under the influence of PEG-1500 (20 g L^{-1}) solution. The maximum AST activation in 4.0–4.6 times was noticed in the maize roots when incubated in 10% PEG solution. However, the stimulation of AST enzymatic activity in maize coleoptiles under water stress was not observed and remained at the control level. There was a closer correlation between ALT and AST activities ($r = 0.97$) in the weekly maize roots than in coleoptiles ($r = 0.76$) under the effect of different strength water deficit.

ALT and AST play a key role in the metabolism of alanine, aspartate and glutamate, from which asparagine and glutamine are synthesized. Therefore, increasing of their activity is a prerequisite for the amide pool accumulation, as NH^{4+} donors for protein synthesis (Beatty et al., 2013). There is a lack of sufficient nitrate in maize seeds, so asparagine and glutamine may be the only source of ammonium while germination. On the other hand, keto acids that are formed in transamination reactions are also required for the renewal of the free amino acid pool and are necessary for the growth and seedlings development (Hildebrandt et al., 2015; Yu et al., 2015; Xin et al., 2018).

This fact is confirmed by the morphometric indicators of maize seedlings (Liu et al., 2015). Water deficiency adversely affected the germination of maize seeds, so the germination energy and laboratory germination of the seeds declined monotonically and according to the increased osmotic potential of the incubated solution. The most significant reduction of maize germination energy by 56.1% and laboratory germination by 50.0% was observed under PEG-1500 (100 g L^{-1}) effect (Table 4).

Table 4. The germination rate of seeds and biometrical parameters of maize's coleoptiles and roots under water deficit, (mean ± *SE*)

Treatments	Energy of germination, %	Laboratory germination, %	Raw weight of 100 un, g		Length of coleoptiles, cm
			coleoptiles	roots	
control H_2O	86.40 ± 3.24	94.17 ± 0.83	10.56 ± 0.08	13.36 ± 1.84	3.58 ± 0.09
PEG-1500 (20 g L^{-1})	87.78 ± 3.04	93.33 ± 1.36	7.97 ± 0.93*	14.12 ± 1.80	3.04 ± 0.12
PEG-1500 (50 g L^{-1})	74.44 ± 3.77*	81.67 ± 2.15*	2.77 ± 0.10*	7.70 ± 0.51*	1.31 ± 0.02*
PEG-1500 (100 g L^{-1})	30.28 ± 6.14*	44.17 ± 2.85*	1.70 ± 0.30*	3.12 ± 0.05*	0.48 ± 0.03*

* compare to control (H_2O treatment) ($P < 0.05$).

The drought induced by PEG is due to osmotic stress resulting from its higher molecular weight than any other similar compounds. The maize seeds cultivated on PEG-1500 (200 g L^{-1}) solution didn't emerge at all, although the swelling of the seeds was observed. It should be noted, that a similar fact was recorded when using PEG-6000 (Maga et al., 2019; Raj et al., 2020).

Osmotically dependent inhibition of growth processes in maize seedlings under water deficiency was observed. If the raw weight of maize coleoptiles is decreased by 24.5% under the influence of PEG-1500 (20 g L^{-1}) solution, the raw weight of roots is decreased by 42.4% only when incubated with PEG-1500 (50 g L^{-1}) solution. The water deficit solution leads to 6.2 times decrease of the raw weight of the coleoptile, and to 4.3 times decrease in the root's raw weight while using PEG-1500 (100 g L^{-1}).

The results of the study indicate a negative correlation between the germination rate of maize seeds and the intensity of peroxidation, the accumulation of proline, and enzyme activity. The inhibition of the growth processes of maize, cereals seedlings, and fruit trees under drought conditions has been shown in a number of papers (Khodarahmpour, 2011; Golabadi et al., 2015; Gerasko et al., 2019; Pawar et al., 2020).

CONCLUSIONS

In conclusion, the development of oxidative stress was observed under conditions of model water deficiency in maize seedlings. The most active inhibition of the soaking process (up to 7.5%) was observed in seeds that were growing in the solution of PEG-1500 (200 g L^{-1}). From the above findings, it can be concluded that TBARS level in the endosperm of seeds, roots and coleoptiles of maize was found higher under water stress affect. The proline content in maize seeds, roots, and coleoptiles maximum exceeded the control values when PEG-1500 (100 and 200 g L^{-1}) was used. It was confirmed that the activation of catalase in coleoptiles in 1.4–1.8 times and roots of maize seedlings in 12.3–13.7 times under water deficit. ALT and AST activities of maize seeds and roots exceeded their activity in the control seedlings significantly under PEG-1500 treatment. These changes should be seen as an adaptive strategy to water deficit.

The result also shows that an increase in water stress level decreased the germination percentage, raw weight of coleoptiles and roots, and coleoptile length of maize. The intensity of maize growth processes in the early stages of development correlates with the level of water deficits. Generally, the maximum reduction in germination, coleoptile length, raw weight of root and coleoptile was observed in the highest water stress given.

The results of this study reveal that further research is required to determine the responses of plants under environmental stresses because plants live simultaneously under the effect of multiple stress factors in natural habitats or fields.

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Migration of herbicides in the soil of agrophytocenoses and the possibility of managing the risk of contamination of environmental components

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Abstract. The migration of residual amounts of sulfonylurea and imidazolinone herbicides under conditions of washing water regime (sum of precipitation in forest and forest-steppe natural zone within 400–600 mm) in practice of intensive agricultural production has been studied. Vertical moisture transport in the soil determines the number of large pores, voids and cracks, and herbicide mobility depends on solubility of the active substance in water and adsorption equilibrium between the soil solution and the soil solid phase. The herbicide was applied to the soil surface, as is the case in practice. Then, pure water (without herbicide) was fed into the column from above, and the movement of water along the soil profile, as well as the adsorption of the herbicide by the soil and the desorption of the active substance by water, were simulated. The water supply to the column was stopped when the portions of water collected after filtering the water through the column contained less than the detection limit of herbicide residues chromatographically. The percentage content of sulfonylurea and imidazolinone herbicides residues in the soil solution varied depending on the nature of the active substance (maximum determined for imazapyr and lowest - metsulfuron-methyl) and soil type (more in acidic soil, compared to neutral and slightly alkaline soil). Effective management of pollution risks of the components of the natural environment (soil, surface and groundwater) provides a set of agrotechnological measures to reduce the number of large pores, voids and cracks in the arable layer.

Key words: pesticide, vertical mobility in soil, sulfonylurea, imidazolinone, plant protection product, pollution of groundwater.

INTRODUCTION

Intensive agricultural production is impossible without the use of chemical protection against pests. Weeds make the greatest contribution to crop yield loss, control of which includes not only agrotechnical methods, but also the use of herbicides. At present, the range of active substances and preparative forms based on them is quite wide and includes a choice of herbicides with different mechanisms of action, method of penetration and movement in the plant, spectrum of action (selectivity), etc. (Larina, 2014; Obraztsov et al., 2018; Sinitskaya, et al., 2018). In recent decades, there has been high interest in herbicides of the class of imidazolinone derivatives and sulfonide-urea derivatives, which have a broad spectrum of action, high biological activity and high selectivity, systemic prolonged action and resistance in biological media (Aichele & Penner, 2005; Paporisch, et al., 2020). But there is also an acute question about the distribution in the soil and the size of the real danger of contamination by residual amounts of herbicides of different classes of natural environment components (surface and groundwater, deep soil layers, etc.). The total balance of pesticide residues in agrophytocenoses consists of 20–65% of the applied pesticide, which is stored and destroyed at the application site, up to 10% accumulates in organic soil layers, 30–55% evaporates, 4–20% penetrates and moves through the plant, up to 5% moves into the lower (mineral) soil layers and can migrate into aquifers (Bauer & Calvet, 1999; Visokova et al., 2019; Larina & Spiridonov, 2000).

There is not enough information about the factors (soil, climate, regional features) that directly or indirectly affect the mobility of residual amounts of modern pesticides in the soil profile of different soils. It is necessary to have an idea about the nature of distribution in the soil and the extent of the real danger of soil (ground) water pollution by residual amounts of chemical plant protection products. Herbicides based on imidazolinone and sulfonylmocene derivatives have been vertical mobility in soil. What are the environmental consequences of their use? How can the risk of hazardous contamination be reduced in soil management practices? Model experiments with soil cores are a convenient tool for modeling herbicide migration under controlled conditions under the influence of a limited number of factors, study the patterns and scales of the vertical migration of the active substance in the soil profile. It is believed that if the solubility of a substance in water exceeds 10 ppm, then it is mainly carried out with the aqueous phase of the runoff. The drug with lower solubility is adsorbed on soil particles and is transported mainly with solid runoff.

The purpose of the study was to evaluate the vertical migration of herbicides in agricultural soils of various compositions under the conditions of a leaching water regime.

MATERIALS AND METHODS

Multi-year experiments in the period 2016–2020 were carried out on the variants of intensive agricultural production - ploughing and fallow land (without plowing for more than 3 years). Mobility of herbicide residues in soil (imazapyr - Imz, imazamox - Imx, imazetapyr - Imr, metsulfuron-methyl - Sfn) in forest and forest-steppe natural zone of the Central region of Russia was estimated. During the year on this territory falls the sum of precipitation equal to 400–600 mm, so there is a risk of deep penetration of herbicide residues down the profile and getting into natural waters. Grain legumes (peas, soybeans, fodder beans) were sown in the fields where imidazolinone preparations were used, while sulfonylurea preparations were used for grain crops (wheat, barley).

During the vegetation season, soil cores were taken from different depths at the experimental plots of ploughing and follow, in which residual amounts of herbicides were identified (Variant_A, field conditions). The plot size is 20 m^2. The repetition is 4 times.

The vertical migration of herbicides was studied using soil columns in laboratory conditions. Two types of columns were used in the laboratory experiment: stuffed (or several sections, disturbed structure) and monoliths (or non-separable, soil of natural structure). Monoliths (height 40 cm and diameter 10 cm) simulate the processes in the topsoil. Column under pressure was hammered into the field and then dug out without breaking the integrity of the soil core (Variant_B, laboratory conditions, undisturbed structure). In the laboratory, the soil column was dried at room temperature. Stuffed column consists of four sections (pipe 10 cm high and 10 cm in diameter) connected in series (Variant_C, laboratory conditions, disturbed structure). The soil was prepared by the thermal method to an air-dry state and passed through a sieve with a hole diameter of less than 10 mm. Each section of stuffed column was lubricated from the inside with silicone grease and stuffed with air-dry soil. The soil was added in small portions and evenly compacted in the column by light tapping on the side surface. In columns with impaired addition, soddy-podzolic soil was used - Variant_C1 or DP (humus 2.5%; pH_{sol} 4.0; mechanical composition – medium loam), leached chernozem - Variant_C2 or ChV (humus 5.1%; pH_{sol} 6.6; mechanical composition - heavy loam). Then outside, at the bottom of the column, a metal mesh, filter paper and cloth (gauze) were sequentially fixed so as not to lose soil. Then the columns were placed in a container with water and saturated with water without pressure to the level of 80% of the field moisture capacity. The following properties of soil cores were also determined: density increased from top to bottom from 1.17 to 1.68 g cm^{-3}, porosity decreased from top to bottom from 49 to 44%, organic matter content decreased from top to bottom from 3.4 to 0.8%. Then the maximum recommended rate of herbicide was applied to the soil surface. A paper filter was placed on its surface to avoid erosion of the upper soil layer and was automatically supplied by a pump distilled water to the upper edge of the column (maintaining the height of the water layer above the soil surface at least 0.5 cm). Total volume of water was equal to the amount of precipitation in the soil-climatic zone (volume of eluate 500 mm). The solubility of herbicides based on sulfonylurea and imidazolinones depends on the properties of water (pH, impurities), therefore, rainwater was not use in the experiments, due to changes (not stability) of its properties depending on the season and other reasons. Filtrate was collected in portions (volume 50 mL day^{-1}) coming from the column from below and the concentration of tested active substance (a.s.) was determined instrumentally. After the concentration of a.s. in the filtrate reached zero, the columns were dismantled. It was established experimentally that the pore water flow rate in Variant_B was lower and was equal to 3.10 ± 0.44 m day^{-1} (0.47 mm min^{-1}), compared to Variant_C - 8.42 ± 1.19 m day^{-1} (1.21 mm min^{-1}).

Table 1. Metrology of the analytical method (HPLC) for the determination of herbicides (*t-test, n* = 16, *P* > 0.05)

Active substance (a.s.)	Code	Matrix	Detection limit, ppm	Separation efficiency, *Ci ± ST*, %
Imazapyr	Imz	soil	0.02	86.4 ± 5.0
		water	0.005	88.2 ± 6.3
imazamox	Imx	soil	0.001	89.7 ± 4.4
		water	0.0002	94.6 ± 3.7
imazetapyr	Imr	soil	0.002	85.0 ± 13.4
		water	0.0004	95.5 ± 3.5
metsulfuron-methyl	Sfn	soil	0.004	75.9 ±1.5
		water	0.005	95.7 ± 1.8

Concentration of the tested a.s in the analyzed substrate (soil, water) was determined by chromatographic methods (Table 1). Based on the results of field studies, profile distribution curves of residual amounts of herbicides by soil layers at 10 cm increments to a depth of 50 cm; based on the laboratory experiments, we plotted (or washout curve) the herbicide residual amounts in successive portions of filtrate (water) from the soil core. The experimental data obtained were systematized and analysed using MS Excel 2013, StatSoft Statistica 2010.

RESULTS AND DISCUSSION

Experimental data on the distribution of residual amounts of herbicides in the soil profile of soddy-podzolic soil in dynamics are shown in Fig. 1 (Variant_A). During the growing season, the average depth with the maximum content of herbicides was 16.2 ± 14.5 cm for imazamox, 18.1 ± 14.3 cm for imazetapyr, and 10.1 ± 7.0 cm for metsulfuron-methyl. In different years of observations at the end of the season the content of residual amounts of herbicides in the arable layer was determined (in the range): for imazamox 17–25% of the applied amount, for imazetapyr 12–31% of the applied amount, for metsulfuron-methyl 10–22% of the applied amount.

Under laboratory conditions the curves of herbicide leaching from soil cores were obtained, which are characterized by a high concentration of a.s. in the first portions of the filtrate (Fig. 2). Further the curves have a decaying wave-like character, which, based on the concepts of classical chromatography, can be explained by the presence of voids in the core and heterogeneity of the material, for example due to natural processes occurring in the soil (podzolization, loessivage, etc.). Experimentally established differences in the distribution of residual amounts of herbicides in portions of filtrate obtained by washing soil cores in Variant_B and Variant_C (as an example, the curves for a.s. from the class of imidazolinone derivatives - imazapyr). The general shape of the curve for the arable layer of soddy-podzolic soil (DP) and leached chernozem (ChV) has a fuzzy and indistinct distribution, with a gradual decrease of herbicide concentration in subsequent portions of the filtrate. The shape of the imazapyr washout curve from the core (fallow) was characterized by a narrow peak in the initial section and a long gentle tail. In the first portions of the filtrate, a high concentration of active substances was determined on average 79 ± 6.2% of the applied herbicide rate, compared to soil (ploughing) - 35 ± 2.7% of the applied herbicide rate. This dependence was established for all studied a.s. from the class of imidazolinone derivatives and sulfonylmochetvina derivatives. It was determined that the concentration of herbicides in the filtrate of acidic and less humusy soils (Variant_C1) grew faster, which is important to consider in agricultural practice when imidazolinone and sulfonylurea preparations are applied to the soil surface in the conditions of washing water regime. As a result, residues of imidazolinone and sulfonylurea herbicides in the collected portions of the filtrate were recorded almost after the full application of more than 400 mL of precipitation. With the exception of imazapyr for which the risk of movement of its residues into deep soil horizons (subsoil layer) during the growing season was determined.

Variant_A

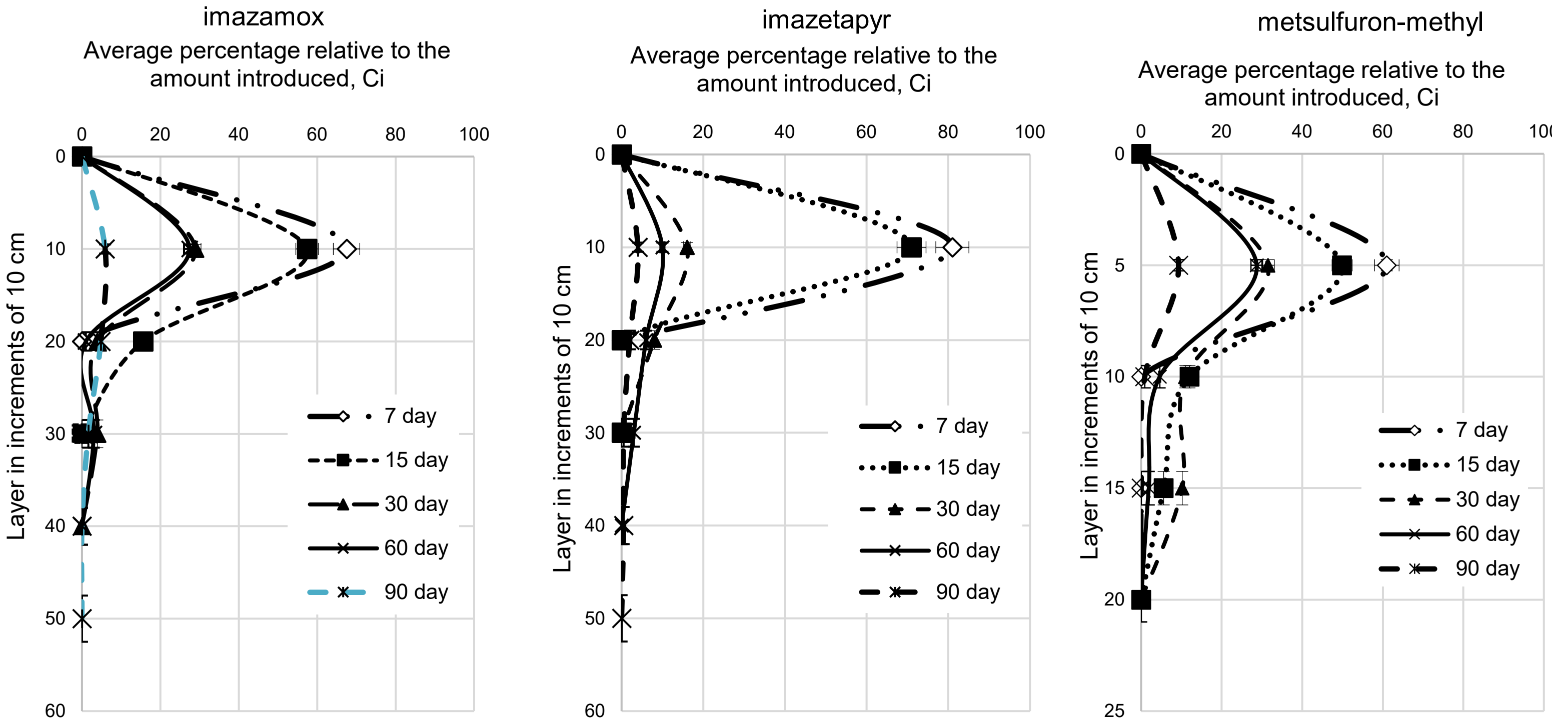

Figure 1. Plots of residual amounts of herbicides at different depths of the soil profile.

The values of adsorption coefficients (L kg^{-1}) for the studied active substances were calculated according to column experiments for different types of soils: *soddy-podzolic soil* - imazapyr (0.200) < imazamox (0.291) < imazethapyr (0.364) < metsulfuron-methyl (2.15); *leached chernozem* - imazapyr (0.023) < imazapyr (0.026) < imazamox (0.029) < metsulfuron-methyl (2.20). The low values of sorption interaction of imazapyr with the soil solid phase confirm the mobility of a.s., as well as the correctness of the assumption about the risks of contamination of natural environment components by residual amounts of this herbicide. In contrast to methsulfuron-methyl, whose residues are highly likely to remain in the place of herbicide application.

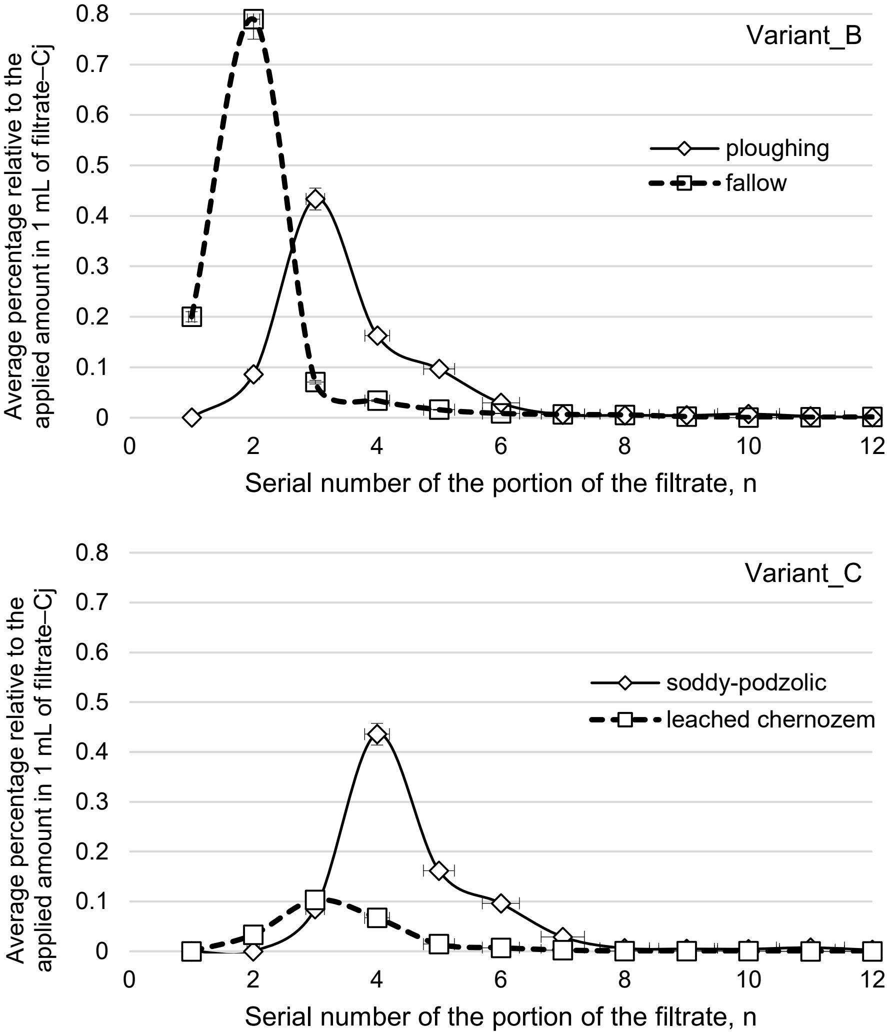

Figure 2. Elution curves of imazapyr from soil cores.

A comparative analysis of the range diagrams showed significant outliers (based on the idea of a normal distribution) for the remains of imazapyr and imazamox in the undisturbed deposit, as well as imazapyr and imazamox in the soddy-podzolic soil and imazapyr in the disturbed chernozem (Fig. 3, a–b). As was revealed in our previous studies, this is due to the heterogeneous composition of the pore space and the presence of cracks and large soil capillaries in the soil from fallow. In the arable layer (Variant_B), the soil has a homogeneous structure after the annual agrotechnical measures (Larina & Spiridonov, 2000; Larina, 2002, 2003). There are practically no cracks and voids in the topsoil, which limits the rapid movement of moisture in the vertical direction along with the herbicide dissolved in it.

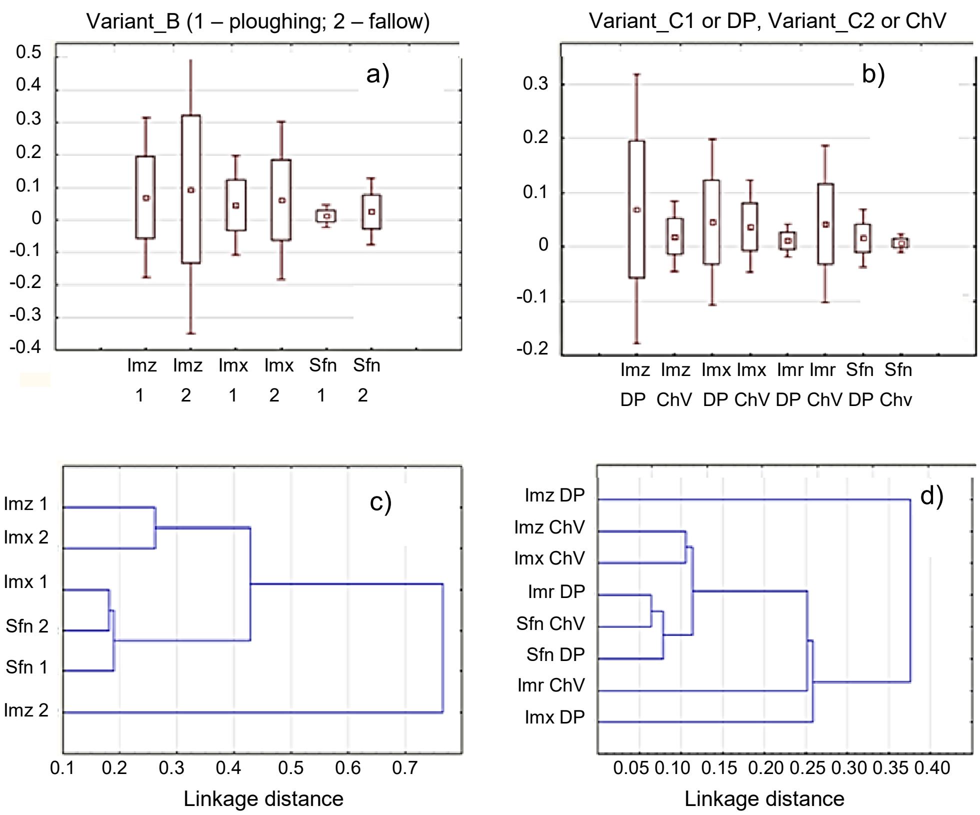

Figure 3. Boxplot (distribution of average values) for the tested herbicides (a–b) and tree diagram for variables (c–d): DP – soddy-podzolic soil, ChV - leached chernozem (*t-test*, $n = 193$, $P > 0.05$).

We carried out a hierarchical classification of the behavior of herbicides in different soils of disturbed and undisturbed addition by the method of a single connection, using the squared Euclidean (Fig. 3, c–d). It is generally accepted that the higher the level of aggregation, the less similarity between members in the corresponding class. It has been established that the behavior of imazapyr in the fallow (Variant_B) and in soddy-podzolic soil (Variant_C1) differs from other options. This fact is explained by the low values of the sorption interaction of imazapyr with the solid phase of the soil and confirms the high risks of pollution of the components of the natural environment with residual amounts over long distances from the place of application.

CONCLUSIONS

The maximum percentage of residual amounts from the applied norm of the herbicide, found in the soil solution, corresponds to imazapyr, the minimum - to metsulfuron-methyl. An intermediate position is occupied by imazamox and imazethapyr. The vertical movement down the profile of water and herbicides dissolved in it depends on the uniformity of the arable layer and the amount of precipitation. In the acidic soddy-podzolic soil, herbicides migrated deeper, in contrast to the weakly alkaline soil (chernozem). The vertical migration of imidazolinone and sulfonylurea preparations was directly dependent on the solubility of the active substance in water and the sorption balance in the soil solution and soil solid phase system. Under the conditions of the leaching water regime (or seasonal showers), the maximum movement of imazapyr residues into the subsoil layer (depth of more than 0.5 m) was observed during the growing season. High environmental risks of pollution of natural (ground) waters by residual amounts of herbicides from the class of imidazolinone derivatives are noted.

Therefore, to manage the environmental risks of the soil, it is necessary to carry out high-quality agrotechnical measures (plowing, harrowing). This will eliminate excessive soil porosity (cracks) and the heterogeneity of the structure of the arable layer and improve the ecological situation of the soil profile.

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Effect of potassium application rate and timing on alfalfa yield and potassium concentration and removal in Tennessee

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Abstract. Alfalfa (*Medicago sativa* L.) is the most important forage crop in the world and potassium plays a significant role in achieving high yields. A field experiment was conducted during the 2012–2014 growing seasons at the University of Tennessee in Springfield. The experimental design was a split-split plot that included four levels of potassium (K) application rates (0, 67.25, 134.50, and 269.00 kg K_2O ha^{-1}) as the main plots and two K application times (green-up and split) as the subplots, and harvest dates as sub-subplots. The results demonstrated that the dry matter yield (DM) increased significantly with each increment in K application rate up to 134.50 kg K_2O ha^{-1}; however, the highest K fertilizer application (269 kg K_2O ha^{-1}) did not result in a significant yield increase relative to 134.50 kg K_2O ha^{-1}, because some luxury consumption of K occurred at the highest rate due to yield leveling off while K_2O uptake continued to rise. Potassium concentration and K removal increased with K fertilizer at rates beyond those that maximized yield, indicating luxury consumption of K. The greatest K concentration and removal were recorded at 269 kg K_2O ha^{-1} in all harvest months. The split application was more beneficial than applying full K at the time of green-up due to higher dry matter, K concentration, and K removal in alfalfa. In conclusion, 134.50 kg K_2O ha^{-1} is adequate for maximizing alfalfa yield; split application of K is sometimes superior to the single dose of K fertilizer in alfalfa production.

Key words: concentration, dry matter yield, fertilizer, *Medicago sativa*, nutrient removal.

INTRODUCTION

Alfalfa (*Medicago sativa* L.), the 'queen of forages', is the premier legume forage and its yield depends on stand establishment, proper harvest times, and fertilization (Dordas, 2006).

Alfalfa has a deep and extensive rooting system that improves soil structure, soil fertility, and soil organic matter content (Bourgeois et al., 1990). An adequate supply of nutrients is important for maintaining high alfalfa forage quality and profitable yields (Moreira et al., 2008). A low potassium level in the soil can result in increased winterkill of alfalfa plants (Jungers et al., 2019). Potassium fertilizer application increases plant K uptake and forage yield if soil test K levels are deficient (Jungers et al., 2019). Potassium uptake by plant is affected not only by the source and application rate, time, and placement of K fertilizer, but also by soil properties (if the fertilizer is applied to soil) and weather conditions including temperature and rainfall. Most of K fertilizers are water soluble and immediately available for plant to take up (Morgan & Connolly, 2013).

Alfalfa has an extremely high requirement for K. It removes more K than any other minerals over time due to high yields or under-intensive alfalfa production (Koenig et al., 2006). Under these conditions, sound K fertility management is essential (Berg et al., 2007). The amount of K fertilizer required depends on the existing level of K in the soil, the tonnage of alfalfa removal from the previous year, and the related soil chemistry (Wolde, 2016). Potassium has a crucial role in alfalfa growth and reproduction and physiological processes within the plant (Lu et al., 2018). Adequate K nutrition increases the long-term productivity and stands longevity of alfalfa (Berg et al., 2007).

Potassium content in crops depends on soil type (if the fertilizer is applied to soil), crop species, doses of K fertilizer, and weather conditions (Askegaard et al., 2004; Khajbullin et al., 2020). Alfalfa can show 'luxury consumption' of K when plants are taking up more K than needed to maximize yield (Macolino et al., 2013). This can lead to a reduction in protein, Ca, Mg, and Na (Pant et al., 2004). As a result, increased K application does not always lead to higher yield (Berg et al., 2018). Split applications of K can lower the risk of alfalfa plants over-consume available K and are considered more effective than a single application of K (Kafkafi et al., 1977).

The effect of harvest time on yield, quality, and profitability of alfalfa may be more important than cultivar choice and other management practices (Orloff & Putnam, 2006). In alfalfa production, yield and quality are inversely related, so if it is harvested at the early maturity growth stages, the level of forage quality increases but the yield decreases (Lamb et al., 2006; Brink et al., 2010). The quality of alfalfa is increased by decreasing cutting intervals (Rimi et al., 2012). However, repeated harvesting of undeveloped alfalfa may lead to reduced yield and plant viability (Kallenbach et al., 2002).

There was inadequate information on K management for maximum alfalfa yield in Tennessee (TN) of the United State (the Mid-South region of the United States). The objectives of this study were to: 1) determine the sufficient and accurate K rate recommendations for alfalfa in TN, 2) evaluate the effects of splitting K applications on alfalfa yield in TN soils.

MATERIALS AND METHODS

A field experiment was conducted at the Highland Rim AgResearch and Education Center of the University of Tennessee in Springfield, Tennessee, United States (N 36° 30' 33.1632", W 86° 53' 5.9928") from 2012 through 2014. The annual average air temperature and precipitation for the area are 15.22 °C and 1,234.44 mm, respectively. Mean monthly temperatures and precipitation for 2012 to 2014 are presented in Table 1. Springfield's climate is classified as warm and temperate. The climate is classified as Cfa by the Köppen-Geiger system.

Table 1. Monthly average air temperature and precipitation and their 30-year means (1984–2014) in Springfield, TN, United States

Months	Precipitation (mm)				Temperature (°C)			
	2012	2013	2014	30-year mean	2012	2013	2014	30-year mean
January	115.3	169.4	81.0	99.8	5.2	3.7	-1.8	1.7
February	40.4	66.0	169.2	104.9	6.2	3.8	1.5	3.7
March	141.2	128.3	95.0	116.3	15.7	4.9	5.9	8.5
April	71.6	248.9	150.4	122.4	15.2	13.4	15.5	13.9
May	200.2	169.9	55.4	138.4	21.5	18.5	20.6	18.6
June	31.0	88.6	67.8	102.9	23.3	23.6	24.9	23.3
July	187.2	230.1	72.4	108.2	27.6	23.4	24.0	25.2
August	77.2	139.7	143.5	82.8	24.1	23.9	25.9	24.7
September	120.4	124.2	30.0	92.7	20.4	21.6	21.5	20.8
October	96.5	83.6	206.2	96.5	13.9	15.3	15.6	14.5
November	36.3	111.3	70.4	104.6	7.4	6.8	5.0	8.8
December	194.6	109.7	79.2	119.4	7.2	4.0	4.8	3.6

The soil series was staser loam soil (Fine-loamy, mixed, active, thermic Cumulic Hapludolls), having a loam texture. The composite soil sample was collected from 6 random locations within each sub-subplot (15.24 cm-depth) using a 1.9-cm steel soil probe for fertility assessment for three years (2012, 2013, and 2014). The samples were dried in a forced-air oven at 60 °C prior to analysis and their final values were reported on a kg ha^{-1} basis. Potassium, calcium, magnesium, and phosphorus were extracted with Mehlich-1 and determined using Inductively Coupled Argon Plasma spectroscopy (ICAP) (Mehlich, 1953) (Table 2).

Table 2. Selected soil properties for the surface 0 to 6-inch layer at the study site in 2012, 2013, and 2014

Year	K rate (kg K_2O ha^{-1})	K (kg ha^{-1})	Ca (kg ha^{-1})	Mg (kg ha^{-1})	P (kg ha^{-1})
2012	0	60.75	45.61	1,767.02	100.54
	67.25	62.20	50.10	1,748.52	91.90
	134.50	56.60	61.08	1,875.40	96.39
	269.00	64.44	113.99	1,760.52	102.55
2013	0	67.81	51.22	3,115.62	133.60
	67.25	63.88	57.38	2,967.42	124.97
	134.50	51.22	65.56	3,247.10	127.44
	269.00	50.99	97.73	2,875.54	116.23
2014	0	77	74.53	2,754.15	122.73
	67.25	61.64	61.08	3,053.19	124.41
	134.50	62.43	76.21	2,480.44	118.24
	269.00	75.65	83.16	2,578.51	121.05

The experimental area consisted of a split-split plot design with four replications. The same plots were evaluated from 2012 through 2014. The entire plot area was seeded with alfalfa (Cropland Consistency 4.10 RR) with 8 inches between rows in April of 2012. The experiment consisted of four levels of K application rates (0, 67.25, 134.50, and 269.00 kg K_2O ha^{-1}) as the main plots and two timings of K application [Green-up (this stage began when the crown buds started to grow in response to warmer temperatures during the spring) and split] as the subplots and harvest dates were the sub-sub plots. Potassium fertilizer was K oxide (K_2O, containing 83% K). The K fertilizer was broadcast applied by hand on the soil surface, and all amounts of K fertilizer were split and brodcast to each designated plot. The phosphorus (134.50 kg ha^{-1} P_2O_5) and boron (1.12 kg ha^{-1} B) were applied each spring at green-up. Sub-subplots measured 3-m ×3-m with the middle 1-m ×3-m harvested. Alfalfa yield estimates were determined from center harvest strips with a carter harvester (Carter Manufacturing Company). The crop was harvested at pre-bloom of each year (approximately 30-d between each harvest) (Table 3).

Table 3. Harvest dates at Springfield from 2012 to 2014

Year	First cut	Second cut	Third cut
2012	July	August	September
2013	May	Jun	July
2014	May	Jun	July

Crop measurements included DM, tissue K concentration, and subsequent calculation of K removal. To determine the crop measurements, grab samples of about 1.1 to 2.2 kg ha^{-1} were taken from every sub-subplot and dried in an air-forced oven at 60 °C for 72 hours then samples were ground and prepared for lab analysis. Alfalfa tissue K concentration was determined with nitric acid digestion and Inductively Coupled Plasma (ICP) spectrometry analysis of the diluted digest. For nitric acid digestion of the sample, 5 mL of 65% HNO_3 was added, and then the mixture was boiled gently over a water bath (90 °C) for 1–2 h or until a clear solution was obtained. Later, 2.5 mL of 65% HNO_3 was added, followed by further heating until total digestion (Zheljazkov & Nielson, 1996). Potassium removal by the plant was calculated by dividing the percent elemental nutrient concentration by 100 and multiplying the quotient by the DM (Murrell, 2008):

K removal (kg K_2O ha^{-1}) = DM (kg ha^{-1}) × [(K concentration(%)) /100] ×1.20

The data were subjected to analysis of variance using the Proc Mixed Model procedure of SAS 9.4 (SAS Systems Inc., Cary, NC) to determine the effects of K rate, the timing of K application harvest time, and their interactions. In the Proc Mixed Model, K rate, the timing of K application, harvest time, and their interaction were considered as fixed effects, while the replicates were set as random effects. The means were separated with the Fisher's protected Least Significant Difference (LSD) method at the 0.05 significance level.

RESULTS AND DISCUSSION

Dry Matter Yield: The analysis of variance for DM showed significant K rate and harvest time effects for all years, but no significant timing of K application effects was observed; on the other hand, the two-way interaction between timing of K application and harvest time was found significant for DM in 2013 (Table 4). The greatest mean DM was observed for the second harvest date (3.83 ton ha^{-1}), while the third harvest resulted in the lowest mean DM yield (2.44 ton ha^{-1}) averaged across all years (Table 5). Third-cut forage generally has lesser digestibility and intake than first and second-cut forage. Under warmer conditions, greater amounts of energy are used to produce cell well components and reproductive tissue, resulting in less digestibility and lower intake potential (Atis et al., 2019).

Table 4. Results of analysis of variance for effects of K rates, K timing, and harvest time on alfalfa DM yield, tissue K concentration, and K removal

		Yield			Tissue K concentration			K removal		
Sources	Df	2012	2013	2014	2012	2013	2014	2012	2013	2014
R	3	0.55**	0.20**	0.32**	0.32**	0.37^{ns}	0.39**	1,656.15**	1,668.89**	2,048.20**
H	2	1.43**	4.98**	13.18**	7.84**	1.56**	0.38*	5,989.19**	11,039.70**	29,905.57**
Error a	6	0.03^{ns}	0.05^{ns}	0.07^{ns}	0.04^{ns}	0.13^{ns}	0.10^{ns}	90.15^{ns}	283.58^{ns}	194.76^{ns}
K	3	0.13**	0.29**	1.22**	4.17**	7.31**	4.16**	2,848.25**	19,738.57**	15,875.96**
H × K	6	0.007^{ns}	0.04^{ns}	0.01^{ns}	0.13*	0.57*	0.30**	132.89^{ns}	1,385.65**	1,479.67**
Error b	27	0.03**	0.06*	0.06^{ns}	0.04^{ns}	0.20^{ns}	0.05^{ns}	49.10^{ns}	494.7^{ns}	142.48^{ns}
T	1	0.04^{ns}	0.02^{ns}	0.28*	0.26*	0.09^{ns}	1.19**	394.74*	1,005.14^{ns}	3,767.33**
H × T	2	0.01^{ns}	0.18**	0.07^{ns}	0.009^{ns}	0.50^{ns}	0.04^{ns}	25.67^{ns}	899.07^{ns}	62.65^{ns}
K × T	3	0.02^{ns}	0.04^{ns}	0.04^{ns}	0.01^{ns}	0.28^{ns}	0.24*	15.84^{ns}	64.21^{ns}	566.81^{ns}
H × K ×T	6	0.009^{ns}	0.02^{ns}	0.01^{ns}	0.007^{ns}	0.35^{ns}	0.12^{ns}	14.40^{ns}	859.78^{ns}	115.82^{ns}
Error	36	0.01	0.03	0.06	0.04	0.17	0.07	69.58	364.84	311.97

Note. Values in this table are mean squares. Replication (R), Harvest time (H), K rates (K), Timing of K application (T). * Significant at the 0.05 probability level. ** Significant at the 0.01 probability level. ns, not significant.

The DM yield of alfalfa was not significantly different between 134.50 and 269 kg K_2O ha^{-1} (Table 5). On average, fertilized plots had a higher DM yield than unfertilized plots, so that, the DM yield was about 24% higher at 269 kg K_2O ha^{-1} than the unfertilized plots (Table 5). The reduced yield in the unfertilized plots in the third year seemed to have been due to the association of no K application resulting in low K content in the soil, as often reported that alfalfa yield depends on soil K fertility (Berrada & Westfall, 2005). The results of Buskiene & Uselis (2008) studies revealed that when the rate of K fertilizers was increased from 90 to 240 kg ha^{-1}, K content in the soil increased to 33%. These yield changes provided a good opportunity to measure the effect of K fertility on alfalfa yield. Our findings confirm the results of other studies, that reported a positive influence of K on alfalfa yield (Lutz, 2008; Lioveras et al., 2001).

Table 5. Mean DM yield affected by K rates and harvest time

Treatments	DM yield (ton ha^{-1})			Average
Harvest date	2012	2013	2014	-
First cut	1.70 b	3.85 b	3.21 b	3.31b
Second cut	2.28a	5.02 a	5.40 a	3.83a
Third cut	1.34c	3.29 c	2.69 c	2.44c
K rate (kg K_2O ha^{-1})				
0	1.63b	3.69b	3.11c	2.80c
67.25	1.68b	4.07a	3.63b	3.11 b
134.50	1.79ab	4.23a	4.10a	3.36 a
269.00	1.99a	4.21a	4.25a	3.47 a

Note. Within a column, means followed by the same letter are not significantly different according to *LSD* (0.05).

Dry Matter yield versus K_2O uptake: Application of high rates of K fertilizer to achieve maximum yields will result in luxury consumption of potassium. The results of the present investigation revealed that some luxury consumption of K occurred at the highest K rate due to yield leveling off while K_2O uptake continued to rise (Fig. 1). The continuing rising K removal seemed to indicate that alfalfa takes up excessive K without increasing DM yield. Therefore, large amounts of K should not be applied to alfalfa as a single dose during the growing season. Our results suggest that a K rate of

134.50 kg ha^{-1} is adequate for maximizing alfalfa yield; higher K rates will not significantly increase yields (Fig. 1).

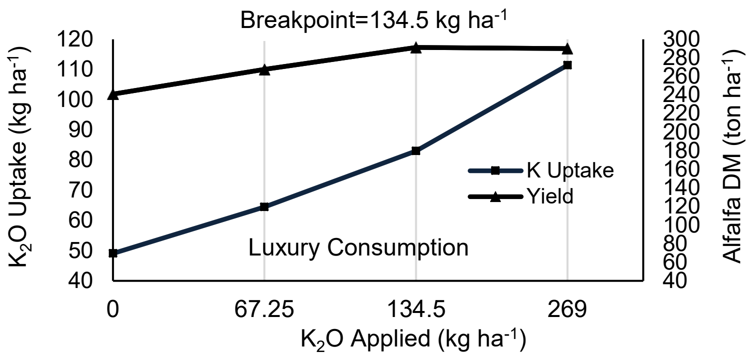

Figure 1. Impact of K rate on DM yield in Springfield, TN from 2012 through 2014.

When K concentration in the soil increases, alfalfa will take up K in proportion to the K concentration in the soil, much higher than the K amount needed for normal growth and development of the crop. This process is often referred to as luxury K consumption. Luxury K consumption often leads to excessive-high K concentration in plant tissues, increased K removal from the field, and reduced economic return (Murrell et al., 2021). Luxury consumption of K by alfalfa confirmed that the yield of alfalfa with K application is improved only to a certain point, after which yield does not increase with additional K fertilizer application (Lioveras et al., 2001; Berg et al., 2018). The result of research by Loide (2004) indicated that as the potassium content increases further, the yield of red clover and ryegrass begins to decrease.

Timing of K Application on DM Yield: The K application time had a significant effect on DM yield only in 2014 (Table 4). The results indicated that split application was more beneficial than applying full K at the time of green-up in terms of DM in one out of three years. The DM yield was around 7% higher in the split application than that under the single application (Table 6). The split application reduces the soil fixation of K and favors the uptake of K during the entire growing season, thereby increasing the yield (Annadurai et al., 2000). Consumption of K rate in split dose reduces competition between microorganisms and plants, luxury consumption, leaching losses, and K fixation processes at the critical plant growth stages (Lu et al., 2014).

Table 6. Mean DM yield, tissue K concentration, and K removal affected by the timing of K application

Timing of K application	Parameters	2012	2014	Average
Green-up	Yield (ton ha^{-1})	-	4.02 b	-
Split		-	4.29 a	-
Green-up	K concentration (%)	1.49 b	1.54 b	1.51 b
Split		1.60 a	1.76 a	1.68 a
Green-up	K removal (kg ha^{-1})	32.03 b	71.67 b	51.85 b
Split		36.58 a	85.71 a	61.01 a

Note. Within columns, means followed by the same letter are not significantly different according to *LSD* (0.05).

Timing of K Application on K Concentration and Removal: The tissue K concentration and removal were significantly affected by the timing of K application in 2012 and 2014 (Table 4). The split application recorded 11% and 18% higher K concentration and K removal than the green-up single application, respectively (Table 6). The continuous supply of K through split application results in higher availability of applied K to the plant (Anji et al., 2018, Sharma & Singh, 2021). The K use efficiency in the split application of K fertilizer is higher than its single application due to the reduction in leaching losses and luxury consumption of K (Tandon & Sekhon, 1988). The benefits of the split application may also be contributed to higher soil buffering capacity with less K fixation (Römheld & Kirkby, 2010; Wani et al., 2014). Split application of K has improved K availability during the growing season, which contributes to better plant metabolic activities, resulting in K uptake and higher yield (Tariq & Shah, 2002; Sheng et al., 2004).

Interactions of K Rates and Harvest Time on Tissue K Concentration and Removal: The interactive effects of K rates and harvest time were significant on both tissue K content and K removal in all three years except K removal in 2012 (Table 4). Potassium concentration for the whole plant increased with increasing K fertilization rates, ranging from 0.71% for the unfertilized treatment in 2012 to 3.46% for the 269 kg K_2O ha^{-1} treatment in 2013 (Fig. 2). Higher application rates of K increased K concentration in forage (Fig. 2).

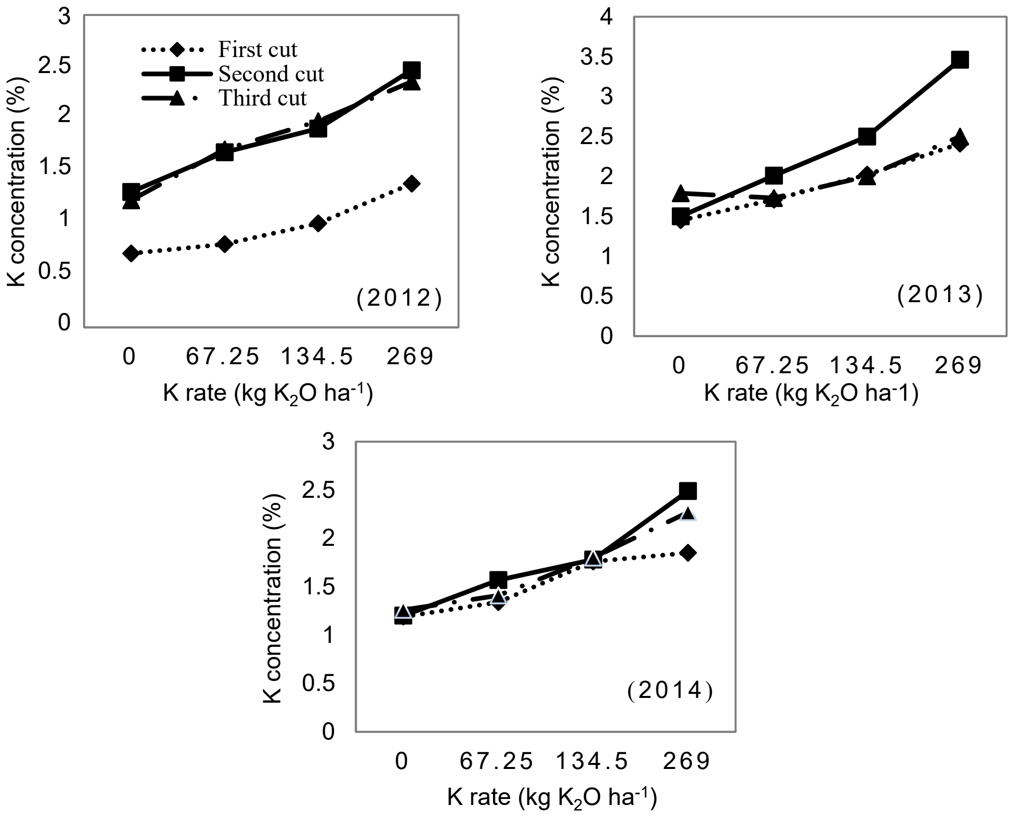

Figure 2. Effects of K rates and harvest time on tissue K concentration in 2012, 2013, and 2014.

Increases in the concentration of alfalfa K with increasing K fertilization have been reported frequently (Snyder & Leep, 2007; Jungers et al., 2019). The increase of K concentration in alfalfa in response to high K_2O application levels may be explained by the luxury consumption of this nutrient (Pant et al., 2004; Snyder & Leep, 2007).

The highest and lowest concentrations of K in plants were observed in the second and third cut in all years, respectively. In 2012, 2013, and 2014, the tissue concentration of K in third cut was 90%, 130%, and 107% higher at the rate of 269 kg K_2O ha^{-1} than those with the unfertilized plots, respectively (Fig. 2). Generally, second cut harvesting resulted in greater K concentration in the biomass than first cut harvesting (Fig. 2). The total 2-yr amount of K removal by harvest increased with increasing K rates (Fig. 3).

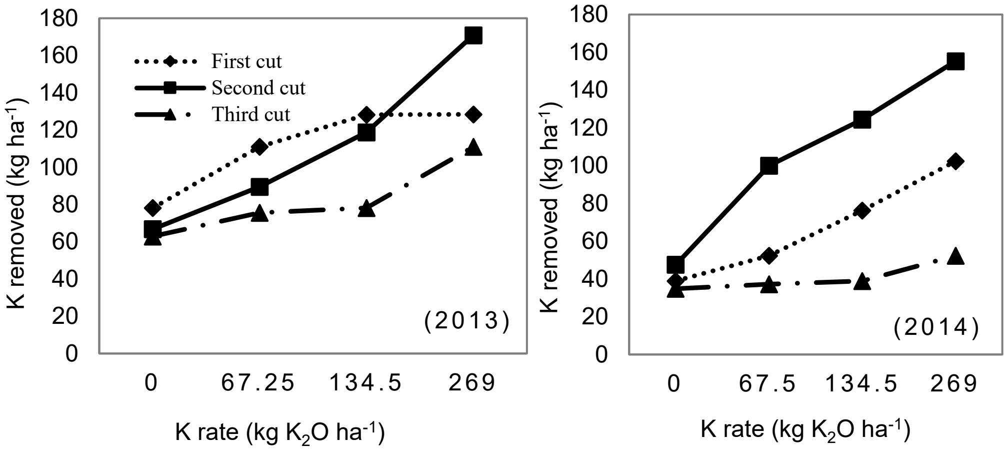

Figure 3. Effects of K rates and harvest time on K removal in 2013 and 2014.

The 2-yr removal of K varied between 34.83 kg ha^{-1} for the unfertilized treatment to 174.11 kg ha^{-1} with the application of 269 kg K_2O ha^{-1} in 2014. The highest value of K removal was recorded with 269 kg K_2O ha^{-1}in the second cut in 2013 and 2014, respectively (Fig. 3).

CONCLUSIONS

The forage concentrations of K were lower from plots unfertilized than those fertilized with 134.50 and 269 kg K_2O ha^{-1}. Meanwhile, in the K-applied plots, K concentration in the forage depended on harvest time and was much higher in the second cutting than in the first cutting. Potassium positively influenced alfalfa yield but should not over-apply K, because plants could engage in luxury consumption, leading to increased tissue K concentrations. The split application of K significantly affected dry matter yield, tissue K concentration, and K removal. The results of the three-year experiment suggest that 134.50 kg K_2O ha^{-1} is adequate for maximizing alfalfa yield; split application of K is sometimes superior to the single dose of K fertilizer in alfalfa production.

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Assessment of the relation between the adaptive potential of oilseed radish varieties (*Raphanus sativus* l. var. *oleiformis* Pers.) and chlorophyll fluorescence induction parameters

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Abstract. The possibility of optimization of the system of varietal identification, particularly of cruciferous crops in the breeding programs an urgent task that needs a scientific solution. A system comparison of a complex model for assessing genotypes for plasticity and stability with indicative basic and derivative indicators of the chlorophyll fluorescence induction curve (CFI) is proposed as a possible solution to this problem. 14 varieties of oilseed radish of different ecological and geographical origin were chosen as the object of research. Widely tested indicators of both methodological approaches were applied, taking into account the agrotechnological aspects of the analysis of oilseed radish plants for uniformity and stability.

The varietal specificity of the basic indicators of the induction of chlorophyll fluorescence of oilseed radish in response to changes in the stress level of the years of the research period was established. The determined interval of correlation-regression dependencies at the level of -0.382–(-0.658) ($p < 0.05$–0.01) between the indicators of genotypic stability (G_p), adaptability (b_i), and selection value (S_v) and the basic indicators of the CFI curve such as minimal fluorescence (F_0), fluorescence of the 'plateau' zone (F_{pl}), maximal fluorescence (F_m) and fluorescence stationary level (F_{st}). Direct correlations were determined at the level of 0.652–0.745 ($p < 0.01$–0.001) in the same comparison system for maximal fluorescence (F_m), fluorescence rise (dF_{pl}), maximum variable fluorescence (F_v), photochemical efficiency (EP), leaf water potential (L_{wp}), plant viability index (RF_d), efficiency of the initial reactions of photosynthesis (K_{prp}), coefficient of decline of the fluorescence (K_{fd}). The indicated pair correlation dependences were confirmed by the results of multiple regression analysis for the value of multiple regression coefficients (R) in the interval of 0.793–0.833 ($p < 0.05$–0.01).

On the basis of the obtained data, an integrated version of the assessment of varieties of cruciferous crops is proposed, which allows optimization of the system of varietal identification, particularly of cruciferous crops, in the breeding programs of their pre- and post-registration study, as well as the system of searching for donors and sources of relevant traits in the breeding hybridization programs at various levels. In terms of further research, it will be promising to apply this variant of the analysis to wild species of cruciferous crops in a single complex with their cultural forms to assess the regularities of the formation of adaptations under the directed trait selection process.

Key words: adaptability, chlorophyll fluorescence, oilseed radish, plasticity, varieties.

INTRODUCTION

Modern approaches to the assessment of plant stress-adaptation are based on determining reliable criteria for assessing their ability to reduce sustainable levels of biological productivity when environmental factors change by a value that differs significantly from the biological optimum for a given plant species (Pandey et al., 2021). There has been a wide range of methodological approaches to identifying, recording, and applying such criteria over many years of research. This range included both indirect methods based on mediated plant responses to the stress factor and direct methods based on explicit morphological and physiological changes in plants caused by the relevant stress factor (Mable, 2019). For example, direct methods include germination of seeds in appropriate osmotic solutions of different concentrations, while associating the nature of liquid uptake and the speed of seed germination with the traits of drought tolerance of a species or genotype (Marcińska et al., 2013). Other methods include cultivation in special drying tanks (Marchin et al., 2020), the use of climatisation chambers, the creation of artificially created germination regimes in a phytotron or greenhouse system (Bartlett et al., 2016; Snowdon et al., 2021).

The range of indirect methods also includes a wide range of methodologies. The main and well-known ones include proline test (Signorelli, 2021; Berka et al., 2022), high and low temperature heat stress protein spectrum analysis (Jacob et al., 2017; Chi et al., 2019; Khan et al., 2021), thermal imaging study of plants with radiation spectral composition analysis using dedicated thermal imaging cameras (Pineda et al., 2020), and a method that has been used for a long time, but which has recently become increasingly popular due to the introduction of devices that give a new approach to its use in experimental work, namely, the chlorophyll fluorescence induction method (Kalaji et al., 2017a). This method is effective in assessing the state of plant stress reactions, which are displayed on the corresponding chlorophyll fluorescence induction curve (CFI) (Saglam et al., 2020). Under these conditions, the indicated reaction curve is effective in determining agrotechnological stress factors as well. The analysis of the dynamics of the curve allows to determine the mechanism of adaptations of the plant organism to changes in the edaphic environment (Kalaji et al., 2017b; Tsai et al., 2019; van Bezouw et al., 2019; Schuback et al., 2021; Valcke, 2021).

It is also noted the importance of identifying crop varieties and hybrids for stress tolerance in terms of plasticity and stability (Najafi et al., 2018; Macholdt et al., 2020; Tryhub et al., 2020; Prysiazhniuk et al., 2021). This will improve both the practical orientation of breeding and ensure food security in the world. An important aspect of modern approaches to genetically marker-assisted breeding with predictive identification is the identification of the relationships between the photosynthetic system of plants and their level of adaptability and stress tolerance (Mihaljevi´c et al., 2021; Hlahla et al., 2022).

Given the presentability of the chlorophyll fluorescence induction methodology in modern scientific research and the extensive approval of methodological approaches to assess varieties and hybrids for plasticity and stability, we considered it relevant to find out the effectiveness of comprehensive assessment of the oilseed radish varieties for adaptability based on a combination of the CFI curve indicators and the main indicators describing the plasticity and stability of the variety. This will make it possible to assess the potential of this approach and the effectiveness of its application in modern breeding practice.

MATERIALS AND METHODS

The six-year cycle of research (2015–2020) is based on dark gray forest soils (in the world soil classification Luvic Greyic Phaeozem soils (IUSS Working Group, 2015) typical for the region. The soil had the following properties: low humus content (2.02–3.20%), low content of mobile forms of nitrogen (67–92 mg kg^{-1}), high phosphorus content (149–220 mg kg^{-1}), average potassium content (92–126 mg kg^{-1}) and slightly acidic reaction of the soil solution (5.5–6.0).

The study covered 14 varieties of oilseed radish of different ecological and geographical origin and different breeding, obtained in cooperation with the National Center for Plant Genetic Resources of Ukraine.

The establishment and methodological support of the study was carried out in accordance with the cruciferous crop experimentation methodology (Sayko, 2011) with a recording plot area of 25 m^2 in a 4-fold replication. Sowing dates corresponded to the end of the first to the beginning of the second decade of April.

Seed productivity was calculated during the brown pod phase (BBCH 85-89) accordingly with the evaluation protocol on homogeneity and stability in oilseed radish (CPVO, 2017). The article presents the results of the different variants of line and wide-row sowing methods applied in the general layout of the experiment, when applying the wide-row sowing method (30 cm row-spacing) with the rate of 1.5 mln.pcs per ha of germinable seeds on the unfertilized soil according to the recommendations for the study of the features of chlorophyll fluorescence induction indicators (Kalaji et al., 2017a, 2017b). The specified variant under the conditions of the research region makes it possible to combine the implementation of the varietal potential of oilseed radish plants and the technology of its application, considering the combination of high levels of individual plant productivity and standing density, which ensures the achievement of potential yield levels (Tsytsiura, 2020).

The analysis of weather conditions and the level of their variability for the period 2015–2020 was carried out on the basis of the coefficient of significance of deviations (C_{sd}) of the elements of the agrometeorological regime of each of the studied years from the multi-year average according to formula 1:

$$C_{sd} = \frac{\left(X_i - X_{av}\right)}{S} \quad (1)$$

where X_{av} – an indicator of the average multi-year value; S – mean square deviation; X_i – an indicator for a particular year. The level of C_{sd}: 0÷ 1 – conditions close to normal; 1 ÷ 2 – the conditions were significantly different from the long-term averages; > 2 – conditions close to extreme.

The hydrothermal coefficient (*HTC*) was determined according to formula 2 (Evarte-Bundere & Evarts-Bunders, 2012):

$$HTC = \frac{\sum R}{0.1 \times \sum t_{>10}} \quad (2)$$

where ΣR (mm) – the amount of precipitation for period with temperatures above 10 °C; $\Sigma_{t > 10}$ – the sum of effective temperatures for the same period.

According to the significance of the deviations of the average monthly value of HTC from the average multi-year data, the years of the research period according to the value of C_{sd} (Table 1) was classified as 2015 - extremely dry, 2016 - dry with significant differences from the average multi-year data, 2017–2020 - conditions close to those typical for the multi-year hydrothermal regime of the research area. The years of research in the order of increasing stress impact on the growth processes of oilseed radish plants were placed in the following order: 2017–2019–2018–2020–2016–2015.

Table 1. Significance of HTC during the growing season of oilseed radish, 2015–2020

Year of research	Months of the vegetation period										The mean value V–IX
	V		VI		VII		VIII		IX		
	X_i	C_{sd}	X_i	C_{sd}	X_i	C_{sd}	X_i	C_{sd}	X_i	C_{sd}	C_{sd}
2015	0.719	-2.637	0.613	-1.264	0.230	-3.693	0.061	-5.961	0.684	0.011	-2.708
2016	1.227	-2.096	0.893	-0.574	0.682	-0.739	0.486	-0.368	0.063	-2.359	-1.227
2017	0.645	-2.716	0.349	-1.914	0.806	0.072	0.563	0.645	1.983	4.969	0.211
2018	0.258	-3.128	3.124	4.921	1.349	3.621	0.349	-2.171	0.680	-0.004	0.648
2019	4.710	1.613	1.555	1.057	1.003	1.359	0.235	-3.671	0.945	1.008	0.273
2020	5.489	2.443	1.474	0.857	0.649	-0.954	0.474	-0.526	1.208	2.011	0.766
X_{av} (1990–2020)	3.195		1.126		0.795		0.514		0.681		–
S (2015–2020)	0.939		0.406		0.153		0.076		0.262		–

The obtained significant differences in the weather regimes of the years during the research period allowed us to apply the system of evaluating the productivity of varieties for plasticity and stability according to the basic indicator of the annual conditions index.

The portable chronofluorometer ‘Floratest’ with a functional measurement period of 90 seconds was used (Romanov et al., 2011).

The measurement was carried out after shade adaptation (10 minutes) of 25 leaves of the middle layer in 4 repetitions per flowering phase of plants (BBCH 61-63). The leaf plate was oriented with its upper part towards the exciting light source and did not contain first-order venation. The obtained fixation points of the chlorophyll fluorescence induction curve were fixed by the device with the processing of the received data and the construction of a curve based on each fixation point. The data was transmitted from the device unit in file format with the extension csv. In the process of analyzing the obtained curves, basic indicators (in relative fluorescence units) were analyzed in accordance with the recommended system of indicator analysis of CFI curves (Brestic & Zivcak, 2013; Kalaji et al., 2017a–c):

F_0 – minimal fluorescence (O level in the O-I-D-P-T nomenclature of CFI curve); F_{pl} – florescence of the ‘plateau’ zone (I level in the O-I-D-P-T nomenclature of CFI curve); F_m – maximal fluorescence (P level in the O-I-D-P-T nomenclature of CFI curve); F_{st} – fluorescence stationary level (T level in O-I-D-P-T nomenclature of CFI curve). The date of the records corresponded to the phenological phase of flowering, which for oilseed radish corresponds to the maximum activation of the assimilation apparatus and its photosystem (Tsytsiura, 2020).

Additionally, relative and calculated indicators to the defined basic indicators (F_0, F_{pl}, F_m, F_{st}) were analyzed (formulas 3–14).

Fluorescence rise (dF_{pl}) (Brestic & Zivcak, 2013; Korneev, 2002; Kalaji et al., 2017a–c):

$$dF_{pl} = F_{pl} - F_0 \quad (3)$$

Maximum variable fluorescence (F_v):

$$F_v = F_m - F_0 \quad (4)$$

An indicator of the influence of exogenous and endogenous factors (Stirbet & Govindjee, 2011; Stirbet et al., 2014, 2018; Sarahan, 2011):

$$\frac{dF_{pl}}{F_v} \quad (5)$$

Photochemical efficiency or quantum efficiency (EP):

$$EP = \frac{F_v}{F_m} \quad (6)$$

Photochemical extinguishing (Q_{ue}) (Larouk et al., 2021):

$$Q_{ue} = \frac{F_0}{F_v} \quad (7)$$

Leaf water potential (L_{wp}) (Larouk et al., 2021):

$$L_{wp} = \frac{F_m}{F_0} \quad (8)$$

Plant viability index (RF_d) (Korneev, 2002; Lichtenthaler et al., 2005; Stirbet & Govindjee, 2011; Stirbe & Govindjee, 2012; Derks et al., 2015):

$$RF_d = \frac{F_m - F_{st}}{F_{st}} \quad (9)$$

Indicator of endogenous stress factors (K_{ef}):

$$K_{ef} = \frac{F_{st}}{F_m} \quad (10)$$

Photochemical quenching of fluorescence (QP) (Korneev, 2002):

$$QP = \frac{F_m - F_{st}}{F_m - F_0} \quad (11)$$

Efficiency of the initial reactions of photosynthesis (K_{prp}):

$$K_{prp} = \frac{F_v}{F_0} \quad (12)$$

The coefficient of decline of the fluorescence (K_{fd}):

$$K_{fd} = \frac{F_m}{F_{st}} \quad (13)$$

Relative change in fluorescence at time t (V_t):

$$V_t = \frac{F_{st} - F_0}{F_m - F_0} \quad (14)$$

The relative value of comparison (%) ($k_{comparison}$) (formula 15) (Rumsey, 2016):

$$k_{comparison} = \frac{k_1}{k_2} \times 100 \quad (15)$$

where k_1 – the value of the indicator in the variant with which it is compared; k_2 – the value of a similar indicator in the variant being compared.

An average seed yield (g plant^{-1}) was calculated for the same plants on which the CFI curve indicators were determined. For this purpose, the specified plants were marked with labels with corresponding numbering.

The parameters of ecological plasticity and stability of oilseed radish varieties were calculated according to the methods of Eberhart & Russel (1966) and Tai & Young (1972).

The following model evaluation of plasticity and stability was considered (formula 16):

$$Y_{ij} = \mu_i + \beta_i I_j + \sigma_{ij} \qquad (16)$$

where Y_{ij} – the variety mean of the i-th variety in the j-th environment ($i = 1, \dots$ v; $j = 1, \dots n$); μ_i – the mean of the i-th variety over all environments; β_i – the regression coefficient, that measures the response of the i-th variety to varying environments; δ_{ij} – the deviation from regression of the i-th variety at the j-th environment; I_j – the environmental index of the j-th environment, was calculated by the formula 17:

$$I_j = \frac{\sum_i Y_{ij}}{\nu} - \frac{\sum_i \sum_j Y_{ij}}{\nu n} \qquad (17)$$

where n – number of years of observations; ν – the number of genotypes in the analysis system (at i = 1, … v; j = 1, … n).

The first parameter of genotype stability (regression coefficient) b_i (formula 18):

$$b_i = \frac{\sum_j Y_{ij} I_j}{\sum_j I_j^2} \qquad (18)$$

The second parameter of stability (S_{di}^2) (deviation from the regression line) (formula 19):

$$s_{d_i}^2 = \frac{\sum_j \hat{\delta}_{ij}^2}{n-2} - \frac{s_e^2}{r} \qquad (19)$$

where s_e^2 – the estimate of the pooled error; r – number of replications.

The following grouping was used to evaluate plasticity (b_i) and stability (S_{di}^2) (according to Eberhart & Russel, 1966; Tai, 1971; Pakudin & Lopatina 1984; Callaway et al., 2003): I $b_i < 1$, $S_{di}^2 > 0$ – had better results under unfavourable conditions, unstable type; II $b_i < 1$, $S_{di}^2 = 0$ – had better results under unfavourable conditions, stable type; III $b_i = 1$, $S_{di}^2 = 0$ – responded well to improving conditions, stable type; IV $b_i = 1$, $S_{di}^2 > 0$ – responded well to improving conditions, unstable type; V $b_i > 1$, $S_{di}^2 = 0$ – had the best results under favourable conditions, stable type; VI $b_i > 1$, $S_{di}^2 > 0$ – had the best results under favourable conditions, stable type. Genotypes with a coefficient of $b_i > 1$ are classified as highly plastic (group average), while $1 > bi = 0$ is classified as relatively low plastic.

The sum of the squares of deviations from the regression line was calculated by the formula 20 (Tai, 1971; Tai & Young, 1972):

$$\sum_j \hat{\delta}_{ij}^2 = (\sum_j Y_{ij}^2 - (\sum_J Y_{ij})^2/n) - (\sum_j Y_{ij} I_j)^2 / \sum_j I_j^2 \qquad (20)$$

A model of analysis of variance with random effects of the environment was considered (formula 21):

$$y_{ijk} = \mu_i + d_i + \varepsilon_j + \gamma_{k(j)} + g_{ij} + e_{ijk} \qquad (21)$$

where y_{ijk} (i = 1, …v, j = 1, … n, k = 1, … r) – the value of the trait of the i-th genotype in the j-th environment in the k-th replication; μ_i – the overall mean; d_i – the effect of the i-th genotype; ϵ_j – effect of the j-th environment; $\gamma_{k(j)}$ – the effect of replicates within environments; g_{ij} – the effect of genotype × environment interaction; e_{ijk} – the residual variation due to replications.

The linear response of the genotype to the effect of the environment (α_i) and the deviation from the linear response (λ_i) was calculated by the formulas 22 and 23 (Tai & Young, 1972):

$$a_i = \frac{MSL(b_i - 1)}{MSL - MSB} \quad (22)$$

$$\lambda_i = \left[\frac{\nu}{\nu - 1}\right]\left[\frac{n-2}{n-1}\right]\frac{s_{d_i}^{\ 2}}{MSE/r} - a_i\left[\frac{b_i - 1}{m-1}\right]\frac{MSB}{MSE} \quad (23)$$

where MSL, MSB, MSE – mean squares, due to environments, replicates within environments and error, respectively; r – number of replications; S_{di}^2 – the second parameter of stability; n – number of years of observations; ν – the number of genotypes in the analysis system; m – degrees of freedom for the error.

The graphical interpretation of the parameters α_i and λ_i according to Pakudin & Lopatina (1984) was made.

The coefficient of homeostaticity (H_{omi}) (stability index) (formula 24) (Khangildin et al., 1979):

$$H_{omi} = \frac{\bar{Y}^2_i}{\sigma_i(\bar{Y}_{i(opt)} - \bar{Y}_{i(lim)})} \quad (24)$$

where σ_i – the standard deviation of the trait for the i-th genotype; $\bar{Y}_i$, $\bar{Y}_{i(opt)}$, $\bar{Y}_{i(lim)}$ – the mean values of the trait for the i-th genotype in all environments, in the optimal and limited environment, respectively.

Agronomic stability coefficient (A_s) was determined as the residual of the difference between the total stability of the genotype and the level of its variation during the evaluation period (Bacsi & Hollósy, 2019; Reckling et al., 2021).

The selection value indicator (S_v) was calculated according to the formula 25 (De Jong, 1994):

$$S_v = \bar{Y}_i \frac{\bar{Y}_{i(min)}}{\bar{Y}_{i(max)}} \quad (25)$$

where $\bar{Y}_i$ – average yield over the evaluation period for a given genotype, $\bar{Y}_{i(min)}$, $\bar{Y}_{i(max)}$ – respectively, the minimum and maximum recorded yields for a given genotype during the evaluation period.

Coefficient of dryness (C_d) was determined as the percentage ratio of the yield of the respective variety in the driest year to its yield in the year with maximum moisture (Becker & Léon, 1988).

Coefficient of stress resistance (C_{sr}) and genetic plasticity (G_p) was determined according to the recommendations of Pakudin & Lopatina (1984).

The coefficient of productivity (C_p) was determined as percentage ratio of the average yield of the variety during the study period to the maximum potential yield shown by the variety during the period of variety production in the conditions of the zone of research (Fernandez, 1991; Fikere et al., 2014).

The evaluation of the variation was carried out according to the gradation of the coefficient of variation (CV_i,%) according to the methodological guidelines (Temesgen et al., 2015; Urruty et al., 2016): up to 10% – low, 11–20% – medium and > 21% – high.

The coefficient of variation of the i-th genotype is actually determined by a standard formula 26 (Snedecor, 1989):

$$CV_i = \frac{\sigma_i}{\bar{Y}_i} \times 100\% \quad (26)$$

where σ_i – the standard deviation of the trait for the i-th genotype; $\bar{Y}_i$ – the mean value of the trait for the i-th genotype in all environments. Genotypes with yield above overall mean yield and CV_i below overall coefficient of variation was considered as more stable than the others.

The degree of integrated connection of plant morphological features was assessed using the weight correlation graph method (Kakade & Foster, 2007; Hajjar et al., 2022) using the formula 27:

$$G = \sum_{|r_{ij}| \geq \alpha} |r_{ij}| \quad (27)$$

where r_{ij} – correlation coefficient between i-th and j-th indicator. Reliable correlation coefficients was considered.

The percentage dependence of the variation of the performance indicator on the influence of the selected factor is determined through the coefficient of determination (d_{xy}) according to formula 28 (Davide et al., 2021):

$$d_{yx} = r_{ij}^2 \times 100 \quad (28)$$

where r_{ij} – correlation coefficient between i-th and j-th indicator.

The periodization of the phenological development of oilseed radish varieties corresponds to the BBCH periodization scale (CPVO, 2017).

To compare the average data in the experimental variants, the indicator of the least significant difference ($LSD_{0.5}$) was used (for the lower limit of the permissible level of significance $p < 0.05$) (Marques de Sá, 2007; Hinnkelmann & Kempthorne, 2019). Standard methods of regression and correlation analyzes using the Statistica 10 framework were also applied (StatSoft – Dell Software Company, USA).

RESULTS AND DISCUSSION

The identification of oilseed radish varieties by plasticity (b) and stability (S_{di}^2) (Table 2) allowed to divide the studied genotypes by the corresponding rank gradations. The first rank includes varieties 'Alfa', 'Iveia', 'Pryhazhunia', 'Raiduha', 'Snizhana', 'Nika', 'Liniia IRHSHI', 'Tambovchanka', 'Sabina'. The VI rank includes varieties 'Olha', 'Ramonta', 'Zhuravka', 'Lybid', 'Fakel'. Varieties of I rank according to the classification given in the methodology section are classified as unstable type with possible manifestation of maximum productivity in years with unfavorable environmental conditions. Varieties of VI rank are referred to a stable type with a positive increase in productivity (in our case, the resulting value of seed yield (SY)) under optimized environmental conditions. Thus, the influence of selective origin of oilseed radish varieties in the expression of their adaptive potential can be traced. This is essentially correct, as most of the varieties under study belong to the Eur asian selection group with the inclusion of local source forms of oilseed radish from the territories of northern and central Ukraine, Russia and Belarus with a certain recombination of breeding forms from Poland and Germany (Blume et al., 2020).

Table 2. Parameters of stability, plasticity, resistance and yield potential of varieties of oilseed radish, (average for 2015–2020)

Variety	SY, t ha^{-1}	C_P, %	CV_i, %	C_{st}	G_p	C_d	A_s	H_{omi}	S_v	b_i	S_{di}^2	a_i	λ_i
Olha	1.81	88.13	23.59	-1.23	1.83	0.70	89.14	2.79	8.96	1.10	2.29	0.46	0.84
Alfa	1.28	69.00	19.58	-0.77	1.26	0.66	89.68	3.81	6.81	0.39	1.56	-0.64	-1.22
Ramonta	2.08	87.62	15.63	-1.00	2.10	0.76	90.97	5.94	12.72	1.09	0.10	-0.13	0.15
Iveia	1.71	67.06	23.25	-1.24	1.67	0.61	86.93	2.65	7.84	0.57	4.23	0.37	-0.47
Pryhazhunia	2.03	78.44	18.84	-1.12	1.91	0.65	88.05	4.31	11.10	0.95	0.48	0.23	0.45
Raiduha	1.82	86.83	17.77	-0.95	1.72	0.63	88.91	4.93	10.32	0.71	1.04	-0.24	0.82
Zhuravka	2.12	87.76	17.37	-1.06	2.05	0.68	88.41	5.19	12.46	1.11	2.39	0.01	2.07
Lybid	1.77	87.71	22.60	-1.23	1.71	0.57	88.62	2.88	8.32	1.08	0.50	0.35	-0.22
Fakel	1.88	88.60	18.08	-1.06	1.82	0.68	92.53	4.39	10.31	1.01	0.69	-0.06	0.68
Snizhana	1.96	82.98	19.50	-1.17	1.94	0.69	83.96	3.84	10.49	1.24	0.26	0.25	0.04
Nika	1.86	73.94	19.35	-1.07	1.93	0.82	89.94	4.10	10.53	0.81	3.17	0.004	1.15
Liniia IRHSHI	1.45	81.10	24.78	-1.06	1.35	0.54	88.87	2.48	6.33	0.65	1.53	0.06	0.77
Tambov-chanka	1.70	69.11	18.09	-0.91	1.58	0.67	87.05	4.62	9.38	0.51	0.95	-0.32	0.83
Sabina	1.72	66.54	17.00	-0.89	1.71	0.72	91.19	5.10	10.10	0.366	3.426	-0.34	-0.23

Parameters (Fisher criterion) for SY (yield, t ha^{-1})	F_f	F_{t05}	LSD_{05} (yield, t ha^{-1})
Variety	284.23	1.82	0.041
Year conditions	111.36	2.46	0.029
Interaction of variety x conditions of the year	9.82	1.48	0.049

Thus, the varieties of Ukrainian selection 'Raiduha', 'Zhuravka', 'Lybid', 'Fakel' were created by repeated individual selection from earlier varieties of German (Skletta) and Polish selection (Bashta, Snopkowska), which in turn were created by population selection from local species forms of oilseed radish common in Central and Eastern Europe (Tsitsiura & Tsitsiura, 2015). The use of positive selection contributed to the selection of genotypes with stable adaptive potential, increasing against the background of optimization of plant growth and development conditions. Oilseed radish varieties of Belarusian selection 'Iveia', 'Pryhazhunia', 'Snizhana', 'Nika', 'Sabina' were created on the basis of hybridization of such varieties as 'Raiduha', 'Tambovchanka', as well as varieties of own selection of an earlier period with varieties of the already mentioned Polish and German selection and subsequent multiple individual selection.

The variety of German selection 'Ramonta' was also created by hybridization. The use of hybridization allowed to increase the overall heterozygous state of the initial population with subsequent stabilizing positive multiple selection. As a result, this contributed to the formation of an unstable type in terms of the ratio of plasticity and stability with a potentially positive reaction of yield growth under the appropriate combination of environmental conditions and genotypic characteristics of the variety. Thus, the use of hybridization in oilseed radish breeding complicates the rank reaction of varieties in the tested model of Eberhart & Russell (1966) with the expansion of the adaptive response of genotypes and at the same time reducing the predictability of such a reaction (increasing the probability of $S_{di}^2 > 0$). This conclusion was confirmed by graphical interpretation of the spatial relationship of the linear response of the genotype to the effect of the environment a_i and the deviation from the linear response λ_i (Fig. 1).

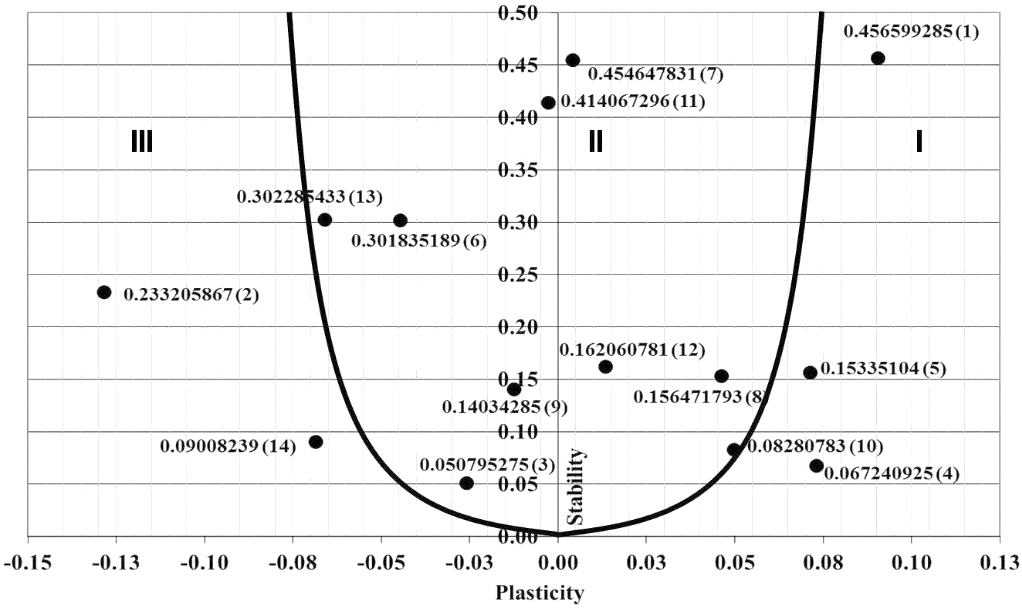

Figure 1. Distribution of varieties of oilseed radish into classes by plasticity (a_i) and stability (λ_i) at a 5% significance level, 2015–2020 (indexation of varieties: 1 – 'Olha'; 2 – 'Alfa'; 3 – 'Ramonta'; 4 – 'Iveia'; 5 – 'Pryhazhunia'; 6 – 'Raiduha'; 7 – 'Zhuravka'; 8 – 'Lybid'; 9 – 'Fakel'; 10 – 'Snizhana'; 11 – 'Nika'; 12 – 'Liniia IRHSHI'; 13 – 'Tambovchanka'; 14 – 'Sabina').

It was found that the varieties of the first (I) zone ('Olha', 'Iveia', 'Pryhazhunia') belong to genotypes with high response to changes in growing conditions. That is, such varieties should be recommended for cultivation in conditions of high culture of agriculture. However, on a low agricultural background, their yields are sharply reduced. In contrast, the varieties whose coordinates are located in the second (II) zone ('Ramonta', 'Raiduha', 'Zhuravka', 'Lybid', 'Fakel', 'Snizhana', 'Nika', 'Liniia IRHSHI', 'Tambovchanka') are more conservative in response to changing environmental conditions. Ecological plasticity of varieties placed coordinately in the third (III) zone ('Alfa', 'Sabina') is at the level of average plasticity, typical for the studied set of varieties. This distribution is consistent with the level of realization of the genetic potential of the variety (productivity coefficient (C_p)) of oilseed radish varieties, which are the result of Ukrainian breeding, in particular 'Raiduha', 'Zhuravka', 'Lybid', 'Fakel' showed the highest levels of this indicator, which is evidence of their adaptation to local conditions and hydrothermal regime of the territory. On the contrary, varieties of the most geographically distant selection 'Alpha', 'Tambovchanka' showed the lowest value of C index in the group. These levels of adaptability for varieties 'Raiduha', 'Zhuravka' and 'Fakel' were confirmed by the values of the coefficient of variation (C_v) at the level of 17.37–18.08 and the coefficient of stress resistance (C_{st}) at the level of -0.95– -1.06 in relation to the effective indicator of seed yield in the applied analysis system. The maximum indices of genetic plasticity (Gp) were determined in varieties Ramonta' and 'Zhuravka', which, taking into account the statements of a number of researchers (Ghanem et al., 2015; Subira et al., 2015), indicates significantly higher values of breeding value of these varieties (Sv) and the level of their homeostasis (H_{omi}) in the general comparison group. As for the level of homeostasis, according to Xu (2016), its value indicates the level of adaptive adaptations of the genotype to the given environmental conditions of the territory of its cultivation. On the basis of this, it was found that for the varieties of oilseed radish 'Olha' and 'Liniia IRHSHI', the breeding centers of which belong to the zone of central Siberia, the level of these adaptations is minimal. At the same time, among the varieties selected in geographically close to the research area, the level of homeostasis is also low, in particular in the varieties 'Lybid' and 'Iveia'. Such results are explained from the point of view of certain properties of the genotype, which are the resultant in the formation of its productivity, as confirmed by Brouziyne et al. (2018). In our case, this is the value of the drought tolerance index by the value of the aridity coefficient (C_d), which for the varieties 'Lybid', 'Iveia' and 'Liniia IRHSHI' was minimal among the studied varieties 0.57, 0.61 and 0.54, respectively.

According to the general combinatorics of indicators characterizing the adaptive properties of the variety, its breeding value and agronomic stability (A_s), the studied varieties of oilseed radish were placed in the following order 'Ramonta', 'Zhuravka', 'Pryhazhunia', 'Nika', 'Snizhana', 'Raduha', 'Sabina', 'Tambovchanka', 'Olha', 'Lybid', 'Iveia', 'Alfa', 'Liniia IRHSHI'. Each of these genotypes according to additional criteria a_i and λ_i showed additional features that determine the variety-specific response to the combination effect of genotype and environmental conditions. This is confirmed by the nature of placement of the studied varieties in the cordinal plane of the ratios of stability and plasticity of varieties (Fig. 1). There are significant differences ($p < 0.05–0.01$) in the ratio of Fisher's criterion (F_f/F_{t05-01}) for the main parameter of determination of varieties - seed yield (SY) allowed to interpret the results from the point of view of genotypic expression of adaptations of varieties and compare them with physiological aspects of growth and photoassimilation processes, in particular with the induction of chlorophyll a florescence. The possibility and feasibility of such a comparison is substantiated in a number of studies (Olivoto et al., 2019; Pour-Aboughadareh, 2022).

The results of accounting of the basic and derivative calculated parameters of the CFI curve (Table 3) showed their significant (at $p < 0.5$) differences within the studied varieties of oilseed radish. At the same time, the share of the influence of environmental factors in the dispersion analysis system was from 21 to 28% for the basic indicators of the CFI curve. Taking into account the share of the year conditions for the seed yield at the level of not less than 25% (Table 2), the significant role of the annual variation in the formation of stability and plasticity of varieties on the resulting trait and in the formation of the CFI curve indicators was proved. Taking into account the results of studies by Hoffmann & Woods (2003), Auld et al. (2010), Mohammadi (2014), Klingenberg (2019), the comparison of varieties evaluation indicators by classical breeding indicators and indicators of chlorophyll a fluorescence induction was applied.

Table 3. The value of the CFI curve indicators for oil radish varieties (relative fluorescence units for flowering phase BBCH 65–67), 2015–2020

Variety	Basic indicators				Estimated indicators and indices											
	F_0	F_{pl}	F_m	F_{st}	dF_{pl}	F_v	dF_{pl}/F_v	EP	L_{wp}	Q_{ue}	RF_d	K_{ef}	QP	K_{prp}	K_{fd}	V_t
Olha	438	619	1,655	523	181	1,217	0.149	0.735	3.78	0.360	2.164	0.316	0.930	2.779	3.164	0.070
Alfa	495	607	1,608	553	112	1,113	0.101	0.692	3.25	0.445	1.908	0.344	0.948	2.248	2.908	0.052
Ramonta	454	681	1,814	518	227	1,360	0.167	0.750	4.00	0.334	2.502	0.286	0.953	2.996	3.502	0.047
Iveia	520	674	1,578	623	154	1,058	0.146	0.670	3.03	0.491	1.533	0.395	0.903	2.035	2.533	0.097
Pryhazhunia	460	633	1,592	525	173	1,132	0.153	0.711	3.46	0.406	2.032	0.330	0.943	2.461	3.032	0.057
Raiduha	522	626	1,534	596	104	1,012	0.103	0.660	2.94	0.516	1.574	0.389	0.927	1.939	2.574	0.073
Zhuravka	438	691	1,808	509	253	1,370	0.185	0.758	4.13	0.320	2.552	0.282	0.948	3.128	3.552	0.052
Lybid	506	623	1,690	570	117	1,184	0.099	0.701	3.34	0.427	1.965	0.337	0.946	2.340	2.965	0.054
Fakel	454	597	1,644	537	143	1,190	0.120	0.724	3.62	0.382	2.061	0.327	0.930	2.621	3.061	0.070
Snizhana	455	617	1,734	526	162	1,279	0.127	0.738	3.81	0.356	2.297	0.303	0.944	2.811	3.297	0.056
Nika	482	641	1,598	529	159	1,116	0.142	0.698	3.32	0.432	2.021	0.331	0.958	2.315	3.021	0.042
Liniia IRHSHI	463	605	1,412	532	142	949	0.150	0.672	3.05	0.488	1.654	0.377	0.927	2.050	2.654	0.073
Tambov-chanka	483	654	1,563	565	171	1,080	0.158	0.691	3.24	0.447	1.766	0.361	0.924	2.236	2.766	0.076
Sabina	497	667	1,619	606	170	1,122	0.152	0.693	3.26	0.443	1.672	0.374	0.903	2.258	2.672	0.097

LSD$_{05}$	F_0	F_{pl}	F_m	F_{st}	The share of influence of experimental factors				
					factors	F_0	F_{pl}	F_m	F_{st}
LSD$_{05}$ factor A (year)	5.96	6.83	5.91	5.25	A	21.369	23.551	28.129	27.884
LSD$_{05}$ factor B (plant species)	6.84	7.92	6.18	4.29	B	48.369	45.274	51.369	49.139
LSD$_{05}$ interaction AB	8.92	10.12	9.24	8.87	AB	30.262	31.175	20.502	22.977

Tukey HSD Test (Signifscant codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1)

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
F_0 RE	13	27,197	2,092	6.75	**
Residuals	84	26,027	310		
F_{pl} RE	13	42,030	3,233	10.80	***
Residuals	84	25,137	299		
F_m RE	13	29,434	2,264	24.74	***
Residuals	84	7,686	92		
F_{st} RE	13	11,450	881	15.08	***
Residuals	84	4,905	58		

The obtained range of values of the basic indicators of the CFI curve (F_0, F_{pl}, F_m, F_{st}) had extremely close intervals of values, which corresponded to a close numerical interval. This is confirmed by graphical interpretation of CFI curves (Figs 2a–2b) in oilseed radish varieties. Based on this, it was concluded that chlorophyll fluorescence induction indices can be used in the processes of genomic identification of plants. In addition to the conclusions of Kalaji & Guo (2008), Flood et al. (2011), Driever et al. (2014) and Flexas & Carriqu´ı (2020), the presented graphical data proved the similarity of the formation of dynamic changes in the process of chlorophyll fluorescence induction in terms of general formation and established the specific nature of the curve sections in terms of ordinal placement and specific formation features in the corresponding sections.

At the same time, with the general similarity of the curves, specific features were noted. This specificity is defined as a factor of special adaptive reactions of the assimilation apparatus of oilseed radish plants due to appropriate responses to environmental stress factors. For example, comparison of CFI curves for significantly different by the established parameters of breeding value varieties of oilseed radish 'Ramonta' (Fig. 2a) and 'Liniia IRHSHI' (Fig. 2b). For the variety 'Ramonta' high maximum ordinate position up to the level of 1800 relative units of fluorescence standard (F_0), intensive decrease of the curve in the area of 33–60 second fixation, complex microrelief character of the curve in the area of 61 second fixation to the level of stationary fluorescence F_{st}.

For the variety 'Liniia IRHSHI' the minimum ordinal position up to the level of 1400 relative units of the fluorescence standard (F_0), slower decrease of the curve in the 33–60 second fixation, smoothed character of the curve in the 61 second fixation to the level of F_{st} were noted.

Taking into account the fact that the photosynthetic apparatus of plants has certain idiosyncratic mechanisms of adaptation to stress, as noted in the studies of Ajigboye et al. (2016) and Araus & Cairns (2014) and genotypic features of the variety determine both the formation of the plant photosystem and the mechanisms of its functioning (Strasser et al., 2004), the obtained data confirm the conjugation of the formation of plasticity and stability of the genotype and the efficiency of its photosystem. In support of this, such a mechanism of formation was noted already at the early stages of formation of juvenile elements of the photosystem of cruciferous crops (Jalink et al., 1998). The possibility of such evaluation approaches was confirmed in the variants of drought tolerance, salinity tolerance and general stress tolerance of rapeseed genotypes (Kauser et al., 2006; Jafarinia & Shariati, 2012; Ayyaz et al., 2021), mustard (Irfan et al., 2015) and radish subspecies (Guo et al., 2005).

A high value of the initial fluorescence index F_0 at the level of 500 relative units of the fluorescence standard was noted, taking into account the results of studies by Gu et al. (2017), according to which the growth of F_0 in the comparison of plant species indicates its sensitivity to an increase in plant density. On the basis of this, the studied varieties of oilseed radish are predictably attributed to those responding to thickening by reducing the level of individual bioproductivity with increasing planting density. Different gradations of the F $index_0$ allowed to hypothetically determine the varieties of oilseed radish with different degrees of agrotechnological response to changes in the range of planting density. Thus, for the varieties 'Raiduha', 'Iveia', 'Lybid', 'Sabina', the optimum standing density should be predicted in a narrower agrotechnological interval than for the varieties 'Olha', 'Zhuravka', 'Fakel', 'Ramonta'.

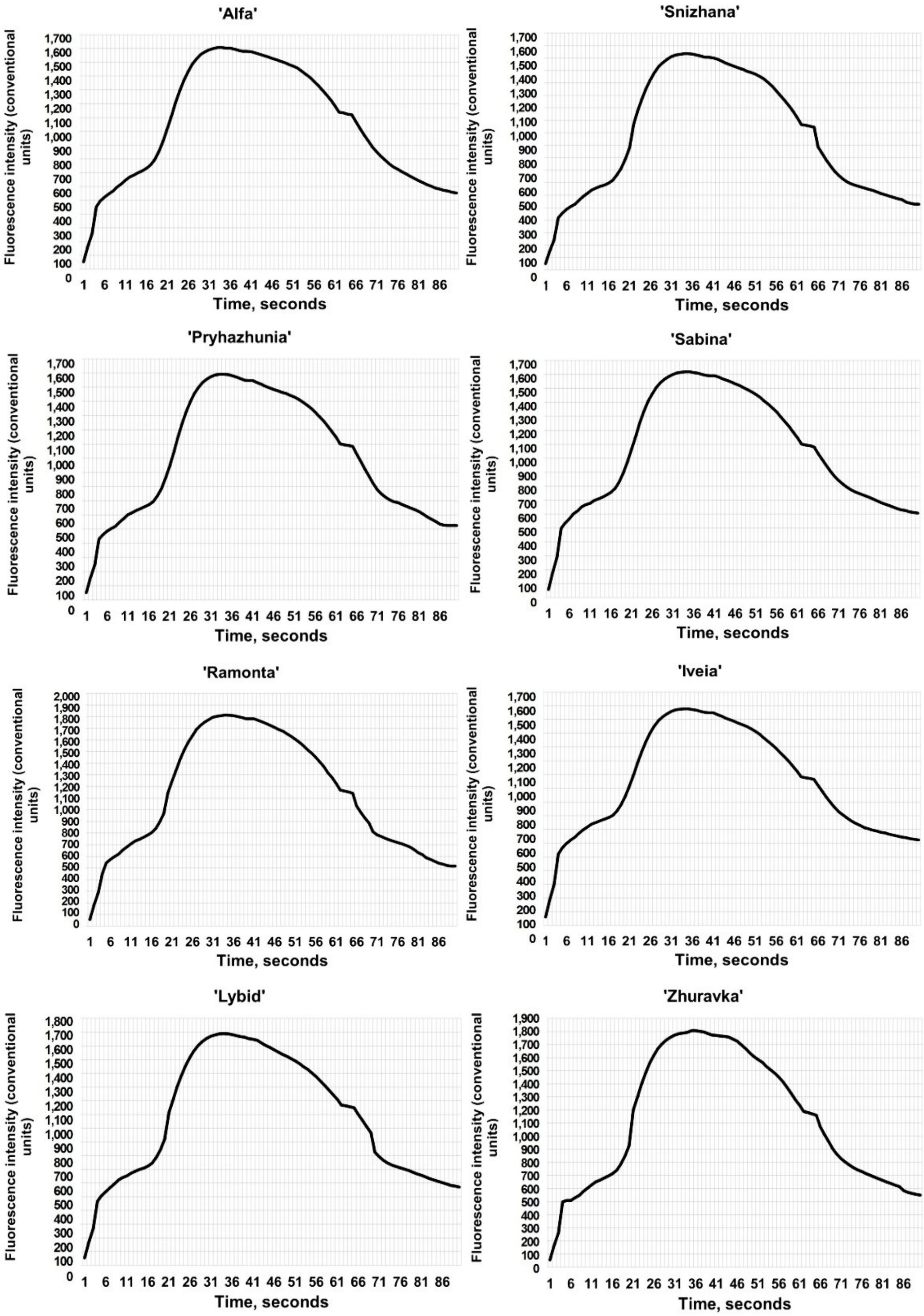

Figure 2a. CFI curves (adjusted for the average multi-year values of the indicator at each fixation point) for oilseed radish varieties based on averaged data from fixation points in the 90 second interval over the 2015–2020 period.

The formation of the 'plateau' zone F_{pl} fluorescence also has few generic as well as varietal differences. Regarding varietal features, oilseed radish was characterized by a dynamically increasing level of formation of this index with a little expressed form of 'plateau' zone at the level of 11–15 seconds of registration with a portable fluorimeter, followed by intensive increase of index of CFI curve up to the value of maximum fluorescence (F_m) (Figs 2a–2b). The varietal specificity of indicator formation should also be noted. Thus, in the varieties 'Alfa', 'Raiduha', 'Iveia', on average over a multi-year period of evaluation, the achievement of the 'plateau' zone in the intensively growing area F_0–F_{pl} was noted. For the varieties 'Zhuravka', 'Fakel', 'Olha', 'Pryhazhunia', the angular slope of the CFI curve in the F_0–F_{pl} area was less.

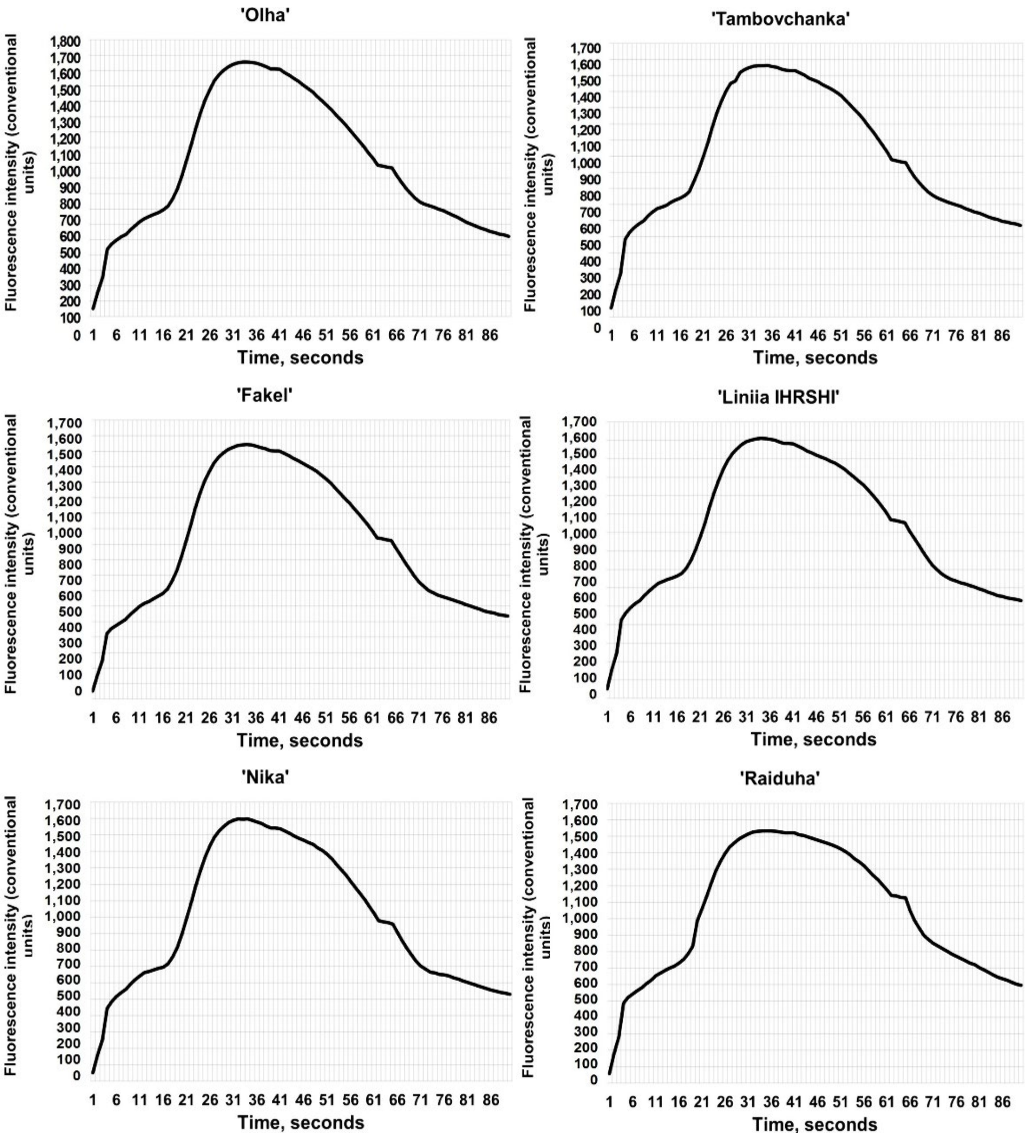

Figure 2b. CFI curves for oilseed radish varieties based on averaged data from fixation points in the 90 second interval over the 2015–2020 period.

Under these conditions, the formation of the F_{pl} indicator was noted on the intensively increasing segment of the CFI curve. At the same time, the nature of such formation was varietal specific. This indicates certain differences in the functioning of the photosystem of different oilseed radish genotypes and similarity in the reaction to a change in the area of plant nutrition. By the way, similar results were obtained in winter wheat (Brestič et al., 2012) and spring barley (Kalaji & Guo, 2008).

A number of peculiarities of oilseed radish varieties in comparison with other cruciferous crops were also found in the area of the CFI curve between F_{pl} and F_m (Figs 2a–2b). The character of the graph in this area had the highest level of visual genotypic differences, which further proved the possibility of varietal identification of oilseed radish on the basis of basic indicators of chlorophyll fluorescence induction. In all years of research, a special area on the CFI curve in the period 61–65 seconds of registration was also recorded, which was typical for all studied varieties of oilseed radish. Similar analogies with different expression of the duration of this area were noted in our studies on spring rape, white mustard and spring rape (Tsytsiura, 2022). The presence of this area is explained by the fact that the nature of the curve formation in the area F_m–F_{st} indicates certain physiological mechanisms of pre-adaptation of the PSII photosystem in the transition to stationary fluorescence, which characterizes the stress sensitivity of the species. The low expression of this activity on the CFI curve of individual cultivars is explained by the higher rate of physiological aging of leaves than in other cruciferous species in the analyzed group, as indicated in the publications of Ward et al. (1995) and Hasanuzzaman (2020).

It was found that the graphical altitudinal position of this transition area for different varieties of oilseed radish was significantly different. Over the long-term study period, it was recorded at the level of 997 relative fluorescence units in the variety 'Olha', 1,020 relative fluorescence units in the variety 'Fakel', and 1125 units in the variety 'Raiduha', respectively (Table 3). In general, with these varietal differences in varieties of oilseed radish, a rather narrow niterval of fixation of this transition in the range of 950–1,150 relative fluorescence units was noted. The very nature of the CFI curve in the area from F_m to the marked characteristic plateau at 61–65 seconds of registration has a pronounced varietal difference from a wide stretched character in the varieties 'Raiduha' and 'Alfa' to a narrow one with an intense decline in the varieties 'Zhuravka', 'Ramonta', 'Nika'. Thus, this section of the curve in cruciferous plant species should be considered as an indicator of appropriate levels of stress resistance. This possibility by the nature of the CFI curve segment F_m–F_{st} in determining the species response to high or low temperatures, drought, soil salinity, heavy metal concentration, lack of nutrients is indicated in a number of studies on different plant species, in particular, Huner et al. (1993), Flexas et al. (2002), Adams & Demmig-Adams (2004), Gonçalves & dos Santos (2005), Urban et al. (2018), Khazaei et al. (2019), McAusland et al. (2019). At the same time, under intense stress, as well as under the conditions of natural stage aging of leaves, the value of F_{st} was significantly higher than F_0 and vice versa under optimal conditions with a shorter time interval for reaching the level of F_{st} on the CFI curve. These generalizations are confirmed by the nature of the plot F_m–F_{st} (Figs 3a–3b). Thus, for the varieties 'Snizhana', 'Fakel', 'Lybid' a consistent slow character of its formation was noted, and for the varieties 'Ramonta', 'Zhuravka', 'Lybid' an intense downward character was observed when reaching the stationary level of F_{st}.

The index dF_{pl} also had significant differences within the studied varieties based on statistically significant difference for the values F_{pl} and F_0 (Table 3). In this case, the value of the indicator between the limit values was 141 conventional units of fluorescence, respectively 253 units in the variety 'Zhuravka' to 112 units in the variety 'Alfa'. It is proved for many plant species that induced stresses (herbicides, thickening, diseases, etc.) reduce the dF_{pl} with simultaneous growth of both F_0 and F_{pl}. However, the growth rate of initial fluorescence outpaces the growth rate of fluorescence of the 'plateau' zone. This has been noted in a number of studies (Goltsev & Yordanov (1997); Lazár et al. (1997); Morales et al. (2000), Klinkovsky & Naus (2004); Papageorgiou & Govindjee (2004); Lazár & Schansker (2009); Rapacz et al. (2015); Ripoll et al. (2016); Østrem et al. (2018); Sánchez-Moreiras et al. (2020). However, the data presented in the case of oilseed radish varieties showed the lack of stable compliance with these patterns. Thus, varieties with different breeding value (S_v) such as 'Pryhazhunia', 'Tambovchanka', 'Snizhana' had similar values of dF_{pl}. Based on this, it was concluded that different varieties of oilseed radish have certain peak values of F_{pl} and F_0, according to the level of physiological capacity of their photosystem. That is, for oilseed radish as for the species as a whole, a narrowed interval of possible genotypic stress response was determined, which is expressed in the formation of an adaptive niche of the reaction of its photosystem.

The results of the correlation analysis between the studied parameters (Table 4) allowed to confirm the previously made conclusions and identify a number of features in the expression of adaptive varietal strategies of oilseed radish in the functioning of their photosystem.

It was determined that the indicator F_m had a close relationship of a direct nature with the parameters SY, G_p, b_i, S_v and H_{omi} and the inverse of CV_i S_{di}^2. For the indicators F_o and F_{st}, the nature of the correlation between the same indicators had the opposite nature of formation. And for the F_{pl} indicator, a heterogeneous impact with a productive direct character was determined.

At the same time, the effective significance of the dependencies of the indicators F_o, F_{pl}, F_m and F_{st} on the size of the correlation graph (G) in the interval of indicators SY–S_v had corresponding differences. According to the size of this correlation graph, the indicators of the impact on the certainty of the main criteria for evaluating varieties for adaptability and stability can be placed in the following ascending order: F_{st} (G = -1,732) – F_0 (-1,925) – F_{pl} (1,946) – F_m (2,459). Thus, the basic criteria for the induction of chlorophyll fluorescence are recommended as predicted for the evaluation and determination of adaptive properties of oilseed radish varieties at the corresponding already announced optimized ratio of b_i and S_{di}^2, according to the grouping ranks of their ratio. It was proved that the oilseed radish variety with higher plasticity and stability will be characterized by higher levels of maximum chlorophyll fluorescence (F_m), lower values of initial fluorescence (F_0) and, as a result, higher values of F_v. At the same time, the indication of dependence was significantly higher for the parameter b_i than for the parameter S_{di}^2. This is explained by the levels of adaptive responses of varieties to environmental stress factors and genotypic features that determine the physiological and anatomical features of the structure and functioning of the plant photosystem. The presence of these physiological and anatomical features in other plant species is reported in the studies of Strasser & Tsimilli-Michael (2001), Lascano et al. (2003), Lepeduš et al. (2012) and Herritt et al. (2020). It should be noted that for the studied varieties of oilseed radish, the range of F_m values was 402 conventional units of fluorescence (1,814 in the variety 'Ramonta' and 1,412 in the variety 'Liniia IRHSHI') (Table 3). In relation to the average long-term value for the studied varieties of 1630 conventional units of fluorescence, it was 24.7%. Taking into account the results of studies by Middleton et al. (2019), Dechant et al. (2020), Quero et al. (2020), Jushkov et al. (2021), where it is noted that the increase in the range of values of basic indicators of fluorescence in a certain species population of plants at a very high gradation indicates its heterogeneity and significantly differentiated physiological stability of plant stress responses. In view of this, the genetically determined mechanisms of adaptive adaptability of oilseed radish varieties to changing environmental conditions was obviously due to the involvement of the original local populations and wild forms of oilseed radish well adapted to reactions to various changes in hydrothermal and edaphic conditions. The effectiveness of breeding to improve varieties in this way is noted in particular in the studies of Li et al. (2006), Long et al. (2006, 2015), Rapacz et al. (2015), Kromdijk et al. (2016), Khazaei et al. (2019) and Zhuang et al. (2020).

Taking into account the direct nature of the relationship between the parameters b_i, S_v and H_{omi} and the inverse relationship between S_{di}^2 and F_m and the inverse relationship with a smaller value of the correlation coefficients with respect to F_0 – the value of ER (F_V/F_m) can be successfully used in breeding programs for evaluating oilseed radish varieties for stability and plasticity. At the same time, given the multidirectional correlation dependencies of ER components, the importance of the criterion F_m in the predicted assessment of the adaptive potential of varieties will be the main one.

Based on the established in studies of varietal characteristics of the CFI curve section F_0–F_{pl}–F_m, it was determined that the correctness of identification of oilseed radish varieties by varietal adaptability will be determined by the physiological response of the genotype at the initial stages of induction of chlorophyll fluorescence in the F_0–F_{pl} section and the speed and intensity of the recovery of the acceptor mechanism of electrons of the PS II photosystem. The tendency of both ratios to the identical reduction reaction at the growth of stress factors causes a less significant level of dependence than in single correlation comparisons between dF_{pl} and F_v, which is clearly confirmed by the data of Table 4.

Genotypic differences in oilseed radish by the rate of change in the induction of chlorophyll fluorescence by V_t were also determined. On the basis of this, in oilseed radish varieties where this indicator was lower, the possibility of expanding the interval of the boundary limits of the corresponding stress factor was established, which provides a wider range of plasticity of the variety and its adaptations. This is confirmed by the inverse relationship between V_t and such criteria as b_i (-0.561, at $p < 0.05$) and the indicator of breeding value of the variety (S_v) (-0.382, $p < 0.05$) and a direct relationship with the indicator of variant stability of the trait, Sdi^2 (0.490, at $p < 0.01$) (Table 4).

The potential possibility of using the vitality index RF_d, (an indicator of the threshold level of exogenous stress (Korneev, 2002)) in identifying the adaptability of oilseed radish varieties on the basis of direct correlations (at $p < 0.01$) with such indicators as seed yield (SY) (0.649), genetic flexibility (G_p) (0.703), breeding value of the variety (S_v) (0.662) and regression coefficient b_i (0.774). At the same time, inverse relationships of medium strength (at $p < 0.05$) were determined with the stability variant Sdi^2 (-0.409) and the coefficient of genotypic variation of the variety CV_i (-0.397).

Table 4. Correlation analysis of dependencies of the system of chlorophyll fluorescence induction indices and adaptive potential of oilseed radish varieties (comparing 28 correlation pairs by averaged indices for the pair of incompatible repeats from the total scheme of 56 observations during 2015–2020 (for Snedecor, 1989))

Indicator	F_{pl}	F_m	F_{st}	dF_{pl}	F_v	dF_{pl}/F_v	EP	L_{wp}	Q_{ue}	RF_d	K_{ef}	QP
F_0	0.031	-0.431*	0.890**	-0.672***	-0.612***	-0.551**	-0.824***	-0.817***	0.824***	-0.777***	0.786***	-0.419*
F_{pl}		0.453*	0.155	0.719***	0.390*	0.702***	0.242	0.284	-0.227	0.225	-0.154	-0.132
F_m			-0.396*	0.635***	0.977***	0.254	0.863***	0.871***	-0.858***	0.858***	-0.836***	0.465*
F_{st}				-0.504**	-0.556**	-0.348	-0.739***	-0.730***	0.741***	-0.810***	0.832***	-0.764***
dF_{pl}					0.714***	0.903***	0.752***	0.778***	-0.741***	0.707***	-0.660***	0.194
F_v						0.352	0.950***	0.955***	-0.945***	0.934***	-0.917***	0.505**
dF_{pl}/F_v							0.445*	0.479**	-0.435*	0.381*	-0.335	-0.058
EP								0.995***	-0.999***	0.964***	-0.960***	0.522**
L_{wp}									-0.991***	0.966***	-0.954***	0.512**
Q_{ue}										-0.960***	0.960***	-0.525**
RF_d											-0.995***	0.712***
K_{ef}												-0.740***

Indices	K_{prp}	K_{fd}	V_t	SY	G_p	CV_i	b_i	S_{di}^2	H_{omi}	S_v
F_0	-0.817***	-0.777***	0.419*	-0.449*	-0.466*	0.096	-0.632***	0.381*	-0.147	-0.413*
F_{pl}	0.284	0.225	0.132	0.461*	0.473*	-0.517**	-0.059	0.440*	0.501**	0.532**
F_m	0.871***	0.858***	-0.465*	0.662***	0.726***	-0.580**	0.638***	-0.231	0.478**	0.689***
F_{st}	-0.730***	-0.810***	0.764***	-0.507**	-0.410*	0.105	-0.658***	0.503**	-0.141	-0.378*
dF_{pl}	0.778***	0.707***	-0.194	0.654***	0.674***	-0.476*	0.396*	0.061	0.473*	0.681***
F_v	0.955***	0.934***	-0.505**	0.685***	0.745***	-0.443*	0.707***	-0.292	0.454*	0.701***
dF_{pl}/F_v	0.470*	0.381*	0.058	0.456*	0.450*	-0.204	0.114	0.248	0.420*	0.467*
EP	0.995***	0.964***	-0.522**	0.658***	0.710***	-0.449*	0.749***	-0.359	0.391*	0.652***
L_{wp}	0.997***	0.966***	-0.512**	0.674***	0.722***	-0.387*	0.762***	-0.343	0.387*	0.680***
Q_{ue}	-0.991***	-0.960***	0.525**	-0.649***	-0.703***	0.385*	-0.744***	0.362	-0.358	-0.643***
RF_d	0.966***	0.967***	-0.712***	0.649***	0.703***	-0.397*	0.777***	-0.409*	0.391*	0.668***
K_{ef}	-0.954***	-0.995***	0.740***	-0.621***	-0.678***	0.388*	-0.775***	0.442*	-0.360	-0.632***

Table 4 (continued)

Indices	K_{prp}	K_{fd}	V_t	SY	G_p	CV_i	b_i	S_{di}^2	H_{omi}	S_v
QP	0.512**	0.712***	-0.995***	0.303	0.352	-0.217	0.561**	-0.490*	0.207	0.350
K_{prp}		0.966***	-0.512**	0.674***	0.722***	-0.383*	0.752***	-0.343	0.387*	0.670***
K_{fd}			-0.712***	0.649***	0.703***	-0.391*	0.774***	-0.409*	0.391*	0.662***
V_t				-0.303	-0.352	0.217	-0.561**	0.490**	-0.207	-0.382*
YS					0.972***	-0.487**	0.731***	-0.225	0.519**	0.914***
G_p						-0.571**	0.734***	-0.145	0.501**	0.906***
CV_i							-0.016	0.175	-0,978***	-0.777***
b_i								-0.540**	0.038	0.524**
S_{di}^2									-0.168	-0.204
H_{omi}										0.809***

* – reliable at 5% significance level; ** – reliable at 1% significance level; *** – reliable at 0.1% significance level.

Table 5. The system of regression dependencies of basic indicators of chlorophyll fluorescence induction and the main criterion estimates of the adaptive value of oilseed radish varieties (based on the combined 2015–2020 data set)

Indexes	Parameters x	Parameters y	Dependence equation	The coefficient of multiple regression (R/R^2)	Assessment of the significance of dependence
F_m	b_i	S_v	$F_m = 2{,}223.9895 – 678.4613x – 3{,}448.9904y + 744.578x^2 – 1{,}395.8739xy + 7{,}748.952y^2$	0.793*/0.629	$F/SS_{total} = 5.18$ ($F_{t05} = 4.67$), ($p < 0.05$)
F_v	b_i	S_v	$F_v = 1{,}671.6984 – 1{,}388.0067x – 57.2714y + 807.98x^2 + 32.3023xy + 3.1041y^2$	0.808*/0.653	$F/SS_{total} = 5.37$ ($F_{t05} = 4.67$), ($p < 0.05$)
EP	b_i	S_v	$EP = 0.8332 – 0.294x – 0.0172y + 0.1742x^2 + 0.0081xy + 0.0008y^2$	0.813*/0.661	$F/SS_{total} = 5.98$ ($F_{t05} = 4.67$), ($p < 0.05$)
L_{wp}	b_i	S_v	$L_{wp} = 5{,}2719 – 3{,}3971x – 0.2887y + 1{,}9941x^2 + 0.0984xy + 0.0148y^2$	0.823*/0.677	$F/SS_{total} = 6.29$ ($F_{t05} = 4.67$), ($p < 0.05$)
Q_{ue}	b_i	S_v	$Q_{ue} = 0.185 + 0.5931x + 0.0289y – 0.3511x^2 – 0.0163xy – 0.0014y^2$	0.803*/0.645	$F/SS_{total} = 5.22$ ($F_{t05} = 4.67$), ($p < 0.05$)
RF_d	b_i	S_v	$RF_d = 3.7205 – 2.7856x – 0.2888y + 1.2207x^2 + 0.1583xy + 0.0115y^2$	0.833*/0.694	$F/SS_{total} = 6.51$ ($F_{t05} = 4.67$), ($p < 0.05$)
K_{ef}	b_i	S_v	$K_{ef} = 0.1805 + 0.3186x + 0.0232y – 0.1401x^2 – 0.0182xy – 0.0007y^2$	0.819*/0.671	$F/SS_{total} = 6.14$ ($F_{t05} = 4.67$), ($p < 0.05$)
K_{prp}	b_i	S_v	$K_{prp} = 4.2627 – 3.4087x – 0.2853y + 1.9895x^2 + 0.1008xy + 0.0145y^2$	0.821*/0.674	$F/SS_{total} = 6.23$ ($F_{t05} = 4.67$), ($p < 0.05$)
K_{fd}	b_i	S_v	$K_{fd} = 4.7205 – 2.7856x – 0.2888y + 1.2207x^2 + 0.1583xy + 0.0115y^2$	0.831*/0.691	$F/SS_{total} = 6.42$ ($F_{t05} = 4.67$), ($p < 0.05$)

* Significant at 5% level.

Indirectly, the physiological state of oilseed radish varieties was assessed by the leaf water potential (L_{wp}), since, according to van der Tol et al. (2009), Vialet-Chabrand et al. (2017) and Zhang et al. (2020), the activity and efficiency of the plant photosystem depends on the total water content of the assimilation surface, its turgor state and water balance. Considering the already mentioned general physiological peculiarity of oilseed radish to intensive reduction of plant leafiness and its different tier physiological stages according to stem height (Tsytsiura, 2022), leaf water potential for oilseed radish cultivars is an important aspect of its photosystem functioning. According to our estimates, the highest value of this indicator in the range of 3.81–4.13 was established for varieties with high breeding value and adaptive properties such as 'Ramonta', 'Zhuravka', 'Snizhana' and confirmed by direct correlations (at $p < 0.01$) with such indicators as seed yield (SY) (0.674), genetic flexibility (G_p) (0.722), breeding value of the variety (S_v) (0.670) and regression coefficient b_i (0.752). That is, the indicators of effective water consumption of plants, the physiological state of the assimilation surface in the vertical profile of leaf placement determine the physiological mechanisms of adaptation of oilseed radish varieties.

Taking into account the calculated derivative of the basic indicators of the CFI curve such as Q_{ue}, K_{ef}, QP, K_{prp}, K_{fd}, its application in the process of evaluating oilseed radish varieties for adaptability, plasticity and stability correlates with the already indicated features of application in terms of basic indicators and certain indices based on them.

The results of multiple regression analysis confirm earlier conclusions and the most statistically significant results are shown in Table 5. Thus, the possibility of effective use of both basic and derivative indicators determined by estimating the chlorophyll fluorescence induction curve for the predicted assessment of both genotypic stability of the variety (b_i) and its breeding value in the system of complex interaction of stressful and non-stressful growing years (S_v) with the level of multiple regression coefficient in the range of 0.793–0.833 (at $p < 0.05$) was proved. The determined level of significance in combination with the results of pairwise correlation (Table 4) according to Pour-Aboughadareh (2022) is sufficient to apply the criteria of the CFI curve of the corresponding variety to identify its adaptive properties.

The established degree nature of the dependence in view of the study by Vaezi et al. (2019) indicates a complex configurational interaction of environmental factors both in relation to the physiological aspects of the photosynthetic activity of oilseed radish plants and in relation to the realization of the genetic yield potential of each variety.

CONCLUSIONS

It was determined that the complex indices of chlorophyll fluorescence induction F_0, F_{pl}, F_m, F_{st} had genotypic differences with the possibility of identifying stress resistance and adaptability of oilseed radish varieties to limiting exogenous environmental factors in the process of determining its breeding value. The possibility of using a comparable systematic analysis of the characteristic areas of the chlorophyll fluorescence induction curve (CFI) of the photosystem of oilseed radish varieties during the period of its maximum activity and identifiers of its plasticity and stability (coefficient of genetic plasticity (G_p), coefficient of agronomic stability (A_s), coefficient of stress resistance (C_{st}), coefficient of homeostaticity (H_{omi})) has been proved. The use of this variant of evaluation is confirmed by the coefficient of determination (d_{xy}) for the inverse nature of the pair correlation (at $p < 0.05$–0.01) at the level of 14.6–53.3% in the comparison of genetic plasticity (G_p), regression coefficient of genetic stability of the variety (b_i), breeding value of the variety (S_v) with such indicators as initial fluorescence (F_0), stationary fluorescence (F_{st}), rate of change of fluorescence in time (V_t). Direct pairwise correlation dependences (at $p < 0.01$–0.001) for the same parameters of genotypic evaluation and a number of CFI curve parameters in the following order of increasing d_{xy} were determined: fluorescence rise (dF_{pl}, d_{xy} = 15.6–46.4%) – maximum fluorescence (F_m, 40.7–52.7%) – photochemical efficiency (EP, 42.5–56.1%) – efficiency of the initial reactions of photosynthesis (K_{prp}, 44.9–56.6%) – maximum variable fluorescence (F_v, 49.1–55.5%) – leaf water potential (L_{wp}, 46.2–58.1%) – coefficient of decline of the fluorescence (K_{fd}, 43.8–59.9%) – plant viability index (RF_d, 44.6–60.4%). Predictability and applicability of these comparisons was confirmed by multiple regression analysis between genotypic stability (b_i), breeding value (S_v) and CFI curve parameters such as F_m, F_v, EP, L_{wp}, Q_{ue} (photochemical extinguishing), RF_d, K_{prp}, K_{ef} (indicator of endogenous stress factors), K_{fd} with the value of multiple regression coefficient (R) in the range 0.793–0.833 (at $p < 0.05$).

From the point of view of prospects for further research, this system of evaluation of initial breeding material should be studied for the possibility of application to other types of cruciferous crops, including its wild forms. This will improve the process of selection of source material and search for an effective variant of its genetic recombination.

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Evaluation of seven barley genotypes under water stress conditions

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Abstract. The evaluation of seven barley genotypes under water stress conditions using drought tolerance indices was investigated during two agronomical seasons in the experimental field of Agricultural University of Athens in Greece. The experimental design was a split-plot layout in four blocks. Four different levels of irrigation were implemented, with the method of escalated distance from the source of water (drip irrigation line). The experimental plots were protected from rain since the experiment was conducted under a rainout shelter. Measurements of water potential index of the plants were conducted, as well as stomatal resistance and stomatal resistance index of leaves, and grain yield of genotypes. A decrease in water potential index (15–25%) and grain yield (35–54%) was observed in all genotypes as soil moisture decreased. On the other hand, stomatal resistance and stomatal resistance index (26–69%) of leaves increased. Grain yield had a strong relationship with the indices of water potential and stomatal resistance of leaves. Grain yield of all genotypes is affected under water stress conditions, with the six-rowed genotypes being more adaptive than the two-rowed ones. It can be concluded that indices of water potential and stomatal resistance of leaves can be effectively used in the evaluation of genotypes under water stress conditions.

Key words: drought tolerance, stomatal resistance index, water potential index, grain yield.

INTRODUCTION

Barley *(Hordeum vulgare* L.) is an important cereal crop grown in arid and semi-arid regions. However, extreme drought and high temperatures can adversely affect plant growthand yield (Panfilova et al., 2019; Goher & Akmal, 2021; Sánchez-Díaz et al., 2002; Samarah, 2005; Mostipan et al., 2021). In these extreme conditions, plants are unable to adequately replace the water they lose due to increased transpiration and therefore, activate mechanisms to response to water stress (Chaves et al., 2009; González & Agerbe, 2010; Bresta et al., 2011, Karabourniotis et al., 2012, Schmid et al., 2015). The identification of morphological, physiological and metabolic parameters as indices of drought tolerance, and their use for the evaluation ofgenotypes is crucial for breeders and has been the subject of many research works (Jamshidi & Javanmard, 2018; Cai et al., 2020; Feiziasl et al., 2022).

Two important indices of drought tolerance are water potential and stomatal resistance of leaves. Water potential as a concept that holds a very important position in plants physiology. On the one hand, it defines the course of movement of water between neighbouring cells or tissues and the environment and on the other hand, it constitutes a measure of plant's water status. Some of the characteristics which form the values of the water potential and consequently the Water Potential Index are: a) the conductance of the stomata and the ability to absorb water from roots (Sibounheuang et al., 2006; Széles et al., 2021), b) the ability to transfer water from roots or sprout to leaves. According to Turner et al. (1984), low hydraulic conductance of the leaves, leads to a decrease in water potential and c) plants' size, either by the size of its leaves or by the number of its secondary stems or even yet by its height. More particularly, Boonjung & Fukai (1996), found that genotypes with large leaf surface had a lesser ability to hold water because of high demands in transpiration. Furthermore, Jongdee et al. (2002) admit that leaf water potential and osmotic adjustment are traits that may be useful as selection criteria for improving drought tolerance.

According to del Moral et al. (2003) and Flohr et al. (2017), cereals grain yield is sensitive to the intensity of dryness during flowering. Karamanos (1981) also refers, stomata respond to a number of environmental factors (Schulze et al., 1972) such as light, relative moisture, CO_2 concentration and temperature of leaves and aquatic status. More particularly the stomatal resistance of leaves1) is decreased by the increasing tension of light 2) is increased with the increase of CO_2 concentration of the surrounding space 3) it is increased with the increase of atmospheric dryness 4) it is reduced with the increase of temperature until an optimum value beyond of which the stomata begin to close and 5) stomata close when leaves reach into a critical dehydration point. From the aforementioned factors, the concentration of CO_2 is considered to have the most important effect on stomatal's movements, whereas light does not necessarily open the stomata (Raschke, 1976), provided that leaf's potential is above a threshold value. If the dehydration continues and the threshold value is reached, then stomata close regardless of the CO_2 concentration.

Considering this background, a two-factorial experiment was designed to investigate the impact of water stress on water potential index, stomatal resistance index and grain yield and their relationship, in seven barley genotypes.

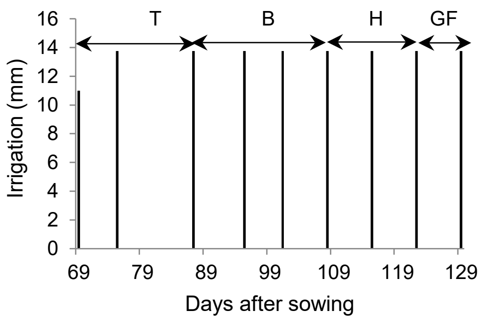
MATERIALS AND METHODS

Site description and field preparations

Two experiments were conducted during 2013–2014 and 2014–2015 agronomical seasons under a rainout shelter with surface of 300 m^2 (30 m × 10 m) and height 2.80 m (minimum) and 3.80 m (maximum), in the experimental field of the Agricultural University of Athens (AUA) in Greece. Soil was clay-loam (34.7% sand, 29.8% clay, 35.5% silt), with a pH of 7.95 and 16% of $CaCO_3$. Sowing was conducted on 14 December 2013 and 12 December 2014, the first and the second agronomical seasons respectively. Before sowing but also during experiments weed control was conducted manually.

Treatments and experimental design

The design of the experiments included a general factorial structure with two treatment factors. The trials followed a split-plot layout in four blocks, in which each of the seven barley genotypes was assigned to the main plots and the four treatments were allocated to the subplots. Each main plot had a surface of 1.8 m^2 (1.5 m × 1.2 m), whereas each subplot was 0.45 m^2 (1.2 m × 0.375 m). Each experimental plot included six rows, 1.2 m long and 20 cm apart and the planting distance within the rows was 3 cm. The irrigation levels were differentiated according to their distance from the source of water (drippers). Irrigation level A (without water stress) was the closest to water source and irrigation level D (high water stress), was in the longest distance from the drippers. The intermediate levels B (low water stress) and C (medium water stress) were found in between the two extremes (A, D). The genotypes consisted of two populations (ANP-233/07, F-002/06) and five varieties (Elassona, Kos, Athinais, Cha-Cha, Grace). The six-row population ANP-233/07 and the two-row F-002/06 are local populations, a remarkable genetic material for study, preserved in the Bank of Genetic Material (NAGREF). Six-row Elassona, two-row Kos and six-row Athinais are Greek varieties adjusted to Greek conditions and they come from the Institute of Cereals (NAGREF). The two-row Cha-Cha and Grace are modern, early, short varieties, of high efficiency, with excellent malting characteristics, breeding achievements of the company Athenian Brewery SA. The irrigation water which was used was supplied with a drip irrigation line system (1–1.5 bar operating pressure, 5 dripper per plot, 10 L per 1 h drippers flow rate and 24 cm distance between drippers). The frequency of irrigation was determined by laboratory measurements of soil moisture as a percentage of its oven-dried weight taken from plot samples. Irrigation was applied when soil water content falls below 30% of field water capacity. The duration of irrigation ranged from 1.5 to 2.5 hours, and the corresponding volume of water from 11 to 13.75 mm. The total volume of water supplied, and the frequency of irrigation is presented in Fig. 1.



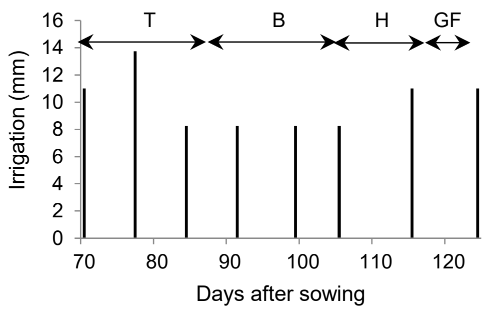


Figure 1. Water treatment after the application of drip irrigation (left) during the first season (2013–2014) and (right) during the second season (2014–2015). T: tillering; B: booting; H: heading; GF: grain-filling.

Plant water status

Two leaves per subplot (irrigation level) and eight leaves in total per main plot were collected as samples at 12 p.m. when the value of water potential reaches its minimum daily value. The youngest fully expanded leaf was sampled until spike emergence (Zadoks stage 58). From that point until maturity, flag leaf was sampled. Leaves were sealed in plastic bags before cutting and transported to the laboratory in closed insulated vessels to avoid water loss. Water potential (*Ψ)* was determined by the Pressure Bomb Technique (Scholander et al., 1964). The pressure chamber was set according to Waring & Cleary (1967). From the time course of *Ψ*, water potential index (WPI) was calculated according to Karamanos & Papatheohari (1999). Water potential index represents plants' water stress history during any period of their growth cycle.

$$WPI = \int_{t=1}^{t} \Psi_t dt/n \qquad (1)$$

where Ψ_t is the water potential at Day *t* within the observation period and *n* is the length of a period in days.

Grain yield

On the first year, harvest was conducted on May 16, 2014 (154 days after sowing) and on the second, on May 10, 2015 (150 days after sowing). From each subplot, 3 plants from the internal lines were chosen as well as 12 plants per main plot in total, in which grain yield per plant (g $plant^{-1}$) was studied. Grain yield effect of barley's genotypes in water stress was assessed by comparing linear regression's coefficients between grain yield and WPI (Karamanos & Papatheohari 1999; Rizza et al., 2004).

Stomatal resistance and stomatal resistance index ($r_{st}I$).

Measurements of stomatal resistance of the lower surface of leaf's margin were conducted. In every replication two leaves per subplot were sampled (irrigation level) and eight leaves in total per main plot. Before cutting the leaves to be transported to the laboratory and to be measured for their water potential, there was a measurement of stomatal resistance by using a porometer (Porometer AP4, Delta-T Devices-Cambridge-U.K). From the time course of stomatal resistance, stomatal resistance index was calculated. The $r_{st}I$ represents leaf's stomatal resistance history during any period of the biological cycle.

$$r_{st}I = \int_{t=1}^{t} r_{st} dt/n \qquad (2)$$

where r_{st} is the stomatal resistance at Day *t* within the observation.

Statistical Analysis

Statistical analysis was performed separately for each agronomical season. There was an initial check for normal distribution with the Shapiro-Wilk test and for homogeneity of variance with the Levene test as, as well as a check for outliers with the Dixon test. The data were subjected to ANOVA and the results are presented as the means ± standard errors. The comparisons of the means were performed using the Tukey HSD criteria with a level of significance of $\alpha = 0.05$. In order to examine and reveal the relationships between grain yield and the indices of water potential and stomatal resistance, bivariate analysis were used.The statistical analysis was done in R 4.1.

Weather conditions

In Fig. 2, weather data from outside of the rainout shelter for the two agronomical seasons are presented respectively. Data include average monthly maximum, mean and minimum temperature, average relative humidity, and finally average intensity of solar radiation.

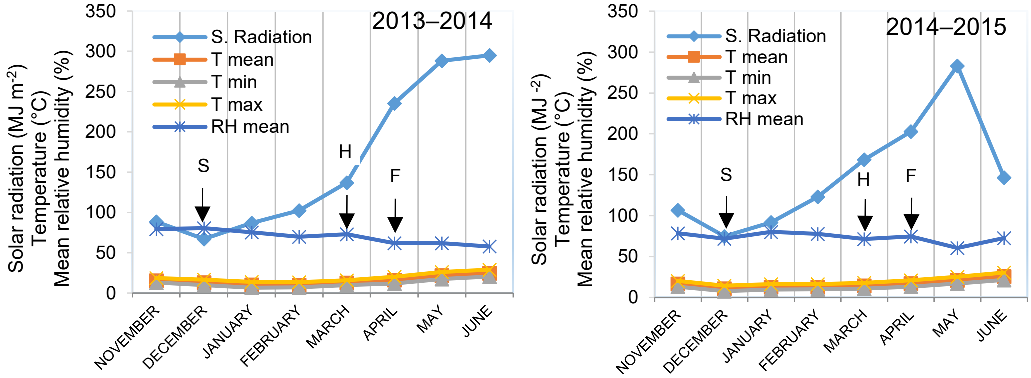


Figure 2. Average monthly maximum, mean and minimum temperature, mean relative humidity and the intensity of the solar radiation, outside of rainout shelter outside of rainout shelter during the first season (2013–2014) (left) and during the second season (2014–2015) (right). The vertical arrows show S: sowing; H: heading; F: flowering. Tmean, Tmin, Tmax: mean, minimum and maximum temperature.

During sowing in the second agronomical season values of minimum, maximum and mean temperature were greater than the first agronomical season by 2.1 °C, 2.5 °C and 2.26 °C, respectively. Both in heading as in flowering, during the first agronomical season values of minimum, maximum and mean temperature, were greater than the ones during the agronomical season. Particularly, during heading, values were greater by 0.95 °C, 2.06 °C and 1.5 °C and in flowering by 1.26 °C, 0.5 °C, and 0.8 °C, respectively. Furthermore, during sowing and heading, on the second season, values of mean relative humidity were greater than the first season by 8.9% and 1.5%, respectively. In flowering, during the first season mean relative humidity was greater by 12.76% from the second one. Also, during sowing and heading on the first season the intensity of the solar radiation was greater than the second season by 6.98 MJ m^{-2} and 31.27 MJ m^{-2}, respectively. On the contrary, during flowering in the second agronomical season, the intensity of solar radiation was greater by 32.64 MJ m^{-2} from the first season. On the first agronomical season during May-June a sharp decline of solar radiation was observed, which was not observed during the second season.

RESULTS

Water Potential Index (WPI)

In both trials, the main effects of genotypes and treatments-irrigation levels were statistically significant while the interaction between the two factors was not significant. The values of WPI for the seven barley genotypes in the four treatments are presented on Table 1. In both trials, all the comparisons of treatments were statistically significant. The genotype Kos has the greatest negative WPI values in high water stress (treatment D), in the first and the second season. The genotypes ANP-233/07 and Grace have the lowest negative WPI values in high water stress, in the first and the second season. The greatest change in WPI values between the extreme water treatments (A–D) during the first season, appeared in genotypes ANP-233/07 (20.2%) and Elassona (17.2%) and in the second season in genotype Elassona (25%). In addition, the smallest change can be observed in genotype Grace, in the first season (15%) and in the second season (15.6%) of the experiments.

Table 1. The means and thetypical errorsof water potential index-WPI (MPa). Means with the same letter are not significantly different from each other (Tukey-HSD test)

Genotypes	Water Treatments				
	A	B	C	D	
	First Season (2013–2014)				Mean Gen.
ANP-233/07	-1.68 ± 0.04	-1.76 ± 0.05	-1.88 ± 0.05	-2.02 ± 0.05	-1.84 a
ATHENAIS	-1.77 ± 0.03	-1.81 ± 0.06	-1.95 ± 0.06	-2.08 ± 0.05	-1.91 ab
GRACE	-1.80 ± 0.02	-1.94 ± 0.02	-1.99 ± 0.02	-2.07 ± 0.02	-1.95 bc
CHA-CHA	-1.79 ± 0.01	-1.94 ± 0.01	-2.02 ± 0.02	-2.08 ± 0.01	-1.96 bcd
F-002/06	-1.86 ± 0.04	-1.95 ± 0.05	-2.06 ± 0.03	-2.18 ± 0.03	-2.01 cde
ELASSONA	-1.86 ± 0.02	-2.00 ± 0.02	-2.07 ± 0.07	-2.20 ± 0.07	-2.03 de
KOS	-1.90 ± 0.03	-2.04 ± 0.03	-2.13 ± 0.04	-2.23 ± 0.04	-2.07 e
Mean W.T.	-1.81 a	-1.92 b	-2.01 c	-2.12 d	G.M[1] = -1.96
	Second Season (2014–2015)				Mean Gen.
ELASSONA	-1.59 ± 0.01	-1.80 ± 0.01	-1.92 ± 0.03	-2.07 ± 0.03	-1.74 a
ANP-233/07	-1.65 ± 0.02	-1.78 ± 0.01	-1.86 ± 0.01	-2.00 ± 0.01	-1.82 b
GRACE	-1.73 ± 0.01	-1.85 ± 0.03	-1.93 ± 0.03	-2.01 ± 0.08	-1.88 c
ATHENAIS	-1.74 ± 0.02	-1.80 ± 0.02	-1.93 ± 0.03	-2.08 ± 0.03	-1.89 cd
F-002/06	-1.71 ± 0.03	-1.84 ± 0.05	-1.93 ± 0.02	-2.08 ± 0.02	-1.89 cd
KOS	-1.76 ± 0.02	-1.84 ± 0.01	-1.95 ± 0.01	-2.13 ± 0.02	-1.92 cd
CHA-CHA	-1.80 ± 0.01	-1.88 ± 0.02	-1.98 ± 0.02	-2.12 ± 0.02	-1.94 d
Mean W.T.	-1.65 a	-1.83 b	-1.93 c	-2.07 d	G.M.= -1.87

[1] G.M. = Grand mean.

Stomatal Resistance (r_{st})

Fig. 3 and Fig. 4 present the changes of stomatal resistance of leaves of the seven barley genotypes over time. The gradually increasing values of stomatal resistance are statistically significant in both trials, for all genotypes and water treatments.

Stomatal Resistance Index ($r_{st}I$)

The values of stomatal resistance index for the seven barley genotypes in the four treatments are presented on Table 2. In first season, the main effects of genotypes and treatments-irrigation levels were statistically significant while the interaction was not significant. All the comparisons of treatments were statistically significant. The genotypes Athinaida, Elassona, ANP-233/07 and Grace have the highest values of $r_{st}I$ in high water stress (treatment D), while the genotypes F-002/06 and Kos have the lowest values. The highest change between the extreme water treatments (A–D) was presented by genotype F-002/06 (55.7%) and the lowest change by the genotype Elassona (48.6%). In second season, the interaction between the two factors was statistically significant. The genotypes Elassona, and Grace have the highest values of $r_{st}I$ in high water stress, wlile the genotypes Athinaida and ANP-233/07 have the lowest values. The highest change between the extreme water treatments (A–D) was presented by genotype F-002/06 (69%) and the lowest change by the genotypes Grace (26.8%) and Cha-Cha (31.1%).

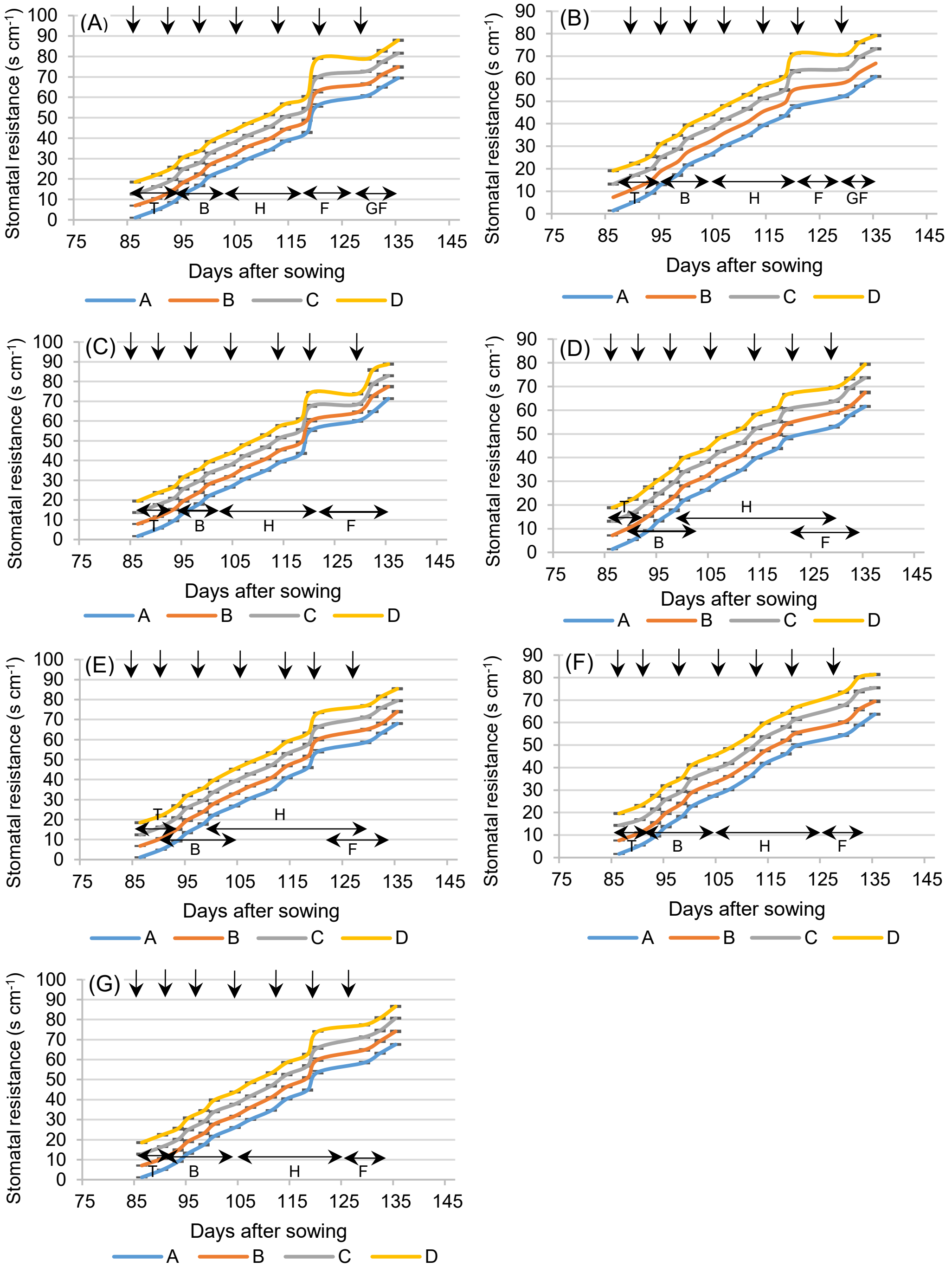


Figure 3. The changes of stomatal resistance of leaves for the barley genotypes during the first season (2013–2014) of experiments. The vertical arrows show each watering point and the horizontal show the growth stage. T: tillering; B: booting; H: heading; F: flowering; GF: grain-filing. A-D: water treatments. (A): ANP-233/-07; (B): F-002/06; (C): Elassona; (D): Kos; (E): Athinais; (F): Cha-Cha; (G): Grace. The vertical bars symbolize the typical error of mean values.

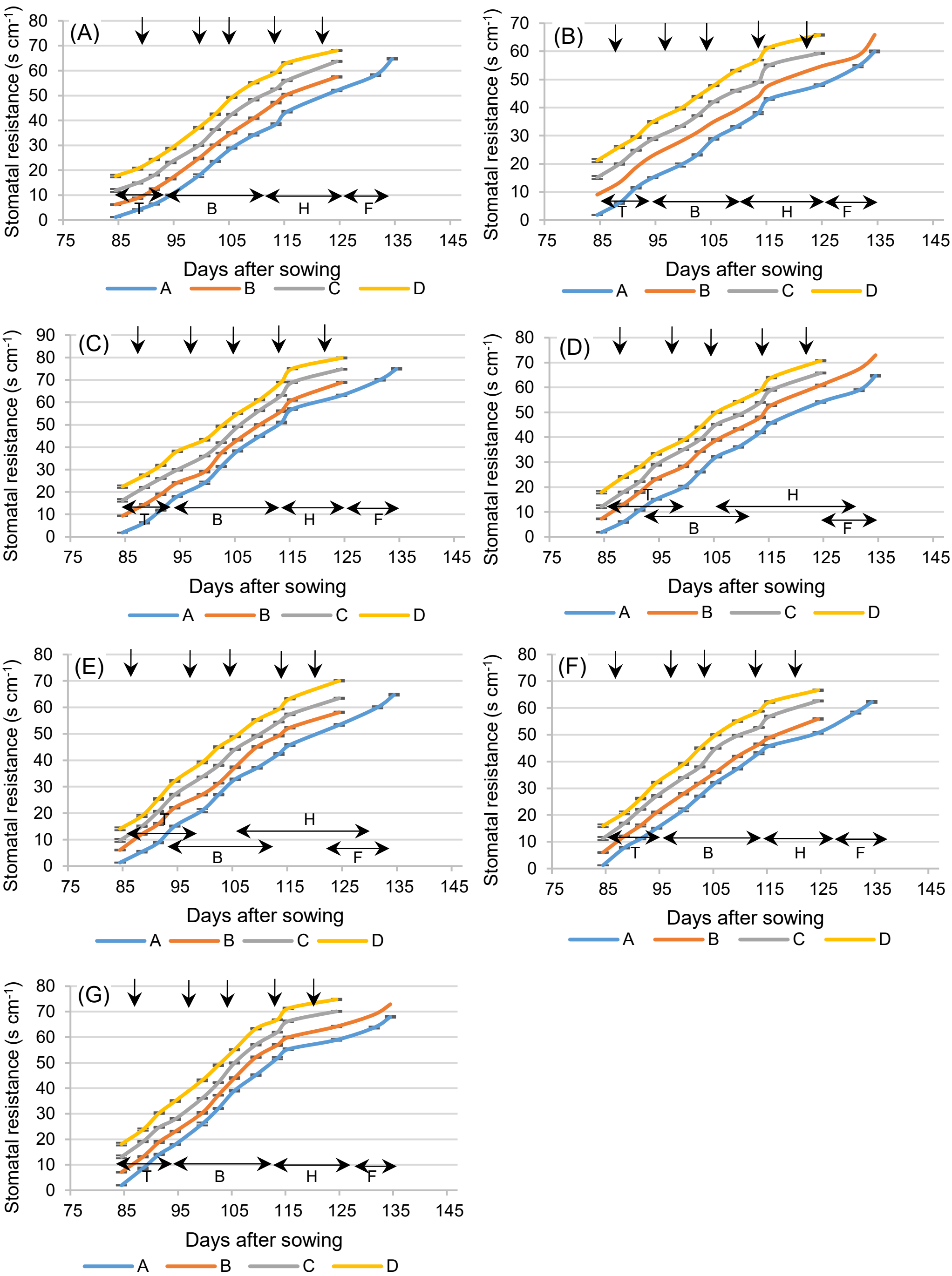


Figure 4. The changes of stomatal resistance of leaves for the barley genotypes during the second season (2014–2015) of experiments. The vertical arrows show each watering point and the horizontal show the growth stage. T: tillering; B: booting; H: heading; F: flowering; GF: grain filing. A–D: water treatments. (A): ANP-233/-07; (B): F-002/06; (C): Elassona; (D): Kos; (E): Athinais; (F): Cha-Cha; (G): Grace. The vertical bars symbolize the typical error of mean values.

Table 2. The means and the typical errors of stomatal resistance index. Means with the same letter are not significantly different from each other (Tukey-HSD test)

Genotypes	Water Treatments				
	A	B	C	D	
	First Season (2013–2014)				Mean Gen.
ATHINAIDA	35.37 ± 0.14	41.52 ± 0.22	47.60 ± 0.21	53.31 ± 0.20	44.45 a
ELASSONA	35.71 ± 0.25	41.43 ± 0.24	47.26 ± 0.27	53.10 ± 0.27	44.37 a
ANP-233/07	35.26 ± 0.19	41.18 ± 0.15	47.17 ± 0.11	53.43 ± 0.12	44.26 a
GRACE	35.01 ± 0.22	41.08 ± 0.18	47.04 ± 0.18	53.25 ± 0.22	44.10 a
CHA-CHA	34.26 ± 0.13	40.17 ± 0.07	46.48 ± 0.18	52.45 ± 0.20	43.34 b
F-002/06	32.82 ± 0.18	38.97 ± 0.18	45.15 ± 0.23	51.11 ± 0.26	42.01 c
KOS	33.23 ± 0.31	39.08 ± 0.27	44.96 ± 0.32	50.72 ± 0.23	42.00 c
Mean W.T.	34.52 a	40.49 b	46.52 c	52.48 d	G.M.[1] = 43.5
	Second Season (2014–2015)				Mean Gen.
GRACE	40.35 ± 0.18 i	45.17 ± 0.31 ef	48.21 ± 0.35 c	51.17 ± 0.20 b	46.23
ELASSONA	36.92 ± 0.29 lm	40.84 ± 0.25 hi	47.01 ± 0.22 cd	53.17 ± 0.27 a	44.49
CHA-CHA	34.52 ± 0.21 n	38.15 ± 0.33 kl	40.62 ± 0.18 hi	45.27 ± 0.16 ef	39.64
KOS	30.06 ± 0.34 q	35.95 ± 0.22 m	41.79 ± 0.35 h	46.21 ± 0.35 de	38.50
F-002/06	27.16 ± 0.31 r	33.12 ± 0.31 op	39.55 ± 0.16 ij	45.92 ± 0.16 def	36.44
ATHINAIDA	28.78 ± 0.12 q	34.09 ± 0.39 no	37.92 ± 0.23 kl	43.37 ± 0.21 g	36.04
ANP-233/07	26.99 ± 0.39 r	32.49 ± 0.23 p	38.65 ± 0.10 jk	44.61 ± 0.19 fg	35.69
Mean W.T.	32.11	37.12	41.97	47.11	G.M. = 39.58

[1] G.M. = Grand mean.

Grain Yield

The values of the grain yield for the seven barley genotypes in the four treatments are presented on Table 3. In both trials, the interaction between genotypes and treatments-irrigation levels were statistically significant. In first season, the highest grain yield in high stress water was observed by genotypes Cha-Cha and Grace and the lowest grain yield by genotypes Elassona and Kos. The highest change between the extreme water treatments (A–D) was observed by genotypes Elassona (46.7%) and Kos (46.7%) and the lowest change by genotypes Cha-Cha (34.5%) and Grace (35.5%). In second season, the highest grain yield in high water stress was observed by genotypes Cha-Cha and Grace and the lowest grain yield by genotypes Elassona and Kos. The highest change in grain yield was observed by genotypes Kos (55.7%) and F-002/06 (54.4%) and the lowest change by genotypes Cha-Cha (38.9%) and ANP-233/07 (36.4%).

Table 3. The means and thetypical errorsof grain yields (g/plant). Means with the same letter are not significantly different from each other (Tukey-HSD test)

Genotypes	Water Treatments				
	A	B	C	D	
	First Crop Season (2013–2014)				Mean Gen.
ANP-233/07	3.51 ± 0.02 d	3.13 ± 0.04 efg	2.75 ± 0.02 ij	2.20 ± 0.04 mn	2.90
ATHINAIDA	3.29 ± 0.03 e	3.02 ± 0.03 g	2.36 ± 0.02 lm	1.95 ± 0.06 o	2.66
CHA-CHA	4.20 ± 0.03 a	4.03 ± 0.03 b	3.22 ± 0.04 ef	2.75 ± 0.03 i	3.56
ELASSONA	2.74 ± 0.03 ij	2.49 ± 0.07 kl	2.03 ± 0.05 no	1.46 ± 0.02 q	2.18
F-002/06	3.12 ± 0.03 efg	2.66 ± 0.02 ijk	2.15 ± 0.02 n	1.68 ± 0.03 p	2.41
GRACE	4.00 ± 0.04 b	3.78 ± 0.03 c	2.99 ± 0.04 gh	2.58 ± 0.01 jk	3.35
KOS	3.10 ± 0.03 fg	2.82 ± 0.03 hi	2.20 ± 0.02 mn	1.65 ± 0.03 p	2.44
Mean W.T.	3.43	3.13	2.53	2.04	G.M.[1] = 2.78

Table 3 (continued)

	Second Crop Season (2014–2015)				Mean Gen.
ANP-233/07	3.20 ± 0.01 d	2.90 ± 0.04 e	2.56 ± 0.02 g	2.04 ± 0.03 j	2.67
ATHINAIDA	2.99 ± 0.02 e	2.72 ± 0.01 f	2.09 ± 0.02 ij	1.66 ± 0.03 l	2.36
CHA-CHA	3.90 ± 0.02 a	3.70 ± 0.02 b	2.92 ± 0.04 e	2.38 ± 0.03 h	3.23
ELASSONA	2.41 ± 0.02 h	2.20 ± 0.03 i	1.78 ± 0.04 kl	1.19 ± 0.02 m	1.89
F-002/06	2.90 ± 0.04 e	2.39 ± 0.03 h	1.74 ± 0.03 kl	1.32 ± 0.02 m	2.09
GRACE	3.75 ± 0.03 b	3.45 ± 0.03 c	2.61 ± 0.02 fg	2.11 ± 0.03 ij	2.98
KOS	2.87 ± 0.03 e	2.66 ± 0.02 fg	1.86 ± 0.03 k	1.27 ± 0.03 m	2.17
Mean W.T.	3.15	2.86	2.22	1.71	G.M. = 2.48

[1] G.M. = Grand mean.

Bivariate Analysis

In both trials the grain yield was significantly positively correlated with the water potential index and significantly negatively correlated with the stomatal resistance index. Linear regression of the response variables of grain yield was performed with the predictor variables of the water potential index and with the stomatal resistance index per genotype and agronomical season (Fig. 5 and Fig. 6). All regression coefficients were significant (Table 4 and Table 5). Also, all regression coefficient comparisons were significant except for the regression coefficients of grain yield and stomatal resistance index during the first season.

Table 4. Regression coefficients between grain yields (g) per plant and water potential index (WPI) during the first and second season

		First Season (2013–2014)				Second Season (2014–2015)			
	Term	Est.	S.E.	Prob>|t|	R^2	Est.	S.E.	Prob>|t|	R^2
ANP-233/07	a[1]	7.64	0.93	<.0001	0.65	8.79	0.30	<.0001	0.96
	b[2]	2.58	0.50	0.0002		3.35	0.16	<.0001	
Athinaida	a	7.78	1.14	<.0001	0.59	9.18	0.57	<.0001	0.96
	b	2.69	0.59	0.0005		3.61	0.30	<.0001	
Cha-Cha	a	12.9	1.33	<.0001	0.78	12.6	0.71	<.0001	0.93
	b	4.79	0.67	<.0001		4.83	0.36	<.0001	
Elassona	a	7.35	1.12	<.0001	0.95	3.88	0.37	<.0001	0.97
	b	2.54	0.55	0.0004		1.14	0.20	<.0001	
F-002/06	a	9.49	0.99	<.0001	0.99	9.17	0.90	<.0001	0.99
	b	3.51	0.49	<.0001		3.75	0.47	<.0001	
Grace	a	13.1	1.40	<.0001	0.77	10.1	1.63	<.0001	0.58
	b	5.01	0.72	<.0001		3.81	0.86	0.0006	
Kos	a	10.0	1.18	<.0001	0.75	10.5	0.55	<.0001	0.94
	b	3.64	0.56	<.0001		4.35	0.28	<.0001	

[1] = intercept; [2]= slope.

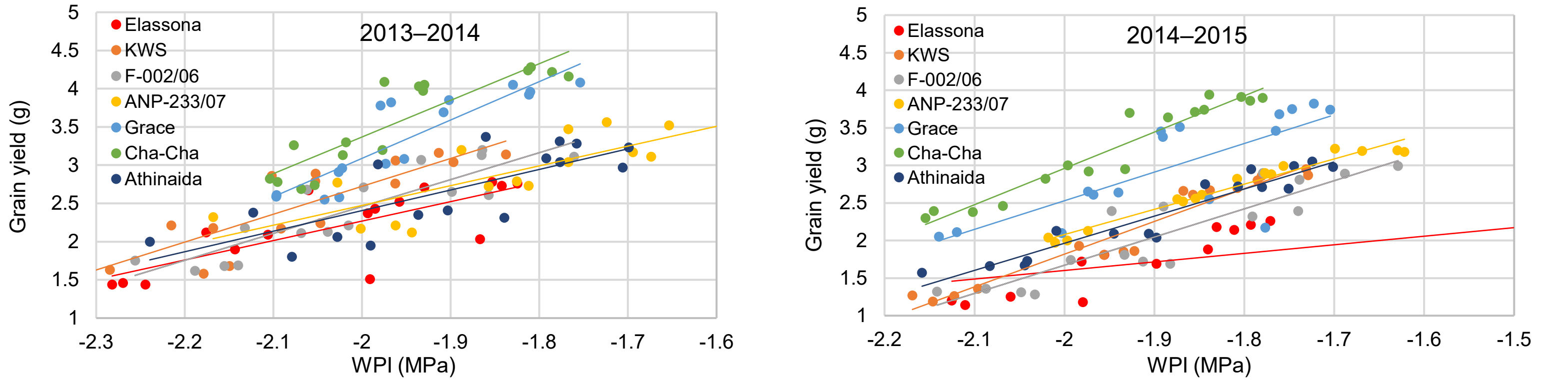


Figure 5. The fitted linear regressions between grain yields (g) per plant and water potential index (WPI) during the first season(left) and second season(right).

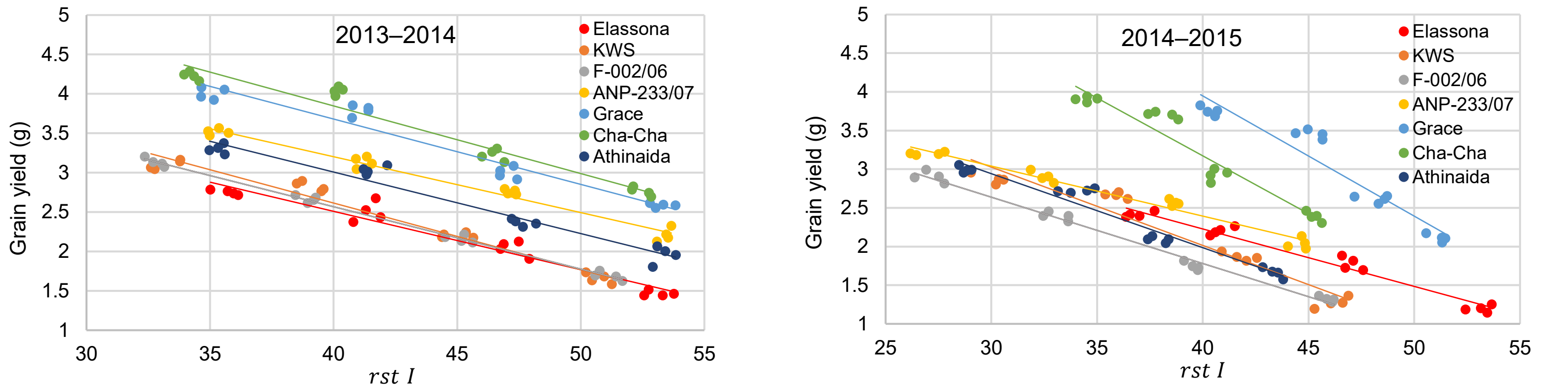


Figure 6. The fitted linear regressions between grain yields (g) per plant and stomatal resistance index ($r_{st}I$) during the first season (left) and second season (right).

Table 5. Regression coefficients between grain yields (g) per plant and stomatal resistance index ($r_{st}I$) during the first and second season

	Term	First Season (2013–2014)				Second Season (2014–2015)			
		Est.	S.E.	Prob>|t|	R^2	Est.	S.E.	Prob >|t|	R^2
ANP-233/07	a[1]	6.03	0.12	<.0001	0.97	4.98	0.11	<.0001	0.97
	b[2]	-0.07	0.01	<.0001		-0.06	0.00	<.0001	
Athinaida	a	6.13	0.19	<.0001	0.96	5.81	0.21	<.0001	0.95
	b	-0.07	0.01	<.0001		-0.10	0.01	<.0001	
Cha-Cha	a	7.28	0.22	<.0001	0.95	9.15	0.45	<.0001	0.97
	b	-0.08	0.01	<.0001		-0.15	0.01	<.0001	
Elassona	a	5.47	0.20	<.0001	0.95	5.20	0.16	<.0001	0.97
	b	-0.07	0.01	<.0001		-0.07	0.00	<.0001	
F-002/06	a	5.73	0.05	<.0001	0.99	5.21	0.08	<.0001	0.99
	b	-0.07	0.01	<.0001		-0.09	0.00	<.0001	
Grace	a	6.99	0.23	<.0001	0.96	10.17	0.59	<.0001	0.91
	b	-0.08	0.01	<.0001		-0.16	0.01	<.0001	
Kos	a	5.99	0.17	<.0001	0.95	6.07	0.28	<.0001	0.93
	b	-0.08	0.01	<.0001		-0.10	0.01	<.0001	

[1] = intercept; [2]= slope.

Discussion

Plants' aqueous status depends on both weather conditions and the irrigation provided. In the first agronomical season the values of the minimum, maximum and mean temperature both in heading and flowering were greater in relation to the second season. Levels of humidity and intensity of the solar radiation did not change dramatically from the one year of experiments to the other (Fig. 2). Moreover, the millimeters of irrigated water were higher during the first season than the second (Fig. 2).

Water Potential is the main and most reliable natural parameter for the estimation of the aqueous status of plants (Karamanos, 1981; Karamanos & Papatheohari, 1999). Values of water potential during the experiments, consequently led to the calculation of the water potential index based on the suggested method of Karamanos & Papatheohari (1999). This particular index provides a fairly reliable indication of the total water stress sustained by a plant during a given season. On the contrary Water Potential provides information for the given time when the sample is taken. The use of Water Potential as an objective index for the estimation of total stress that plants can sustain in a given environment has many advantages and can be used in order to evaluate its genotype drought resistance (Papastavrou et al., 2004). On the first season, WPI index had a greater amount of negative values, meaning that plants were under more intense stress conditions than in the second year. WPI presented an average value of 1.81 MPa, 1.92 MPa, 2.01 MPa and 2.12 MPa for irrigation levels A, B, C, D respectively. On the second year, WPI presented an average value of 1.65 MPa, 1.83 MPa, 1.93 MPa and 2.07 MPa for the four different levels of irrigation, respectively. These results can be easily explained by the alterations in temperature (Vahamidis et al., 2018). Experiments were conducted in a protected environment under the same circumstances so there was no differentiation due to e.g. different wind speeds. Moreover, a common feature of the WPI values of the seven studied barley genotypes in both agronomical seasons was the genotypic differences and clear classification between treatments (Table 1). On the first season, Kos, F-002/06 and Elassona had the greater negative values of WPI. Whereas the less negative value on irrigation level A, was observed for the population ANP-233/07, on irrigation level D, the most negative values appeared on Kos and Elassona and the less negative values on ANP-233/07, Athenais, Cha-Cha and Grace. On the second agronomical season, the most negative values appeared on Cha-Cha and Kos and the less negative value Elassona, on irrigation level A. On irrigation level D, the most negative values appeared on Kos and Cha-Cha and the less negative values on ANP-233/07 and Grace. In conclusion, we ascertain the tendency for more negative values on Kos and less negative on ANP-233/07 and Grace. Intra-genotypic differentiation reflects the corresponding genetic variation they conceal and which is expressed in a many different phenotypic ways (Papastavrou, 2004; Panfilova et al., 2020). The almost complete differentiation of WPI between treatments showed that the seven genotypes during the experimental procedures had different water stress intensities in the four subplots.

The increased stomatal resistance of leaves to water vapor diffusion is one of the immediate and rapid reactions of plants to water scarcity (Reynolds-Henne et al., 2010). The course of stomatal resistance revealed low values during the first growth stages, followed by a rise with the progress of growth stages. Variation in the seven barley genotypes was also observed in the values r_{st} in various irrigation treatments (Figs 3 and 4), with greater stomatal resistance values in treatment D in relation to treatment A. Sezen et al. (2019), ended in similar assumptions in red pepper plants and Nemeskeri et al. (2015), in pea plants. The observed increase in parameter values is attributed to both increased water deficits and growth stage (Karamanos et al., 1983; Gupta et al., 2001). Moreover, according to Teare et al. (1973), Ahmed et al. (2013) and Ghotbi-Ravandi et al. (2014), the closure of stomata on barley plants and the reduction of their conductivity is a general plant reaction to drought in order to prevent dehydration. A similar reaction, namely the closure of the stomata and a decrease in their conductivity was observed in soybean plants (Fenta et al., 2012) in drought susceptible genotypes. The negative effects of drought in the closing of stomata and photosynthesis leadto lower capacity development and reduction of biomass accumulation (Benešová et al., 2012). The effect of water stress was also visible on the stomatal resistance index of leaves in all the seven barley genotypes, with increasingly higher values from treatment A to treatment D (Table 2). Moreover, in the first season plants were more fatigued therefore the values of the stomatal resistance index are higher than in the second season.

In our experiment, in both experimental years, in irrigation levels A and D, the greatest grain yield appeared for the two-rowed varieties Cha-Cha and Grace whereas the smallest for the variety namely Elassona. The reduction on grain yield per plant due to the reduction on soil humidity for both seasons was obvious for all seven of the studied barley varieties (Table 3). The reduction on yield with the reduction of the available water is the most characteristic reaction of all crops (Horváth et al., 2021), barley included. Indicatively, relative reports have been done in the studies of Fischer & Maurer (1978), Brisson & Cassals (2005), Samarah et al. (2009); Hakala et al. (2012); Arshadi et al. (2018) and Zargar et al. (2018), where they tried to explain the effect of different treatments on yield differentiation. Grain yield modification in cereals is a multi-factor dependent process which involves complex procedures. In our case, the intensity of water stress as well as the implemented growth stage play an important role. Three critical stages for the grain yield were defined: from booting till the beginning of the last leaf's sheath swelling, flowering and grain filling. Time before heading has the greatest contribution in the final reduction (Karamanos, 2008). Still, according to del Moral et al. (2003) and Flohr et al. (2017) grain yield is sensitive to water scarcity during flowering.

Moreover, during the experiment it has been observed that the two-row genotypes were more sensitive (higher coefficient b) to water stress. Furthermore, in the first season in which we had more intense water stress (more negative values of WPI), there was a clearer separation of inclination regarding the second one. Additionally, we observed that WPI and $r_{st}I$ presented a strong relationship with the grain yield per plant, which was stronger in the second season. The definition coefficient (R^2) ranged the first season from 0.59 till 0.79 and the second season from 0.59 till 0.96 (Fig. 5). The definition coefficient (R^2) for stomatal resistance index ranged the first season from 0.59 till 0.78 and the second season from 0.91 till 0.99 (Fig. 6).

CONCLUSIONS

For the evaluation of barley's drought resistance, some aqueous status and acclimatization parameters in two populations and five modern varieties were studied. In both populations and varieties, the reduction of the available soil moisture decreased the water potential index (appearance of more negative values). All genotypes showed an increased tendency in stomatal resistance and stomatal resistance index of leaves. In addition, water stress decreased grain yield of all the genotypes. The two-row genotypes Cha-Cha and Grace have statistically significant the highest grain yield in all water stress treatments and the stronger relationship between the grain yield and the variables WPI and $r_{st}I$. Stomatal resistance index has for the first time been introduced in this research and could be applied in order to define plants' water stress. Finally, it can be concluded that indices of water potential and stomatal resistance of leaves can be effectively used in the evaluation of barley genotypes under water stress.

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Potato varieties resistance study to wart *Synchytrium endobioticum* (Schilbersky) Percival and late blight *Phytophthora infenstans* (Mont) de Bary

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Abstract. The results of research on the selection of potato varieties and breeding material from Institute for potato study NAAS and Institute of Agriculture of Carpathian Region NAAS are presented in this paper.
There were selected 12 potato varieties of Ukrainian breeding with high resistance level: 'Aria', 'Glazurna', 'Knyagynya', 'Zheran', 'Zhytnytsia', 'Podolyanka', 'Skarbnytsia', 'Slavyanka', 'Chervona Ruta', 'Fantasy', 'Schedryk' and 'Chortytsia' (1.2–2.4 points (R1) and 2 varieties ('Dyvo' and 'Povin' - 3.4 and 3.0 points (R2)) by the research results for potato assessment to wart in 2020–2021 by the results of field and laboratory studies for determining Ukrainian breeding potato varieties resistance to wart *Synchytrium endobioticum* (Schilb) Perc. There were chosen 6 varieties with relatively high resistance degree to late blight *Phytophthora infenstans* (Mont) de Bary (7.2–8.5 points): 'Aria', 'Dyvo', 'Zhytnytsia', 'Knyagynia', 'Podolyanka' and 'Slavyanka' by the choice. The evaluation results allow to put them into the State Register of plant varieties suitable for dissemination in Ukraine. These potato varieties recommend for implementation in potato disease sources and usage as a parent form for crossing as sources of potato resistance to wart and late blight.

Key words: choice, donor, late blight, resistance, *Solanum tuberosum* L., wart.

INTRODUCTION

Potatoes are one of the most valuable and important agricultural crops with various uses. It has been growing in many countries worldwide. It is ranked fourth after rice, wheat, and maize by growing area. The gross potato harvest in Ukraine (nearly 330 m tones) and the sown area (more than 18 m ha) confirm the importance of crops in global problem of food providing (Zelya, 2009). Potato is a second bread for Ukraine. It takes special place for today during the war in country, when many lands damaged and blown up.

The potato wart causative agent *Synchytrium endobioticum* (Schilb.) Perc. belongs to the kingdom simple fungi and intercellular obligate parasites. It is narrow and specializes in nature. *Synchytrium endobioticum* (Schilb.) Perc. infects plants of the family *Solanaceae*. Among these are potato, tomato, bittersweet nightshade, black nightshade, and physallis. The causative agent may be saved in soil in the form of winter and summer zoosporangium to 46 years old, as per J. Przetackiewicz data (Przetakiewicz, 2014).

Potato warts were first identified in 1888 in Austrian-Hungary (Kiraly et al., 1970). It was determined in England in 1898 (Bojnansky, 1984). Some sources were identified in Scotland, Wales, and Ireland in 1902 (Zelya et al., 2018). Warts appeared in the USA in 1918 (Kiraly et al., 1970). Potato warts were recorded in 1914 in Norway and in 1915 in Sweden (Bojnansky, 1984). The sources were determined in Poland in 2008 (Zelya et al., 2018), they were determined in Turkey in 2009 (Çakir & Demirci, 2017). Potato wart sources have been recorded in 38 countries worldwide (Europe, Africa, North America, and South America) (Van de Vossenberg et al., 2022).

Potato wart has variety specialization towards to basic plant-host-potato.

There were determined 40 different potato wart fungi pathotypes as per data EPPO (European and Mediterranean Plant Protection Organization). The new pathotypes differs from pathotype appearing in Germany, Czech Republic, Slovakia, Poland, Turkey, Greece, Peru, Canada, India, Italy (Obidiegwu et al., 2014; Novikova et al., 2021). The causative agent belongs to List of regulated pests A2) (EPPO Standard, 2004).

Potato wart spread in 5 regions on area 2315.26 ha in Ukraine (Zelia et al., 2020).

Aggressive causative agents of potato warts were recorded in the mountain-Carpathian zone of Ukraine in 1961. They infected potato varieties that were resistant to the common pathotype. New potato wart causative agent pathotypes appear in sources with a high density of potato monoculture, full absence of phytosanitary terms, growing variety mixes with different resistance to warts, favorable climatic and soil conditions for disease development, and not following quarantine rules (Zelia et al., 2020). There are 5 pathotypes of potato wart causative agent: D1-dalem (common) pathotype and 4 aggressive: 11-Mizhirrya; 13-Rachiv; 18-Yasynnia and 22-Bystrets (Zelia et al., 2020).

Late blight is the second most dangerous disease affecting potatoes. Late blight is a potentially dangerous potato disease. This destroys the assimilating plant surface during tuber formation. This causes a serious decrease in yield and tuber decay during storage.

Serious changes in disease development and adaptation of causative agents to external terms have recently been observed. The pathogen virulence spectrum and aggressiveness increased. Races of *Phytophthora infenstans* have been identified based on their ability to circumvent the protection afforded by specific R genes (Black, 1951; Mastenbroek, 1952). Thus, yield loss also increased. The appearance of new *Phytophthora infenstans* population isolates A1, A2 types of compatibility and sex processes with oospore formation may come to be saved in soil for many years (Razukas et al., 2008). This caused the phytosanitary state of potato sown (Valskyte et al., 2003; Holiachuk & Kosylovych, 2018) and chemical plant protection became difficult through the appearance of phytophthora-resistant forms to fungicides.

There are different methods for dealing with warts, late blights, and other potato diseases. Among these are agrotechnical, chemical, and biological. Much attention has been paid to the development of biological ways to combat plant diseases. They are alternatives to chemical means of protection, which has a negative impact on the ecology of agrophytocenosis. Improving the production of the most resistant potato varieties is the most profitable, ecologically friendly, and effective method for controlling potato disease (Zelya et al., 2018).

Ukrainian science-research plant quarantine station IPP NAAS conducted work with the choice of new potato varieties and hybrids on resistance to common and aggressive pathotypes of wart-causative agents determined in Ukraine areas over 84 years.

The joining of field trials for potato resistance-determining diseases with the most exact methods of laboratory diagnostics allows the division of varieties and hybrids based on the degree of resistance (Zelya, 2009).

The breeding evaluation and choice of resistant potato varieties for disease conduct enriching zoned assortment of new potato varieties.

The new potato varieties put into production in sources of disease-causative agents spread favors the potato production increase and improves the phytosanitary state of households.

The purpose research is evaluation and choice of potato varieties of Ukrainian breeding, resistant to wart and late blight for implementation in potato disease sources and usage as a parent form for crossing.

MATERIALS AND METHODS

There were 14 potato varieties of Ukrainian breeding: 'Aria', 'Dyvo', 'Zheran', 'Zhytnytsia', 'Podolyanka', 'Povin', 'Santarka', 'Skarbnytsia', 'Slavyanka', 'Solocha', 'Chervona Ruta', 'Fantasy', 'Schedryk', 'Chortytsia' for resistance study to potato wart causative agent and late blight. The Institute for Potato Study National Academy of Agrarian Sciences of Ukraine and the Institute of Agriculture Carpathean Region National Academy of Agrarian Sciences of Ukraine began potato breeding in 1932 and 1945, respectively. The variety 'Poliska rozheva' used for positive control and for negative 'Glazurna' for wart sensitivity. Registration of potato varieties conducted in Ukraine during 1999–2021 (State register of plant varieties suitable for dissemination in Ukraine, 2022). The variety 'Nezabudka' used for positive control and 'Glazurna' was used as a negative control for late blight sensitivity. The experiments were conducted in the laboratory conditions of laboratory of quarantine pests and diseases in the Ukrainian Science Research Plant Quarantine Station Institute of Plant Protection National Academy of Agrarian Sciences of Ukraine (v. Boiany Chernivtsi district Chernivtsi region) and field trials (settlement Berehomet. Vyzhnytsia district, Chernivtsi region).

The methodological requirements for the evaluation and choice of potato breeding material resistant to warts developed by specialists UkrSRPQSIPP NAAS, harmonized with EU requirements, as per the protocol for *Synchytrium endobioticum* (Schilb) Perc. 7/28 used during potato resistance testing to wart (EPPO Standard, 2004; EPPO Standard, 2017).

Two methods used for laboratory diagnostics:

a) Method potato infecting by potato samples by winter zoosporangia *Synchytrium endobioticum (Schilb) Perc*. soil/pearlite (1:1). The potato varieties were prepared for inoculation in special containers using winter zoospores in the laboratory. They put into specially prepared pearlite (with granule dimension 1.25–4.0 mm, company 'Agrowinn', 'Vinnytsia'). Perlite was used as a favorable medium for tuber growth. The studied varieties were infected in substrate soil/perlite, with infectious loadings of the winter zoosporangia wart causative agent together with winter zoosporangia *Synchytrium endobioticum* (Schilb) Perc. 40–50 zoosporangium /1 g substrate for resistance potato testing to wart and control varieties: positive control varieties infected by potato wart causative agent ('Poliska rozheva') and negative control varieties not infected by any causative agent ('Glazurna'). Containers leaved in climatic chamber for 75 days at 60–80% of humidity, lighting 1,600 lx 12/12 at temperature 17–18 °C. They watered every three days. The reaction of the pathogen in the potato samples was determined over 75 d. The plants dig out of the container, and every potato sample analyzed, as well as with the control varieties.

b) Method potato infecting by summer zoosporangia *Synchytrium endobioticum* (Schilb) Perc. The paper ring was fixed around the stem part of the tuber by heating mixed paraffin and Vaseline (1:1). Distilled water was placed into the ring by adding fresh warts, which consisted of summer zoospores of the causative agent. The samples were incubated in a climatic chamber at 11 °C for infection stimulation. The paper rings put off after from potato tubers for 24 hours and continued incubation at temperature 17–18 °C, humidity 80% for 20 d without lighting. The reaction of the pathogen in the potato samples was determined after a specified period. The potato sprout-infected cuttings were analyzed under a microscope (15×10) mark BioLight 300 (DELTA optical, Poland).

The degree of resistance to wart defined on a per 5 points scale: 1 – high resistance, early necrosis, soruses absence (R1); 2 – resistant, late necrosis, simple soruses (R1); 3 – weak resistant, very late necrosis, to five soruses (R2); 4 – weakly resistant, dense soruses formation with potato sprouts (S1); 5 – strongly susceptible, dense soruses, wart node (S2) (Zelya et al., 2018).

The generally approved techniques by Kirally are used for laboratory terms for infesting potato varieties by causative agent inoculums *Phytophthora infenstans* (Mont) de Bary (Kirally et al., 1970). This technique was modified by the Institute for Potato Study, National Academy of Agrarian Sciences, with the addition (Cherednychenko et al., 2021). Field trials were conducted as per previously described techniques (Razukas et al., 2008).

The infected degree defined using 9 point scale: 9 – very high resistance (symptoms of infected absent); 7 – relatively high resistance (infected tissue takes from 10% to 25% surface and tubers cut); 5 – mid resistance (infected from 25% to 50%); 3 – low resistance (infected from 50% to 75%); 1 – very low resistance (infected more than 75%). (Methodological recommendations for potato studies conducted 2002) (Cherednychenko et al., 2021).

The testing of potato variety resistance was conducted on two different scales of evaluation of resistance degree. The scale for potato testing resistance to wart is 5 points scale and for resistance to late blight (*Phytophthora infenstans* (Mont) de Bary.) is 9 points scale.

There were favorable conditions for the field trails, potato development during the growing period, for example, the air temperature was in the range of 10.1–21.2 °C in

2020 and 10.4–22.6 °C in 2021, and the amount of precipitation was 402 mm in 2020 and 373 mm in 2021 (Table 1).

Table 1. Weather indexes during the growing period (2020–2021 years)

Years	Month	Average temperature (°C)			Precipitation (mm)		
		Rate	Factically	Deviation	Rate	Factically	% from rate
2020	April	9.2	10.1	+ 0.9	47	19	39
2021		9.9	10.4	- 2.5	44	32	73
2020	May	14.9	12.9	- 2.0	76	131	176
2021		15.1	14.2	- 0.9	75	84	112
2020	June	18.0	19.4	- 0.9	75	84	112
2021		18.8	19.4	+ 0.6	93	83	89
2020	July	19.8	20.3	+ 0.5	96	85	89
2021		20.5	22.6	+ 2.1	93	78	84
2020	August	19.1	21.3	+ 2.2	75	27	36
2021		19.9	19.5	- 0.4	66	96	144

Statistics of Tables 3 and 4 show means and their standard deviations (x ± SD). The reliability of differences between samplings was assessed by the dispersion analysis with further evaluation of the least significant difference (*LSD*) using the Statistica 5 software package.

RESULTS AND DISCUSSION

Description of Ukrainian potato varieties

The Table 2 are presented the main potato characters used for potato cultivars description.

Table 2. Pedigree information of Ukrainian potato cultivars

Varieties	Registration in Ukraine	Maturity	Parentage
1.'Aria'	2014	Mid-Early	'Delikat' × 'Tiras'
2. 'Dyvo'	2014	Early	'Izora' × ('Ausonia' × 'Lugovska')
3. 'Zhytnytsia'	2020	Early	'Zdabitok' × 'Santarka'
4. 'Zheran'	2006	Early	'Dobrochin' × 'Post 86'
5. Knyagynia'	2019	Medium	'Slavyanka' × 'BellaRossa'
6. 'Podolyanka'	2006	Early	'Ausonia' × 88.1439-38
7. 'Povin'	2000	Early	76.198/175 × 70.533-38
8. 'Slavyanka'	1999	Medium	KE 78.5053 x'Kondor'
9. 'Skarbnytsia'	2008	Very late	77.583/16 × 'Liu'
10. 'Solocha'	2019	Late	'Umo101117' × 'Tiras'
11. 'Chervona ruta'	2005	Mid-late	'Agria' × 87.127/15
12. 'Fantasy'	2001	Mid-Early	79.534/61 × 'Beloruska 3'
13. 'Schedryk'	2011	Early	85.2391s12 × 'Bagryana'
14. 'Chortytsia'	2020	Very late	'Umo 101117' × 'Santarka'
'Poliska rozheva'	1989	Mid-late	'Nemichaevska iuvileyna' × 'Perlina'
'Glazurna'	2010	Early	'Gorlitsa' × 'Dobrochin'
'Nezabudka'	2022	Early	'Vityazi' × 'Nemichaevska iuvileyna'

Potato variety ‘Aria’ received from crossing parents forms ‘Delikat’ × ‘Tiras’. It is mid-early table variety. It has pink tubers of round-oval shape. The flowers are red-violet The flesh is cream. The starch content is 15.5%. It is resistant against potato wart (1.2 points), relatively resistant against late blight (8.2 points). The variety put into the State Register in 2014 after state testing on resistance to specified diseases. The growing areas are: Polissya, Foreststeppe Ukraine. Yield - is to 36 t ha^{-1}.

Potato variety ‘Dyvo’ received from crossing parent’s forms ‘Izora’ × (‘Ausonia’ × ‘Lugovska’). It is early and table variety. The tubers are white and oval. The flowers are white colour. The starch content is 13–14%. The weak wart resistance degree consisted of 3.0 points; and to late blight was 8.1 points, respectively. The recommended areas are Western Foreststeppe. Yield - is to 38.0 t ha^{-1}.

Potato variety ‘Zhytnytsia’ received from crossing parent’s forms ‘Zdabitok’ × ‘Santarka’. It is early table variety. Tubers are oval. The flesh is white. The starch content is 14.4–15.9%. It has resistance to common patotype (D1) of potato wart. The wart resistance degree consisted of 1.4 points; and to late blight was 8.0 points. The variety put into the State Register in 2020. The recommended areas are: Polissya, Foreststeppe. Yield - is to 48 t ha^{-1}.

Potato variety ‘Zheran’ received from crossing parent’s forms ‘Dobrochin’ × ‘Post 86’. It is first early and table variety. Tubers are oblong and oval form. They are pink colour. The crown of flowers is reddish purple. The starch content is 14–15%. It has resistance to wart (2.2 points); relatively high resistance to late blight (7.2 points). It registered in 2006. The recommended areas for growing: Polissya, Foreststeppe, Steppe. Yield - is to 40 t ha^{-1}.

Potato variety ‘Knyagynia’ received from crossing parent’s forms ‘Slavianka’ × ‘Bellarosa’. It is medium table variety. It has light- pink tubers. The flesh is yellow colour. The flowers are red-violet. The starch content is 14–15%. It is resistant to common pathotype (D1) of potato wart (1.6 points). It has a high resistance against late blight (8.3 points). It registered in 2019. The recommended areas for growing: Polissya, Foreststeppe, Steppe. Yield - is to 52 t ha^{-1}.

Potato variety ‘Podolyanka’ received from crossing parent’s forms ‘Ausonia’ × 88.1439s6. It is early table variety. Tubers are oval yellow. The flesh is yellow-light. The starch content is 15%. It has resistance to wart (1.4 points); high resistance to late blight (8.5 points). It registered in 2006. The recommended areas for growing are: Polissya, Foreststeppe. Yield - is to 37 t ha^{-1}.

Potato variety ‘Povin’ received from crossing parent’s forms 76.198/175× 79.533-38. It is early potato table variety. Tubers are round and red colour. The flowers are red - violet. The starch content is 15–16%. It has weak resistance to wart (3.0 points); relatively high resistance to late blight (7.5 points). It registered in 2000. The recommended areas for growing are: Woodlands, Forest-steppe, Steppe. Yield - is to 42 t ha^{-1}.

Potato variety ‘Slovyanka’ received from crossing parent’s forms KE 78.5053 × ‘Kondor’. This variety is medium and table. Potato tubers are long-oval. The tubers are red violet. The skin is red and smooth. Leaves are big light green. This variety has a high yielding. The strarch content is 12–13%. The cultivar is resistant to wart (2.0 points), resistant to late blight (8.3 points). The variety put into the State Register in 1999. The recommended areas for growing are: Polissya, Foreststeppe. Yield - is to 50 t ha^{-1}.

Potato variety 'Skarbnytsia' received from crossing parent's forms 77.583/16 × 'Liu'. It is very late table. Tubers are oval and yellow colour. The flesh is cream and light-yellow colour. The crown of flowers is reddish purple. The starch content is 12.6–13.0%. It has resistance to wart (1.8 points), relatively high resistance to late blight (7.5 points). It registered in 2006. The recommended areas for growing: Woodlands, Forest-steppe, Steppe. Yield - is to 33.0 t ha^{-1}.

Potato variety 'Solocha' received from crossing parent's forms 'Umo 101117' × 'Tiras'. It is late variety. Tubers are dark-violet colour and round form. The flesh is dark-violet colour. The flowers are white. The starch content is 19.2%. The cultivar is resistant to wart (1.2 points), low resistant to late blight (3.4 points). The variety put into the State Register in 2019. The recommended areas for growing are Woodlands, Foreststeppe. Yield - is to 44 t ha^{-1}.

Potato variety 'Chervona ruta' received from crossing parents forms 'Agria' × 87.127/15. It is mid-late variety. Tubers are short-oval. The flesh is white. The flowers are red-violet. The starch content is 19–20%. The cultivar is resistant to wart (1.8 points), resistant to late blight (7.4 points). The variety put into the State Register in 2005. The recommended areas for growing are: Woodlands, Forest-Steppe. Yield - is to 39 t ha^{-1}.

Potato variety 'Fantasy'. Received from crossing parent's forms 79.534/61 × 'Beloruska 3'. It is mid-early variety. Tubers are red ova and cream colour. The flesh is white. The flowers are red-violet. The starch content is 18-19%. It has resistance to wart (2.4 points) and relatively high resistant to late blight (7.6 points). It registered in 2001. The recommended areas for growing are: Woodlands, Forest-steppe, Steppe. Yield - is to 45 t ha^{-1}.

Potato variety 'Schedryk' received from crossing parent's forms 85.2391s12 × 'Bagryana'. It is early and table variety. Tubers are round and yellow colour. The flesh is white. The flowers are white. The starch content is 13–14%. It is resistant to potato wart (1.2 points), relatively resistant to late blight (7.4 points). It registered in 2011. The recommended areas for growing: Woodlands, Foreststeppe, Step. Yield - is to 48 t ha^{-1}.

Potato varieties 'Chortytsia' received from crossing parent's forms 'Umo101117' × 'Santarka'. It is very-late and table variety. The tubers are red and prolonged form. The flesh is red colour. The flowers are white. The starch content is 19%. It is resistant to potato wart (1.4 points), late blight (7.6 points). The variety put into the State Register in 2020. The recommended areas for growing are: Woodlands, Foreststeppe. Yield - is to 40 t ha^{-1}.

Potato variety 'Poliska rozheva' (positive control of potato warts). It received from crossing parent's forms 'Nemichaevska iuvileyna' × 'Perlina'. This is a mid-late and table variety. The tubers were red and oval. The flesh is white. The flowers are red violet. The starch content is 17–18%. It is resistant to late blight, less resistant to common scab, black rot, and ring rot, and is resistant to warts. The recommended area for growth is woodlands. The variety does not put into the State Register. Yield - is to 38 t ha^{-1}.

Potato variety 'Glazurna' (negative control to potato wart and late blight) received from crossing parent's forms 'Gorlitsa' × 'Dobrochin'. It is early and table variety. The tubers are red and oval. The crown of flowers is reddish purple. The starch content is 15.7%. It has a high nutritious quality. It has resistance to potato wart (1.2 point), late blight (8.2 point). The variety put into the State Register in 2010. The recommended areas are: Woodlands, Step. Yield - is to 36 t ha^{-1}.

Potato variety 'Nezabudka' (positive control to late blight). It received from crossing parent's forms 'Vityazi' × 'Nemichaevska iuvileyna'. It is early and table variety. The rubbers are short - oval and hite colour. The flesh is cream. The flowers are white. The starch content is 13.3–15.3%. It has resistance for potato wart, is susceptible to late blight. There is no in State Register in January, 2022. Yield - is to 30 t ha^{-1}.

Evaluation and choice of potato varieties of Ukrainian breeding of wart causative agent *Synchytrium endobioticum*

By the study's results, the following 14 varieties of Ukrainian breeding 'Aria', 'Dyvo', 'Zheran', 'Zhytnytsia', 'Podolyanka', 'Poivn', 'Santarka', 'Skarbnytsia', 'Slavyanka', 'Solocha', 'Chervona Ruta', 'Fantasy', 'Schedryk', 'Chortytsia' among testing varieties for evaluation and choice of potato varieties resistant to common pathotype (D1) causative agent during 2020–2021 (Table 3).

Table 3. The evaluation results and choice of potato breeding material resistant to common pathotype of potato wart (2020–2021 years)

Nr.	Varieties	Research results on resistance to common pathotype						Resistance group (M ± m)
		Laboratory			Field			
		Total number of plants tested	Number of plants infected	Infection rate (%)	Total number of plants tested	Number of plants infected	Infection rate (%)	
1.	'Aria'	10	0	0	30	0	0	1.2 ± 0.03 (R1)
2.	'Dyvo'	10	0	0	30	0	0	3.4 ± 0.06 (R2)
3.	'Zhytnytsia'	10	0	0	30	0	0	1.4 ± 0.03 (R1)
4.	'Zheran'	10	0	0	30	0	0	2.2 ± 0.03 (R1)
5.	'Knyagynia'	10	0	0	30	0	0	1.6 ± 0.06 (R1)
6.	'Podolyanka'	10	0	0	30	0	0	1.4 ± 0.03 (R1)
7.	'Povin'	10	0	0	30	0	0	3.0 ± 0.03 (R2)
8.	'Slavyanka'	10	0	0	30	0	0	2.0 ± 0.03 (R1)
9.	'Skarbnytsia'	10	0	0	30	0	0	1.8 ± 0.06 (R1)
10.	'Solocha'	10	0	0	30	24	80	1.2 ± 0.03 (R1)
11.	'Chervona ruta'	10	0	0	30	0	0	1.8 ± 0.03 (R1)
12.	'Fantasy'	10	0	0	30	0	0	2.4 ± 0.03 (R1)
13.	'Schedryk'	10	0	0	30	24	80	1.2 ± 0.06 (R1)
14.	'Chortytsia'	10	0	0	30	0	0	1.4 ± 0.03 (R1)
	'Poliska rozheva' (positive control)	10	10	100	30	30	100	5.0 ± 0.03 (S2)
	'Glazurna' (negative control)	10	0	0	30	0	0	1.2 ± 0.03(R1)

The resistance degree to wart consisted of 1 point for the following potato varieties 'Aria', 'Knyagynia', 'Zhytnytsia', 'Podolyanka', 'Skarbnytsia', 'Chervona Ruta', 'Schedryk' and 'Chortytsia' (high resistant, early necrosis, soruses absence (R1) (Table 3). The resistance degree was on the level 2 points (resistant, late necrosis, simple soruses (R1) for potato varieties 'Zheran', 'Slavyanka' and 'Fantasy'. The potato varieties 'Dyvo' and 'Povin' showed a moderate reaction to pathogen infected (3 - weak resistant, very late necrosis, to five soruses (R2). Two varieties ('Solocha' and

‘Schedryk’) of potatoes are in the R1 resistance group. The results of field studies showed that the infection rate is 80%. The laboratory tests conducted in this way on all 14 potato samples (100%), So they showed resistance to common pathotype D1 potato wart S*ynchytrium endobioticum* (Schilb) Perc.

The infection rate of the susceptible control variety ‘Poliska rozheva’ range from 80–100%. The degree of infected consisted of five points (strongly susceptible, dense soruses, wart node (S2). Summer zoosporangia wart-causing agents were observed under a microscope at the top of the infected sprouts. The negative control (resistant variety - ‘Glazurna’) was consisted of 0%. The resistance degree consisted of 1 point (high resistance, early necrosis, sorus absence (R1)). There were no causative agents of potato warts determined in field conditions for any variety, except for the positive control (highly resistant to potato wart ‘Poliska rozheva’). The warts were determined (Fig. 1).

Figure 1. Potato variety ‘Poliska rozheva’ infected by potato wart causative agent *Synchytrium endobioticum* (Schilb) Perc.

Assessment and choice of potato varieties of Ukrainian breeding resistant to late blight *Phytophthora infenstans* (Mont) de Bary

There were chosen 6 potato varieties (‘Aria’, ‘Zhytnytsia’, ‘Knyagynia’, ‘Podolyanka’ and ‘Slavyanka’- breeding of Institute for Potato Study National Academy of Agrarian Sciences of Ukraine; ‘Dyvo’ - breeding of Institute of Agriculture of Carpathean Region National Academy of Agrarian Sciences of Ukraine with high resistance to late blight (8 points - high resistance (surface infected, necrosis takes to 10% of surface and tuber’s cut) among 14 potato varieties of Ukrainian breeding by the results of laboratory testing studies conducting. The following varieties: ‘Zheran’, ‘Povin’, ‘Skarbnytsia’, ‘Chervona Ruta’, ‘Fantasy’, ‘Schedryk’ and ‘Chortitsia’, which consisted of 42.8% from total number. They were infected at 7 points (relatively high resistance (infected tissue takes from 10% to 25% surface and tubers cut).

The variety ‘Solocha’ showed the lowe resistance. This consists of three points. More than 50% of leaves surfaces and potato tubers were removed (Table 4).

We obtained the same results in the field trials. The field experiments conducted on the area of Ukrainian Science- Research Plant Quarantine Station IPP NAAS in village Boyany Chernivtsi district, Chernivtsi region. Potato varieties ‘Aria’, ‘Zhytnytsia’, ‘Knyagynia’, ‘Podolyanka, Slavyanka’ Dyvo’ showed the high field resistance to disease causative agent (in the scope 8 points). The following varieties: ‘Zheran’, ‘Povin’, ‘Skarbnytsia’, ‘Chervona Ruta’, ‘Fantasy’, ‘Schedryk’ and ‘Chortitsia’ showed the resistance on 7 point (relatively high resistance in field conditions). The average point consisted of 8–7 points on resistance to late blight. These potato varieties may serve as parent forms for crossing and receiving resistant descendants. They were infected at 25% of leaves surface and tubers. The variety ‘Nezabudka’ (for positive control) wasinfected to 80% of leaves surface (1 point) and infected tubers (Fig. 2), The potato variety ‘Glazurna’ (for negative control) had the late blight infected degree on the level 5%.

Table 4. Assessment results and choice of potato breeding material resistant to late blight *Phytophthora infenstans* (Mont) de Bary (2020–2021 years)

Nr.	Varieties	Research results on resistance to common pathotype					
		Laboratory			Field		
		Total number of plants tested	Infection rate (%)	Point (M ± m)	Total number of plants tested	Infection rate (%)	Point (M ± m)
1.	‘Aria’	5	10	8.1 ± 0.06	10	10	8.2 ± 0.06
2.	‘Dyvo’	5	5	8.2 ± 0.06	10	10	8.1 ± 0.04
3.	‘Zhytnytsia’	5	5	8.1 ± 0.04	10	10	8.0 ± 0.03
4.	‘Zheran’	5	15	7.0 ± 0.06	10	25	7.2 ± 0.06
5.	‘Knyagynia’	5	10	8.2 ± 0.06	10	10	8.3 ± 0.03
6.	‘Podolyanka’	5	10	8.4 ± 0.04	10	10	8.5 ± 0.06
7.	‘Povin’	5	15	7.4 ± 0.06	10	15	7.5 ± 0.04
8.	‘Slavyanka’	5	10	8.2 ± 0.05	10	10	8.3 ± 0.03
9.	‘Skarbnytsia’	5	15	8.0 ± 0.06	10	15	7.5 ± 0.06
10.	‘Solocha’	5	50	6.0 ± 0.03	10	60	3.4 ± 0.06
11.	‘Chervona ruta’	5	25	7.3 ± 0.06	10	25	7.4 ± 0.03
12.	‘Fantasy’	5	25	7.4 ± 0.03	10	25	7.6 ± 0.06
13.	‘Schedryk’	5	25	7.2 ± 0.06	10	25	7.4 ± 0.06
14.	‘Chortytsia’	5	25	7.5 ± 0.06	10	25	7.6 ± 0.04
	‘Nezabudka’ (pozitive control)	5	80	1.7 ± 0.06	10	80	1.8 ± 0.06
	‘Glazurna’ (negative control)	5	5	8.1 ± 0.06	10	5	8.2 ± 0.03

It is necessary to conduct control inspection every five years on resistance to diseases as per ‘Statement potato testing order on resistance to wart’ (1996) on potato varieties with high yield and tasting qualities included in in State Register of plant varieties suitable for dissemination in Ukraine early (in 2000–2015). The reaction on potato wart infection did not change on the following potato varieties: ‘Aria’, ‘Dyvo’, ‘Zheran’, ‘Glazurna’, ‘Zheran’, ‘Podolyanka’, ‘Povin’, ‘Slovyanka’, ‘Skabnytsya’ and ‘Chervona Ruta’ (Zelya, 2018). Some of them were resistant to late blight. There were chosen 5 potato varieties among the new: ‘Zhytnytsya’, ‘Knyagynia’, ‘Solocha’, ‘Schedryk’ and ‘Chortytsia’.

Figure 2. Potato variety ‘Nezabudka’ infected by late blight *Phytophthora infenstans* (Mont) de Bary.

Potato varieties showed the negative reaction on pathogen infected recommends improving in disease sources and to use in breeding for crossing and to receive the resistant descendants.

The present potato varieties recommend breeders of Ukrainian research institutions in the form of parents' forms for crossing and implementing disease sources. This decreases the infectious load of the present pathogens in the soil and improves the phytosanitary state of agricultural plots.

CONCLUSIONS

The evaluation of potato breeding material using laboratory and field methods allows the determination of potato varieties that are most resistant to diseases of potato wart *Synchytrium endobioticum* (Schilb) Perc. with resistance points 1–2 and late blight *Phytophthora infenstans* (Mont) de Bary with resistance points 7–8.

There were selected 12 potato varieties of Ukrainian breeding with high resistance level: 'Aria', 'Glazurna', 'Knyagynya', 'Zheran', 'Zhytnytsia', 'Podolyanka', 'Skarbnytsia', 'Slavyanka', 'Chervona Ruta', 'Fantasy', 'Schedryk' and 'Chortytsia' (1.2–2.4 points (R1) and 2 varieties ('Dyvo' and 'Povin' - 3.4 and 3.0 points (R2)) by the research results for potato assessment to wart in 2020–2021 by the results of field and laboratory studies for determining Ukrainian breeding potato varieties resistance to wart *Synchytrium endobioticum* (Schilb) Perc. There were chosen 6 varieties with relatively high resistance degree to late blight *Phytophthora infenstans* (Mont) de Bary (7.2–8.5 points): 'Aria', 'Dyvo', 'Zhytnytsia', 'Knyagynia', 'Podolyanka' and 'Slavyanka' by the choice. The evaluation results allow to put them into the State Register of plant varieties suitable for dissemination in Ukraine.

The present potato varieties recommend breeders of Ukrainian research institutions in the form of parents forms for crossing and implementing disease sources. This decreases the infectious load of the present pathogens in the soil and improves the phytosanitary state of agricultural plots.

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