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Subscription information:
Institute of Technology, EMU
Fr.R. Kreutzwaldi 56,
51006 Tartu,
ESTONIA
e-mail: timo.kikas@emu.ee

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Hybridspecific nutrient interactions and their role in maize yield quality

C. Bojtor[1,*], Á. Illés[1], É. Horváth[1], J. Nagy[1] and L.C. Marton[1,2]

[1]University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Land Use, Engineering and Precision Farming Technology 138 Böszörményi St, H-4032 Debrecen, Hungary
[2]Eötvös Loránd Research Network, Centre for Agricultural Research, Agricultural Institute, 2 Brunszvik St, H-2462 Martonvásár, Hungary
[*]Correspondence: bojtor.csaba@agr.unideb.hu

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Abstract. Different fertilization levels can be used according to the intensity of the plant production technology. The interactions among all the essential nutrients, the different ways of antagonisms and synergisms can weaken or strengthen the physiological processes of the plants, affecting significantly the efficiency of the production. Complex mineral profile of the vegetative (stalk, leaves) at V2, V4, V8, VT and R6 stages in 4 replications and generative (grain, cob -R6 stage only) plant parts of maize were determined in accredited laboratory with ICP-MS, ICP-OES microwave-assisted multielement analysis for metal components (P, K, mg, Ca, S, Zn, Fe, Cu, Mn, Ni, Mo) and Dumas method for nitrogen. Results showed that the effect of different nitrogen fertilization levels was significant on grain yield, protein, oil and moisture content. Significant differences were measured between the different maize genotypes in grain yield, protein and starch content. In the experiment all essential nutrient concentrations were measured, and the important nutrient ratios of macro- and micronutrients for the physiological development of maize were calculated. These nutrient stoichiometric ratios were evaluated according to their scale of influence in the yield formation. It can be concluded that different nitrogen fertilization levels affected significantly the essential nutrient ratios of the vegetative and generative plant parts of three maize hybrids in the growth period. Although different variables of nutrient stoichiometric ratios and yield parameters did not highly correlated, Pearson's correlations suggest that K:Mg and K:Zn ratio of stalk would be related with grain yield (R = 0.32; 0.34; 0.39 and 0.35; 0.37; 0.30, respectively) marking them as important parameters for novel nutrient stoichiometry research. Analysing the optimal nutrient ratios related to the yield quality and their interaction with the fertilization practices can give certain recommendations to the farmers to implement hybrid- and site-specific nutrient management strategies, reducing the environmental impact of the over-fertilization.

Keywords: fertilization efficiency, macronutrients, nitrogen use; nutrient ratios, stoichiometry.

INTRODUCTION

Maize (*Zea mays* L.) is currently one of the most important food crops in the world, maximizing its productivity and yield while maintaining the quality is one of the primary goals of corn producers (Xin et al., 2016).

The future of modern multinutrient management strategies is not clear. Cropping systems in the world are diverse and can be divided into at least two different levels of intensification, low and high input fertilization systems (Fischer & Connor, 2018). Optimal nutrient supply is a key circumstance to achieve high yields with good quality parameters. Soil - plant interactions and the complex use of nutrients during the growing season - beside other environmental factors – can determine the plant's health conditions, having a high impact on the dry matter and grain yield accumulation of maize (Bojtor et al., 2020).

Optimum nitrogen concentration in crop plants can improve leaf development as well as photosynthesis, and delay the leaf senescence during the grain filling stages in maize (Liu et al., 2017). It also influences the utilization of phosphorus, potassium, and other minerals in plants. Optimal quantity of these macro- and micronutrients in the soil cannot be utilized efficiently in case of nitrogen deficiency. Therefore, nitrogen deficiency can result in reducing maize yields (Humtsoe et al., 2018). Lamptey et al. (2017) confirmed that the grain yield of maize is sensitive to changes in yield components and nitrogen content in the leaf. According to their results small stresses on these traits can result in significant impacts on grain yield.

High nutrient use efficiency is an economic and environmental goal of sustainable cropping systems (Gastal et al., 2015). There are a lot of factors mediating nutrient use efficiency, such as genotype (Wang et al., 2017), enough precipitation (Hoogmoed et al., 2018) and availability of other essential nutrients (Duncan et al., 2018). Different nutrient ratios are widely used to investigate their limitation for crops. N:P ratio has been used to quantify N and P deficiency in plants (Bélanger et al., 2017). Some studies also investigated the N:S ratio in maize (Salvagiotti et al., 2017; Carciochi et al., 2019). The aim of exploit the site-specific nutrient sources of the soil is one of the primary intentions of the modern agricultural practice. To achieve this goal the use of different maize genotypes that have better nutrient use efficiency is an important strategy with the correct management of the plant's nutritional status. (Ortas, 2018). Analysis of different stoichiometric ratios of the plant's organs of the essential macro- and micronutrients and testing the site-specific characteristics of the different production areas is an important research topic as a genotype-specific nutritional diagnostic tool.

Generally, obtaining relevant information about hybrid-specific nutrient interactions can help the farmers creating an environmentally favorable fertilization process in their farm practice.

The connection between different nutritional levels in the main phenological stages and yield quality parameters can provide the optimal nutritional bases to achieve high quality yield. The main objective of this study was to clarify - through a controlled long-term field experiment - the role of different nitrogen fertilization levels in the interrelationship among the essential macro-meso and micronutrients of maize hybrids during the vegetation period, focusing mainly on the nutrient status of the hybrids and on the way it can refer to the producible yield and its quality parameters at an early development stage.

MATERIALS AND METHODS

Experimental area

The experiment was performed at the Látókép Crop Production Experimental Site of the University of Debrecen (47° 33' N, 21° 26' E, 111 m above sea level) in 2019 with 3 different maize (*Zea mays* L. H1 = FAO360, H2 = FAO420, H3 = FAO490) hybrids and 3 different nitrogen fertilization levels, as a part of a long-term 6-level multifactorial fertilization experiment founded in 1983 (Nagy, 2019). In the experiment, phosphorus (P) and potassium (K) doses were constant at an optimal level for the plant by autumn application (184 kg ha^{-1} and 216 kg ha^{-1}, respectively), and nitrogen (N) varied between 0 and 300 kg ha^{-1} as follows (0, 60, 120, 180, 240 and 300 kg ha^{-1} N), with spatially randomized plots in four replications (Table 1). There was no nutrient replenishment in the soil on the control plots for 30 years.

Table 1. NPK doses of the multifactoral fertilization experiment of the Látókép Crop Production Experimental Site, Debrecen, Hungary. Bold fonts mark the treatments used in the present study

Fertilization level	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Total (kg ha^{-1})
N0	**0**	**0**	**0**	**0**
N1	60	184	216	460
N2	**120**	**184**	**216**	**520**
N3	180	184	216	580
N4	240	184	216	640
N5	**300**	**184**	**216**	**700**

Soil parameters of the experimental area

From the pedological point of view, the area is calcareous chernozem formed on loess ridges, with an average liquid limit (LL = 43–45), average humus content ($Hu_{\%}$ = 2.7–2.8), and with a humus layer thickness of 80 cm. Ammonium lactate soluble P_2O_5 and K_2O are 133 and 240 mg kg^{-1}, respectively, with a bulk density of 1.4–1.45 g cm^{-3} in the cultivated soil layer and 1.25–1.29 g cm^{-3} in the lower layers. In the 0–200 cm soil layer, which is relevant for the water supply of plants, the soil can hold 600–650 mm water, 50–60% of which is disponible for the plants. The average depth of groundwater is 3–5 m, and even in rainy periods it does not rise above 2 m. of depths (Nagy, 2019).

Weather conditions of the experimental area

The experimental area typically has a continental climate, with frequent occurrence of various weather extremes, such as changes in the amount and distribution of precipitation and fluctuations in temperature values within and outside the vegetation period. Only 311.8 mm total precipitation fell during the growing season between 01/04 and 31/10, and there were only 15 days with more than 5 mm, and 4 days with more than 15 mm precipitation (Fig. 1).

Experimental design

The experiment was split-plot design with four replicated plots for the optimal statistical evaluation. The main factor of the experiment was the fertilization (plots), the sub-factors were the hybrids (sub-plots). The size of the experimental plots was 7.2 m^2, with plant density of 73,000 plants ha^{-1} (2 rows x 5 m sized microplots, with approx. 50 plants in each one). Sowing date was 20.04.2019., with a co-application of tefluthrin-based soil disinfectant (15 kg ha^{-1}). In the present study total control plots (0 kg ha^{-1}) a medium, generally common nitrogen dose (120 kg ha^{-1}) and a high nitrogen dose (300 kg ha^{-1}) were selected for plant sampling to examine the effect of nitrogen fertilization on the nutrient ratios of maize. 5 different sampling dates were used in the experiment according to the main phenological stages of maize.

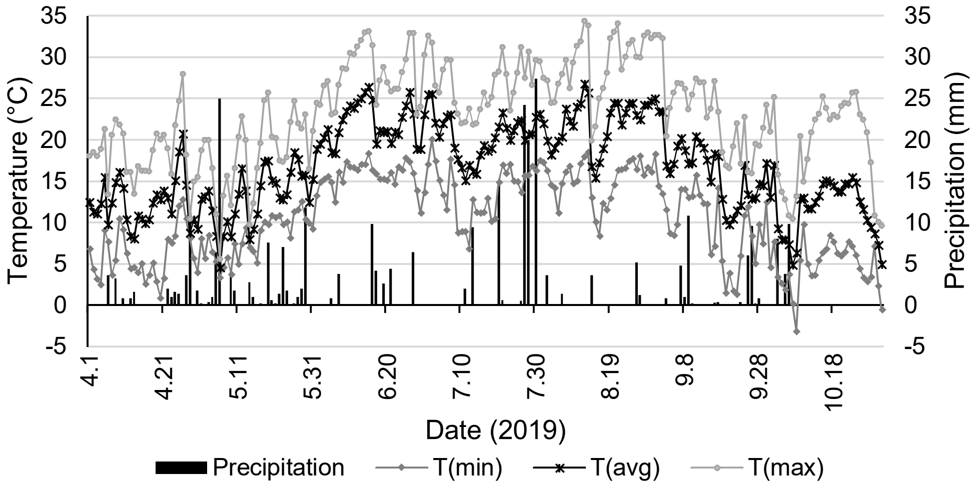

Figure 1. Precipitation and temperature data of the experimental area. 2019, Debrecen, Hungary. T(min) = Daily minimum temperature; T(avg) = Daily average temperature; T(max) = Daily maximum temperature.

Plant sampling

Plant samples were taken 5 times during the growing season, at V2, V4, V8, VT and R6 phenological stages in 4 replication. All plant samples were separated into leaf, stalk, and at the R6 stage into cob and grain too, then dried at 60 °C to a constant weight, weighed to obtain the dry matter of the plants (DM), and ground into fine powder.

Macro- and micronutrient analysis

Complex mineral profile of the vegetative (stalk, leaves) and generative (grain, cob) plant parts were determined in accredited laboratory with ICP-MS, ICP-OES (P, K, mg, Ca, S, Zn, Fe, Cu, Mn, Ni, Mo) using microwave-assisted multielement analysis (Tarantino et al., 2017) and Dumas method for N (Ebeling, 1968).

Sample preparation for the laboratory analysis was as follows: two parallel measurements were made from each sample. 0.4 g sample was weighed, and then 2 mL of high purity water and 4 mL of cc. nitric acid were added to the samples. The adequate digestion program was chosen according to the Milestone Ultrawave microwave system manual (Milestone Inc., USA). Sample extraction with microwave digestion was performed at 200 °C, with a holding time of 10 minutes. ICP-OES analysis was used for the determination P, K, mg, Ca, S, Fe, and Mn concentration. Sample of 5 mL was pipetted into a plastic test tube, and 5 mL of deionized water, 0.2 mL of the acid mixture, and 0.2 mL of 100 ppm Y-containing ISTD (internal standard) were added to the sample. The mixture was homogenized, and then it was put into the 5900 ICP-OES (Agilent Technologies Inc., USA). The Zn, Cu, Mo and Ni concentration of the samples were determined from the homogenized mixture with 7900 ICP-MS

(Agilent Technologies Inc., USA). Extracted sample of 5 mL, 1 mL of the acid mixture, and 4 mL of deionized water were added to the test tube for the analysis. A sample of each matrix type was prepared twice. Blank samples were prepared in each series of measurements by measuring water of the same quantity instead of a sample. Due to possible inhomogeneity, two parallel digests were made from each sample and the final result was calculated from the average of these.

N concentration was measured using the Dumas combustion method. Samples were subjected to oxidative digestion at a high temperature (900 °C) with a controlled oxygen supply The resulting flue gases passed through a copper oxide-platinum catalyst using a CO_2 carrier gas, thus ensuring complete oxidation. After the subsequent reduction processes and the purification of the carrier gas, the nitrogen content remaining in the CO_2 carrier gas was detected in a thermal conductivity detector (VELP NDA 702, Velp Scientific, Italy). The N_2 volume provided an electrical measurement signal, from which the N content of the various burned samples was measured and calculated based on a preprepared calibration curve.

Nutrient concentration ratios for all nutrient concentration combinations were calculated for each sample for the five different development stages, and their interrelationship with different yield parameters was evaluated according to its scale of influence and presented in this study.

Determination of yield parameters

Maize grain yield was determined through a plot-size harvester (Sampo SR2010, Sampo Rosenlew, Finland). The yield quality parameters (protein, starch, oil, and moisture content) were measured with rapid, non-destructive near-infrared transmittance (NIT) technology (Perten DA 7250, PerkinElmer Ltd, Waltham, USA).

Statistical analysis

Data normality was tested by Kolmogorov–Smirnov test. Analysis of variance *(ANOVA)* test was used to evaluate the effect of different nitrogen fertilization doses and different genotypes on the nutrient ratios of the different plant parts. General model of the analyses used fixed effects (Fertilization, Hybrid), interactions (HxF) and random effects (runs nested into 5 times) based on the four replications of each measured parameter. When the F value was significant, *Fisher's LSD* test was used to compare the means and find the least significant difference between them $LSD = t_{\nu,\alpha}\sqrt{MS_{S(A)}\ \frac{2}{S}}$ (Williams & Abdi, 2010). *Pearson's* correlation analysis was used to examine the interactions between the nutrient ratios and the different yield parameters (Nahler, 2009). Significant differences are marked by letters (a, b, c, d) and bold fonts in the manuscript, respectively. Statistical analyses were conducted in R 3.2.4. (Team, 2016a), with RStudio graphical interface (Team, 2016b) and in Minitab Statistical Software.

RESULTS AND DISCUSSION

Simple and multiple effects of fertilization and genotypes on yield parameters

Results of the *ANOVA test* showed that the effect of different nitrogen fertilization levels was significant on grain yield, protein, oil and moisture content. Significant differences were measured between the different maize genotypes in grain yield, protein and starch content. The multiple effects of hybrids and fertilization levels were significant on the oil and moisture content (Table 2).

Increasing nitrogen level can primarily affect the organic matter accumulation and thus, the yield formation of the different crops. However, well-balanced, complex macro- meso and micronutrient replenishment is needed to achieve high yield in more consecutive years, returning the nutrients took away from the production areas with the high yields.

Data evaluation with *Fisher's LSD test* showed the exact differences between the yield parameters. The two higher fertilization levels increased significantly the grain yield of all genotypes, and the results of the H1 genotype was significantly higher at all the three N levels than the values of the other two hybrids.

Table 2 Effect of different N fertilization levels on the yield parameters of the hybrids

	Hybrid	Fertilization	HxF
Grain yield	10.90***	26.78***	0.51
Protein	7.70**	21.91***	2.07
Starch	2.97	8.41**	1.11
Oil	9.93**	1.81	3.26*
Moisture	46.06***	3.06	2.79*

F values of the *ANOVA test* of the yield parameters. H = Hybrids; F = Fertilization. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

At both increased N levels the H2 genotype had statistically proven higher protein and moisture content compared to the control. The highest protein content, 9.08% was measured in H3 at N5 level. Starch content decreased significantly in H1 genotype at both N levels compared to the control. Increasing N levels affected differently the protein and starch content of the grain, causing higher protein and lower starch content, respectively (Table 3).

Table 3. Differences of yield parameters between hybrids and fertilization levels with *Fisher's LSD* method

		Grain yield ($t\ ha^{-1}$)	Protein (%)	Starch (%)	Oil (%)	Moisture (%)
N0	H1	8.39^{b}	8.13^{c}	63.1^{ab}	4.08^{c}	14.0^{c}
	H2	5.49^{c}	6.88^{d}	63.2^{a}	4.13^{bc}	14.9^{a}
	H3	4.67^{c}	8.13^{c}	62.1^{bcd}	4.05^{c}	14.8^{a}
N2	H1	12.37^{a*}	8.63^{abc}	61.8^{cd*}	4.25^{ab}	14.0^{c}
	H2	9.27^{b**}	8.30^{bc***}	62.2^{abc}	4.05^{c}	14.4^{b*}
	H3	8.83^{b**}	8.90^{ab}	61.0^{d}	4.08^{c}	14.8^{a}
N5	H1	11.90^{a*}	8.85^{ab}	61.6^{cd*}	4.30^{a*}	14.0^{c}
	H2	10.29^{ab***}	8.80^{ab***}	61.7^{cd*}	4.10^{c}	14.3^{bc**}
	H3	10.28^{ab***}	9.08^{a*}	61.8^{cd}	4.05^{c}	14.8^{a}

Means that do not share a letter are significantly different. Values marked with asterisks are statistically significant compared to control (N0). $LSD_{(yield)} = 1.76$; $LSD_{(protein)} = 0.38$; $LSD_{(starch)} = 0.67$; $LSD_{(oil)} = 0.15$; $LSD_{(moisture)} = 0.31$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Our results are directly in line with previous findings by Széles et al. (2018), that the higher nitrogen fertilization level can mainly increase grain yield, but the effect on grain protein content was not uniformly significant, it is affected by other agronomic and environmental factors. Plant nutrition has a great impact on yield formation, and the stability of different genotypes is a main parameters influencing the farmer's hybrid choice (Shojaei et al., 2021).

Simple and multiple effects of sampling time, fertilization and genotypes on the nutrient ratios of stalk, leaves, grain and cob of maize

In the experiment all essential nutrient concentrations were measured. Among them, the important nutrient ratios of macro- and micronutrients for the physiological development of maize were calculated and presented in the tables below. Results of the *ANOVA* of the stalk shoved that between the sampling times and the fertilization levels all presented nutrient ratios had significant differences, with exception of one value at $P < 0.001$ level. Between the different maize genotypes, significant differences were measured in the N:Mg, N:Ca, P:S and P:Fe ratios. The interaction of different development stages and genotypes affected significantly the P:S, P:Zn and P:Fe ratios.

While the analysis of fertilization level - sampling time interaction showed statistically verified differences between all presented nutrient ratios, except the N:Mg. Results of this analysis suggest that for the nutrient composition of stalk during the growing season the increasing nitrogen fertilization level is a higher influencing factor than choosing the adequate hybrid (Table 4). Ma et al. (2016) confirmed that grain yield, dry matter production, N and P uptake were affected by the N fertiliser application rate. Significant genotype effect was measured on grain yield, and shoot N and P contents, and also the N:P ratios increased with higher N fertiliser rates. The critical N and P values on the plant parts and their stoichiometric connection to other essential macro- and micronutrients is an important topic to further studies for a nutrient diagnosis system in maize.

Table 4. Effect of different N fertilization levels on the nutrient ratios of stalk

	Stalk						
	ST	H	F	STxH	STxF	HxF	STxHxF
N:Mg	53.60^{***}	3.50^{*}	22.58^{***}	0.77	1.21	0.83	0.44
N:Ca	48.26^{***}	3.33^{*}	15.05^{*}	1.09	2.13^{*}	0.77	0.35
N:Zn	14.44^{***}	0.97	14.09^{***}	1.15	4.73^{***}	0.31	0.73
P:K	47.55^{***}	2.21	17.67^{***}	1.07	2.21^{*}	0.53	0.53
P:Mg	48.90^{***}	0.04	10.19^{***}	1.89	2.23^{*}	0.25	0.24
P:S	19.66^{***}	4.25^{*}	18.35^{***}	2.35^{*}	4.70^{***}	0.98	0.42
P:Zn	27.44^{***}	0.50	19.56^{***}	2.11^{*}	8.12^{***}	1.06	1.37
P:Fe	65.67^{***}	4.10^{*}	12.63^{***}	2.47^{*}	5.99^{**}	1.57	1.34
K:Mg	25.02^{***}	0.68	28.73^{***}	1.26	3.07^{**}	0.84	0.73
K:Zn	30.19^{***}	1.15	27.27^{***}	0.72	4.93^{***}	0.39	0.59

F values of the *ANOVA test*. ST = Sampling times; H = Hybrids; F = Fertilization. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Analysis on the leaf nutrient ratios showed that the different sampling times and fertilization levels and their multiple effects significantly influenced all of them at $P < 0.001$ level, except the N:Mg and N:Ca ratios in their common effect. Between the three genotypes statistically proven differences could be measured in the N:Mg, N:Ca, P:K, P:S, P:Zn and P:Fe ratios.

Determining the adequate genotype for each production area is essential to achieve successful plant production. The nutrient ratios between genotypes, and the genotype - sampling times multiple effects resulted in significant differences in six of the ten important nutrient ratios (Table 5). Ortas (2018) experienced significant increases in grain yield, N, P and K concentrations of leaves as an effect of K fertiliser treatments, and it also increased the K and mg concentrations of seeds. Using different genotypes led to statistically proven higher values in N, K, Fe and Zn concentrations and ratios with increased K fertilization. Qiang et al. (2019) also found significant differences between genotypes in leaf N concentration which parameter contributed greatly to higher yields and increasing nitrogen and water use efficiency (NUE, WUE).

Table 5. Effect of different N fertilization levels on the nutrient ratios of leaves

	Leaves						
	ST	H	F	STxH	STxF	HxF	STxHxF
N:Mg	96.50***	4.08*	32.88***	0.67	1.26	1.32	0.57
N:Ca	317.33***	11.61***	16.59***	4.51***	1.65	0.65	0.80
N:Zn	7.25***	0.39	10.32***	1.19	6.23***	0.83	0.73
P:K	27.74***	5.64**	23.92***	6.04***	7.60***	0.69	0.68
P:Mg	108.82***	0.98	20.84***	1.75	3.96***	0.95	0.49
P:S	25.28***	3.80*	34.93***	3.41**	10.78***	0.04	0.42
P:Zn	40.22***	9.42***	17.14***	5.75***	8.61***	0.75	0.88
P:Fe	43.68***	5.61**	15.98***	5.14***	8.35***	3.15*	1.55
K:Mg	130.10***	2.24	46.22***	1.20	4.25***	0.31	0.42
K:Zn	50.18***	2.44	27.42***	2.41*	6.64***	0.20	0.34

F values of the *ANOVA test* of the nutrient ratios of leaves. ST = Sampling times; H = Hybrids; F = Fertilization. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

The analysis of variance of the grain and cob indicated significant differences between the three maize genotypes in the P:K, K:Mg, K:Zn and P:K, P:Fe, K:Mg and K:Zn ratios, respectively. The increasing N fertilization levels affected the nutrient ratios of grain and cob similarly, causing statistically confirmed differences in the P:S, P:Zn, P:Fe and K:Zn values. Examination of the multiple effects of the two factors showed that in the grain the P:S, in the cob the K:Mg and K:Zn ratios had significant changes, making these nutrient ratios important in the assessment of genotype-specific fertilization management systems (Table 6).

Table 6. Effect of different N fertilization levels on the nutrient ratios of grain and cob

	Grain			Cob		
	H	F	HxF	H	F	HxF
N:Mg	0.95	0.78	0.93	0.14	2.56	0.75
N:Ca	0.51	0.72	0.15	0.21	0.79	0.26
N:Zn	1.12	2.87	0.46	1.43	5.24*	1.03
P:K	9.99***	1.94	0.75	5.96**	0.08	0.94
P:Mg	0.56	2.53	0.12	0.65	1.14	0.35
P:S	0.98	5.24**	3.30**	3.05	6.15**	0.76
P:Zn	0.68	16.33***	1.12	1.07	16.42***	2.41
P:Fe	1.99	5.81**	0.24	6.89**	6.45**	0.86
K:Mg	6.25**	2.46	0.21	3.54*	1.59	3.47*
K:Zn	3.57*	8.26**	0.56	4.99*	10.76***	4.52**

F values of the *ANOVA test* of the nutrient ratios of grain and cob. H = Hybrids; F = Fertilization. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Results of correlation analysis of the vegetative parts of maize with yield parameters

Correlation analysis indicates the relationship between the different nutrient ratios and the yield parameters, how similarly their extent varies. Analysing the hybridspecific nutrient interactions and their connection to yield parameters can contribute to implement fertilization strategies helping the farmers to increase the yield and its quality parameters. The mean nutrient ratios of the five sampling times and three fertilization levels were correlated to the grain yield, protein and starch content of maize. There are high differences between the exact nutrient concentrations during the growing season in the five sampling times, so we intended to identify the significant correlations to find the connections between the yield parameters and values measured during the vegetative growth period.

According to the results of correlation analysis between grain yield and nutrient ratios of stalk, the H1 genotype positively correlated with N:Zn, and K:Zn at $P < 0.01$, and negatively with P:S. The H2 genotype also had positive correlation at $P < 0.01$ with N:Mg, K:Mg and K:Zn, and negative correlation with P:K ratio. H3 grain yield had close positive correlation only with K:Mg ratio. Correlation between K:Mg ratio and grain yield was significant in all three hybrids, marking it as an important parameter for future research. Protein content had important correlation values only with the nutrient ratios of the H2 genotype, significantly positive at $P < 0.01$ with N:Mg, K:Mg and K:Zn, and negative with P:K and P:S, respectively. Starch content of the grain had only negative notable correlation values, N:Zn in H1, and K:Mg, K:Zn in H2 genotype (Table 7).

Table 7. *Pearson's R* values between the means of nutrient ratios of maize stalk with different fertilization levels and the yield parameters

	Grain yield			Protein			Starch		
	H1	H2	H3	H1	H2	H3	H1	H2	H3
N:Mg	0.30*	0.28**	0.22	0.14	0.37**	0.14	-0.27*	-0.32*	-0.11
N:Ca	0.29*	0.18	0.21	0.10	0.27*	0.11	-0.28*	-0.21	-0.06
N:Zn	0.41**	0.23	0.25	0.14	0.27*	0.11	-0.33**	-0.19	0.03
P:K	-0.24	-0.33**	-0.24	-0.29*	-0.35**	-0.13	0.27*	0.28*	0.09
P:Mg	0.16	0.22	0.11	0.12	0.30*	0.19	-0.18	-0.27*	-0.10
P:S	-0.31**	-0.29*	-0.23	-0.21	-0.36**	-0.11	0.28*	0.28*	0.15
P:Zn	0.26*	0.29*	0.21	0.16	0.30*	0.18	-0.25	-0.19	0.05
P:Fe	-0.17	-0.28*	-0.11	-0.15	-0.30*	0.02	0.12	0.22	-0.04
K:Mg	0.32*	0.34**	0.39**	0.23	0.44**	0.28*	-0.31*	-0.41**	-0.20
K:Zn	0.35**	0.37**	0.30*	0.18	0.41**	0.19	-0.32*	-0.31**	-0.03

$^{*}P < 0.05$; $^{**}P < 0.01$.

Gökkuş et al. (2016) also revealed that the high protein and high oil genotypes had stalk and leaves of higher nutritional parameters. It is important to study the exact nutrient characteristics to understand the factors influencing yield quality.

Correlation analysis between the mean nutrient ratios of leaves and yield parameters showed that in grain yield H2 negatively correlated with P:K, P:S and P:Fe, H3 with P:S ratios at $P < 0.01$, and positively with N:Mg, N:Zn, P:Zn and K:Zn ratios at $P < 0.05$ level. Protein and starch content had significant correlation with H2 genotype in case of protein P:K, P:S and P:Fe negatively, and P:S with starch, positively, all of them at $P < 0.01$ level. (Table 8). Shao et al. (2020) reported significant positive correlation between grain yield and N, P and K accumulation and remobilization efficiency during the growing season with pre- and post silking measurements.

Table 8. *Pearson's R* values between the means of nutrient ratios of maize leaves with different fertilization levels and the yield parameters

	Grain yield			Protein			Starch		
	H1	H2	H3	H1	H2	H3	H1	H2	H3
N:Mg	0.21	0.21	0.28*	0.21	0.24	0.27*	-0.27*	-0.19	-0.16
N:Ca	0.07	0.06	0.18	0.13	0.08	0.13	-0.14	-0.05	-0.06
N:Zn	0.16	0.23	0.31*	0.02	0.23	0.20	-0.10	-0.17	-0.18
P:K	-0.20	-0.39**	-0.31*	-0.16	-0.36**	-0.22	0.25	0.32*	0.07
P:Mg	0.14	0.15	0.18	0.21	0.17	0.23	-0.20	-0.13	-0.19
P:S	-0.26*	-0.43**	-0.35**	-0.09	-0.46**	-0.27*	0.22	0.41**	0.05
P:Zn	0.03	0.09	0.27*	0.01	0.07	0.22	-0.06	0.01	-0.26*
P:Fe	-0.10	-0.32**	-0.23	0.17	-0.34**	-0.21	0.00	0.24	0.05
K:Mg	0.19	0.27*	0.25	0.23	0.30	0.26*	-0.29*	-0.24	-0.14
K:Zn	0.15	0.28*	0.31*	0.10	0.29*	0.23	-0.19	-0.20	-0.19

$^{*}P < 0.05$; $^{**}P < 0.01$.

Nitrogen use efficiency, the relationship and co-limitation of nitrogen with other macro- and micronutrients is an important research area in terms of sustainable agriculture, agriecosystem modelling and scailing them into global level (Elser et al., 2010; Khoshgoftarmanesh et al., 2011; Weih et al., 2018). Different studies of the nutrient stoichiometry revealed that N:P, N:S and P:S relationships were allometric and not modified by P deficiency. The lower N:P value observed under N limitation was mainly caused by a higher P content, but the higher N:P value observed under P limitation was mainly caused by a higher N content. The P:S stoichiometric relationship may be used as a 'post-mortem' tool for identifying S responsive sites using grain nutrient analysis (Yan et al., 2016; Salvagiotti et al., 2017; Carciochi et al., 2018). N:S stoichiometry can be used for characterizing plant S nutritional status when N is not a limiting factor for plant growth, eg. in areas or crop production technologies with high nitrogen fertilization supplies (Divito et al., 2016; Arata et al., 2017, Carciochi et al., 2020). Nutrient stoichiometry can be affected mainly by soil microbial activity (Auwal et al., 2021), soil type and its nutrient status (Bruns & Ebelhar, 2006), crop rotation systems (Ma et al., 2003; Xia et al., 2013; Ma & Zheng, 2018) and the plant's water use efficiency (Yan et al., 2016). As a conclusion, the optimal crop nutrition systems have great importance on the morphological parameters, quality and quantity parameters of the plants, which need a well-balanced fertilization practice (Illés et al., 2020; Mousavi et al., 2020; Bojtor et al., 2021) Nutrient stoichiometry can provide a reliable scientific tool to monitor the plant's health status, and make adequate decisions about the optimal fertilization management strategies.

CONCLUSIONS

The findings of this study focused on how the increased nitrogen fertilization levels affected significantly the essential nutrient ratios of the vegetative and generative plant parts of maize. The optimal nutrient supply and ratios of these nutrients of the vegetative plant parts is essential for the optimal dry matter accumulation, which allows to maximize the leaf area and also the photosynthetic capacity, providing the possibility of achieving high yields with remarkable quality parameters. Results of this study could be validated in further novel research and a new perspective of an optimal fertilization dosage could be achieved. Analysing the optimal nutrient ratios related to the yield quality and their interaction with the fertilization practices can give certain recommendations to the farmers to implement hybrid- and site-specific nutrient management systems, reducing the environmental impact of the over-fertilization.

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Effect of replacement of coated barley grain with hulless barley in diet on growth, carcass and meat quality traits of fattening pigs

L. Degola[1,*], I. Jansons[2] and V. Šterna[2]

[1]Latvia University of Life Sciences and Technologies, Institute of Animal Sciences, Liela street 2, LV 3001 Jelgava, Latvia
[2]Institute of Agricultural Resources and Economics, Dizstende, Libagi parish, LV 3258 Talsi County, Latvia
[*]Correspondence: lilija.degola@llu.lv

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Abstract. A amount of experimental pigs were 40 crossbred pigs (Yorkshire × Landrace). The initial body weight of pigs were average 27.0 kg. The goal of research was to assess the effect of replacement of coated barley grain with hulless barley in diet on pig growth, carcass and pork quality indices. Experimental groups of pigs on the holding were conducted according to age and sex. For trial group of pigs, a compound feed with hulless barley (38.9–45.4%) was prepared, for the control with coated barley (39.3–43.3%). The feed recipes made according the pigs age. The other feed ingredients were not changed and were wheat, soybean meal and oil, premivit, and from 20 till 70 kg liveweight also fish meal. Diets were formulated with the same of metabolizable energy and crude protein content. During the study the live weight of pigs was monitored and the feed consumption was counted. At the end of the study all pigs slaughtered, determined carcasses traits and took samples of loin muscle for chemical analyses. The results showed that pig fattening indices (daily liveweight gain were in control pig group 0.686 ± 0.183 and trial 0.716 ± 0.174) did not differ significantly between groups ($P > 0.05$), although its were slightly lower in the control group pigs by 4.37%. Feed consumption for live weight gain in both groups ranged from 3.14 to 3.25 kg. Carcasses scores showed significant differences in lean meat and chops ($P < 0.05$). There were also differences in the backfat thickness. The thickness of backfat was 2.62 mm less in the control pig group, which indicates that when feeding coated barley to fattening pigs, the carcasses have a higher proportion of lean meat (62.1 ± 0.7%). Pigs were slaughtered reaching a live weight of 110 to 114 kg. The meat yield 71.7% and moisture level (70.2–75.2%), as well as protein (22.3–22.9%) indicators showed that fattening pigs are sold at the optimal age. In conclusion, results from this study suggest that feeding hulless barley to fattening pigs results in higher live weight gain. Carcass indicators showed a significantly higher proportion of lean meat and weight of chops when pigs eating coated barley. Chemical composition of pork in groups without significant differences.

Key words: hulless barley, fattening pigs, growth performance.

INTRODUCTION

In a pig farms, feed costs represent 60 to 75% of the total cost of production, therefore, it is important for a producer to formulate diets on a least - cost basis, without reducing production. Pig diets in the Latvia and Europe traditionally are made up of wheat, barley grain and soybean meal. Barley is widely used as a feedstuff for pigs. Barley can be either two - rowed or six - rowed, covered or hulless, awned or awnless. Hullessness is mentioned as one of the desirable characteristics of forage barley, because it has been formed that the hulless - barley has a significant higher content of digestible energy than covered barley. It is mainly connected with the reduced NCP (no-starch polysaccharide) content in hulless barley, because a large part of them is located in hulls (Degola, 2007). The other researchers before considered that hulless barley may be a good alternative energy source for use in pig diets (Jih-FangWu et al., 2000; Degola, 2007). Therefore, the our study was aimed at investigating the effect of hulless barley as replacement of covered barley in pig's diet on growth, carcass and meat quality traits of fattening pigs.

MATERIALS AND METHODS

Animals and experimental design

The experimental 40 crossed pigs (Yorkshire × Landrace) were housed in accordance with the pig welfare requirements Cabinet of Ministers Regulation No. 743. The initial body weight of pigs were average 27.0 kg. The both trial groups completed according to their age and sex, in each group were 20 pigs. Pigs were selected from a commercial pig farm. The experiment lasted 122 days. The dietary treatments were: for trial group of pigs, was prepared a compound feed with local hulless barley, for the control group with local coated barley. The feed recipes made according the pigs age and were three for each group. The other feed ingredients were not changed and were local wheat, soybean meal and oil, as additives, used premivit, and from 20 till 70 kg liveweight also fish meal. The amount of ingredients and choice of mixed feed were adjusted continuously over time depending on the actual weights of pigs are shown in Table 1.

Table 1. Ingredients of compound feed

Ingredients, %	Control group			Trial group		
Pigs liveweights, kg	20–40	40–70	70–105	20–40	40–70	70–105
Wheat	39.31	40.09	43.28	38.96	40.98	45.41
Coated barley	39.32	40.1	43.27	-	-	-
Hulless barley	-	-	-	38.96	40.98	45.41
Extracted soybean meal	15.61	14.28	9.16	16.9	12.78	4.9
Soybean oil	1.0	1.0	1.0	0.5	0.76	1.0
Fish meal	1.0	1.0	-	1.0	1.0	-
Premivit Result or 3% Bacon*	3.76	3.53	3.29	3.68	3.5	3.28

*for growing period used Premivit Result and for fattening period Premivit 3% Bacon.

All nutrients in diets were formulated according the recomendation of NRC (2012). Pigs eated the compound feed *ad libitum*. Each pen was equipped with nipple of drinker. According to the growth phase of the pigs, the feed was changed (Table 1). The live weight of the pigs was monitored during experimental periods. Feed consumption was recorded throughout the all study period and feed efficiency was calculated as the amount of compound feed consumed per unit of liveweight gain.

Chemical analyses

The analysis of the composition of the feedstuffs were done in the accredited Scientific laboratory of Agronomic analysis of Latvia. Samples of feed were milled through a 1-mm screen before analysis. Dry matter (DM), crude protein (CP), crude fiber (CF), fat, calcium (Ca), phosphorus (P), contents were analysed based on standard methodology (Degola et al., 2019). Amino acids were detected using amino analyzer. The identity and quantitave analysis of the amino acids were assessed by comparison with the retention times and peak areas of the standard amino acid mixture. The metabolizable energy (ME) were calculated based on tested parameters in accordance with McDonald et al. (2002). The chemical compositions of the feed mixtures are presented in Table 2. The pork samples were tested by quality parameters - pH, moisture and protein content (LVS ISO 1443:1973), cholesterol content (BIOR-T-012-132-2011), unsatured fatty acids (BIOR-T-012-131-2011) in laboratory of Food and Environmental Investigations (BIOR) in Latvia, but amino acids contents of pork determined in Eurofins GfA laboratory in Germany with methods ISO 13903:2005, IC-UV for all amino acids and for tryptophan used method EU 152/2009, LC-FLD.

Table 2. Chemical composition of compound feed (in dry matter)

Nutrients	Control group			Trial group		
Pigs liveweights, kg	20–40	40–70	70–105	20–40	40–70	70–105
Dry matter, %	89.9	88.7	89.5	89.8	88.7	89.3
Crude protein, %	19.9	17.4	16.8	20.9	18.2	16.7
Crude fiber, %	4.3	3.1	4.5	3.0	3.1	2.5
Fat, %	3.6	3.2	3.6	3.2	2.9	3.1
ME, MJ kg	13.1	13.4	13.3	13.1	13.3	13.2
Ca, g	9.5	9.1	9.1	11.5	8.9	8.8
P, g	5.8	5.2	5.1	6.7	5.2	5.5
Lysine, g 100 g^{-1}	0.95	0.84	0.74	1.04	0.82	0.72
Methionine, g 100 g^{-1}	0.34	0.36	0.29	0.36	0.37	0.28
Cystine, g 100 g^{-1}	0.35	0.27	0.28	0.37	0.28	0.29
Treonine, g 100 g^{-1}	0.59	0.54	0.47	0.65	0.54	0.48
Valine, g 100 g^{-1}	0.71	0.66	0.65	0.76	0.69	0.63

The chemical content in dry matter of used coated and hulless barley in feed mixtures also were tested. The crude protein, β-glucans and starch in coated and hulless barley were with small difference, respectively 13.3 ± 1.0%, 4.0 ± 0.2% and 60.9 ± 0.8% in coated barley, but hulless barley grain contained CP 15.1 ± 0.9%, β-glucans 4.7 ± 0.3% and starch 61.2 ± 0.7%. The amino acids content were - lysine 4.1, methionine 1.7, cystine 2.2, treonine 4.7 g kg DM in coated barley, but hulless barley contained a little bit more, respectively 4.3,1.8, 2.3, 5.0 g kg DM. In the other research the chemical results of different varieties of barley were the same as: levels of total β-glucan, ADF, CP, and starch (90% DM) in the 20 barley samples ranged from 2.7 to 4.5%, 4.5 to 9.2%, 10.8 to 15.1%, and 42.3 to 53.4%, respectively (Fairbairn et al., 1999). The chemical content of barley cultivars influence many factors.

Slaughter and carcass quality measurements

The all experimental pigs at the 110–114 kg were slaughtered in commercial slaughterhouse. Each pig carcass weight was recorded, backfat depth (F) was measured at the head of the last rib, 6 cm from the mid back line, using a Intrescope Optimal Probe (Latvia Regulations of the Cabinet of Ministers Nr.307). The individual percentage of lean meat was calculated by formula: 66.6708–0.3493 × F and estimated by European standard for classification of lean meat in pig carcasses. The letters SEUROP designations are used to refer muscle development. Muscle eye area was measured with the planimeter (Degola &Jonkus, 2018). Carcass yield was calculated by dividing the hot carcass weight by the live body weight. Left side of carcases were divided into parts for determination weight of ham. After 24 hours of pig slaughter, meat samples were taken from the *musculus longissimus lumborum et thoracis*.

Statistical analyses

All data generated in this experiment were subjected to the general linearized model procedures of the SAS/STAT 9.22 software package (2010). The *t-test* was used to compare the means of the indices of control and trial groups. Variability in the data was showed as the pooled standard error. Statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

The growth rate and the efficiency of feed conversion of pigs fed barley rations are often inferior to those of pigs fed diets based on lower fibre grains such as corn or wheat. Influence of our experimental diets on pig performance showed that pig average liveweight gains in research period for both groups were medium high, without significant difference ($P > 0.05$) between pig groups (Table 3), although, the fattening rates were slightly higher by 4.37% of pigs in the trial group which fed hulless barley. Hulless barley, therefore, is of interest and was studied as early as 1924 by Joseph, who found it equal to wheat. Later, the others researchers compared a hulless and covered barley and obtained pig performance equal to that of pigs fed wheat rations, but inferior to corn. Researchers Newman & Eslick, 1970 found no differences between corn, covered and hulless barleys in starter and grower diets of pigs, but corn and hulless barley were better than covered barley during the finishing period (Mitchall et al., 1976).

Table 3. Pig fattening results (n = 40)

Traits	Control group $x \pm SD$	Trial group $x \pm SD$
Liveweight at begining of fatttening, kg	26.9 ± 0.82	27.0 ± 0.88
Liveweight at the end of experiment, kg	110.7 ± 15.18	114.5 ± 14.33
Liveweight gain per fattening period, kg	83.7 ± 15.28	87.4 ± 14.54
Daily liveweight gain, kg	0.686 ± 0.183	0.716 ± 0.741*

*$P > 0.05$.

In the other researhers publications could find the similar results to ours. For example, the average daily gain of pigs fed the hulless barley diet was significantly higher than of pigs fed the corn diet during the grower period, but during the finishing and all trial periods were not significantly different average daily gain (Wu et al., 2000).

Feed consumption in our research (Table 4) in both groups was also similar. There were daily gain from 1 kg feed 0.307 and 0.318 kg. The same results we found in Wu et al., 2000 publication, where the daily feed intake of pigs fed the hulless barley diet was not significantly different from that pigs which fed the corn diet during all periods. Also same results were for feed consumption to liveweight gain in pig growing period (Wu et al., 2000). But during the finishing and over all periods the pigs which fed the hulless barley diet required less feed per unit of liveweight gain than pigs fed the corn diet. Nevertheless, that fattening pigs in our research did not received corn, we got the same results, pigs which fed hulless barley required less feed per 1 kg liveweight gain (Table 4). Advantage of using hulless barley instead of hulled barley as a energy source in pig diets is that a reduction feed costs. In the research of Thacker et al., 1987 we found out, that most cost effective grain to include in pig diets would be hulless barley, assuming a similar purchase price for hulless and hulled barley. This research also demonstrated that level of soybean meal supplementation could be reduced. Feeding different barley cultivars in experiment (Castell & Bowren, 1980), when used pigs from 25 to 93 kg liveweight, growth rates, efficiencies of feed conversion and carcass measurements were not signicantly ($P > 0.05$) affected by cultivar. However, the trend for diets based on two-row cultivars to be superior in feed conversion was supported by their higher apparent digestibilities of energy and of nitrogen in the diets. A palatability study using these diets indicated the pigs preference for two-row over six-row barleys (Castell & Bowren, 1980)

Table 4. Feed consumption

Indices	Control group	Trial group
Fattening days	122	122
Feed consumption for one pig, kg	272.2	274.5
Feed per day, kg	2.23	2.25
Feed conversion, kg	3.25 ± 0.264	3.14 ± 0.062*
Daily gain from 1 kg feed, kg	0.307 ± 0.0221	0.318 ± 0.0158*

*$P > 0.05$.

In the other research before wrote that diets containing 0, 20, 40, 60, or 80% barley were fed to pigs with initial body weight 67.9 kg for 8 weeks, results were: feeding diets with increasing levels of barley resulted in a linear decrease in daily gain ($P < 0.01$) and backfat thickness ($P < 0.01$). Dressing percentage linearly decreased with length of barley feeding ($P < 0.05$), but the concentration of saturated fatty acids in backfat increased (linear, $P < 0.05$) the longer the barley diet was fed, without no effect of barley on loin muscle quality and barley did not consistently change fat color (Beob et al., 2014). In our experiment carcass traits showed (Table 5) significant differences in lean meat and chops weight ($P < 0.05$). There were also differences in the backfat thickness, 2.62 mm less backfat thickness in the control group, which indicates that when feeding coated barley for fattening pigs, the carcass has a higher proportion of lean meat. Carcass yield in both pig groups were 71.7%, but in publication of Wu et al., 2000 the carcass yield and length, backfat thickness and muscle eye area of pigs fed the hulless barley diet were not significantly different from pigs which fed the corn diet.

Table 5. Pig carcass measurements

Measurements	Control group *x* ± *SD*	Trial group *x* ± *SD*
Carcass weight, kg	79.4 ± 6.39	82.1 ± 11.5
Backfat thickness, mm	7.63 ± 3.231	10.25 ± 3.073*
Lean meat, %	62.13 ± 0.701*	61.45 ± 0.683
Muscle-eye area, cm^2	52.3 ± 7.62	51.4 ± 3.73
Ham weight, kg	8.9 ± 0.64	8.0 ± 0.37
Chops weight, kg	2.43 ± 0.091*	2.07 ± 0.182

*$P > 0.05$.

Chemical composition of pork without significant differences (Table 6). Pigs were slaughtered reaching a live weight of 110 to 114 kg, when high meat yield and moisture, as well as protein content indicated that fattening pigs are sold at the optimal (of 5.5 till 6 month) age (Table 6). The same results we found in italian researchers (Prandini et. al., 2015) publication where they evaluated the effect of diets based on hulled or hulless (normal and low amylose) barley varieties on growth performance and carcass characteristics in heavy growing-finishing pigs for the production of protected designation of origin (PDO) Italian products. Four diets were formulated: corn-based diet (control), control diet with 80% of a normal-amylose hulled barley variety named Cometa (Cometa), control diet with 80% of a normal-amylose hulless barley variety named Astartis (Astartis), and control diet with 80% of a low-amylose hulless barley variety named Alamo (Alamo). The researchers found that no difference in carcass characteristics among treatments ($P > 0.05$). This study showed that diets based both on hulled and hulless barley might be suitable for the heavy pig breeding intended to the production of Italian PDO products (Prandini et al., 2015). It indicates that hulled or low-amylose hulless barley could be valuable to support maximum pig growth performance without affecting carcass composition.

Table 6. Influence of diets on pork chemical content

Indices	Control group *x* ± *SD*	Trial group *x* ± *SD*
Moisture, %	70.3 ± 0.2	75.2 ± 0.2
Protein, %	22.3 ± 0.3	22.9 ± 0.3
pH	5.41 ± 0.03	5.44 ± 0.03
Cholesterol, mg 100 g^{-1}	62.6 ± 15.7	62.7 ± 15.7
Unsaturated fatty acids, %	56.8 ± 2.5	49.5 ± 2.1
Tryptophan, g 100 g^{-1}	0.282 ± 0.028	0.301 ± 0.030
Hydroxyproline, g 100 g^{-1}	0.0880 ± 0.018	0.0710 ± 0.014
Tryptophan:Hydroxyproline ratio	3.20 ± 0.016	4.24 ± 0.019

According to the SEUROP carcass classification system of pork, all pig carcasses were evaluated by the (S) class, where the lean meat was more 60 percent.

CONCLUSIONS

Feeding hulless barley to fattening pigs, results in higher live weight gain for pigs, although the differences with the inclusion of coated barley in pig feed are not significant. Carcass indicators show a significantly higher proportion of lean meat and weight of chops when pigs eating coated barley in diets. The chemical composition of pork does not differ significantly.

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Delineation of catchment area for the lake Kisezers for environmental sustainability

J. Dumpis[1,2,*], A. Lagzdins[1] and I. Sics[2]

[1]Latvia University of Life Sciences and Technologies, Department of Environmental Engineering and Water Management, 2 Liela Street, LV-3001 Jelgava, Latvia
[2]Institute of Food Safety, Animal Health and Environment 'BIOR', Lejupes Street 3, LV-1076 Riga, Latvia
[*]Correspondence: janisdumpis94@gmail.com

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Abstract. The study aims to develop a methodology for the delineation of a catchment area. The methodology includes the processing and analysis of LiDAR data, on-field height measurement data, bathymetric data, hydrological data. High definition catchment area maps are successfully constructed. Catchment area influencing factors such as water mass movement and changes in land use are determined. Lake Kisezers was selected as the study site because the location of the lake, the availability of data, the feasibility studies, the economic potential of the catchment area determine the topicality and significance of this study. The lake catchment area covers multiple rivers, urban and rural territories, forests, high and low terrains. In the catchment area of Lake Kisezers many hydrologic monitoring stations with continuous data are situated. In the research area, we can study how those factors interact with the possibility to perform a catchment area delineation. The final result of this study is the catchment area for Lake Kisezers. The research results are high-definition and can be used to understand locations of floodplains, territories with malfunctioning drainage systems. The repetition of this study requires extensive knowledge of cartography, experience in working with terrain and bathymetry data, wide range of GIS knowledge. The research was performed using computer software such as QGIS and GRASS GIS. The application of the methodology used in this study can serve as an example for delineation and analysis of a catchment area for other lakes and rivers. Overall, the study is a success.

Key words: catchment area, GRASS GIS, LiDAR, QGIS, lake.

INTRODUCTION

The previous extensive research on catchment area delineation in Latvia was carried out in the late 20th century. As a result of this historical research, the maps of catchment areas for lakes and rivers were constructed using manual work and techniques, which questions the overall precision of the maps prepared at that time. These maps can be used to compare the shapes of lakes and rivers in of 20th-century, their compatibility, changes in the selected basins, artificial barriers, meanders of rivers (River basin maps, 1975).

Anthropogenic impacts combine with climate change over time might lead to changes in the hydrological network of rivers, hydrological regime, surrounding terrain, depths of lakes and rivers, groundwater levels, and soil moisture conditions. As a result of anthropogenic activities and natural conditions, the characteristics of catchment areas have changed over time and it is necessary to re-identify the catchment areas and their characteristics. This exercise can help to evaluate the functional possibilities of the territory and understand how to ensure sustainable and environmentally friendly management of the territory (Gilberto et al., 2014).

Surface runoff always flows from a higher to a lower point due to gravity. As individual streams merge, converge, and gradually turn into streams, rivers, which in turn flow into the nearest lakes, larger rivers, sometimes rivers flow through the lakes (Leinerte, 1988). In order to study a lake, it is important to identify and analyze the catchment area determine the influencing factors such as morphometry, quality, water movement, and level (Gilberto et al., 2014). It is important to view and identify not only the entire catchment area but also smaller catchment areas of the water body in the entire catchment area of the main lake. The watershed can be relatively thought of as a line drawn along the highest points of the terrain around the lake (Leinerte, 1988). For coastal lakes, the boundary of the catchment area is difficult to see in nature (Melluma & Leinerte, 1992).

Chemical substances as dissolved in water enter the lake from the catchment area. (Shelton, 2009). In Latvia, we need to increase the number and details of research to analyze water quality. The identification and mapping of the catchment area are of particular importance in this type of research (Gilberto et al., 2014). Combining the results of the previous catchment studies with the possibilities provided by up-to-date technologies and geospatial data such as LiDAR, terrain models, observation stations, bathymetry data, land use data, orthophoto maps helps to delineate catchment areas in unprecedented resolution and systematically observe any changes. The areas with an understandable transport of water can be used for economic growth, for example, by identifying the economic potential of the area as agricultural land, it is possible to build irrigation systems that improve the potential for land use (Arslan et al., 2020).

Anthropogenic impacts particularly on territories with minor elevation changes make it challenging to construct the catchment areas because decomposition of the catchment areas by terrain is more time-consuming and requires the application of high cartographic skillsets (Haas et al., 2020). The territories are more suitable for economic activity and have a wide range of uses, for example, for construction, economy. Due to construction activities, felling forests, engaging in agriculture the landscape is changed, as a result of which the size and shape of the catchment area might change as well, which in turn leads to uncontrolled circulation of dissolved chemical substances with little or no control over, for example, accidental spills and pollution. Thus in-depth research is also possible to identify the effects of climate change on the site (Andri et al., 2020).

Water is one of the most important natural resources, and it is important to research its quality, movement, and impact factors (Arseni et al., 2016). Of all ecosystems, lakes are among the most sensitive to changes in the environment, therefore, research into the hydrometry of lakes needs increased attention (Agnieszka et al., 2011). The lakes contain a significant amount of the world's freshwater reserves, so it is important to take care of their water quality. The water quality of lakes affects the quality of drinking water in the area, recreation, fishing (Perivolioti et al., 2017). Lakes are an important part of the ecosystem because lakes collect all water from catchment areas (Schiefer & Klinkenberg, 2004). In the world, the study of lakes in terms of physical, chemical, and biological characteristics has developed rapidly since the beginning of the 20th century (Tundisi, 2012). Lakes are sensitive to climate change (Tan et al., 2017) as well as rising human economic activity. Identifying the physical size of a water body and the movements of water masses is important to understand how best to use water bodies (Hassen et al., 2013). Research on catchment areas of lakes is important because it is possible to understand groundwater and directional runoff flows. Research can provide a better understanding of the origin of waters and the potential risk factors that threaten water quality (Ramachandran et al., 2020). In case of accidents, it is possible to identify and localize pollution sites and act more quickly if river basin maps are available (Davraza et al., 2019). Water resources have remained under threat as a result of globalization (Ramachandran et al., 2020).

Characteristics of Lake Kisezers

The lake is located in the Seaside Lowlands in close proximity to the Gulf of Riga in the flat relief area. The lake is a part of the City of Riga, the capital of Latvia. Lake Kisezers is situated in the Daugava river basin district (Tidrikis, 1995). The lake is located in the depression of the Kisezers-Jugla lake district, in the far part of the Litorina Sea lagoon zone (Kacalova, 1988). Lake Kisezers together with Lake Jugla is located in one valley formed by the former Daugava tributary. Lake Kisezers is a continuous river-type lake (Slaucitajs, 1935; Stiebrins, 2011). Together with Lake Mazais Baltezers and Lake Lielais Baltezers, Juglas lake is interconnected and belongs to the Daugava-Gauja system, which unites 4 lakes and two rivers - Gauja and Daugava. The Daugava-Gauja system was established by the Vidzeme Waterway Improvement Society in 1901–1903 (Kacalova et al., 1962). As Lake Kisezers is located on the outskirts of the City of Riga, its catchment area has been partially reconstructed and significantly affected as a result of artificial land drainage, infrastructure development, construction, and other economic activities. In addition to natural waters, Lake Kisezers also receives storm runoff from the asphalted surfaces like streets and parking lots, quite often storm runoff is not properly treated (Stiebrins, 2011). Due to the inflow of industrial wastewater in Lake Kisezers, there have historically been problems with self-treatment (Kacalova et al., 1962). The greater the anthropogenic impact in the catchment area, the poorer the water quality will be in water bodies (Leinerte, 1988). Analyzing the topographic maps, descriptions of the lake, movement of water, it can be concluded that Lake Kisezers has a contribution from groundwater, precipitation, surface runoff, the impact of Riga Hydroelectric Power plant (HPP) activity, water also is contributed from the sea (LVM, 2020). According to Cabinet Regulation No. 418 'Regulations on risk water bodies' Lake Kisezers is defined as a water body at risk (Cabinet Regulation No. 418, 2011). A water body at risk is a water body for which there is a risk of not reaching water quality goals set by the Cabinet Regulation No. 118 'Regulations on the Quality of Surface and Groundwater' (Cabinet Regulation No. 118, 2002). Moreover, Lake Kisezers is also mentioned as a priority water for fishes, but to set appropriate ecological and chemical quality criteria for Lake Kisezers, it is necessary to identify its depths, which have been studied by the author in the previous study (Dumpis & Lagzdins, 2019). The average depth is a feature according to which lakes are classified by applying appropriate quality standards (Cabinet Regulation No. 858, 2004). As Lake Kisezers is located in the city it is an important recreational object, but at the same time, it is also subject to strong anthropogenic influences such as wastewater discharges causing increased concentrations of nutrients in the water that accelerate eutrophication processes. In its turn, Lake Kisezers is included in the flood risk area according to the flood risk management plan of the Daugava river basin district for 2016–2021 (LEGMC, 2015).

Description of the lakes in Latvia

The lakes - a very characteristic element of the Latvian landscape and an integral part of its ecological chains (Glazaceva, 2004). The lakes observed in the territory of Latvia have formed in the Holocene (Zelcs, 1995). The lakes began to form as the hydrographic network developed. As the waters gradually flowed away, the Baltic Ice Lake was formed, as the glacier melted, the first lakes formed between the hills, disappeared at the end of the Ice Age and re-emerged before the beginning of the Ice Age (Zelcs, 1995). Many of Latvia's lakes are of glacial origin. The pits of Latvian lakes have been formed in the course of complex, usually combined geological processes (Tidrikis, 1995). This can be explained by the last icing about 10 thousand years ago. A large number of lakes makes Latvia a country rich in lakes. The richness of the lakes opens up a wide range of opportunities to exploit this valuable resource. Most lakes are relatively small. There are 843 lakes with an area of more than 10 ha, but only 141 lakes with an area of more than 1,000 ha (Glazaceva, 2004). The area of many lakes is decreasing, it is related to human economic activity and eutrophication. If those lakes with an area of at least 1 ha are counted then there are 2,256 (Tidrikis, 1995). The Lake Kisezers belongs to the coastal lakes, lagoon lakes (Kacalova et al., 1962). Lake Kisezers has been formed in complicated processes. The development from the Baltic Ice Lake to the Baltic Sea has been long and can be divided into several phases, such as the Baltic Ice Lake, the Joldia Sea, the Ancilus Lake, and the Litorina Sea stages (Andrén, 2012).

The seaside lakes have formed relatively later than the lakes in the Latvian highlands and other lowlands. According to P. Sloka Lake Kisezers formed from the ancient bed of the Daugava and Lielupe rivers. The lakes formed from ancient riverbeds are deeper than other coastal lowland lakes that used to be lagoons (Sloka, 1956).

People have long been interested in the origin of the lakes, mythical explanations can be found also in Latvian legends. According to their origin, Latvian lakes can be divided into seven separate types: glacial, coastal, tributary and old-growth, hot and suffusion, moss bog lakes (Leinerte, 1988). The study examines the origin of the lakes of the Seaside Lowlands by paying special attention to the origin of Lake Kisezers.

Problem statement

The main task of this study is to delineate the catchment area for the Lake Kisezers. Delineation of lake Kisezers catchment area is crucial for further research. Further research includes an in-depth analysis of lake Kisezers. This research part focuses on delineation of catchment area and understanding of catchment area influencing factors.

This research aims to develop a methodology for the delineation of catchment areas for lakes using the example of Lake Kisezers.

The following tasks have been set:

– To develop methodology suitable for delineation of a catchment area using open-source software and tools;

– To perform high-definition delineation of the catchment area for Lake Kisezers;

– To evaluate the functionality of the methodology developed during this study and assess the possibilities of further applications.

MATERIALS AND METHODS

The research was implemented using computer software developed for processing and analysis of geospatial data such as QGIS 3.6 and GRASS GIS 7.6.1, which are free-access software. A high-definition methodology for the delineation of the catchment area was developed. After the performing delineation of the catchment area in GRASS GIS software, the quality control of the catchment area created was performed using remote sensing methods in QGIS software. To verify the accuracy of the results, the flat relief areas were inspected manually and it was checked whether the catchment area delineated represents reality. The development of the methodology was time-consuming and requires extensive cartographic knowledge, because the topography is relatively flat in Latvia, and delineation at a high degree of detail requires the processing of long-term data. Territories such as lake Kisezers catchment areas have anthropogenic influences with makes it strenuous to delineate the correct border of the catchment area.

The developed methodology is repeatable, but knowledge of cartography, geography, decoding, work with terrain data, and digitization is required. The methodology only includes open access computer software, as this makes it more accessible and more likely for someone to repeat this exercise.

The basic principles are as follows:

– Research of historical data and contours in the catchment area of interest;

– Digitization work and water connectivity analysis;

– Delineation of the catchment area using GIS tools;

– Correction of the catchment area constructed using LiDAR data, connectivity of rivers, and fieldwork measurements;

– Compilation of the results. The final design of the catchment area and reflection of the results in a map.

Planning and feasibility study

Analysis of available cartographic materials, literature analysis takes a place for understanding specifics of the study area. It has been concluded that extensive and detailed information on the catchment areas in Latvia is not available. Nowadays, the Latvian Environment, Geology, and Meteorology Centre (LEGMC) is engaged in the extraction and analysis of water bodies. Directive 2000/60 / EC of the European Parliament and the Council was adopted on 23.10.2000 to establish a comprehensive framework for the protection of inland, transitional, coastal, and groundwater. The main objective is to maintain and improve the quality of surface water and groundwater. Accordingly, each EU Member State elaborates the River Basin Management Plans. In Latvia, the River Basin District Management Plans are developed by the LEGMC as prescribed by the regulations 'Regulations on river basin district management plans and measures in the program' (Cabinet of Ministers Regulations No. 646, 2009).

When analyzing the information available in the River Basin Management Plans and 2nd cycle Flood Risk Management Plan 22.12.2021–2027. it can be concluded that the catchment areas for the water bodies are not delineated in the highest possible level of detail and for in-depth studies, it would be necessary to create more detailed catchment areas for specific rivers or lakes. It is important to mention that the main task of the LEGMC is to create the catchment areas for the water bodies of interest at a certain scale, this task does not include delineating the catchment areas for every river or lake.

Methodology for catchment area delineation

Using freely available topographic maps of Latvia's State Forests (LST) ap server orthophoto (Table 1), land reclamation, water management, and the catchment maps of the Latvian State Land Reclamation Design Institute from 1975 (scale 1:100000), LiDAR shading available on the map server (scale 1 m), LiDAR data from the Latvian Geospatial Information Agency (LGIA) to construct lake Kisezers full catchment and direct catchment area. When constructing the catchment area, the connection of watercourses and the direction of runoff were studied from orthophoto maps and the previous 1975. year research results. After the construction of the hydrological network, by studying the LiDAR data available on the LVM map server, historical information, cartographic materials, and literature, the catchment area was constructed. Based on Corine Land Cover data for 1990, 2000, 2006, 2012, an analysis of changes in land use in the catchment area was performed. The analysis is necessary to be able to characterize the factors influencing the catchment area and the lake, both natural and anthropogenic. HPPs and other dams in the catchment area have been identified, which impede the natural run-off of water and the flow of substances. Analysis of changes in Lake Kisezers bathymetry from 2017 to 2019 was performed. The mass movement of water was analyzed using the LEGMC observation station Kisezers, Milgravis.

Table 1. Orthophoto maps

Orthophoto cycle	Production yea	Catchment area territory aerial photography year
Orthophoto 1	1994	1997; 1998
Orthophoto 2	2003	2003; 2004; 2005
Orthophoto 3	2007	2007; 2008
Orthophoto 4	2010	2010; 2011
Orthophoto 5	2013	2013
Orthophoto 6	2016	2016; 2018

The study is divided into stages. **The first stage includes** the drawing of the historical catchment area from the 1975 catchment area map of the Ministry of Land Reclamation and Water Management and the Latvian State Land Reclamation Design Institute. This was followed by the construction of the shorelines of the large lakes - Kisezers, Jugla, S. Baltezers, and L. Baltezers and the shorelines of the connecting watercourses from Orthophoto maps. All Orthophoto maps were used for understanding the correct watercourse location. The shorelines of the L. Jugla and S. Jugla rivers and all their tributaries are constructed. Analyzing the cartographic materials, it can be concluded that thanks to the dense network of drainage ditches, nowadays the Kisezers catchment area merges even with the Gauja catchment area. After marking the shores, the connection of watercourses and water bodies was studied, the locations of dams were deciphered. For this step, QGIS is used.

The second stage involves the delineation of catchment area

Using the LiDAR (at leaset 1.5 point to m^2) data and the plotted water contours in the computer program GRASS GIS, the catchment area were constructed. LiDAR data were uploaded to GRASS GIS, then vector to raster function is used. After creating the raster data were inspected using QGIS. Final catchment area borders were calculated using a watershed calculating option. Data were compared with historical data, orthophotos, delineated watercourse connectivity map. Data were compared with the map server DEM model.

Catchment area construction includes relief model construction using rivers and lakes as quality control points. Digitalized river and lake contours need to match with river and lake contours from constructed relief model. After many attempts, parameter fix and work with resolution The error areas were manually edited for the catchment area and its correctness was checked using the cartography computer program QGIS. Fig. 1 reveals relief data used for the construction of lake Kisezers catchment area.

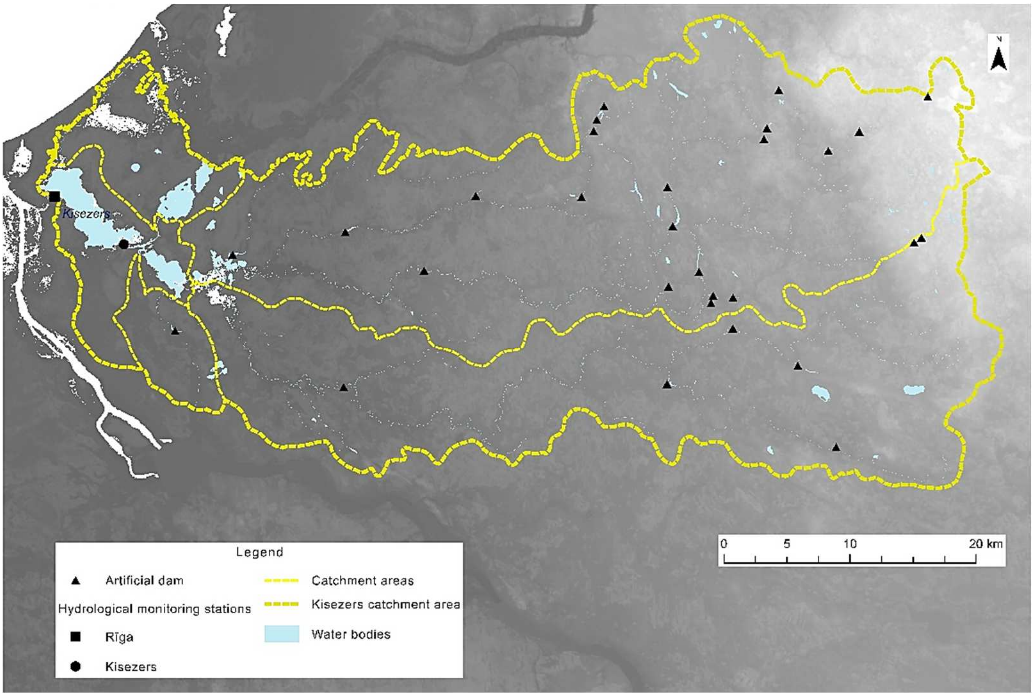

Figure 1. Catchment area shaded relief model.

Field work

In fieldwork with GPS Garmin GPSMAP 66i, obscure places, areas with flat relief, and difficult to understand water location was surveyed. Most of field work take at places with an elevation close to sea lever and places with continuous flat relief. Territories with an undetectable border of catchment area were separated and catchment areas were separated using historical maps.

Field work results are compared with the results of relief analysis data. Lake Kisezers direct catchment area, the common catchment area, the M. Jugla, and L. Jugla catchment areas have been constructed. If necessary the data are sufficient to be able to construct a catchment area for any watercourse and water body belonging to the Kisezers catchment area. For this research main focus is lake Kisezers catchment area, for understanding influencing factors and possible causes of changes in lake Kisezers not only direct catchment area maps were constructed.

The third stage is presentable data maps construction and lake Kisezers catchment area influencing factor specification for further research

The third stage includes the preparation of the catchment area maps, land use analysis based on lake Kisezers catchment area influencing factor specification using Corine Land Cover 1990, 2000, 2006, 2012 data. Water level data at LEGMC observation stations Kisezers (1948–2017), Milgravis (2014–2017), and water mass movement data at observation station Mīlgrāvis were analyzed. Depth changes in the largest water body of the catchment area lake Kisezers have been analyzed from 2017–2019. Depth changes show how the lake changes as sediments settle in it (Elsahabil et al., 2018).

RESULTS AND DISCUSSION

One of the main factors influencing the lake is the catchment area. Lake Kisezers catchment area covers 1,840 km^2 (according to A. Tidriķis data in the encyclopedia 'Latvija nature' - 1,900 km^2). The results of the study prove that lake Kisezers catchment area accurate size is 1903.26 km^2; it consists of the following water bodies:

1) watercourses: Milgravis, Jugla, L. Jugla, Suda, Mergupe, S. Jugla river;
2) lakes: L. Baltezers, S. Baltezers, Juglas, Pecoru, and Plauzu lake.

In addition to the mentioned hydrological objects, lake Kisezers catchment area also includes smaller rivers, such as Pikurga, Smerlupite, and Langa (also known as Garupe). Most of the rivers in the catchment area start in the Vidzeme highlands, flow along the Central Latvian slope and the Ropazi plain (downstream). This condition also determines the variability of the longitudinal slope of rivers in their sections (rapid descent upstream and slow-flowing sections downstream). The river Jugla and the river Langa are also slow-flowing rivers. The river Smerlupite is a typical river flowing through the city, most of which is artificially regulated and discharged underground (Tidrikis, 1995).

According to the research results, lake Kisezers catchment area is 1,903.26 km^2 (Fig. 2). The majority of the catchment area consists of the L. Jugla catchment area 941.59 km^2 and the S. Jugla catchment area 712.16 km^2. The direct catchment area of Kisezers covers 99.96 km^2. The largest bogs in the catchment area are Lielpurvs, Jūgu bog, Getliņi bog, Maltuve bog, Langa's bog, Lielais Kangaru bog, Pecora bog, Suda bog. The largest water bodies are Kisezers, lake Jugla, L. Baltezers, S. Baltezers. According to E. Apsite, Kisezers has a greater role in the regulation of runoff, because the catchment area of the lake significantly exceeds the volume of Kisezers itself and the ratio between the runoff and the area of the catchment area is 111. Usually, the ratio of runoff to basin area is between 5 and 30 (Apsite et al., 2018).

The lake is located in a lowland close to the sea, which results in water from the Gulf of Riga entering the entire catchment area W part. Brackish water from the Gult of Riga true the river Daugava can flow into lake Kisezers, lake Juglas, S. Baltezers, and L. Baltezers because of interactions between Riga HPP and Baltic sea tides. There is a high risk of pollution for Kisezers, as the water enters the lake from the Daugava, the Gulf of Riga, and the catchment area. As a result of the pollution, the consequences would be serious, because the territory is of great economic significance, part of the territory of the Latvian capital Riga is located in the catchment area. In isolated lakes located in the valley, the risk of pollution is less high and can be localized (Davraza et al., 2019), but in the case of Lake Kisezers, the solution to the problem would be an extremely difficult challenge. It is, therefore, necessary to carry out research, model potential risks, and plan measures to address them.

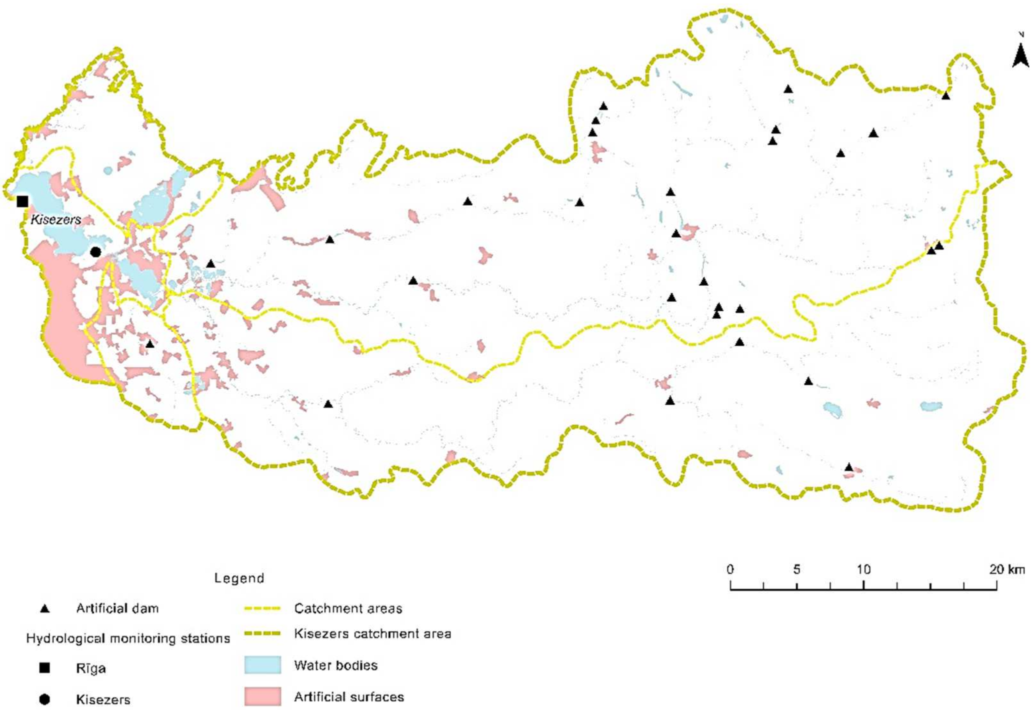

Figure 2. Catchment area map of lake Kisezers.

A total of 6 small HPPs and 20 dams were deciphered due to the construction of the catchment area. Obstacles that interfere with water movements in the catchment area were deciphered for the understanding of the transport of substances in the catchment area. Water exchange and transport between lakes and rivers, the catchment area, is one of the most important processes for maintaining a healthy ecosystem (Yang et al., 2016). By deciphering the locations of the dams, we can conclude that free mass flow of water is not possible in the catchment area. Small dams are not economically profitable in Latvia. In Latvia in general all small HPPs produce energy that is not profitable and economically advantageous for the Latvian population. Disproportionate damage is caused to nature (Timmermanis, 2001). Rapid water level changes thru-out a day is a threat to environmental sustainability in an ecosystem. In the direct catchment area of Kisezers since 1968. until 2005, 2006 in the Jugla River near Lake Jugla. there were locks built to prevent brackish water from entering lake Jugla and lake L. Baltezers (Glazaceva, 2004). Nowadays locks are dismantled.

During the floods in lake Kisezers, water flow can be observed (Kacalova et al., 1962), as a result of which the lake self-cleanses. From research, we can conclude that the lake self-cleanses frequently. Lake Kisezers can be polluted more because of water movements which bring water from Daugava and Baltic sea into lake Kisezers. Kisezers is characterized by a mixed water supply. The lake water extraction is from melting snow, rain, and groundwater, as well as diversity in terms of terrain, climate, and water regime. The long-term average precipitation layer P according to A. Pastor's 1987 study in the Kisezers catchment area varies from more than 850 mm in the NE part to 600 mm in the SW part of the catchment area. In most parts of the catchment area, the runoff layer is from 750 mm to 850 mm (Ziverts, 2004).

Land Use analysis of lake Kisezers catchment area

The land use analysis of the Kisezers catchment area has been performed (Fig. 3). Identifying land use patterns helps to understand changes in the catchment area, to identify potential problems in the future (Matsushita et al., 2006). To better understand the data, land use is divided into generalized groups which are artificial surfaces, agricultural areas, forest, and semi-natural areas, wetlands and water bodies. Areas are simplified and combine several land-use groups. The simplified breakdown is designed for a better understanding of land use in the catchment area, detailed land use data is hard to read, detailed data is used for data processing, not for visualization purposes. Detailed land use maps are not reflected in the results of the study, because research results in an analysis of catchment area impact factors not an analysis of mosaic land use where every slight difference is distributed. In the research land use is analyzed as an impact factor on large scale. Land use is one of the most important factors influencing the catchment area (Matsushita et al., 2006).

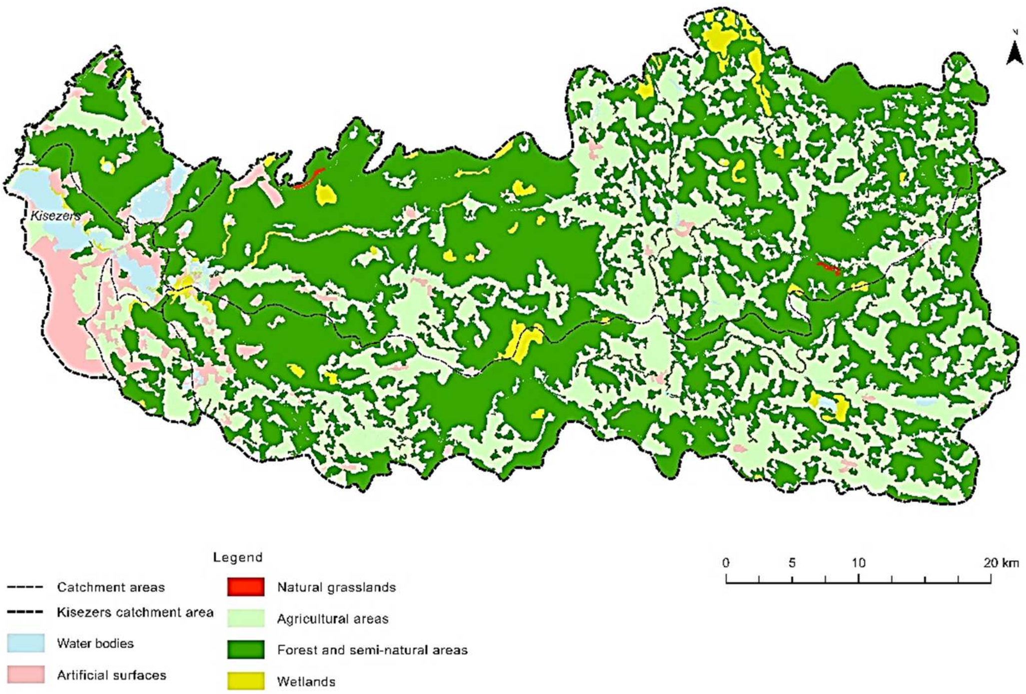

Figure 3. Catchment area land use map (Prepared by the author using Corine Land Cover 2012 data).

The main impact factors are anthropogenic land use areas (artificial surfaces). Urban fabric, industrial, commercial, and transport units, mine, dump, and construction sites, artificial, non-agricultural vegetated areas are divided into areas of anthropogenic impact (artificial surfaces). Arable land, pastures, heterogeneous agricultural areas were allocated as agricultural area covers more than 30% of the catchment area. It is observed that more areas of anthropogenic impact area in the NW part of the catchment area. Anthropogenic impact areas make it difficult to designate catchment areas and identify water mass movements (Haas et al., 2020), which in turn leads to the conclusion that areas with anthropogenic impact are areas at risk of pollution that are difficult to control and predict. Most artificial surface areas are located near Lake Kisezers, which creates an increased impact on the water body. Agricultural areas are mostly observed in part E of the catchment area. Forests, scrub and herbaceous, open spaces with little or no vegetation are combined under forests and semi-natural areas without separating forest types. Forests in the catchment area can be observed in the whole territory, coniferous forests dominate in the west part, but mixed forests in the E part. Forests and semi-natural areas are dominated land-use types covering more than half of the catchment area (Table 1). Wetlands, water bodies have been identified for understanding catchment area soil humidity. Natural grasslands are preserved on the map for understanding how endangered are natural territories in the catchment area.

The study's analysis of land use has changed between 1990 and 2012. In the period from 1990 to 2000, land use has changed relatively in large territories (Table 2). Most changes in artificial surface territories. Agricultural areas are spread. Forest territories suffer from deforesting. Wetlands are increasing in size because of beaver activities. Water bodies' territories are shrunk because of groundwater changes and small creek, melioration canal drainage.

Table 2. Land use in the Kisezers catchment area (Prepared by the author using Corine Land Cover data)

Land-use type	Area 1990 (km^2)	Area 2012 (km^2)
Artificial surfaces	65.43	98.31
Agricultural areas	558.02	633.81
Forest and semi-natural areas	1,138.73	1,088.99
Natural grassland		1.21
Wetlands	86.49	430.73
Water bodies	54.13	37.85

In total there have been changes in the area of 6,457.022 ha. Changes have taken place in the forested areas, with forests turning into shrubs, which indicates logging. Overgrazing and land reclamation have taken place. In the period from 2000 to 2006, changes took place in an area of 4,096.61 ha. According to the nature of the changes, it can be concluded that forestry is developing in the territory, the number of urban areas is increasing, and mining is taking place in quarries. In general, an increase in the anthropogenic load of the area can be observed. In the period from 2006 to 2012, changes took place in 8,388.27 ha of territory. Similar to the development of forestry from 2000 to 2006, the number of urban areas and the extraction of minerals in quarries are increasing. The trend towards anthropogenic influences continues.

Water mass movement analysis of lake Kisezers catchment area

The significant changes in the water level observed in Kisezers can be mentioned as a significant impact factor. The recording of the water level at the observation station Kisezers started on 01.04.1929, a constant data series has been available since 1948. The observation station is located at the SE end of lake Kisezers at the mouth of the river Jugla in Kisezers. Observation recording at the observation station Riga started on 01.01.2014. The station is located at the NW end of Kisezers, at the source of the Milgravija canal from Kisezers. Lake Kisezers has a runoff to the Daugava along the Milgravis canal, which was built in the 13th century. The canal functions as leakage of lake water to the Daugava, but changes in the lake level are affected by changes in the Daugava water level regime, which after 1974 is additionally determined by the Riga HPP operation regime during the day, season, and year. Another factor that can determine significant changes in the lake level from time to time is the inflow into the Gulf of Riga from the Baltic Sea through the Irbe Strait caused by the prevailing NW, N, SW, and NW winds, but water inflows - E, ENE, NE, NNE, NNW, and NW winds (Glazaceva, 2004).

Analyzing the movement of water masses, it can be concluded that Milgravis channel and Jugla river, which connect Kisezers and lake Jugla, tend to change the direction of the current in the opposite direction, as well as the Bukulti canal, which discharges the S. Baltezers and L. Baltezers waters into lake Kisezers. We can conclude that complex water mass movements are observed at the lower end of the catchment area and more water flow and level observation stations would be needed. Thus, it is difficult to correctly separate Kisezers catchment area, because as a result of complicated natural conditions and anthropogenic influence, Milgravis sometimes flows out of Kisezers, but sometimes flows into Kisezers. The movement of water masses is significant, the largest flow was observed on 06.12.2017. It has been 698.28 m^3 s. The largest negative flow was observed on 26.10.2017. it has been 1,251.34 m^3 s. Thus if the Riga HPP had been operating at a full capacity of 3,600 m^3 s. at this time (Pastors, 1990), approximately one-third of the Riga HPP runoff would end up in Kisezers. Unfortunately, there are no data available on the flow and operation schedule of the Riga HPP that would confirm this conclusion. The main factor influencing the significant flow fluctuations in 2017 is the Riga HPP. For further research is mandatory to start monitoring water levels and mass movements in canals connecting lake Juga, lake Kisezers, lake Mazais Baltezers, and lake Lielais Baltezers.

Lake Kisezers bathymetry changes

In the research bathymetric survey was performed in 2017. And in 2019. Bathymetric maps were constructed (Fig. 4). Research results are identical and lakes bathymetry has not changed. For detecting changes in bathymetry regular bathymetric survey has to be done. Lake Kisezers deepest point is 21.5 m, average depth is 1.99 m. Data were collected by the author using echosounder Lowrance HDS Carbon 9 and GNSS Trimble catalyst with a precision of 1 cm.

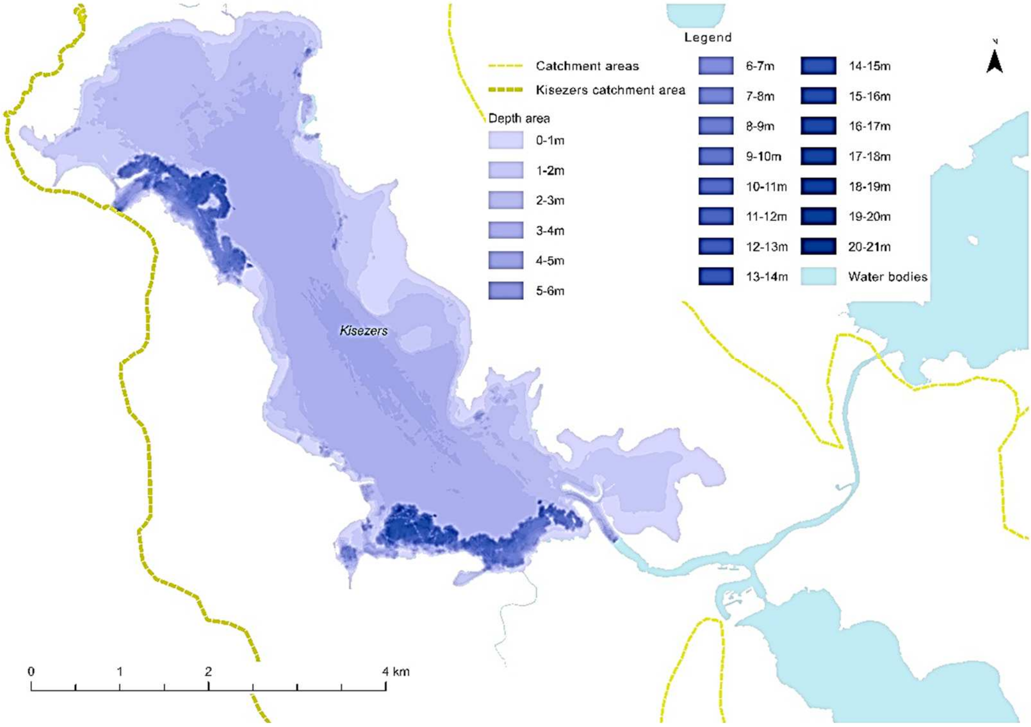

Figure 4. Lake Kisezers bathymetric map 2019. (Prepared by the author using personal field work data).

CONCLUSIONS

After successful completion of the aim and tasks of the research, a high-definition lake Kisezers catchment area map and definite influencing factors. The research was challenging and acquires special care, but its results prove that in Latvia is possible to achieve the state of the art results using open-source technologies. It would be simpler to repeat the study in another body of water because it is rare to find such a complicated situation with many influencing factors, not everywhere is that high-degree data availability. The missing water level and flow data from the Bukulti canal and the mouth of the Jugla river in Lake Jugla can be mentioned as shortcomings and collecting data for future research will improve the research result level of detail. To make the constructed catchment area even more accurate, it would be necessary to monitor groundwater flows and locate observation stations in the entire catchment area. To assess how the catchment has changed, it would be necessary to obtain LiDAR data after a few years (Elsahabi et al., 2018) and repeat the catchment design process. To assess the sediment flow, it is necessary to continue the bathymetry monitoring of Kisezers, the possibility to perform bathymetry monitoring in all large basin waters should be considered.

The prepared and described methodology can be used for the delineation of the catchment area and provides high-detail results. In order to repeat the study, the high-resolution orthophoto and LiDAR data are required.

The delineation of the catchment area for Lake Kisezers was challenging and time-consuming, as the area has a large population and is subject to anthropogenic influences, which in turn has changed the landscape and it is difficult to construct the catchment area. The methodology can be used in various hydrological, geological, chemical studies where the object of research is a lake or river because without the catchment area it is not possible to characterize water quality and quantity for a lake or river in high quality.

According to the author's observations, it can be concluded that the channels have frequent and rapidly changing water levels, current speed, and direction. In the past, the hydrographic network changed only in natural processes, but after the establishment of the city of Riga, as the city developed and expanded, changes in the hydrographic network were also facilitated by human activities.

Changes in land use in the Kisezers catchment area are mainly determined by the increase in anthropogenic load, which can be explained by the proximity of the Riga the development of the city, and its periphery. Land use analysis allows seeing in detail how rapidly territory changes. Using land cover data it is possible to determine with changes have the most significant impact. For example, if the territory is deforested and construction industrials site is built it is bad for environmental sustainability because on this landmass newer will be forest again. Thous territories from nature become polluting areas.

Factors influencing the catchment area have been analyzed, as one of the most significant are the changes in the water level and the irregularity of the movement of water masses. As well it is important to perform a high level of detailed land use analysis for the determination of potential risks in catchment areas. We need to understand the significance of anthropogenic load to the research territory and plan for solutions for making lake Kisezers catchment area environmentally sustainable.

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Community/shared solar power option: a pathway to sustainable rural electrification in Nigeria

I. Dunmade*

Mount Royal University, Faculty of Science & Technology, Department of Earth & Environmental Sciences, 4825 Mount Royal Gate SW, Calgary, AB T3K 0C3, Canada
*Correspondence: idunmade@mtroyal.ca, israel_dunmade@yahoo.ca

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Abstract. Nigerian governments at all levels have been making concerted effort to extend electricity supply to the rural areas. Among the several efforts by various governments are formulation of rural electrification policies, creation of agencies for the administration of rural electrification projects, installation of diesel power generators, and development of public-private partnership strategies in solving rural power supply problems. While significant progress has been made, several rural communities are yet to have access to electricity supply. Rugged terrain and limited financial resources are among the constraints to extension off-grid to the rural areas. However, electricity supply, being one of the drivers of economic development and social well-being, is a problem that has to be tackled head-on. The current climate change concern and many other environmental issues of our time necessitate finding a sustainable solution to the problem. Consequently the goal of this study was to examine the potentials of community solar power option as a sustainable rural electrification approach in Nigeria. Other goals included identifying potential hick-ups and solutions to lifecycle management of community solar. Moreover, the study was to determine the suitable configuration for efficient and sustainable community solar power management in Nigeria. The study methodology involved intensive literature survey, historical data collection and case studies on rural electrification in Nigeria as well as examples of community solar projects in Nigeria. Preliminary results revealed that community solar power would be a sustainable approach to rural electrification in Nigeria if a number of conditions are satisfied. Some of the conditions to be satisfied include devolving the management of a community solar system to a participative committee of stakeholders representatives, and incorporating community capacity building in the plan. Other conditions are government, corporate and municipalities' collaboration in funding such projects from scratch till after one or two years of operation, and incorporation of monitoring and intervention strategies for continuous power supply and further improvement.

Key words: community solar, lifecycle management, renewable energy, rural electrification, shared solar, sustainable energy.

INTRODUCTION

Rural electrification in Nigeria

Nigeria is a developing country of about 200 million population. According to the world bank's 2018 estimate, 49.66% of the population live in rural areas (indexmundi, 2021). Like many other developing countries, Nigeria is faced with acute shortage of electric energy supply. While the problem is prevalent in most parts of the country, rural communities experience more outages and epileptic power supply. Several sparsely populated rural towns, especially those that are located in rugged remote terrain are not connected to the public power supply system. According to Umana (2018), only 36% of the rural population are connected to the national grid. This erratic power supply situation has caused serious setbacks to socioeconomic development in the rural communities (Akpan et al., 2013; Umana, 2018). Individuals and businesses have attempted solving the problem by turning to fuelwood and petroleum powered electric generators for their energy needs. Utilization of these energy sources have been causing enormous noise pollution and greenhouse gas releases with the attendant environmental and human health problems.

Governmental and Non-governmental efforts on Rural Electrification in Nigeria

Governmental and non-governmental organisations (NGOs) have been grappling with the efforts aimed at solving Nigeria's rural electricity supply problems for several years. These efforts by governments include formulation of rural electrification policies, creation of agencies for the administration of rural electrification initiatives/projects, and development of public-private partnership strategies in solving rural power supply problems. There are also a number of Inter-agency and inter-sectoral collaborations that brought governmental agencies, development partners and the private sector together to foster off-grid energy supply systems' development, promote synergy and prevent duplication of functions. Examples of some recent collaborations include the U.S. Agency for International Development (USAID)'s Nigeria Power Sector Support Program (NPSP), All On, and Nigeria's Rural Electrification Agency on an initiative called Energizing Economies Initiative (EEI). The initiative, according to USAID (2019), is aimed at 'rapid deployment of off-grid electricity solutions to provide access to over 80,000 retail shops, empower 340,000 micro, small and medium sized enterprises, and create over 2,500 jobs while serving over 18 million Nigerians'. All On is a Shell-funded impact investment company that brings together investors and access-to-energy providers to roll out solutions that are scalable and commercially sustainable (All On, 2020). Similarly, it was reported that in 2020, Nigeria's Rural electrification Agency (REA) implemented several projects across the six Nigerian geo-political zones. Among the implemented rural electrification projects are: 218 grid extension projects, 10 solar mini-grid projects, three injection substations projects, and 197 solar street lights (The Nation (2021)). Some of the projects have been completed, while others are at different levels of completion. In 2020, the World Bank and the African Development Bank (AfDB) supported Nigerian government energy development drive with a $350 million grant and $200 million respectively. The goal of the project was to increase electricity access to 105,000 households, 20,000 Nigerian Micro, Small, and Medium Enterprises (MSMES), and 8 federal universities across Nigeria. Others include the deployment of 12 Mini-grids and over 19,000 Solar Home Systems through Nigerian rural electrification fund.

While these are significant/commendable efforts in the right direction by various governments and NGOs, there are several rural communities that are yet to have access to electricity supply. Although difficult/rugged terrain and small population dispersed over large areas that make electric transmission investment uneconomic are genuine reasons for poor electric energy supply to the rural areas, it is essential to find cheap and sustainable energy solutions that enable all and sundry to have access to stable and affordable electric energy supply. This is because electric energy is needed for smooth and effective operation of many of our socioeconomic activities such as our healthcare facilities, economic activities, and recreational facilities. Umana (2018) buttressed this point by stating that 'Sufficient energy supply is indispensable for sustainable economic development of Nigeria and any nation'. The current climate change concern and many other environmental issues of our time necessitate finding sustainable and affordable solutions to the problem. All sustainable energy options should be considered to address the dire need. Seeing that community/shared solar has not been reported in any literature as one of the options being considered for rural electrification in Nigeria, it is the goal of this study to bridge the research gap. The contribution of this research is in the evaluation of the characteristics of community solar, effectiveness of its use, and its potential utilization as a sustainable rural energy source for Nigeria.

MATERIALS AND METHODS

The study methodology involved an intensive literature survey and case studies on rural electrification in Nigeria. More than 50 Scholarly journals, government and non-governmental websites, and news media were searched for statistical data, history and trends. Approaches to rural electrification in Nigeria and their outcomes were also examined. There was also a literature survey on community solar, shared solar and their use for rural electrification in various countries. The criteria adopted in determining which article to include or exclude are fitness into the searched terms, and publication within the period of 1990–2021. The searched terms are 'rural electrification in Nigeria', 'rural electrification agency', 'community solar', and 'shared solar'. In addition, there was an assessment of potential use of community solar in Nigeria and their potentials for successful implementation. Three common community solar adoption models were examined. Collaborative lifecycle management based stakeholders operated community solar system is proposed as a sustainable community solar model for Nigeria. The proposal was based on personal experiences growing up in a rural community of Kwara State, Nigeria; previous research experience, and data gathered from the literature reviewed.

This paper was arranged as follows: The next session details the results and discussion on the findings regarding the trends in rural electrification in Nigeria. This is followed by the proposed sustainable community solar energy model for rural communities in Nigeria. Potential challenges to community solar implementation in Nigeria as well as possible solutions were then discussed before making conclusions and recommendations on the way forward to achieving sustainable energy supply for rural communities in Nigeria.

RESULTS AND DISCUSSION

Features and Characteristics of Community Solar

Community solar, according to the Solar Energy Industries Association (SEIA (2021)), refers to 'local solar facilities shared by multiple community subscribers who receive credit on their electricity bills for their share of the power produced'. Putting it in a simpler way, Energysage (2020) described a community solar project as 'a solar power plant whose electricity is shared by more than one property'. Community solar is known as community shared solar or simply shared solar. The main objective of community solar is to enable members enjoy the opportunity of sharing in the benefits of electricity generated from solar, even when due to one reason or another they could not install solar panels on their own property. One advantage of community solar is that the electricity generated is cheaper than the one from the utility company. Community solar energy supply systems are increasingly being utilized all over the world. For example, there have been reported cases of community solar use in Australia (Hansen et al., 2020); Hungary (Deutsch & Berényi, 2020); South Korea (Yang et al., 2021), USA (Michaud, 2020), UK and other European Countries (Peters et al., 2018). Adoption of community solar as an alternative energy source has been growing over the years, especially in rural America. According to SEIA, 2,579 megawatts of community solar have been installed in the U.S. through 2020. To illustrate the feasibility of community shared solar in meeting energy needs, SEIA projected that in the next five years the U.S. community solar market will add up to 3.4 GW. That energy is enough to power about 650,000 homes. According to Klein et al. (2021), 'Community solar farms (CSF) have the potential to expand solar access and improve financial viability compared to traditional residential and commercial solar options.' There has been some discussions regarding the merits and demerits of community solar. The main issues against community solar involve finding appropriate sized sunshine receptive land areas for the solar farm to be installed (Daware, 2021). This is not expected to be an issue for Nigerian rural communities where there are low-cost expanse of underutilized lands that could be used for the project (Global Solar Atlas, 2021). Another issue is the cost.

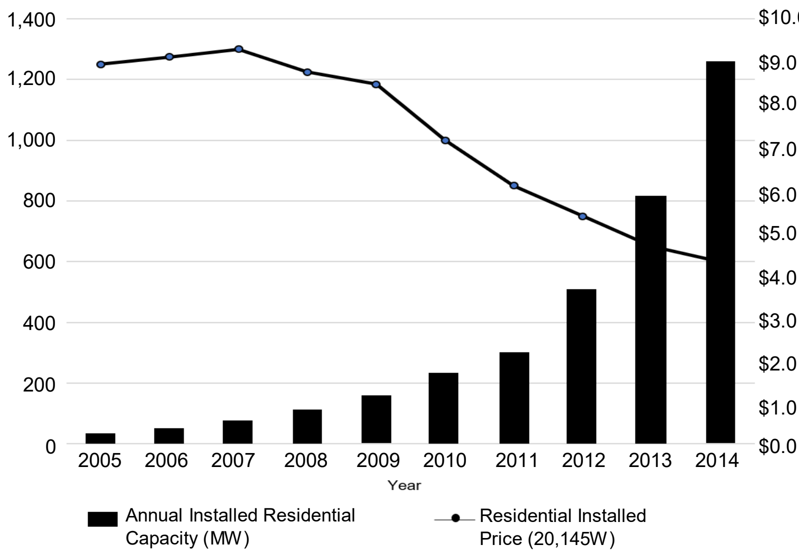

Figure 1. US annual residential installed capacity and price changes from 2005 and 2014. [Source: Augustine & McGavisk, 2016].

The cost of solar systems have been decreasing since 2007. Fig. 1 shows the US annual residential solar installed capacity and price changes from 2005 and 2014. One could see that the price has dropped by more than 50% over the period. That is the general trend all over the world. Another crucial consideration is in selecting a suitable management approach for the effective operation and maintenance of the community solar system. This would not be a big challenge with appropriate organization and supportive governmental policies (Augustine & McGavisk, 2016; Dunmade, 2016; Theophilus, 2017; Hansen et al., 2020; CCSA, 2021).

Potentials of community and shared solar power as a sustainable rural electrification approach in Nigeria

Nigeria, is endowed with abundant renewable energy resources in the form of solar radiation, biomass, wind and water resources that could be harnessed for small hydro energy supply. For example, Nigeria enjoys an estimated 8 hours/day of intense sunshine all year round. Fig. 2 shows the Nigerian solar irradiation and PV power potential map. In addition, Iwayemi (2008) put the estimate of Nigeria's hydro resources, solar power potential and wind energy potential at 14,750 MW, 7.0 KW m^{-2} per day, and 150,000 TJ yr^{-1} and 144 million tonnes per year respectively. Simonyan & Fasina (2013) also estimated the Nigerian biomass resources from residues and wastes at 47.97 Million tonnes of oil equivalent (MTOE), that is about 2008 PJ energy. These renewable energy resources can be harnessed to solve the perennial epileptic energy supply problems. Transitioning to the use of renewable energy sources from fossil energy is important not only because that is a global trend now but also because of its numerous benefits and applications (Pikk & Annuk, 2014; Papez & Papezova, 2016; Vinnal et al., 2020). As earlier highlighted, governments and other agencies have been making efforts at harnessing them to solve rural electricity supply problems. However, there has not been any reported utilisation of community and shared solar as one of the options being used for rural electrification in Nigeria.

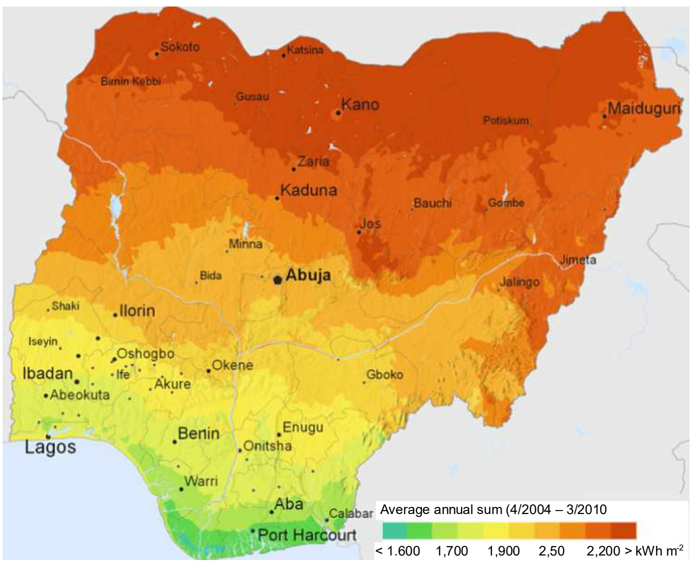

Figure 2. Nigerian solar irradiation and PV power potential map.
Source: https://globalsolaratlas.info/global-pv-potential-study

A valid concern regarding introduction of a new technology, a new program or initiative is whether the desired goal(s) will be achieved. There may also be the question regarding the sustainability of such endeavours.Reports (Sunmetrix, 2013; Energysage, 2020; YSG Solar, 2020; Elevateenergy, 2021; Etelson, 2021; Gagne & Aznar, 2021; Global Solar Atlas, 2021) have shown the usability of community solars for electric power supply all over the world wherever there is unobstructed sunshine over some hours of the day. There is also reported evidence of community solar's installations in remote locations and rugged terrains. Utilization of community solar is environmentally sustainable as it is a renewable energy source with lower ecological footprint. Community solar is also economically sustainable as it is scalable to the capability of the subscribers. In addition, it is a feasible and far cheaper option than the connection of remote and sparsely populated small villages to the national grid (Radl et al., 2020). Similarly, it is socio-culturally inclusive as community solar provides opportunity for all citizens of various socioeconomic status to participate in the transition from the use of environmentally harmful energy sources to a renewable one. It is also affordable as individuals can choose the extent they wish to be invested in the project and they can increase their involvement over time.

Approaches to community/shared solar development and management organisation

Community solar as an electricity supply option would really impact all aspects of life of rural communities in Nigeria in a positive manner. There are several possible benefits of its incorporation into the Nigerian rural electrification equation. Among the many benefits of community solar is that, in view of its smaller capital outlay requirement, it is a viable alternative to the epileptic and expensive Mega-Scale electric energy distribution system (Radl et al., 2020). It is also not only renewable but also scalable to the needs of the community being served. With its location in the tropical region, almost all areas of Nigeria receives abundant sunlight all year round. This provides a great potential for sufficient power generation to meet the needs of the population. Furthermore, community solar is amenable to local involvement in the planning, development and management operations of the system. This is an added advantage for its sociocultural sustainability.

Development and management of community solar systems are often structured on the bases of financial and organizational expediency. There are three common community solar business models, namely: utility-owned community solar, developer-owned community solar, and special purpose entity-owned community solar. Utility-owned community solar was reported to be the most common of the three. According to Elevateenergy (2021), utility-owned community solar model is a situation where the solar array is owned by the local utility. The utility then sells or leases the solar panels to subscribers or sells a certain amount of solar electricity at a fixed price for a specified time period. Participation in the community solar project involves purchasing a share of the project while receiving credits on the electricity bills. In the developer owned community solar model, a solar developer is responsible for the entirety of everything about the solar system right from the design to the operation and maintenance of the system. However, the developer enlists the services of a subscriber management organisation who helps him/her in getting subscribers and managing them. The third approach is the special purpose entity model where the community solar is owned by a business entity. The business entity could be a for-profit or non-profit organisation. The advantage of this third model is that the benefits of the community solar project are retained in the community (Etelson, 2021; Gagne & Aznar, 2021). Fig. 3 is an illustration of the three common types of community solar.

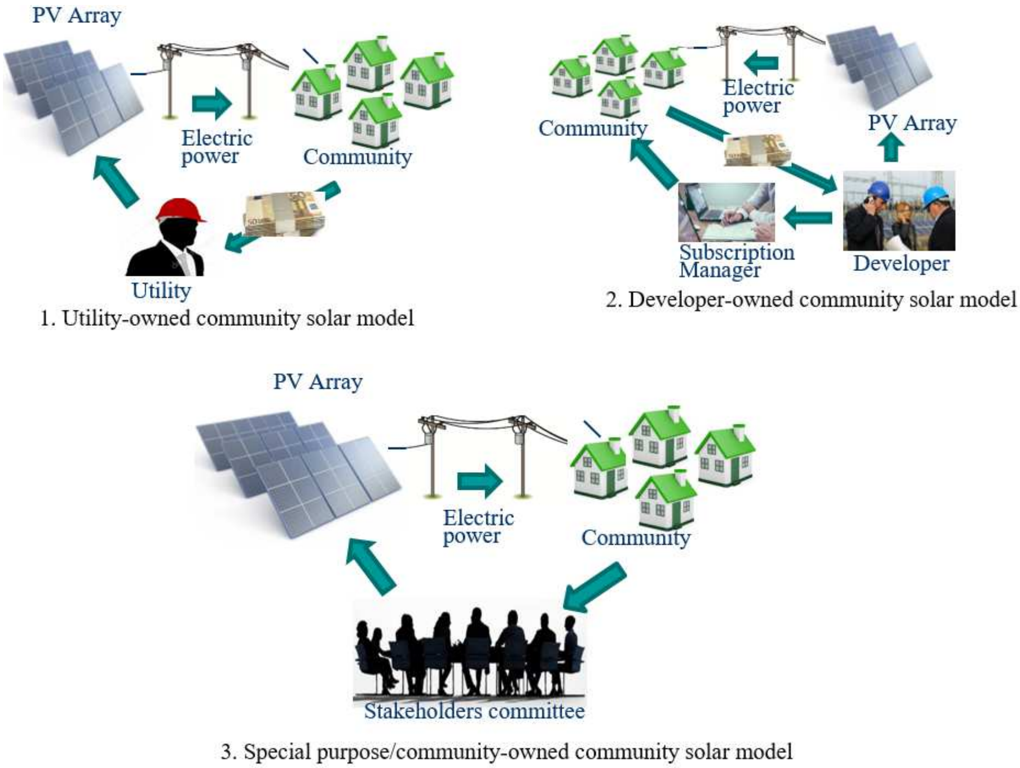

Figure 3. The three common types of community solar.

The development and management of a sustainable community scale solar energy supply system require supportive regulatory policies. According to Wiseman & Bronin (2013), supportive policies for a sustainable community scale solar energy supply system would need to include 'land use planning, acquisition, and installation of renewable equipment. It would also need to cover maintenance and operation of the equipment, and the sale of energy (Furthermore, Wiseman & Bronin (2013) stated that communities must be able to form business enterprises that govern the purchase, installation, operation, and maintenance of generation infrastructure and that manage the sale of energy produced.'

Suitable configuration for efficient and sustainable community solar power management in Nigeria

Our study, based on literature reviewed and personal experience of Nigeria, revealed that community solar power would be a sustainable approach to rural electrification in Nigeria if it is appropriately configured for efficiency. A sustainable approach to community solar deployment in Nigeria would require devolving the lifecycle management of the community solar system to a participative/collaborative committee that involves all stakeholder groups' representatives right from the planning stage throughout the entire lifecycle of the system (Chan et al., 2017; Hansen et al., 2020; Hess & Lee, 2020; Michaud, 2020). Fig. 4 is a schematic diagram of the proposed community solar system while Fig. 5 is an illustration of the process that could be used to achieve a suitable configuration for an efficient and sustainable community solar power management system. It emphasizes participation of all the stakeholders throughout the life of the project. It also recommended assessment of needs, evaluation of local community situation, development of supportive policy, sustainable design and manufacturing of the power supply system, and evaluation of the system for suitability determining factors (Palm, 2016; Michaud, 2020). The adoptable plan also included community capacity building for effective transfer of the technology to the locality. Such capacity building would need to cover areas of operation, repairs, component fabrication and management of the system. These would essentially require the development of programs for the training of various levels of manpower that would be needed to locally design, manufacture, operate, maintain, upgrade and manage the system in environmentally-, socially- and economically sustainable manner.

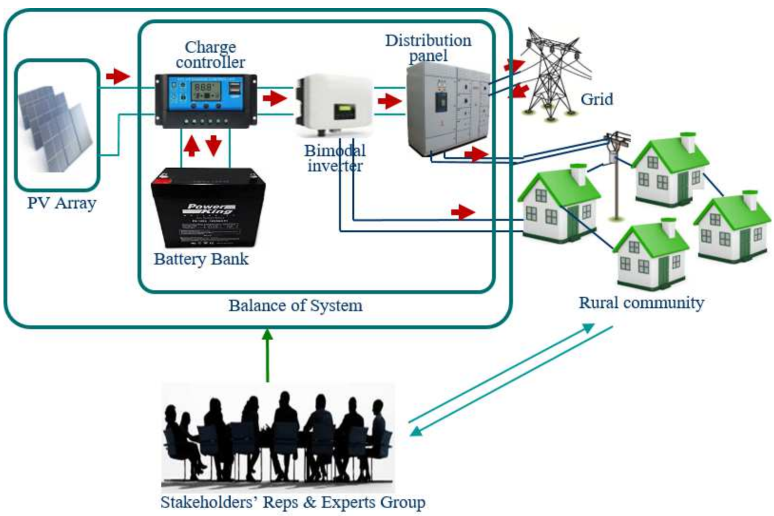

Figure 4. A schematic diagram of the proposed the community solar system.

Other conditions for effective and sustainable community solar projects are government, corporate and municipalities collaboration in funding some demonstrative community solar power supply projects, and incorporation of monitoring and intervention strategies for continuous power supply and further improvement (Wiseman & Bronin, 2013; Awad & Gül, 2018; Salihu et al., 2020). Approaching the introduction of community solar as a rural electrification option will enhance availability of electric power for rural development and improve the standard of living for rural communities in Nigeria. It will drastically reduce losses of fruits, vegetables and many other agricultural products from the villages that get spoiled due to lack of modern processing and storage facilities that require electric power. Introduction of community solar as an electricity supply option for rural Nigeria would not only positively impact their agriculture, the commerce sector will also benefit by making it possible for them to use electricity powered machines to process their agriculture products to various value-added commodities. This would lead to improved earnings and thereby reduce poverty and improve their standard of living. In addition, community solar would enable students to comfortably read into the night and to have access to new learning tools thereby improve their education. Improvement in their education will give them access to new opportunities in the future and facilitate breaking the cycle of poverty. Furthermore, the introduction of community solar would facilitate cleaner air in the homes as electric light will eliminate the use of kerosene lamps that contribute to air pollution and negatively impact human health. Similarly, rural healthcare facilities would be able to use electric powered medical devices and enable them to attend to night emergency services more comfortably. They would also be able to store certain medicines that require electricity dependent special care (Independent Evaluation Group, 2008; Torero, 2015; Syed et al., 2020; Alliance for Rural Electrification, 2021).

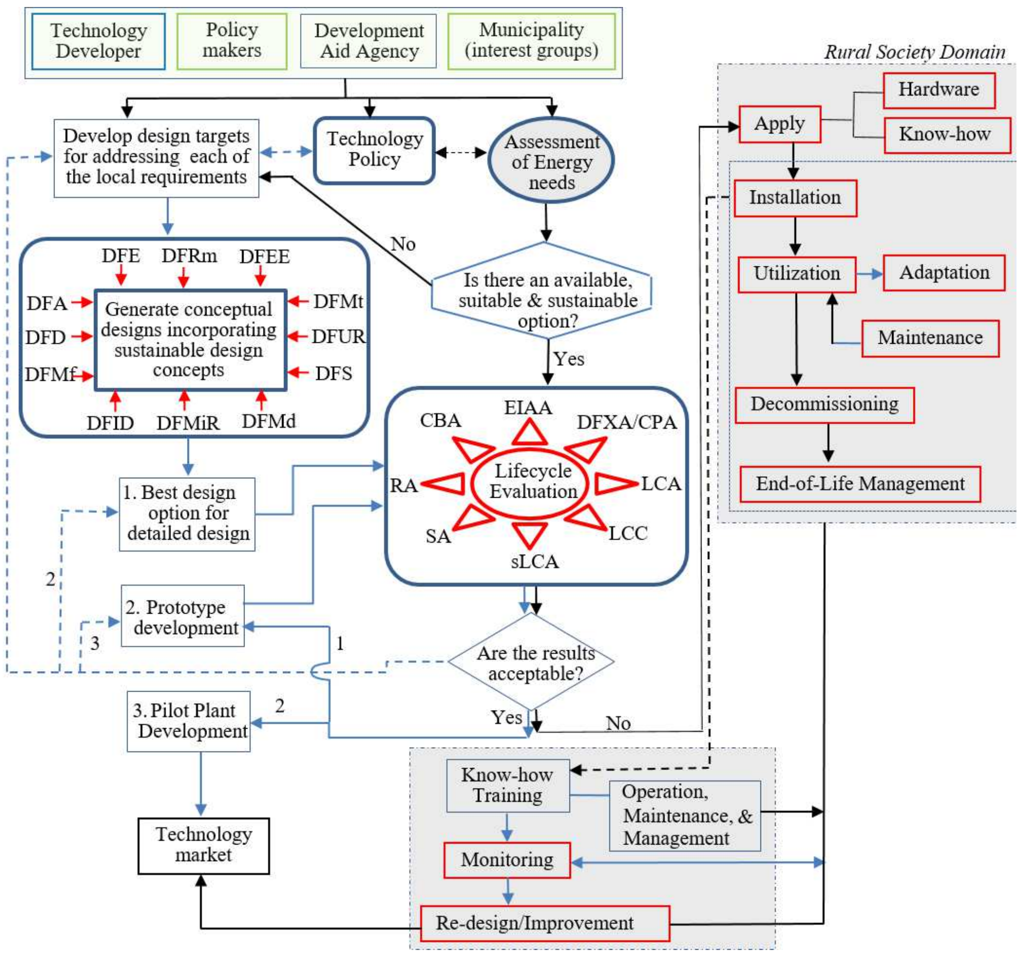

Figure 5. A framework for the evolution of suitable configuration for efficient and sustainable community solar power development and management in Nigeria.

Comparison of community solar adoption in some countries and the economic impacts

Community solar is most widely adopted in USA as an alternative means for rural electrification, especially in sparsely populated regions and areas with rugged terrain (Augustine & McGavisk, 2016). According to Guidehouse (2021), the total installed capacity of community solar programs in 2018 was '734 MW, with approximately 387 MW installed in 2017'. Adoption of community solar is widespread across 36 states in USA. However, community solar utilization in Europe is limited, despite its over 20 years of electricity liberalization. Although there is an existing European regulatory framework for its adoption, actual community solar projects are only recently launched in some European localities like London. Deutsch & Berényi (2020) also did a case study on the economic potentials of community solar models in Hungary. They reported that community financed model is the best of the three community solar models that are commonly used in the country. Furthermore, although provinces like Alberta, Ontario and Saskatchewan have been making significant progress in developing the solar market, adoption of community solar in Canada is not widespread (Oyasolar, 2020). Majority of the successful adoption of community solar is recorded in the USA.

There are several economic and environmental benefits attributed to the development and adoption of community solar across various jurisdictions. These include reduction in the electricity bill of the subscribers and creation of new employment opportunities (Alliance for Rural Electrification, 2021). For example, Oyasolar (2020) reported that since 2006, community solar programs has led to 52% growth in solar market and creation of over 260,000 jobs across the US. This makes it a major contributor to the economy. Oyasolar (2020) also reported that adoption of community solar have helped in reducing ecological footprint of communities and is facilitating the achievement of the climate goals.

Potential hick-ups in the successful deployment of community solar in Nigeria and suggested solutions

The first anticipated challenge involves selling the ideas to investors and appropriately educating the public regarding the suitability of the system for their community. Another challenge is in addressing the political and economic barriers in the region. Yet another possible hick-up in the sustainable deployment and operation of community solar electric power supply systems to rural communities in Nigeria is in ensuring effective transfer of the technology to the local technicians that would have to repair and maintain the system (Augustine & McGavisk, 2016; Peters et al., 2018).

Governments and intergovernmental agencies, like they are doing in other infrastructure projects, can help in preventing the aforementioned- and other problems by sponsoring public education programs and bankrolling the first set of demonstrative projects all across the country. Government can also encourage citizens and corporate organisations to participate in the project by developing supportive regulations and providing incentives. The incentives could include subsidies and tax rebates. Government can also provide scholarships for the training of manpower needed for local proliferation, adaptation, and maintenance of the technology.

CONCLUSIONS

This paper presented results of an assessment regarding the potentialities of adopting community solar as a sustainable electric power supply option for rural communities in Nigeria. Implementation of the proposed framework will facilitate the development and operation of an r efficient and sustainable community solar power supply. The deployment of community solar power supply systems to rural communities in is expected to result in significant improvement in rural economy, education, healthcare, supply of goods and services and improved standard of living for the rural population in. Adoption of the proposed framework is also expected to result in the smooth running of community solar power systems in Nigeria. Other developing nations in the same situation would be able to adopt the same approach to tackling the problem of rural electrification in their region. Consequently, taken such steps would lead to reduction in poverty, progression towards zero hunger, improvement in health and wellbeing, and facilitate the achievement of several sustainable development goals in rural communities.

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Some features of cultivating different *Chamaenerion angustifolium* (L.) Scop. forms *in vitro*

D.A Egorova[1], O.I. Molkanova[1], Yu.N. Gorbunov[1], A.A. Gulevich[2] and E.N. Baranova[1,2,*]

[1]N.V. Tsitsin Main Botanical Garden of Russian Academy of Sciences, Botanicheskaya street 4, RU127276 Moscow, Russia
[2]All–Russian Scientific Research Institute of Agricultural Biotechnology, Timiryazevskaya street 42, RU127550 Moscow, Russia
[*]Correspondence: greenpro2007@rambler.ru

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Abstract. *Chamaenerion angustifolium* (L.) Scop. characterized by a wide range of economically useful properties. White-flowered form of *Ch. angustifolium* (L.) Scop. Is extremely rare in nature. At the same time, it is promising as a source of biologically active substances and as a highly decorative plant. The optimal way to reproduce this form is clonal micropropagation. Methods for obtaining *Ch. angustifolium in vitro* were developed, as well as the optimal selection timing of starting material for micropropagation was determined. In addition, the effect of a mineral composition of nutrient medium and plant growth regulators on the regeneration of microshoots was studied. The highest values of morphometric parameters were achieved on MS medium (Murashige & Skoog, 1962) supplemented with 0.5 mg L^{-1} BAP. The multiplication factor of the lilac–flowered form was 8.4 ± 0.2, of the white-flowered form - 9.2 ± 0.6. Comparative analysis of morphometric parameters during cultivation of *Ch. angustifolium* showed no significant difference between the lilac-flowered and white-flowered forms. The effect of antioxidants on the growth and development of regenerants has been shown. The most optimal nutrient medium for clonal micropropagation of the lilac-flowered form was MS medium (Murashige & Skoog, 1962) containing 0.5 mg L^{-1} of BAP, 50 mg L^{-1} of ascorbic acid and 50 mg L^{-1} of citric acids and for micropropagation of the white–flowered form it was the medium, containing 100 mg L^{-1} PVP.

Key words: clonal micropropagation, phenolic exudation, plant growth regulators, fireweed.

INTRODUCTION

Fireweed or *Chamaenerion angustifolium* (L.) Holub is a long-rhizome perennial. The stem of this herb reaches a height of 2 m. Leaves are alternate, narrow-lanceolate, pointed, whole-edged or with indistinct denticles. Inflorescence is a long raceme. The flowers are large, four-petal, pink or purple. The area covers a large territory of the Northern Hemisphere. In Europe, the fireweed is distributed throughout the all forest zone (Adamczak et al., 2019).

D.P. Syreishchikov (2020), in addition to the widespread, typical narrow-leaved, lilac-flowered form, noted the presence of two rare forms (modifications) of fireweed: f. *macrophyllum* Hausskn (large-leaved, characterized by oval leaves) and f. *albiflorum* Hausskn (white-flowered). In a garden a rare white coloured form is used for creating a solitaire plant, background plantings, flower beds as an alternative to delphinium and stock–rose. Traditionally *Chamaenerion angustifolium* (L.) Holub cultivar 'Album' (*Epilobium angustifolium* cv. 'Album') is cultivated (Mayer et al., 2017).

Various organs of fireweed contain a whole complex of chemical compounds: essential oil, tannins, rich elemental composition, carbohydrates (starch, sugars, pectin), flavonoids, vitamin C, amino acids and other substances. The rich chemical composition determines the broad biological activity of the plant. *Ch. angustifolium* has antioxidant, vasoconstrictor, anti-inflammatory, analgesic, antipyretic, antimicrobial and antiviral effects (Kosalec et al., 2013; Frolova et al., 2014; Shrivastava et al., 2014; Tsarev et al., 2016).

Ch. angustifolium is also used in traditional and folk medicine for a variety of diseases (for headaches, stomach ulcers, gastritis and colitis, as a hypnotic and sedative, etc.). It is a very promising medicinal plant that can be used in official medicine (Kosalec et al., 2013; Tsarev et al., 2016; Kadam et al., 2018). In recent decades, special attention has been paid to the antitumor effect of fireweed preparations (Maruška et al., 2017; Gryszczyńska et al., 2018). The white-flowered form of fireweed is especially promising as a raw material for the production of these drugs. The absence of pigments in flowers can facilitate the release of pharmaceutical substances for the drug, most of which are represented by phenolic compounds. This form can be in demand both as a valuable pharmaceutical raw material and as an exclusive variation of ornamental garden culture. The uniqueness of this form necessitates its introduction *in vitro*.

Fireweed has also a nutritional value. In Northern Europe, the herbal drink obtained by brewing fermented leaves of *Ch. angustifolium* was known for a long time, and was popular with the general population (Svanberg, 2012). In recent years, it has regained its former popularity as herbal tea (Kalle et al., 2020). Fireweed rhizomes were using as a vegetable, they are suitable for baking bread. Root sprouts and young stems were eaten like asparagus and cabbage (Adamczak et al., 2019). The leaves are suitable for preparing salads and as a seasoning for meat dishes and broths (Svanberg, 2012; Kalle et al., 2020). Rhizomes and young shoots are promising for use in food fresh and pickled. Fireweed shoots in terms of protein content are close to high-protein legumes, and the protein composition indicates its high quality (Tsarev et al., 2016).

Fireweed is one of the best honey plants among wild plants. Nectar production from 1 ha of its thickets reaches 500–600 kg, and in terms of sugar - 250–300 kg. Fireweed honey has a pleasant odor, delicate taste, greenish colour, and it is very sweet (Adamczak et al., 2019).

It can be stated that willow herb is a very promising plant with great potential for integrated use. Hence, it becomes necessary to introduce it into a wider culture. The proposed technologies of seed and vegetative propagation (by rhizomes and green cuttings) are economically unprofitable. They do not allow obtaining a large number of plants in a short period. Also *Ch. angustifolium* is characterized by interspecific hybridization, which complicates the production of genetically homogeneous material (Molkanova et al., 2018). The most promising method for obtaining a large number of fireweed planting material is clonal micropropagation. Clonal micropropagation of plants is a tissue culture method that ensures the preservation and multiplication of a large number of cultivars of valuable decorative and medicinal plant species, widely used for commercial propagation (Rout et al., 2000). An important advantage of this method is the ability to carry out breeding work throughout the year.

MATERIALS AND METHODS

The research was carried out at the Plant Biotechnology Laboratory at the N.V. Tsitsin Main Botanical Garden of the Russian Academy of Sciences. The studied plants were provided by the Department of Cultivated Plants (Fig. 1) (Egorova et al., 2016).

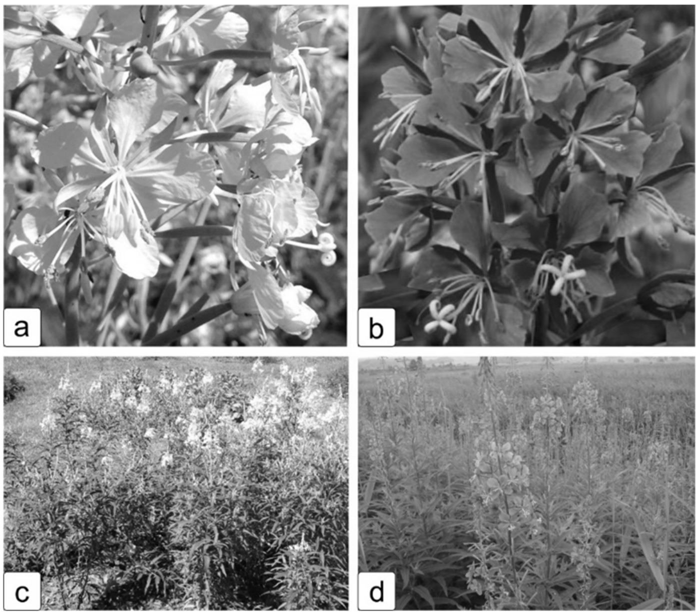

Figure 1. The source material of two varieties of purple and white fireweed (*Ch. angustifolium*).

Cultivation of plants *in vitro* was carried out according to generally accepted biotechnological methods and techniques developed in our laboratory (Egorova et al., 2016; Mitrofanova et al., 2018.; Molkanova et al., 2018). At the initiation stage, axillary meristems located on different parts of the shoot at different times of the vegetation phase were used as explants. Explants were kept in a 2–4% solution of a systemic fungicide with difenoconazole, then they were immersed in 70% ethyl alcohol for 1–2 min and transferred to 7% calcium hypochlorite solution and stirred for 5–10 min.

MS (Murashige & Skoog, 1962) and QL (Quoirin & Lepoivre, 1977) nutrient media with addition of 0.1–1.5 mg L^{-1} 6-benzylaminopurine (BAP) were used at the stage of micropropagation. Media were adjusted to a pH 5.7–5.8 with 0.1 N KOH and autoclaved at 120 °C for 20 min. Citric and ascorbic acid in a concentration of 50 and 100 mg L^{-1}, a combination of ascorbic and citric acids (in a concentration of 25 and 50 mg L^{-1}), as well as PVP (polyvinylpyrrolidone) in a concentration of 50 and 100 mg L^{-1} were added to reduce phenolic exudation. Cultures were placed in the growth chamber at 25 ± 2 °C and 16 h photoperiod under cool–white fluorescent lights. Plantlets were subcultured after

30–40 days. Multiplication factor (mean of new axillary shoots produced per microshoot) and mean length of shoots were recorded after 30 days of culture. Explants were also visually evaluated for leaf necrosis, hyperhydricity and chlorosis. The experiments were carried out in threefold replicate, 10 explants in each variant. The length of microshoots was measured and multiplication factor was calculated at the propagation stage. Results are presented as means ± standard error. Statistical analyses were done using ANOVA followed by the Tukey's Honestly Significant Difference test. Statistical significance was defined as $P \leq 0.05$ and marked with different letters in superscript: a, b, c, d. Statistical data were processed in the data analysis software packages - PAST (PAleontological STatistics).

RESULTS AND DISCUSSION

In the process of studying the effect of different explant sterilization timing on the viability of developing microshoots of various fireweed forms it was found that the highest indicator of shoot viability (70–90%) was typical for plants in the period from April to early June. When studying the effect of various exposures of calcium hypochlorite on the process of axillar meristems disinfection, it was found that exposure of 5–6 min was not enough to obtain aseptic fireweed explants. The highest yield of aseptic explants of the white-flowered form (93.3 ± 2.0%) and the lilac–flowered form of *Ch.angustifolium* (86.36 ± 9.9%) was observed at an exposure of 7 min. Increased exposure resulted in decreased contamination, but increased the number of non-viable explants (Fig. 2).

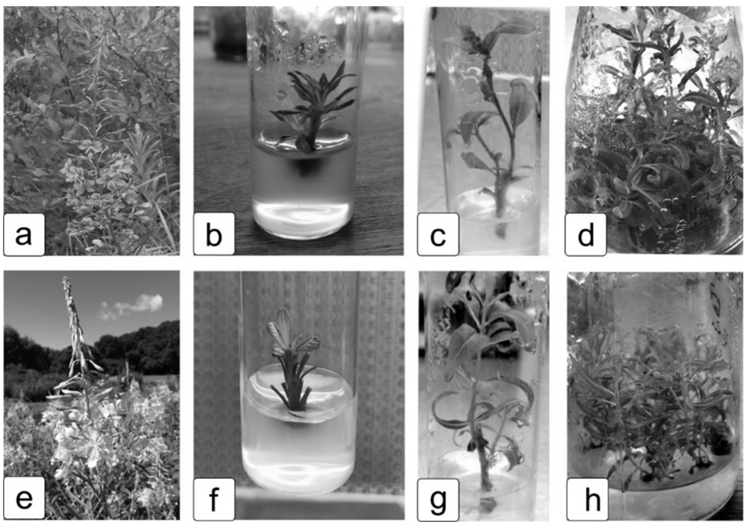

Figure 2. Micropropagation of lilac- and white-flowered forms of *Ch. angustifolium*; a – parent plant of fireweed, lilac form; b – phenolic exudation of lilac form at the initiation stage; c – the beginning growth of axillar buds of lilac form; d – the multiplication of lilac form on MS medium supplemented with 0.5 mg L^{-1} BAP, 50 mg L^{-1} ascorbic acid, and 50 mg L^{-1} citric acid; e – parent plant of white form of *Ch. angustifolium*; f – the beginning growth of axillar shoot of white form; g – the beginning growth of axillar buds of lilac form; h – the multiplication of white form on MS medium supplemented with 0.5 mg L^{-1} BAP and 100 mg L^{-1} PVP.

One of the main factors influencing the processes of morphogenesis and the intensity of micropropagation is the mineral content of nutrient medium (Dreger & Wielgus, 2015). Initially, nutrient media differing in mineral composition (MS and QL) have been compared (Table 1).

Table 1. Influence of nutrient medium on microshoots length and multiplication factor of different *Ch. angustifolium* forms

Ch.angustifolium form	Nutrient medium	Microshoot length, mm	Multiplication factor
Albiflorous form	MS	39.6 ± 5.5^a	4.1 ± 0.6^a
	QL	15.6 ± 4.3^b	2.4 ± 0.5^b
Lilac form	MS	38.6 ± 4.2^a	4.0 ± 0.4^a
	QL	14.2 ± 3.3^b	2.4 ± 0.5^b

*Values represent mean + standard error. Means followed by the same letter within the column do not differ significantly ($P \leq 0.05$) according to a Tukey's Honestly Significant Difference test.

The MS medium turned out to be the supreme better result in all respects. An active plant growth was observed and new microshoots were formed on this medium. The multiplication factor of lilac-flowered and white-flowered forms was 4.0 ± 0.4 and 4.1 ± 0.6, respectively. Plant growth was retarded on QL medium; sometimes plants were dying immediately after passage. Also, a plant chlorosis was observed 10–15 days after transplantation on this medium. It should be noted that a chlorosis was also observed in the case of long-term cultivation (more than 40 days) on MS medium.

Plant growth regulators have a significant effect on various morphophysiological processes in plants, including growth and development. The proper choice and optimal ratios of growth regulators (cytokinins and auxins) are essential for the *in vitro* enhanced growth of microshoots (Trettel et al., 2020). In the course of the study, the most optimal concentrations of exogenous cytokinin for enhanced growth induction of fireweed microshoots were identified (Fig. 3–4).

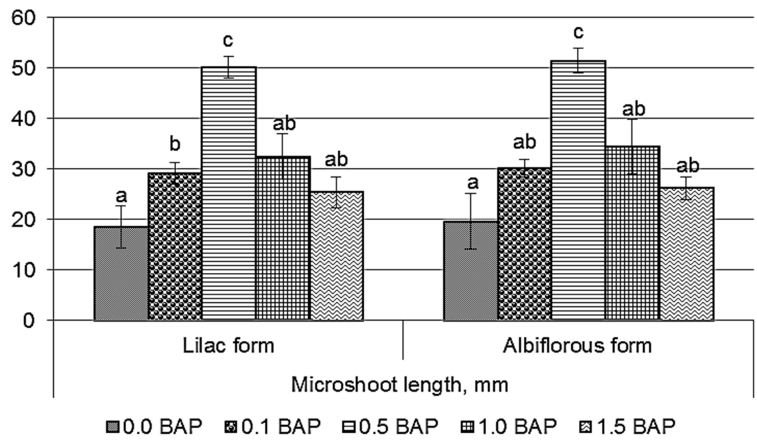

Figure 3. Influence of different BAP concentrations on *Ch. angustifolium* microshoots length.

*Values represent mean + standard error. Means followed by the same letter within the column do not differ significantly ($P \leq 0.05$) according to a Tukey's Honestly Significant Difference test.

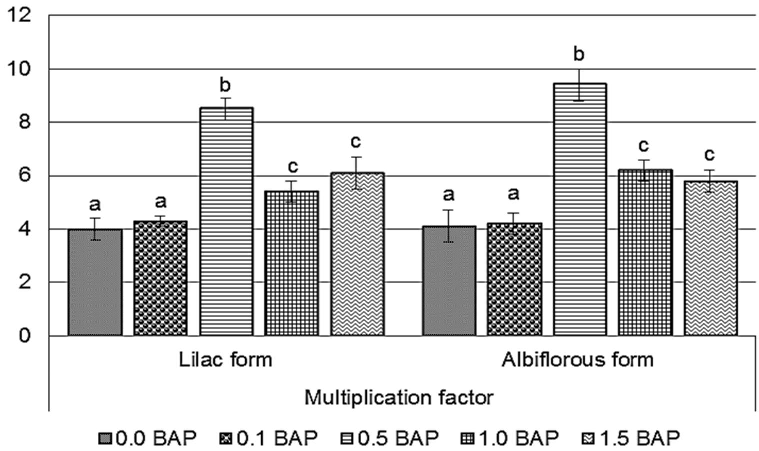

Figure 4. Influence of different BAP concentrations on *Ch. angustifolium* multiplication factor.
*Values represent mean + standard error. Means followed by the same letter within the column do not differ significantly ($P \leq 0.05$) according to a Tukey's Honestly Significant Difference test.

BAP concentrations from 0.1 to 0.5 mg L^{-1} was found to provide an increase in the multiplication factor. But a higher concentration caused tissue hydration, overgrowth of callus and a decrease in the microshoots proliferation. Under the influence of 0.5 mg L^{-1} BAP, the length of microshoots of white-flowered and lilac-flowered forms reached the maximum values: 50.2 ± 2.2 mm and 51.5 ± 2.5 mm, respectively. The multiplication factor also reached its maximum: 8.4 ± 0.2 in white–flowered form and 9.2 ± 0.6 in lilac-flowered form. Comparative analysis of morphometric parameters during micropropagation of *Ch. angustifolium* showed no significant difference between the lilac-flowered and white-flowered forms. Thus, the optimal medium for the cultivation of fireweed turned out to be a medium supplemented with 0.5 mg L^{-1} BAP, which showed the highest morphometric parameters.

Comparative analysis of the obtained data showed that nutrient media of different composition are required for two forms of *Ch. angustifolium* in order to increase their morphogenic potential (Table 2).

Table 2. The effect of antioxidants *on Ch. angustifolium* morphometric parameters

Antioxidants	Concentration of antioxidants, mg L^{-1}	Microshoot length, mm		Multiplication factor	
		Lilac form	Albiflorous form	Lilac form	Albiflorous form
–	–	8.5 ± 0.7^{a}	6.2 ± 0.6^{a}	2.1 ± 0.3^{a}	1.2 ± 0.4^{d}
Ascorbic acid	50	13.1 ± 1.1^{b}	23.9 ± 0.8^{d}	3.4 ± 0.3^{b}	4.0 ± 0.4^{b}
	100	18.0 ± 1.4^{c}	24.0 ± 1.1^{d}	4.0 ± 0.4^{b}	4.1 ± 0.4^{b}
Citric acid	50	14.1 ± 1.4^{b}	10.1 ± 1.6^{b}	3.1 ± 0.4^{b}	2.3 ± 0.3^{a}
	100	12.5 ± 0.8^{b}	12.1 ± 1.1^{b}	3.4 ± 0.3^{b}	2.5 ± 0.4^{a}
Ascorbic acid + citric acid	50	16.9 ± 1.1^{c}	23.2 ± 1.4^{d}	4.0 ± 0.3^{b}	4.0 ± 0.3^{b}
	100	23.3 ± 1.1^{d}	18.0 ± 1.1^{c}	5.2 ± 0.3^{c}	4.2 ± 0.4^{b}
PVP	50	14.6 ± 1.6^{b}	24.5 ± 1.4^{d}	3.7 ± 0.4^{b}	4.5 ± 0.3^{b}
	100	12.5 ± 1.7^{b}	28.4 ± 0.8^{e}	3.2 ± 0.3^{b}	5.5 ± 0.4^{c}

*Values represent mean + standard error. Means followed by the same letter within the column do not differ significantly ($P \leq 0.05$) according to a Tukey's Honestly Significant Difference test.

Different concentrations of various antioxidants showed to be effective in growth stimulation of microshoots. So, the maximum length (23.3 ± 1.1 mm) of the lilac-flowered form was observed on a medium containing 50 mg L^{-1} of ascorbic and citric acids, and the one of the white-flowered form on a medium containing 100 mg L^{-1} PVP (23.2 ± 1.4 mm). The smallest value of the length (6.2 ± 0.6 mm and 8.5 ± 0.7 mm) was observed in the control variant (without antioxidants), which was characterized by partial death of explants due to high phenolic exudation, as well as a slowdown in the growth and development of microshoots.

It was found that all the studied antioxidants and sorbents contributed to an increase in the multiplication factor. In the lilac-flowered form, this indicator significantly exceeded the values obtained using other concentrations and amounted to 5.2 ± 0.3 on a medium with the addition of ascorbic acid and citric acid (50 mg L^{-1} each). The highest multiplication factor of the white-flowered form of *Ch. angustifolium* was observed on culture medium containing 100 mg L^{-1} PVP (5.5 ± 0.4). In other variants, the multiplication factor varied slightly.

The induction of morphogenesis and the ways of the morphogenetic potential expression in fireweed, like other plants, depend on the genotype, initial explant, composition of the nutrient medium and cultivation conditions (Dreger et al., 2020). One of the most crucial stages in plants introduction *in vitro* is a selection of the explant isolation timing. Different explants were used for *in vitro* reproduction of fireweed. In study of Turker et al. (2008) was shown that root explants produced more shoots than other explants. In Dreger's work (2016), the best shoot regeneration was obtained from stem fragments (96%) and root explants (60%), which formed many shoots. In our study, only axillary buds at stem fragments regeneration were used, and the percentage of regeneration was 98%. Thus, propagation of fireweed plants by means of axillary buds gives a better result in comparison with other explants.

The browning of tissues of the primary explants and nutrient media due to the exudation of phenolic compounds from the cut surface often represents a significant difficulty for obtaining a sterile culture and further successful clonal micropropagation of medicinal plants. To solve this issue, antioxidants (ascorbic, citric acids) and sorbents (polyvinylpyrrolidone, activated charcoal) are added into the nutrient medium, which prevent the activation of hydrolytic enzymes and the death of explants (Nayanakantha et al., 2010). Ascorbic and citric acids detoxify reactive oxygen formations. The antioxidant impact of organic acids manifests itself by a tendency to increase the adaptive potential of plants and to optimize the processes of growth and productivity (Ulianych et al., 2020). Phenolic exudation in explants from *Ch. angustifolium* plants has also been observed in other studies. Turker et al. (2008) were able to overcome this problem only by adding 100 mg L^{-1} of ascorbic acid into the medium. However, the content of phenolic compounds depends on many factors, including the genotype. It is possible that in our study the colour mutation in the white-flowered form somewhat mitigated the intensity of the synthesis of phenolic compounds, which favourably affected the decrease in the amount of phenols neutralized by organic acids.

CONCLUSIONS

Thus, the optimal timing for isolating *Ch. angustifolium* explants is the initial stage of the growing season: from April to early June. We also report that a 7% solution of calcium hypochlorite in 7 minutes exposure was the most effective surface sterilization procedure for the viability of explants. MS nutrient medium with the addition of 0.5 mg L^{-1} BAP is the most efficient at the propagation stage. There were no differences in a morphogenic potential between lilac-flowered and white-flowered *Ch. angustifolium* forms. Combination of ascorbic acid and citric acid (50 mg L^{-1}) was effective in reduction of phenolic exudation, but the best result was achieved for MS medium containing 100 mg L^{-1} PVP.

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Decision three to predict respiratory rate of piglets submitted to cold conditions

P.F.P. Ferraz[1,*], V.G. Cadavid[2], G.A.S. Ferraz[1], J.A.O. Saraz[2], G. Rossi[3] and M. Barbari[3]

[1]Federal University of Lavras, Agricultural Engineering Department, Campus Universitário, PO Box 3037, CEP 37200-000 Lavras, Minas Gerais, Brazil
[2]Universidad Nacional de Colombia Sede Medellin, Facultad de Cuencias Agrarias, Departamento de Ingenieria Agricola y de Alimentos, PO Box 050034 Medellín, Colombia
[3]University of Florence, Department of Agriculture, Food, Environment and Forestry, 13 Via San Bonaventura, IT50145 Firenze, Italy
[*]Correspondence: paricia.ponciano@ufla.br

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Abstract. Pigs subjected to thermal conditions outside their comfort zones may show altered physiological and behavioural responses, which may consequently cause productive losses. For these reasons, the aim of this paper is to develop a decision tree for the prediction of respiratory rate (RR, mov min^{-1}) of piglets exposed to different thermal situations. The experiment was carried out in an experimental pig farm of the Universidad Nacional de Colombia Campus Medellin, located at the San Pablo Agraria Experimental Station located in the eastern sector of the department of Antioquia, during August 2019. A database containing the raw data for dry bulb temperature - tdb (°C), and relative humidity - RH (%) as input variables, and RR (mov min^{-1}) of six piglets were assessed every two hours as output variable for piglets was generated. The experimental database was composed of 78 observed data. The decision trees were developed to conditions of tdb between 19.2 to 29.5 °C and RH between 50.2 to 88.4%. In the experimental period, RR of piglets submitted to tdb higher than 27.1 °C the RR was around 60 mov min^{-1}, tdb smaller than 27.1 °C the RR varied from 36 to 46 mov min^{-1}. These low values of physiological responses may indicate that the piglets are not in a comfortable situation, so their development, welfare and production can be affected. The decision tree developed can be useful to provide a quick understanding of the piglet's welfare condition based on the environmental variables and physiological responses.

Key words: animal welfare, pig, physiological responses, thermal stress.

INTRODUCTION

The newborn piglet, despite being neurologically developed, is still physiologically immature. Some important changes occur early in piglets' life may enabling them to survive. In an intensive production system, the environment can be one of the responsibles for the comfort and welfare of pigs (Cecchin et al., 2019). Pigs subjected to thermal conditions outside their comfort zones may show altered behavioural responses physiological parameters, such as body temperature, respiratory rate and cardiac movements, which may consequently cause losses related to the productive responses of these animals. The first days are the most critical in a piglet's life and the physiological changes occur mainly in this period. According to Damasceno et al. (2019) in the first weeks of life, pig's thermoregulatory system is still inefficient in maintaining their homeotherm effectively. When the dry bulb temperatures (tdb) are below the thermoneutral zone (TNZ), part of the animal's feed energy intake that could be used for growth or production is diverted to thermoregulation to maintain homeostasis (Mujahid, 2010).

Under severe low tdb, homeothermic animals can suffer with decreased alertness hypothermia, and behavioural and physiological problems (Ferraz et al., 2017). Physiological variables can be an indicative of the thermal comfort of the animals. Any variation in physiological responses can indicate if the animal is or not under thermal stress (Nascimento et al., 2012). The physiological variable of easy measurement is the respiratory rate (RR), which can be measured by visual analysis.

Data mining techniques can transform raw data into valuable information, and support for the decision-making process, as well as might promote the discovery of knowledge in the field of pig production (Fonseca et al., 2020). Techniques of decision trees can be used as reliable techniques for the development of predictive models to support decision-making. This technique is an hierarchical graphical structures that can be easily understood and applied. Decision trees are characterized by segmenting heterogeneous data according to their similarities such that the data become more homogeneous about the target variable (Vieira et al., 2018).

For those reasons, the objective of this paper is to develop decision trees for predicting the respiratory rate (RR, mov min^{-1}) of piglets exposed to cold thermal situations.

MATERIALS AND METHODS

The experiment was carried out at the maternity facility of an experimental pig farm on the Nacional University of Colombia, Campus Medellin, located at the San Pablo Agraria Experimental Station located in the eastern sector of the department of Antioquia, Rionegro municipality, during August 2019. The raising system evaluated involved intensive confinement, and the animals did not have access to the outside of the pig house.

The experimental facility has a width of 8.0 meters and a length of 12.0 meters, built in brick without thermal insulation, with a right foot 2.0 meters high with a concrete floor and biogas heating systems (Fig. 1, a). The evaluated piglets from commercial genetic lines (Fig. 1, b)

In order to characterize the thermal environment inside the house, dry-bulb temperature (tdb, °C) and relative humidity (RH, %) at 1.0 m height inside the house were measured. For the collection of respiratory rate (RR) was adopted methodology used by Cecchin et al. (2019), where is made the measurement and counting of the movements of the animal for 15 seconds. So, the value is multiplied by four to get the amount of movement per minute. The RR was evaluated with the aid of a digital timer (± 0.01 s). The environmental variables (tdb and RH) and physiological response (RR) of six piglets from commercial genetic lines from 0 until the first 24 h of life were assessed every two hours, totalizing 13 times during a day. A database was generated considering as input variables the raw data: tdb (°C), RH (%), and as output variable RR (mov min^{-1}) for piglets. The experimental database was composed of 78 observed data.

a)  b) 

Figure 1. The experimental maternity facility for pigs (a) and the evaluated piglets (b).

The dataset was randomly distributed among two subsets using random sampling as data partition method. The first subset (70% of the data) was for training the decision trees, and for the second subset (30%) was used for validation, following the methodology proposed by Hernández-Julio et al. (2019). For the prediction of RR of piglets exposed to different situations of dry bulb temperature (tdb, °C) and relative humidity (RH, %) 60 decision trees models were developed. The model with the best performances was selected. The decision trees were developed to conditions of tdb between 19.2 to 29.5 °C and RH between 50.2 to 88.4%.

RESULTS AND DISCUSSION

Fig. 2 shows the box plot analysis of tdb (Fig. 2, a), RH (Fig. 2, b) and RR (Fig. 2, c) results for the evaluated period. The mean values were 22.18 °C, 75.25% and 49 mov min^{-1} for tdb, RH and RR, respectively. According to Ferreira (2015) thermoneutral zone for piglets from 0 until 2 days of live is 32 to 34 °C and the lower critical effective temperature is 22 °C. Ferreira et al. (2007) studied physiologic parameters of the first 24 hours of sucking pigs life and found values of 41 to 63 mov min^{-1}.

Based on this information it is possible to observe that the piglets were submitted to a thermal discomfort situation. In a stress condition, in which there is a higher demand for thermal maintenance energy, the energy contribution to the productive functions is reduced, generating health imbalances, reduced production rates and changes in animal behaviour (Huynh et al., 2005; Fonseca et al., 2020). According to Ferreira (2015) the recommendable RH for piglets is 60–70%.

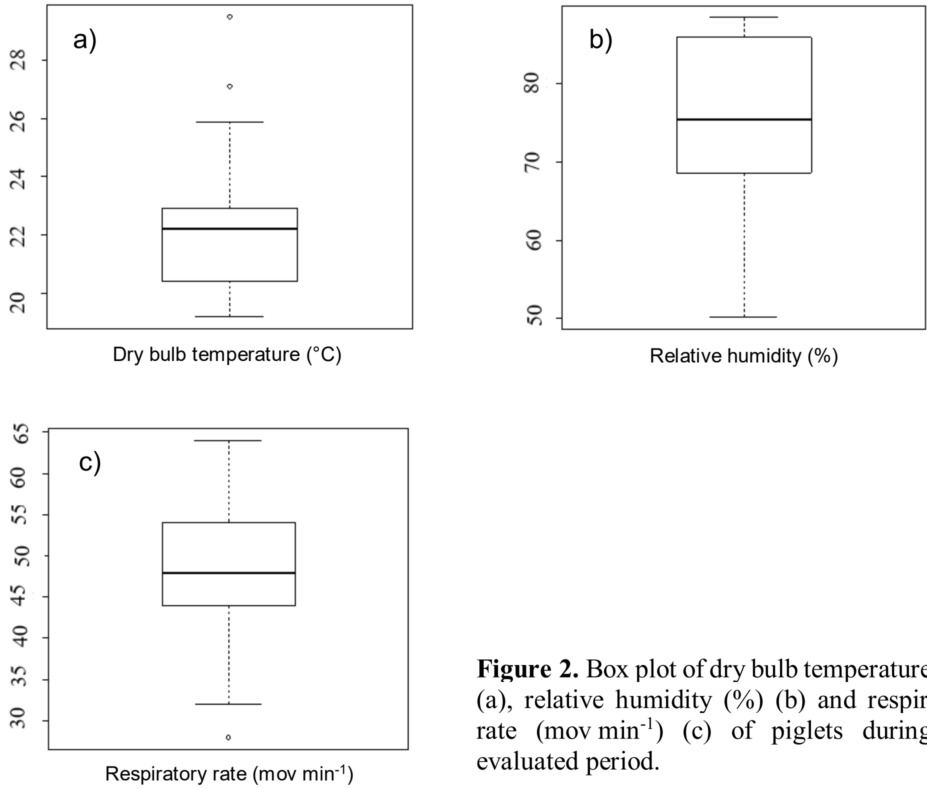

Figure 2. Box plot of dry bulb temperature (°C) (a), relative humidity (%) (b) and respiratory rate (mov min^{-1}) (c) of piglets during the evaluated period.

Principal component analysis was used to associate the RR, tdb, and RH data. Fig. 3 shows that the RR were positively correlated with the tdb and negatively correlated with the RH. Thus, the data suggest that an increase in tdb will increase the piglets' RR. Conversely, an increase in RH will decrease the piglets' RR.

To evaluate the level of comfort and welfare of animals in confinement systems, practical and easy to apply technologies are being used. The average structure of the RR decision tree is described in Fig. 4. The first split is related to tdb that is the most important factor influencing the RR and the second split is related to RH. According to the experimental data, this decision tree is related to tdb values of 19.2 to 29.5 °C and RH between 50.2 to 88.4%, which can be considered a cold condition for piglets.

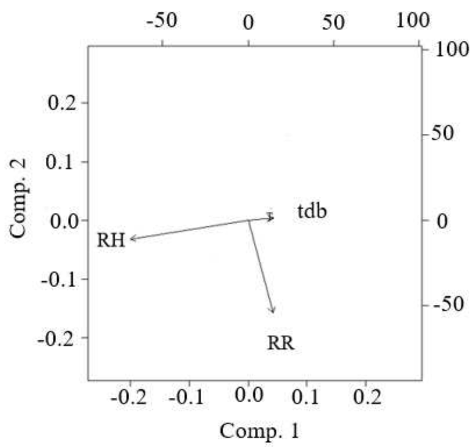

Figure 3. Principal component analysis of the respiratory rate (RR), dry bulb temperature (tdb) and relative humidity (RH).

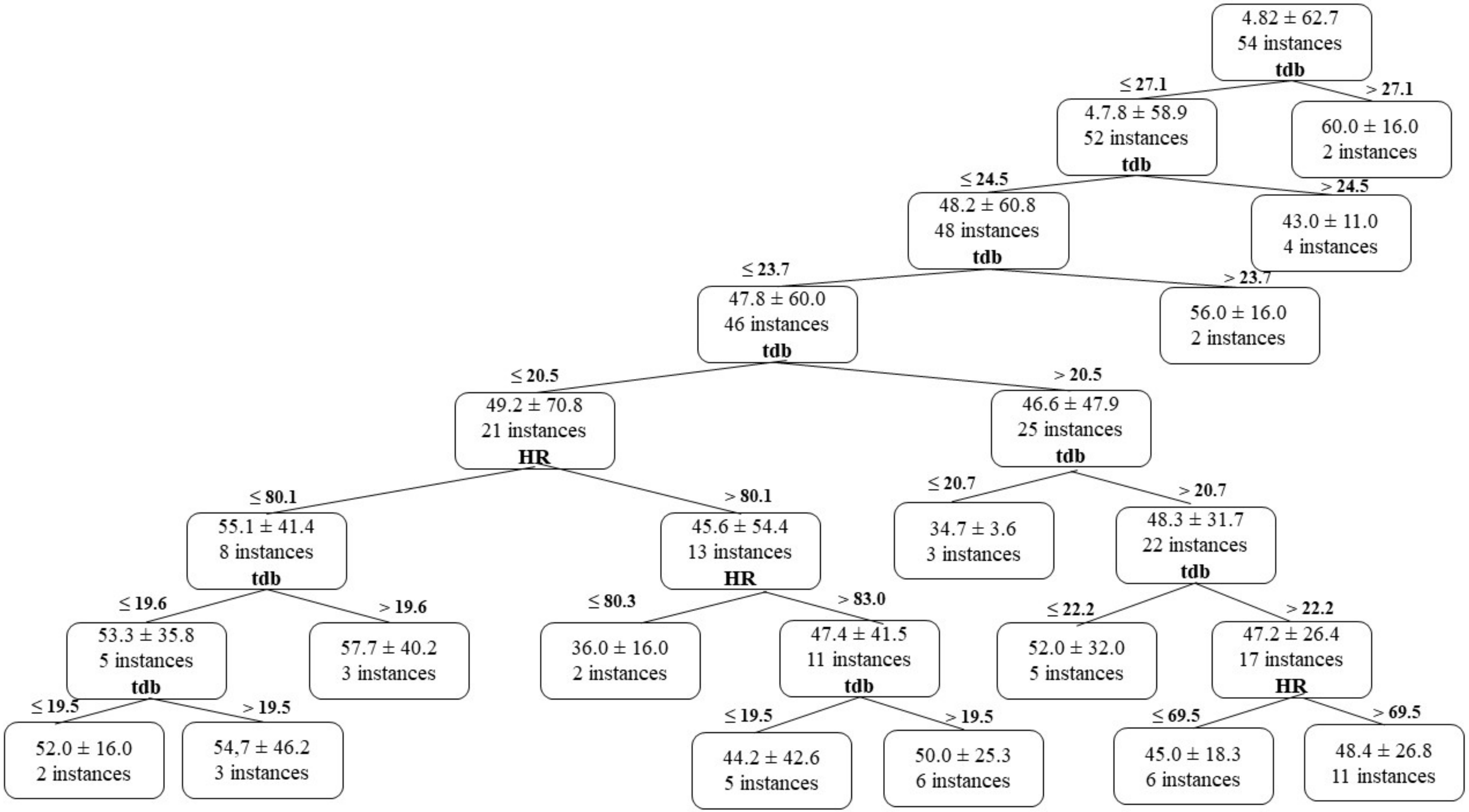

Figure 4. Visual decision tree for the respiratory rate output variable (mov min^{-1}).

The first split for tdb occurs at approximately 27 °C. The decision tree model chosen presented R^2 of 0.997, which indicates an excellent adjustment to predict the RR.

The variation of pig's RR can be considered one of the first physiological adjustments to maintain thermoregulation (Oliveira et al., 2019). Besides, this physical property has the advantage of being measured through visual analysis.

Pigs, due to their physiological characteristics, have difficulties in adapting to environmental thermal fluctuations. The temperature range for your comfort varies with age. In the experimental period, RR of piglets submitted to tdb higher than 27.1 °C the RR was around 60 mov min^{-1}, tdb smaller than 27.1 °C the RR varied from 36 to 46 mov min^{-1}. These low values of physiological responses may indicate that the piglets are not in a comfortable situation, so their development, welfare and production can be affected. When the tdb values are below the thermoneutral zone, part of the feed energy intake is diverted to thermoregulation to maintain homeostasis (Mujahid, 2010). On the other hand, high environmental temperatures negatively affect pigs, causing changes in their rectal temperature, skin surface and respiratory rate (Manno et al., 2005).

The decision tree developed can be used in field situations to provide a quick understanding of the piglet's welfare condition based on the environmental variables and physiological responses, as RR, for example. This information can be obtained in real-time, and it may help the suines breeder in decision making to get a satisfactory environmental condition for the piglets. The decision tree models were successfully employed to predict the RR, obtaining the ranges of the input variables values and the decision-making rule base from the data. Thermal variables (as tdb and RH) and physiologic variables (as RR) could be considered as knowledge-based management subsystem in a decision support system. These variables can provide information about data and their relationships can help in decision making.

CONCLUSIONS

Using the proposed decision tree models it was possible to predict respiratory rate successfully, based on environmental variables (such as tdb and RH).

Environmental variables and physiological responses as, RR can be evaluated as a knowledge-based management subsystem in a decision support system, which provides information about data and potential relationships to assist in decision-making.

In the experimental period, RR of piglets submitted to tdb higher than 27.1 °C the RR was around 60 mov min^{-1}, tdb smaller than 27.1 °C the RR varied from 36 to 46 mov min^{-1}.

The propoed model exhibited a good fit between the observed and predicted data. Therefore, the decision trees can support decision-making to avoid thermal stress conditions.

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Storage stability of rapeseed methyl ester stored in a sealed barrel for seven years

S. Heikkilä*, K. Sirviö, C. Nuortila and S. Niemi

University of Vaasa, School of Technology and Innovations, P.O. Box 700, FIN-65101 Vaasa, Finland
*Correspondence: sonja.heikkila@univaasa.fi

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Abstract. Storage stability is one of the main quality parameters related to fatty acid methyl esters (FAME) biofuels. The deterioration of biofuels´ properties during storage is a more serious issue than with conventional fuels. In particular, lengthy storage threatens the oxidative stability of FAME fuels because factors such as the presence of air, elevated temperatures or presence of metals promote the oxidation process. Consequently, the acceptable storage time for FAME fuels is generally regarded to be regrettably short, at no more than six to12 months. However, storage conditions play an important role in determining actual storage stability. This study aimed to investigate and evaluate any deterioration in the quality of rapeseed methyl ester (RME) fuel that has been stored for as long as seven years in adequate storage conditions. The fuel was stored in the dark, contained in a sealed steel barrel in an insulated shipping container outdoors. The temperature of the container varied with seasonal fluctuation, but the fuel never froze during storage. The study analysed six key fuel properties of the RME: ester content; water content; density; kinematic viscosity; oxidation stability index; and acid number. The analyses were conducted immediately after opening the barrel, and again after two months of storage in a laboratory. The results were compared to those measured for the fresh fuel, seven years earlier. The comparison of the results indicate that the fuel quality had suffered no serious deterioration during the seven-year period.

Key words: biodiesel, RME, shelf life, storage stability.

INTRODUCTION

Most of energy worldwide is produced from non-renewable sources such as oil, coal and natural gas. These fossil energy sources are depleting and there is an urgent need for new sources of clean and sustainable energy. Increasing the renewable energy share from cleaner sources also helps reduce emissions of toxic pollutants and greenhouse gases. One renewable option is to use sustainably produced biodiesel to replace petroleum diesel as a power source in compression-ignition engines. Not only does biodiesel have the advantage of containing oxygen, but also emissions such as carbon monoxide (CO), hydrocarbons (HC) and particulate matter are lower when combusting biodiesel than when burning petroleum diesel (Ovaska et al., 2019). In Europe, fossil automotive diesel fuel can contain maximum 10 mg kg^{-1} (10 ppm) of sulphur (EN 590:2013, 2013). Generally, biodiesel contains lower levels of sulphur than fossil diesel and only low sulphur oxide (SO_x) emissions are formed when biodiesel is burned. Even though oils and fats can contain significant amounts of sulphur, the sulphur content is reduced when the oils and fats are processed to biodiesel. Most of the biodiesel samples studied by He et al. (2009) contained sulphur at less than 10 ppm.

Biodiesel is composed of methyl esters of long-chain fatty acids. Primary raw materials of biodiesel are waste vegetable oils and animal fats. The production process is based on transesterification of the triglycerides with sodium methoxide or sodium hydroxide and methanol (Varghese et al., 2021).

Despite the advantages of biodiesel, its use is problematic. Biodiesels are prone to oxidation and this may cause major problems in engines´ fuel systems and in fuel storage tanks and pumps. The storage stability of biodiesel also is a matter of concern. The acceptable storage time of FAME fuels currently is reckoned to be only six to 12 months (Bouaid et al., 2007). The tendency to degrade due to oxidation is associated with the chemical composition of biodiesel and so therefore it depends on the fuel´s raw material. FAME made from raw materials with larger amounts of unsaturated fatty acids is considered to be more susceptible to chemical degradation. Vegetable oils usually contain more unsaturated fatty acids than are found in animal fats. The fuel´s degradation becomes more rapid as the storage time lengthens. Other factors that affect the storage stability are humidity, exposure to air and heat (de Siqueira Cavalcanti et al., 2019; de Sousa et al., 2021).

This study set out to investigate any deterioration in the quality of rapeseed methyl ester fuel that had been stored for seven years in adequate, real life storage conditions. The fuel had been purchased for research purposes. One barrel had remained unopened in a fuel storage and this barrel's fuel was investigated in the current study. The fuel was contained in the sealed full steel barrel, stored in the dark in an insulated shipping container outdoors in Vaasa, Finland. The container is a designed fuel storage and contains e.g. catchment basin for the fuels. The temperature inside the container varied due to seasonal fluctuation, but the variation was not followed precisely.

Six properties of the RME were analysed: ester content; water content; density; kinematic viscosity; oxidation stability index; and acid number. The analyses were conducted after opening the barrel and then were repeated after two months of storage in a laboratory. The three-litre fuel sample was stored in the laboratory in a glass bottle inside a fume cupboard. The results from the two sets of analyses were compared to those measured for the fresh fuel seven years earlier. The research question was to establish if the properties of the RME biodiesel had changed during the seven-year storage in a sealed barrel, and whether any further changes would occur if the sample subsequently is exposed to air and light in a laboratory.

MATERIALS AND METHODS

Fuel

The University of Vaasa purchased the RME fuel at the end of 2012. The supplier was Archer Daniels Midland Company (ADM) in the USA. As a fresh fuel, the RME fulfilled the requirements of the prevailing European Standard EN 14214:2012 for FAME biodiesel.

Methods

The RME was stored in a real life fuel storage; in an insulated shipping container outdoors. The temperature inside the container varied due to seasonal fluctuation, but the variation was not followed precisely. The annual average temperature in Vaasa region is +4–+5 °C (Finnish Meteorological Institute, 2021). A radiator ensured that the temperature inside the container remained above 0 °C during the winter. Ambient summer temperatures in Finland are between approximately +10 and +30 °C: the temperature inside the insulated container has been close to that range. The RME barrel had remained sealed until the sample was taken. The sample was taken with a siphon from the middle of the full barrel. Contents were stirred with a rod prior to sampling.

Six key properties of the RME fuel were measured, using the following methods and equipment.

The ester content was measured with a PerkinElmer Clarus 580 gas chromatograph. Methyl heptadecanoate is used as an internal standard for this method. It is suitable for biodiesels containing methyl esters between C14 and C24, and when the ester content is higher than 90 m-%. The ester content, C (m-%), is calculated in compliance with Eq. 1,

$$C = \frac{\left(\sum A\right) - A_{EI}}{A_{EI}} \times \frac{C_{EI} \times V_{EI}}{m} \times 100\% \quad (1)$$

where $\sum A$ is the total peak area from the methyl ester in C14 to that in C24:1; A_{EI} is the peak area corresponding to methyl heptadecanoate; C_{EI} is the concentration of the methyl heptadecanoate solution (mg mL^{-1}); V_{EI} is the volume of the methyl heptadecanoate solution (mL); and m is the mass of the sample (mg). The method is described in European Standard EN 14103 (EN 14103, 2003).

Water content is measured according to the coulometric Karl Fischer titration method. The in-house procedure used is based on international standard ISO 12937 (ISO 12397, 2000).

Kinematic viscosity and density were measured with a Stabinger SVM 3000 rotational viscometer, which uses torque and speed measurements to determine viscosity. The device calculates the dynamic viscosity, η (mPas), from the rotor speed by applying Eq. 2

$$\eta = \frac{K}{\left(n_2 / n_1 - 1\right)} \quad (2)$$

where K is constant; n_1 is the speed of the measuring rotor (mm s^{-1}); and n_2 is the speed of the measuring tube (mm s^{-1}).

The viscometer also has a density-measuring cell which utilises the oscillating U-tube principle. The kinematic viscosity, KV (mm^2 s^{-1}), was calculated automatically based on these measurements, according to Eq. 3

$$KV = \frac{\eta}{\rho} \quad (3)$$

where η is dynamic viscosity (mPas); and ρ is density (g cm^{-3}) (Novotny-Farkas et al., 2010).

The oxidation stability index (OSI) was measured by a Metrohm 873 Biodiesel Rancimat instrument. This uses an accelerated method to determine the oxidation stability of biodiesel, blowing a stream of air through a heated fuel sample. Vaporising compounds from the sample drift with air into water and the consequential change in the water´s conductivity is measured. The end point is achieved when the conductivity increase is at its highest. This method is described in European Standard EN 14112 (EN 14112, 2003).

The acid number was analysed with a Metrohm Titrando 888 titrator, which uses a potentiometric titration method. The sample is diluted with iso-propanol and titrated with potassium hydroxide. The acid number, AN, is reported according to Eq. 4

$$AN = \frac{56.1 \times V \times c}{m} \tag{4}$$

where V is the volume of the standard volumetric potassium hydroxide solution used (mL); c is the exact concentration of the standard volumetric potassium hydroxide solution used (mol L^{-1}); m is the mass of the sample (g); and 56.1 is the molecular mass of potassium hydroxide. Results are expressed as mg KOH g^{-1} (mg of potassium hydroxide per g of sample). The measurement was performed according to European Standard EN 14104 (EN 14104, 2003).

The relative standard deviations for the measurements were: ester content < 1%; kinematic viscosity < 1%; oxidation stability 4.5%; acid number 7.9%; and density < 1%. The relative standard deviation for water content was not measured.

RESULTS AND DISCUSSION

The results are presented in Table 1 and Figs 1–6.

The ester content (Table 1, Fig. 1) of fresh RME was 98.6%. The ester content of the samples stored for seven years and for seven years and two months was 98.3% in both cases, thus remaining compliant with EN 14214, which stipulates a minimum value of 96.5%.

Table 1. Ester content, water content, density, kinematic viscosity, oxidation stability index and acid number

RME sample	Ester content (%)	Water content (mg kg^{-1})	OSI (h)	Density (15 °C) (g cm^{-3})	Kinematic viscosity (40 °C) (mm^2 s^{-1})	Acid number (mg KOH g^{-1})
Fresh	98.6	132	12	0.89	4.5	0.2
Stored for 7 years	98.3	200	12	0.88	4.5	0.2
Stored for 7 years and two months	98.3	170	11	0.88	4.5	0.2
EN 14214	> 96.5	< 500	> 8	0.86–0.90	3.5–5.0	< 0.5

The water content (Table 1, Fig. 2) of the fuel stored for seven years was higher (200 mg kg^{-1}) than it was for the fresh fuel (132 mg kg^{-1}) or the sample stored for seven years and two months (170 mg kg^{-1}). Nevertheless, all three results are comfortably within the EN 14214 maximum limit of 500 mg kg^{-1}. Biodiesels are more prone to degrade when visible water is present or air humidity is high (Yang et al., 2017). In this study, it can be stated that in respect of free water, the storage conditions have been dry and correct.

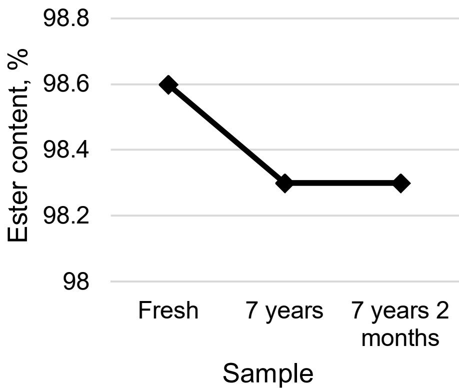

Figure 1. Ester content of the samples.

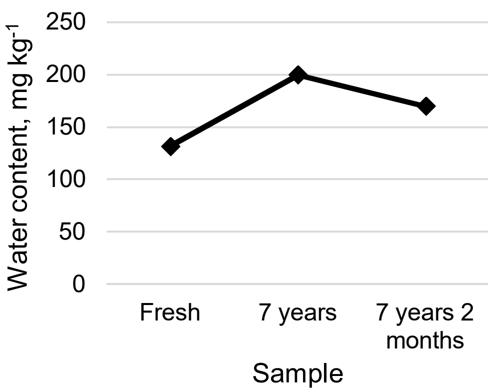

Figure 2. Water content of the samples.

The kinematic viscosity (Table 1, Fig. 3) of all the samples was 4.5 $mm^2 s^{-1}$. Thus, no change was detected and all three results fall within EN 14214´s acceptable range (3.5–5.0 $mm^2 s^{-1}$).

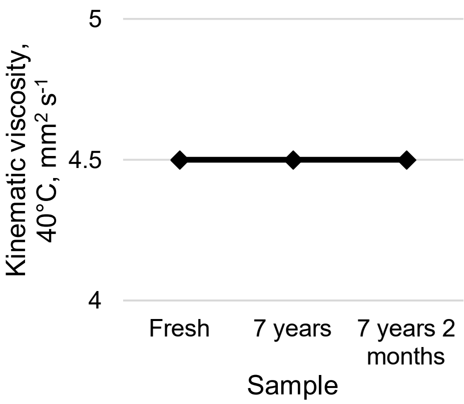

Figure 3. Kinematic viscosity of the samples.

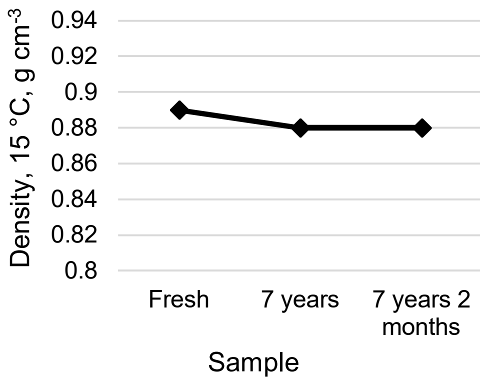

Figure 4. Density of the samples.

The density (Table 1, Fig. 4) for the fresh sample was 0.89 g cm^{-3} and for the samples after seven years and after seven years and two months of storage it was 0.88 g cm^{-3}. The change is very small and all the results are within the limitations of the EN 14214, which sets a minimum of 0.86 and a maximum of 0.90 g cm^{-3}.

The OSI value (Table 1, Fig. 5) of the fresh sample was 12 h, and exactly the same result was obtained for the sample after seven years of storage, so there had been no measurable deterioration in oxidation stability. The OSI value of the sample stored for seven years and two months was 11 h. The minimum limit for OSI in EN 14214 is 8 h, so all three samples comfortably exceeded that requirement. Apparently, the RME contained antioxidant, although the exact quantity was unknown. Antioxidants inhibit the autoxidation process and can be used as additives to extend the storage time of biofuels (Knothe, 2007). Several published articles related to biodiesel antioxidants indicate that relatively high concentrations of antioxidants are needed to meet the requirements set for fuel stability (Das et al., 2009; Yang et al., 2017).

The acid number (Table 1, Fig. 6) of biodiesels must be below 0.5 mg KOH g^{-1} to comply with EN 14214. The acid number for all three samples was 0.2 mg KOH g^{-1} and so the FAME standard limit presented no difficulties.

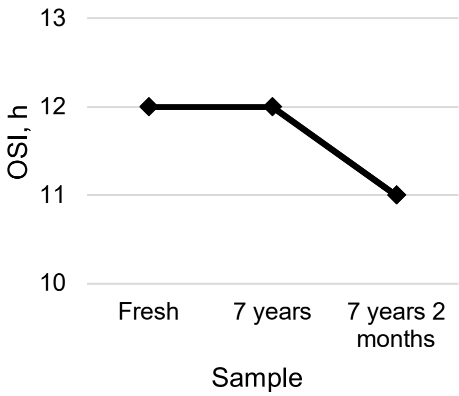

Figure 5. OSI of the samples.

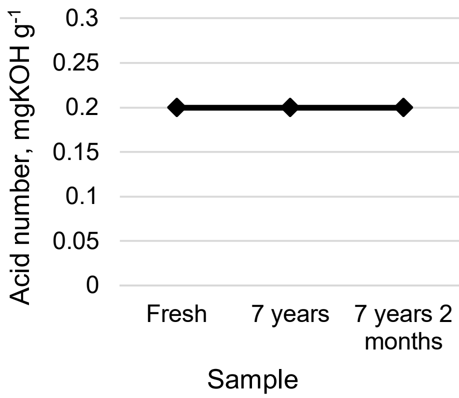

Figure 6. Acid number of the samples.

All the analysed results still fulfilled the requirements set in European Standard EN 14214, indicating that the fuel maintained its quality in a sealed barrel. The exposure to air after opening the barrel did cause an 8% reduction in the fuel´s oxidation stability index. Nevertheless, the fuel quality was still within the specification of EN 14214.

The transesterification process is well known to be reversible (Pullen & Saeed, 2015). No significant reversion occurred in the studied conditions, and nor was there any significant oxidation reaction, i.e., the chemistry of biodiesel seemed to be stable for the studied period. Storage stability in terms of oxidation has been studied widely but there are relatively few recorded results concerning storage stability in relation to ester content.

Thompson et al. (1998) studied RME's properties for 24 months under various storage conditions. The fuel was in glass and steel containers stored at room temperature at approximately +23 °C, and outdoors at ambient conditions with an annual average temperature of +8.3 °C. The samples were vented to the atmosphere. According to their results, the acid number of all the samples remained below the EN 14214 limit of 0.5 mg KOH g^{-1} for approximately 15 months. Their density rose evenly to reach 0.89 g cm^{-3} at the end of the 24-month storage period. The RME´s viscosity was relatively high even at the beginning of the test, being above 5.5 mm^2 s^{-1}. At the end of the 24-month storage period it was above 7 mm^2 s^{-1} for all the samples. The samples stored indoors degraded slightly faster than the ones stored outdoors. No antioxidants were used. (Thompson et al., 1998).

Mittelbach & Gangl (2001) also studied the storage stability of RME. They stored samples for 170–200 days, at between 20 and 22 °C in polyethylene bottles or tin cans. Some of the samples were exposed to air and daylight. The OSI of their original samples was poor, measured at just 5.6 h at the beginning of the test. The sample exposed to air and daylight had oxidised totally after 170 days and the other samples also lost their oxidative resistance during the storage time. This was shown in the OSI and acid number results. The authors of this study did not mention whether the fuel contained any antioxidants (Mittelbach & Gangl, 2001).

Published studies of biodiesel storage stability have focused on soybean-based fuels far more frequently than on biodiesel made from rapeseed oil. For example, de Siqueira Cavalcanti et al. (2019) studied three commercial Brazilian biodiesels, storing them in carbon steel containers in subtropical conditions. One of the biodiesels was made of soybean oil, one was of beef tallow and the third was a blend of 65% soybean and 35% beef tallow. The samples were initially stored for 60 days in their original storage conditions. The containers were opened after 60 days and the samples were then stored for a further 90 days in conditions where the temperature varied between 24 and 34 °C and the relative air humidity varied from 30 to 80%. De Siqueira Cavalcanti et al. found that biodiesel made solely of soybean oil showed limited shelf life: its water content and oxidation stability results were particularly poor. The two other biodiesels maintained their quality during the storage time.

Storage conditions worldwide differ considerably. The fuel in the present study was much older than in other studies but the storage temperatures were notably lower than in the Brazilian study. The results obtained by de Siqueira Cavalcanti et al. (2019) also indicate that raw material is the key factor in storage stability of biodiesels. The amount of unsaturated fatty acids in soybean methyl ester is much greater than in rapeseed methyl ester.

Additionally, the fuel quality at the beginning of the storage stability research also seems to be important. This is evident by comparing the results from the present study with those obtained by Thompson et al. (1998) and Mittelbach & Gangl (2001). If some of the fuel properties are out of specification at the start, this seems to exacerbate the degradation of biodiesel during storage. Antioxidants are needed for good oxidation stability. Clean, dry and proper storage conditions also play an important role in storage stability, and the biodiesel should be used as soon as possible after the barrel is opened. However, if the barrel remains sealed, the results of this study show that the fuel will maintain its quality for a surprisingly long time.

CONCLUSIONS

The aim of this study was to find out if the quality of rapeseed methyl ester has deteriorated when the fuel has been stored for seven years in adequate storage conditions. The RME was stored in a sealed barrel in a real life fuel storage; in an insulated shipping container outdoors. The temperature inside the container varied due to seasonal fluctuation, but the variation was not followed precisely. A radiator ensured that the temperature inside the container remained above 0 °C during the winter. A fuel sample was taken from the middle of the full barrel after the content had been stirred with a rod.

The properties measured were ester content, water content, kinematic viscosity, density, oxidation stability and acid number. One sample was analysed straight after opening the barrel and another one after two months of storing in the laboratory. The results were promising: after seven years, all the measured properties still fulfilled the requirements for FAME biodiesel set in European Standard EN 14214, being as followed (seven years and two months results in brackets):

- The ester content 98.3% (98.3%)
- The water content 200 mg kg^{-1} (170 mg kg^{-1}.)
- The kinematic viscosity 4.5 mm^2 s^{-1} (4.5 mm^2 s^{-1})
- The density 0.88 g cm^{-3} (0.88 g cm^{-3})
- The OSI value 12 h (11 h).

The exposure to air after opening the barrel did cause an 8% reduction in the fuel´s oxidation stability index. The exposure to air did not cause decrease in the fuel quality in other measured properties.

If biodiesel made of rapeseed oil is of good quality, contains sufficient antioxidant and is stored in dry conditions, without an exposure to air and at temperature above 0 °C and below 30 °C (annual average around +5 °C), the quality of RME seems to remain constant for several years. Nevertheless, the recommendation is to ensure the fuel´s quality before use in engines.

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Comparative effect of different amount of inulin and symbiotic on growth performance and blood characteristics 12 weeks old calves

A. Ilgaza and A. Arne*

Latvia University of Life Sciences and Technologies, Faculty of Veterinary Medicine, K. Helmana street 8, LV-3004 Jelgava, Latvia
*Correspondence: astra.arne@gmail.com

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Abstract. The study was focused on assessment of the effect of prebiotic inulin (from Jerusalem artichoke (JA) powder it contain ~50% of inulin) and mixed with probiotic preparation call synbiotic thereof on growth performance and blood parameters of milk calf. In total, 70 milk calves (50+/-5 kg; 23+/-5 d.old) were used in a 57-d experiment. The seven dietary treatments consisted of the control diet (CoG); diet CoG supplemented with different amount of prebiotics (PreG6; PreG12; PreG24; accordingly 6g, 12 or 24 g of JA) and three different amount of synbiotics (SynG6; SynG12; SynG24; accordingly inulin and probiotic 0.25 g Enterococcus faecium (2×10^9 CFU g^{-1}). Throughout the study prebiotic (PreG) and synbiotic (SynG) groups calf average daily gain (ADG) was increased ($p < 0.01$) than CoG, the end of study the highest ADG reached PreG12 than SynG12 and it was ($p < 0.01$) compare with CoG. At the end of te study PreG6 and SynG6;12 hematocrit was higher ($p < 0.05$), but PreG12;24 and SynG24 ($p < 0.01$) than CoG. Hemoglobin PreG and SynG12;24 was higher ($p < 0.01$) compare with CoG. WBC was lower ($p < 0.05$) PreG24, but there were no differences between synbiotic groups (SynG) compare CoG. PLT was ($p < 0.01$) higher PreG and SynG supplemented groups than CoG. Total protein PreG12; SynG6;12 ($p < 0.01$) higher compare CoG. Glucose PreG6;12 is lower ($p < 0.05$) than CoG. In conclusion, 12g and 24g of JA powder and the same amount ja powder mixed with probiotic Enterococcus faecium can improve the 4 to 12 weeks old calf performance and health status. However, new synbiotic didn't improve inulin action.

Key words: calf, inulin, *Enterococcus faecium,* growth, blood.

INTORDUCTION

Newborn ruminants are known to encounter potentially stressful situations during the first months of life, including morbidity, transportation, and weaning (Chashnidel et al., 2020). Stress can reduce immunity and increase the risk of disease (Salak-Johnson & McGlone, 2006), and for calves diarrhea is one of the most common causes of falls behind in growth and mortality (Gulliksen et al., 2009). To reduce this consequence there is search in different directions how to improve calf health. There was time when antibiotics were added to feed, but they stimulate the development of antibacterial resistance both in humans and in animals, and so the alternatives are searched (Jansons et al., 2011; Jonova et al., 2021). As some of the alternatives for several years are being investigated, prebiotics, probiotics and synbiotics, whose feeding to food-producing animals has increased in recent years (Hamasalim et al., 2016; Dar et al., 2017; Markowiak & Śliżewska, 2018; Tóth et al., 2020).

The prebiotic inulin is a polysaccharide belonging to the fructan group and is one of the most commonly used prebiotic in research. In the digestive tract, inulin is hydrolyzed and fermented to a substrate, which is used by the beneficial intestinal bacteria for their growth, so the numbers of those bacteria -such as Bifidobacteria and some species of bacteria Lactobacilli are increased (Han et al., 2014; Gupta et al., 2019; Jonova et al., 2021). Studies in ruminants have shown that inulin increases weight gain and induces a change in physiological blood measurements (Masanetz et al., 2010, 2011). There are studies showing that the use of prebiotics in ruminants substantially influence blood physiological parameters Chashnidel et al. (2020), reported that there is a significant ($p < 0.05$) difference in the amount of glucoses, BUN, total protein, albumin, globulin and IgG, also, Dar et al. (2017) observed substantially higher number of lymphocytes in the blood of calves which were fed prebiotics.

Combining prebiotics with probiotics call synbiotics that possible work better than prebiotics or probiotics alone (Shim, 2005; Hamasalim, 2016).

The aim of our study was to find out the impact on dairy calf daily gain as well as various blood hematological and biochemical parameters when using synbiotic got from the combination of inulin (obtained from Jerusalem artichoke) with probiotic (*Enterococcus faecium).*

MATERIALS AND METHODS

Study Herd, Housing and Feeding

Seventy randomly selected, 23 (± 5) days old clinically healthy male Holstein crossbreed calves, weighing 50 kg (± 5 kg), were randomly allocated to 7 groups. The study started when the animals had reached 4 weeks of age. The calves were kept in pen of 5, under the same +conditions and were fed twice a day, ~3.5 liter of whole milk per feeding. Calves were free-fed whole milk from a trough providing approximately 7L per calf daily. The pen for 5 calves was 25m^2. Water and hay were freely available 24 hours per day, and fodder was added two weeks after the start of the study when the animals were 6 weeks old. Bedding material was straw and pens were cleaned manually one time per day.

The calves were divided into 7 groups: control group (CoG, n = 10) was fed only whole milk, 3 with whole milk and different amount of prebiotic inulin (PreG6 = 6 g; PreG12 = 12g; PreG24 = 24 g of Jerusalem artichoke powder (JA) it contain ~50% of inulin) and 3 different synbiotic amount groups (SynG6, n = 10, SynG12, n = 10, SynG24, n = 10) in which the calves received 3 different amounts of the prebiotic inulin mixed with 0.25 g of the probiotic *Enterococcus faecium* (2×10^9 CFU g^{-1} (Protexin International Ltd., South Petherton, UK)). Inulin powder and probiotic were added to the first one of the two daily milk feedings.

The 4 week old calves were weighed at the beginning of the study and every two weeks during the study (6, 8, 10, 12 weeks). There were no serious health problems observed during the experiment.

Blood sampling

The blood samples of calves were taken from *v.jugularis* externa on the 0, 28th and 56th day of the research before morning feeding. The level of total protein, albumin and glucose as well as blood morphology: red blood cells, white blood cells, platelets, hemoglobin and hematocrit were determined.

Blood samples for biochemical examination were taken from the calves into vacuumed sample tubes and samples were centrifuged at 3,500 rpm for 10 minutes to separate the serums. Resultant serums were then transferred to Eppendorf tubes and preserved at -23 °C until the time of analysis in the laboratory of Veterinary Hospital of Latvia University of Life Sciences and Technologies. Blood glucose measurements were performed on blood samples without preservatives by the *Freestyle Optium*. A drop of blood samples was placed on the test strip. Single-use reagent strips were used. The study lasted 8 weeks or 56 days.

Statistical analyses

Descriptive statistics for resultant data were expressed in means and standard errors. MS Excel and the R-Studio were used for the data analysis of live weight given as the mean ± standard error (SE). Significance was tested by applying the *Student t-test*. Values of less than 0.05 *($p < 0.05$)* were considered significant.

RESULTS

Effects of prebiotic and synbiotic supplements on performance of Holstein calves are provided in Table 1. Calves receiving prebiotic inulin (PreG) in amount 3, 6 and 12 g day^{-1} per head and mixed with probiotic call synbiotic (SynG) showed clearly higher ($P < 0.01$) body weight in first 12 weeks of life than calves from control group (CoG) (Table 1). It was confirmed by higher daily weight gains of calves that whole milk were supplemented by prebiotic and synbiotic (Table 1).

Table 1. Average daily weight gain of calves in different research periods

Group	Average weight of calves (kg) at 3 research intervals			Average daily weight gain (kg)		
	0.–28. day	28.–56.day	0.–56. day	0.–28. day	28.–56. day	0.–56. day
CoG	18.2 ± 5.06	12.4 ± 5.09	31.2 ± 8.44	0.67 ± 0.20	0.44 ± 0.18	0.56 ± 0.15
PreG6	26.5 ± 1.90**	18.2 ± 3.58**	44.7 ± 4.03**	0.95 ± 0.07**	0.65 ± 0.13**	0.80 ± 0.07**
PreG12	29.0 ± 1.88**	23.3 ± 5.09**	52.3 ± 3.68**	1.03 ± 0.06**	0.83 ± 0.17**	0.93 ± 0.07**
PreG24	25.2 ± 7.36*	22.9 ± 5.09**	48.1 ± 4.18**	0.90 ± 0.26**	0.82 ± 0.18**	0.86 ± 0.08**
SynG6	24.4 ± 3.68**	24.7 ± 4.69**	49.1 ± 2.81**	0.86 ± 0.19**	0.88 ± 0.16**	0.88 ± 0.03**
SynG12	25.7 ± 1.57**	25.6 ± 2.32**	51.3 ± 2.71**	0.91 ± 0.12**	0.92 ± 0.08**	0.92 ± 0.02**
SynG24	22.5 ± 1.65	26.1 ± 3.70**	47.6 ± 4.28**	0.93 ± 0.13**	0.93 ± 0.13**	0.85 ± 0.03**

* compare to CoG ($p < 0.05$); ** compare to CoG ($p < 0.01$).

Started with 28th research day hematocrit (HCT) was higher ($p < 0.01$) all prebiotic groups animal compare to CoG, in research 56th day PreG6 group was higher ($p < 0.05$) HCT, but PreG12, PreG24 ($p < 0.01$) higher HCT compare to CoG. Control group hemoglobin (HGB) in 28th research day was lower ($p < 0.01$) than PreG24 and lower ($p < 0.05$) than PreG6 group. End of the study in day 56th all prebiotic groups HGB was higher ($p < 0.01$) compare to CoG. Highest erythrocytes (RBC) level was group PreG12 and in 28th and 56th research day was higher ($p < 0.01$) than CoG. PreG24 group RBC was higher ($p < 0.01$) in the 28th research day and ($p < 0.05$) end of the research (Table 2).

Table 2. Blood hematology variables of prebiotic groups calves on 0; 28th and 56th research days (mean ± *SD*)

	Research day	Group			
		CoG	PreG6	PreG12	PreG24
HCT (%)	0	22.5 ± 4.26	24.1 ± 4.51	23.8 ± 3.51	24.1 ± 4.48
	28	18.4 ± 5.53	24.0 ± 4.02**	24.3 ± 2.34**	26.8 ± 1.56**
	56	18.6 ± 3.29	22.6 ± 4.62*	25.8 ± 2.30**	25.7 ± 1.01**
HGB (g/dl)	0	9.1 ± 1.21	9.0 ± 1.55	9.2 ± 1.30	9.2 ± 1.29
	28	8.2 ± 1.68	9.8 ± 1.41*	9.2 ± 0.81	10.2 ± 1.71**
	56	8.6 ± 0.75	9.8 ± 1.12**	9.7 ± 0.71**	10.5 ± 1.79**
RBC (x1012/L)	0	6.9 ± 1.51	7.6 ± 1.49	8.0 ± 0.70*	7.7 ± 0.94
	28	6.7 ± 1.20	8.5 ± 1.22*	7.9 ± 1.09**	8.2 ± 0.93**
	56	7.2 ± 0.91	7.6 ± 1.29	8.3 ± 0.45**	7.8 ± 1.26*
WBC (x109/L)	0	8.9 ± 1.07	9.1 ± 1.41	8.9 ± 1.13	8.8 ± 0.92
	28	9.5 ± 1.46	9.3 ± 1.44	9.7 ± 1.14	8.8 ± 1.80
	56	10.9 ± 1.29	11.7 ± 1.67	9.5 ± 1.77*	8.7 ± 1.15**
PLT(x109/L)	0	812.9 ± 216.66	774.6 ± 258.98	566.5 ± 251.79**	714.8 ± 187.80
	28	992.2 ± 88.04	592.9 ± 180.35**	657.8 ± 173.69**	660.5 ± 113.87**
	56	687.1 ± 211.78	591.7 ± 124.80**	649.0 ± 113.68**	601.8 ± 110.61**

* compare to CoG ($p < 0.05$); ** compare to CoG ($p < 0.01$).

Leucocytes (WBC) significantly difference was in 56th research day were PreG12, Pre24 higher ($p < 0.05$) compare to CoG.

Platelets was influenced by dietary treatments. Control group platelets (PLT) was significantly higher ($p < 0.01$) starting from 28th study day (Table 2, 3).

Table 3. Blood hematology variables of synbiotic groups calves on 0; 28 and 56 research days (mean ± *SD*)

	Research day	Group			
		CoG	SynG6	SynG12	SynG24
HCT (%)	0	22.5 ± 4.26	22.5 ± 2.96	23.9 ± 3.63	25.9 ± 3.46*
	28	18.4 ± 5.53	22.5 ± 3.87*	23.2 ± 5.98*	25.1 ± 4.72**
	56	18.6 ± 3.29	21.8 ± 3.60*	22.7 ± 5.31*	24.9 ± 4.39**
HGB(g dl^{-1})	0	9.1 ± 1.21	8.8 ± 1.01	9.1 ± 1.24	10.2 ± 1.04*
	28	8.2 ± 1.68	9.3 ± 0.70*	10.1 ± 0.92**	10.2 ± 0.74**
	56	8.6 ± 0.75	8.4 ± 0.85	9.1 ± 1.11**	9.9 ± 0.86**
RBC(x1012/L)	0	6.9 ± 1.51	7.4 ± 1.44	7.6 ± 1.81	8.0 ± 1.77
	28	6.7 ± 1.20	7.4 ± 0.83*	7.9 ± 1.21**	9.1 ± 0.82**
	56	7.2 ± 0.91	7.5 ± 1.23	7.4 ± 1.60	7.7 ± 1.56
WBC (x109/L)	0	8.9 ± 1.07	9.2 ± 1.56	9.9 ± 1.57*	9.9 ± 1.69
	28	9.5 ± 1.46	11.9 ± 1.81*	10.9 ± 2.52	9.8 ± 1.51
	56	10.9 ± 1.29	8.6 ± 1.73**	9.7 ± 2.50	10.2 ± 2.70
PLT(x109/L)	0	812.9 ± 216.66	870.1 ± 221.61	928.5 ± 168.51	668.3 ± 212.35
	28	992.2 ± 88.04	921.0 ± 202.85	716.7 ± 210.14**	673.4 ± 185.26**
	56	687.1 ± 211.78	703.0 ± 195.95*	666.2 ± 203.74**	649.0 ± 218.28**

*$p < 0.05$ compare to CoG; $p < 0.01$ compare to CoG.

All synbiotic groups HCT starting from 28th research day was higher ($p < 0.05$) compare with CoG, SynG24 it was significantly ($p < 0.01$) higher, but SynG6;12 ($p < 0.05$) higher than CoG (Table 3). In the end of the research. SynG6;12 it was ($p < 0.05$) higher, but SynG24 ($p < 0.01$) higher than CoG. HGB SynG12;24 starting from 28th research day was significantly higher ($p < 0.01$). In the middle of the research RBC all synbiotic groups was higher ($p < 0.05$) compare to CoG, but in 56th RBC was not affected by treatment (Table 3).

Leucocytes SynG6 group has higher ($p < 0.05$) than CoG in day 56th it was lower ($p < 0.01$) compare to CoG.

PLT was significantly ($p < 0.01$) influenced by inclusion of middle and high amount of synbiotic (SynG12, SynG24) (Table 3). Inclusion of middle (6 g) and high (12 g) amount of inulin and it combination with probiotic resulted ($p < 0.01$) in a significant increase in total protein (TP) concentration (Table 4).

Table 4. Blood clinicochemical variables of calves on 0, 28th and 56th research days (mean ± *SD*)

	Research day	Group KoG	PreG6	PreG12	PreG24	SynG6	SynG12	SynG24
TP	0	60 ± 1.5	59± 2.9	56 ± 3.32**	56 ± 3.8**	58 ± 2.7	60 ± 2.3	59 ± 2.3
($g\ L^{-1}$)	28	54 ± 3.8	54 ± 4.6	51 ± 10.9	58 ± 2.9**	54 ± 3.9	55 ± 2.7	54 ± 4.0
	56	58 ± 2.2	57 ± 2.4	63 ± 1.5**	61 ± 2.2**	58 ± 2.9	62 ± 2.5**	63 ± 3.2**
Alb	0	41 ± 1.1	42 ± 1.5	39 ± 1.9*	39 ± 1.2*	40 ± 2.5	42 ± 0.6**	41 ± 1.2
($g\ L^{-1}$)	28	37 ± 2.6	37 ± 2.8	35 ± 1.0**	36 ± 1.7	35 ± 0.7*	39 ± 1.5	37 ± 2.3
	56	39 ± 2.1	38 ± 1.1	37 ± 1.2*	38 ± 1.9	38 ± 1.5	38.1 ± 1.7	39 ± 1.9
Gl	0	5.5 ± 0.6	5.3 ± 0.6	5.0 ± 0.5	5.9 ± 1.5	5.7 ± 0.4	5.8 ± 0.3	5.3 ± 0.5
($mmol\ L^{-1}$)	28	5.9 ± 0.7	5.8 ± 0.6	4.5 ± 0.4**	4.6 ± 0.8**	5.8 ± 0.4	4.9 ± 0.4	4.6 ± 0.4
	56	4.9 ± 0.7	4.7 ± 0.8	4.1 ± 0.4**	4.5 ± 0.9*	4.8 ± 0.6	4.6 ± 0.5	4.2 ± 0.3**

*$p < 0.05$ compare to CoG; $p < 0.01$ compare to CoG.

As given in Table 4, serum Albumin (ALB) concentration was not influenced by prebiotic and synbiotic dietary treatments. Additionally, substitution of 6 g inulin markedly ($p < 0.01$) decreased the glucose level in the 28th and 56th day of research. High dose of inulin (12 g) decrease ($p < 0.05$) glucose level in the 28th and 56th day of the research. As given in Table 4, glucose concentration was not influenced by synbiotic dietary treatments, omitting high dose synbiotic group (SynG24) it was significantly ($p < 0.01$) decrease compare to CoG in the end of the study.

DISCUSSION

This study proved that adding inulin and inulin combination with the *Enterococcus faecium* bacteria to the feed, significantly greater weight gain occurred in all the prebiotic and synbiotic groups as compared to the control group. The present results are similar with author research (Adams et al., 2008; Masanetz et al., 2010; Król, 2011; Markowiak & Śliżewska, 2018; Jonova et al., 2021).

In our and other researchers' studies, the age of calves is variable, so, as has written Klinkon & Ježek (2007), the hematological and biochemical calves. Blood parameters are especially affected at the time when dry food (hay, concentrate) is fed after milk feeding. The main function of erythrocytes is gas transportation, supplying cells with oxygen and ensuring that they have sufficient hemoglobin levels (Awodi et al., 2005). In our study, number of erythrocytes in calves. PreG12;24 increased, which could be explained in a way that inulin effects the attraction of iron ions to cells (Tako et al., 2008). Throughout the study, control group animals generally had lower levels of hematocrit, erythrocytes, and hemoglobin than animals in the prebiotic groups. The lowest hemoglobin levels in control group were observed on day 28th of study, which coincides with studies by other authors (Mohri et al., 2007; Dar et al., 2017). Also in their study the lowest hemoglobin levels in calves were observed at the same age, which then tended to increase again. Such trend was not observed in calves of the prebiotic groups, which could indicate the better iron absorption. In CoG animals the HCT at 28th and 56th day is below normal, indicating lower oxygen saturation to cells in this group of animals. Masentez et al. (2011) in his study had not found significant differences in the total number of red blood cells between the prebiotic group and control group animals, but there was significantly higher level of hemoglobin in the groups were inulin was fed in comparison with control groups were lactulose was used. This coincides with the results of our study, where the medium and high doses of inulin feeding increases the level of HGB level.

Also, Król (2011) study with calves which received inulin 3 g day^{-1}, on day 56th the level of hemoglobin in the blood was significantly ($p \leq 0.05$) higher than the other groups, which are similar to the results of our study and approve the inulin intake positive effect on HGB raising in calves.

In our study, leukocyte counts showed the most stable results in the PreG24 group, CoG and PreG6 animals, with the most in the final stage of the study on the 56th day. At the end of the study, animals of the PreG24 group had less than the control group, but there were no significant differences observed during the rest of the study, what is similar to (Roodposhti & Dabiri, 2012). Also, Król (2011) in his study where was fed inulin 6 g day^{-1} on the 56th day of the study obtained a higher ($p \leq 0.05$) amount of leukocytes than in the other groups, indicating that feeding higher doses of prebiotics increases the number of leukocytes in the body. This may be due to an inflammatory response in the intestines to a high dose of prebiotics. Also El-Mehanna1et al. (2017) in their study found that the total white blood cell count is higher in the prebiotic and synbiotic group than in the control group, which would confirm this theory.

The thrombocytes count in control group animals were significantly higher throughout the study compared to the prebiotic and synbiotic group. Lower platelet counts reduce the risk of blood clots and cardiovascular disease, which is due to the intake of high-fiber foods such as prebiotics (Bagger et al., 1996). Król (2011) found that the control group animal's platelet count is three times higher than for calves which were fed prebiotics.

During postnatal development, the age of calves is cited as a major factor influencing total protein levels, emphasizing that it is lower in calves than in cows (Shil et al., 2012). Tao et al. (2015) fed beta glucans to calves and concluded that they did not affect total protein level, as well as albumin. In contrast, our study showed that calves that received a middle dose of inulin (12 g day^{-1}) has a higher ($p < 0.01$) total protein level than CoG, at the end of the study PreG12, SinG6 and SinG12 groups ($p < 0.01$) was higher than CoG. Albumin, in our study, reaches the lowest point on the day 28th which coincides with the time of fecal mass dilution, which is one of the possible causes of the decrease of albumin.

It is observed that glucoses in blood serum in calves has higher than in cows, which could be explained by the fact that in calves the stomach initially functions similarly to monogastric animals. When calves become older, the level of glucoses is decreasing (Shil et al., 2012), which is similar in our study. Feeding different doses of prebiotics or probiotics, several authors mention that no significant differences in serum glucose levels were observed compared to control group and experimental groups (Adams et al., 2008; Dar & Para, 2019). However, in our study, the animals of PreG12 group on the days 28^{th} and 56^{th} had ($p < 0.01$) lower glucose levels than control group animals. Similarly, in SinG24 it is significantly ($p < 0.05$) lower than in control group. El-Mehanna1 et al. (2017) in studies with lambs fed by FOS and synbiotics, hadn't observed, significant differences ($p > 0.05$) in glucose level. Similarly, Ghorbani et al. (2002) combining the use of *Propionibacterium* and *Enterococcus faecium* did not have any significant differences with in comparison with the control group.

CONCLUSIONS

By feeding inulin 6 and 12 g day^{-1}, it is possible to achieve a higher weight gain, which is significantly higher than in control group. Medium and high doses of inulin affect the levels of red blood cells, hemoglobin and hematocrit in the blood of animals. Inulin feeding can affect blood parameters of calves of transitional feeding age so also do the synbiotics. However, the results with feeding synbiotics were not providing conclusive results on the improvement of animal's blood parameters.

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The ethical concerns of this study, animal protection and wellbeing were reviewed by Latvia University of Life Sciences and Technologies conducted LL Animal Welfare and Protection Ethics Council. Permission for this study was granted (Nr DZLAEP-2017/2).

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Evaluation of the combining ability of CMS lines in crosses with samples of grain sorghum and Sudan grass

O. Kibalnik*, S. Kukoleva, D. Semin, I. Efremova and V. Starchak

FSBSI Russian Research and Design-Technological Institute of Sorghum and Corn, 1-y Institutskiy Passage, 4, RU410050 Saratov, Russia
*Correspondence: kibalnik79@yandex.ru

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Abstract. The versatility of use, species diversity, and high drought resistance have ensured the demand for sorghum among agricultural crops in the world. Currently, the most promising direction of breeding is the creation of F1 sorghum hybrids, which is based on the identification and selection of parental forms with high combining ability. In this paper, the combining ability analysis crosses of two hybridization schemes: grain×grain sorghum and grain sorghum×Sudan grass was carried out using topcross method. Gene action governing inheritance of a particular trait can be measured in terms of general and specific combining ability estimates, where general combining ability effects are mostly indicative of additive gene action ($ms_{(GCA)}/ms_{(SCA)}>1$)and specific combining ability effects - non-additive ($ms_{(GCA)}/ms_{(SCA)}<1$). Genes with an additive effect participate in the genetic control of breeding-valuable traits, which is proven by$ms_{(GCA)}/ms_{(SCA)}$ ratios changing within1.10-28.01 range. It is advisable to involve CMS-lines A3 Feterita 14, A2 KVV 114 and A2 O-1237 to create high-yielding hybrids of grain sorghum; highly productive sorghum-sudangrasshybridss - lines L-106, L-143 and Anastasiya, Kinelskaya 100, Elegiya variety samples; with a high share of grain in the total biomass – Allegoriya, Krasnodarskaya 75 and Zonalskaya 6 variety samples. CMS lines are distinguished by high and average CA values both in crosses with samples of grain sorghum and Sudan grass: by weight of 1,000 grains - A2 O-1237; by seed yield and weight of 1,000 grains - A2 KVV 114.

Key words: sorghum, Sudan grass, CMS lines, CMS types, F1 hybrids, GCA effects, SCA dispersion, agronomic traits.

INTRODUCTION

Sorghum is one of the most popular agricultural crops in the world, such as wheat, rice, corn, and barley. In Africa and Asia, sorghum is not only a forage crop, but also a food crop (Hariprasanna & Rakshit, 2016). In Russia, most of the acreage under sorghum is formed in the Volga, Southern and North Caucasus Federal Districts. The main use of grain sorghum is feeding, grass and sugar sorghum - forage. This crop is characterized by drought resistance, ability to form a sufficiently high productivity in the conditions of climate change in the direction of aridization (Pannacci & Bartolini, 2018; He et al., 2020). Therefore, the genetic improvement of the source material for use in increasing the productivity of grain and biomass remains relevant, as well as the quality of the resulting products, especially in arid regions around the world.

Currently, breeding work is aimed at creating heterotic F1 hybrids. It should be noted that sorghum hybrids are characterized by superiority over the parental forms for productivity and other important agronomic traits. The heterosis effect has been widely used by breeders for a long time(He et al., 2020). For large-scale production of hybrids, it is necessary to use lines with cytoplasmic male sterility (CMS). Therefore, the determination of the general (GCA) and specific combining ability (SCA) of the components of the cross scheme is an important stage of breeding for heterosis (Justin et al., 2015; Kibalnik, 2017). GCA is understood as the average value of the parent line in combinations of crosses with its participation. The effect of GCA is determined by the action of additive genes. SCA evaluation allows for the characterization of each hybrid combination separately, when they turn out to be worse or better than expected based only on the effects of the GCA of the parent forms in the crossing scheme; it is controlled by the dominant and epistatic action of genes (Kumar et al., 2013; Khotyleva et al., 2016). It is noted that the components of crosses with high values of GCA effects are sources of agronomically valuable traits. Moreover, such parental forms are more adapted to the growing conditions (Fasahat et al., 2016). Analysis of SCA effects allows to identify promising hybrid combinations. In sorghum breeding, test and diallel crosses are the most commonly used methods for assessing the combining ability (Justin et al., 2015; Patil & Kute, 2015; Rocha et al., 2018; Da Silva et al., 2020). Thus, the analysis of male and female forms makes it possible to include the working collection more effectively in breeding programs on identification of heterotic hybrid combinations, to determine the inheritance of the analyzed traits and the action of genes/groups of genes that control the studied traits (Zhuzhukin et al., 2017; Oliveira et al., 2019).

Special attention should be paid to the use of different CMS sources in breeding in order to expand the genetic diversity of first-generation hybrids (Kibalnik & Semin, 2018). Cytoplasm A1 (*milo*) is the main and most common type of sterility in sorghum, used in breeding programs for the creation of grain and feed hybrids (Reddy et al., 2009; Aruna et al., 2012). Alternative types of CMS are included in the crossing schemes less frequently and are not fully studied (Kibalnik, 2017). Therefore, the purpose of the research was to evaluate the combining ability of CMS lines based on sterile cytoplasms A1, A2, A3, A4, 9E, M-351A in test crosses with samples of grain sorghum and Sudan grass.

MATERIALS AND METHODS

Field studies were conducted at the experimental field of the Institute in 2015–2018 (city of Saratov, Russia). The experimental area is characterized by the extreme continental climate. The temperature regime and water availability conditions are uneven. Frequent occurrence of soil and air drought is a peculiarity of the summer period. Average yearly precipitation varies between 250 and 450 mm, evaporation for the period from April to October amounts to from 450 to 770 mm. An average of 170–175 mm of precipitation falls within the vegetation period of sorghum. Yearly average air temperature amounts to +4.8 °C. The absolute temperature minimum is observed in January (- 40 °C), maximum - July-August (+ 42 °C). The sum of active temperatures (over +10 °C) for sorghum active vegetation period amounts to 2,400–3,100 °C. In 2015, the hydrothermal coefficient was 0.41, which indicates a strongly pronounced lack of natural moisture supply for plant development. In 2016–2018, the vegetation of hybrids and parental forms took place in 'arid' conditions (HTC = 0.64-0.90). The sum of active temperatures during the study period varied in the range of 2,613.1–2,805.0 °C, and the amount of precipitation - 106.2–178.3 mm. Weather conditions for the study seasons were different (Fig. 1).

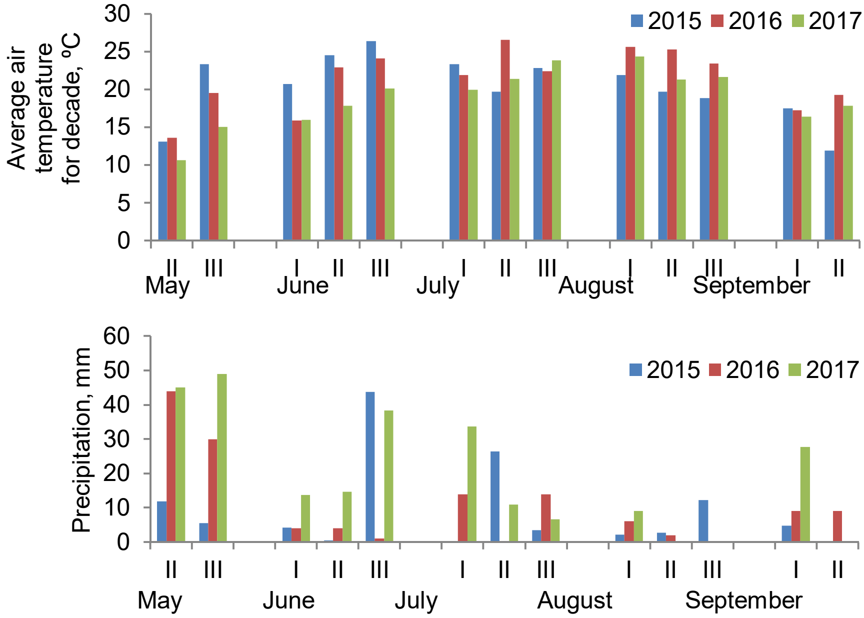

Figure 1. Meteorological conditions during the growing season of sorghum (2015–2017).

The soil of the experimental site is represented by southern medium-loamy chernozem. The humus content in the arable layer is 3.5%. Nitrification ability - 7.7 mg kg^{-1}; phosphorus - 34.2–35.7 mg kg^{-1}, potassium (in a carbon ammonium extract) - 349–378 mg kg^{-1}. Reaction of the soil environment pH = 6.3–6.4. Content of mobile trace elements, mg kg^{-1}: sulfur - 6.0–6.1, manganese - 4.6–5.5, copper - 0.07–0.11, zinc - 0.25–0.27; boron - 1.60–1.75.

The cultivation technology included the main technological operations: plowing, pre-sowing cultivation, sowing, post-sowing rolling, three row-to-row cultivations (the last one with hilling), harvesting. The predecessor is fallow. F_1 hybrids and parental forms were sown in a wide-row method with a row spacing of 70 cm in the third decade of May. The repetition in the experiment is threefold. The placement of plots with an area of 7.7 m^2 is randomized. The plant stand was set manually - 100 thousand pl. ha^{-1}. Traits' evaluation and the yield accounting were carried out according to the method of the state testing of agricultural crops (Methods of state variety testing of agricultural crops, 1989). The combining ability of the parental forms was determined by the topcross method (Savchenko, 1973). The following traits were analyzed: plant height, panicle length, panicle stem extension, weight of 1,000 grains, seed and biomass yield.

In this paper, two schemes of test crosses were considered. The first hybridization scheme (2015–2017) is aimed at creating a highly productive hybrid of grain sorghum. F1 hybrids (49 in total) were obtained on the basis of seven CMS lines: A1 O-Yang 1, A2 KVV 114, A2 O-1237, A3 Feterita 14, A4 KP 70, M-35-1A Pishchevoe 614, 9E Pishchevoe 614 (Elkonin et al., 1997). Seven varieties were used as pollinators (testers): Mercury, Ogonyok, Avans, Topaz, Volzhskoe 615, Pishchevoe 35, Volzhskoe 4.

In order to develop a productive sorghum-Sudangrass hybrid, a second hybridization scheme was created (2016–2017), including three CMS lines (testers) of grain sorghum - A2 O-1237, A2 KVV 114, A1 Efremovskoe 2 and 17 pollinators of Sudan grass - Zonalskaya 6, Chishminskayarannyaya, Krasnodarskaya 75, Kinelskaya 100, Zernogradskaya, Yaktash, Yubileynaya 20, Saratovskaya 1183, Zemlyachka, Allegoriya, Ambitsiya, Anastasiya, Faina, Elegiya, L-106, L-143, MEV-728.

Statistical processing of experimental research results was performed by a factor analysis of variance using the program 'Agros 2.09'.

RESULTSANDDISCUSSION

First generation hybrids' testing is carried out to obtain results on parental forms' combining ability (Khotyleva et al., 2016).The determination of the combining ability of the parental forms by test crosses method takes place in two stages: first, the differences between the hybrids in this crossing scheme are determined, and then the effects of GCA and SCA dispersion in the cross components (Savchenko, 1973; Zhuzhukin et al., 2017). There sults of the dispersion analysis in the first scheme of crosses showed significant differences of F1 hybrids for all traits except for seed yield. However, it was established that it indicated asignificant in fluence of the pollinator on the manifestation of all the studied traits ($p \leq 0.05$). There was no significant influence of the CMS-line and the interaction of factors (CMS-line×pollinator) on the formation of grain yield (Table 1).When calculating the combining ability of parental forms, variability of F1 hybrids obtained on their basis was also reflected in a number of publications (Justin et al., 2015; Kibalnik, 2017; Oliveira et al., 2019).

Table 1. Variance analysis of the combining ability of sorghum CMS-lines for economically valuable traits (2015–2017)

Source	df	Plant height	Panicle length	Panicle stem extension	Weight of 1,000 grains	Seed yield
$F_{(hybrid)}$	48	8.03*	2.11*	2.50*	33.80*	0.90
$F_{(CMS\text{-}line)}$	6	10.98*	2.35*	3.09*	6.44*	1.49
$F_{(pollinator)}$	6	43.88*	10.59*	11.38*	18.24*	2.20*
$F_{(CMS\text{-}line\times pollinator)}$	36	1.56*	0.66	0.92	1.28	0.59
ms		87.06	9.48	17.51	8.34	2.26
$F_{(GCA\ CMS\text{-}line)}$	6	43.90*	10.59*	11.40*	18.35*	2.20*
$F_{(GCA\ pollinator)}$	6	10.98*	2.36*	3.09*	6.46*	1.48
$F_{(SCA)}$	36	1.56*	0.66	0.92	1.27	0.59
$ms_{(GCA)}$		1,274.32	33.50	66.54	51.08	1.66
$ms_{(SCA)}$		45.49	2.09	5.37	3.55	0.45
$ms_{(GCA)}/ms_{(SCA)}$		28.01	16.03	12.39	14.39	3.69

Note: * $p \leq 0.05$.

This table also contains significant effects of sterile lines' GCA by five main breeding traits ($p \leq 0.05$). The $ms_{(GCA)}/ms_{(SCA)}$ ratios varied in the range of 3.69–28.01, which indicates the predominant influence of additive genes over non-additive ones in the genetic control of breeding-valuable traits.

To a greater extent, test crossings by top cross methodal low to allocate parental forms with high GCA, where as the system of diallelic crossings gives the fullest information on combining ability (Khotyleva et al., 2016). High GCA components effects of crossings indicate their valueas a parental form and possess the most practical interest in the creation of perspective hybrids (Patil & Kute, 2015). In our research, CMS line on the basis of sterile A2 cytoplasm with positive GCA effects by a number of economic traits was allocated. The female form A2 O-1237 is characterized by an average common general combining ability in plant height (4.17), panicle stem extension (0.89), weight of 1,000 grain (0.36), seed yield (0.63), but low for panicle length (-0.49) (Fig. 2). It should be noted that literary data also contain information on Sudan grass CMS lines with high GCA on a complex of main selection traits -panicle length, panicle mass, weight of 1,000 grains, seed yield (Rafiq et al., 2002; Kibalnik, 2017).

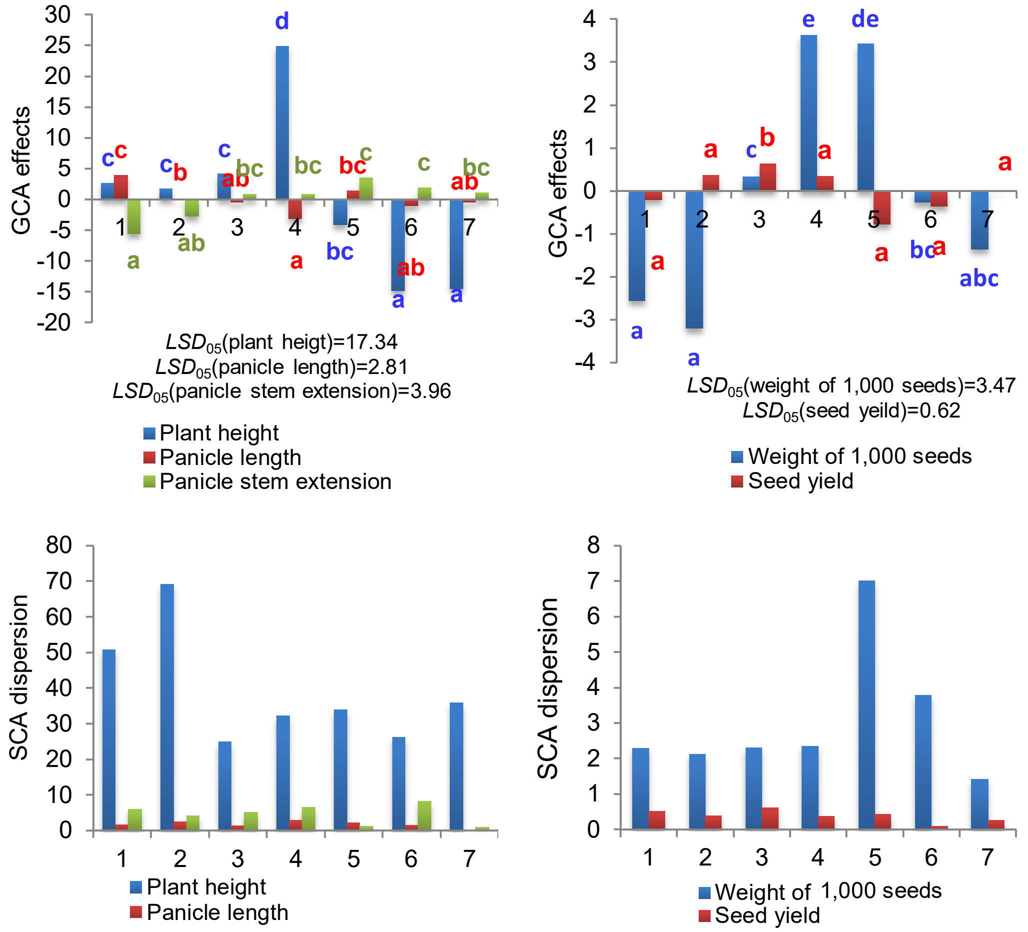

Figure 2. Effects of GCA and SCA dispersion of sorghum CMS-lines (2015–2017).

Note: 1. A1 O-Yang 1; 2. A2 KVV 114; 3. A2 O-1237; 4. A3 Feterita 14; 5. A4 KP 70; 6. M35-1A Pishchevoye 614; 7. 9E Pishchevoye 614.Data followed by the same letter did not differ significantly ($p \leq 0.05$) according to Duncan Multiple Range Test.

High SCA dispersion values for most of the studied traits were observed in CMS-lines A1 O-Yang 1 and A2 KVV 114. Grain sorghum hybrids when used on grain forage should be cultivated in a region with a plant height of no more than 130–150 cm. Therefore, the parental forms A4 KP 70, M-35-1A and 9E Pishchevoye 614 with low GCA effects (-14.78 – -4.11) are promising for such hybrid breeding programs. The breeding value of the CMS-line A4 KP 70 also lies in the fact that in crosses with individual male forms, the offspring has a fairly large grain: the SCA dispersion for weight of 1,000 grains was 7.02.Evaluation of the combining ability of CMS-lines showed that A3 Feterita 14 is characterized by high GCA effects in plant height (24.88), weight of 1,000 grains (3.62) and seed yield (0.34) and average SCA dispersions - 32.28; 2.34; 0.38, respectively (Fig. 2).

The results of the variance analysis confirm the significant differences between sorghum-Sudangrass hybrids ($p < 0.05$), as well as the significant influence of the male and female forms, their interaction for the manifestation of all the studied traits annually. It was found that in the genetic control of traits (weight of 1,000 grains, seed yields and biomass yield) genes with an additive effect are involved: the ratio of standard deviations varied in the range of 1.10–6.60 (Table 2).

Table 2. Variance analysis of the combining ability of CMS-lines of grain sorghum and variety samples of Sudan grass by economically valuable traits (2016–2017)

Source	df		Biomass yield		Weight of 1,000 grains		Seed yield	
	2016	2017	2016	2017	2016	2017	2016	2017
$F_{(hybrid)}$	41	35	154.03*	121.59*	129.22*	82.10*	29.11*	101.07*
$F_{(CMS\text{-}line)}$	2	2	345.10*	3.48*	864.24*	386.49*	40.40*	138.34*
$F_{(pollinator)}$	13	11	220.62*	174.03*	108.62*	104.09*	30.41*	227.73*
$F_{(CMS\text{-}line \times pollinator)}$	26	22	106.03*	106.11*	82.98*	43.44*	27.59*	34.35*
ms	82	70	0.51	0.63	0.23	0.43	0.16	0.05
$F_{(GCA\ pollinator)}$	13	11	220.44*	173.96*	108.64*	104.01*	30.49*	228.56*
$F_{(GCA\ CMS\text{-}line)}$	2	2	344.81*	3.50*	864.47*	386.28*	40.48*	139.35*
$F_{(SCA)}$	26	22	105.95*	106.06*	82.99*	43.40*	27.66*	34.54*
$ms_{(GCA)}$			37.11	36.59	8.19	14.80	1.66	3.43
$ms_{(SCA)}$			17.84	22.31	6.25	6.18	1.51	0.52
$ms_{(GCA)}/ms_{(SCA)}$			2.08	1.64	1.31	2.39	1.10	6.60

Note: * $p \leq 0.05$.

Sorghum-Sudangrass hybrids are used not only for green forage, but also for grain storage, silage. For this purpose, the yield of F1 hybrids is accounted in the wax ripeness phase. Of the male forms, the high combining ability for the productivity of the entire biomass yield for 2016–2017 was established in the line of Sudan grass L-106 of the institute's breeding: the GCA effects were 5.66–7.84, the SCA dispersion - 4.25–57.68 (Table 3). The highest SCA dispersion in some years were also characterized by the variety samples Anastasiya, Kinelskaya 100, Elegiya (21.68–116.39) and the line L-143 (71.07). If the sorghum-Sudangrass hybrid is intended for grain-haylage, then it is necessary to take into account the share of seed productivity. In general, during the study period, a high combining ability for seed yield was established in the variety samples of Allegoriya, Krasnodarskaya 75 and Zonalskaya 6: the GCA effects varied in the range of 0.08–1.46; the SCA dispersion - 0.33–3.05. The variety samples of Zemlyachka, Chishminskayarannyaya and the line MEV-728 in 2016 were characterized by a high specific combining ability (2.86–3.58). In 2016–2017, the variety sample Saratovskaya 1183 was distinguished by an average combining ability by weight of 1,000 grains: the GCA effects - 0.17–3.49, and the SCA dispersion - 0.55–4.03. In some years of the test, high CA for this trait was observed in Yaktash, Zemlyachka, and Elegiya: 1.44–2.62 and 7.31–25.29, respectively.

Table 3. Combining ability of components of sorghum-Sudangrass hybrids crosses according to the main agronomic traits, 2016–2017

Variety sample	Biomass yield				Weight of 1,000 grains				Seed yield			
	2016		2017		2016		2017		2016		2017	
	GCA[1]	SCA[2]	GCA	SCA	GCA	SCA	GCA	SCA	GCA	SCA	GCA	SCA
Variety samples of Sudan grass												
Saratovskaya 1183	1.97	2.76	-2.38	0.05	0.17	4.03	3.49	0.55	-0.74	1.63	2.35	0.81
Zonalskaya 6	0.04	4.01	–	–	0.27	7.56	–	–	0.92	0.52	–	–
Yubileynaya 20	-4.76	5.10	-4.06	8.26	0.84	4.33	-0.08	6.93	-0.44	0.16	0.10	0.02
Ambitsiya	2.70	36.24	–	–	0.64	11.03	–	–	0.13	0.22	–	–
Allegoriya	2.34	0.64	-1.23	5.80	0.31	0.84	-0.88	6.59	0.76	0.33	1.46	2.42
Elegiya	–	–	-0.80	116.39	–	–	2.62	25.29	–	–	-1.53	0.33
L-143	-2.59	71.07	-2.85	1.05	1.21	2.31	-1.81	0.06	-0.67	1.56	0.42	0.01
Faina	–	–	2.92	16.48	–	–	0.62	3.62	–	–	-0.90	0.46
MEV-728	-0.53	8.02	2.71	1.77	-1.13	13.76	1.49	12.01	-0.54	3.14	-0.20	0.02
L-106	7.84	4.25	5.66	57.68	-2.19	12.06	-4.78	8.93	1.23	0.29	-0.09	0.30
Chishminskaya rannyaya	-3.59	21.36	–	–	-0.76	1.57	–	–	0.46	3.58	–	–
Krasnodarskaya 75	0.84	19.66	-0.82	13.69	-1.16	3.95	0.79	1.10	1.00	3.05	0.08	0.35
Kinelskaya 100	-2.44	54.42	–	–	-3.76	1.11	–	–	-0.93	0.26	–	–
Zernogradskaya	-4.62	2.69	-0.24	0.09	2.23	1.01	-1.94	1.41	-0.65	0.29	-0.94	0.004
Yaktash	-0.63	1.37	–	–	1.87	7.31	–	–	-0.04	1.63	–	–
Zemlyachka	3.43	0.27	-4.58	2.45	1.44	10.39	-0.54	1.32	-0.51	2.86	-0.79	0.06
Anastasiya	–	–	5.67	21.68	–	–	1.02	0.14	–	–	0.04	0.91
CMS-lines of grain sorghum												
A2 O-1237	-2.23	5.42	0.02	20.63	2.49	5.10	1.99	3.90	-0.45	0.92	0.45	0.26
A2 KVV 114	0.47	12.57	-0.26	9.38	-1.17	3.80	0.28	4.95	0.16	0.96	-0.09	0.52
A1Efremovskoe 2	1.76	17.68	0.24	14.61	-1.32	3.57	-2.27	3.50	0.29	1.13	-0.37	0.26

Note:[1] GCA – GCA effects; [2] SCA – SCA dispersion.

In addition, it should be noted that sorghum-Sudangrass hybrids based on A1 Efremovskoe 2 differed in biomass yield productivity every year. The positive GCA effects of the CMS-line were 0.24–1.76, and the SCA dispersion was 14.61–17.68. Hybrids based on A2 O-1237 were distinguished by weight of 1,000 grains. This line has high GCA effects (1.99–2.49) and SCA dispersion (3.90–5.10). CMS-line A2 KVV 114 was characterized by an average combining ability for productivity, and the manifestation of the GCA effects depended on the growing conditions.

The selection of cross components in breeding for heterosis is of exceptional importance since the hybrid productivity depends on the genetic potential of the parental forms. Therefore, the study of the combinational ability of the source material is a fundamental step in hybrid breeding. It should be noted that hybrid sorghum breeding in most cases is based on the use of CMS lines with a high combinational ability for morphological traits and yield elements. Nevertheless, they were obtained on the A1 cytoplasm (Mahdy et al., 2011; Patil & Kute, 2015). To expand the genetic diversity of the source material, it is proposed to involve sterile lines with different CMS types as parental forms (Kibalnik, 2017). Currently, a great number of CMS-inducing cytoplasms are known in sorghum (Reddy et al., 2005). The discovery of new sterility sources requires a study of their breeding value. This direction is reflected in the few works of researchers, in which the assessment of the combining ability of CMS lines based on three types of cytoplasms - A1, A2 and A3 is considered (Mohammed, 2009; Reddy et al., 2009; Aruna et al., 2012; Zhou et al., 2021).

The presented experimental data demonstrate the expedient inclusion of the studied CMS lines obtained on the basis of the following sterility sources - A1, A2, A3, A4, 9E and M-35-1A - into the crossbreeding programs for the breeding of heterotic F1 sorghum hybrids. As a result of the analysis of combining ability, parental forms with high GCA effects and SCA variances were identified. Involvement in hybridization of grain sorghum and Sudanese grass samples as paternal forms made it possible to identify two CMS lines with the greatest significance in practical breeding - A2 O-1237 and A2 KVB 114.Thusfemale lineA2 O-1237 has high indicators of GCA effects (0.36–2.49) and SCA dispersions (2.31–5.10) by weight of 1,000 grains; A2 KVB 114 has average values for seed yield (-0.09–0.63 and 0.52–0.96, respectively) and weight of 1,000 grains (-1.17–0.33 and 2.31–4.95, respectively). Understanding of the inheritance of agronomic traits is also necessary to create hybrids with given traits. In our study, it was revealed that genetic control of breeding-valuable traits in these hybridization schemes is carried out by genes with an additive effect, as evidenced by the values of the ratios of standard deviations 1.10–28.01 (Tables 1 & 2). There is also information in the literature that productivity and its elements (for example, weight of 1,000 grains) are under the control of additive genes (Akata et al., 2017; Chikuta et al., 2017; Kibalnik, 2017).

CONCLUSIONS

Hybrids of sorghum crops using cytoplasmic male sterility are distinguished by high yield of biomass and grain, which is in demand by the agricultural branches of different regions of sorghum production. The analysis of the combining ability of the parental forms of grain sorghum and Sudan grass of the two crossbreeding programs allowed to identify components with a high general and specific combining ability. It was found that in both directions of test crosses, the genetic control of the studied breeding-valuable traits is carried out by genes with an additive effect. It is notable that the sterile lines A2 O-1237 and A2 KVV 114 were characterized by a high combinational ability in terms of productivity elements both in crosses with grain sorghum and Sudanese grass samples, which indicates their significant breeding value.

It is reasonable to create highly productive hybrids of grain sorghum on the basis of CMS-lines - A3 Feterita 14, A2 O-1237, A2 KVV 114. To breed a sorghum-Sudangrass hybrid with high biomass productivity, it is recommended to use two lines L-106, L-143 and three variety samples Anastasiya, Kinelskaya 100, and Elegiya as the male form. When using a hybrid for grain-haylage, growing with a high proportion of grain in the total biomass, it is advisable to include the varieties Allegoriya, Krasnodarskaya 75 and Zonalskaya 6 in the hybridization program. To increase the seed size, it is necessary to involve the variety samples Saratovskaya 1183, Yaktash, Zemlyachka, and Elegiya in the crosses. It should be noted that the greatest practical significance in crosses with samples of grain sorghum and Sudan grass was established in two CMS-lines: A2 O-1237 has indicators of GCA effects (0.36-2.49) and SCA dispersions (2.31–5.10) by weight of 1,000 grains; A2 KVB 114 has average values for seed yield (-0.09–0.63 and 0.52–0.96, respectively) and weight of 1,000 grains (-1.17–0.33 and 2.31–4.95, respectively).

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Influence of the structural and functional characteristics of the seeding material on the yield structure elements and resistance to leaf diseases of spring soft wheat

L.E. Kolesnikov[1,*], N.S. Priyatkin[2], M.V. Arkhipov[2], I.E. Razumova[1], D.Yu. Radishevskiy[1] and Yu.R. Kolesnikova[3]

[1]Saint–Petersburg State Agrarian University, Faculty of Agrotechnologies, Soil science and Ecology, Department of Plant Protection and Quarantine, Petersburgskoe Shosse, 2, RU196601 St-Petersburg - Pushkin, Russia
[2]Agrophysical Research Institute (ARI), plant biophysics division, Grazhdanskiy ave. 14, RU195220 St-Petersburg, Russia
[3]Federal Research Center N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR), genetic resource of wheat department, plant introduction department, Bolshaya Morskaya ul. 42–44, RU190000, St-Petersburg, Russia
[*]Correspondence: kleon9@yandex.ru

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Abstract. The high-quality grain use when sowing is a necessary condition for obtaining a high yield. Along with the standard tests regulated by the ISTA (International Seed Testing Association), there are promising introscopic techniques for the seed material quality controlling - methods of microfocus radiography and gas discharge visualization (electrophotography). The effect of structural and functional characteristics of the seeding material on the wheat productivity and diseases resistance was studied out on the experimental field of the Vavilov All-Russian Institute of Plant Genetic Resources. Ten accessions of soft wheat with the 'parametric passport' (including more than thirty optical parameters, including gas discharge images, morpho- and densitometric analysis of X-ray patterns) were used as an experimental seeding material. Unviable wheat seeds, in comparison with healthy ones, were characterized mainly by a smaller area, form coefficient, standard deviation of three-dimensional fractality by isoline, entropy by isoline, higher brightness and standard deviation of the isoline radius of the gas discharge images. Morpho- and densitometric indices of unviable seeds differed in reduced values of the circle factor, roundness, minimum and maximum average brightness, but in greater elongation and optical density of the X-ray patterns projection. The intensity of wheat affection by diseases has varied depending on the structural and functional characteristics of seeds. It was noted, that the brown rust development decreased with an increase in the entropy by isoline, the contour irregularity and the average radius of the isoline.

Key words: disease resistance, gas discharge imaging, microfocus radiography, seed material, soft wheat, yield structure.

INTRODUCTION

The environmental safety of food production is one of the main requirements for agricultural products in modern conditions of the global agro-industrial sector. In Russia the concept of agricultural production development are revising and shifting it towards reducing the external anthropogenic impact on agrocenoses and creating favorable conditions for realizing their own potential (Pavlyushin & Lysov, 2019).

Smart farming system - this is an innovative technology that helps to improve the quality and quantity of agricultural products in the country (Bharath et al., 2020). A necessary condition for obtaining high yields of wheat and improve its quality is use complete grain when seeding (Šramkováa et al., 2009; Shpilev et al., 2018; Ricachenevsky et al., 2019). At the same time, the seed material is often of poor quality now. The part of grain crops' substandard seeds can reach up by 40% (Arkhipov et al., 2013a). In this regard, there are a number of standard tests regulated by ISTA (International Seed Testing Association), as well as promising introscopic methods for seed quality control (Pearson et al., 2007; Huang et al., 2015; Abud et al., 2018; Arruda et al., 2018; Severiano & Pinheiro, 2018).

The problem of the grain harvest preserving and protecting it from harmful organisms during storage is very relevant in agriculture, since a large number of agricultural products are stored without providing the necessary conditions for this. An important aspect of obtaining high-quality products is to determine the degree of grain contamination before it is stored by direct visual observation or using instrumental methods that reveal the hidden forms of seeds infection by diseases and damage by pests (Dumitru et al., 2020).

It is obvious that the phytosanitary control of crops with the use of advanced technologies and modern plant protection products is a necessary condition for high grain yields obtaining. It is notable that the seed material' phytosanitary condition, which would prevent the infectious diseases emergence and development and contributed to the improvement of crops, needs for the most attention (Gagkaeva et al., 2009; Firsova et al., 2019).

Instrumental high-tech analysis methods comply with modern requirements for identification of the seed material quality. They take into account the features and defects of the internal structure on which the viability of seeds depends. The possibilities and advantages of such methods and their extended informativeness are described in detail in the work of Musaev et al., 2017. The method of microfocus radiography through the seeds internal structure visualizing allows us to identify such signs as: the presence of internal injury, the biological degree of the germ and endosperm plumpness, the presence of diseases infection, infestation and damage by pests. For vegetable crops and their selection without germination, it is possible to determine the separateness, singleness, self-fertility. Differences between varietal-population and hybrid seeds (experiments with radish) are also well detected using microfocus radiographs by the degree of plumpness of internal structures.

The analysis of digital images of agricultural crops' seeds is an important tool not only for the study of their heterogeneity, but also serves as the basis for the creation of automatic seed sorters (separators) that allow to obtain the seed material with improved sowing qualities compared to the initial ones (Sandeep et al., 2013). The analysis of a combine grain yield monitoring system is given in the work of Risius (2014). The developed system comprised a yield meter, GNSS receiver and a computer installed with customized software, which, when assembled on a local rice combine, mapped real-time rice yield along with grain moisture content (Sirikun et al., 2021).

The method of soft-beam microfocus radiography has been successfully used for many years both in Russia (Arkhipov et al., 2016; Potrakhov et al., 2019; Priyatkin et al., 2019) and abroad (Burg et al., 1995; Moreira et al., 1999; Gomes-Junior et al., 2012; Silva et al., 2012 and 2013; Franco et al., 2015). X-ray densitometry is a promising method for non-destructive evaluation of the seed's internal morphology of various crops and has great potential for development in the field of seed testing (Gomes-Junior et al., 2012). The Agrophysical Research Institute has developed highly informative methods for conducting radiography of seeds and plants, as well as modern technical means for their implementation (Musaev et al., 2020). Image analysis has been used recently both to the seed quality assess and to some aspects of seed physiology clarify. X-rays can be used successfully to identify physical abnormalities and their occurrence correlation with the physiological potential of seeds (Pereira da Silva et al., 2020). It can be used to detect various structural seeds defects, such as crack, enzymomycosis depletion, internal germination, hidden pest infestation, mechanical injuries and germ defects, empty grain, etc. (Haff & Slaughter, 2004; Panchal et al., 2014).

A promising direction for assessing the biological objects' general condition is the gas-discharge visualization (GDV) method, which allows recording and analyzing the gas-discharge glow induced in biological objects and their structures. This method gives the opportunity to control the quality of plant raw materials, in particular, fruits, seeds and grains (Priyatkin et al., 2006a).

The gas-discharge visualization technique is an important part of a complex methodology for the seed material quality assessing, as it combines together the seeds assessment using morphometric and automated radiographic methods (Arkhipov et al., 2014). It allows to obtain the data about the biological and economic suitability of seed material, to predict the field germination of seeds and their potential productivity (Priyatkin et al., 2006b).

A preliminary analysis of the characteristics of the seeds' gas-discharge glow is an essential addition to the main standardized methods for the seed quality assessment (Arkhipov et al., 2016). The study of the seed surface characteristics is carried out in the wavelength ranges from near ultraviolet to near infrared, including using several light sources with different wavelengths, which allows multispectral images obtaining (Olesen et al., 2015). In the last 10 years, data have emerged on the possibility of using the terahertz imaging method to determine the varietal purity of seeds (Lu et al., 2005), the quality of seed material (Ge et al., 2014), as well as the early prediction of laboratory seed germination (Jiang et al., 2016).

The data on the possibility of using the terahertz imaging method to determine the varietal purity of seeds (Lu et al., 2005), the quality of seed material (Ge et al., 2014), as well as the early prediction of laboratory seed germination (Jiang et al., 2016) have obtained for the last 10 years.

However, the system analysis studies of changes in wheat productivity and resistance to pathogens depending on the seeds structural and functional characteristics have not been conducted. Earlier, the effectiveness of wheat stimulating treatments with microbiological biopreparations, was evaluated using the above methods of introscopic analysis of wheat grain, and the quality of the newly harvested crop and changes in wheat diseases resistance were analyzed (Kolesnikov et al., 2019, 2020a, 2020b).

The aim of the work is to reveal the dependence of the soft wheat productivity elements and diseases resistance on the structural and functional characteristics of seeds.

The indicators of the grains' structural and functional activity, associated with the hidden heterogeneity of the seed material, for the first time in the Leningrad region conditions were analyzed, and the interrelations in the change of the productivity elements and wheat diseases resistance were revealed by more than 30 optical parameters, including gas-discharge glow, morpho- and densitometric analysis of radiographs. The practical value of the work lies in the development of tools for programming the yield and increasing the wheat diseases resistance through the selection of high-quality seed material when determining its latent heterogeneity by microfocus radiography and gas-discharge visualization.

MATERIALS AND METHODS

The field experimental study the influence of structural and functional characteristics of soft wheat seed material on the wheat productivity and diseases resistance was carried out at the scientific and production base 'Pushkin and Pavlovsk Laboratories of VIR' of the Vavilov All-Russian Institute of Plant Genetic Resources (VIR). 10 cultivars of spring wheat (*Triticum aestivum* L.) from the collection of the wheat genetic resources Department of VIR were used as plant experimental material: Omskaya 18, k-58220; Kolkhoznitsa, k-30177; Saratovskaya 29, k-40599; Leningradka, k-47882; Moskovskaya 35, k-48762; HD 2329, k-58775; Leningradskaya 97, k-62935; Tulunskaya 12, k-64361; Rostan', k-64391; Ester, k-64544.

All grains of each of the 10 wheat cultivars had a 'parametric passport' (for more than 30 optical parameters, including gas - discharge glow, morpho- and densitometric analysis of X-rays) when assessing the impact of seed quality on wheat productivity and disease resistance. The sample size for each cultivar was 105 grains (Fig. 1).

Figure 1. Soft wheat grains used as seed material with a 'parametric passport'.

To take into account the individual characteristics of the seeds, a separation mesh was used. The area of the accounting plot for one sample was 1.3 m^2. Samples for plots were sown manually by an rows seeding method with row spacing of 15 cm and a distance in a row of 1–2 cm. Seed depth: 5–6 cm (Fig. 2).

Gas-discharge and X-ray characteristics of wheat seeds used as seed material were determined on the experimental equipment of the Plant Biophysics sector of the AFI Federal State Budgetary Institution.

Hardware and software of the method of gas-discharge visualization (electrophotography) of wheat seeds is presented by the serial device 'GRV-Camera' analysis of digital gas-discharge images 'GRV Scientific Laboratory'.

Figure 2. The beginning of the field experience for the study of the wheat yield and disease resistance dependence on the structural and functional characteristics of wheat seeds.

The method of gas-discharge visualization (electrophotography) of wheat seeds was carried out by the use of serial device 'GRV-Camera' in complete with software for the analysis of digital gas-discharge images 'GRV Scientific Laboratory'. Developer and manufacturer of hardware and software complex - LLC 'Biotechprogress'. This method allows to record and quantify the characteristics of the corona discharge that occurs when the seed is placed in a high-intensity electromagnetic field (Fig. 3).

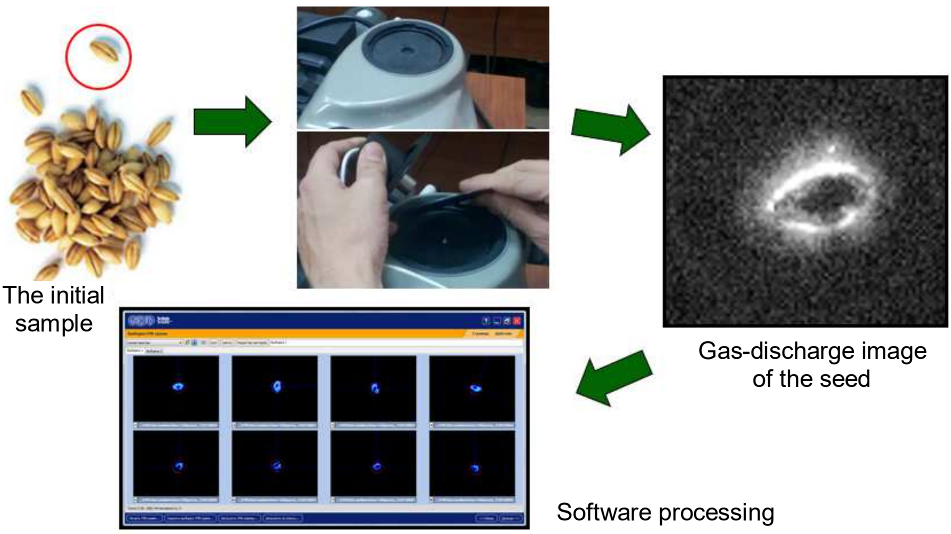

Figure 3. The algorithm for wheat seeds analysis by gas-discharge visualization.

When using the gas-discharge visualization method, wheat seeds were characterized by the following indicators: glow area (cm^2), total glow intensity (relative units), shape coefficient (relative units), average contour radius (pixels), normalized standard deviation of the contour radius (pixels), contour length (pixels), entropy calculated from the contour (relative units), fractality calculated from the contour (relative units), X-ray images were obtained on a serial mobile X-ray diagnostic unit PRDU-02 (CJSC 'ELTECH-Med'). The analysis of digital X-ray images of wheat seeds was performed by the use the tablet scanner EPSON Perfection V200 Photo and software 'Agrus-Bio' produced by LLC 'ArgusSoft' (Fig. 4).

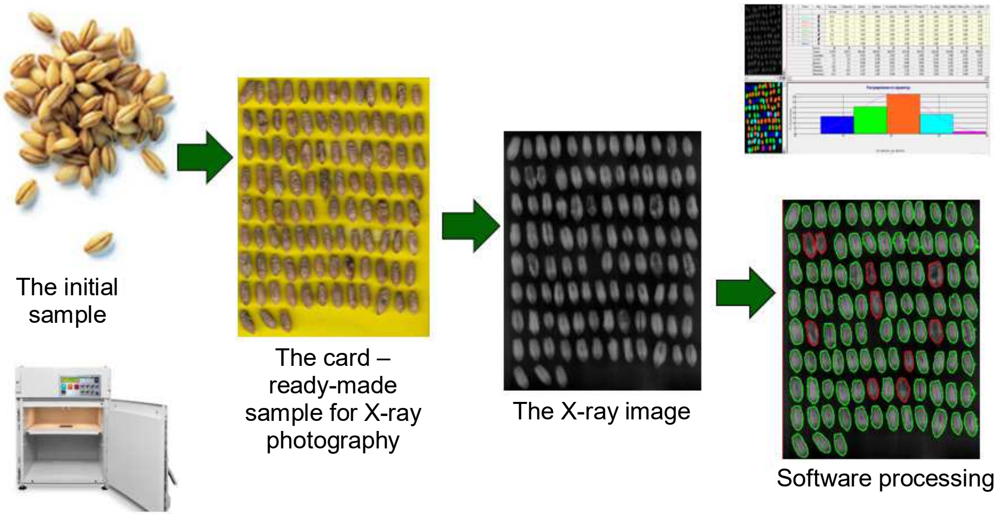

Figure 4. The algorithm for wheat seeds analysis by microfocus radiography.

When using the microfocus X-ray method, wheat seeds were characterized by the following indicators: the area of the X-ray projection of the seed (cm^2), the perimeter of the X-ray projection of the caryopsis (cm), the length of the X-ray projection of the seed (cm), the width of the X-ray projection of the seed (cm), the roundness of the X-ray projection of the seed (relative units), the elongation of the X-ray projection of the seed (relative units), the deviation of the brightness of the X-ray projection of the seed (units of brightness), the optical density of the X-ray projection of the seed (relative units) and the integral optical density of the X-ray projection of the seed (relative units).

Wheat productivity was studied in three times replication in the phases of germ shoot development, earing-flowering and maturation according to a set of indicators. In the germ shoot development phase, wheat cultivars were evaluated according to the generally accepted indicator - field germination (%). In the ear–flowering phase, the complex of plant indicators: the productive and total bushiness (pcs.), the plant phase (score, on the Zadox scale (Eucarpy), the flag and pre-flag leaf area (cm^2), the plant height (cm), the spike length (cm), the spikelets number per spike (pcs.), the spike weight (g) was studied. In addition, the number and length of roots (main germ root, germ and coleoptile roots) extending from the epicotyle were determined. The number and length of wheat nodal roots were taken into account. The roots' and plants' vegetative part weight was calculated (Kolesnikov, Kremenevskaya et al., 2020; Kolesnikov, Novikova et al., 2020).

In the maturation phase (phase of full ripeness) the wheat yields structure was studied by the indicators: the number of spikelets per spike, pcs.; the spike length, cm; the spike weight; the grains number per spike, pcs.; the grains weight, the 1,000 grains weight. The potential (biological) yield of a single wheat plant was calculated by the indicators: the productive bushiness and grain weight per one plant spike (g plant). The wheat cultivars' potential yield (Y_p) in relation to the sowing area (t ha^{-1}) was calculated by the productive bushiness, the grains weight per spike and the number of plants sown per 1 m^2. The assessment of the plant damage degree by diseases was carried out in the main phases of wheat ontogenesis (Kolesnikov, Kremenevskaya et al., 2020; Kolesnikov, Novikova et al., 2020).

The size of pathogens' infectious structures formed on leaves during pathogenesis (spots, pustules, etc.) was calculated using ocular and objective micrometers. The pustule area values for rust fungi and the spots area for powdery mildew were determined by the ellipse area formula (Fig. 5).

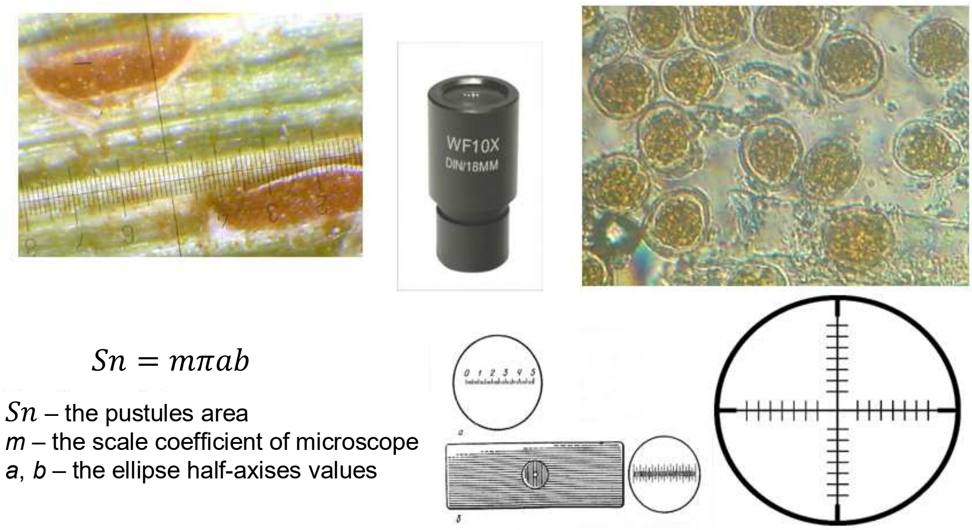

Figure 5. The wheat brown rust uredopustules' size calculation.

Statistical analysis of the experimental results was carried out in the PC programs SPSS 26.0, Statistica 10.0, Excel 2019. The calculations were performed by the use of parametric statistical methods (based on the *mean M* and their *standard errors* ± *SEM*, 95% confidence intervals, and Student's *t-test*). The relationships between the indicators were analyzed using *Spearman's rank correlation*. When identifying the most important factors that affect the dependent variable and constructing numerical dependencies, the method of nonlinear regression analysis was used.

RESULTS AND DISCUSSION

The possibility of the wheat seeds quality controlling using non - destructive testing methods: gas-discharge visualization and microfocus radiography for programming wheat yield and predicting changes in the intensity of wheat damage by diseases was studied as a result of the conducted research.

Identification of soft wheat non-viable and healthy seeds by gas-discharge visualization

The area of gas-discharge characteristics of soft wheat seeds was compared with the following signs of field germination: the seeds did not shoots (non - viable seeds - NVS); the seeds have shoots (viable (healthy, fulfilled) seeds -VS); the seeds have shoots, but the plants died (conditionally viable seeds - CVS). A slight decrease in the area of the gas-discharge image (GDI) of non-viable seeds that did not germinate, compared with viable seeds that sprouted, was found in 40% of the studied wheat cultivars (Saratovskaya 29, k-40599 - by 0.9%; Leningradskaya 97, k-62935 - by 8.0%; Tulunskaya 12, k-64361 - by 0.8%; Ester, k-64544 - by 0.1%). The greatest differences in the seeds GDI area change in the direction of decrease were noted in the CVS compared to the VS. This tendency was found in 80% of cultivars. The CVS was characterized by the GDI smaller area compared to VS (significantly by 12.0%, $t = 2.4$ - Saratovskaya 29, k-40599; by 14.0%, $t = 2.6$ - Leningradka, k-47882; by 6.7%, $t = 9.0$ - Tulunskaya 12, k-64361; by 19.0%, $t = 3.1$ - Ester, k-64544, and by 1.01% - Moskovskaya 35, k-48762; by 0.9% - HD 2329, k-58775; by 0.6% - Leningradskaya 97, k-62935; by 1.2% - Rostan', k-64391).

Compared with viable seeds, non-viable seeds were characterized mainly by large values of the brightness index of the gas-discharge image. This pattern was found in 70% of cultivars. The decrease in the average intensity of the gas discharge image brightness was revealed on the UVS of 30% cultivars (Moskovskaya 35, k-48762 - 2.1%; Leningradskaya 97, k-62935 - 1.2%; Tulunskaya 12, k-64361 - 5.0%) and on the CVS of 70% cultivars (significantly by 3.6%; $t = 2.9$ - Leningradskaya 97, k-62935; 7.8%; $t = 12.0$ - Tulunskaya 12, k-64361; 15.1%; $t = 3.0$ - Ester, k-64544, and also by 1.2% - Saratovskaya 29, k-40599; by 3.4% - Leningradka, k-47882; by 0.9% - Moskovskaya 35, k-48762). It should be noted that in the work of Arkhipov et al (2016), a negative correlation between this indicator and the parameters: seedling length (cm), the wheat flag leaf area, was found. The revealed regularity makes it possible to use the brightness indicator of the gas-discharge visualization for express selection of seeds with the best sowing qualities (Cater & Batic, 1998; Zanco, 2016).

The GDI shape coefficient may reflect the 'thinness' of grains caused by enzymomycosis depletion (Arkhipov et al., 2016). The shape coefficient is the ratio of the GRV image glow external contour' perimeter in a square to its area. In 40% of cultivars a decrease in the shape coefficient values on the CVS, compared to the VS, was recorded (significantly by 2.8%; $t = 2.8$ - Leningradka, k-47882; by 8.9%; $t = 2.8$ - Omskaya 18, k-58220; by 2.1%; $t = 3.0$ - Ester, k-64544, and also by 1.1% - Saratovskaya 29, k-40599). The shape coefficient values on the CVS decreased in 40% of cultivars compared to VS (by 10.3% - Kolkhoznitsa, k-30177; by 13.1% - Saratovskaya 29, k-40599; by 9.5% - Moskovskaya 35, k-48762; by 3.1% - HD 2329, k-58775. In the work of Arkhipov et al (2016), the positive correlation with the parameter 'GDI shape' and 'the 1,000 seeds weight' was found.

In comparison with VS, a decrease in three - dimensional fractality along the isoline of the gas-discharge image was found on 40% of the NVS cultivars (significantly by 2.8%; $t = 2.9$ - Leningradka, k-47882; by 8.9%; $t = 7.7$ - Omskaya 18, k-58220; by 2.1%; $t = 3.1$ - Ester, k-64544, and also by 1.1% - Saratovskaya 29, k-40599). A similar tendency was found in 40% of cultivars with the CVS (by 10.3% - Kolkhoznitsa, k-30177; by 13.1% - Saratovskaya 29, k-40599; by 9.5% - Moskovskaya 35, k-48762;

by 3.1% - HD 2329, k-58775). Supposedly, the fractal parameter depends on the seeds size characteristics. In the work of Arkhipov et al (2016) a positive correlation between this indicator and the 1,000 seeds weight was found. No such studies have been conducted in the world.

The standard deviation (SD) of GDI fractality was reduced in NVS (70% of cultivars) and CVS (60% of cultivars) compared to healthy seeds.

Reliable reduction in the gas discharge image contour irregularity of the NVS compared to VS was detected in 50% of cultivars (by 33%; t = 13.8 - Leningradka k-47882; 29.7%; t = 18.3 - Moskovskaya 35, k-48762; 19.9%; t = 12.5 - Omskaya 18, k-58220; 3.2%; t = 2.8 Tulunskaya 12, k-64361; 3.1%; t = 2.5 - Ester, k-64544) and 30% cultivars with CVS (by 84,5%; t = 5.0 - HD 2329, k-58775; 34.4%; t = 2.5 - Leningradskaya 97, k-62935; 19.9%; t = 3.2 - Tulunskaya 12, k-64361.

The isoline average radius displays the 'width' of the glow around the object, the normalized SD of the isoline radius makes it possible to estimate the unevenness of the glow width along the contour (Arkhipov et al., 2016).

In NVS of 40% of cultivars, a decrease in the isoline average radius in comparison with VS was observed (significantly by 6.0%; t = 2.3 - Moskovskaya 35, k-48762; by 4.7%; t = 3.6 - HD 2329, k-58775; by 12.9%; t = 5.1 - Leningradskaya 97, k-62935; by 4.3%; t = 2.5 - Ester, k-64544). This tendency was also revealed in the remainder of 60% of cultivars when analyzing the isoline average radius of the CVS compared to the VS (significantly by 24.0%; t = 7.5-Leningradka, k-47882; by 13.4%; t = 2.3-HD 2329, k-58775; by 9.2%; t = 2.3-Leningradskaya 97, k-62935; by 28.4%; t = 19.1 - Tulunskaya 12, k-64361; by 10.0%; t = 8.3 - Rostan', k-64391, and also by 6.4% - Ester, k-64544).

The increase in values of normalized SD GRI isoline radius on the CNV was detected in 60% of cultivars (significantly by 10.2%; t = 2.8 to Omskaya 18 k-58220; 11.3%; t = 2.5 - Leningradka, k-47882 and 2.8% - Kolkhoznitsa, k-30177; 2.2% - Saratovskaya 29, k-40599; 5.4% - Tulunskaya 12, k-64361; 4.4% - Rostan', k-64391) and on the CVS in 30% cultivars, compared to VS (by 11.3% - Kolkhoznitsa, k-30177; 8.9% - Saratovskaya 29, k-40599; 12.4% Moskovskaya 35, k-48762).

The entropy values along an isoline were reduced on the NVS and the CVS, compared to healthy seeds. This regularity was revealed in 60% of the cultivars on the NVS (significantly by 3.5%; t = 3.8 Kolkhoznitsa, k-30177; 0.8%; t = 4.1 - Saratovskaya 29, k-40599; 1.1%; t = 3.1 - Omskaya 18 k-58220; 0.7%; t = 6.8 - Leningradskaya 97, k-62935; 1.1%; t = 2.4 - Tulunskaya 12, k-64361) and in 30% of the cultivars on the CVS (2.5% Moskovskaya 35, k-48762; 0.9% Leningradskaya 97, k-62935; 4.9% - Ester, k-64544). In the work of Arkhipov et al. (2016) the positive correlation between this indicator and the seed germination energy was revealed.

The isoline length is an important indicator of seed quality. In our experience, the greatest decrease in the indicator was registered in the group of CVS, compared with the VS. This pattern was found in 60% of cultivars (significantly by 11.6%; t = 2.8 - Leningradka, k-47882; by 9.7%; t = 2.3 - HD 2329, k-58775; by 5.2%; t = 7.6 - Tulunskaya 12, k-64361; by 3.6%; t = 4.7 - Rostan', k-64391; by 5.7%; t = 2.7 - Ester, k-64544). Only 30% of cultivars were characterized by reduced values of the indicator on NVS. It should be noted that in the work of Arkhipov. et al (2016) a positive correlation between the isoline length and the 1,000 seeds weight was noted.

Previously, the GDI characteristics, obtained on wheat seeds that had no visible signs of damage - 'seemingly healthy', having a weak and strong degree of infection caused by the spike fusariose agent *Fusarium* spp., was studied by Priyatkin and co-authors (2006). It was revealed that 'seemingly healthy' seeds were characterized by the maximum values of the GDI parameters: brightness distribution, shape coefficient and three-dimensional fractality compared to infected seeds. GDI of 'seemingly healthy' seeds were distinguished by a more rugged contour and a variety of brightness spectrum than infected grains. When studying the seeds of common spruce (*Picea abies* L.) by gas-discharge imaging, it was noted that the NVS did not shown a gas-discharge glow, in contrast to the seeds that subsequently sprouted, which can be explained by the fact that the gas-discharge imaging method is sensitive to humidity and electrical conduction of the object. In NVS, humidity and electrical conduction are lower due to the absence of the germ and endosperm, so the initialization of gas-discharge glow under the specified modes of the GRV-Camera device did not occur (Arkhipov et al., 2013).

Identification of non-viable and viable soft wheat seeds by microfocus soft-beam radiography

One of the indicators, characterizing the 'endosperm fullness', is of the seed projection area. The most decrease in the grain projection area on the CVS compared to the VS was recorded. This regularity was found in 50% of cultivars (significantly by 5.4%; t = 2.8 - Leningradka, k-47882; by 8.3%; t = 2.4 - Moskovskaya 35, k-48762; by 12.3%; t = 11.4 - Tulunskaya 12, k - 64361, by 4.2% - Saratovskaya 29, k-40599, by 2.2% - Leningradskaya 97, k-62935). A decrease in the projection area of NVS compared to VS was recorded in 40% of cultivars (significantly by 3.3%; t = 2.4 - Saratovskaya 29, k-40599; by 2.1%; t = 2,3 - Omskaya 18, k-58220; by 5,6%; t = 2.2 - Leningradskaya 97, k-62935; by 2.6% - Rostan', k-64391).

The most expressed decrease in the seed projection perimeter on the CVS compared to the VS was registered. This pattern was found in 50% of cultivars (significantly by 7.8%; t = 12.3 - Tulunskaya 12, k-64361; by 3.1%; t = 2.1 - Leningradskaya 97, k-62935; by 0.8% - Saratovskaya 29, k-40599; by 0.1% - Leningradka, k-47882; by 1.6% - Moskovskaya 35, k-48762). A decrease in the NVS projection perimeter compared to the VS was registered only in 30% of cultivars (significantly by 0.7%; t = 2.1 - Omskaya 18, k-58220; by 2.7%; t = 3.2 - Leningradskaya 97, k-62935, and also by 0.9% - Saratovskaya 29, k-40599).

The most reduction in the seed projection length was recorded in the CVS compared to the VS. This pattern was found in 40% of cultivars (significantly by 9.5%, t = 2.5 - Leningradka, k-47882; by 12.5%, t = 2.5 - Moskovskaya 35, k-48762; by 6.2%, t = 9.0 - Tulunskaya 12, k-64361, and also by 1.8% - Leningradskaya 97, k-62935). A decrease in the NVS projection length in comparison with healthy seeds was also recorded on 40% of cultivars (significantly by 1.0%, t = 4.3 - Saratovskaya 29, k-40599; by 1.0%, t = 2.3 - Leningradka, k-47882; by 7.7%, t = 14.3 - Omskaya 18, k-58220; by 8.4%, t = 22.3 - Rostan', k-64391).

The most decrease in the seed projection width was registered in the CVS compared to the VS. This was found on 50% of cultivars (significantly by 7.2%, t = 9.4 - Moskovskaya 35, k-48762; by 2.7%, t = 5.1 - Tulunskaya 12, k-64361; by 5.0% - Saratovskaya 29, k-40599; by 1.5% - Leningradka, k-47882; by 2.4% - HD 2329, k-58775, by 0.2% - Leningradskaya 97, k-62935). A decrease in the NVS projection width compared to VS was also recorded on 50% of cultivars (significantly by 2.5%, t = 18.0 - Saratovskaya 29, k-40599; by 0.5%, t = 2.6 - Omskaya 18, k-58220; by 1.9%, t = 16.3 - HD 2329, k-58775; by 2.9%, t = 12.2 - Leningradskaya 97, k-62935; by 2.9%, t = 6.2 -Rostan', k-64391).

The most decrease in the seed projection' average chord was registered in the CVS compared to the VS. This pattern was found on 60% of cultivars (significantly by 10.2%, t = 9.3-Saratovskaya 29, k-40599; by 6.4%, t = 2.4 - Leningradka, k-47882; by 3.4%, t = 2.2 - Leningradskaya 97, k-62935; by 8.0%, t = 15.5 - Tulunskaya 12, k-64361; by 3.11%, t = 6.5 - Rostan', k-64391). A decrease in the NVS projection' average chord, compared with healthy seeds, was also recorded on 30% of cultivars (significantly by 3.8%, t = 26.2 - Kolkhoznitsa, k-30177; by 3.4%, t = 9.5 - Leningradskaya 97, k-62935; 6.8%, t = 44.1 kHz - Saratovskaya 29, k-40599).

70% of the NVS wheat cultivars in was characterized by greater elongation of the seed projection compared to VS (significantly by 1.8%, t = 24.9 - Saratovskaya 29, k-40599; 2.1%, t = 5.3 - Leningradka, k-47882; 2.4%, t = 31.6 - Moskovskaya 35, k-48762; 1.6%, t = 16.9 - HD 2329, k-58775; 2.8%, t = 8.9 - Tulunskaya 12, k-64361; 0.5%, t = 2.2 - Rostan', k-64391; 0.8% t = 5.2 - Ester, k-64544). A decrease in the of the CVS projection elongation, in comparison with the VS, was recorded in 50% of the cultivars.

The most decrease in the seed projection size was recorded on NVS. This pattern was found in 50% of cultivars (significantly by 1.5%, t = 9.8 - Saratovskaya 29, k-40599; by 0.3%, t = 22.7 - Omskaya 18, k-58220; by 2.1%, t = 2.8 - HD 2329, k-58775; by 0.1%, t = 5.6 - Leningradskaya 97, k-62935; by 5.9%, t = 12.8 - Rostan', k-64391). A decrease in the CVS size was shown by 30% of cultivars.

Smaller values of the NVS projection' circle factor compared to VS were detected in 70% of wheat cultivars (significantly by 1.3%, t = 9.8 - Kolkhoznitsa, k-30177; 1.5%, t = 8.8 - Saratovskaya 29, k-40599; 0.3%, t = 9.8 - Leningradka, k-47882; 0.3%, t = 9.1 - HD 2329, k-58775; 0.2%, t = 9.8 - Leningradskaya 97, k-62935; 0.1% t = 12.8 - Tulunskaya 12, k-64361; 0.3%, t = 13.8 - Rostan', k-64391). In the case of CVS, the tendency of circle factor decrease was observed only in 40% of cultivars.

The most increase in the values of the seed projection ellipse factor was registered on the CVS compared to the VS. This tendency was found in 60% of cultivars. In the same time, the NVS were characterized by lower values of the indicator in 50% of cultivars.

A decrease in the values of the seed projection roundness on the NVS and CVS, compared with the VS, was revealed. This trend was identified in 60% (reliably by 2.7%; t = 85.9 - Kolkhoznitsa, k-30177; by 1.9%; t = 104,8 - Saratovskaya 29, k-40599; 5.9%; t = 40.4 - Moskovskaya 35, k-48762; 0.6%; t = 3.9 - Leningradskaya 97, k-62935; 0.5%; t = 3.4 - Tulunskaya 12, k-64361; 3.2%; t = 103.2 - Ester, k-64544) and 70% of cultivars (significantly by 5.2%; t = 3.3 - Leningradskaya 97, k-62935; 3.3%; t = 5.7 - Tulunskaya 12, k-64361; 22.1%; t = 42.1 - Rostan', k-64391, by 5.0%; t = 5.7 - Kolkhoznitsa, k-30177; by 6.2% - Saratovskaya 29, k-40599; by 7.0% - Leningradka, k-47882), respectively.

A lower average brightness of the NVS projection was marked in 40% of wheat cultivars compared to the VS (significantly by 0.8%, t = 2.2 - Saratovskaya 29, k-40599; 1.7%, t = 3.7 - Omskaya 18 k-58220; 3.7%, t = 2.6 - Rostan', k-64391 and reliably by 6.4%, t = 2.6 - Moskovskaya 35, k-48762; 3.7%, t = 11.3 - Rostan', k-64391; 7.0%, t = 0.9 - Leningradka k-47882; 1.5%, t = 2.9 - Saratovskaya 29, k-40599).

The NVS and CVS were distinguished by a greater SD of the seed projection' average brightness compared to VS. This dependence revealed in 40% of the cultivars (significantly by 2.7%, t = 4.0 - Moskovskaya 35, k-48762; 3.6%, t = 5.9 - Leningradskaya 97, k-62935; 3.3%, t = 7.5 - Tulunskaya 12, k-64361; 5.6%, t = 14.6 - Rostan', k-64391 and 13.6%, t = 2.4 - Leningradka, k-47882; 6.9%, t = 2.6 - Leningradskaya 97, k-62935; 4.8%, t = 2.3 - Saratovskaya 29, k-40599).

The decrease in the minimum average brightness of the NVS projection compared to the VS was revealed in 60% of cultivars (significantly by 0.5%, t = 6.0 to Saratovskaya 29, k-40599; 0.2%, t = 2.3 - Leningradka k-47882; 2.4%, t = 5.5 - Omskaya 18 k-58220; 1.9%, t = 2.7 - HD 2329, k-58775; 9.8%, t = 2.3 - Rostan', k-64391; 0.6%, t = 10.9 - Ester, k-64544, and by 0.7% Moskovskaya 35, k-48762). The tendency of the minimum average brightness decreasing in 30% of cultivars on the CVS in comparison with VS was identified.

In 50% and 60% of wheat cultivars with NVS and CVS, seed projections were characterized by a lower maximum brightness compared to VS.

A decrease in the brightness interval in the NVS and CVS (compared to the VS) was registered. This trend was identified in 50% (significantly by 0.6%, t = 14.5 - Leningradskaya 97, k-62935, by 8.6% - Saratovskaya 29, k-40599; 2.7% Moskovskaya 35, k-48762; 0.6% - Tulunskaya 12, k-64361; 0.3% - Ester, k-64544) and in 70% of cultivars (significantly by 20.5%, t = 2.6 - Moskovskaya 35, k-48762; 22.3%, t = 4.6 - Leningradskaya 97, k-62935; 10.9%, t = 7.8 - Tulunskaya 12, k-64361; 2.4%, t = 2.6 - Rostan', k-64391, by 9.5% - Saratovskaya 29, k-40599; 10.5% - Leningradka k-47882; by 0.9% - Ester, k-64544).

An increase in the optical density of the NVS and CVS projection in comparison with VS was determined. This tendency was revealed in 50% (0.4% - Saratovskaya 29, k-40599; 0.2% Moskovskaya 35, k-48762; 0.7% - in Omskaya 18 k-58220; 0.1% HD 2329, k-58775; 1.6% - Rostan', k-64391) and 40% of the cultivars (significantly by 2.4%, t = 2.3 - Moskovskaya 35, k-48762; 1.5%, t = 12.0 - Rostan', k-64391, and 0.7% - Saratovskaya 29, k-40599; 2.9% - Leningradka k-47882).

A growth of the integral optical density values on the NVS compared to the VS in 60% of cultivars was marked (significantly by 8.0%, t = 2.5 - Moskovskaya 35, k-48762; 5.7%, t = 2.8 - Tulunskaya 12, k-64361, by 0.6% - Kolkhoznitsa, k-30177; 4.0% - Leningradka k-47882; 1.8% - HD 2329, k-58775; 2.1% - Ester, k-64544). In 40% of the cultivars with CVS, a tendency to increase the integral optical density in comparison with VS was revealed.

The identification of wheat productivity and intensity of diseases development dependences on gas-discharge characteristics of seed material

The data about the yield structure and diseases development intensity on wheat cultivars of different origin are presented in the Table 1. For the period of phytosanitary monitoring of wheat as diseases causative agents were identified: brown rust (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Eriks.), wheat leaf blotch (*Stagonospora nodorum* Castell.et Germano) and powdery mildew (*Blumeria graminis* Speer.). Samples from the European part of the Russian Federation had better indicators of yield structure than samples from the Asian part of the Russian Federation. The maximum grain weight per plant was marked on the sample Rostan', k-64391 from Eastern Europe (Belarus) - 2.36 ± 0.15 g plant.

Table 1. The yield structure and the intensity of plant pathogens development on cultivars with different origin

Origin	Spike length (cm)	Number of spikelets (pcs.)	Number of grains per spike (pcs.)	Grain weight per plant (g)	Development of brown rust (%)	Development of wheat leaf blotch (%)	Development of powdery mildew (%)
Russia, the European part	7.60* ± 0.06 (7.49–7.71)	13.61 ± 0.08 (13.44–13.78)	19.80 ± 0.26 (19.31–20.29)	1.73 ± 0.08 (1.59–1.87)	28.40 ± 2.13 (24.93–31.86)	80.89 ± 1.46 (78.21–83.55)	1.34 ± 0.13 (0.97–1.71)
Russia, the Asian part	6.53 ± 0.06 (6.41–6.66)**	12.73 ± 0.10 (12.54–12.91)	18.09 ± 0.26 (17.53–18.66)	1.69 ± 0.08 (1.52–1.87)	17.76 ± 1.77 (13.31–22.20)	86.48 ± 1.69 (83.22–89.73)	2.63 ± 0.29 (2.17–3.08)
Eastern Europe (Belarus)	6.95 ± 0.08 (6.75–7.15)	15.27 ± 0.14 (14.98–15.57)	24.38 ± 0.51 (23.49–25.27)	2.36 ± 0.15 (2.08–2.64)	14.84 ± 2.29 (7.48–22.19)	86.14 ± 1.89 (80.79–91.48)	0.02 ± 0.02 (0.00–0.77)
South Asia (India)	6.48 ± 0.11 (6.18–6.77)	11.39 ± 0.20 (10.95–11.83)	16.44 ± 0.62 (15.12–17.74)	1.66 ± 0.14 (1.22–2.11)	0.35 ± 0.16 (0.10–12.38)	89.56 ± 3.13 (80.96–98.16)	5.80 ± 1.54 (4.50–7.09)

* – Mean and standard error of mean; ** – 95%-confidence interval.

The cultivars from the European part of the Russian Federation in comparison with the samples from the Asian part had the best indicators of yield structure and the highest values of the GDI area and the of the seeds GDI glow average intensity (Fig. 6).

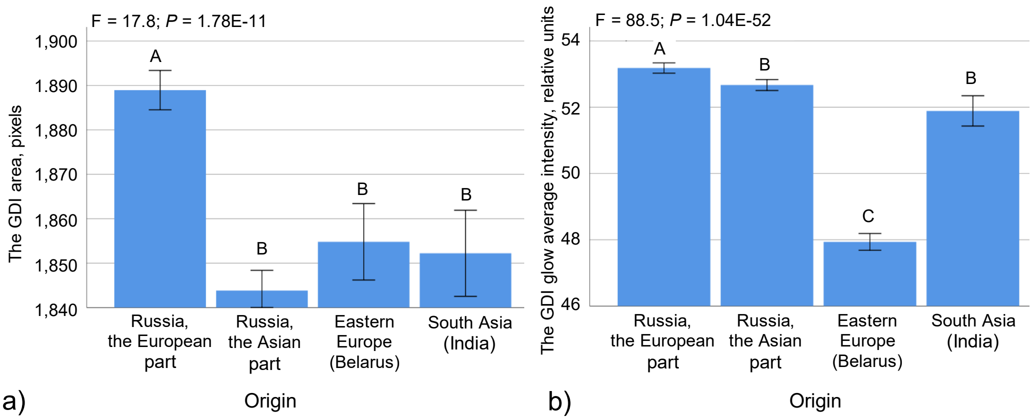

Figure 6. The area (a) and the average glow intensity of GDI (b) of soft wheat seeds with different origin. The graphs show the average values of the indicators and standard error of mean. The same letters mark the values of the indicator that are not significantly different according to the Scheffe criterion. F – Fisher criterion according to the single-factor analysis of variance.

The highest values of the area and the glow average intensity of the seeds GDI were found in wheat cultivars from the 2^{nd} climatic zone of the Russian Federation, adapted to warmer growing conditions, compared with samples from the 3^{rd} climatic zone (Table 2). Natural and climatic factors can have a significant impact on the intensity of the wheat diseases development (Kolesnikov et al., 2009), to modify the plant resistance manifestation, in particular, associated with both the expression of plant resistance genes and pathogens virulence genes (Naseri & Sasani, 2020a; Naseri & Sabeti, 2020b). Naturally changing environmental conditions can develop a genetic adaptation to these conditions in plants. Our studies have shown that the least development of brown rust was recorded on cultivars from the third climate zone of the Russian Federation, which can be explained by the low virulence and aggressiveness of local phytopathogen populations in relation to wheat cultivars from another climatic zone.

Table 2. The yield structure and the intensity of plant pathogens development on cultivars from different climatic zones

Climatic zones	Spike length (cm)	Number of spikelets (pcs.)	Number of grains per spike (pcs.)	Grain weight per plant (g)	Development of brown rust (%)	Development of wheat leaf blotch (%)	Development of powdery mildew (%)
The second climate zone of the Russian Federation (Leningrad, Moscow, Saratov region).	7.60 ± 0.06* (7.49–7.71)**	13.61 ± 0.08 (13.44–13.78)	19.80 ± 0.26 (19.31–20.30)	1.73 ± 0.08 (1.58–1.87)	28.40 ± 2.13 (24.93–31.86)	80.89 ± 1.46 (78.21–83.56)	1.34 ± 0.13 (0.97–1.71)
The third climate zone of the Russian Federation (Irkutsk, Omsk region, Krasnoyarsk region)	6.53 ± 0.06 (6.41–6.61)	12.73 ± 0.10 (12.54–12.91)	18.09 ± 0.26 (17.53–18.66)	1.69 ± 0.08 (1.52–1.86)	17.76 ± 1.77 (13.31–22.20)	86.48 ± 1.69 (83.22–89.73)	2.63 ± 0.29 (2.17–3.08)

* – Mean and standard error of mean; ** – 95%-confidence interval.

The tendency of increase of most indicators of wheat productivity with a reduction in the GDI area, SD of isoline radius of seeds GDI, average chord, and elongation with the increase of entropy along isoline GDI, the isoline GDI length was revealed by the correlation analysis method. In particular, the grains number per spike of the cultivar HD 2329, k-58775 increased with a growth the GDI brightness average intensity ($r = 0.6$; $P = 0.01$), the entropy along isoline ($r = 0.4$; $P = 0.03$), the isoline average radius ($r = 0.4$; $P = 0.01$) and decrease in shape coefficient ($r = 0.6$; $P = 0.01$), normalized SD of the isoline radius ($r = –0.6$; $P = 0.004$). The spikelets number per spike of the HD 2329, k-58775 cultivar increased with a growth in the GDI brightness average intensity ($r = 0.4$; $P = 0.02$) and a decrease in the isoline radius SD ($r = –0.5$; $P = 0.008$), the shape coefficient ($r = –0.6$; $P = 0.001$). A positive dependence was found between the GDI area of wheat grain used as a seed material and the flag leaf area of HD 2329, k-58775 wheat cultivar. This pattern is typical for 42% of the seed material and reflects the associated growth of indicators.

The data on the equity distribution of positive and negative correlation coefficients that characterize the relationships between wheat productivity indicators, diseases development intensity and gas-discharge characteristics of wheat seeds were obtained during the research.

In particular, the increasing tendency in 45.4% of productivity indicators with a decrease in the seeds GDI area, was revealed. Wheat affect by brown rust have increased with the growth of the GDI area. In the same time, for wheat leaf blotch and powdery mildew the opposite trend was revealed. A positive correlation between 36.3% of productivity indicators and the average intensity of seed glow dominated. Inverse relationship was typical for 18.1% of the indicators. The wheat affect by brown rust have increased with the increase of the optical index, while affect by wheat leaf blotch and powdery mildew declined.

The increase in the seeds GDI shape coefficient values led to a growth of 36.3% of the indicators: plant height, plant vegetation phase, productive tillering, total tillering and a decrease of 45.5% of the indicators - spikelets number per spike, spike length, spike weight, grains number per spike and biological yield. The development intensity of the wheat leaf blotch and the brown rust pustules number have decreased with the indicator increasing, while powdery mildew –increased.

An increase in the entropy values along the seeds GDI isoline caused an increase in the values of 63.6% of productivity indicators and a decrease in the intensity of brown rust development, but led to an increase in plant damage by wheat leaf blotch. A decrease in the values of fractality along the seeds GDI isoline caused an increase of 63.6% of productivity indicators and an increase in plant damage by powdery mildew and wheat leaf blotch, but a decrease in damage by brown rust.

There was a tendency of increase of 36.4% of wheat productivity indicators with a decrease in the values of SD of fractality along the seeds GDI isoline. The development intensity of powdery mildew and wheat leaf blotch was negatively correlated with the values of this indicator, and brown rust was positively correlated.

A positive correlation was found between the brown rust development and the optical index. The opposite trend was revealed for the powdery mildew development on wheat.

The predominance of the growth tendency of 54.5% of productivity indicators with a decrease in the normalized SD of seeds GDI isoline radius was noted. A similar positive correlation for 36.4% of wheat productivity indicators was revealed. The wheat damage by brown rust decreased, while powdery mildew affect increased with the optical index growth.

There was a tendency of the predominance of growth of 36.4% of productivity indicators with an increase in the size of the GDI contour irregularity. The wheat damage by powdery mildew and leaf blotch decreased with the optical index increase, while brown rust affection increased.

An increase in the length of the seeds GDI isoline accompanied by a decrease in the values of 81.8% of productivity indicators, an increase in the brown rust development and a decrease in leaf blotch and powdery mildew.

Identification of the productivity and wheat diseases intensity dependence on the X-ray characteristics of seed material

The plants yield of the Saratovskaya 29, k-40599 cultivar increased with the growth in the seed projection width (r = 0.3; P = 0.02), the brightness deviation (r = 0.4; P = 0.01) and the integral brightness (r = 0.5; P = 0.01). On the Ester, k-64544 cultivar, an increase in plant yield with a decrease in the maximum and interval seeds brightness was noted (r = –0.5; P = 0.01 and r = –0.4; P = 0.01). The spike weight grew with an increasing of the seed projection width (Saratovskaya 29, k-40599 - r = 0.5; P = 0.003), of the values of average brightness deviations (Saratovskaya 29, k-40599 r = 0.4; P = 0.01; Tulunskaya 12, k-64361 - r = 0.3; P = 0.04).

The number of seedlings leaves of the Ester cultivar, k-64544 increased with a growth of the average chord (r = 0.4; P = 0.03) and a decrease of the seed projection optical density (r = 0.5; P = 0.001). An increase in the values of the circle factor of seeds projection of the Omskaya 18, k-58220 cultivar led to an increase in plant height (r = 0.4; P = 0.03).

The spike length increased with a growth in the seed projection perimeter and width (Kolkhoznitsa, k-30177: r = 0.5; P = 0.02; r = 0.6; P = 0.03, respectively) and a decrease in the seed projection maximum and interval brightness (Ester, k-64544: r = 0.4; P = 0.03; r = 0.3; P = 0.04, respectively).

The grains number per spike increased with a growth in the ellipse factor values (Kolkhoznitsa, k-30177 - r = 0.4; P = 0.03; Rostan', k-64391 - r = 0.4; P = 0.04) and decreased with an increase in the seeds roundness (Rostan', k-64391 - r = –0.5; P = 0.01).

The spikelets number per spike increased with a growth of length (Omskaya 18 k-58220 - r = 0.6; P = 0.008) and width of seeds (Saratovskaya 29, k-40599 - r = 0.5; P = 0.03) and reduction in the maximum brightness (Ester, k-64544 - r = –0.7; P = 0.001).

Plants of the Ester, k-64544 cultivar grew faster with decreasing of the brightness deviation, the maximum brightness, interval brightness of seed (r = –0.4; P = 0.03; r = –0.7; P = 0.004; r = –0.4; P = 0.03), while on cultivars Kolkhoznitsa, k-30177 - with decreasing the seeds elongation (r = –0.4; P = 0.04), Saratovskaya 29, k-40599 - with decreasing the integral brightness (r = –0.5; P = 0.02).

The total and productive bushiness of plants of the Ester, k-64544 cultivar decreased with a growth of maximum and integral brightness (r = –0.6; P = 0.01; r = –0.4; P = 0.03 and r = –0.5; P = 0.03; r = –0.6; P = 0.02, respectively). A decrease in the productive bushiness of the Leningradka, k-47882 cultivar with increasing of integral luminance was observed (r = –0.5; P = 0.03).

The flag leaf area of the HD 2329, k-58775 cultivar decreased with a growth of the roundness (r = –0.6; P = 0.02) and the optical density of seeds (r = –0.4; P = 0.01).

Wheat seeds can be both a direct source of infection during the growing season, and have an indirect effect on the complex of plants adaptive reactions to environmental factors, including resistance to diseases. The genes of plant defensins, which inhibit, in particular, the development of phytopathogenic micromycetes, are expressed in various plant organs, including seeds (Andersen et al., 2018). The powdery mildew development increased with a decrease in the seed projection width (Kolkhoznitsa, k-30177 - r = –0.3; P = 0.04), the average chord (Leningradskaya 97, k-62935 - r = –0.5; P = 0.02), the brightness deviation (Saratovskaya 29, k-40599 - r = –0.6; P = 0.01) and with an increase in the optical density of the seed projection (Saratovskaya 29, k-40599 – r = 0.4; P = 0.02), and in the integral brightness (Saratovskaya 29, k–40599 - r = 0.6; P = 0.01).

The brown rust development decreased with an increase in the seed ellipse factor (Saratovskaya 29, k-40599: r = –0.5; P = 0.03), roundness (Ester, k-64544: r = –0.6; P = 0.01) and with a decrease in the average brightness, brightness deviation, and integral brightness of seeds (HD 2329, k-58775 - $r = 0.7$; $P = 0.003$; $r = 0.5$; $P = 0.02$; $r = 0.4$; $P = 0.04$, respectively). The pathogen's pustules number increased on wheat leaves with an increase in average brightness, brightness deviation, and integral brightness (HD 2329, k-58775 - $r = 0.6$; $P = 0.04$; $r = 0.6$; $P = 0.01$; $r = 0.5$; $P = 0.03$; respectively) and decreased with an increase in the optical density of seeds (HD 2329, k-58775–$r = -0.7$; $P = 0.004$).

The equity distribution of positive and negative correlation coefficients that characterize the dependences between the wheat productivity indicators, the intensity of diseases development and the X-ray characteristics of wheat seeds were analysed during the experiment.

Positive correlations dominated in relation to the seed projection area and to 63.6% of wheat productivity indicators. The perimeter of the seed projection influenced on the increase and decrease of 45.4% of wheat productivity indicators. The wheat leaf blotch development in most cultivars increased with a growth of the indicator values, while powdery mildew and brown rust (according to the pustules number) - decreased. The dominant tendency was an increase in the values of 54.5% of wheat productivity indicators with an increase in the seeds length. The wheat leaf blotch development increased with a growth in the optical index values in 70% of cultivars, while brown rust and powdery mildew - decreased in 80% of cultivars.

A significant increase in the values of 81.8% of productivity indicators with an increase in the width of the seed projection was revealed. In particular, the wheat yield increased with a growth in the optical index in 60% of cultivars. The wheat leaf blotch development in most cultivars increased with a growth of the indicator values, while powdery mildew and brown rust (according to the pustules number) - decreased.

An increase of 63.6% of productivity indicators with a decrease in the values of the seed projection' average chord was observed. The wheat leaf blotch development in most cultivars increased with a growth of the indicator values, while powdery mildew and brown rust (according to the pustules number) - decreased. A predominantly inverse correlation between 54.5% of productivity indicators and the seed projection' elongation was found. The yield of 70% of cultivars decreased with an increase in the optical index value. The growth of the average size of the seed projection caused an increase in the values of 81.8% of productivity indicators. The yield of 70% of cultivars increased with a growth in the value of this optical indicator. The development of wheat diseases in most cultivars increased with increasing the indicator values.

A positive correlation was found between of 45.5% of productivity indicators and the values of the seed projection circle factor. An increase in wheat yield was observed in 70% of cultivars with a growth in the values of this optical indicator. The brown rust development decreased with an increase in the indicator of 60% of cultivars, and powdery mildew increased in 70% of cultivars. An increase in the values of the seed projection' circle factor caused an increase in 72.7% of productivity indicators. A tendency of increase the wheat biological yield with a growth in the optical index in 80% of wheat cultivars and an increase in plant damage by powdery mildew was revealed. An increase in the seed projection roundness led to a decrease of 45.5% of wheat productivity indicators. Besides this, negative correlations between this optical indicator and biological yield were found in 60% of cultivars. The degree of wheat powdery mildew damage decreased with an increase in the seed projection roundness, which was found in 60% of cultivars.

The prevailing tendency was a decrease of 54.5% of wheat productivity indicators with an increase in the average brightness of the seed projection. The wheat biological yield increased with a decrease in the values of the optical index, which was typical for 70% of the studied cultivars. The wheat leaf blotch and powdery mildew development increased with an increase in the average brightness of the seed projection. This dependence was typical for 80% of the studied cultivars.

The nature of the SD of the seed projection brightness dependence on the productivity indicators varied. Positive dependences prevailed in relation to the plant development phase, the flag leaf area and the spike weight, negative ones - to the plants height, the spike length. Wheat damage caused by leaf blotch and powdery mildew increased with a growth in the brightness of the seed projection, while by brown rust (by the pustules number) - decreased.

The growth of the minimum brightness of the seeds projection was accompanied by an increase of 36.3% of the productivity indicators: the productive bushiness (marked on 80% of cultivars), the total bushiness (marked 90% of cultivars), the spike length (noted on 60% of cultivars), the biological yield (noted by 60% of cultivars) while reduced of 45% of the indicators: the number of seedlings leaves, the plants height, the flag leaf area, the spikelets number per spike, the grains number per spike. The development of wheat leaf blotch and brown rust mainly increased with a growth in the minimum brightness of the seed projection, while powdery mildew decreased.

A predominantly inverse correlation between of 45.5% of productivity indicators and the maximum brightness of the seed projection was determined. The yield of 60% of cultivars decreased with an increase in the value of the optical index. The plants damage by leaf blotch mainly increased with a growth in the indicator, while by powdery mildew decreased.

For 72.7% of productivity indicators, inverse correlations with the integral brightness of the seed projection were marked. At the same time, a decrease in biological yield with an increase in the optical index was recorded in 70% of cultivars. The intensity of wheat affects by leaf blotch increased with a growth in the integral brightness of the seed projection, while by powdery mildew - decreased.

The predominance of positive correlations between the values of the integral brightness of the seed projection and of 45.4% of productivity indicators was noted. This dependence is also characteristic of the wheat biological yield, which increased in 60% of cultivars with a growth in the integral brightness of the seed projection. The brown rust and powdery mildew development decreased with an increase in the optical index, while leaf blotch increased.

CONCLUSIONS

The application of the latest achievements of biophysics, including instrumental methods of non - destructive testing of the structural and functional characteristics of seeds-gas - discharge imaging and soft-beam X-ray microscopy, was analyzed. Nonviable wheat seeds in compare to healthy (completed) differed mainly smaller area, shape factor, standard deviation (SD) of three-dimensional fractality along the isoline, entropy along the isoline, higher brightness and standard deviation of the gas discharge image (GDI) isoline radius. Morpho - and densitometric parameters of non-viable wheat seeds were characterized mainly by reduced values of the circle factor, roundness, minimum and maximum average brightness, but by greater elongation and optical density of the X-ray projection. Wheat seeds, which gave the seedlings, died later, had a smaller area, SD of three-dimensional fractality along the isoline, average radius of the isoline, entropy along the isoline, GDI isoline length, average chord and roundness of the radiographs projection, but greater values of ellipse factor of the projection radiographs. The revealed tendency of increase of most of the wheat productivity indicators with a reduction in area GDI, SD of the seeds GDI isoline radius, the chord average, elongation, the average brightness of the projection, the integral brightness of the projection and with the increase of entropy along the GDI isoline, the length of the GDI isoline, area, size, length and width of seeds, the factor of projection circle.

The intensity of wheat diseases varied depending on the structural and functional characteristics of wheat seeds.

In particular, it was noted, that the development of brown rust decreased with an increase in the entropy along the isoline, the contour irregularity and the average radius of the isoline. An increase in the values of the average GDI intensity, fractality along the contour, and irregularity of the seed contour led to a decrease in the intensity of powdery mildew development. The development of wheat leaf blotch increased with a decrease in the average radius of the isoline, the irregularity of the GDI contour, and an increase in the normalized SD of the isoline radius, the SD of fractality. The obtained data indicate the possibility of more effective cultivation and protection of soft wheat from diseases using a complex of introscopic methods for seed material assessment (microfocus radiography, gas-discharge visualization).

The experimental data indicate the possibility of more effective cultivation and protection of soft wheat in forecasting the biological usefulness of vegetative plants using the methods of microfocus radiography and gas discharge visualization of seed material.

As a result of the conducted research, the complex of indicators of the seeds gas-discharge glow, morphometric and optical parameters of their radiographs, which are an additional tool for the express assessment of the quality of seed material, has been identified. This information makes it possible to obtain a more complete description of the biological and economic suitability of the seed material, and, possibly, in the future, to predict the seeds field germination and potential yield, identify the main seeds defects, predict the development of diseases and possible changes in plant resistance to diseases.

The question of the relationship between the characteristics of gas-discharge glow with the indicators of plant bioproductivity and the mechanisms of a gas discharge formation when placing a seed in a high-intensity electromagnetic field still needs careful study with the involvement of scientists in the field of physics of living systems.

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DEM modelling of tillage tools in sand and verification of draft forces in the soil box

J. Kuře[1,2,*], M. Linda[1], R. Chotěborský[2], B. Černilová[1] and M. Hromasová[1]

[1]Department of Electrical Engineering and Automation, Faculty of Engineering, Czech University of Life Sciences Prague, Kamýcká 129, CZ165 21 Prague – Suchdol, Czech Republic
[2]Department of Material Science and Manufacturing Technology, Faculty of Engineering, Czech University of Life Sciences Prague, Kamýcká 129, CZ165 21 Prague – Suchdol, Czech Republic
[*]Correspondence: kure@tf.czu.cz

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Abstract. Soil resistance is still being important parameter during tillage. By reducing the soil resistance during processing, greater efficiency and cost reduction can be achieved. With the correct design of the shape of the tillage tools, reduction in the force required for tillage can be achieved. New tool designs must be tested in field conditions to determine the effect. Using DEM (Discrete element method) modelling, individual designs can be compared without the need for field tests. However, the accuracy of the model must first be verified on real tests. The paper deals with the creation of a mathematical model of sand, which is used for testing tillage tools in the soil box. The models are focused on tests of various shapes of wings on tillage tools. Draft forces are compared, and the correctness of the model is verified.

Key words: DEM, modelling, tillage tools, soil box, draft force.

INTRODUCTION

The construction of the usable mathematical model of bulk material depends on the correct setting and use of the correct method. The basic models can be divided into models without adhesive forces (Cundall & Strack, 1979; Walton, 1993), that correspond to materials without significant moisture (seed, sand, etc.) and into models with adhesive forces (Pasha et al., 2013; Pasha et al., 2014) that correspond to materials such as soil, wet sand, etc.

In agriculture, these materials can be modelled using the DEM method (Ucgul et al., 2014). This method is suitable for materials such as agricultural crops (Boac et al., 2014; Kanakabandi & Goswami, 2019) or for sand and soil (Shmulevich et al., 2007; Milkevych et al., 2018; Ucgul et al., 2018). These models usually do not respect the diversity of the soil environment and are limited to a maximum of two different types according to the simulation. The solution is usually performed in an EDEM environment. Using the model, it is possible to simulate the passage of the tool through the soil and then evaluate, for example, the forces acting on the tool or the behaviour of the soil itself (Ucgul et al., 2017; Ucgul & Saunders, 2020). However, the individual models must be verified experimentally. The validated model can then be used to investigate other tillage processes. By using the model, it is possible to predict the behaviour of the modelled material or the interaction between the tools and the material and immediately reject inappropriate designs. This can save work and money in designing real prototypes of new tools and equipment.

The Rocky DEM environment enables the creation of the mathematical models with respect to the action of adhesive forces and models without adhesive forces (Fonte et al., 2015). To set up the material model and its interaction with solids in solving the problem, it is necessary to set the boundary conditions, material model and kinematics of solids (Yan et al., 2015; Kuře, Hájková et al., 2019), that can be obtained from experimental results. For example, some Ucgul's DEM models of soil with interaction of agricultural tools were created according to Fielke's experimental results. It is possible to use results from field measurements such as soil resistance. When designing a soil model, a 1:1 scale tool model can be created for interactions. The parameters of the model can be set in any variances until the results of the model are comparable with the results of field tests. Subsequently, the model can be declared suitable.

The boundary conditions of the model are usually obtained using partial models and verified on the basis of real tests (Kuře, Chotěborskýet al., 2019) or field tests (Kešner et al., 2019). The modelling can be used in agriculture as a tool for the design of new tillage tools or innovation of new geometry and determining their properties in tillage. The model verification can be performed on real models of tillage tools in the soil box model (Kuře et al., 2020).

The 3D printing can be used to design new tool shapes. When used in a soil box model, individual tools can be changed easily and quickly. In the case of using a model printed on a 3D printer, the material properties must be also verified (Hnízdil et al., 2020). Especially if a tool model is created using the finite element method. Different types of sand are often used as a filler in soil boxes. Sand has very similar mechanical properties to a soil and is much easier to keep in a consistent condition compared to a soil.

The aim of this paper is to create a mathematical model of sand, which is used to testing of tillage tools in a soil box model. The model is tested on various shapes of wings, which are equipped on tillage tools. The model verification is performed based on a comparison of the draft force of the model and of the real test.

MATERIALS AND METHODS

To create the model, it is necessary to perform a set of measurements and obtain default settings. The basic settings of the model of silica sand were chosen on the basis of already known data (Schellart, 2000; Katinas et al., 2021) and own measurements. Each model must be verified on a real test to verify its authenticity. The sand model was verified for test in the soil box. The soil box is used for testing tillage tools. The soil box is designed for tools at a scale of 1:2. The tensile force is the main value obtained from tests. The soil box is designed for testing different geometries of tillage tools before full field tests.

The dimensions of the box are height 500 mm, width 500 mm and length 1,500 mm. Silica sand with fraction 0.1–0.3 mm was used as filling of the box. The moisture content of silica sand was 0%. The subject of interest was the active length. The active length expresses the area, where the tool is already fully recessed and correspond to normal field work. The active length of the box depends on the used tool, its shape, and the length of the tool engagement. For tests was chosen tool with changeable wings. In the case of tillage tool with variable wings, the active length was 200 mm. The schema of the soil box is shown in Fig. 1. In figure is shown active length l_m (mm), draft force F (N) and variable depth of the tool d (mm). Tillage tools used in the soil box were printed on a 3D printer. Material used for printing was Pet-G. The advantage of plastic materials is fast and cheap production of many structural designs and their verification, in contrast to the expensive production of steel prototypes. Although plastic has a different value of interaction with particulate matter (e.g. Static Friction, Dynamic friction, Adhesive stiffness), when comparing design solutions that are made of a single material, changes in results (such as a decrease or increase in forces) will correlate with a metal tool.

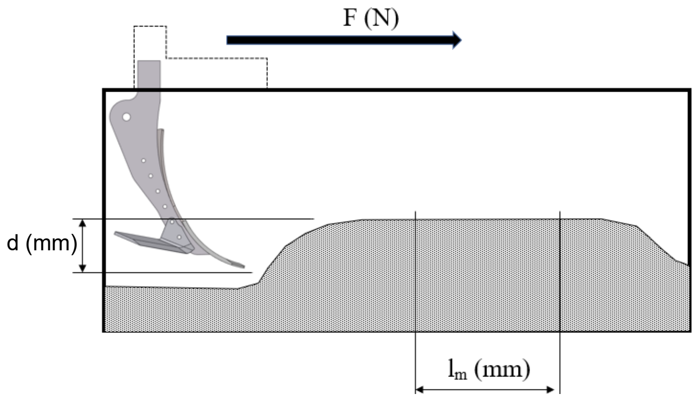

Figure 1. Schema of soil box (Kuře et al., 2020).

The verification of the model was performed on already obtained data within the measurement of individual wing geometries (Kuře et al., 2020). Data from the active length measurements at a depth of 10 cm were used. Measurement was performed for four different types of wings with the same width. Measured data were taken from (Kuře et al., 2020)

For the simulation were made geometry of the soil box, tillage tool and four types of wing. All of geometries were made in ratio 1:2 to origin tools. These models were imported into the Rocky DEM environment ('Rocky DEM Particle Simulator', 2018). Rocky DEM software is an environment for creating models using the DEM method. The positions of the geometries, velocities and movements of the models corresponding to the real measurement conditions were set. The entire length of the soil box was used in the model (in the original testing, the length of the box was shortened by a camera, which was placed inside the box). The velocity of tillage tool was set to 0.1 m s^{-1}. For each test, the tool with the appropriate wing shape was imported. Wing shapes are shown in Fig. 2.

The main prerequisite for the individual designs was the preservation of the projected area of the wings. Type 0 is the basic type of wing with an angle of inclination of 45°. For the Type 1 wing, the initial inclination was changed to 60° and for the Type 2 wing to 30°. For the last Type 3, the shape was designed using a curve. In all cases, the projected areas are preserved.

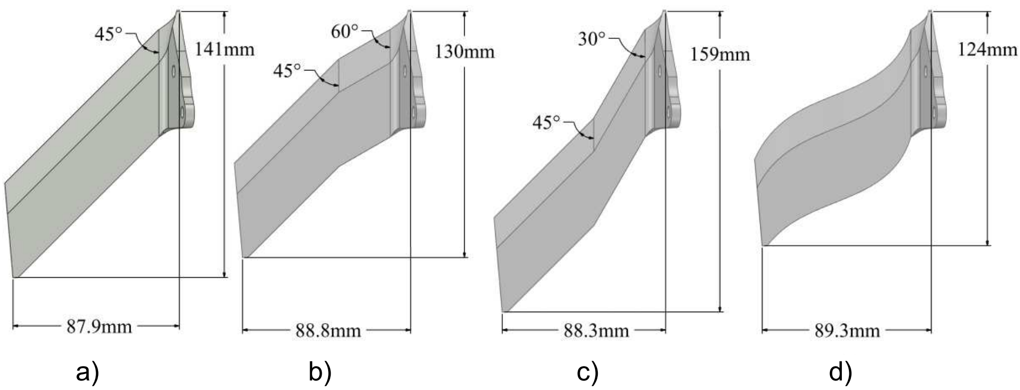

Figure 2. Schema of wings types: a) type 0 b) type 1 c) type 2 d) type 3.

After importing the geometries, it was necessary to set the DEM model of the particulate matter (silica sand). The shape of the particle was chosen as a sphere. The shape of the sphere can be used to simplify the shape of the sand and speed up the calculations. The basis of the model was set according to Table 1. Initial values of material model were obtained experimentally (Bulk density was determined by weighing material of known volume). The parameters having a great influence on the change of results were determined. These parameters were used in the subsequent design of experiment (DoE) analysis. The principle of DoE was in the selection of input parameters significantly influencing the results of the model. The values of these parameters were systematically set to extreme values and, based on the change of the result, adjusted to the optimal setting. The individual combinations of settings are listed in Table 3 below. The Young Modulus of silica sand was included in the DoE analysis due to the large variance of the measurement results.

Table 1. Settings of material model of silica sand

Material			Particles	
Bulk Density (kg m^{-3})	Young Modulus (MPa)	Poisson's ratio (-)	Size (mm)	Rolling Resistance (-)
1533	DoE	0.3	10	DoE

In the case of setting interactions, the sand / sand and sand / tool relationship had to be set. The individual settings were measured on sand shear tests. Based on these tests, the values for static and dynamic friction were determined. Friction between sand and tool (Pet-G material) was included in the DoE analysis. The remaining values were left in the original model settings. The basic settings for model interactions are listed in Table 2.

Table 2. Settings of interactions between sand / sand and sand / PetG

Relation	Static Friction (-)	Dynamic Friction (-)	Tangential stiffness r. (-)	Restitution coef. (-)
Sand / Sand	0.7	0.6	1	0.3
Sand / Pet-G	DoE	DoE	1	0.3

The total number of particles used in the one model was 97,072. Simulated time of one model was 12 seconds. The Timestep for saving data was set to 0.05 seconds. The Internal Timestep of solver was $2.93764.10^{-5}$ seconds. The model was optimized to save time in the calculation. The optimization included the shape and size of the DEM particles. The calculations were performed on an Nvidia Tesla P100 graphics card. The calculation time for one model was 1 hour.

The model was calibrated according to the test using the first type of tillage tool (model contained wing type 0). Gradually, the input parameters were set according to the DoE analysis and the model calculations were performed. The resulting draft force of model was determined and compared with the results of real tests. In case of differences in results, parameters were adjusted and calculation was performed again. The procedure is shown in Fig. 3.

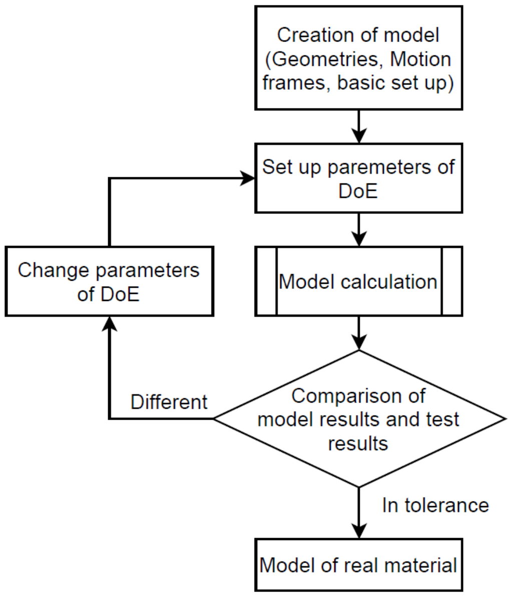

Figure 3. Scheme of the model creation process.

The results were analyzed from individual model settings. The force in the Z axis was determined, which was compared with the measured values. The comparison was determined for the active working part (active tool depth). The average value of force in this working part was compared with the measured data. The model setting that corresponded to the best match between the compared values was also used for the other three types of tools (with different wings). Subsequently, the individual results were evaluated and compared.

RESULTS AND DISCUSSION

For each model, the parameters according to DoE were changed and the results were compared. The aim was to set the individual parameters so that the resulting model corresponded to real tests. Table 3 lists the individual parameters that were used for each model. Each model was set individually and after the calculation the output data (draft force) were evaluated. The output data are listed in the table in the last column and corresponds to the settings in the relevant row. The best match was achieved when setting up model 17. The results show that it is possible to rely on material models designed for other types of computational tasks such as for example wear (Katinas et al., 2021).

Table 3. Settings of interactions between sand / sand and sand / Pet-G

	Set Up				Results
Number of DoE	Young Modulus (MPa)	Rolling Resistance (-)	Static Friction (-)	Dynamic Friction (-)	*F in active l. (N)*
1	8	no	0.5	0.5	*375.4*
2	6	no	0.5	0.5	*344.6*
3	4	no	0.5	0.5	*330.4*
4	2	no	0.5	0.5	*256.1*
5	2	0.15	0.5	0.5	*66.5*
6	2	0.17	0.5	0.5	*70.6*
7	2	0.2	0.5	0.5	*78.6*
8	1.9	0.15	0.5	0.5	*66.5*
9	1.9	0.17	0.5	0.5	*70.9*
10	1.9	0.17	0.5	0.3	*64.4*
11	1.9	0.17	0.4	0.3	*63.8*
12	2	0.15	0.4	0.3	*61.4*
13	2	0.14	0.4	0.3	*59.5*
14	1.9	0.14	0.4	0.3	*58.2*
15	1.8	0.14	0.4	0.3	*58.6*
16	1.8	0.12	0.4	0.3	*55.1*
17	**1.7**	**0.14**	**0.4**	**0.3**	***56.2***
18	1.7	0.12	0.4	0.3	*54.7*

Fig. 4 graphically shows a simulation of the passage of a tool with wings of type 0. The particles are marked according to depth.

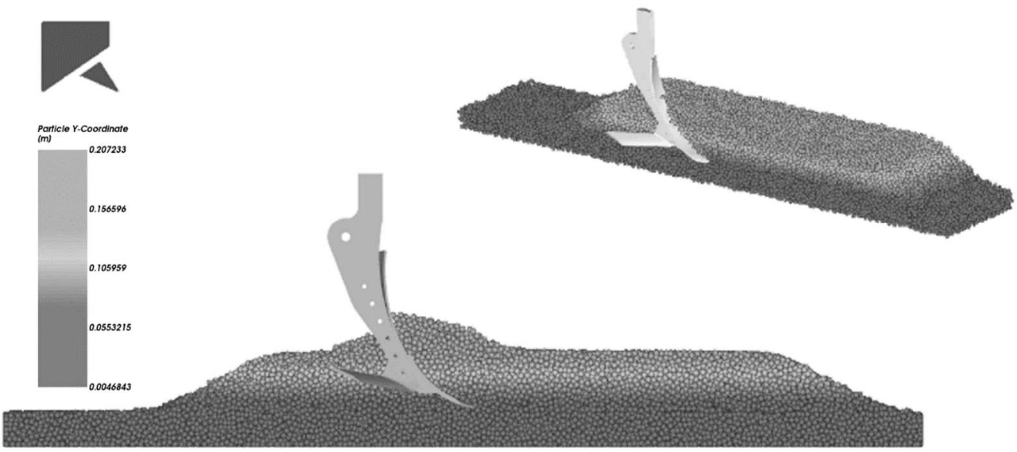

Figure 4. Graphical results of model from Rocky DEM.

The evaluation of the data from the model is shown in Fig. 5. The course of the draft force was determined from the model. Only the part corresponding to the active length (where the force is constant) was selected. The average force from this part was compared with the already measured results for the given type of tillage tool.

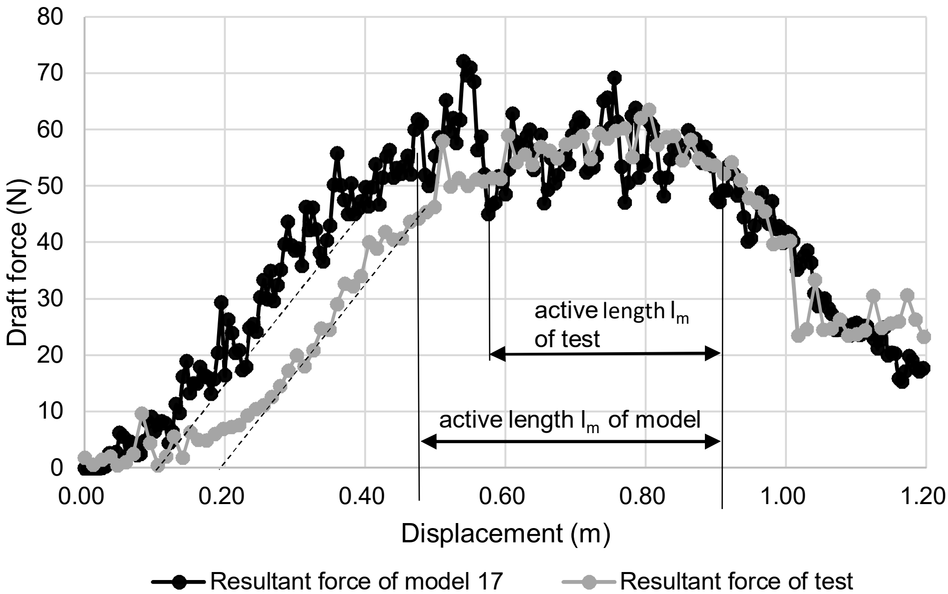

Figure 5. Comparison of model results with measured values.

Fig. 5 shows a comparison of the measured force in the active length during real tests and the course of the draft force obtained from the model. The data of force from active length in the graph corresponds to the results of model number 17. The optimal results were achieved. The large scatter of force in the active part was caused by the size of the model particles. This size was chosen to optimize the time in the calculation. However, the result is not otherwise affected. It is also evident in the graph that the increase and decrease of the force measured in the real test correspond to the results obtained from the model. The active length during the measurement was reduced by 0.1 m due to the placement of the camera in the box. Nevertheless, the slopes of the increasing force are the same. It was possible to reduce the active length. When increasing the active length and maintaining the conditions (same depth, material, etc.), the average value of the force always stabilizes (Kešner et al., 2019). For this reason, it is not necessary to examine the whole course but only steady values.

After obtaining the optimal setting, it was necessary to compare other types of wing geometries. The same setting was selected (according to model 17) and models for individual geometries were calculated. The behaviour of the model in interaction with the tool must be the same even if the ambient condition changes as the speed of the tool or tool shape (Shmulevich et al., 2007). The resulting draft forces were compared with the measured data. The comparison is shown in Fig. 6.

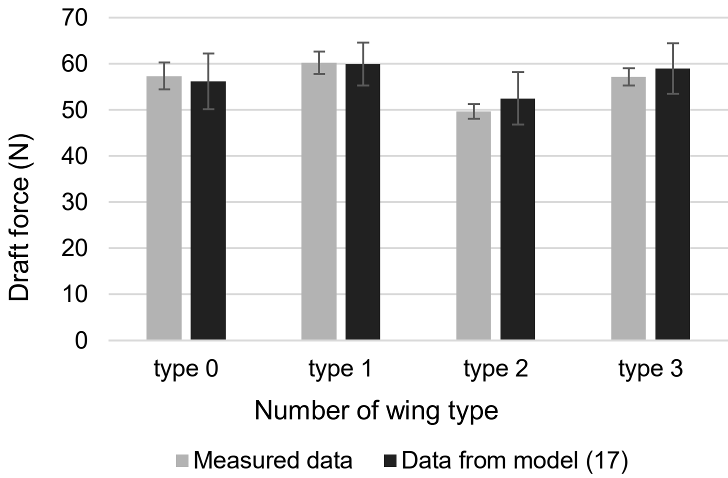

Figure 6. Comparison of measured draft force and model draft force.

The graph shows the individual average forces measured in the active length for both real tests and the model. Individual results were evaluated using statistical methods. An *F-test* was performed to determine the consensus of the variances of the individual results. In all cases, the result p-value was less than 0.05. The hypothesis of equality of variances was rejected. For this reason, an unpaired *T-test* for different variances was used to determine the concordance of the results. *P-values* of *T-tests* are shown in Table 4. In all cases except wings type 2, statistical consensus was achieved.

Table 4. *T-test* results for individual draft forces

	Wings type 0	Wings type 1	Wings type 2	Wings type 3
Measured force (N)	57.3 ± 2.9	60.2 ± 2.4	49.7 ± 1.6	57.1 ± 1.8
Model force (N)	56.2 ± 6.1	59.9 ± 4.6	52.5 ± 5.6	58.9 ± 5.5
p-value of *T-test* (> 0.05)	0.38	0.75	$0.2e10^{-3}$	0.14

From the data according to the graph (Fig. 6) the results for individual geometries correspond to individual models. The smallest measured draft force was found for a tillage tool with wings type 3. The largest draft force was found for a tillage tool with wings type 2. These dependences were same for individual models.

It seems that the DEM, after the correct setting, corresponds very well to the real conditions. Therefore, it is possible to obtain the additional information from the results, which simply cannot be obtained by measurement. The examples could be all forces and moments on the geometry, position of the centre of gravity, position of the forces, etc. This information can be combined with the FEM model of the machine and optimize or innovate individual parts or the whole machines for agriculture.

CONCLUSIONS

The DEM model of the silica sand for filling the soil box was created. The created model was verified for various geometries. The draft force was compared for verification. This parameter is suitable for the model verification for the purpose of determining the force effects. In all cases (with the same DEM model settings) relatively identical results were obtained. These results were compared using statistical methods. In most cases, a consensus of the results was found. It was found that with the correct setting of the DEM model, results corresponding to real conditions can be obtained. The model settings described in this paper can be used for a silica sand with zero moisture content and a fraction of 0.1–0.3 mm.

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The effect of cultivation technology on the plant development of organically grown garlic

L. Lepse[1,*], S. Zeipiņa[1], I. Missa[1] and A. Osvalde[2]

[1]Institute of Horticulture, Graudu iela 1, LV–3701 Dobele, Latvia
[2]Institute of Biology, University of Latvia, Ojāra Vācieša iela 4–201, LV–1004 Rīga, Latvia
[*]Correspondence: liga.lepse@llu.lv

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Abstract. The new technological solutions for the hardneck garlic production were tested to prevent the influence of unfavourable soil and climatic conditions on the rooting, sprouting and wintering ability of hardneck garlic - factors that affect significantly the hardneck garlic production in Latvia. Field studies were carried out at the experimental field of the organic farm, located at the Koknese district, Latvia, during the seasons of 2018/2019 and 2019/2020, on sandy loam soil using hardneck garlic cultivar `Liubasha` and local clones. Two variants of garlic growing were compared - traditional planting in the autumn in the field as control, and planting in the trays as an innovative solution. Results indicated that low temperature treatment (below +7 °C) for the period of at least 50 days initiates cloves primordia development. The using of trays is effective technology to prevent unfavourable agroecological conditions on the field in the case if controlled conditions are available and technically feasible in the farm.

Key words: *Allium sativum* var. *ophioscorodon* (Link) Döll, bulb, clove, stalk development.

INTRODUCTION

Garlic (*Allium sativum* var. *ophioscorodon* (Link) Döll) is a bulbous plant of relatively short vegetation season (90–140 days from the sprouting in the spring until harvest). The part of plant consumed in the food is a bulb consisting of a number of lateral buds transformed into storage organs (cloves). The clustered bulb, enveloped by layers of dry thick leaf bases, consists of one or more whorls of cloves; each one is made of a vegetative bud embedded inside a thick storage leaf enveloped by an external dry, protective cylindrical leaf sheath (Mann, 1952; De Mason, 1990). Cloves are placed on the bulb basal disc around a long flower stalk (scape) producing topsets (inflorescence bulbils). Hardneck or bolting garlic tend to have four to twelve cloves in each bulb, depending on the genotype.

Young cloves develop from the buds initiated from the base of planted (mother) clove. Cloves initiating is determined by environmental factors, such as temperature (in the storage period prior planting and after planting) and light conditions (Rahim & Fordham, 1988). The optimal storage temperature for cloves prior planting is between

10–12 °C (Baumane, 1973). Higher storage temperature reduces clove development and promotes vegetative growth of the plant.

In order to ensure optimal plant development conditions, traditionally hardneck garlic in boreal climatic zone is planted in the autumn (September - October). Such planting time ensures vernalisation conditions for the cloves, thus initiating the formation of new cloves in the planted mother clove and subsequent bulb development. According to different authors, the initiation of cloves takes place in the period of 6–8 weeks at a temperature of 4 to 7 °C (Rahim & Fordham, 1988; Põldma et al., 2005). Exposing the bulbs to low temperatures stimulates cytokine and gibberellin accumulation, modifying the hormonal balance and leading the bulb development (Resende et al., 2011). Thus, in the field conditions two-month period of temperature between 0 and 10 °C is sufficient for proper bulb development. In order to ensure proper rooting of planted cloves and increase plant ability to survive in low temperature, the period of cloves rooting for 1–1.5 months should be ensured in the field prior temperature drops below 0 °C (Põldma et al., 2005).

Changing climate conditions often burdens proper garlic development or even planting in the autumn so endangering yield (Muska & Saksone, 2019. In recent years, more intense precipitation has been observed in the autumn period (data from the Latvian Environment, Geology and Meteorology Centre), which makes it difficult or impossible to plant garlic in the field. Also often thaws during the winter period worsen plants overwintering ability. In order to overcome the changing climate conditions, and implement precisions agriculture, the innovative method of garlic planting using controlled conditions was tested in the project 'The modernization of winter garlic cultivation to ensure guaranteed yield in the organic production system' in the period of 2018–2020. The aim of the study was to develop the growing technology ensuring proper plant development conditions and efficiently use available resources.

MATERIALS AND METHODS

The field trials were performed in the farm 'Lazdiņas Aagro', Koknese district, Latvia (56°39'21.6"N 25°29'31.2"E). The experiment was carried out in sandy loam soil without irrigation possibilities. A hardneck garlic cultivar of Ukrainian origin Liubasha and local Latvian garlic clone were used as planting material.

In the season 2018/2019 using traditional technology, garlic was planted at the beginning of November, 2018. Uniformed cloves were planted with a 3-row configured planter where the distance between rows was 0.6 m and cloves were planted at a distance of 0.15 m in the row. To test the innovative technology, garlic cloves were planted in the 3rd decade of March, 2019 in trays (cell size 73×38 mm) filled with substrate prepared from the mixture of sphagnum peat and manure. Trays were placed in the refrigerated dark room, where air moisture was between 70 and 80%. Trays irrigation was not performed, as the substrate well kept the humidity. Due to technical problems, trays with plantlets were exposed to continuous low-temperature of 4.0–7.0 °C for only 20-days period. During the remaining period until the planting (30 days), the temperature ranged between 2 and 18 °C (Fig. 1).

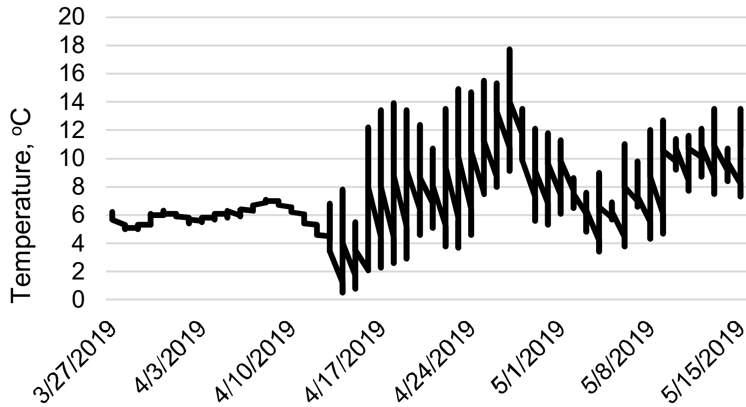

Figure 1. Temperature conditions in 2019 for plantlets grown in trays.

In 2019, container plantlets from trays were planted on the field in the 2nd decade of May at phenological growth stages BBCH 101-104, according to Lopez-Bellido et al. (2016) (Fig. 2).

Figure 2. Trays with garlic plantlets.

In the season of 2019/2020, in the traditional technology, garlic was planted on the field at the beginning of December, 2019, using the same technology as in the previous year. In the innovative technology variant, garlic cloves were planted in trays in the 3rd decade of March, 2020. The substrate was similar to that used in the previous year. Trays were exposed to low-temperature of 4–7 °C for 50 days and garlic plantlets were planted on the field in the 1st decade of May, at BBCH 104-106. Planting density was the same as for the control plants planted in the autumn.

As overwintering of control variant garlic on the field was good, there were no drop-off plants observed for both winters. Therefore overwintering of control variant plants was not measured.

Plant development in trays was evaluated by measurements of root length and plantlet height in the day of planting in the field (15.05.2019 and 10.05.2020), and plant height measurement in the 1st decade of July on the field. Plant measurement was performed also for the plants growing in the field accordingly to the traditional growing technology at the same days. Stalk development for plants of both variants was evaluated in July for both years, to assess bulb development completeness.

Clove initiation for plantlets grown in trays and for field-grown plants was inspected microscopically in 21.05.2019 and 12.05.2020. The number of clove primordia was registered for each inspected plant.

All measurements were performed in three replications. Garlic yield in each technological variant was evaluated after harvest and expressed in t ha^{-1}.

The data were subjected to analysis of variance (ANOVA) and *Duncan's* criteria, $p < 0.05$, to evaluate the differences in plant development and yield between garlic cultivation methods.

RESULTS AND DISCUSSION

The results obtained in the two-year investigation proved the possibility to obtain satisfactory yield by using innovative trays method for garlic production in controlled conditions.

The initiation of cloves primordia and complete plant development cycle, yielding in well-developed bulbs and stalk, for the plants grown in trays took place only in the case if plantlets were subjected to low temperature for appropriate period. This is supported by the data obtained in two different seasons - 2018/2019, when the low temperature was not ensured for necessary period (20 days), and season of 2019/2020, when the low temperature period was of sufficient length (50 days) (Tables 1 and 2).

Table 1. Parameters characterizing bulb development in two seasons of the trial

Growing method	Variety/ clone	Clove primordia, pc 1st decade of May		Stalk development 1st decade of July	
		2019	2020	2019	2020
Traditional	Liubasha	4.6 ab*	5.3 a	yes	yes
	Clone No 2	7.3 b	7.3 b	yes	yes
Innovative	Liubasha	0.6 a	5.0 a	no	yes
	Clone No 2	2.0 a	7.0 b	no	yes

* Means in a column followed by same letter (s) are not significantly different for growing technology factor.

The data presented in Table 1 clearly indicated the linkage between clove and stalk development. When clove initiation did not occur in the 1st year of the trial due to a too short period of low temperature, it also negatively affected stalk development.

In the study of given genotypes, we observed that stalk development is the indicator of appropriate bulb development i.e. cloves formation. This feature was used as indicator of complete bulb development. In the 2nd year, clove primordia and stalk were initiated under appropriate temperature conditions, thus the proper development of the bulb took place.

Adequate yield formation followed and sufficient garlic yield was harvested (Fig. 3).

Morphological parameters of garlic plants, such as plantlet height and root length on the day of planting and plant height later in summer, in the 1st decade of July, at the maximum plant size prior maturation, were compared with the same parameters for traditionally field-grown plants (Table 2).

Table 2. The garlic plants height measurements in two seasons of the trial

Growing method	Variety/clone	Plant height, m			
		1st decade of May		1st decade of July	
		2019	2020	2019	2020
Traditional	Liubasha	0.38 a*	0.24 a	0.58 c	0.61 b
	Clone No 2	0.38 a	0.26 a	0.62 d	0.61 b
Innovative	Liubasha	0.41 b	0.20 a	0.29 b	0.35 a
	Clone No 2	0.42 b	0.21 a	0.24 a	0.34 a

* Means in a column followed by same letter (s) are not significantly different for growing technology factor.

As root length did not differ significantly for the tested variants, it was not included in the table. The plant height parameters were used to evaluate overall development of plant, its ability to produce vegetative part, thus influencing production of assimilates for bulb development. It was clearly indicated in the 1st season, when plants were kept too long in trays prior planting out, at the planting day they were significantly longer than plants growing in the field conditions. After planting out, they delayed in development, as suffered from transplanting stress, the leaves partially were destroyed, and finally in July, transplanted plants had significantly smaller canopy in comparison to traditionally grown plants and in comparison to their size at the transplanting. In the 2nd season, when plantlets in trays were stored in appropriate low temperature for 50 days, the plants were shorter, more compact. Thus they less suffered from transplanting and had higher ability to adapt in the field conditions. In the mid-season, they were smaller in size than traditionally grown plants, but completely developed.

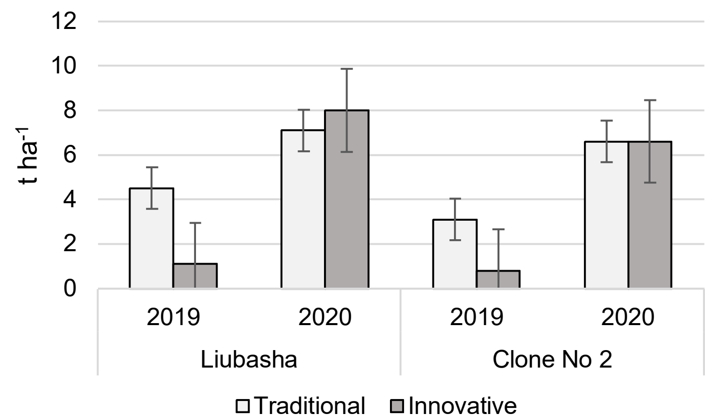

Figure 3. Garlic yield in both growing variants, in 2019 and 2020.

Consequently, the garlic yield in 2019 was relatively low in comparison to average hardneck garlics yield in the region - it fluctuated between 0.8 and 4.5 t ha^{-1} (Fig. 3). The yield outcome of 2019 was affected by insufficient precipitation during the garlic active vegetation season (March - August), when total precipitation reached only 288 mm and the drought periods overlapped with critical periods of garlic yield formation (May – June).

The garlic yield in 2020 was much more appropriate, reaching up to 8.0 t ha^{-1}. This vegetation season had higher precipitation (336 mm), which had a positive effect on the yield development.

The yield of 2020 is of average level for the region, as it is referred by others (Põldma et al., 2005; Juškevičiene et al., 2016), having between 4.3 and 16.8 t ha^{-1}.

There is clear evidence of the effect of clove initiation intensity on the garlic yield formation. In 2019, when cold treatment in the innovative growing variant was of insufficient period according to Resende et al. (2011), thus negatively influencing clove initiation, the yield in this variant was negligible - it did not exceed 1.1 t ha^{-1}. In turn, the yield of 2020 in the innovative technology variant, when appropriate period of low temperature was ensured for plantlets grown in the trays, was insignificantly higher (for cultivar Liubasha) or similar (for local clone) to the traditional technology reaching up to 8 t ha^{-1} for Liubasha and 6.6 t ha^{-1} for local clone. The yield for Liubasha in traditional growing technology was 7.1 t ha^{-1} and for local clone there were 6.6 t ha^{-1} harvested.

It is evident, that in the given seasons the innovative technology variant did not show significantly superior yield to traditional technology. The yield results of 2018/2019 season were negatively influenced by the inappropriate cold storage conditions and extreme drought in the vegetation season of 2019 which influenced transplanted plant establishment and yield formation. Whereas the insignificant yield difference between variants in 2019/2020 season was caused by the very good overwintering conditions in the field, therefore the innovative method could not show superior yield to traditional technology. We assume, that in harder winter when field overwintering conditions would be harsh for garlic, the yield difference would be for the sake of innovative technology.

CONCLUSIONS

The results of performed trial confirm initial idea that innovative hardneck garlic growing technology by using controlled conditions is appropriate for obtaining safe yield in changing climate conditions. The period of 50 days with temperature of 4.0–7 °C ensured clove initiation and complete bulb development.

In the study of given genotypes we observed that stalk development is the indicator of appropriate bulb development i.e. cloves formation.

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The effect of bean flour addition on the rheological properties and baking quality of a triticale flour blends

M. Maradudin[1], I. Simakova[1,*] and A. Fedonnikov[2]

Saratov State Medical University named after V.I. Razumovsky, Faculty of Pharmacy, B. Kazach'ya Str., 112, RU410012 Saratov, Russia
[2]Saratov State Agrarian University named after N.I. Vavilova, Department of Veterinary Medicine, Biotechnology and Food Technology, B. Sadovaya Str., 220, RU410005 Saratov, Russia
[*]Correspondence: simakovaiv@yandex.ru

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Abstract. The aim of this research work was to study and compare the main parameters of the rheological state of the dough made from triticale flour (a variety of George selection by the FSBIS Agricultural Research Institute of the South-East), dough made from flour of white and red bean seeds, as well as parameters of dough from flour of composite mixtures based on them. The rheological properties of the dough were determined using a Mixolab device according to the GOST ISO 17718-2015 method. The mixing ability of the blends was additionally tested by the SDS sedimentation method. It was found that the moment of force, which characterizes the gelatinization process, correlates well with the SDS sedimentation index. To a lesser extent, this indicator correlates with the values of the moments of force characterizing the process of 'starch retrogradation' and the energy intensity of the dough formation process. The water absorption capacity of flour highly correlates with the moment of force during the liquefaction phase and with the moments of force characterizing the minimum and maximum consistency of the dough during the 'starch retrogradation' phase. The correlation between the SDS sedimentation rate and water absorption capacity was found to be rather low. The rheological parameters were also significantly influenced by the type of beans. Taking into account the results of studies of the rheological state of the dough, test baking of bread with various mass fractions of components was carried out. The results obtained confirmed the improving effect of bean flour.

Key words: beans, triticale, composite mixtures, rheological properties, bakery qualities.

INTRODUCTION

One of the directions for creating functional products with an improved chemical composition is the development of multicomponent flour blends enriched with high-grade proteins, vitamins, minerals, and dietary fiber. The basis of such mixtures, as a rule, is cereal flour (mainly wheat and rye), which is supplemented with whole-ground or fermented flour, wheat germ, wheat bran, various types of cereal flakes, flax seeds, sunflower seeds, sesame seeds, and legume flour (mainly soybeans), etc. (Baturina & Muzalevskaya, 2010; Matveeva & Koryachkina, 2012). There is quite a large number of theoretical and experimental studies of the effect of the ingredients of composite mixtures on the carbohydrate-amylase and protein-proteinase complexes of such mixtures, on the basis of which new technologies for the production of bread with increased nutritional value have been developed. However, it is noted that, while possessing certain advantages, both wheat and rye have their own disadvantages, which include both the deficient amino acid composition of wheat and the low gluten capacity of rye. In this regard, triticale is a more promising crop as the basis of the composite mixture, which combines the best properties of its progenitors, wheat and rye, namely, a high protein content with its best amino acid composition. In this regard, Triticale flour is a more promising culture as the basis of a composite mixture combining the best properties of its predecessors, wheat and rye, namely the high protein content with its best amino acid composition. It is noted that due to the increased content of the most complete vegetable proteins, sugars, vitamins, macro - and microelements, grain triticale has a high biological value.It is noted that due to the increased content of the most complete protein substances, sugars, vitamins, fiber, macro- and microelements, triticale grain has a high biological value (Koryachkina et al., 2012; Matveeva & Koryachkina, 2016).

As an additional raw material in the production of functional bakery and flour confectionery products, it is proposed to use legumes, which, due to their unique biochemical composition, occupy a special place among food raw materials of plant origin. Due to the high content of protein, micro- and macro elements, as well as other equally important nutrients, they can be widely used as one of the main raw materials in the production of multicomponent flour mixtures (MFM) with a high content of plant protein, thereby compensating for the lack of animal proteins (Baturina & Muzalevskaya, 2010; Matveeva & Koryachkina, 2012).

Among all the variety of legumes, one of the most attractive crops, as an additional component for composite mixtures, is beans. The flour obtained from bean seeds has a high protein content and a balanced amino acid composition. The protein content ranges from 23.2 to 33.4%, essential amino acids vary from 8,384 to 12,147 mg, the predominant amino acids were leucine and lysine. In addition, it has a significant content of vitamins (thiamine, riboflavin, niacin, vitamin E). The total amount of ash is 2.6–3.7%, while the flour contains potassium, calcium, magnesium, sulfur, phosphorus, iron, copper, manganese Gorbatovskaya et al., 2015; Korshenko & Chizhikova, 2015). Thus, a composite mixture of triticale and bean flours is undoubtedly promising for creating functional bakery products.

Thus, a composite mixture of triticale flour and beans is undoubtedly promising for creating functional bakery products.

The aim of the research work was to study the effect of bean flour addition, on the rheological properties of dough from a composite mixture, to confirm the possibility of its use in bakery production. The impact was determined by establishing a correlation between the qualitative characteristics of composite flour from triticale with bean and the rheological properties of dough based on it. The objects of research were triticale flour of the promising selection line of the FSBIS Agricultural Research Institute of the South-East and whole-ground flour from white and red beans (GOST 7758-75), obtained by successive grinding of beans in the grinding mechanism of a multifunction kitchen machine (MKM) and a laboratory mill Quadrumat Junior (Brabender), as well as flour blends based on them in percentage ratio (triticale:bean): 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90.

MATERIALS AND METHODS

The rheological properties of the dough were determined using a Mixolab device (Mixolab, Chopin, France) according to the GOST ISO 17718-2015 method (GOST ISO 17718-2015et al., 2015). This device, based on the Chopin + protocol, in real time, measures the rotation moment in $N \cdot m$ (Nwm) that occurs between two kneading blades when mixing dough out of flour and water for several successive kneading phases due to different temperatures. This provides complete information that provides a comprehensive assessment of the technological properties of flour and objectively determines its intended use (Dubat & Risev, 2008; Kulevatova et al., 2017; Kazantseva et al., 2018).

The main parameters of the rheological state of the dough were analyzed, including water absorption capacity (WAC,%), stabilization time (T_2, min), moment of force during the liquefaction phase (C_2, $N \cdot m$), moment of force during the gelatinization phase, (C_3, $N \cdot m$), moments of force characterizing the minimum (C_4, $N \cdot m$) and maximum (C_5, $N \cdot m$) consistency of the dough during the phase of starch retrogradation, as well as the energy absorbed during the dough formation (P, $\frac{W \cdot h}{kg}$), which were compared with the indicators of the water absorption capacity for the flour of the initial components and composite mixtures.

In order to test the mixing ability of the composite mixture, we used the SDS sedimentation method (dodecyl sulfate sedimentation) modified by V.M. Bebyakina, M.V. Buntina (Bebyakin & Buntina, 1991).

The bread baking of flour blends was carried out in the laboratory of the FSBIS Agricultural Research Institute of the South-East according to the method of state crop variety testing of agricultural crops. During the research, we used composite mixtures based on triticale flour (TrF) and white bean flour (WBF) and red bean flour (RBF) in a ratio of 90:10; 80:20; 70:30; 60:40, 50:50, 40:60, 30:70. 20:80, 10:90. Experimental dough samples were prepared in accordance with the recipe for the unpaired method of laboratory baking with intensive dough kneading in fivefold repetition (Methodology… et al., 1991).

The correlation between the studied parameters was determined using Microsoft Excel programs. Critical values of the correlation coefficient (r) at 5% significance were identified by the method of V.M. Dospekhov (Dospekhov, 1985).

RESULTS AND DISCUSSION

The research results of the rheological properties of the dough and the correlation coefficients between them and the water absorption capacity are presented in Table 1.

The data obtained (Table 1) confirmed the effect of the content of bean flour in the flour blend on the rheological properties of the dough from composite mixtures based on triticale with white and red bean flour. At the same time, for all indicators, the correlation between water absorption capacity and rheological properties had a significant character (at a 5% significance level $r = 0.553$ (Dospekhov, 1985).

Table 1. Parameters of mixolabograms of dough made from blends of triticale (TrF) white bean flour (WBF) or red (RBF) bean flour

	Composite mixture, %	SDS Sedimentation Index, mm	BWAC, %	T_2, min	C_2	C_3	C_4	C_5	PA, $\frac{W \cdot h}{kg}$
					$N \cdot m$				
		On the basis of White bean flour (WBF)							
1	triticale flour 100%	40	55.8	2.5	0.38	1.15	2.38	4.83	123.88
2	TrF 90%+10% WBF	36	55.7	3.83	0.33	0.93	2.14	4.68	120.65
3	TrF 80%+20% WBF	35	54.7	3.80	0.36	0.96	2.42	5.26	132.80
4	TrF 70%+30% WBF	32	55.7	4.80	0.36	0.61	2.38	4.96	127.99
5	TrF 60%+40% WBF	30	55.3	5.00	0.34	0.50	2.44	5.16	129.29
6	TrF 50%+50% WBF	29	55.4	5.50	0.36	0.46	2.24	4.75	108.53
7	TrF 40%+60% WBF	29	58.4	3.60	0.37	0.45	2.00	4.26	108.02
8	TrF 30%+70% WBF	28	56.2	3.0	0.43	0.46	1.73	4.40	102.30
9	TrF 20%+80% WBF	29	57.2	7.63	0.47	0.53	1.58	4.25	99.09
10	TrF 10%+90% WBF	29	59.5	7.75	0.51	0.53	1.21	3.87	82.18
11	WBF 100%	28	61.6	5.95	0.48	0.50	0.76	2.59	56.50
12	SDS correlation coefficient	1.0	**0.25**	**0.01**	**0.24**	**0.95**	**0.32**	**0.25**	**0.35**
13	WAC correlation coefficient (*r*)	-	**1.0**	**0.23**	**0.58**	**0.18**	**0.84**	**0.92**	**0.85**
		On the basis of Red bean flour (RBF)							
1	triticale flour 100%	40	55.8	2.5	0.38	1.15	2.38	4.83	123.88
2	TrF 90%+10% RBF	37	56.4	3.33	0.35	0.99	2.23	4.24	114.80
3	TrF 80%+20% RBF	32	56.1	4.15	0.34	1.40	2.43	5.14	132.32
4	TrF 70%+30% RBF	30	56.9	4.18	0.31	0.67	2.29	4.25	112.39
5	TrF 60%+40% RBF	27	54.7	4.73	0.29	0.44	2.24	4.48	113.20
6	TrF 50%+50% RBF	26	55.3	4.48	0.30	0.45	2.15	4.14	108.53
7	TrF 40%+60% RBF	25	56.1	4.87	0.31	0.46	1.90	4.06	100.49
8	TrF 30%+70% RBF	24	56.1	2.87	0.32	0.45	1.75	3.27	79.74
9	TrF 20%+80% RBF	25	57.7	3.55	0.37	0.42	1.22	2.10	52.72
10	TrF 10%+90% RBF	25	59.4	3.55	0.43	0.47	1.03	2.0	45.84
11	RBF 100%	24	56.9	3.0	0.46	0.53	0.65	0.02	29.84
12	SDS correlation coefficient	1.0	**0.07**	**0.07**	**0**	**0.58**	**0.36**	**0.39**	**0.43**
13	WAC correlation coefficient (*r*)	-	**1.0**	**0.09**	**0.45**	**0.03**	**0.45**	**0.47**	**0.46**

It was found that with the increase in the amount of bean flour in the flour blend from 10 to 90%, the SDS-sedimentation indicator steadily decreases from the maximum to the minimum value (1.24 for WBF and 1.48 for RBF). Moreover, this indicator is highly correlated with the moment of force C3, which characterizes the gelatinization process. Since the SDS Sedimentation Index is a measure of the quality and quantity of gluten, therefore, increasing the bean flour content in the composite mixture is guaranteed to reduce the amount of gluten.

In addition, with the increase in the amount of bean flour in the composite mixture from 10 to 90%, the water absorption capacity increases by 6.4% (from 55.7% to 59.5%) when using white bean flour and by 5.1% (from 56, 4% to 59.4%) when using red bean flour. This confirmed the previously obtained data on the effect of morphological features of bean products addition on the water absorption capacity (Maradudin & Simakova, 2019; Simakova et al., 2019).

The change in the content of bean flour in the composite mixture affected the stabilization time (C_2), however, since this parameter changed abruptly, the correlation dependence between the WAC parameters and the stabilization time was not significant. Moreover, up to a certain ratio (60:40) of the components, the stabilization time increased (for both white and red beans), and then this indicator decreased. It is known that increasing the stabilization time has a positive effect on the dough, providing a good rise in the bread during proofing. Therefore, it can be expected that increasing the content of bean flour in the composite mixture in the range of 40–50% will not significantly reduce the rise of the baked goods.

The correlation between water absorption capacity and moment of force during the liquefaction phase (C_2) (r = 0.58 and r = 0.45) was more significant. This parameter characterizes the process of activating proteolytic enzymes, leading to a decrease in the consistency of the dough due to the rupture of hydrogen bonds in the protein molecules that hold the protein molecular chains together. Degradation of gluten proteins and liquefaction of the dough occurs. Moreover, the lower the moment C_2 is, the higher the volumetric bread yield becomes. Since in our case the opposite process was observed, we can expect a decrease in the volumetric yield of baked products as the content of bean flour in the flour blend increases.

The increase in the amount of bean flour in the composite mixture affected the change in the moment of force (C_3), which characterizes the properties of starch and amylolytic activity in the analyzed sample (Gorbatovskaya et al., 2015; Korshenko & Chizhikova, 2015). High values of C_3 characterize a weak enzymatic activity, and low values, on the contrary, characterize high enzymatic activity (GOST ISO 17718-2015 et al., 2015).

However, since the change in this parameter had an abrupt character, and the relationship between the water absorption capacity and the moment C_3 is insignificant (r = 0.18 and r = 0.03), then, taking into account the available data, it can be assumed that the varietal characteristics of beans have a more significant influence on the gelatinization process. This is indicated by a high correlation between the sedimentation index and the moment C_3 (r = 0.95 and r = 0.58).

It was also noted that the bean flour content increase in the composite mixture affects the change in the moments of force characterizing the minimum (C_4) and maximum (C_5) dough consistency during the 'starch retrogradation' phase. However, this impact is ambiguous due to the varietal characteristics of the beans.

The moments of force at the extremum points C_3, C_4, C_5 characterize the carbohydrate-amylase complex of the studied system and the processes occurring in it. Low values of these parameters, characterizing high autolytic activity, ensure the formation of a finely dispersed crumb structure during baking. Bakery and confectionery products obtained from composite mixtures with low values of the C_5 moment are distinguished by greater resistance to staleness, and, therefore, increased shelf life. However, for composite mixtures based on triticale, with the bean flour content increase in the mixture from 10% to 40%, the increase in the moments of force is observed from the minimum to the maximum value. With a subsequent increase in the content of bean flour in the mixture, the C_5 moment decreases. In a smoother mode, bean flour content is 80%. When there is a sharper decrease, a further increase of bean flour content to 90%

is observed. This indicates a more complex interaction of the protein-carbohydrate complex of triticale and beans, and the need for a deeper study of these systems.

The results of triticale-bean bread baking test (Fig. 1, a; b) are shown in Table 2.

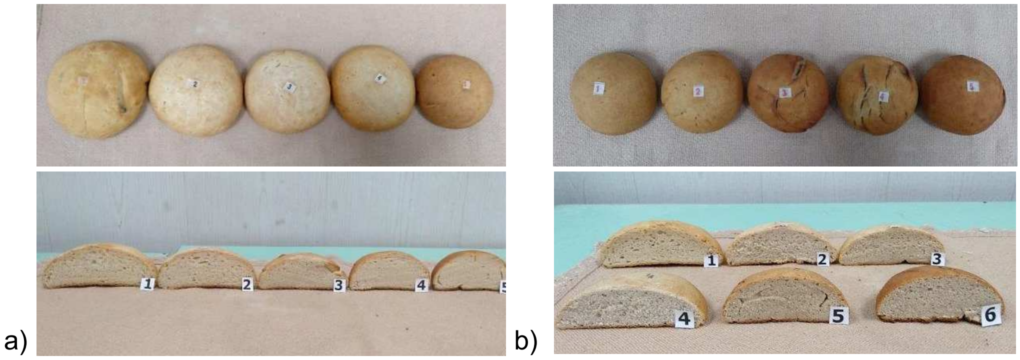

Figure 1. Samples of test bread baking from composite mixtures with different mass fractions of components: a) with the use of white bean flour (WBF); b) with the use of red bean flour (RBF).

Table 2. Selected quality indicator of flour-blend and bread made from flour blends based on triticale flour and white bean flour (WBF) and red bean flour (RBF)

No.	Bean flour content in mixture	SDS Indicator, mm	Weight of loaf, g	Diameter, mm	Height of a loaf, mm	Ratio, H d^{-1} cm^3	Loaf volume	Porosity, points	Acidity, degree
1	TrF 100%	40	134.5	125.0	47.0	0.38	410	4.8	3.0
2	10% WBF	36	137.6	111.3	45.0	0.40	350	4.6	2.2
3	20% WBF	35	137.8	107.7	40.0	0.37	300	4.4	2.9
4	30% WBF	32	138.0	103.3	44.3	0.43	275	4.1	3.1
5	40% WBF	30	138.2	140.0	43.7	0.31	270	4.0	3.7
6	50% WBF	29	150.2	95.2	41.7	0.44	260	4.0	2.5
7	60% WBF	29	149.1	92.3	44.0	0.48	270	4.0	2.9
8	70% WBF	28	152.2	90.4	46.3	0.51	270	4.0	3.6
9	80% WBF	29	152.4	91.2	49.0	0.54	270	3.8	3.2
10	90% WBF	29	151.8	89.3	51.7	0.59	280	3.8	3.5
11	100% WBF	*28*	155.7	91.0	52.0	0.57	295	3.5	3.3
	R. correlation coefficient	1.0	**0.71**	**0.34**	**0.08**	**0.37**	**0.80**	**0.21**	**0.21**
1	TrF 100%	40	134.5	125.0	47.0	0.38	410	4.8	3.0
2	10% RBF	27	137.0	116.0	42.0	0.36	380	4.8	3.4
3	20% RBF	32	137.4	113.3	39.7	0.35	355	4.8	3.3
4	30% RBF	30	138.8	110.0	33.7	0.31	300	4.6	3.7
5	40% RBF	27	140.3	94.7	39.0	0.41	255	4.3	3.9
6	50% RBF	26	141.9	94.7	38.3	0.40	220	4.3	4.2
7	60% RBF	25	145.4	86.0	43.0	0.50	220	4.0	3.4
8	70% RBF	24	146.0	85.3	42.0	0.49	225	4.0	3.0
9	80% RBF	25	146.6	82.2	45.0	0.54	220	4.0	3.6
10	90% RBF	25	148.0	82.1	47.0	0.57	220	3.8	4.0
11	100% RBF	24	148.1	83.0	48.0	0.58	250	3.8	4.8
	R. correlation coefficient	1.0	**0.67**	**0.73**	**0.0**	**0.37**	**0.67**	**0.58**	**0.23**

Table 2 data show that the ratio of flour triticale and flour beans significantly affects the bakery properties of the composite mixture. Moreover, the qualitative characteristics of baked bread samples are well correlated with an indicator of SDS-sedimation (for weight R = 0.67, for the diameter R = 0.73, for the volume R = 0.67 and for porosity R = 0.58). With the increase of bean flour amount in the composite mixture from 10 to 90%, an increase in weight, height and the ratio of height to diameter of the baked loaves is observed. At the same time, the decrease in the SDS-sedimentation index is proportional to the diameter, volume and porosity of the baked loaves decrease. This indicates a significant effect of bean flour on the protein-proteinase complex of the composite mixture.

CONCLUSIONS

The improving effect of bean flour lies in the fact that even with a high mass fraction of bean flour in the composite mixture (60–100%), it was possible to obtain a full rheological profile of this system, which indicates the preservation of the optimal dough structure. In addition, dosed use of bean flour as a component in a flour composite mixture or a complete replacement of triticale flour with bean flour, increasing the protein content in bakery, pasta and confectionery products, allows one to influence the shape stabilization of the final product:

– when the content of bean flour in the mixture is up to 10%, the stabilization time changes insignificantly, therefore, the shape and volume of bakery products (tin bread) will not change significantly either;

– when the content of bean flour is up to 50%, the stabilization time increases 2.2–1.8 times, which indicates a more significant effect on the shape stabilization of the final product, and, therefore, such mixtures can be recommended for functional food products, for which he content of the final product is more important than its shape;

– when the content of bean flour is more than 70%, the stabilization time is reduced 3–4 times, and, therefore, such mixtures can be used for low-gluten food products of functional purpose with an unfixed shape.

At the same time, a significant difference in the tendencies of changes in the rheological properties of the dough made from composite mixtures with triticale, in comparison with the rheological properties of the dough based on wheat, indicates a more complex interaction of the components that requires a more careful research study.

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Dendrological collections of the Stavropol Botanical Garden: introduction and development prospects

T.V. Nezhentseva*, A.F. Koltsov and E.N. Grishchenko

Federal State Budget Scientific Institution North-Caucasian Federal Agricultural Research Center, Lenin street 478, RU355029 Stavropol, Russia
*Correspondence: sbsconifers@mail.ru

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Abstract. Botanical gardens perform active introductory work, carry out educational and academic activities, preserve the gene pool of the red book species, and serve as a source of enrichment plant resources. The leading role in solving these problems is played by dendrological collections, on the basis of which botanical research is carried out. The main part of the woody collections is located in the Stavropol Botanical Garden (SBG) arboretum. Work on the construction of the SBG arboretum began in 1959. Initially, it was planned to collect various species, natural and cultural forms of woody and shrubby plants from the temperate, and partly northern and subtropical zones. In addition, four models of forest vegetation formations of the Stavropol Krai and Karachay-Cherkessia were created on the territory of the SBG. The article presents the main stages of the introduction work on the creation of dendrological collections, the methods used. Most of the collections were formed in a short time due to the preliminary selection and mobilization of planting material. The long period of introduction made it possible to judge the advantages and disadvantages of the chosen method of genus complexes. The modern composition of the collections is analyzed, the role of the introduction process in the conservation of biodiversity is noted. The species that are promising for use in the landscaping of the region are listed. In the future, work with dendrological collections implies the attraction of new species, the introduction of information technologies, continuation of study and monitoring of plants listed in the Red Books of the Stavropol Krai, Russia and the Red List of the International Union for Conservation of Nature.

Key words: dendrological collections, arboretum, botanical garden, plant introduction, angiosperms, gymnosperms.

INTRODUCTION

In the modern world, botanical gardens play an important role. They perform active introduction work, carry out educational and academic activities, and preserve the gene pool of red book species. The dendrological collections are of particular value in the botanical gardens. Their formation takes a long period of time and requires careful long-term research. Trees and shrubs play a landscape-forming role in the park areas of the gardens (Kuzevanov & Sizykh, 2005). Arboretums of botanical gardens serve as centers for the formation of a new development level, as a source of enrichment with new types of plant resources (Hu et al., 2020). Arboretums have accumulated a rich gene pool of woody plants *ex situ*, which can be used in forest and green construction, as well as for rare species reintroduction (Andreev, 2003).

Work on the Stavropol Botanical Garden (SBG) arboretum construction began in 1959 - the year of the foundation of the garden. Initially, it was planned to collect various species, natural and cultural forms of woody and shrubby plants from the temperate, and partly northern and subtropical zones. The employees were tasked with studying these species, identifying the most promising ones for further use in various branches of forestry and green economy. The project of the arboretum was an example of park art with the use of landscape and architectural elements. A clear layout was worked out for permanent tree and shrub plantings in the initial period of construction of the garden. Taken together, these plantings made up the entire park part of the garden and represented the final result of all its introduction work. Forest formations were also laid on the territory of the SBG for the better representation of dendrological collections.

With the growth of cities and an increase in the volume of landscape gardening in recent years, there is an increasing need for woody plants, which must have: high decorative qualities, durability, resistance to pests and diseases, as well as unfavorable environmental factors, economically useful properties. The introduction work carried out in SBG for 60 years allows expanding the modern assortment of trees and shrubs used in the landscape design of the region.

The aim of the research, based on the historical analysis of the formation of the SBG dendrological collections, is to evaluate the advantages and disadvantages of the introduction method used, and to suggest ways for further development of the collections.

MATERIALS AND METHODS

Soil and climate characteristics. The Stavropol Botanical Garden is located in the western part of the Stavropol Upland, which is a large geomorphological area and has a significant impact on the climate of the Fore-Caucasus. The Botanical Garden is located between the forestlands of the Krugly Les and the Russkaya Lesnaya Dacha at an altitude of 620–640 m above sea level. The flat terrain of the garden has a slight slope in the direction from south to north. The temperate continental climate with unstable humidification of the research area is formed under the influence of the Main Caucasian Ridge. The hydrothermal coefficient of heat and moisture supply is 0.9–1.1. The average annual precipitation is 500–600 mm. In recent decades, there has been a trend towards an uneven distribution of precipitation throughout the year. Winter is moderately mild, the coldest month is January, with an average monthly temperature of -1.9 °C. On average, the snow cover height is 10–12 cm. Often, a stable snow cover for the winter is not established at all (Badakhova et al., 2014). The duration of the frost-free period is 180–190 (up to 210) days. The number of days with thaws is 45–48. The warmest month is July, the average monthly temperature in July is +20–25 °C. There is an unfavorable dynamics of weather conditions (especially for the shoots of woody plants) - late spring frosts, drought in April, in August-September, dry winds, strong winds. The number of days with dry winds is 50–60 (Agroclimatic resources of the Stavropol Krai, 1971; Badakhova & Knutas, 2007).

The soils of the territory of the botanical garden and the landscape arboretum in particular are represented by leached thick low-humus light-clay chernozems with a humus content in the upper part of the profile of 5.22%. The availability of mobile phosphorus in the upper horizon is increased (34 mg kg^{-1}). The exchangeable potassium content is 174 mg kg^{-1}, which characterizes the soil as low-supplied with this element. The water suspension reaction is slightly acidic in humus horizons. The humus content is 5–6%, the average thickness of the humus horizon is 63 cm, the humus reserves in the meter layer average 499 t ha^{-1} (Kuprichenkov & Kopeykin, 1988). The use of groundwater by plants is difficult, since they lie at a depth of 10 m.

The Stavropol upland is a forest-steppe. Forests and shrubs predominate on wet slopes, at altitudes of 500–600 m above sea level. The forest also grows on uplands. On the territory of the Stavropol heights there are quite large forestlands: Krugly, Tatarsky and Mamaysky, Russkaya and Tamanskaya Lesnaya Dacha. The parent rocks of the cenoses from wet to dry ecotopes are *Fagus orientalis*, *Quercus robur*, *Q. calcarea, Carpinus betulus*, *Fraxinus excelsior*, hawthorn, rose hips, blackthorn *(Prunus spinosa)*, Russian almond *(Amygdalus nana),* Pallas buckthorn *(Rhamnus pallasii)*.

The study was conducted by analyzing the data presented in the report documentation of the Stavropol Botanical Garden for the entire period of its existence.

The object of research is the dendrological collections of the SBG.

Research methods. The research was carried out by analyzing the data presented in the reporting documents of the past years, and modern data obtained using geoinformation technologies. The current state of plants in the collections was characterized using a visual assessment method (Lapin & Sidneva, 1973).

RESULTS AND DISCUSSION

Founders of the garden the chief forester of the region A.A. Klopov and D.B.Sc. V.V. Skripchinsky were at the origins of the creation of woody plants collections. M.A. Lesunova (1959–1964), L.V. Boyko (1961–1986) took an active part in the collection, replenishment and study of representatives of the tree flora. Thanks to the long-term work of A.K. Chikalina (collection of gymnosperms, 1964–2006) and M.A. Koltsova (collection of angiosperms, 1972–2015), dozens of species and intraspecific taxa were attracted, and numerous introduction studies were conducted. A.F. Koltsov (1984–2020), E.V. Bachurina (1986–1993), E.V. Pyatko (1992–2014), E.N. Obshchiya (1994–2003), T.V. Nezhentseva (curator of the gymnosperms collection since 1993) has made a contribution in the study of dendrological collections in different years (Bardakova et al., 2020).

The main part of the collections of woody plants according to the plan is concentrated in the arboretum, which is divided into two components: regular and landscape. In addition, the attraction of introduced species made it possible to create several models of forest formations on the territory of the botanical garden (Fig. 1), which also became part of the dendrological collection.

The *regular arboretum,* covering an area of 13 hectares, is the first permanent plantings, with which the construction of the botanical garden began in 1959. In 1960–61, the entire network of alleys was planted. In 1962, tree plantations were planted with border shrubs, and along with this, the replacement of sick and dead trees in the alleys was carried out. At the moment, it consists of 24 alleys, 11 of which have partially or completely lost their decorativeness and require restoration measures (Khrapach & Gudiev, 2020).

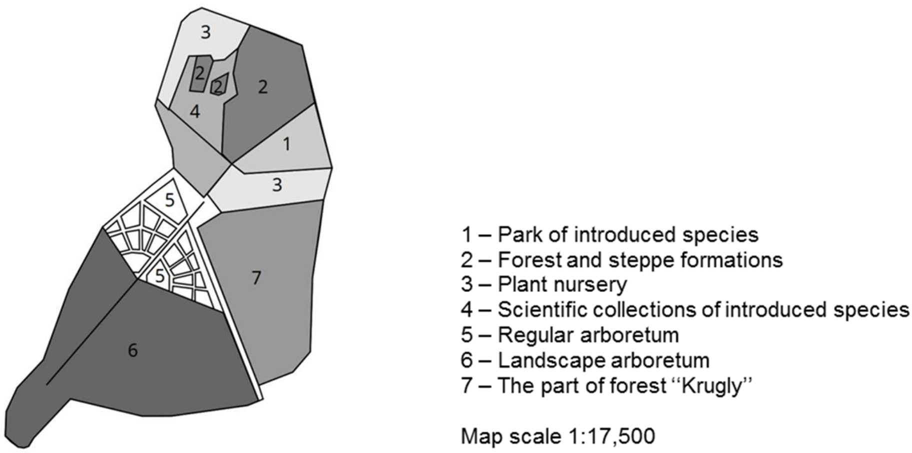

Figure 1. Schematic map of the Stavropol Botanical Garden.

In 1961, the *landscape arboretum* was laid after the preparatory work. This year, 345 plant species from 76 genera and 28 families were planted here, with a total of more than 2.5 thousand seedlings. By the end of 1962, the landscape arboretum contained 19 species from 7 genera of gymnosperms (*Picea*, *Pinus*, *Abies*, *Larix*, *Thuja*, *Juniperus, Biota)* and 556 species and forms of angiosperms. In 1964, *Taxodium distichum* was planted in a permanent place in the arboretum, in 1969 - *Pseudotsuga menziesii.* They are among the oldest specimens of the gymnosperm collection. Angiosperms were most widely represented by the families *Rosaceae* (87 species) and *Fabaceae* (43 species), as well as such genera as *Lonicera* (41 species), *Berberis* (25), *Rosa* (23), etc. (Report on the work of the Stavropol Botanical Garden, conducted in 1962).

On the territory of the botanical garden, there are expositions of forest formations representing the main tree species of vegetation of the Stavropol Krai (beech) and the Karachay-Cherkess Republic (birch, spruce, fir, pine) (Report on the work of the Stavropol Botanical Garden, conducted in 1962). Models of birch, beech, fir-spruce and pine forests were created on thin soil with a thickness of 20–30 cm on a slab of shell limestone.

Birch forest. In 1961, 1000 birch saplings were planted, harvested by Zelenchuksky forestry in the places of its natural growth. By the end of 1961, only 559 plants were preserved on an area of 0.6 hectares. In order to further expand the area of the birch grove and bring it to the design size, 1,280 pieces of natural birch seedlings of different ages were harvested by 1963 in the floodplain of the Teberda river. The model of the birch forest has been preserved to this day, but the plants do not reach the height observed in nature. The exposition is quite decorative.

The *beech forest* began to be laid in 1961 on an area of 0.3 hectares with seedlings of oriental beech (*Fagus orientalis*) grown in the Stavropol Botanical Garden nursery, and in 1962 this work was continued - seedlings from the Stavropol mechanized forestry enterprise were planted on an area of 0.73 hectares. The model corresponds to natural cenoses of the same age in their condition, self-seeding is observed by a woodsidet, decorative effect increases.

The *fir-spruce forest* was planted on an area of 0.8 hectares by three-year-old seedlings of Caucasian fir (*Abies nordmanniana*) and Oriental spruce (*Picea orientalis*) in 1961. As a result of the prevailing weather conditions, most of the plants died. In the following years, the restoration was carried out with wild fir seedlings harvested in the Teberdinsky Nature Reserve. Currently, the area of the spruce-fir forest is 0.39 hectares. When creating this model of cenosis, the method of shading was used, corresponding to the natural change of communities - conifers were planted together with taller birch seedlings, which were then cut down. The state of the model is the same as that of the birch forest exposition. Abundant self-seeding firs are formed even under the forest canopy, but their ability to replace old plants is unlikely.

The *pine forest* was planted in 1962 with two-year-old seedlings of hamated pine (*Pinus sylvestris* var. *hamata*), obtained from Kislovodsk and grown in the botanical garden nurseries. Currently, the exposition is located on an area of 0.32 hectares. The state of the model corresponds to natural cenoses, but self-seeding is almost not observed.

Thus, the most successful models were formed of beech and pine forests, they are easier to save from overgrowth by local species - ash and hornbeam. The created models of forest communities are always popular with tourists, have decorative, educational and scientific significance, thus performing important functions of botanical gardens.

The introduction work carried out in the SBG during the formation of collections consists of 4 stages. The first stage is preliminary selection, which is based on the most common method of climatic analogs (Mayr, 1909). The second stage is the mobilization of species diversity and the formation of collections directly, in which the method of (phylogenetic) generic complexes is used (Rusanov, 1950). The third stage is the analysis of plant adaptation (methods of visual observation - phenology, growth dynamics, methods of reproduction). The fourth stage is summing up the results of the implementation, which consists in assessing the prospects and possibilities of using the studied plants in ornamental gardening, forestry and in environmental protection measures. The implementation process is long and continuous.

Analysis of the geographical origin of woody plants, carried out at the first stage, showed that in the temperate zone of the North Caucasus, the main sources of collection fund replenishment are dendroflora of the East Asia (from the northern and mountainous regions of China, Korea, Japan, the forest-steppe of the Russian Far East), North America, European-Siberian flora, and to a lesser extent - the flora of the Mediterranean (Takhtajyan, 1978).

At the second stage, the main source for the mobilization of the species diversity of the local flora were expeditions. The plant material collected in this way was used for planting in nurseries and permanent plantings. In addition, the number of species, varieties and cultivars in the collections has increased significantly due to established relations with botanical organizations of the former USSR. Thanks to the creation of the Council of Botanical Gardens in the early 60s and the seed exchange system (delectus) there was an active replenishment with new introducers. Botanical gardens and arboretums of Moscow, Kiev, Alma-Ata, Vladikavkaz, Minsk, Sochi, St. Petersburg and other cities became the sources of planting material. A special place in the dendrological collections of the botanical garden is occupied by plants grown from seeds obtained from foreign botanical gardens in Germany, Poland, Japan, Canada, Hungary, etc. (Kozhevnikov et al., 2000).

In the first years of laying dendrological collections, the main work was focused on the collection, cultivation of planting material and care of plantings. The received species were initially tested in botanical garden nurseries, the formation of which was carried out in parallel with active introduction work with taxa from different climatic zones. The creation of shady areas made it possible to start mass reproduction of coniferous and deciduous species. Plantings in the arboretum were almost annually replenished with new species and forms.

According to the systematic principle of the Rusanov method, genera of the same family are located in a single array. Within the exposition of each genus, species, varieties, forms and hybrids are presented, from which genus diversity is composed.

In modern conditions, the importance of phenological studies and especially long continuous series of observations is increasing (Zheng et al., 2002; Firsov et al., 2010). In the SBG in different years and up to the present time, phenological data are collected according to the method developed in the Main Botanical Garden (Moscow) (Alexandrova et al., 1975). The results obtained make it possible to judge the degree of compliance of introduced species with new growing conditions. In the course of research work, winter hardiness, drought resistance, methods of reproduction, characteristics of decorative and economic features of introduced species are studied, which will further allow assessing the results of introduction.

About 90 species of trees and shrubs from different angiosperm families were recommended for reproduction for economic purposes as the first preliminary result of the introduction in 1967: *Berberis thunbergii*, *Betula populifolia*, *Castanea sativa*, *Cercis siliquastrum*, *Corylus avellana*, *Forsythia ovata*, *Quercus rubra*, *Spartium junceum*, *Viburnum lantana*, etc. (Report on the topic No. 15, 1967). In 1970, the prospects of representatives of the *Rosaceae* family consisting of 28 genera and 280 species were evaluated. As a result, 211 species of the most promising plants were identified, which accounted for 77% of the representatives of the family. Numerous species and forms of the genera *Crataegus*, *Sorbus*, *Malus*, *Photinia*, *Physocarpus*, *Rosa*, etc. are among the economically valuable ones for the Stavropol Krai. In the period from 1970 to 1973, attention was paid to representatives of the local flora - 35 species from 41 genera and 18 families were planted in the arboretum. More than 1,000 species and forms of woody and shrubby plants were tested by the end of the 70s (Report on the work of the Stavropol Botanical Garden, conducted in 1976).

In the 70s, the staff of the garden set up an experiment on growing a salt-resistant form of scots pine (*Pinus sylvestris*) for testing on saline soils of the Sengileevskaya depression. As a result, the survival rate of seedlings was 80% (Report on the work of the Stavropol Botanical Garden, conducted in 1974). Experimental studies on determination of the optimal conditions for gymnosperms (shading, watering, mulching) have become relevant. As a result, recommendations were formulated on agricultural techniques for growing seedlings of Oriental spruce (*Picea orientalis*) and Caucasian fir (*Abies nordmanniana*), taken from under the forest canopy. A long-term series of experiments on vegetative propagation by stem cuttings of ornamental forms, species and cultivars of the Cypress family (*Cupressaceae*) deserves attention. A special feature of the experiment was a detailed study of the factors that affect the rhizogenesis process (various substrates, temperature, humidity, rooting time, growth stimulators) (Nezhentseva & Kozhevnikov, 2018). Long-term researches of the garden staff were aimed at identifying the dynamics features of growth and development, the introduction possibilities of species, natural forms and cultivars of gymnosperms. The results obtained allow to select adapted plant genotypes for use in forestry, which is consistent with modern methods of rational nature management (Jansons et al., 2020).

In the period from 1981 to 1985, the generic complexes of the *Oleaceae* family were studied: *Fraxinus*, *Syringa*, *Ligustrum*, *Forsythia*. Based on the integrated assessment of the prospects for the introduction of plants of the genus *Fraxinus*, recommendations were developed for the use of various types of ash in landscaping, depending on the soil and climatic conditions in the regions of the Stavropol Krai (Koltsova et al., 1980). Subsequent years were devoted to the study of ecological and biological features, accelerated methods of reproduction of such genera and species as *Sorbus*, *Salix*, *Crataegus*, *Chaenomeles*, *Pentaphylloides daurica* and *P. fruticosa*, *Exochorda racemosa*, *Aronia*, *Cornus mas* (varieties), *Asimina triloba*, etc. (Koltsova et al., 2009; Pyatko, 2010; Koltsova et al., 2014). Since 1994, the SBG has been working on the introduction of tree and shrub exotics. A significant part of these plants have a natural range in the warm-temperate and subtropical climate zone. Therefore, special attention was paid to their winter hardiness. The prospect group of some representatives of the families *Hamamelidaceae*, *Magnoliaceae*, and *Styracaceae* was determined (Khrapach et al., 2009).

In 2015, a large-scale inventory of the arboretum's ancestral complexes using information technologies was launched. The creation of a detailed map using modern software using the layout scheme of the arboretum of previous years (Fig. 2), the creation of a map of the area using satellite navigation, and the work with the electronic database of the complete list of arboretum species served as a prerequisite for further research in this direction (Petin & Nezhentseva, 2011; Kotenko, 2016).

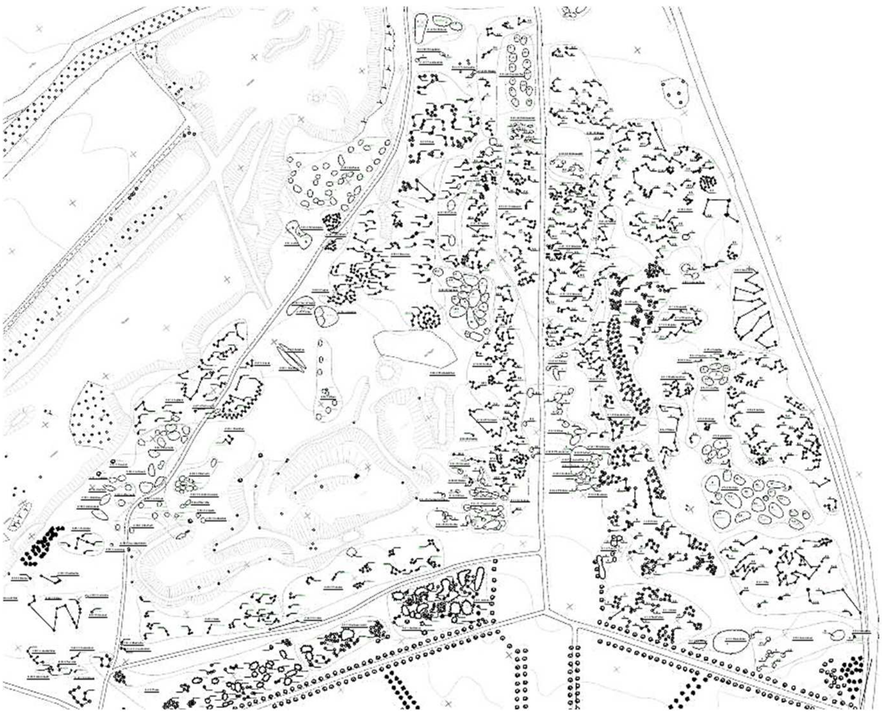

Figure 2. Schematic map of the SBG arboretum (fragment).

Inventory of species composition for such an object as a landscape arboretum is a labor-intensive, long-term task and will be continued in the coming years. Currently, the arboretum collection contains about 580 species of cultivars and forms of Angiosperms *(Magnoliophyta)* and 164 - Gymnosperms *(Pinophyta)* plants (Nezhentseva & Shchegrinets, 2018; Bardakova et al., 2020). The generic complexes of the family *Rosaceae* are still the most widely represented in the arboretum collection: *Crataegus*, *Sorbus*, *Malus*, from other families – *Betula*, *Fraxinus*, *Acer*. The largest families of Gymnosperms are *Pinaceae* and *Cupressaceae* (Figs 3 and 4). Despite the fact that most of the plants in these complexes are more than 50 years old, a significant part of them are in good or satisfactory condition, but many of them require a lot of work to update and repair, for some of them it is necessary to verify the taxonomic affiliation.

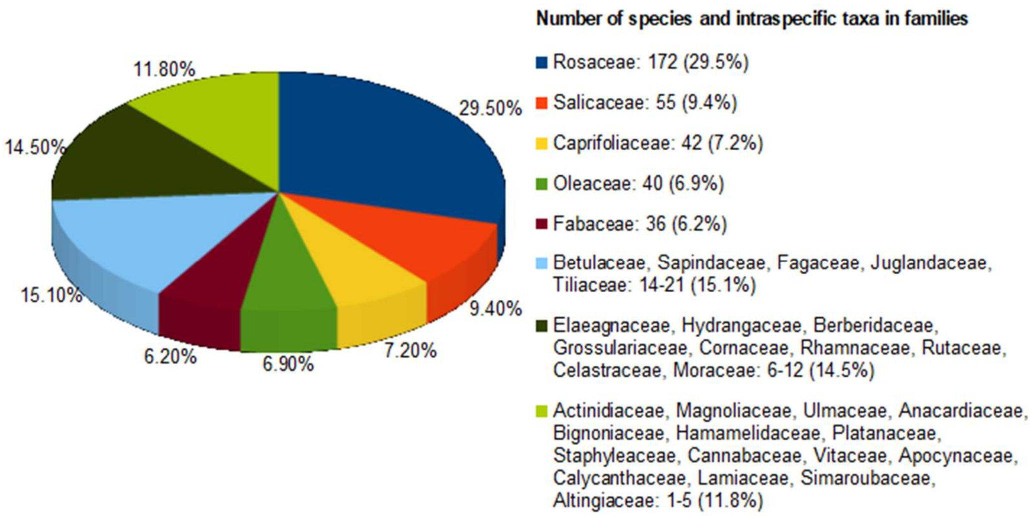

Figure 3. Current state of the angiosperms collection of the arboretum.

In the period 2006–2020, more than 100 promising species and forms from different families of angiosperms and gymnosperms were recommended from the SBG dendrological collection for use in landscape design (Koltsova et al., 2006; Nezhentseva & Kozhevnikov, 2018). During the entire period, part of the research work was to monitor the growth and development of plants transferred to the landscaping of the city of Stavropol and more than 40 settlements of the region. Many introduced species and varieties have received a good biological and economic assessment. As an assessment scale, we used a method for assessing the prospects for the introduction of woody plants based on visual observation data (Lapin & Sidneva, 1973). The main criteria characterizing the state of introduced species were: the degree of annual ripening of shoots, winter hardiness, preservation of habitus, shoot-forming ability, regularity of shoot growth, ability for generative development, methods of reproduction. Currently, the most popular species in the landscaping of the region are such species as *Thuja occidentalis* 'Fastigiata', *Juniperus chinensis* 'Pfitzeriana', *Pinus pallasiana, P. mugo, Taxus baccata, Betula pendula, Tilia cordata, Cercis canadensis, Paulownia tomentosa, Catalpa bignonioides, Aesculus hippocastanum, Castanea sativa, Corylus colurna.*

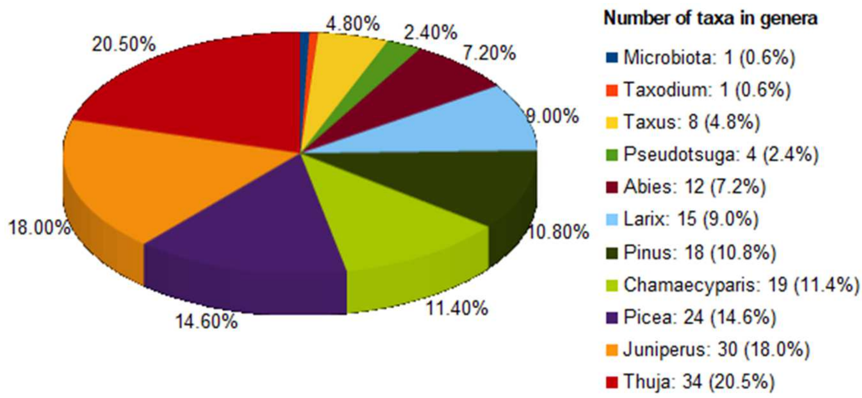

Figure 4. Current state of the gymnosperms collection of the arboretum.

Another important area of work with dendrological collections in SBG is the *ex situ* conservation of plant biodiversity. This has acquired particular relevance after the adoption of the International Strategy for the Conservation of the Gene Pool of Rare and Endangered Plant Species (Andreev, 2003; Wyse Jackson, 2009). The SBG dendrological collection currently includes 68 angiosperm taxa and 15 gymnosperms with different rarity status. Of these, 28 species are included in the Red Data Book of Russia, 49 species are included in the Red Data Books of the regions of Russia and neighboring countries, and 6 species are included in the IUCN Red List (Bardakova et al., 2020). Monitoring of the state of rare and endangered species in the SBG collections and in nature is carried out annually, and the methods of their reproduction are studied. As a result of this work, promising species recommended for landscaping were identified: *Picea omorica, Pinus pallasiana, Taxus baccata, Betula raddeana, Acer laetum.*

CONCLUSIONS

In SBG, as a result of planned introduction work carried out since 1961, representative collections of woody plants have been created that have scientific and practical significance. Currently, the landscape arboretum contains all the variety of species of trees and shrubs introduced in the botanical garden. Most of the taxa have entered the generative phase of development and are producing viable seeds, which indicates their acclimatization at the point of introduction. The collected seed material makes it possible to expand research on seed reproduction of these species.

As a result of the integrated assessment, it was found that the most appropriate is the further introduction of fast-growing and rather durable species and cultivars of poplars, related to white poplar (*Populus alba*) and aspen (*P. tremula*), drought-resistant species of maple, birch, mountain ash from the *Lobatae* section, from slow-growing ones - species of oak, cultivars of magnolia kobus. In the long term, it is possible to introduce plants that do not tolerate local soil conditions (species of rhododendrons, except for *Rhododendron lutea*, which successfully grew in the birch forest model, and the highly ornamental Xanthoceras *(Xanthoceras sorbifolia*)).

Analysis of the history of the formation of woody collections of SBG outlined the main stages of introduction research. Most of the collection was formed in a short time due to the preliminary selection and mobilization of planting material. A long period of research has made it possible to judge the advantages and disadvantages of the chosen method of generic complexes. Positive aspects: the visibility of the species diversity within each complex, the possibility of comparative studies in similar ecological conditions. The negative side: the limited spatial distribution of species of the same genus leads to closely related crosses, which affects the purity of the species.

In the future, work with dendrological collections implies the attraction of new species, the continuation of information technologies introduction. The increased needs of the population for a variety of highly ornamental plants create prerequisites for the development of new directions in the study of dendrological collections. It is planned to reconstruct the lost decorative elements and create new expositions. It is planned to continue studying and monitoring plants listed in the Red Books of the Stavropol Krai, Russia and the Red List of the International Union for Conservation of Nature.

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Leaf nutrient status of tomatoes in coconut coir medium – differences in cultivars, impact on yield and quality

A. Osvalde*, A. Karlsons and G. Cekstere

University of Latvia, Institute of Biology, Laboratory of Plant Mineral Nutrition, O. Vaciesa street 4, LV-1004 Riga, Latvia
*Correspondence: anita.osvalde@inbox.lv

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Abstract. Coconut coir as an alternative to rockwool is increasingly used as a substrate for soilless hydroponic greenhouse production of tomatoes. However, little is known about the nutrient status of tomatoes in coconut coir, especially under intensive production conditions. The aim of this study was to investigate the nutrient status of different tomato cultivars (orange plum 'Organza F1', red cherry 'Daltary RZ F1', red large fruit-sized 'Securitas RZ F1' and pink large fruit-sized 'Fujimaru F1') under industrial greenhouse production, using coconut coir as a substrate to reveal nutrient imbalances, their impact on tomato yield and quality, and cultivar differences. Essential nutrient and leaf SPAD value was detected for the youngest fully developed leaves and the old still vital leaves twice per month from April to August 2020. The total yield, marketable and non-marketable yield was regularly determined. During the crop cycle, the content of most of the nutrients in tomato leaves corresponded to the standard range reported for tomatoes. Only some imbalances were found: all cultivars were characterized by low Zn and high S levels in both young and old leaves. The obtained results identified several differences between the cultivars: cherry tomatoes 'Daltary' had significantly higher N, K, Fe and Zn in leaves compared to other cultivars. The lowest N, Fe and Cu were determined for large fruit-sized 'Securitas'. Regardless of the nutrient status and microclimate conditions, the marketable yield of 'Daltary', 'Organza' and 'Securitas' was almost 100%, indicating on high suitability of these cultivars for hydroponic cultivation in coconut coir.

Key words: different-age leaves, different colour and fruit-size cultivars, natural lighting, *Solanum lycopersicum*, SPAD indices.

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) are one of the most widely produced and consumed vegetables in the world with high economic, nutritional and health values (Souri & Dehnavard, 2017). Soilless hydroponic greenhouse production has been extensively used in tomato cultivation during the last 30–40 years. Thus, in Europe and Canada, 95% of greenhouse tomatoes are produced in a soilless culture system (Savvas & Gruda, 2018). In general, rockwool medium is the most commonly applied due to its good structure, water holding capacity and porosity. However, rockwool as inorganic material is non-biodegradable and its disposal or recycling after cultivation is expensive

(Gruda, 2019). As an alternative to rockwool, coconut coir as an organic substrate is increasingly used as a growing medium for the greenhouse tomato industry. Coir is the material consisting of the dust and short fibers derived from the mesocarp of the coconut fruits (*Cocos nucifera* L.) and is one of the most abundant organic waste materials in many tropical and subtropical countries (Carlile et al., 2015). Due to its good water retention and aeration properties, coconut coir provides a favorable air and water balance for plant roots (Barrett et al., 2016).

Although mineral nutrition is one of the key factors in determining the yield and quality of vegetables (Ahmadi & Souri, 2018; Souri & Dehnavard, 2018; Souri et al., 2018), little is known on the nutrient status of tomatoes in coconut coir under hydroponic conditions. Only a few studies have evaluated this media type in relation to the provision of nutrients during the growing cycle of vegetables (Kleiber et al., 2012; Xiong et al., 2017; Xing et al., 2019). Adapting fertilizer management to the specific conditions of the substrate as well as to the specific requirements of the cultivars can ensure the production of tomatoes with high yields and quality.

The chlorophyll content is an important indicator of leaf photosynthetic capacity and plant health (Hatamian et al., 2020; Zargar Shooshtari et al., 2020). The determination of chlorophyll content by rapid non-destructive methods is well developed for many fields and greenhouse crops, including tomatoes (Alsina et al., 2016; Jiang et al., 2017), providing valuable diagnostic information for purposes such as climate control, especially lighting, and nutrient assessment. One of the most known and used portable chlorophyll meters is SPAD-502, which offers easy operation in both research and agricultural settings. Although several nutrients are involved in ensuring a successful process of photosynthesis (Hochmal et al., 2015; Mohammadipour & Souri, 2019), SPAD indices are mainly used for real-time assessment of plant nitrogen status and control of nitrogen management (Xiong et al., 2015; Costa et al., 2018). It is based on a high correlation between the green color intensity and chlorophyll, as well as the nitrogen content of the leaves. Little is known about the possibilities of using the SPAD values to determine the status of such nutrients as Mg, S, Fe, Mn and others, whose deficiency can also cause leaf chlorosis, inhibition of photosynthesis and, consequently, reduced yields.

In greenhouse production, several factors are important in the choice of tomato cultivars, such as potential yield, resistance to abiotic and biotic stresses, etc. On the other hand, market-oriented trends and consumer preferences can also be crucial. Different studies showed that diversification of tomato color, taste, shape and texture is required to satisfy consumers, especially for fresh consumption (Casals et al., 2019; Jürkenbeck et al., 2019). In Latvia, historically the consumers prefer large fruit-sized tomatoes, associating them with good quality. However, cherry and plum tomatoes, as well as orange and pink tomatoes also become very popular.

Overall, only a few reports have examined the effects of cultivar differences on nutrient content and chlorophyll meter measurements for vegetables grown under the same fertilization conditions, especially in terms of yield and quality (Waterland & Moon, 2017; Souza et al., 2020). To our knowledge, no comparative study has been published showing differences in the nutrient status of different tomato cultivars under commercial greenhouse conditions in coconut coir substrate.

We hypothesized that the use of a single nutrient solution for different cultivars, widely used in modern soilless greenhouse production of tomatoes, could lead to nutrient imbalances for a particular cultivar. Therefore, the objective of this study was to investigate the dynamics of nutrient status of different tomato cultivars under industrial greenhouse production conditions using coconut coir as a growing substrate to reveal nutrient imbalances, their impact on tomato yield and quality, and cultivar differences.

MATERIALS AND METHODS

The study was carried out in the commercial greenhouse of the farm 'Kligeni', located in Cesis (57°18'N, 25°16'E, sub-boreal climatic zone), Latvia, during the spring-summer season of 2020, without artificial lighting. The greenhouse was equipped with computer-controlled fertilization, irrigation and partly climate system. Four tomato cultivars with different fruit weight, color and shape were used: orange plum 'Organza F1' (average fruit weight 45–50 g), red cherry 'Daltary RZ F1' (average fruit weight 16 g), red large fruit-sized 'Securitas RZ F1' (average fruit weight 260–270 g) and pink large fruit-sized 'Fujimaru F1' (average fruit weight 180 g).

Tomato seedlings were transplanted into coconut coir slabs in mid-February. The planting density was 2.5 plants m^{-2}. All crop management measures were performed in accordance with the current recommendations for tomato growing (Heuvelink, 2018). During the crop cycle, the solar radiation ranged from 248–2643 J cm^{-2} day^{-1}, and the average day/night temperature was at 20.9/18.2 °C, respectively. Pollination was ensured by the use of bumblebees (*Bombus terrestris* L.). Nutrient solutions of the following chemical composition (in mg dm^{-3}): 10–15 N-NH_4, 200 N-NO_3, 40–50 P, 370–450 K, 200–250 Ca, 60–65 Mg, 100–140 S-SO_4, 1.8–3.0 Fe, 0.2–0.6 Mn, 0.40–0.70 Zn, 0.05–0.10 Cu, 0.05 Mo, 0.35 B was used. The pH of the solution was adjusted to 5.40 and the electrical conductivity (EC) was in the range of 2.60–3.20 mS cm^{-1}, according to the growth stage of the tomatoes. Corrections in the micronutrient (Fe, Mn, and Zn) concentrations of the nutrient solution, done till the middle of the crop cycle, were based on the results of leaf analyses. Thus, Fe content was gradually increased from 1.8 mg dm^{-3} to 3.0 mg dm^{-3}, Zn from 0.4 mg dm^{-3} to 0.70 mg dm^{-3}, but Mn content decreased from 0.60 mg dm^{-3} to 0.20 mg dm^{-3}.

To diagnose nutrient status, samples for chemical analysis of 12 essential nutrients (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, and B) were collected and chlorophyll content (in SPAD values) was measured for the youngest fully developed leaves and the old still vital leaves twice per month from April 2020 to August 2020. There were 10 sampling times: 1 – 03.04.2020; 2 – 23.04.2020; 3 – 08.05.2020; 4 – 21.05.2020; 5 – 04.06.2020; 6 – 18.06.2020; 7 – 06.07.2020; 8 – 22.07.2020., 9 – 06.08.2020., 10 – 27.08.2020. One average, sample included 20 leaves collected from 10 plants. Collected tomato leaves were quickly rinsed with distilled water, dried at 60 °C in a laboratory dryer with forced air circulation and ground. For the determination of K, P, Ca, Mg, Fe, Cu, Zn, Mn, Mo and B, plant material was dry-ashed in concentrated HNO_3 vapours and re-dissolved in 3% HCl. For detection of total N, wet digestion was done in conc. H_2SO_4, for S - in conc. HNO_3. After mineralization of the plant samples, chemical analyses were done using the following methods: the levels of K, Ca, Mg, Fe, Cu, Zn, and Mn were estimated by microwave plasma atomic emission spectrometer (MP-AES) 4210 Agilent Technologies, the levels of P, Mo, N, and B were determined by colorimetry: P by ammonium molybdate in an acid-reduced medium, Mo by thiocyanate in reduced acid medium, B by hinalizarine in sulfuric acid medium, N by modified Kjeldal method using Nesler's reagent in an alkaline medium, S by turbidimetry by adding $BaCl_2$, with a spectrophotometer JENWAY 6300 (Rinkis et al., 1989).

Non-destructive measurement of the chlorophyll content of 10 tomato leaves at all sampling times for all cultivars was performed with a portable chlorophyll meter SPAD 502 (Minolta, Warrington, UK).

From the beginning of the harvest in mid-April until the end of the harvest in mid-October 2020, the total yield, the percentage of marketable and non-marketable yield per m^2 was determined once a week for all tomato cultivars.

Statistical analyses (standard errors, Student's t-test, $p < 0.05$, Pearson's correlation) were performed to determine differences in nutrient status of cultivars and leaves of different age, as well as to reveal correlations between nutrient content, chlorophyll content, and percentage of tomato marketable yield using MS Excel 2016. To find out the complex differences between the tomato cultivars, the PCA analysis was performed by PCord-6 software (McCune & Grace, 2002).

RESULTS AND DISCUSSION

Despite the significant differences between the tomato cultivars and the differences in macro- and micronutrient content in the leaves found between the cultivars, no symptoms of deficiency or excess of certain nutrients were observed during the tomato cultivation in coconut coir substrate.

The obtained data indicated that, in general, there was no difference between the cultivars in the distribution of nutrient concentrations between new and old tomato leaves. On average, the concentration of only two nutrients - N and Cu, was significantly higher in younger leaves, while significantly higher content of P, Ca, S, Mn, Mo, and B was found in older leaves. There was no difference for different-age leaves regarding Mg, Zn, Fe, and partly K (Tables 1, 2, Fig. 1). In the case of K, lower contents in younger leaves were recorded for cultivars Daltary and Organza. In general, this distribution pattern is closely related to nutrient mobility in plants.

In this study the content of most nutrients in tomato leaves (Tables 1, 2, Fig. 1) corresponded to the standard range for tomatoes reported by Brust (2013), Haifa (2020) and Campbell (2000). According to these references, there was a sufficient level of N, P, K, Ca, Mg, Fe, Cu, Mo and B in tomato leaves for all cultivars.

However, some imbalances were found: all cultivars were characterized by low Zn and high S levels in both young and old leaves. Among the macronutrients, S along with Ca, showed the most striking differences in the distribution within the different aged plant leaves, with the highest concentrations in the older ones. The concentration of these nutrients in young leaves showed and increasing trend during the growing period till mid-crop cycle in June.

Sufficient S content in greenhouse tomato leaves according to various references (Hochmuth, 2018; Campbell, 2000) ranges from 0.4 to 1.0 mg kg^{-1}. According to the data presented in Table 1, the results of our study showed higher than recommended S levels not only in the older but also in the younger leaves for all tomato cultivars. As plants can generally tolerate quite high concentrations of S in the growing media, wide use of Mg, K, Mn, Zn, and Cu fertilizers in the form of sulphates (Hochmuth, 2018), as well as the use of S-containing products for plant disease management (Llorens et al., 2017) is common in greenhouse vegetable production. Although high levels of S may act as antagonists to other nutrients, our study did not reveal a significant negative correlation between S and other nutrients in young and old tomato leaves ($p < 0.05$). This suggests that increased accumulation of S in the leaves could not adversely affect tomato cultivation in coconut coir substrate. This is consistent with studies of Xiong et al., 2017, also showing high uptake of S and K by tomato under coconut coir.

Table 1. Mean macronutrient concentration (mass %, dry matter) in young and old tomato leaves during the crop cycle from April 2020 to August 2020. Sufficiency range in leaves for greenhouse tomatoes was indicated according to Brust (2013), Haifa (2020) and Campbell (2000)

Tomato cultivar	Young leaves		Old leaves		Sufficiecy range
	Range	Mean ± *SE*	Range	Mean ± *SE*	
	N				
Daltary	4.20–5.80	4.70 ± 0.15b[1]B[2]	3.75–4.55	4.10 ± 0.09aB	3.50–6.00
Organza	3.30–5.05	4.19 ± 0.18aA	3.30–4.40	3.87 ± 0.11aB	
Fujimaru	3.70–5.15	4.31 ± 0.15bA	3.20–3.90	3.60 ± 0.08aA	
Securitas	3.45–4.45	4.03 ± 0.11bA	3.00–4.02	3.53 ± 0.11aA	
	P				
Daltary	0.27–0.66	0.43 ± 0.03aA	0.36–0.49	0.43 ± 0.01aA	0.30–1.00
Organza	0.36–0.78	0.51 ± 0.04aA	0.51–0.92	0.72 ± 0.05bB	
Fujimaru	0.48–0.66	0.54 ± 0.02aB	0.47–0.91	0.72 ± 0.05bB	
Securitas	0.44–0.69	0.57 ± 0.03aB	0.63–0.90	0.77 ± 0.03bB	
	K				
Daltary	2.87–4.99	4.18 ± 0.19aB	3.67–7.78	4.94 ± 0.38bB	3.50–6.00
Organza	3.02–4.29	3.59 ± 0.14aA	3.28–5.11	3.98 ± 0.17bA	
Fujimaru	3.37–4.86	4.10 ± 0.13aB	3.12–6.69	4.41 ± 0.34aB	
Securitas	3.51–4.49	3.87 ± 0.09aA	3.27–5.80	3.87 ± 0.26aA	
	Ca				
Daltary	1.46–4.28	3.13 ± 0.29aA	4.44–6.20	5.43 ± 0.17bA	1.20–4.00
Organza	2.04–3.67	2.81 ± 0.21aA	4.57–5.69	5.11 ± 0.14bA	
Fujimaru	1.88–4.44	2.96 ± 0.26aA	4.85–6.18	5.51 ± 0.14bAB	
Securitas	2.52–4.40	3.11 ± 0.19aA	5.19–6.37	5.82 ± 0.12bB	
	Mg				
Daltary	0.17–0.54	0.41 ± 0.03aB	0.19–0.77	0.49 ± 0.05aA	0.30–1.00
Organza	0.28–0.42	0.34 ± 0.01aA	0.27–0.61	0.41 ± 0.04aA	
Fujimaru	0.23–0.56	0.39 ± 0.03aAB	0.30–0.72	0.46 ± 0.04aA	
Securitas	0.27–0.51	0.34 ± 0.02aA	0.32-0.78	0.42 ± 0.05aA	
	S				
Daltary	0.70–2.13	1.39 ± 0.14aA	2.00–3.13	2.50 ± 0.12bA	0.40–1.00
Organza	0.90–2.13	1.28 ± 0.14aA	1.50–3.38	2.32 ± 0.18bA	
Fujimaru	0.85–2.00	1.41 ± 0.12aA	1.63–3.50	2.43 ± 0.17bA	
Securitas	1.10–2.50	1.45 ± 0.14aA	1.88–3.50	2.72 ± 0.14bA	

Values with different letters differ significantly ($p < 0.05$), according to Student's *t*-test; [1]For rows, lowercase letters compare young and old leaves of cultivar for each nutrient (a < b); [2]For column, uppercase letters compare cultivars for each nutrient (A < B).

Although the initial concentration of Zn (0.4 mg dm^{-3}) in nutrient solution was generally in the standard level of 0.3–0.4 mg dm^{-3} (Heuvelink, 2018), Zn deficiency was found from the second plant sampling time (mid-April) which coincided with the start of the tomato harvest (Fig. 1, a). Based on the results of leaf analyses, until the middle of the crop cycle (06.06.2020.), the Zn content in the nutrient solution was gradually increased from 0.4 mg dm^{-3} to 0.70 mg dm^{-3}. This measure resulted in an increase in leaf Zn content and in most cases for young leaves the lower sufficiency level was reached. However, some differences in cultivars were found - for old and young leaves of 'Securitas', and old leaves of 'Fujimaru' and 'Organza', Zn levels do not reach 25 mg kg^{-1} even after increasing Zn in the nutrient solution, or it happened very gradually. As Zn has many biochemical functions in the plant and is required for activation of enzyme system, chlorophyll production, auxin metabolism, water stress tolerance, etc. (Mengel & Kirkby, 2001), attention should be paid to the optimal supply of Zn for tomato growing in coconut coir substrate.

Table 2. Mean micronutrient concentration (mg kg^{-1}, dry matter) in young and old tomato leaves during the crop cycle from April 2020 to August 2020. Sufficiency range in leaves for greenhouse tomatoes was indicated according to Brust (2013), Haifa (2020) and Campbell (2000)

Tomato cultivar	Young leaves		Old leaves		Sufficiecy range
	Range	Mean ± *SE*	Range	Mean ± *SE*	
	Cu				
Daltary	6.40–13.40	9.86 ± 0.64bA	6.20–9.80	7.88 ± 0.33aAB	6.0–25.0
Organza	8.00–18.60	11.04 ± 0.96bA	5.40–11.20	8.62 ± 0.51aB	
Fujimaru	7.60–13.60	10.58 ± 0.58bA	6.80–9.40	8.36 ± 0.27aB	
Securitas	5.40-13.40	9.56 ± 0.82bA	3.80–8.80	6.78 ± 0.58aA	
	Mo				
Daltary	1.00–3.00	2.23 ± 0.20aA	1.80–3.90	2.98 ± 0.20bA	1.0–5.0
Organza	1.60–3.00	2.19 ± 0.13aA	2.08–5.20	3.15 ± 0.37bA	
Fujimaru	1.40–3.40	2.10 ± 0.18aA	1.80–5.20	3.00 ± 0.30bA	
Securitas	1.40–2.72	1.92 ± 0.14aA	1.80–4.40	2.57 ± 0.28bA	
	B				
Daltary	25–50	36.5 ± 3.14aAB	24–66	48.2 ± 3.92bA	25–75
Organza	23–44	30.1 ± 1.98aA	35–68	48.9 ± 3.34bA	
Fujimaru	25–50	33.2 ± 2.40aA	33–70	45.9 ± 3.75bA	
Securitas	23–48	30.6 ± 2.25aA	36–62	48.4 ± 2.65bA	

Values with different letters differ significantly ($p < 0.05$), according to Student's *t*-test; [1]For rows, lowercase letters compare young and old leaves of cultivar for each nutrient (a < b); [2]For column, uppercase letters compare cultivars for each nutrient (A < B).

A rapid increase in Mn concentrations from the beginning of the growing season was observed in both young and old leaves (Fig. 1, b). To limit this, a reduction of Mn concentrations in the nutrient solution was initiated. From the end of April to the beginning of June, the Mn concentration in nutrient solution was gradually reduced from 0.60 mg dm^{-3} to 0.20 mg dm^{-3} and remained the same until the end of the season. This measure stopped the almost linear increase of Mn concentrations in the older leaves and ensured the decrease of Mn in the new leaves - their concentrations reached values within the normal range for all cultivars. High Mn concentrations exceeding 500 mg kg^{-1} can be toxic and may adversely affect the uptake of other nutrients (Mengel & Kirkby, 2001; Kleiber, 2014).

Although the Fe concentration in the leaves was generally in the standard range for tomatoes, the rapid increase in Mn concentration during the crop cycle resulted in an unfavorable Fe: Mn ratio in the leaves, with manganese significantly exceeding the Fe content. Therefore, along with the reduction of the Mn, the increase of the Fe concentration in the nutrient solution was started from 1.8 mg dm^{-3} to 3.0 mg dm^{-3}. As a result, the Fe content of the tomato leaves increased only for cultivars ‘Daltary’ and ‘Fujimaru’, while the ‘Securitas’ and ‘Organza’ had lower Fe levels throughout the season in both new and old leaves, which did not change even after increasing the Fe content in the nutrient solution (Fig. 1, c).

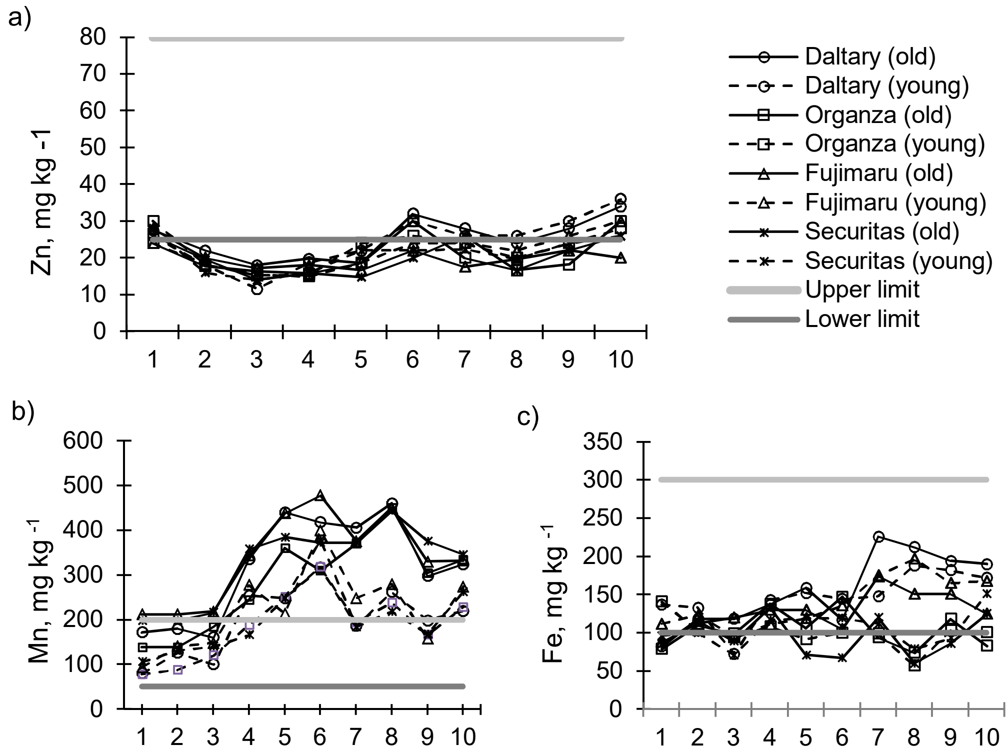

Figure 1. Nutrient content in young and old tomato leaves during the crop cycle from April 2020 to August 2020. Sufficiency range in leaves for greenhouse tomatoes was indicated according to Brust (2013), Haifa (2020) and Campbell (2000). Sampling time: 1 – 03.04.2020; 2 – 23.04.2020; 3 – 08.05.2020; 4 – 21.05.2020; 5 – 04.06.2020; 6 – 18.06.2020; 7 – 06.07.2020; 8 – 22.07.2020; 9 – 06.08.2020; 10 – 27.08.2020.

The obtained results identified several differences between the cultivars in the leaf nutrient content. Thus, cherry tomatoes 'Daltary' had significantly higher mean concentrations of N, K, Fe, Zn, but lower P in leaves compared to most other tomato cultivars. In general, the lowest N, Fe and Cu were determined for the leaves of large fruit-sized ‘Securitas’. The highest response to changes in nutrient solution composition was found for tomato cultivars 'Daltary' and 'Fujimaru'. Indeed, these cultivars accumulated additional Zn and Fe in leaves more efficiently than ‘Organza’ and ‘Securitas’.

Many studies have reported almost linear relationships between chlorophyll meter values and leaf N content of vegetable crops, including tomato (Dehnavard et al., 2017; Jiang et al., 2017; Souri et al., 2017; Padilla et al., 2018). However, the results obtained in our study showed that under optimal N supply conditions no significant correlation was found between the N content in the tomato leaves and the SPAD readings ($p < 0.05$). A narrow range of relatively high N content in tomato leaves may have been responsible for the weak relationships between SPAD measurements and plant N content. Such a plateau response when chlorophyll meters can become partially saturated at high N and chlorophyll contents has also been reported for greenhouse-grown cucumbers (Padilla et al., 2017) and basil (Walters & Currey, 2018). In tomato, in two cases it is possible to have low SPAD value induced by leaf N status: one when N is in the deficient range in leaf due to low application of N or its uptake and another due to ammonium nutrition and associated toxicity that generally results in leaf senescence and yellowing (Souri et al., 2009; Souri & Roemhald, 2009; Dehnavard et al., 2017).

In contrary to N, our results showed a significant positive correlation between leaf SPAD value and the concentrations of S in young tomato leaves for all cultivars except 'Organza'. Of the macronutrients, Ca in the new leaves also positively correlated with SPAD in all cultivars except 'Securitas' (Table 3). A significant positive correlation was also found between SPAD indices and concentrations of micronutrients Fe, Mn, Zn and B in tomato leaves, especially in young leaves of all studied cultivars. Previously, significantly higher levels of chlorophyll in vegetable leaves have been reported for foliar Ca, Fe, Zn and Mn application (Roosta & Mohsenian, 2012; Bin et al., 2020), convincingly demonstrating the important role of these nutrients in chlorophyll biosynthesis and photosynthetic processes in plants. Therefore, to ensure intensive photosynthesis as a prerequisite for high yield, it is important to monitor the nutrient status of tomato plants and make timely adjustments by optimizing their supply, especially for micronutrients.

Table 3. Pearson's correlation coefficients between leaf nutrient concentrations and SPAD values for young leaves of tomato cultivars (n = 10; $p < 0.05$; $r > 0.578$; ns – not significant)

	Ca	S	Fe	Mn	Zn	B
Daltary	0.800	0.621	0.795	0.770	0.756	0.901
Organza	0.585	ns	ns	0.721	ns	0.678
Fujimaru	0.697	0.819	0.955	ns	0.832	0.698
Securitas	ns	0.783	0.689	0.69	0.578	0.685

The data of total yield during crop cycle were as follows: for 'Daltary' - 21.56 kg m^{-2}, 'Organza' - 30.41 kg m^{-2}, 'Fujimaru' 36.41 kg m^{-2}, 'Securitas' - 46.71 kg m^{-2}. Overall, the total yield for cherry tomato 'Daltary', obtained without artificial lighting, can be regarded as high and comparable with the yields obtained in the Netherlands and Italy with the same tomato topology and supplemental lighting: e.g. 21–25 kg m^{-2} (Dueck et al., 2012; Palmitessa et al., 2020). While the average yields for beefsteak tomatoes in the Netherlands and other leading tomato-growing countries reach 50–60 kg m^{-2} and more (Heuvelink, 2018). Therefore, the yield obtained for large fruit-sized 'Securitas' in coconut coir substrate is generally in line with the cultivar and growing conditions, although improvements are possible.

The study included 4 tomato cultivars with different fruit size and weight. Therefore, the percentage of marketable fruit production in the total yield was compared. The results revealed that regardless of the nutrient content, for the cultivars 'Daltary', 'Organza' and 'Securitas', the proportion of marketable yield was close to 100% and amounted to 99.95%, 96.65% and 95.43%, respectively. This indicates the high suitability of these cultivars for industrial greenhouse cultivation. However, higher yields could potentially be achieved by addressing some nutrient imbalances.

Since the nutrient concentrations in leaves had a significant impact on photosynthesis, chlorophyll content and tomato yield, all these data have been used in the PCA analysis. The 1st axis explained 34.44% and 23.54% of the total variances, the

2nd axis - 21.13% and 17.39% of the total variances for young and old leaves, respectively (Fig. 2, a, b). PCA confirmed that the chemical composition of 'Daltary' generally differed from other cultivars. It was particularly pronounced for old leaves. Overall, 'Daltary' had a higher content of N, Zn, Fe and marketable fruit production. Individual sampling points for young leaves of different cultivars were more scattered in the ordination space, mainly grouped by sampling time, which indirectly indicates plant age, the period of a growth cycle, and the possible impact of microclimate. Thus, PCA indicated on substantial differences in leaf nutrient concentrations by course of time in the crop cycle.

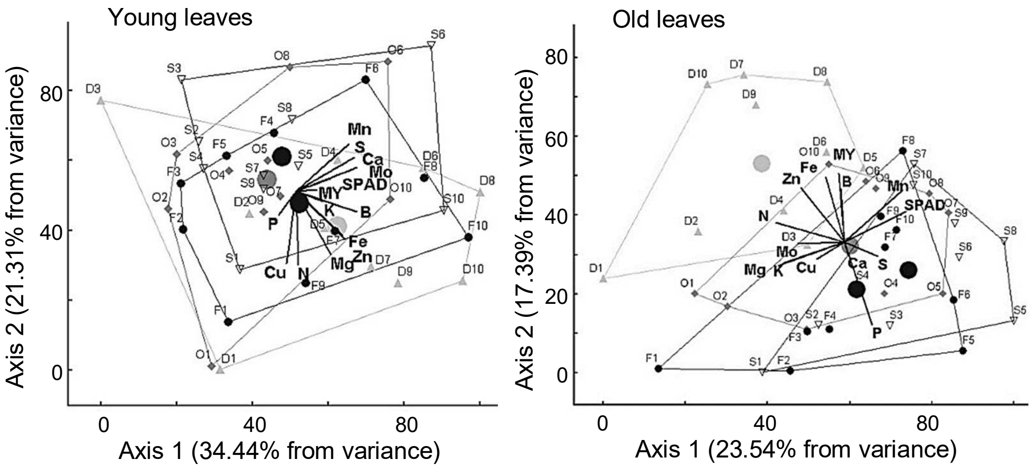

Figure 2. Distribution of four studied tomato cultivars within the axes of principal component analysis (PCA) of dataset of leaf chemical results, SPAD values and tomato marketable yield. a – young leaves, b – old leaves (D – 'Daltary'; O – 'Organza'; F – 'Fujimaru'; S – 'Securitas'; MY – marketable yield).

Analysis of correlation between marketable yield, leaf nutrient content and SPAD values was performed for 'Fujimaru', the only cultivar for which a significant proportion of non-marketable tomato yield (on average 13.53%) was found. The obtained results confirmed the importance of Ca, S, Fe, Zn, and B in ensuring high quality of fruit yield (Table 4). A positive correlation between these nutrients in young leaves indicated some synergy in the uptake processes. Thus, our research revealed a significant positive correlation between Ca and S, Mn, B; S and Fe, Mn; Fe and Zn, B; Zn and B ($0.601 < r < 0.825$, $p < 0.05$).

Table 4. Pearson's correlation coefficients between leaf nutrient concentrations, SPAD values and marketable yield of tomato cultivar 'Fujimaru' ($n = 10$, $p < 0.05$, $r > 0.578$)

Young leaves		Old leaves	
Nutrient	*r*	*Nutrient*	*r*
Ca	0.688	K	-0.580
S	0.734	B	0.588
Fe	0.854	SPAD	0.739
Zn	0.805		
B	0.853		
SPAD	0.884		

Although K has an undeniable positive impact on the quality of tomatoes preventing such physiological damage as uneven fruit coloring and incomplete ripening (Schwarz et al., 2013; Hernández-Pérez et al., 2020), the only significant negative correlation was found for K in older leaves and marketable fruit yield of ‘Fujimaru’. A significant negative correlation was also found between SPAD indices and K concentration in old leaves of ‘Fujimaru’. According to various studies, competitive interaction between nutrients occurring among Ca, Mg, and K are a widespread phenomenon in tomato cultivation, sometimes leading to the choice of either increasing the supply of K to improve fruit quality in terms of storage or taste or reducing K levels by reducing the risk of physiological Ca disorders (Pujos & Morard, 1997; Fanasca et al., 2005; Sambo et al., 2019). Although our study did not reveal Ca and mg deficiency nor a negative correlation between K-Ca and K-Mg in ‘Fujimaru’ leaves, a high K: mg ratio was found both for young and old leaves: 10.0 and 11.3, respectively, comparing to optimum range 6–8, reported by Brust, 2013. As a similar K: mg ratio in the leaves was typical also for the other studied tomato cultivars with almost 100% fruit quality, K-Mg antagonism was unlikely possible. In general, physiological disorders such as blossom-end rot (BER) and blotchy ripening were not observed for ‘Fujimaru’ fruits, thus suggesting that the non-marketable crop production was not related to shortcomings in the supply of K, Ca, and Mg.

The tomato quality problems that led to the non-marketable part of ‘Fujimaru’ harvest were related to the non-compliance of the fruit with the average weight of the cultivar, the irregular shape of the fruit and the deformation of the tomato fruit called 'catface'. The incidence of such fruit disorders are generally attributed to the microclimate in a greenhouse, mainly low temperatures and light levels during flowering, as well as incomplete pollination (Peet, 2009). Our study found a significant positive correlation between the percentage of marketable yield and day temperature, 24-hour average temperature, and solar radiation 6 weeks before harvest ($0.414 < r < 0.658$, $p < 0.05$). The largest deviations from the recommended optimal daily temperature range (19 to 20 °C), as well as the lowest light intensity level were observed between February and mid-April, which also determined a higher percentage of defective fruit at the beginning of the harvest. Cultivation of moderate-climate vegetables, such as tomatoes during spring months without artificial lighting in the geographical latitudes of Latvia is complicated and may not provide a high and 100% quality yield. However, our study revealed cultivar differences - ‘Daltary’, ‘Organza’ and ‘Securitas’ were less sensitive to deviations from optimal microclimate conditions.

CONCLUSIONS

- During the crop cycle, the content of most of the nutrients in the tomato leaves corresponded to the standard range reported for tomatoes. Only a few imbalances were found: all cultivars were characterized by low Zn and high S levels in both young and old leaves, as well as by high concentrations of Mn in older leaves.
- Several differences were identified between the cultivars. Thus, cherry tomatoes 'Daltary' had significantly higher mean concentrations of N, K, Fe, and Zn in the leaves compared to other tomato cultivars, The lowest N, Fe and Cu were determined in the leaves of large fruit-sized ‘Securitas’. The highest response to changes in nutrient solution composition was found for 'Daltary' and 'Fujimaru' - these cultivars accumulated additionally Zn and Fe in leaves more efficiently than ‘Organza’ and ‘Securitas’.
- Regardless of the nutrient status and microclimate conditions in a greenhouse without artificial lighting, for ‘Daltary’, ‘Organza’ and ‘Securitas’, the proportion of

marketable yield was close to 100%, indicating on high suitability of these cultivars for industrial greenhouse cultivation in coconut coir.

- Tomato defects as non-compliance of the fruit with the average weight of the cultivar and the deformation of the fruit indicated that 'Fujimaru' was more sensitive to deviations from optimal microclimate in a greenhouse.

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Enhanced assimilation rate due to seaweed biostimulant improves growth and yield of rice bean (*Vigna umbellata*)

P.R.L. Pascual[1,2,*], D.E. Carabio[1], N.F.H. Abello[2], E.A. Remedios[1] and V.U. Pascual[3]

[1]Cebu Technological University, Crop Science Department, College of Agriculture, - Barili Campus, Cagay, Barili 6036, Cebu, Philippines
[2]Cebu Technological University, Crop Biotechnology Unit, Center for Studies in Biotechnology, - Barili Campus, Cagay, Barili 6036, Cebu, Philippines
[3]Cebu Technological University, Department of Agricultural Economics and Development Studies, - Barili Campus, Cagay, Barili 6036, Cebu, Philippines
[*]Correspondence: petroey.pascual@ctu.edu.ph

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Abstract. Rice beans are traditionally planted as intercrop to corn or as the main crop during dry season when corn production is difficult. The use of biostimulants is widely studied to ameliorate the adverse effects of biotic and abiotic stresses. Three possible fermented biostimulants: seaweed, bamboo shoot, and Japanese snail were compared to a commercial organic liquid fertilizer (10 mL L^{-1}) based on morphological, photosynthetic, and yield responses. Fermented seaweed-treated rice bean registered the greatest average vapor pressure deficit (VPD) at 4.33 KPa on the first month and is comparable to the highest average VPD of 4.39 KPa registered by plants applied with fermented Japanese snail on the second month. This interestingly, did not result in difference of transpiration rate (µmol H_2O m^{-2} s^{-1}). Such could be attributed to the plants reduced stomatal aperture when applied with fermented seaweed at 406.80 µmol CO_2 mol stomatal conductance and 38.59 Pa total conductance on the second month. Despite this, the average carbon dioxide assimilation rate of rice beans still increased in both the first (15.26 µmol CO_2 m^{-2} s^{-1}) and second (16.51 µmol CO_2 m^{-2} s^{-1}) month. This increased assimilation rate of fermented seaweed-treated rice beans resulted to about 12 cm increase in height at 128.53 cm (R^2 = 0.894), 0.02 g pod^{-1} (R^2 = 0.978) heavier and 0.90 seeds pod^{-1} (R^2 = 0.978) more when compared to those applied with the commercial liquid organic fertilizer. Thus, by limiting stomatal conductance, despite the differences in VPD, transpiration rate was not affected while significantly increasing assimilation rate to improve production of rice beans, thereby taking full advantage of available seaweed by-products.

Key words: japanese snail liquid fertilizer, morphological responses, organic foliar fertilization, photosynthetic responses.

INTRODUCTION

Rice bean is a neglected crop cultivated in small areas by subsistence farmers in Nepal, Northern India and Southeast Asia. However, it can be grown in diverse conditions and is well known among farmers because of its wide adaptation and productivity even in marginal lands, drought-prone sloping areas, and flat rainfed tars (Joshi et al., 2007). The nutritional quality of rice beans is higher than many other legumes of the Vigna family. The plant has appreciable levels of crude protein with 59–93% protein digestibility, and all essential amino acids (especially methionine, tryptophan, lysine, tyrosine, and valine), minerals, vitamins, and a relatively high proportion of healthy, unsaturated fatty acids make it a nutritious health package (Katoch, 2012).

Farmers commonly use inorganic fertilizers because these are known to increase yield, which also increases income. All the same, it is observed to have severe and detrimental effects on soil, the environment, and other species. For that reason, many farmers turn back to using organic fertilizers. Nevertheless, the organic matter application is essential in improving and sustaining land productivity by enhancing soil's physical, chemical, and biological properties (Lasmini et al., 2019).

Seaweed biostimulant is one of the known organic fertilizers. It is a beneficial marine resource that is affordable and is also rich in various bioactive compounds. Some of those are lipids, proteins, carbohydrates, mineral nutrients, and antimicrobial compounds (Raghunandan et al., 2019). It increases the biochemical constituents of every plant, and this possesses environmental stress mitigating potential. Application of seaweed liquid fertilizer to soil improves soil health by enhancing micronutrient quantity and quality, and microbial activity.

Net assimilation rate (NAR) is used to determine the photosynthetic or growth efficiency in plants. NAR has been used as a growth predictor for some woody and herbaceous plants (Poorter & Nagel, 2000; Shipley, 2006). Photosynthesis results from the assimilation of water and carbon dioxide. The plant absorbs them and converts them into a plethora of organic molecules directly in the plant's numerous cells. A reduced assimilation rate meant that CO_2 and water are less utilized for the production of essential biomolecules. Carbon dioxide assimilation in plants is the most critical key for crop production (Lawlor, 2002).

Thus a study to determine the physiological, morphological and overall yield performance of rice beans is deemed necessary considering that Japanese snail is a major pest of most crops; bamboo shoot is widely abundant in the Philippines; and seaweed by-product is abundant for fermentation since the University is engaged in the production of wellness blends from seaweed. Moreover, rice bean is an integral component in crop rotation mostly utilized as legume in the farming system used by corn farmers in Cebu, Philippines.

MATERIALS AND METHODS

Collection, Preparation, and Fermentation of Organic Fertilizers

The materials used for fermentation were collected at Cebu Technological University - Barili Campus, Barili, Cebu, Philippines. All of the materials were washed to remove dirt.

The seaweed pulp by-product from wellness blend production was added with molasses. A ratio of 1:1 was used following the procedure of Rosit et al. (2015). After seven days of fermentation, all materials were squeezed and filtered through a strainer. Lastly, it was stored in a cool, dry place.

The outer covering of bamboo shoots were peeled off and were chopped into approximate less than 1 cm per piece. Next, water was added at a 1:1 ratio. After 24 hours of soaking, these were squeezed and filtered through a strainer. Lastly, the material was stored in a proper place.

The Japanese snails were smashed, including the shell. These were placed in a container and added with molasses at a ratio of 1:1. The mixture was stirred thoroughly. The container was covered with manila paper and was placed in a cool, dry place. After seven days of fermentation, the mixture was squeezed and filtered through a strainer.

Experimental Site and Plant Samples

The research was conducted inside the greenhouses of Cebu Technological University - Barili Campus (10°7'53" N, 123°32'45" E) with an area of 8.95 m × 6 m. Rice bean seeds, used as the planting material, were secured from a local farmer. To manage possible leakage of the applied foliar fertilizer, 2.5 kg garden soil as growing media were placed in pots spaced 30 cm apart.

Experimental Design and Treatments

This research used a randomized complete block design with four treatments at 15 samples per treatment and replicated three times. The treatments include T0 – commercial natural liquid fertilizer, T1 – fermented seaweed, T2 – fermented bamboo shoot, and T3 – Japanese snail. The concentration of 10 mL L^{-1} biostimulant at a weekly rate used was based on the study of Pascual et al. (2020). Data were analyzed using Analysis of Variance (ANOVA), and a further test was done using Tukey's test at $p < 0.05$ to test for differences between means of treatments using SPSS version 18.

Data Gathered

Li-6800 Portable Photosynthesis System was used to measure plant photosynthetic parameters such as; average vapor pressure deficit, average transpiration rate, average stomatal conductance, average total conductance and average assimilation rate. Morphological parameters such as plant height (from the base to the tip of the plant held vertically), leaf length (from the base of the petiole to the tip of the leaf blade of the middle leaf), and the number of leaves were also recorded together with the yield parameters of grain weight (the weight of grain per pod), pod diameter (the diameter of the base of the pod), number of pods (counting the pods per plant), number of seeds per pod and pod length (from the base to the tip of the pod).

RESULTS AND DISCUSSION

Growth and Yield Responses

Based on Table 1, seaweed biostimulant application significantly produced the tallest plants at 128.53 ± 3.9 cm. at week 8. This resulted by a 12 cm difference in height with the commercial liquid organic fertilizer. Yield response (Table 2) with the application of seaweed biostimulant significantly increased the grain weight at 0.72 ± 0.09 g, number of pods per plant at 95.80 ± 2.14, number of seeds per pods at 9.5 ± 0.31, and pod diameter 0.53 ± 0.31 cm. This result coincides with Pascual et al. (2020) study that the length of shoots outperformed other treatments due to the auxins present in the seaweed extracts that have an influential role in cell division and enlargement. In the study of Das

& Prasad (2015), 18% of the total indole-3-acetic acid (IAA) was present in *Kappaphycus alvarezii* seaweed, responsible for the enhanced growth of the plant. This is the essential auxin in plants, which controls many important physiological processes that include cell enlargement and division, tissue differentiation, and responses to light and gravity (Leveau & Lindow, 2005).

Table 1. Effects on Plant Height (cm), Leaf Length (cm), and Number of Leaves (± *SD*) on Rice beans with the Application of Different Foliar Fertilizers

Parameters	Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Plant Height	T0	12.30 ± 0.12	21.09 ± 0.80	27.90 ± 2.60 a	34.60 ± 1.22 b	92.40 ± 10.23 a	106.33 ± 10.44	107.60 ± 6.92 b	116.00 ± 6.97 b
	T1	12.09 ± 0.13	19.03 ± 1.10	23.40 ± 2.08 ab	30.47 ± 0.70 c	67.27 ± 5.22 b	96.13 ± 3.74	123.80± 3.67 a	128.53 ± 3.91 a
	T2	12.41 ± 0.64	20.82 ± 0.88	26.37 ± 1.59 a	39.93 ± 0.42 a	97.60 ± 2.88 a	113.40 ± 7.8	114.60 ± 1.56 ab	117.33 ± 2.23 b
	T3	11.95 ± 0.50	18.78 ± 0.83	20.67 ± 0.31 b	28.27 ± 2.58 c	66.53 ± 2.87 b	100.33 ± 5.60	113.27 ± 3.70 ab	118.30 ± 1.31 ab
Leaf Length	T0	6.61 ± 0.53 a	7.73 ± 0.37	9.79 ± 0.69 ab	12.77 ± 0.86	13.37 ± 1.20 a	11.40 ± 0.10 ab	11.70 ± 0.35	11.27 ± 0.29
	T1	6.05 ± 0.15 ab	6.88 ± 0.19	8.43 ± 0.32 b	10.46 ± 3.00	11.28 ± 0.48 ab	10.87 ± 0.15 ab	11.43 ± 1.21	10.60 ± 0.56
	T2	6.49 ± 0.03 ab	7.14 ± 0.19	9.99 ± 0.57 a	12.37 ± 0.25	12.63 ± 0.29 ab	11.63 ± 0.55 a	12.10 ± 0.62	10.60 ± 0.56
	T3	5.97 ± 0.19 b	6.85 ± 0.54	8.60 ± 0.57 ab	10.64 ± 0.55	10.67 ± 1.17 b	10.50 ± 0.44 b	10.79 ± 0.61	11.23 ± 0.83
Number of Leaves	T0	4.73 ± 0.23	10.73 ± 0.31	16.87 ± 1.70	25.53 ± 1.81 ab	33.33 ± 1.70	59.13 ± 3.31 b	66.13 ± 5.03 c	69.27 ± 5.61 a
	T1	4.47 ± 0.50	9.73 ± 0.90	15.60 ± 3.47	24.33 ± 2.66 ab	34.60 ± 3.49	61.33 ± 3.20 ab	83.00 ± 1.56 b	85.00 ± 3.49 b
	T2	4.87 ± 0.23	9.33 ± 1.14	24.40 ± 12.41	28.53 ± 1.72 a	35.93 ± 3.10	71.47 ± 3.52 a	103.27 ± 2.53 a	97.07 ± 7.40 b
	T3	4.40 ± 0.53	8.93 ± 0.92	15.13 ± 2.66	22.27 ± 2.73 b	31.53 ± 2.72	51.13 ± 8.33 c	72.57 ± 4.10 c	116.73 ± 5.22 c

Means within a column followed by a common letter is not significantly different from each other at 5% level of significance using Tukeys *HSD*.

Legend: T0 – Commercial Natural Liquid Fertilizer; T1 – Fermented Seaweed; T2 – Fermented Bamboo Shoot; T3 – Fermented Japanese Snail.

Table 2. Growth and Yield Components of Rice bean (± *SD*) as Affected by Different Liquid Organic Fertilizer

Treatments	Grain weight per pods (g)	Number of pods per plant	Number of seeds per pods	Pod diameter (cm)	Pod length (cm)
T0	0.68 ± 0.03a	122.03 ± 3.20a	8.60 ± 0b	0.45 ± 0.12b	8.94 ± 1.2b
T1	0.72 ± 0.09a	95.80 ± 2.14b	9.5 ± 0.31a	0.53 ± 0.31a	9.42 ± 2.90ab
T2	0.61 ± 0.05b	129.47 ± 2.32a	9.47 ± 0.46a	0.44 ± 0.2b	9.57 ± 3.25a
T3	0.52 ± 0.02c	129.36 ± 3.29a	9.00 ± 0.35a	0.46 ± 0.2b	9.11 ± 2.57ab

Means within a column followed by a common letter is not significantly different from each other at 5% level of significance using Tukeys *HSD*.

Legend: T0 – Commercial Natural Liquid Fertilizer; T1 – Fermented Seaweed; T2 – Fermented Bamboo Shoot; T3 – Fermented Japanese Snail.

Moreover, Mazhar et al. (2020) stated in their study that the application of seaweed extracts significantly increase the bulb weight of onion cultivars by 5.8% (Lambada), 5.4% (Red Bone), 2.4% (Phulkari), and 2.0% (Nasar Puri) at 0.5% SWE, respectively. This may be due to the seaweed's auxin, which improved cell division, elongation, and differentiation and the uptake of higher proteins and nucleic acid reserves, leading to an increased grain weight. In addition, nutrient manipulation - such as soil drenches, fertilization, foliar sprays, and systemic nutrient implants - is one way and commonly used to enhance plant health and, as a result, minimize disease severity. (Nadeem et al., 2018). Ahmed et al. (2012) mentioned in their study that the use of seaweed extract drastically improved the growth and yield components of snap beans through spraying or foliar application. His research also stated that the highest values of photosynthetic pigments, N, P, K, and Mg content of leaves, were obtained by spraying seaweed extract at a higher rate.

Bamboo shoot significantly enhanced the leaf length at 12.10 ± 0.62 cm and pod length at 9.57 ± 3.25 cm. This is because bamboo shoots have a high potassium content. Potassium is an essential fertilizer ingredient that is needed for plant development. This factor aids in the development of a good root system and the retention of water. This is in line with Alimento et al. (2021) findings that foliar application of 10 g per 1 L potassium improves soybean yields compared to other treatments. Bamboo shoot is believed to have a plant growth regulator such as gibberellin, stimulating and influencing cell division. Auxin and gibberellins promote vessel tissue growth and cell division, resulting in stem cell enlargement. The study of Carabio et al. (2021) supported this result, where bamboo shoot-based liquid fertilizer alone produced the longest leaves that which is also comparable with the control. It was reported by Gamuyao et al. (2017) that the young tissues of bamboo shoots were reported to have an enrichment of genes associated with the synthesis of DNA and DNA precursors, the cell cycle, cell division, and cell organization kinesins and microtubules. The leaf primordium in plants grows mainly through cell proliferation which will be replaced with an alternative mode of cell cycle activity and endoreduplication that enhances endopolyploidy linked to increased cell size (Gonzalez et al., 2010). This might be the reason why bamboo liquid fertilizer resulted to increased leaf length compared with the other treatments.

For the number of leaves, Japanese snail fertilizer has the highest among all the treatments with 116.73 ± 5.22. In the study of Jatto et al. (2010), the Japanese snail's shell has a chemical composition that includes proteins (amino acids), carbohydrates, fats, and minerals such as iron, zinc, and copper, all of which are essential for the growth as well as the number of leaves. Catubis et al. (2013) found that applying a minimal amino acid (100 ppm) to a native tomato garnered the most leaves on unflooded and minimally flooded conditions. This is also found to be rich in phosphorus, an essential element affecting plants' growth like plant height, leaf number, and shoot dry biomass (Malhotra et al., 2018). The application of organic fertilizer is of great help to soil with crop rotation implemented. In addition, biological means are taken to fight diseases and pests, thus resulting in improved plant growth (Česonien & Rutkoviene, 2009).

Physiological Responses

Result showed based on Fig. 1, A, fermented seaweed-treated rice beans registered the greatest average vapor pressure deficit at 4.33 KPa on the first month and is comparable to the highest average VPD of 4.39 KPa registered by plants applied with fermented Japanese snail. This, interestingly, did not result in differences in average transpiration rate (µmol H_2O m^{-2} s^{-1}) (Fig. 1, B). However, in the study reported by Schoppach et al. (2017) the limited whole-plant transpiration under high VPD in wheat has resulted in advantageous water conservation and crop yield increase which relates to this study's result in which plant treated with seaweed biostimulant increased the plant height with high VPD. This was also supported by Gholipoor et al. (2010) in which studies in species other than wheat also indicate that limited transpiration at high VPD in water-limited environments results in yield increases. Limiting transpiration in this situation will help the plant to conserve water for later use in the crop growing seasons when drought develops (Mura & Vangimalla, 2018).

Such could be attributed to a reduced stomatal aperture of rice beans applied with fermented seaweed at 406.80 µmol CO_2 mol average stomatal conductance (Fig. 1, C) and 38.59 Pa average total conductance (Fig. 1D) on the second month. The feedback hypothesis states that stomatal conductance decreases as VPD increases because of an increase in transpiration that lowers the leaf water potential. The results available for wheat are not consistent with stomatal closure at high VPD being a response to an increased whole leaf transpiration rate or lower leaf water potential. The lack of conductance response to VPD in CO_2 - free air suggests that ABA may mediate the response (Medina et al., 2019). This was also supported by Grossiord et al. (2020), where it was stated that there is an abundance of evidence that suggests that stomatal conductance declines under high VPD, just the same with the total conductance to CO_2.

Photosynthetic carbon assimilation (Fig. 1, E) is directly related to stomatal conductance. Still, this relationship is mediated by the intrinsic water-use efficiency (iWUE = A/gs), so that the response of photosynthesis to VPD depends on the stomatal sensitivity to VPD but also on the extent to which iWUE itself changes as VPD rises. In this case, this study's assimilation rate increases because vapor pressure deficit was also higher in this treatment. The average assimilation rate of rice beans was increased in both the first (15.26 µmol CO_2 m^{-2} s^{-1}) and second (16.51 µmol CO_2 m^{-2} s^{-1}) when applied with fermented seaweed.

This increased assimilation rate of fermented seaweed treated rice beans resulted in about 12 cm taller plants at 128.53 cm (R^2 = 0.894), 0.02 g pod^{-1} (R^2 = 0.978) heavier and 0.90 seeds pod^{-1} (R^2 = 0978) more when compared to those applied with the commercial liquid organic fertilizer, thereby showing strong positive correlation (Fig. 2). In the result conducted by González et al. (2013), Oligo-carrageenans stimulate growth of 3-year-old *Eucalyptus globulus* trees by increasing photosynthesis, nitrogen assimilation, and basal metabolism. This means that application of seaweed liquid organic fertilizer increases assimilation rate which indeed is the reason for the increase on plant height of Rice Bean. In the study of Li et al. (2016), forest trees always had high net assimilation rates and the individuals with high assimilation rates always grew fast. It is one of the reasons why it was reported that net assimilation rate and sustainable growth rate were strongly positively associated with both maximum photosynthetic rate and leaf N concentration, especially when expressed on the basis of leaf area.

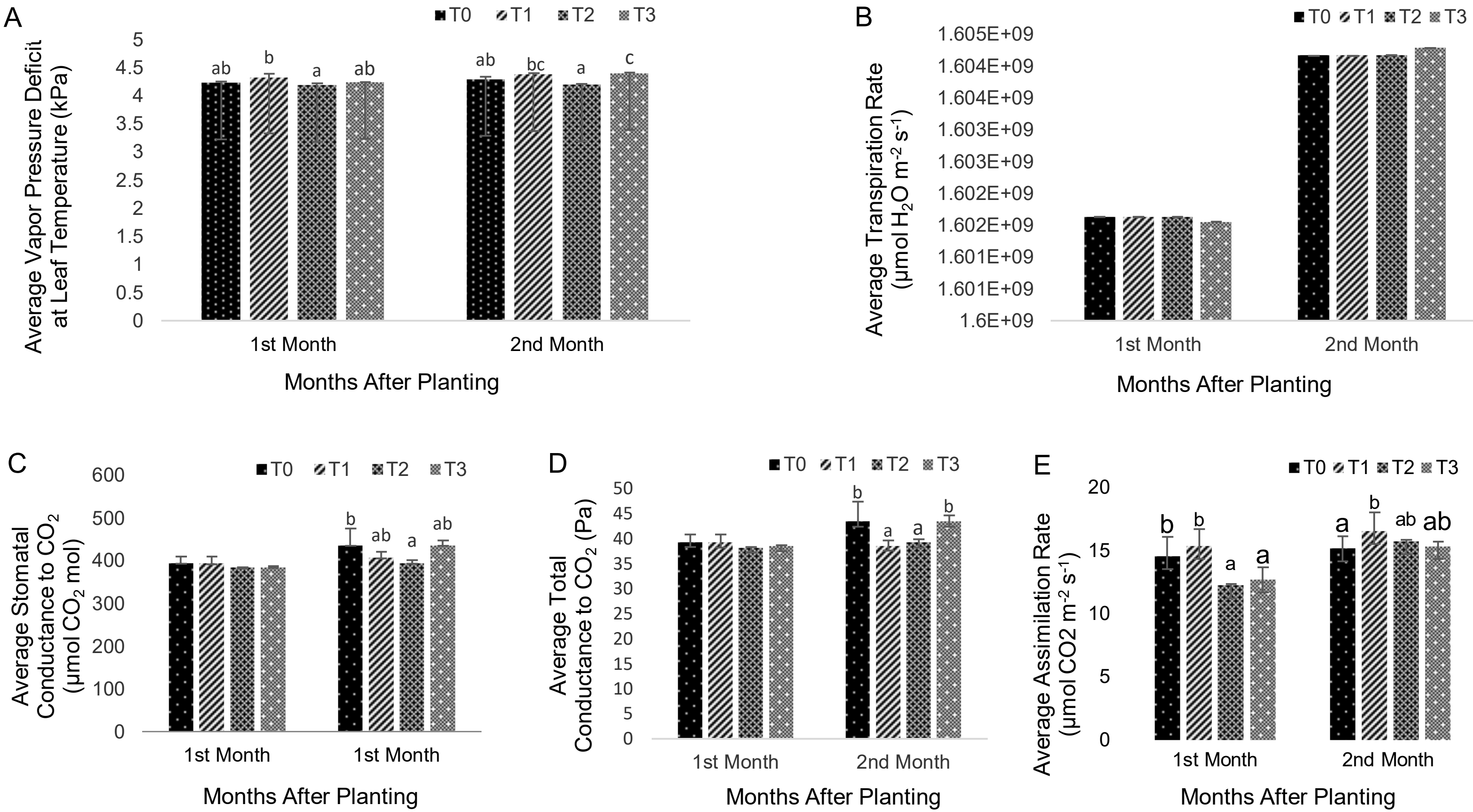
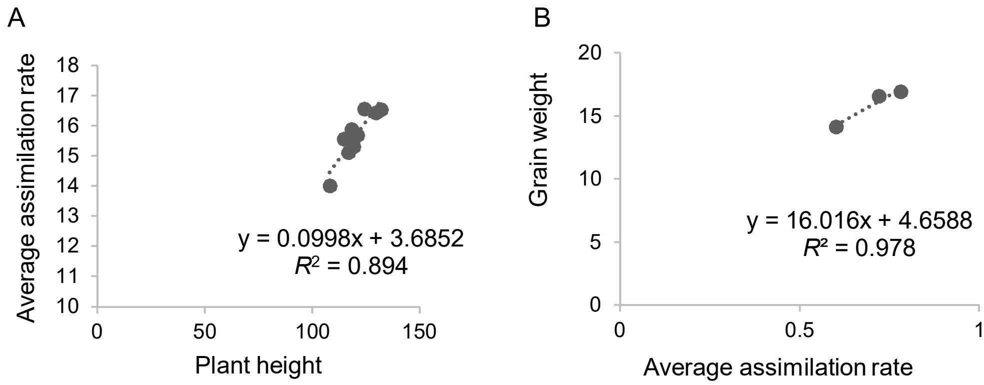


Figure 1. Photosynthetic Responses after application of different treatments on Corn: (A) Average vapor pressure deficit at leaf temperature (B) Average Transpiration Rate (C) Average Stomatal Conductance to CO_2 (D) Average Total Conductance to CO_2 (E) Average Assimilation Rate.



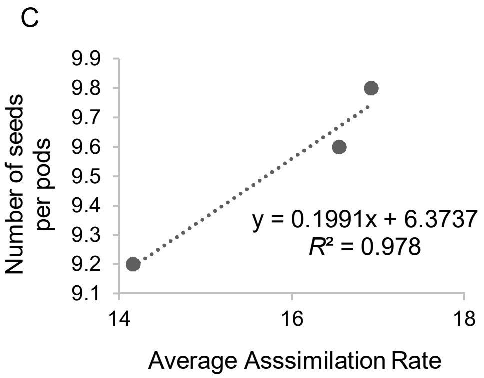


Figure 2. Relationship of Assimilation after the application of Seaweed liquid organic fertilizer; (A) plant height, (B) grain weight and (C) number of seeds per pods.

CONCLUSION

Application of Seaweed biostimulant significantly enhanced the assimilation rate of rice beans thereby resulting to increase in height, heavier pods and more seeds per pod. Bamboo shoot extract produced longer leaves and pods. Fermented Japanese snail increased the number of leaves.

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Sowing time effect on yield and quality of field beans in a changing meteorological situation in the Baltic region

I. Plūduma-Pauniņa[1,2,*], Z. Gaile[1] and G. Bimšteine[1]

[1]Latvia University of Life Sciences and Technologies (LLU), Faculty of Agriculture, Institute of Soil and Plant Science, Liela street 2, Jelgava LV–3001, Latvia
[2]LLU, Faculty of Agriculture, Research and Study Farm 'Pēterlauki', Platone parish, LV–3021, Latvia
[*]Correspondence: ievapluuduma@inbox.lv

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Abstract. As field beans (*Vicia faba* L.) need a lot of moisture to germinate, growers believe that they should be sown as early as possible in the spring. Field trial was carried out at the LLU RSF 'Pēterlauki', from 2018 to 2020. Following factors were researched: A) sowing time (early, medium and late), B) variety ('Laura', 'Boxer', 'Isabell'), C) sowing rate (30, 40, 50 germinable seeds m^{-2}), D) fungicide application (without and with application of fungicide at the GS 61-65). Meteorological conditions during the study had the greatest impact on the results as they were contrasting. Adverse meteorological conditions for field bean growing were observed in 2018 and in spring and early summer of 2019. The best year for bean yield formation was 2020, when temperature and precipitation was moderate. The highest average three year been yield was obtained sowing beans at the medium sowing time, however, equivalent yield was obtained sowing beans also in early sowing time. Fungicide application increased average three year yield significantly ($p = 0.007$) and independently of the sowing time. Influence of variety and sowing rate on average three year yield was insignificant, and it was not proved that any variety or sowing rate could be more suitable in a specific sowing time. Average three-year values of crude protein content, thousand seed weight and volume weight were affected by sowing time significantly ($p < 0.001$). Trial year, variety and fungicide application also affected all quality parameters significantly ($p < 0.05$), but the effect of sowing rate was insignificant ($p > 0.05$).

Key words: *Vicia faba* spp. *minor*, sowing time, variety, sowing rate, fungicide application.

INTRODUCTION

Field beans (*Vicia faba* L.) are well known all over the world. Mostly they are used for food and feed consumption. Although field bean sowing area has not considerably changed overall the world for more than thirty years (around 2.5 million ha each year[1]), in Baltic countries the sowing area has significantly grown during the last decade. Last decade showed not only a significant increase in sowing area, but also an increase of

[1] FAOstat data base: http://www.fao.org/faostat/en/#data/QC.

bean seed yield (from 0.27 t ha^{-1} in Estonia, 2012; up to 3.68 t ha^{-1} in Latvia, 2017)[2]. The same tendency is observed in the European and world data. It could mean that farmers are interested to make beans' growing more profitable, adjusting agrotechnical measures for obtaining higher seed yields. This presumption was confirmed by a farmers' survey conducted within the framework of SusCrop - ERA-NET project LegumeGap 'Increasing productivity and sustainability of European plant protein production by closing the grain legume yield gap'. In Latvia, farmers mentioned the following as the most important factors influencing field beans yield: sowing time, depth and rate, pest control measures; as important factors also variety choice, as well as interval before re-cultivation of field beans in the same field were mentioned (Klūga, 5 November 2020, oral report in scientific seminar, held by Faculty of Agriculture of LLU).

Growers in Baltic region, specifically in Latvia, believe, that higher field bean seed yield could be obtained by sowing beans in the earliest possible timing, which is connected with beans' high water demands and tolerance to comparatively low temperature during germination. High seed yields (5.8–7.3 t ha^{-1}) were obtained sowing beans in late March or first decade of April in previous our researches carried out in Latvia (Plūduma-Pauniņa et al., 2018), but at the same time germination of the beans (from GS 00 until GS 11) took from 36 to 44 days depending on the trial year (Plūduma-Pauniņa et al., 2019). In contrast, sowing time of the beans often occurs in the last decade of April or even in early May in the production conditions, despite the opinion that beans must be sown as early as possible. For instance, beans' demonstration trial was sown in the third decade of April, and obtained seed yield varied from 2.9 to 3.3 t ha^{-1} (Mellere, 2016), but another production occasion tells about field beans' sowing in the first decade of May, and the obtained seed yield in this case was 3.8 t ha^{-1} (Bartuševics, 2014).

Several researches have been carried out all over the world about sowing time effect on field bean yield and quality in diverse climatic conditions. Most of the research results gave evidence that earlier sowing time of beans ensures higher yield, if compared to sowing them in late sowing timing (Loss & Siddique, 1997; Tawaha & Turk, 2001; Hassan, 2008; Ibrahim et al., 2009; French, 2010; Badr et al., 2013; Alharbi et al., 2015; Raymond et al., 2016). However, some research results gave the opposite conclusion (e.g., Landry et al., 2016) about sowing time effect on seed yield.

Most of the above mentioned researches about field beans have been carried out in Australia, USA or in the Middle East (Egypt, Iran, Jordan, and Turkey). Unfortunately, it was not possible to find any recently published scientific results obtained in the Baltic region on field beans' sowing time and its interaction with other agro-technical elements such as variety, sowing rate and fungicide application for disease control.

Our research was aimed to clarify the influence of sowing time together with other agrotechnological factors on field bean yield and quality in the changing meteorological conditions.

MATERIALS AND METHODS

Research was carried out in three-year period: from 2018 to 2020. Field trials were performed at the Research and Study Farm 'Pēterlauki' (56°32'31.2"N 23°42'57.6"E) of the Latvia University of Life Sciences and Technologies. Four factors were researched

[2] EUROstat data base: https://ec.europa.eu/eurostat/data/database.

each year: factor A - sowing time (early, medium and late, Table 1); factor B - variety ('Laura', 'Boxer', 'Isabell'); factor C - sowing rate (30, 40 and 50 germinable seeds per 1 m^2); factor D - fungicide application (without fungicide and with fungicide Signum (boscalid, 267.0 g kg^{-1}, pyraclostrobin, 67.0 g kg^{-1}) application at the GS 61-65). Each year 54 variants in 4 replications were sown. In the data mathematical processing trial year was considered as the fifth factor because of the annual meteorological conditions' differences. Plot size was 16 m^2 (1.6 × 10 m).

Each year the early sowing was performed on the earliest possible date, which depended on meteorological conditions; and around 10 day interval was maintained between sowing timings (Table 1).

In the field trial, varieties were chosen based on their popularity between farmers in Zemgale region of Latvia; they are also widely used in all Baltic countries and Northern Europe. Varieties 'Laura' and 'Boxer' are well known for their high productivity, but variety 'Isabell' - for high crude protein content in seeds. Another reason of variety preference was the use of the same varieties in our previous research (Plūduma-Pauniņa et al., 2018, 2019).

Table 1. Field bean sowing dates in all three trial years, Pēterlauki, Latvia

Sowing time	Trial year 2018	2019	2020
Early	21 April	05 April	28 March
Medium	29 April	15 April	07 April
Late	08 May	25 April	17 April

The soil at the trial site was silt loam, Endocalcaric Abruptic Luvisol (World Reference Base, 2014). Depending on each year trial location site, soil agrochemical indices was as follows: pH_{KCl} - 6.5–6.9; organic matter (%) - 3.0–3.5; P_2O_5 (mg kg^{-1}) - 104–181; K_2O (mg kg^{-1}) - 150–207. Traditional soil tillage was used - ploughing in the autumn and soil cultivation before each sowing time. Fertilizing and spraying of plant protection products performed as needed, according to the rules of good agricultural practice (Table 2). Fungicide Signum (dose 1.0 kg ha^{-1}) was used based on the trial scheme at GS 61-65 (flowering).

Table 2. Used agro-technology in field beans' trial, 2018–2020, Pēterlauki, Latvia

Agro-technology	Trial year 2018	2019	2020
Fertilizer NPK 15-15-15 + 11 S (BS), kg ha^{-1}	250	200	250
	37.5	30.0	37.5
	37.5	30.0	37.5
	37.5	30.0	37.5
Foliar fertilizers			
YaraVita™ Brassitrel Pro, L ha^{-1}	1.0	2.0 + 1.0	–
Boron, L ha^{-1}	1.0	2.0 + 1.0	–
Herbicides			
Pendimethalin (330 g L^{-1}) (GS 07), L ha^{-1}	2.0	2.0	2.0
Bendioxide (480 g L^{-1}) (GS 14), L ha^{-1}	–	2.0	2.0
Cycloxydim (100 g L^{-1}) (GS 39), L ha^{-1}	1.0	2.0	–
Quizalofop-P-etil (50 g L^{-1}) (GS 30), L ha^{-1}	–	–	1.2
Insecticides			
Alpha–cypermethrin (50 g L^{-1}) (GS 61), L ha^{-1}	–	0.25	–
Thiacloprid (100 g L^{-1}), deltamethrin (10 g L^{-1}) (GS 61-65), L ha^{-1}	0.75	0.75	–

Notes: BS – before sowing; foliar fertilizers were given together with insecticides (in 2019 also with herbicide; GS 39) at the start of flowering (GS 61-65).

During vegetation, main phenological phases were observed, but severity of diseases was noted each week after emerging of the first symptoms (both are not analysed in detail in this paper).

Yield was harvested (Table 3) from each plot by small trial combine (Sampo 130) at the GS 89, weighted and recalculated to the standard moisture (14%) and 100% purity. Seed samples were taken for quality analysis from each plot's yield. Only crude protein (CP) content (% on dry matter basis) in seeds was detected using analyser Infratec™ NOVA (FOSS), but seed volume weight (VW) (g L^{-1}) (LVS EN ISO 7971-3: 2011) and thousand seed weight (TSW) (g) (LVS EN ISO 520: 2011) was detected according to the standard methods.

Table 3. Field beans' harvest dates, Pēterlauki, Latvia, 2018–2020

Sowing time	Trial year 2018	2019	2020
Early	13 August	29 August	04 Septemb
Medium	13 August	29 August	04 Septemb
Late	04 September	05 September	04 Septemb

April of 2018 was characterised with high average air temperatures and lot of precipitation (Table 4). High soil moisture delayed field bean sowing significantly (Table 1). In general, the season was atypically dry and hot (Table 4), thus resulting with low field beans' yields.

Table 4. Meteorological conditions during research period in Pēterlauki, Latvia, 2018–2020

Month	Average air temperature, °C				Precipitation, mm			
	2018	2019	2020	Norm	2018	2019	2020	Norm
March	-2.0	3.0	3.1	-1.5	10.8	29.6	27.0	31.3
April	9.0	8.1	6.1	5.3	69.5	3.0	9.2	40.0
May	16.1	12.4	9.9	11.7	12.0	57.0	30.0	51.4
June	16.8	19.4	18.7	15.4	16.0	32.0	140.0	75.3
July	20.7	16.8	17.0	16.6	56.5	93.5	48.0	81.7
August	19.4	17.6	17.7	16.2	34.0	37.8	65.0	73.7
September	14.9	12.7	14.9	11.5	25.4	53.6	24.0	62.7
Per season	13.6	12.9	12.5	10.7	Σ224.2	Σ306.5	Σ343.2	Σ416.1

Note: Norm means long-term average observations.

April 2019 started with almost no precipitation. Average air temperature was lower than that in previous year during the same date. In May, average air temperature was optimal, and the amount of precipitation sufficient, but June was hot and with lack of moisture. Stabilization of meteorological conditions for field bean growing in July could not recover the development delay at the beginning of growing season fully. In 2020, average air temperature at the end of March (1.6 °C on average per lasts ten-day period), April and May (Table 4) was lower than that in the previous two trial years. At the same time, moisture conditions were suitable for soil tillage and early field beans' sowing. June and the rest of the vegetation period was warmer, and with high precipitation amount, thus it was enough to develop high field beans' yield.

The General Linear Model Univariate Procedure was used for analysis of variance for factorial design using SPSS 15 software. For the comparison of factors' means Bonferroni test was used. Variants are considered significantly different when $p \leq 0.05$. As a significant effect of year conditions was observed on the studied parameters during the trial period (Table 5), results of each year were analysed also separately.

RESULTS AND DISCUSSION

The average per trial year **field beans' yield** was 2.49 t ha^{-1} in 2018; 6.32 t ha^{-1} in 2019, and 6.75 t ha^{-1} in 2020. Conditions of the trial year had the greatest impact on average field beans' yield and its quality ($p < 0.0001$) according to the test of between subjects' effect (Table 5). The factor with the next largest effect on the studied parameters was the sowing time ($p < 0.0001$). Used field bean variety did not affect the average seed yield significantly ($p = 0.9$), but a significant effect of it was observed on crude protein (CP) content in seeds, volume weight (VW) and thousand seed weight (TSW) ($p < 0.0001$). Sowing rate did not affect neither average seed yield ($p = 0.123$), nor any of the previously mentioned quality indicators (respectively: $p = 0.725$, $p = 0.827$, $p = 0.817$) significantly.

Table 5. Type III Sum of squares for researched factors per whole trial period, 2018–2020

Researched factors	Researched results			
	Yield	CP content	VW	TSW
Trial year*	2373.099	70.036	169848.476	1570370.313
Sowing time	139.680	26.832	23575.525	160470.451
Variety	0.908	55.665	17020.012	85460.588
Sowing rate	18.045	0.749	213.718	1599.551
Fungicide application	31.142	5.593	6830.776	175668.786

*– conditions in trial year; CP – crude protein, VW – volume weight, TSW – 1,000 seed weight.

Fungicide application (Table 5) had a significant impact on seed yield ($p = 0.007$), as well as on CP content in seeds ($p = 0.028$), VW and TSW ($p < 0.0001$). Four field beans' diseases, which needed to be controlled, were observed in the trial every year (more detailed information is given by Bankina et al., 2021). Chocolate spot (caused by *Botrytis* spp.) and *Alternaria* leaf blight (caused by *Alternaria* spp. and *Stemphylium* spp.) were the most important field beans' leaf diseases. Rust (caused by *Uromyces viciae-fabae*) and downy mildew (caused by *Peronospora viciae*) did not reach a significant level. The highest severity of leaf diseases was observed in 2020 for both main leaf diseases, but the lowest - in 2019. Early sowing time essentially promoted the development of both diseases. Fungicide application decreased severity of both diseases significantly, and in result improved yield and quality of field beans.

As the conditions of the trial year had the most significant impact on the results, the yield of each year and its quality will be analysed separately.

In 2018, the field beans' yield was the lowest per trial period regardless of the beans' sowing time (0.94–3.21 t ha^{-1}, Table 6), due to hot and dry weather that had rarely been observed for the last 100 years[3]. The average field beans' seed yield was significantly affected only by sowing time ($p < 0.0001$) and fungicide application ($p = 0.014$) (Table 6). The effect of variety ($p = 0.236$) and sowing rate ($p = 0.299$) on average seed yield was insignificant. Sowing time had the biggest impact on the average

[3] Latvian Environment, Geology and Meteorology Centre. https://www.meteo.lv/lapas/laika-apstakli/klimatiska-informacija/laika-apstaklu-raksturojums/2018/gads/2018-gads-sausakais-noverojumu-vesture?id=2374&nid=1177 [in latvian].

field beans' seed yield, and the highest yield was obtained when beans were sown in medium sowing time (3.33 t ha^{-1}), but it did not differ significantly from that obtained when sowing crop in early sowing time (3.21 t ha^{-1}) ($p = 0.214$). Yield obtained from variants sown in late sowing time was significantly lower (Table 6), and the yield decrease, if compared to the variants sown in the early and medium timings, was 2.27 t ha^{-1} (by 71%) and 2.39 t ha^{-1} (by 72%), respectively. Application of fungicide gave average yield increase by 0.39 t ha^{-1}. This tendency was noted for variants sown in all sowing times; the biggest yield increase by fungicide application was obtained when beans were sown in medium sowing time (+ 0.57 t ha^{-1}) ($p < 0.0001$).

Table 6. Field beans' yield (t ha^{-1}) depending on researched factors in 2018, Pēterlauki, Latvia

Factor	Sowing time ($p < 0.0001$)			Average
	early	medium	late	
Variety ($p = 0.236$)				
Laura	3.27^{a}	3.38^{a}	0.92^{ab}	2.52^{A}
Boxer	3.38^{a}	3.48^{a}	1.06^{a}	2.64^{A}
Isabell	2.97^{b}	3.13^{a}	0.83^{b}	2.31^{A}
Sowing rate (germinable seeds m^{-2}) ($p = 0.299$)				
30	2.98^{b}	3.12^{b}	0.90^{a}	2.33^{A}
40	3.22^{ab}	3.36^{ab}	0.93^{a}	2.50^{A}
50	3.42^{a}	3.52^{a}	0.97^{a}	2.64^{A}
Fungicide application ($p = 0.014$)				
F0	3.00^{b}	3.05^{b}	0.85^{b}	2.30^{B}
F1	3.42^{a}	3.62^{a}	1.02^{a}	2.69^{A}
Average	3.21^{A}	3.33^{A}	0.94^{B}	×

F0 – without fungicide application; F1 – with fungicide application.

Significantly different means are labelled with different letters in superscript: A, B – significant difference for average yields of three sowing times and means of factors' gradations; a, b – significant difference in a specific sowing time.

The second trial year (2019) was characterised with slightly better meteorological conditions for field beans' growth and development. Although the start of vegetation was cooler and some drought was observed, later conditions improved (Table 4), and field beans could form high yield (Table 7). The field beans' yield was significantly affected by three of four investigated factors except variety ($p = 0.113$), and the variety effect on seed yield was insignificant regardless of the sowing time ($p = 0.191$; $p = 0.798$; $p = 0.373$, respectively) (Table 7). The significantly highest field beans' seed yield was provided sowing beans in medium sowing time (6.54 t ha^{-1}) ($p < 0.0001$). Lower and similar seed yields ($p = 0.543$) were obtained sowing beans in early and late sowing timings. It could be explained with meteorological conditions in June - when beans sown in early sowing time started to flower, air temperature was high, but precipitation amount was low, therefore beans produced less flowers and later - pods.

Table 7. Field beans' yield (t ha^{-1}) depending on researched factors in 2019, Pēterlauki, Latvia

Factors	Sowing time ($p < 0.0001$)			Average
	early	medium	late	
Variety ($p = 0.113$)				
Laura	6.34^{a}	6.56^{a}	6.23^{a}	6.38^{A}
Boxer	6.32^{a}	6.59^{a}	6.20^{a}	6.37^{A}
Isabell	6.14^{a}	6.48^{a}	6.22^{a}	6.22^{A}
Sowing rate (germinable seeds m^{-2}) ($p < 0.0001$)				
30	6.13^{a}	6.32^{b}	5.85^{b}	6.10^{B}
40	6.29^{a}	6.51^{ab}	6.24^{a}	6.35^{A}
50	6.38^{a}	6.80^{a}	6.38^{a}	6.52^{A}
Fungicide application ($p < 0.0001$)				
F0	6.19^{a}	6.20^{b}	5.86^{b}	6.08^{B}
F1	6.34^{a}	6.89^{a}	6.46^{a}	6.56^{A}
Average	6.27^{B}	6.54^{A}	6.16^{B}	×

F0 – without fungicide application; F1 – with fungicide application.

Significantly different means are labelled with different letters in superscript: A, B – significant difference for average yields of three sowing times and means of factors' gradations; a, b – significant difference in a specific sowing time.

These conditions did not cause stress for beans sown in late sowing time as their flowering started two weeks later when conditions improved. Only a small decrease in beans' yield was observed in this study year when beans were sown in early and late sowing timings, if compared with the variants sown in the medium sowing time (by 4% and 6%, respectively).

In 2019, detailed analysis of sowing rate effect on beans' seed yield showed that it differed depending on sowing timing. The highest yield always was obtained sowing 50 germinable seeds m^{-2}, but this yield did not differ significantly from any other sowing rate variant in early sowing time and from variant where 40 germinable m^{-2} seeds were sown in medium and late sowing timings (Table 7).

The average seed yield was affected also by fungicide application ($p < 0.0001$), like in 2018. In medium and late sowing time yield increase by fungicide application was significant ($p < 0.0001$). On average, fungicide application gave 0.48 t ha^{-1} (by 8%) seed yield increase.

The third trial year (2020) can be characterized as the best for field beans' yield formation according to meteorological conditions despite cold April. In 2020, the average yield was significantly affected by all four researched factors ($p < 0.0001$) (Table 8). The medium sowing time showed the best results for field beans' yield (6.95 t ha^{-1}) formation, but similarly to 2018, average yield obtained in variant sown in this sowing time did not differ significantly ($p = 0.102$) from that harvested in early sown variant (Table 8). Decrease of yield sowing beans in late sowing timing was 0.38 t ha^{-1} (by 5.5%), if compared with the variant sown in the medium timing, and 0.20 t ha^{-1} (by 3%), if compared with the variant sown in the early timing.

Table 8. Field beans' yield (t ha^{-1}) depending on researched factors in 2020, Pēterlauki, Latvia

Factors	Sowing time ($p < 0.0001$)			Average
	early	medium	late	
Variety ($p < 0.0001$)				
Laura	6.65^{a}	6.71^{b}	6.48^{b}	6.61^{B}
Boxer	6.81^{a}	6.92^{ab}	6.41^{b}	6.71^{B}
Isabell	6.85^{a}	7.21^{a}	6.83^{a}	6.96^{A}
Sowing rate (germinable seeds m^{-2}) ($p < 0.0001$)				
30	6.44^{b}	6.69^{b}	6.34^{b}	6.49^{B}
40	6.90^{a}	7.07^{a}	6.52^{b}	6.83^{A}
50	6.96^{a}	7.07^{a}	6.86^{a}	6.97^{A}
Fungicide application ($p < 0.0001$)				
F0	6.55^{b}	6.69^{b}	6.34^{b}	6.53^{B}
F1	6.99^{a}	7.20^{a}	6.81^{a}	7.00^{A}
Average	6.77^{A}	6.95^{A}	6.57^{B}	×

F0 – without fungicide application; F1 – with fungicide application.

Significantly different means are labelled with different letters in superscript: A, B – significant difference for average yields of three sowing times and means of factors' gradations; a, b – significant difference in a specific sowing time.

In 2020, the variety 'Isabell' provided the significantly ($p < 0.05$) highest average yield (Table 8) in contrast not only to previous trial years of current research, but also in contrast to our previously published results (Plūduma-Pauniņa et al., 2018). The variety effect on beans' yield was more expressed in medium and late sowing timings (Table 8). The sowing rate showed a significant impact on field beans' yield. The highest yield was observed mostly when the highest sowing rate (50 germinable seeds m^{-2}) was used (Table 8), and this effect was significant in variants sown late. On average, the yield did not differ significantly between sowing rate variants 50 and 40 germinable seeds m^{-2} similarly to results obtained in 2019, and to our previously obtained results (Plūduma-Pauniņa et al., 2018). In 2020, fungicide application has increased field beans' average seed yield significantly ($p < 0.0001$) in similar amount (by 0.47 t ha^{-1}) as in previous years.

Bonelli et al. (2016) concluded that the choice of a suitable sowing time could minimize impact of some biotic and abiotic factors on plant phenological development. A significant effect of sowing time on plant growth and development together with the yield can be observed especially in field beans. Our results showed that early and medium sowing timing can provide similar bean yields. In Australia, in absolutely different conditions, if compared to those in Baltic region, a two-year trial in two locations with two different varieties and three sowing times was carried out, and Manning et al. (2020) concluded that regardless of the variety used, the trial year and location, the significantly highest field beans' yield was obtained in medium sowing time (3.05 t ha^{-1}). Similarly to our results, a decrease of field beans' seed yield with later sowing times was found also in other researches (Turk & Tawaha, 2002; Badr et al., 2013; Alharbi et al., 2015; Raymond, 2016; Wakweya et al., 2016; Zeleke & Nendel, 2019).

A significant impact of other researched factors on the field beans' yield was found in some previous researches carried out in Latvia. For example, in our previous work (Plūduma-Pauniņa et al., 2018), the variety 'Boxer' provided the significantly highest yield on average in two from three trial years. In other trials carried out in Latvia, the yield depended on the trial site, and varieties 'Laura' and 'Isabell' gave the highest yield in different sites (Zute, 2014). Our current research showed good results of all three mentioned varieties, which were not dependent from the sowing time, but rather from the conditions of the trial year in general. Sowing rate increase positively impacted field beans' yield. Our current results and previous work (Plūduma-Pauniņa et al., 2018) showed that yield was higher when 40 and 50 germinable seeds m^{-2} were sown, but evidence that different sowing rates are needed in different sowing timings was not obtained. Similarly, researches showed a diverse impact of sowing rate on beans' seed yield in different climatic condition. Loss et al. (1998) found in three-year trial that the increase of field beans' sowing rate increases the obtained yield. But it does not happen indefinitely - there is an optimum sowing rate, and, in their trial, it was 45 germinable seeds m^{-2}, which differed significantly from previously recommended sowing rate in Australia - 30 germinable seeds m^{-2} (Loss et al., 1998). Lopez-Bellido et al. (2005) also concluded that there is a maximum point up to which we can increase sowing rate for obtaining a higher yield. Research results in Mediterranean conditions showed completely opposite results - higher field beans' yield was obtained using lower seeding rate (larger row spacing) (Thalji, 2006; Yucel, 2013b). Kikuzawa (1999), and Yucel (2013a) found that too high sowing rate can decrease field beans' yield, which is based on the self-regulation of plant density.

Significant field bean yield increase using fungicide was obtained regardless of the sowing timing variant. Chemical control methods of the field beans' diseases can increase seed yield, when used in the right time, according to the rules of good agricultural practice (Stoddard et al., 2010; Kora et al., 2016; Plūduma-Pauniņa et al., 2018).

Crude protein (CP) content in seeds was affected by the trial year (Table 5), but observed fluctuation was within 1% (30.96% (2019) - 31.67% (2018)). On average per all three trial year's, the highest CP content in seeds was obtained when beans were sown in late sowing time (31.61%), and it was slightly (by 0.47%) higher, if compared with the lowest result obtained on average in plots sown in early sowing time. Similarly, the average CP content fluctuation depending on variety was within 1% (the highest CP content was provided by the variety 'Isabell' (31.81%), but the lowest - by the variety 'Boxer' (31.10%). Sowing rate did not give a significant effect on average three year CP content, but fungicide application gave a slight decrease of it (by 0.18%).

CP content in **2018** was significantly affected only by sowing time and field beans' variety (Table 9). On average, the highest ($p < 0.0001$) CP content in seeds was obtained using the variety 'Isabell' (32.26%). The same tendency was obtained sowing beans in early and medium sowing times, but the variety 'Laura' (32.39%) showed the insignificantly ($p = 0.428$) highest CP content when sown in the late sowing time. The highest CP content in seeds was observed sowing field beans in late and medium sowing times (Table 9).

In the **second trial year (2019)**, CP content in seeds was affected only by variety and fungicide application. In 2019, the impact of sowing time on CP content in seeds was insignificant ($p = 0.561$). Similarly to results in 2018, the highest CP content in seeds was noted if beans were sown in the late sowing time, but it was only slightly higher, if compared with that when beans were sown in the early sowing time.

Variation of CP content in seeds depending on variety was similar to that observed in the first trial year (Table 9). Fungicide application affected the CP content in seeds negatively, i.e., caused CP decrease in beans' seeds in all the variants; when beans were sown in early and medium sowing times this decrease was insignificant ($p = 0.174$; $p = 0.471$, respectively); in variants sown in late sowing time - even though the decrease was small (by 0.45%), it was mathematically significant ($p = 0.003$).

Table 9. Crude Protein content (%) in field beans' seeds depending on researched factors, 2018–2020

Factors	Year and sowing time								
	2018 ($p < 0.0001$)			2019 ($p = 0.561$)			2020 ($p = 0.850$)		
	early	med*	late	early	med	late	early	med	late
Variety ($p_{2018} = 0.0001$; $p_{2019} < 0.0001$; $p_{2020} < 0.0001$)									
Laura	30.57^{b}	31.63^{b}	32.39^{a}	31.08^{a}	31.06^{a}	30.97^{ab}	31.52^{ab}	31.34^{b}	31.64^{a}
Boxer	30.22^{b}	31.56^{b}	31.89^{a}	30.60^{b}	30.54^{b}	30.77^{b}	31.40^{b}	31.38^{b}	31.56^{a}
Isabell	31.83^{a}	32.88^{a}	32.07^{a}	31.04^{a}	31.25^{a}	31.31^{a}	32.00^{a}	32.08^{a}	31.85^{a}
Sowing rate (germinable seeds m^{-2}) ($p_{2018} = 0.858$; $p_{2019} = 0.570$; $p_{2020} = 0.945$)									
30	30.81^{a}	31.92^{a}	32.06^{a}	30.93^{a}	30.89^{a}	30.91^{a}	31.83^{a}	31.50^{a}	31.61^{a}
40	31.26^{a}	32.08^{a}	31.83^{a}	30.89^{a}	31.10^{a}	31.06^{a}	31.61^{a}	31.61^{a}	31.75^{a}
50	30.54^{a}	32.06^{a}	32.46^{a}	30.90^{a}	30.86^{a}	31.07^{a}	31.47^{a}	31.69^{a}	31.68^{a}
Fungicide application ($p_{2018} = 0.160$; $p_{2019} = 0.037$; $p_{2020} = 0.347$)									
F0	31.09^{a}	32.06^{a}	32.27^{a}	30.99^{a}	30.90^{a}	31.24^{a}	31.86^{a}	31.64^{a}	31.59^{a}
F1	30.65^{a}	31.98^{a}	31.96^{a}	30.82^{a}	31.00^{a}	30.79^{b}	31.42^{b}	31.56^{a}	31.78^{a}
Average	30.87^{B}	32.02^{A}	32.12^{A}	30.91^{A}	30.95^{A}	31.02^{A}	31.64^{A}	31.60^{A}	31.68^{A}

*med – medium; F0 – without fungicide application; F1 – with fungicide application.

Significantly different means are labelled with different letters in superscript: A, B – significant difference for average yields of three sowing times in a specific year; a, b – significant difference in a specific sowing time.

In the **third trial year (2020),** CP content in seeds was significantly affected only by one researched factor - the variety ($p < 0.0001$), and the highest CP content regardless of the sowing time was provided by the variety 'Isabell' (Table 9).

Kondra (1975) concluded that, although sowing date did not affect CP content in seeds significantly, the tendency was to decrease CP content within later sowing dates (from 29.2% to 26.9%). It is in contrast with the tendency we have observed, whereas, Rowland (1978) concluded that CP fluctuation among sowing timing variants is rather random. In currently described research, the impact of sowing rate on CP content was insignificant. It is in contrast with our previous research results (Plūduma-Pauniņa et al., 2018), when we found that CP content in seeds tended to be higher when higher sowing rate was used. The same tendency was observed also in trial carried out in Egypt (Bakry et al., 2011). The effect of variety on CP content is similar to our previous results (Plūduma-Pauniņa et al., 2018), and mainly 'Isabell' provided the highest CP content in seeds. Fungicide application showed decreasing effect on CP content, that is in contrast with the previously obtained results (Micek et al., 2015; Plūduma-Pauniņa et al., 2018). As CP content fluctuation was within 1%, this variation could be explained by interaction of researched factors with meteorological conditions in every specific study.

The volume weight (VW) on average was significantly (p < 0.0001) affected by the trial year (736 g L^{-1} (2020) - 774 g L^{-1} (2019); Table 5). On average per all three trial year's, the highest VW was obtained sowing field beans in early sowing time (763 g L^{-1}), and VW decreased in variants of every next sowing time (medium sowing time - 761 g L^{-1}, late sowing time - 750 g L^{-1}). A significant effect on average VW was showed also by the variety (p < 0.001; the highest average VW was provided by 'Isabell' - 765 g L^{-1}), and fungicide application (p < 0.001; in variants with fungicide application VW was 761 g L^{-1}). The impact of sowing rate on the average result of this indicator was insignificant (p = 0.238).

In **2018**, the VW was significantly affected only by the sowing time (p < 0.0001). Significant impact of other researched factors on VW was observed only in variants sown in some specific sowing times (Table 10).

Table 10. Volume weight (g L^{-1}) in field beans' seeds depending on researched factors, 2018–2020

Factors	Year and sowing time								
	2018 (p < 0.0001)			2019 (p < 0.0001)			2020 (p < 0.0001)		
	early	med*	late	early	med	late	early	med	late
Variety (p_{2018} = 0.123; p_{2019} < 0.0001; p_{2020} < 0.0001)									
Laura	780^{b}	769^{b}	734^{a}	757^{c}	775^{c}	772^{b}	740^{ab}	718^{c}	734^{b}
Boxer	782^{b}	773^{b}	731^{a}	763^{b}	779^{b}	776^{b}	736^{b}	728^{b}	730^{b}
Isabell	791^{a}	780^{a}	735^{a}	775^{a}	786^{a}	783^{a}	746^{a}	741^{a}	749^{a}
Sowing rate (germinable seeds m^{-2}) (p_{2018} = 0.997; p_{2019} = 0.711; p_{2020} = 0.173)									
30	784^{a}	772^{a}	735^{a}	767^{a}	780^{a}	778^{a}	737^{a}	728^{a}	735^{a}
40	784^{a}	775^{a}	733^{a}	765^{a}	779^{a}	776^{a}	741^{a}	730^{a}	737^{a}
50	785^{a}	774^{a}	732^{a}	765^{a}	781^{a}	777^{a}	743^{a}	729^{a}	742^{a}
Fungicide application (p_{2018} = 0.186; p_{2019} = 0.001; p_{2020} < 0.0001)									
F0	783^{a}	771^{b}	732^{a}	763^{b}	779^{a}	773^{b}	737^{b}	723^{b}	732^{b}
F1	786^{a}	777^{a}	735^{a}	768^{a}	781^{a}	781^{a}	744^{a}	735^{a}	744^{a}
Average	784^{A}	774^{B}	734^{C}	765^{B}	780^{A}	777^{A}	740^{A}	729^{B}	738^{A}

*med – medium; F0 – without fungicide application; F1 – with fungicide application.

Significantly different means are labelled with different letters in superscript: A, B, C – significant difference for average yields of three sowing times in a specific year; a, b, c – significant difference in a specific sowing time.

Just like in the first trial year, the volume weight in the **second year (2019)** was affected by sowing time ($p < 0.0001$). Only in this year, the highest volume weight was obtained sowing beans in medium sowing time (on average 780 g L^{-1}) (Table 10). Volume weight was significantly affected also by the variety. The highest VW was always provided by the variety 'Isabel', but the differences of VW between 'Boxer' and 'Laura' were significant ($p < 0.0001$) when they were sown in early and medium sowing times (Table 10). Fungicide application increased the volume weight of field beans, but sowing rate did not have a significant ($p = 0.711$) impact on field beans' VW, similarly to results in 2018.

In the **third trial year (2020),** the VW was significantly affected by three researched factors: sowing time, variety, and fungicide application ($p < 0.0001$) (Table 10). The highest VW was observed for beans sown in early sowing time, but it did not differ significantly from VW observed when sowing beans in late sowing time ($p = 0.831$). Unexpectedly, significantly lower VW was observed sowing beans in medium sowing time ($p < 0.0001$) (Table 10). VW of 'Isabell' was always the significantly highest, but VW differences and their significance of 'Boxer' and 'Laura' depended on sowing time (Table 10). Fungicide application increased VW significantly ($p < 0.0001$).

It was not possible to find other research results in studied literature illustrating VW dependency on sowing date. However, we obtained similar results to our previous findings (Plūduma-Pauniņa et al., 2018) regarding the impact of variety, research year and sowing rate on the VW.

Average **thousand seed weight (TSW)** was significantly affected by the trial year (Table 5; $p < 0.001$), and on average it was 511 g in 2018, 626 g in 2019, and 537 g in 2020. On average per all three trial years, sowing time, variety, and fungicide application affected TSW significantly ($p < 0.001$). The highest TSW was obtained sowing beans in early sowing time (579 g), but it decreased in succeeding sowing times, showing diverse results depending on the trial year. The variety 'Boxer' (574 g) provided the highest average TSW, and the average TSW was increased in variants with fungicide application. The impact of sowing rate on TSW was insignificant ($p = 0.143$) on average.

TSW during the **first trial year (2018)** was significantly affected by sowing time and variety (Table 11). The highest TSW on average (540 g) was observed in variants sown in early sowing time regardless of the variety used (Table 11). Sowing rate did not impact neither the average TSW ($p = 0.667$), nor that obtained in any of sowing time variants significantly. Although, the highest TSW in all cases was observed using 30 germinable seeds m^{-2} (on average 514 g). Fungicide application affected TSW in every sowing time variant significantly ($p < 0.01$), but these differences were not equipollent. Due to this, a significant impact of this factor on average TSW was not observed ($p = 0.256$; Table 11). However, fungicide application increased TSW when beans were sown in early and medium sowing time.

Despite the fact that the average TSW was the highest in the **second trial year (2019),** we observed similar impact of researched factors on this indicator. Sowing rate did not impact the average field beans' TSW ($p = 0.966$) significantly, but the effect of sowing time, variety and fungicide application was significant ($p < 0.0001$). Similarly to results a year before, the highest TSW was provided by variants sown in early sowing time, and TSW of the variety 'Boxer' was the highest on average (646 g). Fungicide application increased field beans' average TSW (652 g; + 52 g) significantly ($p < 0.0001$), and that in all sowing time variants (Table 11).

Table 11. Field beans' 1,000 seed weight (g) depending on researched factors, 2018–2020

Factors	Year and sowing time								
	2018 ($p < 0.0001$)			2019 ($p < 0.0001$)			2020 ($p < 0.0001$)		
	early	med*	late	early	med	late	early	med	late
Variety ($p_{2018} = 0.001$; $p_{2019} < 0.0001$; $p_{2020} < 0.0001$)									
Laura	543^{b}	481^{b}	509^{a}	630^{b}	626^{b}	573^{a}	544^{b}	516^{b}	531^{a}
Boxer	556^{a}	492^{a}	514^{a}	672^{a}	667^{a}	600^{a}	573^{a}	555^{a}	539^{a}
Isabell	519^{c}	475^{b}	509^{a}	642^{b}	644^{b}	579^{a}	535^{b}	513^{b}	525^{a}
Sowing rate (germinable seeds m^{-2}) ($p_{2018} = 0.667$; $p_{2019} = 0.966$; $p_{2020} = 0.429$)									
30	541^{a}	484^{a}	516^{a}	651^{a}	644^{a}	579^{a}	556^{a}	532^{a}	533^{a}
40	540^{a}	481^{a}	511^{a}	648^{a}	648^{a}	585^{a}	550^{a}	528^{a}	535^{a}
50	538^{a}	483^{a}	506^{a}	645^{a}	645^{a}	588^{a}	547^{a}	524^{a}	527^{a}
Fungicide application ($p_{2018} = 0.256$; $p_{2019} < 0.0001$; $p_{2020} < 0.0001$)									
F0	533^{b}	473^{b}	520^{a}	629^{b}	627^{b}	545^{b}	529^{b}	511^{b}	507^{b}
F1	546^{a}	493^{a}	502^{b}	667^{a}	665^{a}	623^{a}	572^{a}	545^{a}	557^{a}
Average	540^{A}	483^{C}	511^{B}	648^{A}	646^{A}	584^{B}	551^{A}	528^{B}	532^{B}

*med – medium; F0 – without fungicide application; F1 – with fungicide application.

Significantly different means are labelled with different letters in superscript: A, B, C – significant difference for average yields of three sowing times in a specific year; a, b, c – significant difference in a specific sowing time.

Similar effect of researched factors' on TSW was observed also in the **third trial year (2020)**, and it was significantly affected by sowing time, the field beans' variety used and fungicide application ($p < 0.0001$) (Table 11). Like in previous two trial years, sowing rate did not have a significant impact on the obtained TSW ($p = 0.429$).

Wakweya et al. (2016) concluded that TSW increased with later sowing time, however, significant differences of TSW were not observed between four sowing time variants. While Manning et al. (2020) research resulted in the opposite direction - with later sowing dates the TSW decreased like in our study. In the two-year trial carried out in Jordan, Al-Rifaee et al. (2004) studied sowing rate effect on TSW, and obtained contradictory results. In the first trial year, significant difference between TSW depending on sowing rate was not found, but in the second trial year, using lower seeding rates, the TSW was higher. Similar result was obtained in some other researches (Stringi et al., 1988; Turk & Tawaha, 2002); our previous results about TSW dependency on sowing rate were contradictory (Plūduma-Pauniņa et al., 2018).

CONCLUSIONS

The changing meteorological conditions in three trial years had a significant and the highest impact on field beans' yield and quality. Although the earliest possible sowing time and, consequently, the medium and late sowing time varied between the different experimental years, the factor 'sowing time' had a significant effect on all the estimated parameters on average and mainly on all - in the given trial year.

The best yield on average was obtained, sowing beans in medium and early sowing timings, which conforms partly to previous recommendation to sow beans as early as possible. Crude protein (CP) content, although dependent on sowing time, varied within 1%, and it is not possible to conclude that there is a possibility to increase it importantly by choosing a specific sowing time. Higher volume weight (VW) and 1,000 seed weight (TSW) was obtained sowing beans in early sowing time.

The effect of the used variety on the obtained results varied depending on evaluated parameter. All three varieties gave similar yield on average per trial period, the variety 'Boxer' presented higher TSW, but the variety 'Isabell' had higher CP content in seeds and VW. It is not proved that a specific sowing time could be more suitable for performance of a certain variety.

In most cases the sowing rate affected only field beans' yield significantly, but the necessity for various sowing rates depending on sowing time was not proved. The highest yield was mostly obtained using the rate 50 germinable seeds per m^{-2}.

Fungicide application increased the obtained field beans' yield, VW and TSW significantly, but slightly decreased CP content in seeds.

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The efficiency of nitrogen stabilizer at different soil temperatures on the physiological development and productivity of maize (*Zea mays* L.)

D. Rácz[1,*], B. Gila[2], É. Horváth[1], Á. Illés[1] and A. Széles[1]

[1]University of Debrecen, Faculty of Agriculture and Food Sciences and Environmental Management, Institute of Land Use, Technology and Regional Development, Böszörményi út 138, H-4032 Debrecen, Hungary
[2]University of Debrecen, Faculty of Science and Technology, Department of Molecular Biotechnology and Microbiology, Egyetem tér 1, H-4032 Debrecen, Hungary
[*]Correspondence: racz.dalma@agr.unideb.hu

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Abstract. Nitrogen (N) stabilizer containing nitrapyrin inhibitor is responsible for slowing the activity of *Nitrosomonas* sp. bacteria down which oxidize ammonium to nitrite ions, thus, N-loss resulting from nitrate leaching can be reduced. Although prior studies have shown its effectiveness in the pre-sowing application in maize, considering that it disturbs the activity of *Nitrosomonas* bacteria which is the most intense between 25 °C and 30 °C, soil temperature may significantly influence the efficiency of nitrapyrin. Besides, nitrapyrin aims to enhance N-use efficiency in high N-demanding plants, such as maize, which demands N at the most during stalk elongation, which lays down the reason for its subsequent application. This study focuses on the efficiency of nitrapyrin at different soil temperatures and its impacts on the physiological development and productivity of maize. In a laboratory test, 10 °C, 15 °C, 20 °C, and 25 °C temperature soils were treated with nitrapyrin and change of nitrate content was monitored to observe the nitrification dynamic. Results show that as the soil temperature elevated, the inhibition efficiency increased. In a field experiment with maize, nitrapyrin was applied in 13 °C and 25 °C temperature soil. Results suggest the later treatment enhanced N-use efficiency, as, during the high N-demanding growth stage, more N-forms were available in the soil. This resulted in significantly higher relative chlorophyll concentration in the leaves and laboratory leaf analysis confirmed the prevention of N deficiency. Results of further measurements on parameters indicating biomass production such as root mass, stalk diameter, ear size, 1,000-kernel weight indicate that the nitrapyrin application should be timed later.

Key words: corn, nitrapyrin, nitrification inhibitor, nitrogen use, *Nitrosomonas*.

INTRODUCTION

The efficiency of nitrogen (N) fertilisation can be enhanced by nitrification inhibitors, which have been widely used in agriculture to reduce N-loss (Liu et al., 2017). Although nitrification inhibitors decrease primarily nitrate (NO_3^-) leaching from the root zone, which is mainly responsible for N-loss (Zhong-Quing et al., 2020), a growing body of studies have been published recently that N-stabilizer containing nitrapyrin also reduces nitrous oxide (N_2O) greenhouse gas (GHG) emission (Futó et al., 2016; Gilsanz et al., 2016; Thapa et al., 2016; Thomas et al., 2017; Monge-Muñoz et al., 2021). Thus, nitrapyrin has attracted wide attention due to its environmental benefits.

Nitrapyrin (2-chloro-6-(trichloromethyl)-pyridine) that was first reported as a nitrification inhibitor in 1962 by Goring (Goring et al., 1962) aims to block the activity of *Nitrosomonas* sp. nitrifying bacteria involved in the nitrification mechanism, which are responsible for the transformation of ammonium (NH_4^+) to nitrite (NO_2^-) ions through inhibition of the ammonia monooxygenase (AMO). Thus, stable ammonium-N forms will be available to crops for a longer period (Degenhardt et al., 2016; Futó et al., 2016; Woodward et al., 2019) and N-use efficiency can be improved (Degenhardt et al., 2016; Martins et al., 2017; Niu et al., 2017; Ren et al., 2017).

Despite the wide-range and split N-fertilisation, the idea that N-fertilisation should be still enhanced is not purposeless. As a consequence of climate change, several stress factors endanger and pose threat to the health condition of the crops (Okoro et al., 2019).

Higher daily temperatures, more frequent droughts, and intense precipitation events are all adverse weather conditions that lead to even greater N-loss (Congreves et al., 2016). Accordingly, split N-fertilisation has been implemented in practice to reduce nitrate leaching and run-off that contaminate underground waters causing serious risks to human health (Huang et al., 2017; Dimpka et al., 2020). Since nitrate leaching has the greatest responsibility for N-loss and it moves freely in the soil, heavy rains increase leaching, particularly in high-permeability soils such as sand (Piccini et al., 2016; Hess et al., 2020), resulting in significant yield loss in crops. Thus, using nitrapyrin seems to be a promising and profitable solution to enhance N-use efficiency in arable crops with high N-demand, such as maize, sunflower, and rapeseed (Futó et al., 2016).

Despite prior studies have shown its efficiency with the pre-sowing application in maize (Gupta et al., 2020), considering that nitrapyrin disturbs selectively the activity of *Nitrosomonas* sp. bacteria living in the soil which is the most intense between 25 °C and 30 °C (Taylor et al., 2019), soil temperature may significantly influence the inhibitory effect of nitrapyrin. On the other hand, the impact of N-application timing on the yield and physiological development of maize has been widely studied, but results tend to vary. A study by Davies et al. (2020) has reported that split applications of N could significantly improve N management and increase maize yield, however, Sawyer et al. (2016) summarized that it should not be an expected occurrence on all soils or in all years, as in-season N application, by itself, does not improve yield unless there is deficient N in the soil system and the crop can respond.

However, maize accumulates approximately 70% of the total N uptake by silking (R1) (Woli et al., 2016), and its N demand is the highest from V6–V18, during rapid stalk elongation (English et al., 2017). Hence, since maize has low N demand at the early growth stage (Panison et al., 2019), nitrapyrin might be more effective if it would not be applied before sowing. Sangoi et al. (2016) also reported that maize takes up only 5–10% of total N from crop emergence to floral primordium that occurs at the stage of five-six expanded leaves. It follows from the foregoing that nitrapyrin application would be preferable to enhance its efficiency when maize enters the critical, rapid growth stage and soil temperature elevates. In terms of practice, nitrapyrin is recommended to be incorporated into the soil (Degenhardt et al., 2016; Vetsch et al., 2017), otherwise, low efficiency should be expected as nitrapyrin can rapidly volatilize and cannot affect bacteria in the soil. Thus, it is favorable to perform nitrapyrin co-application with liquid N-fertiliser when spring side-dress fertilisation is planned using a cultivator. With manufacturers recommendation, if incorporation technically is not possible, at least 10–15 mm precipitation must be fallen within two weeks after its application.

While nitrapyrin treatment has been optional up to the present due to its high cost, concerns are growing about global warming and its irreversible effects, and pressure is mounting on the agricultural stakeholders as strict regulations are expected worldwide related to N-fertiliser treatments. This will have a major impact on nitrification inhibitor usage as well, thus, testing technologies that mitigate the harmful effects of climate change has been never so important before.

This study focuses on the effect of nitrapyrin applied at different soil temperatures on the physiological development and productivity of maize. Nitrapyrin effectiveness was observed under laboratory conditions and through field trials as well. We hypothesized that late nitrapyrin treatment will increase N-availability in the soil during the rapid growth stage of the maize compared to its pre-sowing application, providing better conditions for effective growth.

MATERIALS AND METHODS

Soil characteristics

The soil type of experiment was loam. The plasticity index according to Arany was 38, with an average pH_{KCl} 6.83 value (slightly acidic soil), which is optimal for the nutrient uptake of crops. Humus content was 2.91%, carbonated lime content 0.9% (lacking in calcium), the AL-soluble P_2O_5 content was 481 mg kg^{-1}, AL-soluble K_2O was 310 mg kg^{-1}, KCl-soluble $N\text{-}NO_3^-$+ $N\text{-}NO_2^-$ content was 2.16 mg kg^{-1}. (HL-LAB Environmental and Soil Testing Laboratory, Debrecen, Hungary).

Soil incubation studies

Under laboratory conditions, changes of nitrate content in the soil were studied in 300-mL plastic plant pots at different temperatures to observe nitrification dynamic indirectly. There were 8 treatments in total and each treatment was applied on 15 pots. Soil samples were collected from the same place to avoid distortion effect (Demonstration Garden of Institute of Plant Protection (University of Debrecen, Debrecen, Hungary; 47°33'07.9"N, 21°36'03.0"E). 200 g soil was treated with 12 g ammonium-sulfate per sample to activate nitrification, and 1 mL nitrapyrin solution (3 mg mL^{-1}) (Powell & Prosser, 1986), then incubated at 10 °C, 15 °C, 20 °C and 25 °C temperatures for three weeks in CLN 240 laboratory incubators (Pol-Eko-Apparatura, Poland). Nitrate content was measured once a week (3 pots per treatment) with Nitrat 2000 soil kit (Stelzner, Germany) according to the steps recommended by the manufacturer.

Design of field experiment

In a field experiment with maize (Zea mays L.) (Armagnac, FAO 490, Kite Zrt, Hungary) nitrapyrin efficiency was tested in 2020. The experiment was located in the Demonstration Garden of the Institute of Plant Protection (University of Debrecen, Debrecen, Hungary; 47°33'07.9"N, 21°36'03.0"E). Soil type and characteristics were just the same that was used for the laboratory trial. The amount of organic manure applied to the soil was 25 t ha^{-1}. The experiment was designed in 3 treatments where nitrapyrin was applied two different times and a control field was left.

N-stabilizer containing nitrapyrin was first incorporated (6–8 cm depth) in the soil with 1.7 L ha^{-1} dose (300 g L^{-1}) before maize sowing (22 April 2020), in 13 °C soil temperature, and later was sprayed at the stage of 6–7 leaves of maize (BBCH 17), when soil temperature was 25 °C (13 June 2020). Since nitrapyrin is only effective in the soil, spraying was timed based on the forecast and within two weeks 45 mm precipitation fell (Hungarian Meteorological Service (HMS), 2020).

Weather conditions

Uneven precipitation distribution characterized the vegetation period (2020).

The initial development of the maize was hampered by severe drought in April and May (only 51.7 mm precipitation fell in these two months). Development of maize was still delayed despite the smaller rainfall in June, however, in mid-June, a series of precipitations has begun that boosted the growth (June: 117.9 mm; July: 96.7 mm precipitation). When maize entered the tasseling period, the optimal amount of precipitation supported yield production. The general Growing Degree Days (GDD) was 1,574 °C (HMS, 2020).

Nitrate content measurement

To observe the effect of nitrapyrin on N-use efficiency, changes in soil nitrate content were monitored. Soil samples were collected at six points per treatment randomly, taking into account heterogeneity, from 0–30 cm depth, about every 2–3 weeks as weather conditions allowed (during intensive, heavy rainfalls, sampling was not possible). Samples were always collected at a specific time of the day (between 8 and 10 am) to avoid the distorting effect of different results on samples taken at different times. Nitrate concentration was measured with nitrate electrode (Nitrat, 2000) according to steps recommended by the producer (Stelzner, Germany).

Relative leaf chlorophyll measurement

Since several prior studies have confirmed that chlorophyll concentration is closely linked to the N-supply and the physiological development of the maize (Kalaji et al., 2017; Yuan et al., 2016), measurements were performed on 6 June, using SPAD-502 chlorophyll meter (Konica Minolta, Japan) Both adult leaf (close to the maize ear) and young (top) leaf were measured (on 20 crops per treatment) to obtain information about the physiological condition. For both leaves, five chlorophyll readings were conducted on each leaf at and the mean of the measurements was retained as a value.

Laboratory leaf analysis

Adult leaf samples were collected (20 leaves per treatment) to determine the accurate nutrient content through laboratory leaf analysis (HL-LAB Environmental and Soil Testing Laboratory (Debrecen, Hungary). During the process, SLW 240 drying oven (Pol-Eko-Aparatura, Poland) was used for the preparation of samples from which extraction was performed with HNO_3-H_2O_2 blend with MARS 6 microwave digester (CEM Corporation, USA). The N content of the extracts was determined on UDK 139 Semi-Automatic Kjeldahl Distillation Unit (Velp Scientifica Srl, Italy) and the other nutrients were determined on iCAP 6300 Radial View ICP-OES spectrometer (Thermo Fisher Scientific, USA).

Stalk diameter measurement

To observe the impact of nitrapyrin on the biomass growth of the maize, the diameter of stalks above the lower three nodes was measured (20 stalks per treatment) with a caliper and the mean of the three measurements was retained as a value.

Root mass measurement

Root mass also indicates biomass production. To prevent distortion effect due to size difference, 15 cm^3 of soil counted from the surface was taken out and root samples (20 roots per treatment) were cleaned from soil residues and dried out at 60 °C. Then, clean and root samples dried to constant weight were measured.

Parameters of maize yield

Maize ear diameter was measured (20 ears per treatment) with a caliper at half of the ear's length. After determining the number of rows in each ear, kernel number per row, 1,000-kernel weight, quality parameters, such as protein, oil, and starch content of kernels were determined using Infratech 1241 Grain Analyzer (Foss, Denmark).

Statistical analysis

The statistical analysis was conducted by the R programming language (v4.0.2; R Core Team, 2020) and 'agricolae' R package (v1.3-3; De Mendiburu, 2020). Student's t-test and its non-parametric version (Mann-Whitney U test) and Duncan's multiple comparison test were used to examining the means at a significance level of 5%.

RESULTS AND DISCUSSION

Under laboratory conditions, the efficiency of nitrapyrin was observed at different soil temperatures. Results suggest that as the soil temperature elevated, the inhibitory effect of nitrapyrin was more intensive since a greater difference was observed between nitrate content in nitrapyrin treated and untreated soil when the temperature was higher (Fig. 1). Gu et al. (2018) also highlighted that the availability of nitrification inhibitors in the soil is strongly influenced by soil temperature. Our results are in line with the study by Dawar et al. (2021), who tested nitrapyrin efficiency under hot climatic conditions and concluded that nitrapyrin significantly increased yield total N uptake and N response efficiency. Despite the results achieved in this research, previous studies have referred to the fact that nitrapyrin goes under chemical hydrolysis (half-life) that increases when soil temperature raises (Woodward et al., 2016; Vetsch et al., 2017; Giacometti et al., 2020), furthermore, Giacometti et al. (2020) refers when soil temperature increases, nitrapyrin effectiveness decreases. This may be due to the transformation of nitrapyrin into degradation products, such as 6-chloropicolinic acid (6-CPA). Thus, Woodward et al. (2016) recommended the later timing of nitrapyrin application, such as fall or spring when the soil temperature is near 10 °C.

One possible explanation for the results obtained in this study could be that elevated temperature soil induced accelerated nitrification. This is in accordance with several studies on the impact of increased temperature which have also confirmed it (Yates et al., 2016; González-Camejo et al., 2019; Dai et al., 2020; Zhongmin et al., 2020). Thus, although the amount of *Nitrosomonas* species in the soil was not an included parameter in our measurements, we strongly suspect that elevated soil temperature induced an abundant amount of active *Nitrosomonas* species. Our assumption relies on a study by Chen et al. (2018), in which *Nitrosomonas* species were more abundant at higher temperature (20 °C) compared to *Nitrosospira* species which were the most dominant only at 10 °C.

These findings conclude that although nitrapyrin efficiency is likely to decrease in elevated soil temperature, probably due to intensified nitrification and an abundant amount of *Nitrosomonas* species in the soil. The inhibitory effect of nitrapyrin acting on them was proved to be stronger. These results may justify the later timing of nirapyrin treatments in soils.

In a field experiment with maize, nitrapyrin application was performed before sowing (13 °C soil) and later, when soil temperature elevated (25 °C). Results of nitrate content changes in the soil suggest that due to later nitrapyrin treatment, despite heavy rainfalls during the rapid, high N-demanding growth stage of the maize, more N forms were accessible in the soil (Fig. 2). In a previous study, Singh & Nelson (2019) have also found a common line that during wet vegetation, nitrification inhibitors were able to limit N-loss and make N more available during the maize growing season. Thus, late treatment (N2) proved to be more favorable

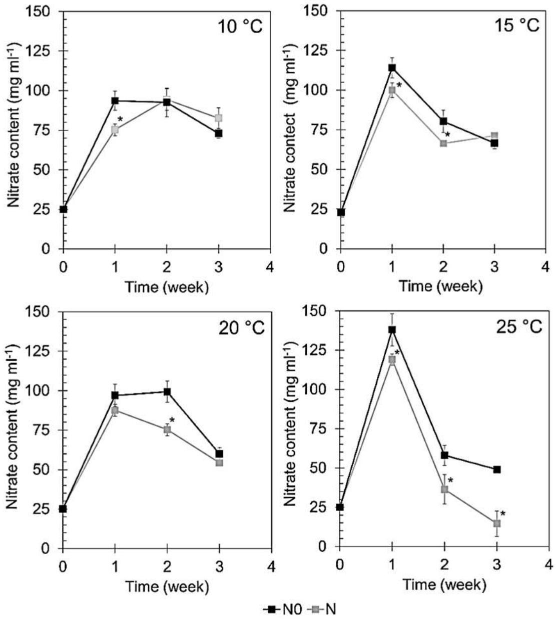

Figure 1. Nitrate content changes of soil samples depending on temperature. N: nitrapyrin treatment. N0: untreated control;. Asterisks (*) denote statistically significant differences (Student's *t*-test, $p < 0.05$) between nitrapyrin treated and untreated soils.

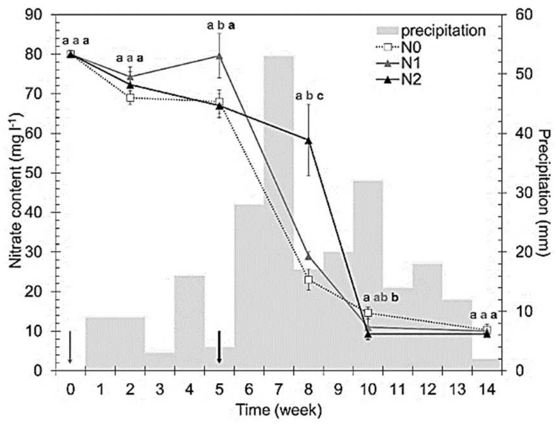

Figure 2. Nitrate content changes in soils. N0: untreated control; N1: early nitrapyrin treatment; N2: late nitrapyrin treatment. The grey and black arrows indicate the dates of nitrapyrin application. Different lowercase letters represent statistically significant differences (Duncan's multiple comparison test, $p < 0.05$) between nitrapyrin treated and untreated soils.

for the maize since the application before sowing seems to be too early and highly unstable, particularly when abundant rainfalls can be expected after its application. Pittelkow et al. (2017) also reported that fall (early) nitrapyrin application did not decrease N-loss or increase yield.

Therefore, nitrapyrin application seems to be more effective if it is timed when maize soon enters the rapid growth stage (approx. week 8.) to provide a sufficient amount of N forms during this critical period.

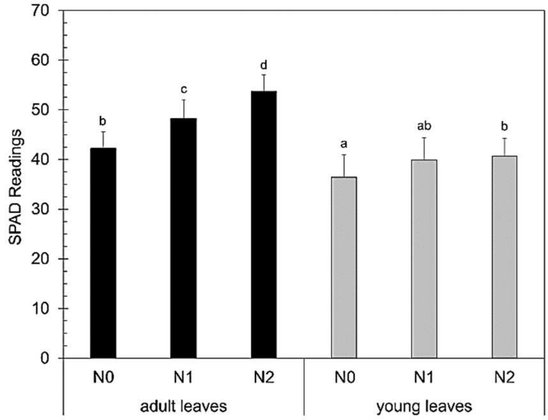

Figure 3. Changes in relative chlorophyll content of adult and young maize leaves due to nitrapyrin treatments. N0: untreated control; N1: early nitrapyrin treatment; N2: late nitrapyrin treatment; Different lowercase letters denote statistically significant differences (Duncan's multiple comparison test, $p < 0.05$) between treatments.

Results of relative chlorophyll measurements suggest that due to later nitrapyrin treatment, significantly higher chlorophyll concentration was detected in adult leaves (Fig. 3), which can be explained by the more accessibility of N in the soil. Several previous studies have also emphasized that nitrapyrin increases plant chlorophyll content due to better nutrients supply and increased photosynthesis capacity, resulting in increased leaf SPAD value (Singh & Nelson, 2019; Nozari et al., 2020; Ren et al., 2020; Taherianfar et al., 2020). Ren et al. (2020) have also reported effectively improved photosynthetic characteristics of maize due to nitrapyrin. Since increased chlorophyll concentration refers to intensified photosynthesis and improved N-supply (Taherianfar et al., 2020), late nitrapyrin treatment contributed to provide favorable conditions for maize.

Table 1. N content of maize leaves due to early and late nitrapyrin treatment. Data represent means and standard deviation (SD)

Treatment	Nitrogen content in leaves (mg kg^{-1})
N0	23,900 ± 1,195
N1	26,700 ± 1,335
N2	28,100 ± 1,405

N0: untreated control; N1: early nitrapyrin treatment; N2: late nitrapyrin treatment.

To detect accurate N content in maize leaf tissues, laboratory leaf analysis was conducted. Results confirmed that due to later nitrapyrin treatment, N content in the leaves has improved (Table 1). Moreover, untreated maize suffered from severe N-deficiency since its value was below the critical minimum value (Nitrogen: 28,000 mg kg^{-1}; Elek & Kádár, 1980) and deficiency symptoms could be observed in older leaves. Improved N-supply in maize treated with late nitrapyrin (N2) can be explained by the better N conditions in the soil provided by the subsequent nitrapyrin treatment as despite the heavy rainfalls, more available N-forms were provided for the crops. Ren et al. (2017) referred that due to excessive rainfalls, more intense nitrate leaching must be expected which leads to the decreaseof available mineral N concentration in soil, and the reduction of root absorption and utilization of N. Thus, N-deficiency should be expected.

However, nitrapyrin can inhibit the oxidation of ammonium to nitrate ions and results in N in the form of ammonium ions remaining in the soil for long periods.

Several previous research studies have confirmed the close correlation between the quality of N-management and SPAD-value (Suresh et al., 2017; Habibullah et al., 2018; Edalat et al., 2019). These findings are in good agreement with our present study which suggests that late nitrapyrin (N2) treatment created beneficial conditions for N-accessibility in the soil due to improved N concentration in leaf tissues.

As for indicators of biomass production, stalk diameter and root mass were measured. Besides, the impact of nitrapyrin on different yield parameters (agronomic characters), such as maize ear length, ear diameter, row number in each ear, kernel number in each row, 1,000-kernel weight, as well as protein, oil, and starch content of kernels were measured. Results are demonstrated in Table 2.

Table 2. The effect of nitrapyrin treatments on different parameters. Data represent means and standard deviation (*SD*)

Treatment	N0	N1	N2
Root mass (g)	14.72 ±5.63	28.06 ± 6.68	45.30 ± 8.13
Stalk diameter (mm)	17.65 ± 1.39	20.67 ± 1.18	45.30 ± 8.13
Ear length (mm)	19.13 ± 2.35	19.64 ± 0.83	21.90 ± 0.85
Ear diameter (mm)	48.55 ± 2.94	49.13 ± 2.16	51.65 ± 1.66
Row number per ear	15.10 ± 1.52	15.60 ± 0.84	16.40 ± 0.84
Kernel number per row	32.60 ± 7.34	35.70 ± 2.98	41.90 ± 2.02
1,000-kernel weight (g)	370.46 ± 38.5	419.28 ± 26.34	415.11 ± 26
Protein content (%)	5.65 ± 0.43	5.64 ± 0.25	6.12 ± 0.36
Oil content (%)	2.85 ± 0.34	3.17 ± 0.35	3.10 ± 0.42
Starch content (%)	64.12 ± 0.80	64.20 ± 0.49	64.07 ± 0.83

N0: untreated control; N1: early nitrapyrin treatment; N2: late nitrapyrin treatment.

For root mass and stalk diameter, a significant difference was measured between early (N1) and late nitrapyrin treatment (N2). Due to subsequent nitrapyrin (N2) application, more beneficial developments were detected. Thus, greater root mass and thicker stalk diameter reflected that later nitrapyrin treatment (N2) induced more intense biomass growth. These findings are in consistent with previous reports on improved biomass and grain yield of maize due to nitrapyrin treatment (Dawar et al., 2021), improved dry matter accumulation (Ren et al., 2017), and the positive correlation between nitrapyrin and biomass production (Zhou et al., 2017; Cai et al., 2018). Cai et al. (2018) have also stated that nitrapyrin increased net photosynthetic area resulting in a remarkable biomass increment.

Results of maize ear parameters (agronomic characters) measurements, such as ear length, diameter, number of rows in each ear, kernel number per row suggest that due to late nitrapyrin treatment, beneficial changes were observed for all characters since a significant difference was detected compared to untreated (N0) and early nitrapyrin treatment (N1).

In terms of 1,000-kernel weight, we hypothesized that both early (N1) and late nitrapyrin treatment (N2) will contribute to significant yield growth. Results are in consistent with a previous study which has shown that nitrapyrin-treated maize had a significantly improved kernel number and 1,000-kernel weight (Ren et al., 2017). In this current research, despite both nitrapyrin treatments significantly increased 1,000-kernel weight, the timing of treatment was not an influencing factor because no difference was observed between the early (N1) and late nitrapyrin treatments (N2). This reflects that although nitrapyrin contributed to yield growth, it was not significantly influenced by the timing of treatment.

For quality parameters, such as protein, starch, and oil content of yield, only slight changes were measured. While protein and oil content were hardly grown, no difference occurred in the change of starch content due to nitrapyrin treatment. Similar findings have been also identified by Singh & Nelson (2019) when only protein content was higher compared to non-treated control which could be due to higher grain N concentration since N is a fundamental component of protein. Nozari et al. (2020) also confirmed that N is a key nutrition of molecules such as protein and reported a significant increase in protein content due to nitrapyrin. In a previous study by Rácz & Radócz (2020), only an increase in protein content was measured due to nitrapyrin, while no significant changes were determined in oil and starch content. These findings draw attention to the appropriate quality of N-management since it has a significant impact on the protein content of the yield.

CONCLUSIONS

This present study aimed to examine how the soil temperature influences the inhibitory effect of nitrapyrin. Results of the laboratory experiment revealed that the inhibitory effect of nitrapyrin increased with increasing soil temperature. The most likely explanation for this is that elevated soil temperature induced an abundance of active *Nitrosomonas* sp, resulting in a greater inhibitory effect of nitrapyrin acting on them. Results of the field experiment with maize suggest that subsequent nitrapyrin treatment contributed to providing more favorable conditions for improved physiological development and productivity of maize as, during the rapid growth stage, more N-forms were available in the soil. In contrast to the pre-sowing application of nitrapyrin, its late treatment proved to be effective at preventing severe N-deficiency, which was confirmed by laboratory leaf analysis and significantly higher chlorophyll concentration in leaves. Besides, the impacts of late nitrapyrin treatment on biomass production manifested in greater root mass and thicker stalk diameter. For maize yield agronomic characters, such as ear length, ear diameter, the number of rows in each ear, kernel number per row, later treatment contributed to improving these parameters as well. These findings can be explained by the more favorable N-conditions in the soil provided by late nitrapyrin. However, the timing of nitrapyrin treatment did not significantly influence the 1,000-kernel weight and quality parameters of the yield.

From the outcome of our investigation, our findings are of direct practical relevance that nitrapyrin treatment is a promising technology to enhance N use efficiency of maize, and thereby, improving physiological development and productivity. Although these results suggest that nitrapyrin application can be preferable to be timed later when maize soon enters the high N-demanding, critical growth stage, we emphasize that this may not be the case in all agricultural areas, as each soil type and climatic factor may have a different effect on treatment effectiveness. Hence, several other questions remain to be addressed and further study of the issue would be of interest.

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Influence of mineral fertilizers on yielding capacity and quality of soft spring wheat grain

M.V. Radchenko[1,*], V.I. Trotsenko[1], Z.I. Hlupak[1], E.A. Zakharchenko[2], O.M. Osmachko[3], V.V. Moisiienko[4], V.Z. Panchyshyn[4] and S.V. Stotska[4]

[1]Sumy National Agrarian University, Faculty of Agrotechnology and Nature Management, Plant Growing Department, 160 G. Kondratieva street, UA40021 Sumy, Ukraine
[2]Sumy National Agrarian University, Faculty of Agrotechnology and Nature Management, Department of Arable farming, soil science and agrochemistry, 160 G. Kondratieva street, UA40021 Sumy, Ukraine
[3]Sumy National Agrarian University, Faculty of Agrotechnology and Nature Management, Department of Plant Protection named after Associate Professor Mishnev A.K., 160 G. Kondratieva street, UA40021 Sumy, Ukraine
[4]Polissia National University, Agronomy Faculty, Plant Growing Department, 7 Staryi Buljvar street, UA10008 Zhytomyr, Ukraine
[*]Correspondence: radchenkonikolay@ukr.net

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Abstract. The aim of the study is optimization of nutrition system for soft spring wheat plants through the use of mineral fertilizers in order to obtain high quality grain with simultaneous yield increase. Different doses of mineral fertilizers were tested in the study. The object of study was a variety of soft spring wheat 'Uliublena'. The structure of the crop was determined by the method of selecting sheaf samples from each accounting area. The leaf surface area was determined by calculation method. According to the results of research, yield capacity of soft spring wheat on average ranged from 2.43 to 4.51 t ha^{-1}. The highest index of gluten amount was obtained in the variant with fertilizers dose $N_{64}P_{64}K_{64}$ - 28.19%, which is higher than in the variants with fertilizer doses $N_{16}P_{16}K_{16}$ and $N_{32}P_{32}K_{32}$ by 6.11 and 0.15%. The highest increase in the yield of soft spring wheat grain (2.08 t ha^{-1}) was obtained with application of $N_{64}P_{64}K_{64}$, slightly lower yield increase was obtained with application of $N_{32}P_{32}K_{32}$ - 1.64 t ha^{-1}, and $N_{16}P_{16}K_{16}$ - 0.99 t ha^{-1} comparing with the control. With fertilizer dose of $N_{64}P_{64}K_{64}$ soft spring wheat provided the maximum yield - 4.51 t ha^{-1}, gluten content - 28.19% and protein content - 14.21%.

Key word: fertilization, ear, yield, protein, gluten.

INTRODUCTION

Attention to grain production is stipulated by its strategic importance within agro-industrial complex of Ukraine, as its products are critically important as key food for support of population lives (Klochan et al., 2018). Spring wheat is a crop with a fairly wide range of use. It is the only equivalent insurance crop in case of winter wheat loss

(Kolyuchiy, 2006). The quality of spring wheat grain is assessed by a number of features that together characterize its nutritional, physical and chemical, and technological properties (Kolyuchiy et al., 2007).

High potential of modern varieties of spring soft wheat can be realized by growing them in technologies that involve complex application of fertilizer. Exactly they should be the basis of modern environmentally friendly and resource-saving technologies for growing spring cereals (Polishuk & Antko, 2020). One of the ways to increase competitiveness of cereals can be achievement of high level of yield capacity and productive prime cost of spring cereals (Tomashevska & Shevchenko, 2019). Rational application of fertilizers allows to control the production process of wheat crops and get high yields, increase economic efficiency of growing crops (Mazur et al., 2020). Violation of cultivation technology leads to indivertible consequences such as loss of yield and grain quality. Many studies have shown that fluctuations in spring wheat yields are connected with its high sensitivity to environmental conditions (Kirichenko et al., 2009).

Adaptation of agricultural crops to conditions of growing, first of all, is determined by correctly chosen agrotechnological measures of cultivation - predecessors, soil tillage, sowing dates, sowing norms, fertilizers, varieties, etc (Popov et al., 2000; Muhamedyarova et al., 2020). Modern varieties of spring wheat have a high potential of yield capacity (in experiments up to 5.0–5.5 t ha^{-1}, under production conditions - about 3.0–3.5 t ha^{-1}). However, the average yield in recent years under conditions of Forest-Steppe was only 2.0–2.5 t ha^{-1}. One of the reasons for low yield capacity is insufficient study of the conditions for effective fertilizer application with taking into account the level of moistening and nutrient content of soils. Identification of regularities of mineral fertilizer influence on soil fertility and crop yield capacity - is an important condition for development of a scientifically substantiated fertilizer system (Sviderko et al., 2004; Melnyk et al., 2006).

It is determined that at the end of vegetation of spring grain crops the content of movable nutrients in the soil decreases significantly. It is connected with the usage of NPK for productivity formation of the studied crops and their partial redistribution in soil profile (Gamayunova et al., 2019). Application of mineral fertilizers contributes to increasing of microorganism number of main ecological-trophic, functional and systematic groups and rising of physiological and biochemical activity of representatives of certain microorganism groups (Malynovska, 2018).

Fertilizer equilibrium is important as high accumulation of nitrogen has a negative effect on the consumption of other nutrients, especially potassium (Hamnér et al., 2017). In its turn, potassium deficiency significantly reduces resistance of plants to drought. It is especially noticeable for genetically more tolerant varieties, ‘as experimentally proved by Wei et al. (2013)’. ‘The study by Yan Deng et al. (2018) also prove the presence of genotypic specificity of phosphorus consumption in different varieties of wheat at morphological, physiological and molecular level’. Nutrition of wheat with nitrogen is especially important as it can increase protein content without reducing yields (Gyuga et al., 2002; Balla et al., 2011; Litke et al., 2019).

Increasing of mineral fertilizer doses had a positive effect on increase of spring wheat yield capacity. It was also found that accumulation of movable compounds of nitrogen, phosphorus and potassium in the soil reduces negative impact of weather conditions and contributes to yield stabilization (Kochmarskiy et al., 2011). Most of experiments concerning the study of mineral fertilizer effect on nutritional regime of soil and yield capacity of spring wheat were conducted in Polissya area and, partially, under conditions of steppe irrigation (Lebid et al., 2006). An issue of growing spring wheat in the Forest-Steppe is still not fully studied.

The aim of the study is optimization of nutrition system for soft spring wheat plants through the use of mineral fertilizers in order to obtain high quality grain with simultaneous yield increase under conditions of the Forest-Steppe of Ukraine.

MATERIALS AND METHODS

The research was conducted in 2016–2020 on the basis of educational and scientific production complex of Sumy National Agrarian University according to described methods of Dospekhov (1985) and Pidoprygora & Pisarenko (2003).

The object of the study was a variety of soft spring wheat Uliublena. Predecessor - soybeans. Sowing was carried out after physical readiness of soil at the temperature of 6–8 °C in the depth of 5 cm. with the help of seeder Klen - 1.5 to a depth of 3–4 cm. with sowing rate of 6.0 million similar seeds per 1 ha. Various doses of mineral fertilizers were tested. Mineral fertilizers were applied to pre-sowing cultivation in the form of nitroammophoska. Nitroammophoska is a complex nitrogen-phosphorus-potassium fertilizer. Mass fraction of nitrogen (N) 16%, phosphorus (P) 16%, potassium (K) 16%. Form of fertilizers - granular.

The total area of the plot was 50 m^2 (4.5 m × 11 m), accounting area was 30 m^2, repetition of the experiment - three times. The placement of plots is systematic.

Scheme of the experiment:

1. Without fertilizers (control);
2. 100 kg ha^{-1} ($N_{16}P_{16}K_{16}$ of active substance per 1 ha);
3. 200 kg ha^{-1} ($N_{32}P_{32}K_{32}$ of active substance per 1 ha);
4. 400 kg ha^{-1} ($N_{64}P_{64}K_{64}$ of active substance per 1 ha).

During phenological observations, the growth and development phase of spring wheat was considered to be beginning with appearance of at least 10% of plants, and the phase was considered to be complete with appearance of 75% of plants.

The dynamics of overground mass growing was determined in the main phases of growth and development by selecting 25 plants in typical places of the plots in two incompatible repetitions. The structure of the crop was determined by the method of selecting sheaf samples from each accounting area. The leaf surface area was determined by calculation method. Amount of gluten was determined according to STST 13586.1-68 Grain. Methods for determining quantity and quality of wheat gluten. Amount of protein was determined according to STST 10846-91 Grain and products of its processing. Method for determination of protein. Statistical analysis of experimental data was conducted according to Dospekhov (1985) with usage of Microsoft Excel.

The soil of experimental field is a typical powerful heavy-loamy and medium humus black soil, which is characterized by the following indices: humus content in arable layer (according to I.V. Tiuryn) - 4.0%, reaction of soil solution is close to neutral (pH 6.5), the content of easily hydrolyzed nitrogen (according to I.V. Tiuryn) 9.0 mg, movable phosphorus and exchangeable potassium (according to Ph. Chyrikov), accordingly, 14 mg and 6.7 mg per 100 g of soil.

Conditions in 2016 were characterized by a slightly higher average daily (average annual) air temperature, namely, 9.5 °C. That is 2.1 °C higher than the long-term index (7.4 °C). Its absolute maximum - 37.0 °C above zero was observed in the third decade of August, the minimum - 24.0 °C below zero was observed in the third decade of January. But amount of precipitation was 792.0 mm, that is 199.0 mm more than long-term norm (593 mm). In spring, average daily temperature was 2.1 °C higher than for many years (8.1 °C). Precipitation was 248.8 mm (188.0%) at a norm of 132 mm. The sum of active air temperatures above +10 °C for the spring period was 795 °C (long-term 620 °C). The average daily air temperature during summer was 21.5 °C, that is 2.1 °C higher than the long-term average one. Precipitation was 250.6 mm, that is 125% at a norm of 200 mm. Total number of days with precipitations for summer period was 25 while a long-term index is 40 days. The sum of active air temperatures above +10 °C during summer period was 1,982 °C, with a long-term index of 1,790 °C.

Conditions in 2017 were characterized by a slightly higher average daily (average annual) air temperature, namely 8.1 °C, that is 0.7 °C higher than the long-term index (7.4 °C). Its absolute maximum - 35.0 °C above zero was observed in the second decade of August, the minimum - 24.0 °C below zero was observed in the first decade of February. But precipitation amount was 449.0 mm, that is 144.0 mm less than the long-term norm (593 mm). In spring, the average daily temperature was 1.5 °C higher than the one for many years (8.1 °C). Precipitation was 54.4 mm (41.0%) at a norm of 132 mm. The sum of active air temperatures above +10 °C for spring period was 553 °C (long-term 620 °C). The average daily air temperature during summer was 21 °C, that is 1.7 °C higher than the long-term average one. Precipitation was 126.0 mm, that is 63% at a norm of 200 mm. Total number of days with precipitations for summer period was 22 while a long-term index is 40 days. The sum of active air temperatures above +10 °C during summer period was 1,937 °C, with a long-term index of 1,790 C°.

Conditions in 2018 were characterized by a slightly increased average daily (average annual) air temperature, namely, 9.4 °C, that is 2.0 °C higher than the long-term index (7.4 °C). Its absolute maximum - 35.0 °C above zero was observed in the second decade of August, the minimum - 22.0 °C below zero was observed in the third decade of February. But amount of precipitation was 539.0 mm, that is 54.0 mm less than the long-term norm (593 mm). During spring period, the average daily air temperature was 9.2 °C, that is 1.1 °C higher than the one for many years (8.1 °C). Precipitation was 150.6 mm (114%) at a long-term norm of 132 mm. The sum of active air temperatures above +10 °C for spring period was 920 °C (long-term - 620 °C). The average daily air temperature during summer was 22.4 °C, that is 3 °C higher than the long-term average one. Precipitation was 100.1 mm, that is 50% of a long-term index of 200 mm. Totally, there were 14 days of precipitation during summer. The sum of active air temperatures above +10 °C during summer period was 2,061 °C, with a long-term index of 1,790 °C.

Conditions in 2019 were characterized by a slightly higher average daily (average annual) air temperature, namely, 9.6 °C, that is 2.2 °C higher than the long-term index (7.4 °C). Its absolute maximum - 35.5 °C above zero was observed in the first decade of August, the minimum - 20.0 °C below zero - in the first decade of January. But amount of precipitation was 409.0 mm, that is 184.0 mm less than the long-term norm (593 mm). During spring period, the average daily air temperature was 10.7 °C, that is 2.6 °C higher than the one for many years (8.1 °C). Precipitation was 102 mm - 77% at a long-term norm of 132 mm. The sum of active air temperatures above +10 °C for spring period was 786 °C (long-term - 620 °C). The average daily air temperature during summer was 22.4 °C, that is 3.0 °C higher than the long-term average one. Precipitation was 78.7 mm, that is 39% at a norm of 200 mm. Totally, there were 14 days of precipitation during summer. The sum of active air temperatures above +10 °C during summer period was 2054 °C, with a long-term index of 1,790 °C.

Conditions in 2020 were characterized by a slightly higher average daily (average annual) air temperature, namely, 10.2 °C, that is 2.8 °C higher than the long-term index (7.4 °C). Its absolute maximum - 35.0 °C above zero was observed in the third decade of July, the minimum - 14.0 °C below zero - in the first decade of February. But amount of precipitation was 466.0 mm, that is 127.0 mm less than the long-term norm (593 mm). During spring period, the average daily air temperature was 8.9 °C, that is 0.8 °C higher than the one for many years (8.1 °C). Precipitation was 120 mm - 91% at a long-term norm of 132 mm. The sum of active air temperatures above +10 °C for spring period was 462 °C (long-term - 620 °C). The average daily air temperature during summer was 22.1 °C, that is 2.7 °C higher than the long-term average one. Precipitation was 126 mm, that is 63% at a norm of 200 mm. Totally, there were 13 days of precipitation during summer. The sum of active air temperatures above +10 °C during summer period was 2027 °C, with a long-term index of 1,790 °C.

In general, the most favorable years for the formation of crop yields were 2016, 2018, 2020. Drought conditions were observed in 2017 and 2019 and characterized by low precipitation amount and extreme deviation of air temperature during vegetation period.

RESULTS AND DISCUSSION

Germination phase is determinant one at quantity formation of productive stem-standing. As not all sown seeds give viable seedlings, it affects the index of field germination. 'Alimov & Shelestov (1995) and others (Bebyakin et al., 2003; Altukhov, 2008) believe that one of the most important elements of crop sowing is the number of plants per unit of area. This number can vary during vegetation period'.

'The experiments of Kalens'ka & Suddenko (2016) revealed the influence of soft spring fertilizer system on the level of field germination and survival rate. Field germination of experimental varieties Elegiya myronivska and Simkoda myronivska increased by 0.7–4.0% depending on mineral nutrition. Plant survival rate increased from 1.7 to 4.8% depending on the system of fertilization and protection'.

The results of conducted research allowed to establish the fact that field germination of spring wheat seeds varied depending on a variant of experiment (Table 1). The lowest field germination was observed in the control 81.7%. The highest field germination of spring wheat seeds was observed in the variant with application of mineral fertilizers at sowing time in a dose of $N_{64}P_{64}K_{64}$ - 91.3%.

Plant standing density in the experiment increased from 490 to 548 pcs m^2. Thus, the lowest density of standing was formed in the control - 490 pcs m^2. The variant with fertilizer dose of $N_{16}P_{16}K_{16}$ is 17 pcs m^2 more than in the control. The variant with fertilizer dose of $N_{32}P_{32}K_{32}$ is 50 pcs m^2 more than in the control. The variant with fertilizer dose of $N_{64}P_{64}K_{64}$ is 58 pcs m^2 more than in the control.

Rate of plants preservation during vegetation period ranged from 70.8 to 76.6%. Thus, the highest preservation rate of plants was obtained in the variant with fertilizer dose of $N_{64}P_{64}K_{64}$ - 76.6%. In the control, preservation rate of plants during vegetation period was 70.8%, in the variant with fertilizer dose of $N_{16}P_{16}K_{16}$ - 75.5%, with fertilizer dose of $N_{32}P_{32}K_{32}$ - 75.4%. (Table 1).

Table 1. Standing density of soft spring wheat depending on fertilizer (average for 2016–2020)

Fertilizer dose	Field germination, %				Standing density, pcs m^2				Preservation rate of plants during vegetation period, %			
	I	II	III	average	I	II	III	average	I	II	III	average
Control (without fertilier)	82.4	80.1	82.6	81.7	482.0	495.0	493.0	490.0	70.9	71.3	70.1	70.8
$N_{16}P_{16}K_{16}$	86.0	83.1	84.4	84.5	512.0	501.0	508.0	507.0	74.6	75.0	75.2	75.0
$N_{32}P_{32}K_{32}$	89.1	91.8	89.1	90.0	536.0	548.0	536.0	540.0	75.0	74.8	76.3	75.4
$N_{64}P_{64}K_{64}$	92.6	90.2	91.1	91.3	556.0	544.0	544.0	548.0	76.6	76.6	76.6	76.6
LSD_{05}	-	-	-	2.6	-	-	-	12.5	-	-	-	1.0

It is known that crop yield amount depends on the efficiency of plant photosynthesis in crops. The largest and best quality crop yields can be obtained only in crops which have optimal leaf area size and processes of their formation (Gorodniy, 2004). The value of these indices is influenced by both biological characteristics of a variety and environmental conditions, which include light intensity, water and air regimes, temperature, as well as plant provision with nutrients (Zinchenko, 2005).

Therefore, it is necessary to create crops with optimal leaf area. Both with insufficient leaf area and under conditions of highly developed leaf area, a decrease in solar energy consumption is observed (Svydynyuk *&* Yula, 2004; Kolyuchiy et al., 2007). 'In the experiments of Kudriavytska (2016) it was established that application of mineral fertilizers significantly influenced the increase in leaf surface area of spring wheat plants. The highest index in leaf area increase of spring wheat (in amount of 51.8 thousand m^2 ha^{-1}) was determined in the variant where fertilizer norm Background + $N_{110}P_{120}K_{120}$ were applied on the background of manure aftereffect - 30 t ha^{-1}'.

The studies have shown that leaf area increase in the first vegetation period was slow. In the phase of tube entering intensity of its growth increased sharply and reached maximum before earing phase in all variants of the experiment (Fig. 1).

Thus, in the tillering phase, the largest leaf surface area of soft spring wheat was formed in the variant with fertilizer dose of $N_{64}P_{64}K_{64}$ and amounted to 9.5 thousand m^2 ha^{-1}. Leaf surface area decreased to 6.4 thousand m^2 ha^{-1} with fertilizer dose of $N_{32}P_{32}K_{32}$. It also decreased to 5.2 thousand m^2 ha^{-1} with fertilizer dose of $N_{16}P_{16}K_{16}$ and to 4.3 thousand m^2 ha^{-1} in the control (LSD_{05} = 0.6). These established differences persisted during the following vegetation phases of soft spring wheat. Thus, in the tube entering phase the area of leaf surface was: 40.4, 30.4, 25.2, 21.3 thousand m^2 ha^{-1}, accordingly (LSD_{05} = 1.8).

The largest leaf surface area was formed in the earing phase in the variant with fertilizer dose of $N_{64}P_{64}K_{64}$ - 43.2 thousand m^2 ha^{-1}. It is 7.8 thousand m^2 ha^{-1} more than in the variant $N_{32}P_{32}K_{32}$ (35.4 thousand m^2 ha^{-1}), 15.4 thousand m^2 ha^{-1} more than in the variant $N_{16}P_{16}K_{16}$ (27.8 thousand m^2 ha^{-1}), 16.7 thousand m^2 ha^{-1} more than in the control (26.5 thousand m^2 ha^{-1}) (LSD_{05} = 1.3) (Fig. 1).

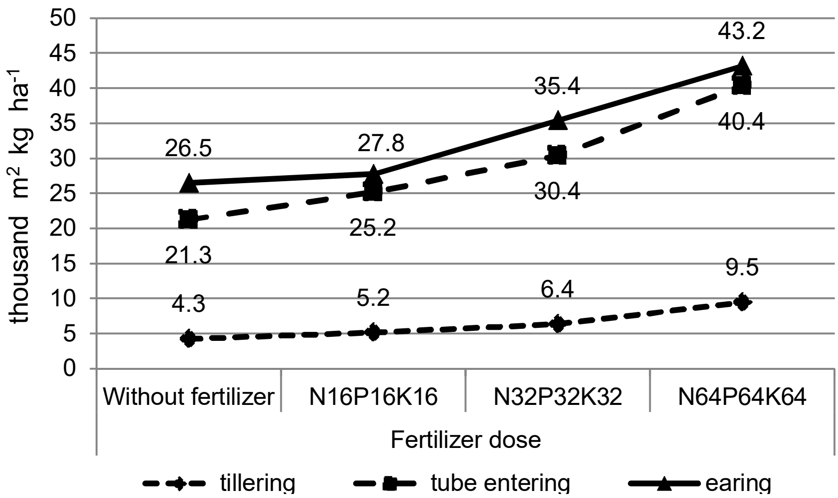

Figure 1. Leaf surface area of soft spring wheat by phases of development depending on fertilization (average for 2016–2020).

Plants of tall varieties have much larger leaf surface area, which provides better supply of assimilants into the ear and allows to form grain yield with lower level of mineral nutrition. It is established that the most favorable combination of morphological features and internal biological processes for the formation of high grain yield is in the plants with height of 85.2–97.8 cm.

In the tillering phase, the highest plant height was observed in the variant with fertilizer dose of $N_{64}P_{64}K_{64}$ - 28.8 cm. Slightly lower height was fixed in the variants with fertilizer dose of $N_{32}P_{32}K_{32}$ - 24.0 cm, with fertilizer dose of $N_{16}P_{16}K_{16}$ - 22.2 cm, in the control – 20.8 cm (LSD_{05} = 1.4).

In the phase of tube entering on average during the years of research, the maximum height was again obtained in the variant with fertilizer dose of $N_{64}P_{64}K_{64}$ - 65.4 cm. Smaller values of plant height were established both in the variants with fertilizer dose of $N_{32}P_{32}K_{32}$, $N_{16}P_{16}K_{16}$, and the control - 57.4, 49.5, 43.0 cm, accordingly (LSD_{05} = 3.7).

In the earing phase, on average during the years of research, the highest plant height was observed in the variant with fertilizer dose of $N_{64}P_{64}K_{64}$ - 97.8 cm, which is 12.6, 17.2, 25.0 cm higher (LSD_{05} = 4.0) than in the variants with fertilizer dose of $N_{32}P_{32}K_{32}$, $N_{16}P_{16}K_{16}$, the control, accordingly (Fig. 2).

'Insufficient provision of winter wheat plants with mineral nutrition in the studies of Zhuk (2016), assisted to reduce ear length (from 0.4 cm to 0.7 cm) of main and sidelong shoots. With deficiency of nutrition the number of grains in the ears of sidelong shoots decreases more significantly (from 7.0 to 8.0 grains) than in the main shoot (from 3.0 to 4.0 grains). It is primarily stipulated by reduction of lower and upper spikelets of the ear or flowers in them, underdevelopment of central grains of spikelet'.

According to the research, an average plant weight of soft spring wheat was from 1.8 to 2.7 g. The highest plant weight was observed in the variant with fertilizer dose of $N_{64}P_{64}K_{64}$ - 2.7 g. On average, during the years of research, maximum ear weight of soft spring wheat plants was in the variants with fertilizer doses of $N_{32}P_{32}K_{32}$ and $N_{64}P_{64}K_{64}$ - 1.7 g. Slightly less ear weight was determined in the control - 1.1 g, and in the variant with fertilizer dose of $N_{16}P_{16}K_{16}$ - 1.6 g.

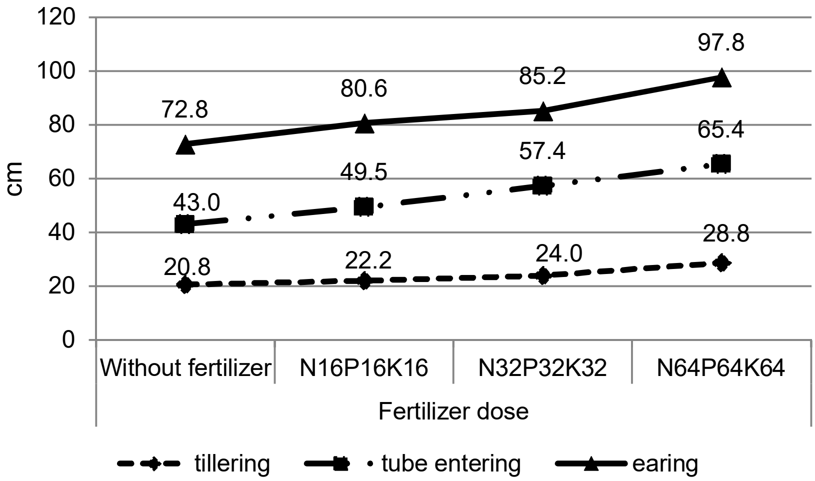

Figure 2. The height of soft spring wheat plants by phases of development depending on fertilizers (average for 2016–2020), cm.

Ear length of soft spring wheat also varied depending on the elements of cultivation technology. The largest index of ear length was obtained in the variant with fertilizer dose of $N_{64}P_{64}K_{64}$ - 9.8 cm and it decreased to 9.4 cm with fertilizer dose of $N_{32}P_{32}K_{32}$, to 9.0 cm with fertilizer dose of $N_{16}P_{16}K_{16}$ and to 8.1 cm in the control (Table 2).

Table 2. Weight of a plant and ear, ear length of soft spring wheat depending on fertilizers (average for 2016–2020)

Fertilizer	Plant weight, g				Ear weight, g				Ear length, cm			
dose	I	II	III	average	I	II	III	average	I	II	III	average
Control (without fertilier)	1.85	1.77	1.78	1.80	1.07	1.15	1.08	1.10	8.02	8.14	8.14	8.10
$N_{16}P_{16}K_{16}$	2.12	1.94	1.94	2.00	1.54	1.62	1.64	1.60	8.84	9.12	9.04	9.00
$N_{32}P_{32}K_{32}$	2.56	2.45	2.49	2.50	1.72	1.68	1.70	1.70	9.38	9.45	9.37	9.40
$N_{64}P_{64}K_{64}$	2.74	2.67	2.69	2.70	1.69	1.72	1.69	1.70	9.86	9.72	9.82	9.80
LSD_{05}	-	-	-	0.12	-	-	-	0.07	-	-	-	0.17

Determining factor for yield capacity is regulation of growth and development processes as important manifestations of organism vital activity. ‘According to the results of research conducted by Zhemela & Shakaliy (2012) it is established that the most rational norm of fertilizer application is - $N_{85}P_{96}K_{51}+N_{30}$. This norm contributes to effective increase of yield structure indices’.

As a result of the research it was found that the largest mass of grain in the ear of soft spring wheat plant was formed with fertilizer dose of $N_{64}P_{64}K_{64}$. The mass was 1.1 g. Slightly lower indices were obtained in the control - 0.7 g, in the variants with fertilizer dose of $N_{16}P_{16}K_{16}$ and $N_{32}P_{32}K_{32}$ - 0.9, 1.0 g, accordingly. The number of grains in the ear varied from 20.9 to 30.0 pieces. Sowing of soft spring wheat without fertilizers significantly reduced the number of grains per ear and amounted to 20.9 pcs. The highest number of grains per ear was obtained in the variant with fertilizer dose of $N_{64}P_{64}K_{64}$ - 30.0 pcs. This index is higher than in the variants with fertilizer doses of $N_{16}P_{16}K_{16}$ and $N_{32}P_{32}K_{32}$ by 3.9 and 2.3 pcs, accordingly (Table 3).

The weight of 1,000 pieces of grain plays a significant role in the formation of grain yield capacity, as this index correlates with the size of grain, which is a varietal trait, but depends on the influence of weather, soil and various technological factors. The plants have acquired optimal values when applying average fertilizer norms of $N_{45}P_{45}K_{45}$ - $N_{60}P_{90}K_{90}$ (Radchenko et al., 2018; Voloshchuk et al., 2019).

Depending on fertilizer dose, the weight of 1,000 grains differed significantly and varied from 34.48 to 36.67 g. The weight of 1,000 grains was trustworthy high depending on fertilization (LSD_{05} = 0.32). Thus, in the control, the mass of 1,000 grains was 34.48 g, in the variants with fertilizer dose of $N_{16}P_{16}K_{16}$ - 35.00 g, $N_{32}P_{32}K_{32}$ - 36.10 g, $N_{64}P_{64}K_{64}$ - 36.67 g. The largest mass of 1,000 grains was in the variant with fertilizer dose of $N_{64}P_{64}K_{64}$ - 36.67 g (Table 3).

Table 3. Structural indices of soft spring wheat plants depending on fertilization (average for 2016–2020)

Fertilizer dose	Mass of grains per ear, g				Number of grains per ear, pcs				Mass of 1,000 grains, g			
	I	II	III	average	I	II	III	average	I	II	III	average
Control (without fertilier)	0.68	0.75	0.67	0.70	21.6	20.2	20.9	20.9	34.75	34.28	34.41	34.48
$N_{16}P_{16}K_{16}$	0.92	0.87	0.91	0.90	26.7	25.9	25.7	26.1	34.92	35.15	34.93	35.00
$N_{32}P_{32}K_{32}$	1.02	0.99	0.99	1.00	27.8	28.0	27.3	27.7	36.21	36.02	36.07	36.10
$N_{64}P_{64}K_{64}$	1.13	1.07	1.10	1.10	29.6	30.2	30.0	30.0	36.78	36.52	36.71	36.67
LSD_{05}	-	-	-	0.08	-	-	-	1.05	-	-	-	0.32

Fertilization is one of the most effective and fast-acting factors of increasing wheat yield and improving its quality. Yield capacity increase occurs due to fertilizers by 50%, varietal properties by 25%, improvement of agrotechnology system by 20–25% (Zhemela, 1991).

Mineral fertilizers increase yield capacity of soft spring wheat under conditions of the Northern Forest-Steppe depending on the elements of cultivation technology on average from 0.1 to 1.0 t ha^{-1}. Application of $N_{30}P_{30}K_{30}$ in comparison with the variant without fertilizers promotes productivity increase by 15–16%, while increase of fertilizer dose to $N_{60}P_{60}K_{60}$ - promotes productivity increase by 4–15%, and increase of fertilizer dose to $N_{90}P_{90}K_{90}$ promotes productivity increase only by 2–7% (Antal et al., 2016).

According to the results of research, yield capacity of soft spring wheat ranged on average from 2.43 to 4.51 t ha^{-1} (LSD_{05} = 0.30). Analysis of yield data shows that the highest yield increase of soft spring wheat grain (2.08 t ha^{-1}) was obtained with application of fertilizer in a dose of $N_{64}P_{64}K_{64}$. Slightly lower yield increase was obtained with application of fertilizer in a dose of $N_{32}P_{32}K_{32}$ - 1.64 t ha^{-1}, the lowest yield increase was obtained in the variant with fertilizer dose of $N_{16}P_{16}K_{16}$ - 0.99 t ha^{-1} in comparison to the control (Fig. 3).

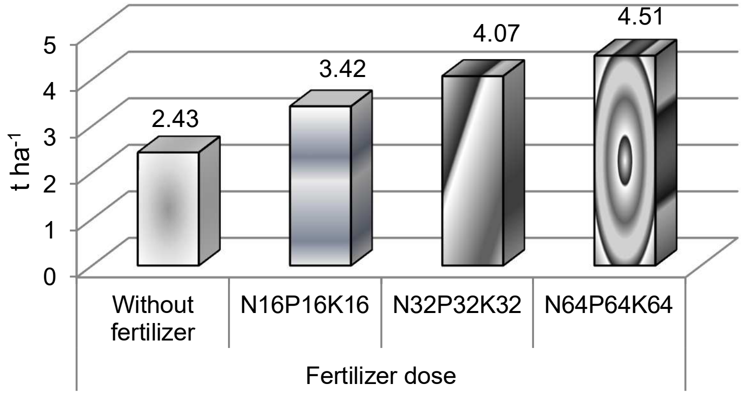

Figure 3. Yield capacity of soft spring wheat grain depending on fertilizer (average for 2016–2020), t ha^{-1}.

The quality of spring wheat grain is assessed by a number of features which together characterize its nutritional, physico-chemical and technological properties (Kolyuchiy et al., 2007; Kulyk et al., 2020). The greatest impact on the quality of wheat grain have mineral fertilizers, and especially nitrogen, its doses and timing of application, as well as ripening conditions and harvesting times. High-quality grain of strong wheat must be healthy and have high baking properties. It is established that the more gluten the grain contains the better baking properties the wheat has (Jula & Drozd, 2015).

Manifestation of quality characteristics is influenced not only by a variety but also by its ecological and geographical origin, in particular in Slovakia varieties from Austria and Hungary as well as varieties from Great Britain, Germany, Poland and Czech Republic dominated over the local ones (Mikulikova et al., 2009).

The content of gluten and protein in the grain of soft spring wheat was changed significantly from fertilization. With increasing doses of mineral fertilizers increases the content of gluten and protein in wheat grain.

The content of crude gluten in wheat grain during the years of research ranged from 18.13 to 28.19% (LSD_{05} = 0.42). In the variant without fertilizers gluten content was the lowest - 18.13%. The highest amount of gluten was obtained in the variant with fertilizer dose of $N_{64}P_{64}K_{64}$ - 28.19%, that is 6.11% more than in the variant with fertilizer dose of $N_{16}P_{16}K_{16}$ and 0.15% more than in the variant with fertilizer dose of $N_{32}P_{32}K_{32}$ (Table 4).

Table 4. Grain quality of soft spring wheat depending on mineral nutrition (average for 2016–2020)

Fertilizer dose	Gluten content, %				Protein content, %			
	I	II	III	average	I	II	III	average
Control (without fertilier)	18.52	18.00	17.87	18.13	10.83	10.08	10.65	10.52
$N_{16}P_{16}K_{16}$	22.15	21.88	22.21	22.08	11.92	11.48	11.61	11.67
$N_{32}P_{32}K_{32}$	27.95	28.09	28.08	28.04	14.38	14.10	14.12	14.20
$N_{64}P_{64}K_{64}$	28.22	28.13	28.22	28.19	14.25	14.12	14.26	14.21
LSD_{05}	-	-	-	0.42	-	-	-	0.56

The content of protein in wheat grain ranged from 10.52 to 14.21% (LSD_{05} = 0.56). The largest amount of protein in the grain of soft spring wheat was obtained in the variant with fertilizer dose of $N_{64}P_{64}K_{64}$ - 14.21%. Slightly lower protein content was obtained in the other variants. Thus, in the variant without fertilizers it was 10.52%, in the variant with fertilizer doses of $N_{16}P_{16}K_{16}$ - 11.67%, and in the variant with fertilizer doses of $N_{32}P_{32}K_{32}$ - 14.20% (Table 4).

CONCLUSIONS

According to the results of research it was found that the best conditions for growth, development and crop formation of soft spring wheat were formed in the variant with fertilizer dose of $N_{64}P_{64}K_{64}$. With this fertilizer dose, soft spring wheat provided maximum plant weight - 2.7 g, ear weight - 1.7 g, grain weight in the ear - 1.1 g and ear length - 9.8 cm. The number of grains in the ear was 30.0 pcs. with the weight of 1,000 grains - 36.67 g. The highest yield increase of soft spring wheat grain (2.08 t ha^{-1}) was obtained with application of $N_{64}P_{64}K_{64}$, slightly lower yield increase was obtained with application of $N_{32}P_{32}K_{32}$ - 1.64 t ha^{-1}, and with application of $N_{16}P_{16}K_{16}$ - 0.99 t ha^{-1} in comparison with the control.

The maximum amount of gluten - 28.19% with protein content of 14.21% in the grain of soft spring wheat was registered in the variant with fertilizer dose of $N_{64}P_{64}K_{64}$.

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Responses of onion growth and yield to different planting dates and land management practices

H. Salari[1,*], R.S. Antil[2] and Y.S. Saharawat[3]

[1]Amity University Uttar Pradesh, Amity Institute of Horticulture Studies and Research, Amity Road, Sector 125, Noida, IN 201301 Uttar Pradesh, India
[2]Amity University Uttar Pradesh, Amity Food and Agriculture Foundation, Amity Road, Sector 125, Noida, IN 201301 Uttar Pradesh, India
[3]ICAR - Indian Agriculture Research Institute, Department of Soil Science, Pusa road, New Delhi, IN 110012 Delhi, India
[*]Correspondence: h.salari@yahoo.com

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Abstract. Local varieties of *onion* (*Allium cepa* L.) are believed to be well adopted to agroecological conditions of their respective growing region but their lower productivity is a concern to be addressed. The variety '*Safid e Paisaye'* was selected for this investigation due to its long storability and higher market demand. The present study was carried out at Agriculture Research Farm of Kabul University to study the influence of land management practices and planting dates on growth and yield of onion. Different agronomic traits including number of leaves per plant, leaf length, leaf area per plant, leaf area index, normalized difference vegetation index (NDVI), maturity period, marketable yield and total yield were studied in these trials. The recorded data were statistically analysed with R software. The planting dates had significant influence on growth and yield of onions. At 90 days after sowing, the highest number of leaves per plant (7.18), leaf length (30.07 cm), leaf area per plant (277.43 cm^2), leaf area index (0.93) and NDVI (0.29) were recorded for the first planting date (10th May). Similarly, the longest maturity period (176.44 days) and highest marketable yield (37.01 t ha^{-1}) and total yield (40.08 t ha^{-1}) were also observed under the first planting date. Land management practices did not have significant effect on growth and maturity period of onions. However, the marketable yield was influenced by tillage depth and land preparation. The deep tillage and flatbed recorded highest marketable yield of 38.58 t ha^{-1} and 26.9 t ha^{-1}, respectively. The results of the study indicate that, early planting was more appropriate to get vigorous and high yielding onions. The deep ploughed flatbeds were appropriate to get higher marketable yield of onions.

Key words: *Allium cepa* L., growth response, Normalized Difference Vegetation Index (NDVI), planting date, tillage depth, yield response.

INTRODUCTION

Onion (*Allium cepa* L.) is a biennial herbaceous plant belonging to the *Alliaceae* family. Local varieties are believed to be well adopted to agroecological conditions of their respective growing region, but their lower productivity is a concern to be addressed.

Central Asia comprising Afghanistan is thought to be the origin of onion crop (Salari et al., 2020). Both improved and local varieties of onions are commercially grown in different parts of the country. *Safid e Paisaye* is a local onion variety grown in central region of Afghanistan especially in *Ghorband* valley. The bulbs of this variety are white-coloured and has flat round shape (Salari et al., 2020). This variety is famous for its long storability and high demand in the local market (Salari et al., 2020). The main problem of *Safid e Paisaye* onion is small size of bulb which causes lower productivity.

Land preparation and planting date determine the growth and yield of agricultural crops. Tillage depth alone do not have significant influence on growth and yield of onions (Jabro et al., 2010; Gronle et al., 2015) however, deep tillage in combination with other agricultural practices increases the yield (Gami et al. (2013). In several regions, commercial onions are produced on raised beds (Shanmugasundaram & Kalb, 2007). The raised beds are efficient in saving irrigation water but its influence on yield and quality of onions is not significant (Hatterman-Valenti & Hendrickson, 2006).

The planting date of onion vary widely among regions, this reflects the differential environmental conditions of the growing regions. It is important to identify the optimum planting dates for onion in order for it to express its full agronomic potentials (Kerpauskas et al., 2009; Deepak et al., 2014; Horváth et al., 2021). The onions grown in early planting dates produces vigorous plants and larger bulb with higher fresh weight and neck and bulb diameters (Bosekeng & Coetzer, 2013; Bosekeng & Coetzer, 2015; Ali et al., 2016; Aboukhadrah et al., 2017; Singh & Singh, 2000).

The previous studies showed that, land preparation and planting date influenced the growth and yield of onions. How these factors influence the growth and yield of *Safid e Paisaye* is yet to be determined. Consequently, the present study was conducted to find the optimum depth of tillage, land preparation method and date of planting for improved plant growth and yield of onion variety *Safid e Paisaye*.

MATERIALS AND METHODS

These trials were carried out at the Agriculture Research Farm of Agriculture Faculty of Kabul University during year 2018/19 and 2019/20 cropping seasons. The trial field falls under dry temperate climatic zone of Afghanistan (Salari et al., 2020). The common growing season in Kabul is from April to November. The average monthly weather data on temperature (°C), relative humidity (%), day length (hours) and rainfall (mm) during the experimental period is presented in Table 1.

The study was laid out in Split-Split Plot Randomized Complete Block Design (RCBD) with eighteen treatments each replicated three times. The depth of tillage at two level (25 cm and 10 cm) was allocated to main plots. The methods of land preparation at three level (flatbed, single row raised bed, double row raised bed) were allotted in sub plots. The dates of planting at three level (10^{th} May, 1^{st} June and 20^{th} June) were applied randomly in sub-sub plots.

Considering the details of experiment, the plots were ploughed to the depths of 10 cm and 25 cm. The beds of the plots were prepared in form of 1) flatbed, 2) raised beds with the height and width of 10 and 20 cm respectively - a single row of onion plants was cultivated on top of raised bed and 3) raised beds with the height and width of 10 and 40 cm respectively - two rows of onion plants were cultivated on top of it. The seeds were raised in nursery for 8 weeks prior to transplanting to the field. For the transplanting dates 10 May, 1 June, and 20 June, the seeds were sown in nursery on 10 March, 1 April, and 20 April, respectively.

Table 1. Average monthly weather data of Kabul, Afghanistan During 2018 and 2019 ('POWER Data Access Viewer' n.d.) and *('Sunrise and Sunset in Afghanistan' n.d.)

Month	Maximum Temperature (ºC)			Minimum Temperature (°C)			Relative Humidity (%)			Day length (hours)*			Rainfall (mm)		
	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean
January	6.9	2.2	4.5	-4.0	-8.7	-6.4	33.0	61.8	47.4	10.1	10.1	10.1	6.5	92.7	49.6
February	7.3	1.0	4.2	-3.7	-9.9	-6.8	50.3	72.8	61.5	11.0	11.0	11.0	27.7	69.7	48.7
March	14.9	7.4	11.2	1.4	-3.5	-1.0	45.6	66.4	56.0	11.6	11.6	11.6	17.4	51.2	34.3
April	19.7	17.3	18.5	4.4	4.1	4.3	38.1	60.8	49.4	13.1	13.1	13.1	13.8	40.5	27.2
May	21.8	21.4	21.6	6.4	5.5	6.0	37.0	42.8	39.9	14.0	14.0	14.0	22.3	29.1	25.7
June	28.2	25.2	26.7	10.9	8.4	9.6	23.7	32.6	28.2	14.3	14.3	14.3	3.6	4.2	3.9
July	30.8	31.1	31.0	13.1	13.4	13.2	20.9	25.5	23.2	14.2	14.2	14.2	4.0	8.4	6.2
August	28.7	29.1	28.9	10.8	11.8	11.3	23.1	24.7	23.9	13.3	13.3	13.3	7.8	12.7	10.2
September	25.0	27.2	26.1	7.7	9.3	8.5	24.2	21.7	23.0	12.3	12.3	12.3	18.3	0.9	9.6
October	17.4	18.2	17.8	2.7	4.3	3.5	34.1	38.6	36.3	11.2	11.2	11.2	12.1	16.7	14.4
November	11.7	9.6	10.7	-0.6	-1.7	-1.2	41.8	51.8	46.8	10.3	10.3	10.3	7.5	39.9	23.7
December	7.5	7.4	7.5	-4.5	-3.8	-4.2	38.5	43.2	40.9	9.5	9.5	9.5	33.8	7.1	20.5

The recommended dosage of inorganic fertilizer (nitrogen at 90 kg ha^{-1}, phosphorus at 60 kg ha^{-1} and potassium at 45 kg ha^{-1}) and farm yard manure at 15 t ha^{-1} were applied to all the plots. The plots were irrigated using common flood irrigation. Considering the climatic conditions, the frequency of irrigation was decided once in each 7–10 days. The plants were grown with rows spaced 0.2 m apart and an in-row plant distance of 0.12–0.15 m. The weeds were controlled manually by hand weeding. To control fungal diseases especially powdery mildew, the leaves were sprayed with 0.2% Mancozeb fungicide solution especially during the rainy season.

The data on growth parameters was recorded at 90 and 120 days after sowing. The numbers of leaves per plant in the selected three plants were counted in each treatment. The length of the leaf was measured in cm from the base to tip of the leaf. Leaf area was recorded from randomly labeled three plants in each treatment and was presented as square centimeter per plant. Leaf area index (LAI) was estimated by dividing the actual leaf area per plant by land area occupied by the same plant (spread of the plant) by using the formula (1) as given by Watson, (1952).

$$LAI = \frac{\text{Leaf area per plant (cm}^2\text{)}}{\text{Land area occupied by each plant (cm}^2\text{)}} \qquad (1)$$

The Normalized Difference Vegetation Index (NDVI) for each treatment was recorded using green seeker. For maturity period, the number of days from sowing to harvest for each treatment were counted and presented in days. Marketable yield and total yield were recorded in kg per plot and presented in metric tons per hectare.

At maturity, the bulbs were harvested and then cured for one month under ventilated conditions, which, were then used for recording the yield data.

The recorded data were statistically analyzed with R software. ANOVA was calculated according to split-split plot RCBD and main effects were separated using Least Significant Difference (*LSD*) at $P = 0.05$. The results are explained for significant interactions.

RESULTS AND DISCUSSION

Number of leaves per plant

Tillage depth, land preparation, date of planting and their interactions affected number of leaves per plant at 90 and 120 days after sowing (Table 2).

Table 2. Number of leaves per plant as effected by depth of tillage, land preparation and date of planting of Safid e Paisaye onion

Treatment	Days after sowing					
	90			120		
	2018	2019	Mean	2018	2019	Mean
Tillage Depth (A):						
Deep tillage (25 cm)	6.57 ± 0.20	5.78 ± 0.19	6.18 ± 0.19	10.48 ± 0.26	10.60 ± 0.21	10.54 ± 0.15
Shallow tillage (10 cm)	6.62 ± 0.17	5.69 ± 0.18	6.16 ± 0.17	10.97 ± 0.30	10.72 ± 0.2	10.85 ± 0.18
F-test	NS	NS	NS	NS	NS	NS
Land Preparation (B):						
Flatbed	6.61 ± 0.26	5.80 ± 0.25	6.21 ± 0.25	10.03 ± 0.29	10.85 ± 0.25	10.44 ± 0.17
Raised bed - Single row	6.72 ± 0.24	5.78 ± 0.24	6.25 ± 0.24	11.22 ± 0.39	10.70 ± 0.27	10.96 ± 0.23
Raised bed - Double row	6.45 ± 0.17	5.63 ± 0.18	6.04 ± 0.17	10.92 ± 0.32	10.43 ± 0.23	10.68 ± 0.19
F-test	NS	NS	NS	NS	NS	NS
Planting Date (C):						
10th May	7.59 ± 0.16 a	6.76 ± 0.16 a	7.18 ± 0.16 a	9.90 ± 0.26 b	11.24 ± 0.19 a	10.57 ± 0.18 b
1st June	6.06 ± 0.12 b	5.22 ± 0.12 b	5.64 ± 0.12 b	11.00 ± 0.26 a	9.50 ± 0.13 b	10.25 ± 0.15 b
20th June	6.13 ± 0.17 b	5.22 ± 0.15 b	5.68 ± 0.16 b	11.27 ± 0.42 a	11.24 ± 0.15 a	11.26 ± 0.21 a
F-test	**	**	**	**	**	**
LSD	0.41	0.39	0.38	0.67	0.54	0.41
CV (%)	9	9.9	9	9.1	7.3	5.5
Interaction: A×B	NS	NS	NS	NS	NS	NS
A×C	NS	NS	NS	*	NS	NS
B×C	NS	NS	NS	**	NS	**
A×B×C	NS	NS	NS	NS	NS	NS

**, * and NS indicate highly significant, significant and non-significant respectively. Each value is the mean ± *SE*. Values with the common letter in the same column for each factor do not differ significantly, as per *LSD* at 0.05 level.

However, the interaction between tillage depth and planting date was found to be significant at 120 days after sowing. The planting date had highly significant effect

($P < 0.01$) on number of leaves per plant. Based on mean values, the highest number of leaves per plant (7.18) at 90 days after sowing were recorded for first planting date (10th May) and the lowest (5.64) for second planting date (1st June). However, after 120 days of sowing the third planting date (20th June) recorded the highest number of leaves per plant (11.26) and the lowest (10.25) for second planting date (1st June). The combination of shallow tillage and third planting date recorded the highest number of leaves per plant (11.96) while the lowest (9.67) were recorded for the combination of deep tillage and first planting date (Fig. 1).

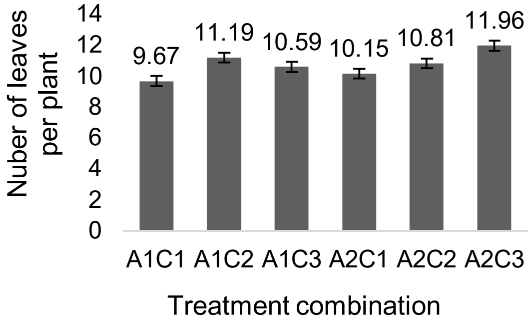

Figure 1. The number of leaves per plant of Safid e Paisaye onion as influenced by significant interaction between tillage depth (A) and planting date (C) at 120 days after sowing during season 2018.

The combination of single row raised bed and third planting date (20th June) recorded the highest number of leaves per plant and the combination of flatbed and second planting date (1st June) recorded the lowest number of leaves per plant (Fig. 2).

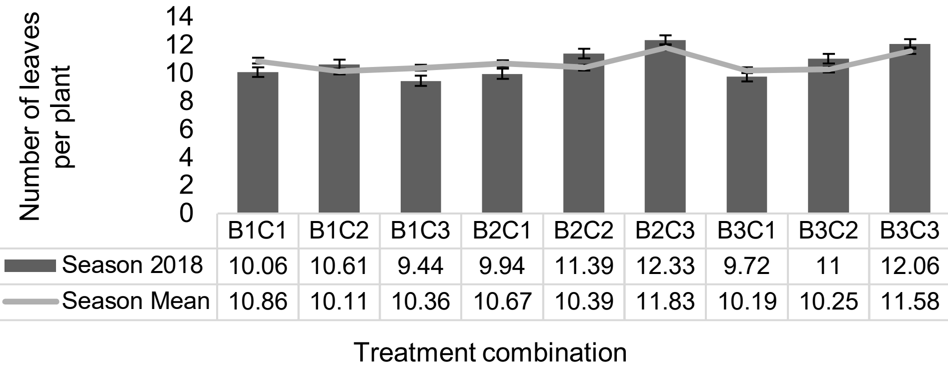

Figure 2. The number of leaves per plant of Safid e Paisaye onion as influenced by significant interaction between land preparation methods (B) and planting date (C) at 120 days after sowing.

Onion has shallow root system which might be the cause for non-significant effect of tillage depth and land preparation methods on number of leaves per plant. Jabro et al., 2010; Parvin et al., 2014 and Feitosa et al., 2020 also reported non-significant effect of tillage depth and land preparation on plant growth. Previous studies (Khokhar, 2017; Sekara et al., 2017) show that, onion growth is influence by weather conditions specially temperature and day length. The increase number of leavers per plant in first and third planting date might be associated with the ideal weather for plant growth. Because, during the month of March the seedling for first planting date were produced under plastic tunnel and hence the ideal weather was ensured. The transplants for second and third planting dates were produced in open field and they produced the lowest number of leaves. Authors (Ali et al., 2016 and Prasad et al., 2017) also reported that onion plants grown in early dates produced more leaves per plant.

Raised bed planting method reduces water losses and shallow tillage decreases the soil temperature which both leads to increased water use efficiency. The efficient use of irrigation water under the combinations of shallow tillage and third planting date and single row raised bed and third planting date might be the possible reason for increased number of leaves per plant. The authors (Aboukhadrah et al., 2017; Ewis et al., 2017 and Peter & Miglena, 2020) reported similar findings.

Leaf length

The effect of planting date on leaf length was highly significant ($P < 0.01$) at 90 days after sowing. The highest leaf length (30.07 cm) was recorded for first planting date (10th May) and lowest for third planting date (20th June) (Table 3).

Table 3. The leaf length (cm) of onion variety Safid e Paisaye as effected by depth of tillage, land preparation and date of planting

Treatment	Days after sowing					
	90			120		
	2018	2019	Mean	2018	2019	Mean
Tillage Depth (A):						
Deep tillage (25 cm)	26.76 ± 0.97	23.60 ± 0.92	25.18 ± 0.94	38.03 ± 0.77	32.96 ± 1.14	35.50 ± 0.66
Shallow tillage (10 cm)	25.48 ± 1.01	22.87 ± 0.89	24.18 ± 0.95	36.09 ± 0.90	33.84 ± 1.22	34.97 ± 0.71
F-test	NS	NS	NS	NS	NS	NS
Land Preparation (B):						
Flatbed	25.61 ± 1.03	23.00 ± 0.91	24.31 ± 0.97	37.8 ± 0.96	34.31 ± 1.64	36.06 ± 0.66
Raised bed - Single row	26.97 ± 1.31	23.98 ± 1.26	25.48 ± 1.28	35.7 ± 1.08	33.19 ± 1.50	34.44 ± 0.99
Raised bed - Double row	25.77 ± 1.33	22.73 ± 1.14	24.25 ± 1.22	37.7 ± 1.06	32.69 ± 1.20	35.20 ± 0.82
F-test	NS	NS	NS	NS	NS	NS
Planting Date (C):						
10th May	31.72 ± 0.94 a	28.41 ± 0.85 a	30.07 ± 0.89 a	38.81 ± 1.03	30.56 ± 0.99	34.69 ± 0.61
1st June	24.25 ± 0.81 b	21.51 ± 0.67 b	22.88 ± 0.73 b	36.03 ± 1.07	34.40 ± 1.60	35.22 ± 1.11
20th June	22.39 ± 0.44 b	19.80 ± 0.39 b	21.10 ± 0.39 b	36.35 ± 0.95	35.23 ± 1.48	35.79 ± 0.73
F-test	**	**	**	NS	NS	NS
LSD	2.37	2.12	2.21			
CV (%)	13.2	13.3	13.1			
Interaction: A×B	NS	NS	NS	NS	NS	NS
A×C	NS	NS	NS	NS	NS	NS
B×C	NS	NS	NS	NS	NS	NS
A×B×C	NS	NS	NS	NS	NS	NS

** and NS indicate highly significant and non-significant respectively. Each value is the mean ± *SE*. Values with the common letter in the same column for each factor do not differ significantly, as per *LSD* at 0.05 level.

The shallow root system of onion crops could be the possible reason for its neutral response to tillage depth and land preparation method. The authors (Khokhar, 2017; Sekara et al., 2017 and Ikeda et al., 2020) reported that short day length encourages vegetative growth of onion while longer day lengths encourage bulb formation in onion. The suitable weather especially shorter day lengths during initial three months of plant growth for the first plant date might be the cause for longer leaf length. The onion in third planting date is less exposed to short days and they tend to produce bulbs instead of completing vegetative growth. Similar results were reported by(Ali et al., 2016; Aboukhadrah et al., 2017 and Prasad et al., 2017).

Leaf area per plant

The effect of planting date on leaf area per plant was highly significant ($P < 0.01$) at 90 days after sowing (Table 4).

Table 4. The leaf area per plant (cm^2) of onion variety Safid e Paisaye as effected by depth of tillage, land preparation and date of planting

Treatment	Days After Sowing					
	90			120		
	2018	2019	Mean	2018	2019	Mean
Tillage Depth (A):						
Deep tillage (25 cm)	184.97 ± 18.30	151.51 ± 17.03	168.24 ± 17.60	862.85 ± 41.82	560.72 ± 25.41	711.79 ± 22.13
Shallow tillage (10 cm)	181.73 ± 17.54	150.56 ± 16.43	166.15 ± 16.90	826.28 ± 51.08	615.36 ± 28.76	720.82 ± 30.31
F-test	NS	NS	NS	NS	NS	NS
Land Preparation (B):						
Flatbed	177.77 ± 18.57	144.15 ± 16.32	160.96 ± 17.33	808.62 ± 47.05	601.82 ± 31.17	705.22 ± 27.10
Raised bed - Single row	196.15 ± 25.67	165.72 ± 25.05	180.94 ± 25.27	816.11 ± 62.6	608.73 ± 38.52	712.42 ± 32.76
Raised bed - Double row	176.12 ± 21.37	143.24 ± 19.28	159.68 ± 20.26	908.97 ± 59.65	553.57 ± 30.81	731.27 ± 37.5
F-test	NS	NS	NS	NS	NS	NS
Planting Date (C):						
10th May	297.43 ± 15.81 a	257.42 ± 14.92 a	277.43 ± 15.09 a	839.30 ± 66.00	577.52 ± 29.17	708.41 ± 33.37
1st June	134.11 ± 5.75 b	104.89 ± 5.28 b	119.50 ± 5.29 b	846.17 ± 47.24	543.37 ± 31.52	694.77 ± 30.89
20th June	118.50 ± 6.05 b	90.80 ± 5.43 b	104.65 ± 5.63 b	848.23 ± 58.89	643.22 ± 36.93	745.73 ± 32.95
F-test	**	**	**	NS	NS	NS
LSD	28.84	25.24	26.37			
CV (%)	22.9	24.3	22.9			
Interaction: A×B	NS	NS	NS	NS	NS	NS
A×C	NS	NS	NS	NS	NS	NS
B×C	NS	NS	NS	NS	NS	NS
A×B×C	*	NS	*	NS	NS	NS

**, * and NS indicate highly significant, significant and non-significant respectively. Each value is the mean ± *SE*. Values with the common letter in the same column for each factor do not differ significantly, as per *LSD* at 0.05 level.

The interaction between tillage depth, land preparation and date of planting was significant for leaf area per plant at 90 days after sowing. The highest leaf area per plant (277.43 cm^2) was recorded for first planting date (10th May) and the lowest (104.65 cm^2) for third planting date (20th June).

The combination of deep tillage, single row raised bed and first planting date (10th May) recorded the highest leaf area per plant. The lowest leaf area per plant was recorded for the combination of shallow tillage, flatbed and third planting date (Fig. 3).

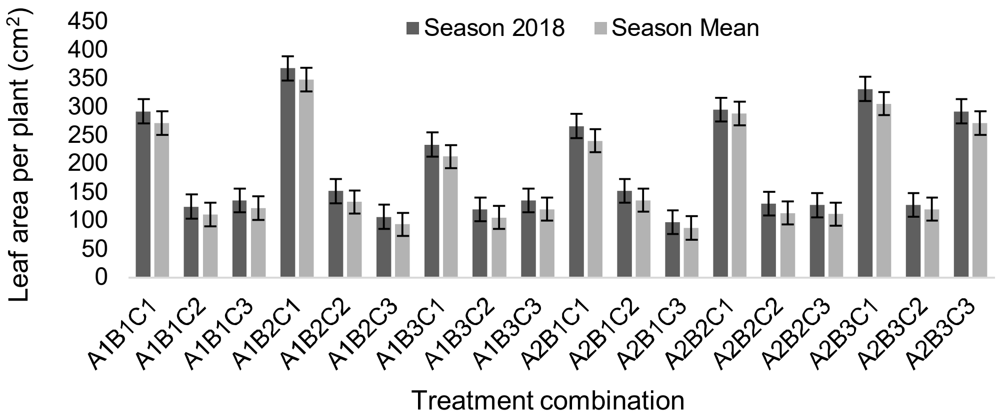

Figure 3. The leaf area per plant of Safid e Paisaye onion as influenced by significant interaction between tillage depth (A), land preparation (B) and planting date (C) at 90 days after sowing.

Leaf area per plant is dependent on number of leaves per plant, leaf length and leaf diameter. Since both leaf number and leaf length at 90 days after sowing were recorded higher for first planting date thereby it is obvious that, leaf area per plant would also be higher for the same planting date. The suitable climatic conditions in first planting date might be the possible cause for increased leaf area per plant. The improved water use efficiency under combination of deep tillage, single row raised bed and first planting date might be the reason for increase leaf area per plant. Similar results are recorded by (Hatterman-Valenti & Hendrickson, 2006; Aboukhadrah et al., 2017; Ahmed et al., 2017 and Ewis et al., 2017).

Leaf area index

The effect of planting date on leaf area index was highly significant ($P < 0.01$) at 90 days after sowing. The interaction between tillage depth, land preparation and date of planting was significant for leaf area index at 90 days after sowing. The highest leaf area index (0.93) at 90 days after sowing was recorded for first planting date (10th May) and the lowest (0.35) for third planting date (20th June) (Table 5).

The combination of deep tillage, single row raised bed and first planting date (10th May) recorded the highest leaf area index at 90 days after sowing. The lowest leaf area index after 90 days of sowing was recorded for the combination of shallow tillage, flatbed and third planting date (Fig. 4).

Table 5. Leaf area index of Safid e Paisaye onion as effected by depth of tillage, land preparation and date of planting

Treatment	Days after sowing					
	90			120		
	2018	2019	Mean	2018	2019	Mean
Tillage Depth (A):						
Deep tillage (25 cm)	0.62 ± 0.06	0.51 ± 0.06	0.57 ± 0.06	2.87 ± 0.14	1.87 ± 0.08	2.37 ± 0.07
Shallow tillage (10 cm)	0.61 ± 0.06	0.50 ± 0.05	0.56 ± 0.06	2.75 ± 0.17	2.05 ± 0.10	2.4 ± 0.10
F-test	NS	NS	NS	NS	NS	NS
Land Preparation (B):						
Flatbed	0.59 ± 0.06	0.48 ± 0.05	0.54 ± 0.06	2.69 ± 0.16	2.01 ± 0.10	2.35 ± 0.09
Raised bed – Single row	0.65 ± 0.08	0.55 ± 0.08	0.60 ± 0.08	2.72 ± 0.21	2.03 ± 0.13	2.38 ± 0.11
Raised bed – Double row	0.59 ± 0.07	0.48 ± 0.06	0.54 ± 0.07	3.02 ± 0.20	1.85 ± 0.10	2.44 ± 0.12
F-test	NS	NS	NS	NS	NS	NS
Planting Date (C):						
10th May	0.99 ± 0.05 a	0.86 ± 0.05 a	0.93 ± 0.05 a	2.79 ± 0.22	1.93 ± 0.10	2.36 ± 0.11
1st June	0.45 ± 0.02 b	0.35 ± 0.02 b	0.40 ± 0.02 b	2.82 ± 0.16	1.81 ± 0.11	2.32 ± 0.10
20th June	0.40 ± 0.02 b	0.30 ± 0.02 b	0.35 ± 0.02 b	2.82 ± 0.20	2.14 ± 0.12	2.48 ± 0.11
F-test	**	**	**	NS	NS	NS
LSD	0.1	0.08	0.09			
CV (%)	22.9	24.3	23.0			
Interaction: A×B	NS	NS	NS	NS	NS	NS
A×C	NS	NS	NS	NS	NS	NS
B×C	NS	NS	NS	NS	NS	NS
A×B×C	*	NS	*	NS	NS	NS

**, * and NS indicate highly significant, significant and non-significant respectively. Each value is the mean ± *SE*. Values with the common letter in the same column for each factor do not differ significantly, as per *LSD* at 0.05 level.

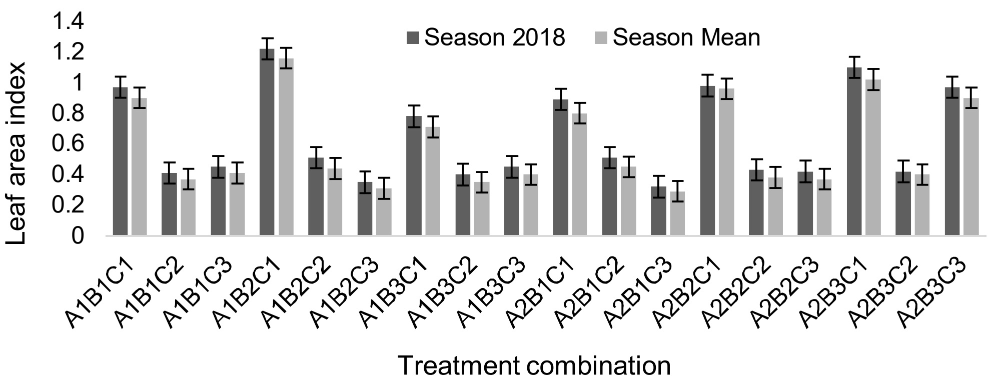

Figure 4. The leaf area index of Safid e Paisaye onion as influenced by significant interaction between tillage depth (A), land preparation (B) and planting date (C) at 90 days after sowing.

The leaf area index, shows that land is most efficiently used under first planting date as compare to later planting dates. Ali et al. (2016), Aboukhadrah et al. (2017) and Prasad et al. (2017) also reported higher plant growth for early planting of onion.

Normalized difference vegetation index (NDVI)

The effect of tillage depth on NDVI was significant at 120 days after sowing. The highest NDVI (0.493) was recorded for deep tillage and the lowest (0.474) for shallow tillage (Table 6). Planting date had highly significant ($P < 0.01$) effect on NDVI at both 90 and 120 days after sowing. The highest NDVI (0.29) at 90 days after sowing was recorded for first plating date and the lowest (0.13) for second planting date. At 120 days after sowing the highest NDVI (0.49) was recorded for second planting date which was on par with third planting date (0.47) (Table 6).

Table 6. Normalized Difference Vegetation Index (NDVI) as effected by depth of tillage, land preparation and date of planting of Safid e Paisaye onion

Treatment	Days after sowing					
	90			120		
	2018	2019	Mean	2018	2019	Mean
Tillage Depth (A):						
Deep tillage (25 cm)	0.24 ± 0.02	0.19 ± 0.02	0.22 ± 0.02	0.49 ± 0.01 a	0.44 ± 0.02	0.47 ± 0.01
Shallow tillage (10 cm)	0.22 ± 0.01	0.17 ± 0.01	0.20 ± 0.01	0.47 ± 0.01 b	0.45 ± 0.02	0.46 ± 0.01
F-test	NS	NS	NS	*	NS	NS
LSD				0.01		
CV (%)				2.3		
Land Preparation (B):						
Flatbed	0.24 ± 0.02	0.18 ± 0.02	0.21 ± 0.02	0.50 ± 0.01	0.44 ± 0.02	0.47 ± 0.01
Raised bed – Single row	0.23 ± 0.02	0.18 ± 0.02	0.21 ± 0.02	0.46 ± 0.01	0.46 ± 0.02	0.46 ± 0.01
Raised bed – Double row	0.23 ± 0.02	0.18 ± 0.02	0.21 ± 0.02	0.49 ± 0.01	0.43 ± 0.02	0.46 ± 0.01
F-test	NS	NS	NS	NS	NS	NS
Planting Date (C):						
10th May	0.31 ± 0.02 a	0.26 ± 0.01 a	0.29 ± 0.01 a	0.48 ± 0.01	0.37 ± 0.01 b	0.43 ± 0.01 b
1st June	0.16 ± 0.01 c	0.10 ± 0.00 c	0.13 ± 0.00 b	0.50 ± 0.01	0.49 ± 0.02 a	0.49 ± 0.01 a
20th June	0.23 ± 0.01 b	0.17 ± 0.01 b	0.20 ± 0.01 c	0.47 ± 0.01	0.47 ± 0.02 a	0.47 ± 0.01 a
F-test	**	**	**	NS	**	**
LSD	0.04	0.03	0.03		0.05	0.03
CV (%)	22.1	25.4	23.1		15.3	8.6
Interaction: A×B	NS	NS	NS	NS	NS	NS
A×C	NS	NS	NS	NS	NS	NS
B×C	NS	NS	NS	NS	NS	NS
A×B×C	NS	NS	NS	NS	NS	NS

**, * and NS indicate highly significant, significant and non-significant respectively. Each value is the mean ± *SE*. Values with the common letter in the same column for each factor do not differ significantly, as per *LSD* at 0.05 level.

The NDVI is dependent on vegetation in the field which increases with increase in plant growth. Feng et al. (2019) and Tan et al. (2020) also reported similar results.

Maturity period

Planting date had highly significant ($P < 0.01$) effect on maturity period. Onions produced in first planting date (10th May) took longer period (176.44 days) to reach maturity and those in third planting date took shortest period (153.83 days) (Table 7).

The hot and dry weather during later planting dates may accelerates the plant growth and development and decrease the maturity period. Potopová et al. (2015), Sekara et al. (2017), Yoon & Choi (2020) reported that early planting of crops in spring increases the length of growing season.

Table 7. Maturity period (days) of onion variety Safid e Paisaye as effected by depth of tillage, land preparation and date of planting

Treatment	Year		
	2018	2019	Mean
Tillage Depth (A):			
Deep tillage (25 cm)	154 ± 2.88	174.96 ± 1.87	164.65 ± 1.97
Shallow tillage (10 cm)	154 ± 3.03	172.74 ± 1.70	163.54 ± 2.01
F-test	NS	NS	NS
Land Preparation (B):			
Flatbed	154 ± 3.76	173.56 ± 2.38	163.81 ± 2.59
Raised bed – Single row	155 ± 3.66	173.67 ± 1.65	164.14 ± 2.18
Raised bed – Double row	154 ± 3.57	174.33 ± 2.54	164.33 ± 2.61
F-test	NS	NS	NS
Planting Date (C):			
10th May	173 ± 0.38 a	180.33 ± 1.30 a	176.44 ± 0.66 a
1st June	155 ± 0.28 b	169.28 ± 2.19 b	162.00 ± 1.12 b
20th June	136 ± 0.28 c	171.94 ± 2.10 b	153.83 ± 1.05 c
F-test	**	**	**
LSD	1.05	6.08	4.03
CV (%)	1	5.1	2.7
Interaction: A×B	NS	NS	NS
A×C	NS	NS	NS
B×C	NS	NS	NS
A×B×C	NS	NS	NS

** and NS indicate highly significant and non-significant respectively. Each value is the mean ± *SE*. Values with the common letter in the same column for each factor do not differ significantly, as per *LSD* at 0.05 level.

Yield

The depth of tillage had significant ($P < 0.05$) influence on marketable yield. The deep tillage recorded higher marketable yield (38.58 t ha^{-1}) as compare to shallow tillage (35.10 t ha^{-1}) (Table 8). The land preparation method significantly ($P < 0.05$) influenced the marketable yield. The highest (26.9 t ha^{-1}) and lowest (22.51 t ha^{-1}) marketable yield was recorded under flatbed and single row raised bed respectively (Table 8). The effect of planting date was highly significant ($P < 0.01$) on both marketable and total yield. The highest marketable yield (37.01 t ha^{-1}) and total yield (40.08 t ha^{-1}) were produced under first planting date (10 May) and the lowest marketable yield (23.21 t ha^{-1}) and total yield (24.22 t ha^{-1}) under third planting date (20 June) (Table 8).

Table 8. The marketable yield (t ha^{-1}) and total yield (t ha^{-1}) as effected by depth of tillage, land preparation and date of planting of Safid e Paisaye onion

Treatment	Marketable yield (t ha^{-1})			Total yield (t ha^{-1})		
	2018	2019	Mean	2018	2019	Mean
Tillage Depth (A):						
Deep tillage (25 cm)	25.50 ± 1.31	38.58 ± 2.28 a	32.04 ± 1.74	26.95 ± 1.34	40.98 ± 2.45	33.97 ± 1.81
Shallow tillage (10 cm)	23.43 ± 1.87	35.10 ± 2.36 b	29.26 ± 1.61	24.95 ± 1.89	38.56 ± 2.89	31.76 ± 1.87
F-test	NS	**	NS	NS	NS	NS
LSD		0.68				
CV (%)		1.6				
Land Preparation (B):						
Flatbed	26.90 ± 2.04 a	36.42 ± 2.08	31.66 ± 1.68	28.49 ± 2.09	38.62 ± 2.37	33.56 ± 1.77
Raised bed – Single row	22.51 ± 1.91b	34.06 ± 2.18	28.29 ± 1.86	23.97 ± 1.92	38.62 ± 3.19	31.30 ± 2.30
Raised bed – Double row	23.99 ± 1.95 ab	40.04 ± 3.89	32.02 ± 2.54	25.39 ± 1.96	42.07 ± 4.10	33.73 ± 2.67
F-test	*	NS	NS	NS	NS	NS
LSD	3.15					
CV (%)	16.7					
Planting Date (C):						
10th May	32.54 ± 1.55 a	41.48 ± 2.77 a	37.01 ± 1.7 a	34.25 ± 1.56 a	45.90 ± 3.30 a	40.08 ± 1.81 a
1st June	24.19 ± 1.43b	39.28 ± 2.68 a	31.74 ± 1.75b	25.61 ± 1.40 b	42.97 ± 2.68 a	34.29 ± 1.72 b
20th June	16.67 ± 0.58 c	29.75 ± 2.41b	23.21 ± 1.24 c	18.00 ± 0.58 c	30.44 ± 2.66 b	24.22 ± 1.38 c
F-test	**	**	**	**	**	**
LSD	3.93	6.23	3.84	3.7	6.93	4.04
CV (%)	23.4	24.6	18.2	20.7	25.3	17.9
Interaction: A×B	NS	NS	NS	NS	NS	NS
A×C	NS	NS	NS	NS	NS	NS
B×C	NS	NS	NS	NS	NS	NS
A×B×C	NS	NS	NS	NS	NS	*

**, * and NS indicate highly significant, significant and non-significant respectively. Each value is the mean ± *SE*. Values with the common letter in the same column for each factor do not differ significantly, as per *LSD* at 0.05 level.

The total yield was significantly influenced by combination of tillage depth, land preparation and planting date. The highest total yield (41.44 t ha^{-1}) was recorded under combination of deep tillage, single row raised bed and first planting date (10th May) and the lowest (20.87 t ha^{-1}) under the combination of shallow tillage, single row raised bed and third plating date (20th June) (Fig. 5).

Jabro et al. (2010) reported that deep tillage reduces soil bulk density, increases water intake, improves aeration, and improves response to nitrogen uptake which might be the possible reason for increased marketable yield under deep tillage conditions. The optimum soil type (silty loam) might be the reason for proper bulb enlargement on flatbeds. The genotype and onion bulb shape are also important factors which determines the response of onion variety to environment including soil management. The variety used in this research is flat and round in shape and its equatorial diameter is almost double of its polar diameter. The bulb shape of this variety might be the possible reason for its better response to flatbed, because small raised beds may not provide enough soil coverage to the bulb.

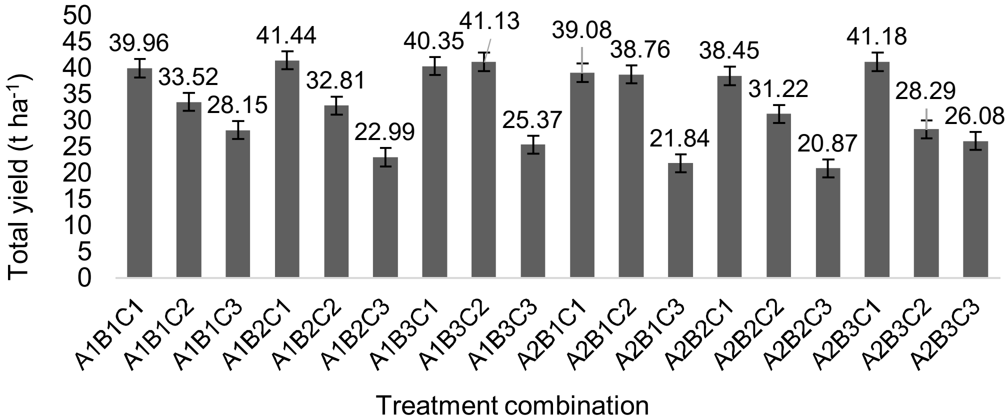

Figure 5. Total yield (t ha^{-1}) of Safid e Paisaye onion as influenced by significant interaction between tillage depth (A), land preparation (B) and planting date (C) during mean of season 2018 and 2019.

The ideal weather might be the possible reasons for higher yield of onion under early planting dates. The authors (Singh & Singh, 2000; Abdulsalam & Hamaiel, 2004; Bosekeng & Coetzer, 2013 and Caruso et al., 2014) reported that delay in planting date reduces yield of onion. Improved soil structure, better water use efficiency and ideal weather might be the possible reason for increased marketable yield under combination of deep tillage, single row raised bed and first planting date. Similar results were also reported by (Singh & Singh, 2000; Jabro et al., 2010; Caruso et al., 2014 and Ewis et al., 2017).

CONCLUSIONS

The tillage depth does not influence the total yield of onion however, deep tillage enhanced the marketable yield. Flatbed increased the marketable yield of *Safid e Paisaye* onions as compare to raised beds. Delay in planting date significantly reduced plant growth and yield and early planting date was more appropriate to get vigorous plants and higher yield of onion variety *Safid e Paisaye*.

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Digestate application with regard to greenhouse gases and physical soil properties

P. Šařec[1], J. Korba[1,*], V. Novák[1] and K. Křížová[1,2]

[1]Czech University of Life Sciences Prague, Faculty of Engineering, Department of Machinery Utilization, Kamýcká 129, CZ165 00 Prague 6, Czech Republic
[2]Crop Research Institute, Division of Crop Protection and Plant Health, Drnovská 507/73, CZ161 06 Prague, Czech Republic
[*]Correspondence: korba@tf.czu.cz

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Abstract. The article deals with the method of application of digestate with regard to the environment, soil properties and utilization of nutrients by plants. The aim is to monitor the dependence of the emission gas leakage and the dose of applied fertilizer. With the current expansion of biogas plants, a large amount of waste product, especially digestate, is being generated. This product is most often used as a liquid organic fertilizer because it contains substances important for plant growth. The disadvantage of this fertilizer is the release of greenhouse gases into the air. The digestate contains mainly ammonia, nitrogen in the residual organic matter and is a fertilizer with rapidly releasing nitrogen. The ammonium nitrogen contained in the digestate is easily subject to air losses. Therefore, a method of application for a certain crop is sought, where the smallest leaks of gases into the air occur. Different amounts of doses for the same route of administration are compared. To measure the amount of emission gases, a wind tunnel was placed on each variant of the application, taking air above the soil surface, which is discharged to the gas analyser. The monitored greenhouse gases are CH_4, NH_3 and CO_2. Furthermore, physical properties of soil were monitored in order to verify the conditions of the experiment. One of the parameters measured was the soil bulk density of the soil by taking intact soil samples. The penetration resistance of the soil was also determined, which indicates the degree of compaction. The use of nutrients was assessed through the condition of the stand on each variant by monitoring vegetation indices using remote sensing of the earth.

Key words: digestate, greenhouse gases, remote sensing, physical soil properties, ammonia.

INTRODUCTION

In today's age of intensive agriculture, we can grow enough food on a smaller area of agricultural land than before, but the soil must be supplied with fertilizers in order to provide enough nutrients. On the area remaining, we can grow plants for other uses. One of such uses relates to biogas plants, where some surplus of agricultural crops can be processed, alongside with the waste generated during livestock and food production. Previously, the input biomass originated from lower quality agricultural crops and plant wastes. Today, when a large amount of input biomass is needed, there is a targeted cultivation of agricultural crops most suitable for biogas production (Černá et al., 2016; Mazur et al., 2020).

Biogas is formed during anaerobic digestion of organic matter in a fermentation tank. The resulting gas contains methane, carbon dioxide and other gases. After the removal of unwanted gases from the biogas, the gas is burned in a cogeneration unit, which produces electricity using a generator (CZ Biom, 2010). The by-product is then a heat, which is partially used in the fermenter to keep it in ideal conditions. Methane is particularly suitable for energy use. This method of electricity generation is currently supported because biomass is a renewable resource (Bloch-Michalik & Gaworski, 2015). Biogas production produces a large amount of digestate, which is a waste product of anaerobic digestion. This product is usually used as a liquid organic fertilizer because it contains a certain amount of nutrients and organic substances (Massaccesi et al., 2013). Less often, the digestate separates into a liquid component and a dry matter (Černá et al., 2016). The digestate is produced in the biogas plant continuously throughout the year. The biogas plant will generate about two tons of digestate per megawatt hour of electricity produced. The exact amount depends on the composition of the input biomass and the process conditions in the fermenter (Ditl et al., 2017). Due to the agronomic deadlines for application, it must be stored for up to several months. The disadvantage of this fertilizer is the release of emissions into the air (Holm-Nielsen et al., 2009). The digestate contains mainly ammonia, nitrogen in the residual organic matter and is a fertilizer with rapidly releasing nitrogen. The ammonium nitrogen contained in the digestate is easily subject to air losses. Agriculture is a major polluter of the air with this gas. The monitored gases are among the greenhouse gases that currently negatively affect the global warming of the planet. Agriculture is one of the biggest air pollutants of this gas. Methods for measuring ammonia emissions in animal production are well developed, but methods for measuring this gas after application are not uniform (Šimek & Cooper, 2002; Dietrich et al., 2020). The concentration of ammonia, nitrous oxide, methane and carbon dioxide in the air above the soil surface is high after application but decreases over time (Češpiva & Zabloudilová, 2016; Dietrich et al., 2020), therefore the measurement should be performed as soon as possible after application.

The aim is to measure the amount of emissions produced after application and incorporation into the soil depending on the dose of digestate, because it is important to optimize the amount of organic fertilizer to minimize the amount of greenhouse gases emitted. When looking for the optimal dose, we must not forget to supply enough nutrients to the plants. Another goal is to compare the physical properties of the soil for different doses of application.

MATERIAL AND METHODS

On the experimental plot on 27th August 2020, digestate was applied by a tank with a disc application unit after the pre-crop had been harvested. Cattle slurry, corn silage and grass silage were used as raw input materials in the biogas plant. The experimental plot was located near the locality Čechtice in the Central Bohemian Region, Czech Republic (GPS 49° 37'07"N 15° 04'04" E). According to USDA, soil texture of the field was sandy loam. Each variant had a width of 24 m, and a length of 100 m. Samples of below mentioned variables were taken in a rectangular grid corresponding to the respective numbers of samples per variant. The digestate was applied by a disk application unit at different rates on four variants, with the fifth variant as control without digestate application, but with the same soil tillage treatment. The depth of digestate incorporation into soil reached approximately 10 cm. The respective digestate rates were 10, 20, 30, 40 t ha^{-1}. The maximum dose of 40 t ha^{-1} was chosen because this dose contains approximately the maximum recommended amount of nitrogen per a single dose. The measurement of emissions and physical properties of soil followed immediately after the digestate application. The monitored emission gasses were CO_2, CH_4 and NH_3.

An INOVA 1412 gas analyser was used to measure the emissions of the gases. Due to the dimensions of the instrument and the battery, the device was located in a transport vehicle. From the wind tunnels (Fig. 1) located at the monitored places, special Teflon hoses lead, through which the analysed air was supplied. In the analyser, a sample of air in a chamber is exposed to UV rays of a given frequency. There is a resonance of the molecules of the monitored gas and subsequent transformation into an oscillating motion of the molecules. No resonance occurs with the other components of the sample. The oscillating motion of the particles is detected by sensitive sensors. Wind tunnels consist of a plastic block that does not have a 35 × 50 cm wall on the underside (Loubet et al. 1999, Yang et al., 2018). The wind tunnel has two ventilation openings on the opposite sides. One opening is equipped with a fan with the possibility of speed control, which ensures the exact speed of air flow out of the wind tunnel, see Fig. 1. The other opening acts as air intake, where the air flow rate is measured and recorded by an anemometer. Inside, there is a thermometer that continuously monitors and records the temperature at which the measurement takes place. When using the INNOVA 1309 option, gas measurements can be performed simultaneously on up to five variants. After an hour, the wind tunnels were moved to another location within the variant, monitoring took place for over approximately three hours. All measured data were recorded and then transferred to a PC.

Figure 1. Emissions measuring wind tunnel.

Simultaneously with the above mentioned measurement, undisturbed soil samples were taken in order to determine soil bulk density by means of Kopecky cylinders (V = 100 cm^3). Soil samples were taken at a depth of 5 to 10 cm. Five samples were taken for each variant. The measurement served as informative on the homogeneity of the field.

Utilizing a penetrometer, another parameter concurrently monitored was the penetration resistance. This method is indirect, because the soil resistance depends not only on the porosity and bulk density but also on soil texture and moisture. It is advisable to compare the measured results only within one plot or with plots of similar properties. This measurement can detect excessive soil compaction. Penetration resistance was evaluated at the depths of 4, 8, 12, 16 and 20 cm in ten samples per variant.

Satellite data of Sentinel-2 (European Space Agency; ESA) was used for crop status evaluation. Five cloud-less images were selected and processed to gather 20–24 pixels (resolution 10 m/px) for each variant. Besides the commonly used Normalized Difference Vegetation Index (NDVI), Green Normalized Difference Vegetation Index (GNDVI) was derived. GNDVI is trusted to be suitable to evaluate plant nitrogen content and is also more sensitive to chlorophyll concentration than NDVI. With regard to the sparse vegetation cover, Soil Adjusted Vegetation Index (SAVI) was also calculated. This spectral index was designed to suppress the influence of pixels representing soil. The correction factor for SAVI equation was set to 0.5. Equations for all three used indices are provided in Table 1. The data was processed using open-source software ESA SNAP, QGIS and R. Yields can be predicted by long-term monitoring of crop stands using remote sensing (Tunca et al., 2018).

Table 1. Vegetation Indices emloyed

Index	Abbreviation	Formula	Authors
Green Normalized Difference Index	GNDVI	$\frac{NIR - GREEN}{NIR + GREEN}$	(Gitelson et al., 1996)
Normalized Difference Vegetation Index	NDVI	$\frac{NIR - RED}{NIR + RED}$	(Rouse et al., 1974)
Soil Adjusted Vegetation Index	SAVI	$\frac{(1 + L) \cdot (NIR - RED)}{(NIR + RED + L)}$	(Huete, 1988)

Statistical analysis of data was performed using the Statistica 12 software. The ANOVA test was employed to evaluate gas emissions and remote sensing. The paired Wilcoxon test was used to evaluate penetration resistance.

RESULTS AND DISCUSSION

In Table 2, average temperature and humidity values within wind tunnels are presented for the individual plots where the measurement took place. Under similar conditions, the physical properties of the soil were measured. The weather was sunny with with the breeze. The average speed of air flow through the opening in the wind tunnels was set to 0.9 m s^{-1}.

Table 2. Average air temperature (°C) and relative humidity for the field near Čechtice during the measurement for individual trial plots

Dose variant (t ha^{-1})	40	30	20	10	0
Average temperature (°C)	24.29	24.92	26.41	27.55	27.75
Relative humidity (%)	58.74	60.52	45.14	40.82	40.01

Fig. 2 shows the ammonia emission balanced values of the individual variants. At the same time, these values are small up to the edge of measurability. Negatively displayed values are the consequence of the measurability limit of the measuring instrument. These small emission concentrations can be attributed to the method of application. The disc applicator mixes the digestate into the entire treated soil layer, leaving a minimal amount on the soil surface, which is subject to a rapid release into the air. Therefore, the measured NH3 concentration is significantly lower than (Wolf et al., 2014), where the digestate was incorporated in a slightly different way. The measured values show approximately slightly decreasing emissions during the measurement period, which is confirmed by other works (Dietrich et al., 2020; Rosace et al., 2020).

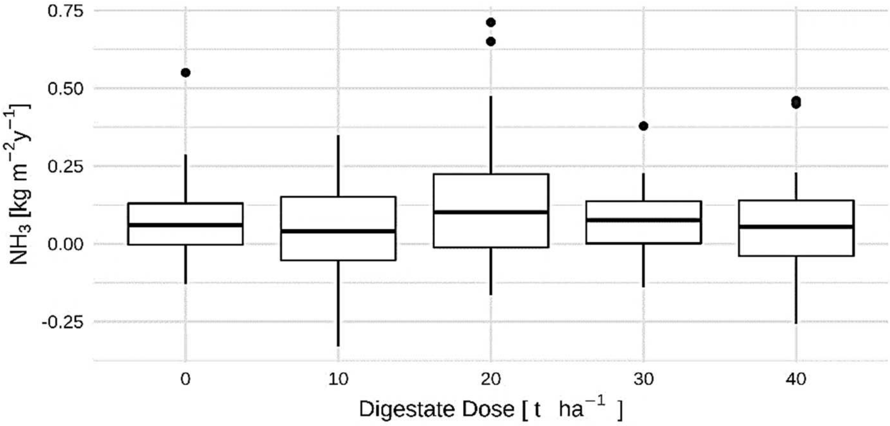

Figure 2. Ammonia emission (kg m^{-2} y^{-1}) for individual digestate dose (t ha^{-1}).

The measured values for methane (Fig. 3) are similarly balanced as for ammonia emissions without statistically significant differences. The same reasons for balanced results apply for methane as for ammonia. When comparing the same time from application, the measured emissions of all application variants are higher than those found by Czubaszek & Wysocka-Czubaszek (2018).

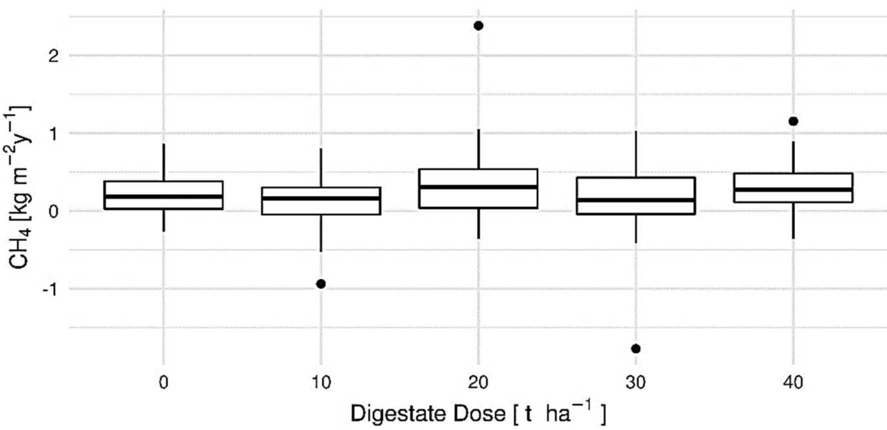

Figure 3. Methane emission (kg m^{-2} y^{-1}) for individual digestate dose (t ha^{-1}).

Differences can be seen in the measurement of carbon dioxide emissions (see Fig. 4), although the measured values have a higher variance. Statistically significant differences were discovered between the 10 t ha^{-1} dose and the control variant without application, then between doses of 10 t ha^{-1} and 30 t ha^{-1}, and finally between doses of 40 t ha^{-1} and 30 t ha^{-1}. The differences were not statistically significant among the other variants. The measured values in all variants were higher than in Rosace et al. (2020), this difference may be due to a different measurement method, although the course of emission leakage over time is similar. When comparing the results of Czubaszek &

Wysocka-Czubaszek (2018) immediately after application, our measured results were smaller, but when compared with their measured results the day after application, our emissions were greater.

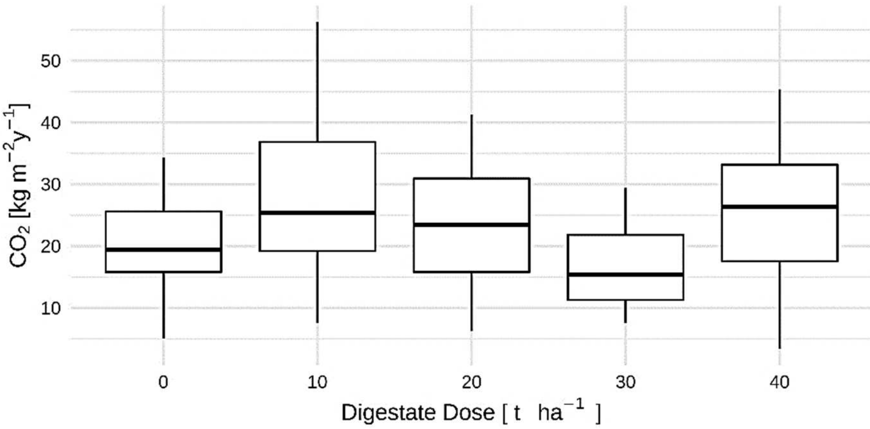

Figure 4. Carbon dioxin emission (kg m^{-2} y^{-1}) for individual digestate dose (t ha^{-1}).

The measurements of the penetration resistance (Fig. 5) in the upper part of the soil, in particular at 4 and 8 cm, can be influenced by the tillage after application of the digestate. At a depth of 12 cm, there are obvious differences, but even here it could be affected by soil tillage. The differences in penetration resistance were then evident in the depth of 16 cm, where the penetration resistances within the variants with a dose of 10 and 20 t ha^{-1} were lower than in the other variants. With smaller differences, a situation like the previous measurement depth can be seen at a depth of 20 cm. Similar resistance values at depths of 16 and 20 cm, which were comparable, were achieved by Beni et al. (2012). Depths of 4–12 cm cannot be exactly compared, because this layer of soil was tilled when applying the fertilizer by the disk application unit.

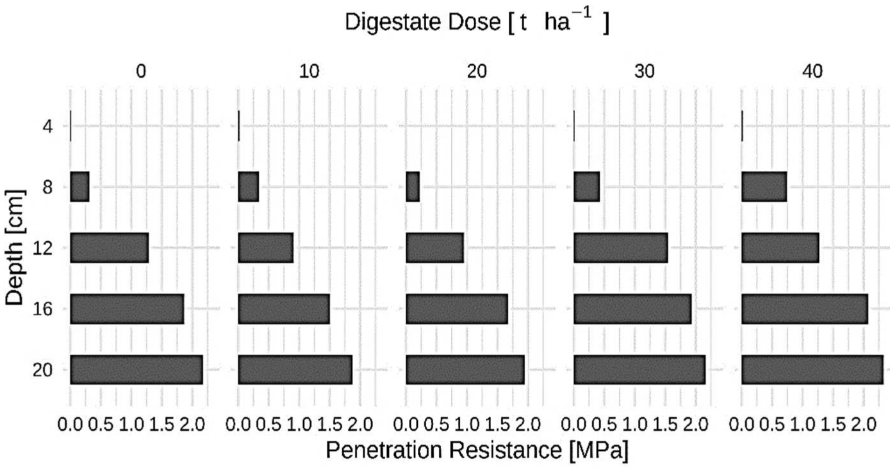

Figure 5. Penetration resistance (MPa) at selected depths.

Fig. 6 shows the highest measured average bulk density value for the variant with a dose of 40 t ha^{-1}. On the contrary, in the variant with a dose of 30 t ha^{-1}, the soil bulk density was the smallest. According to Pokorný et al. (2007), the measured values were below the limit of excessive compaction for a given type of soil. When compared with their first year of measurement, similar values of soil bulk density were reached after application of similar doses of digestate Jaša et al. (2019).

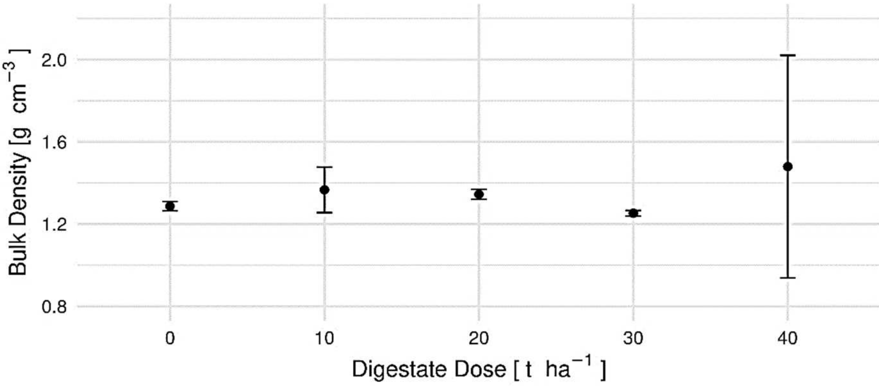

Figure 6. Soil bulk density (g cm^{-3}) for individual digestate doses (t ha^{-1}).

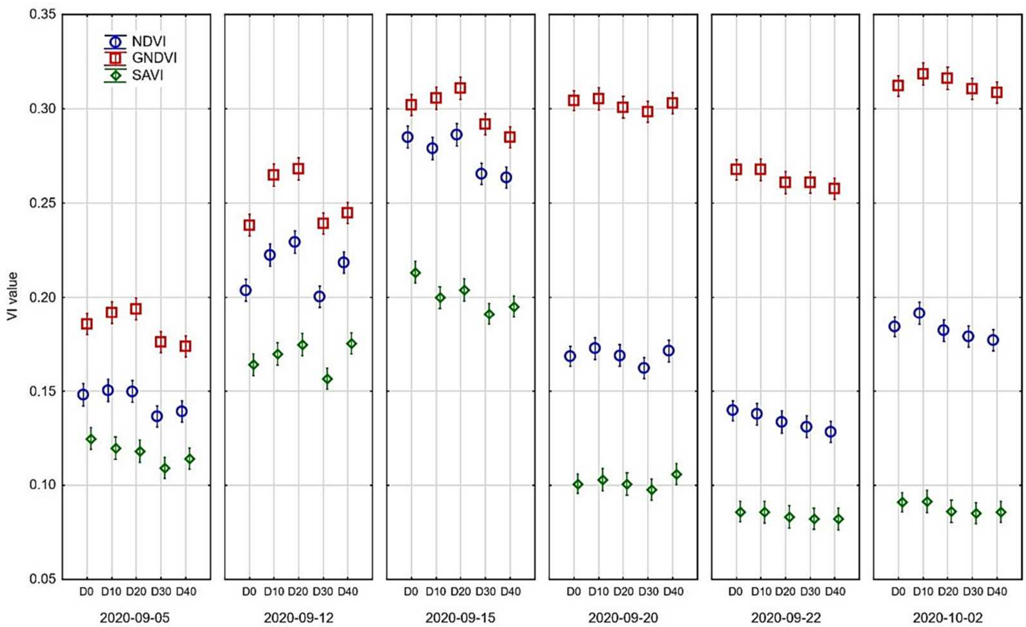

Figure 7. Vegetation indices of individual variants (digestate doses) in the experimental field near Čechtice.

Fig. 7 shows the growth of the intermediate crop after the application of digestate, thus increasing the values of vegetation indices. The differences between the variants were already apparent during the first shooting. Apart from the SAVI index, which decreases approximately with dose, the highest values were reached at a dose of 20 t ha^{-1} but did not differ statistically from the control variant and the dose of 10 t ha^{-1}. The results can only be affected by growing vegetation. A similar result can be seen in the next evaluated date, where the highest vegetation indices were attained by the dose of 20 t ha^{-1}, but statistically the result did not differ from dose 10 and 40 t ha^{-1}. On the third monitored date, the SAVI index reached the highest value in the control variant but did not differ statistically with the dose variants of 10 and 20 t ha^{-1}. For the other two indices, except for the dose of 40 t ha^{-1}, the statistical analysis did not find any differences. On the fourth control date, the indices were balanced. The GNDVI index reached its maximum for the variant of 10 t ha^{-1}, but without significant differences compared to the others. The NDVI index reached the highest value at 10 t ha^{-1}, but except for the variant 30 t ha^{-1} without any statistical difference. The SAVI index reached the highest value at 40 t ha^{-1} but except for the variant 30 t ha^{-1} without any significant difference. On the fifth date of observation, in addition to the SAVI index, there were differences among the groups of variants. The control variant worked best. The last evaluated date presented the best variant with dose as of 10 t ha^{-1}, but except for the variant with dose 40 t ha^{-1} without any significant difference. In this case, the growth was not yet sufficient for the growth phase to make an accurate estimate.

CONCLUSION

Measurements of emissions after digestate incorporation showed differences among variants only in CO_2 concentrations. The concentrations of other monitored gases were discovered without any significant differences. After application, lower values of penetration resistance at depths of 16 and 20 cm were measured for variants with a dose of 10 and 20 t ha^{-1} than for the other doses. Concerning soil bulk density, no significant differences were found. Remote sensing of the stand showed only small differences among vegetation indices of individual variants. Generally low measured levels of emissions from the soil surface were probably caused by the digestate having been incorporated into the soil. Therefore, this method of application can be recommended.

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Micromorphological features of the leaf epidermis of the evening primrose cultivars of the VILAR biocollection

O.M. Savchenko*, S.A. Totskaya and M.Yu. Gryaznov

All-Russian scientific research institute of medicinal and aromatic plants, agrobiology laboratory, 7 Grina Str., RU 117216 Moscow, Russia
*Correspondence: nordfenugreek@gmail.com

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Abstract. Novelty of the work. For the first time, a comparative study of the epidermis of the leaf plates of four cultivars of *Oenothera biennis* L. was carried out. **The aim of the work**. Study and comparison of morpho-anatomical signs of the epidermis of the leaf plate of the cultivars of evening primrose to reveal their potential ecological plasticity. **Materials and methods**. There were used leaves of plants of the second year of life in the phase of mass flowering. Micromorphological study of the epidermis of leaf plates included: determination of the shape of the main cells of the epidermis, the size and number of stomata per 1 mm^2, the type of stomatal apparatus, the presence and parameters of trichomes. The number of trichomes was calculated per 1 mm^2. **Results.** In the *Genoteros* cultivar, the stomata were smaller; their number on the lower epidermis was the lowest. Two types of trichomes were classified. At cultivar *Genoteros* unicellular covering trichomes were mainly located in the upper epidermis of the leaf. No covering trichomes were found in the *Svetlyachok* cultivar. The *Tverskoj* cultivar differs in the length of the hairs: from 774 to 790 microns. Papillary single-celled trichomes on the leaves of the studied cultivar of evening primrose are concentrated on both sides of the epidermis, mostly in the middle part of the leaf, less often - in the mesophyll space. **Conclusion.** Based on the obtained data, the authors established the potential ecological plasticity of the studied varieties of evening primrose for the subsequent study of the influence of stress factors on the described cultivars. The results obtained may be important taxonomic for the correct identification of problematic *Onagraceae* taxa and in further breeding work.

Key words: *Oenothera biennis* L., leaf plate, cultivars, epidermis, stomata, trichomes.

INTRODUCTION

The evening primrose (*Oenothera biennis* L.) is a biennial herb of the evening-primrose family (*Onagraceae* L.). It grows in the central part of the European part of the Russian Federation, in the Crimea, in the Caucasus, in the Ussuriysk Territory, in the Middle Urals, and the Far East (Brem, 2007; Totskaya & Gryaznov, 2019).

The evening primrose has been cultivated in VILAR since 1989. It is well adapted to the conditions of the Nonblack Earth region of the Russian Federation. The evening primrose fruit is a promising source of medicinal raw materials (Vandyshev et al., 2002; Totskaya & Gryaznov, 2019). Seed cultivation technology has been developed for the

Oenothera biennis L. in the Nonblack Earth region of the Russian Federation, along with investigation of the methods of fatty oil separation (Klimakhin et al., 2006; Zalepugin et al., 2006).

Based on the multi-year research results, it is possible to cultivate evening primrose as an oilseed crop in the Nonblack Earth region of the Russian Federation. There are about 50 cultivars of (*Oenothera biennis* L., *Oenothera fruticosa* L., *Oenothera macrocarpa* Nutt.) in the world for various uses (Totskaya & Gryaznov, 2019; UPOV).

The evening primrose is considered as infrequently distributed crop in Russian Federation. Nowadays, there exist four cultivars of the evening primrose (*Oenothera biennis* L.) of all-Russia scientific research institute of medicinal and aromatic plants (FGBNU VILAR, Russia, Moscow) selection (Klimakhin et al., 2014; Gryaznov et al., 2015; Gryaznov et al., 2016; Gryaznov et al., 2021).

Previous studies were conducted to analyze micromorphological peculiar properties of the aboveground and underground organs of *Oenothera biennis* L. in which have been determined the type of leaf space position, the shape of the cells, and the type of trichomes and stomata (Popov et al., 2009; Cheryatova, 2014). Previous researchers have not conducted a comparative study of samples of *Oenothera biennis* L. of different geographical origin. The existing data on the micromorphological features of the epidermis of the leaves of *Oenothera biennis* L. do not reflect the completeness of intraspecific differences. However, the metrological characteristic of the studied epidermal structures of *Oenothera biennis* L. cultivars has not been carried out earlier.

Foliar epidermal features were based on the micromorphology of trichomes types, epidermal cells and stomatal complex. Even though each feature has its own limited taxonomic value but collectively these characteristics may be systematically important especially for the discrimination and identification of complex and problematic taxa (Saba Gul et al., 2019).

Abiotic stress is one of the main limiting factors in crop cultivation worldwide (Asma Ayaz et al., 2021). In the course of breeding work, which consists in creating highly productive and resistant varieties, knowledge about the mechanisms of plant resistance to stress factors is important (Sunera Amna et al., 2020; Fiza Liaquat et al., 2021). The use of morphological features associated with the manifestation of resistance to adverse environmental factors will be important in the selection process. These morphological features include stomata (size, number); leaves (area, shape, growth, orientation, aging, pubescence, wax content in the cuticle) and many other aspects (Lonbani & Arzani, 2011).

It was also noted that among the vegetative organs of plants, the leaf is the most sensitive to changes in abiotic environmental factors. The characteristics of the anatomical structure of the leaf plate may indicate the potential adaptability of the plant to adverse environmental factors (Kuznetsova, 2015).

Therefore, the leaf blades were chosen as the object of study.

This work aims to investigate the leaf plate epidermis anatomical features of the evening primrose cultivars selected by the FGBNU VILAR.

Research objectives:

1. Perform an anatomical analysis of the leaf plate's epidermis of the cultivars;
2. To determine the micromorphological differences of the leaf plates epidermis of the cultivars;
3. To determine the potential ecological plasticity of the studied varieties

MATERIALS AND METHODS

The object of the study was the two-year-old cultivars of the evening primrose plants of the VILAR FGBNU: *Tverskoj*, *Genoteros*, *Svetlyachok*, *Fonarik*.

Seeds of the original population of the cultivar *Genoteros* were obtained from the USA, *Tverskoj* - from the Tver region of Russia, *Fonarik* - from the UK, *Svetlyachok* - from the Czech Republic.

As a result of long-term observations, it was found that even with a successful introduction of the plant, the *Oenothera biennis* L. continue to retain some morphological features inherent in their natural habitats (Mishurov et al., 2005; Totskaya & Gryaznov, 2019; Totskaya & Gryaznov, 2020).

The *Tverskoj* cultivar is based on the native population. The all cultivars of the evening primrose were obtained by individual selection (Fig. 1, a–d). *Svetlyachok* cultivar is approved for decorative use in the State Register of Breeding Achievements. *Fonarik* cultivar of the evening primrose was intended for decorative use (Klimakhin et al., 2014; Gryaznov et al., 2016; Totskaya & Gryaznov, 2019; Gryaznov et al., 2021).

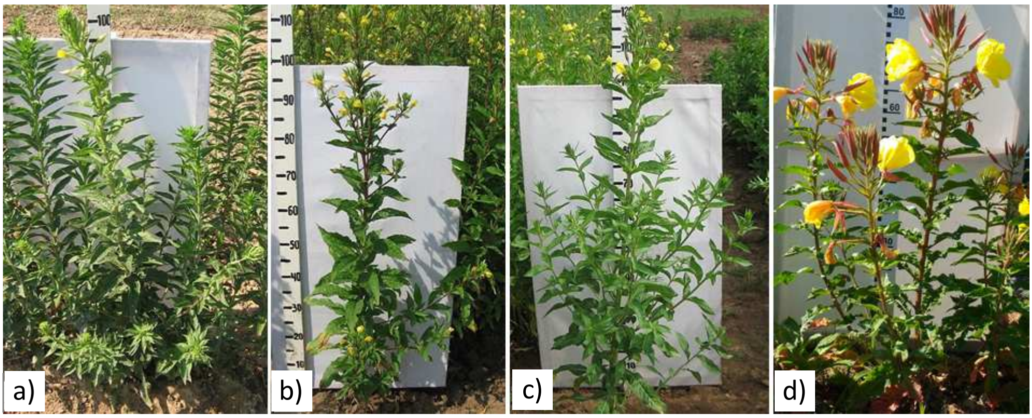

Figure 1. Cultivars of evening primrose. The flowering phase: a) – *Genoteros*; b) – *Tverskoj*; c) – *Svetlyachok*; d) – *Fonarik* [Compiled by the authors].

The research material was collected in the phase of mass flowering, on plants grown from seeds at the collection site of the FGBNU VILAR. The research was carried out on leaf plates of plants of the second year of life. The leaves of the middle part of the shoots were taken from plants in the generative state. Areas of the lower and upper epidermis of the middle part of leaves were studied using temporary specimens (Vorob'yova & Basargin, 2013). The cultivars were examined under light microscope LOMO MIKMED-1. Qualitative and quantitative foliar epidermal anatomical features were examined for both adaxial and abaxial surfaces. Qualitative characters like epidermal cell shape, trichomes type, stomata type and stomata position were examined. Quantitative characters like the length and width of leaf epidermis, stomata, stomatal pore, subsidiary cell and trichomes for both adaxial and abaxial surfaces were studied and measured (Fazal Ullah et al., 2021). The number of cells, stomata, and trichomes in the field of view (0.785 mm^2) was recalculated per 1 mm^2 (Tamahina. & Ahkubekova, 2018). The quantitative parameters and length of trichomes were determined using a

9x Ernst Zeits Wetzlar eyepiece micrometer and an OM-P object micrometer with the main scale length of 1 mm. The samples were prepared according to the methods for light microscopy (Barykina et al., 2004). The description of the evening primrose varieties has been compiled according to the UPOV methodology.

Analysis of microscopic characters of raw materials, morphometric and histochemical studies were carried out according to the methods of the State Pharmacopoeia of the Russian Federation XIV edition and photographed with a 14.0 Mp USB 2.0 C-Mount camera. The sample consisted of 10 measurements; statistical processing was performed in Microsoft Excel. For each studied character, its mean value (M), the error of the mean (m), and the coefficient of variation (Cv) were determined. The anatomical parameters of the epidermis are considered low variable if the coefficient of variation Cv is less than 20%, moderately variable - with Cv > 20%, highly variable - with Cv > 40%. The types of stomatal apparatus were determined according to the special classification (Baranova, 1985), the description of trichomes was done according to the methodology (Aneli, 1975).

RESULTS AND DISCUSSION

It was previously established that the leaves of evening primrose are dorsoventral, amphistomatic. The upper epidermis of the leaf has polyhedral cells, the anticline walls are slightly branched, the trichomes are simple single-celled hairs with a rosette at the base of 7–8 elongated cells and smaller hairs in the shape of a head. Stomata are quite common; the type is anomocytic and anisocytic. The lower epidermis has strongly convoluted anticlinal walls, stomatal apparatus of the anomocytic and anisocytic types, and a large number of simple unicellular hairs were found (Cheryatova, 2014).

The walls of the abaxial epidermis cells of the leaf plates of the evening primrose studied varieties are of rounded-sinuous shape. The cells of the adaxial epidermis are rounded-elongated. The stomatal apparatus is of an anomocytic type. Leaf plates of *Genoteros*, *Tverskoj*, *Fonarik* cultivars are covered with agranular trichomes on the lower and upper epidermis. Trichomes are simple, unicellular, conical in shape, 80 to 190 microns long, located mainly along the veins. Epidermal cells forming a rosette are located around the attachments of cone-shaped pointed trichomes. In some areas of the interveinal space of the *Genoteros* and *Tverskoj* cultivars, a dense localization of trichomes was noted (12–15 pcs mm^{-2}). Papillary unicellular trichomes were also found in the interveinal space of the central part of the evening primrose leaves of *Genoteros, Svetlyachok*, and *Fonarik* cultivars.

Genoteros. The walls of the epidermal cells are sinuous. The size of the stomata on the upper epidermis of the leaf is 30.21 ± 2.76 µm long, 26.34 ± 2.15 µm wide. The number of stomata per 1 mm^2 is 458.67 ± 38.81 pcs. The size of the stomata on the lower epidermis of the leaf has a length of 31.72 ± 3.38 µm and width of 22.18 ± 2.45 µm. The number of stomata per 1 mm^2 comprises 560.57 ± 39.18 pcs.

On the abaxial side of the leaf, papillary unicellular trichomes (2–4 units mm^{-2}) and unicellular cone-shaped trichomes (6–8 units mm^{-2}) 300–500 µm long were found. On the adaxial side of the leaf, cone-shaped trichomes (10–12 pcs mm^{-2}) up to 600 µm in length were revealed. Papillary unicellular trichomes are detected on the lower epidermis (Fig. 2, Tables 1 and 2).

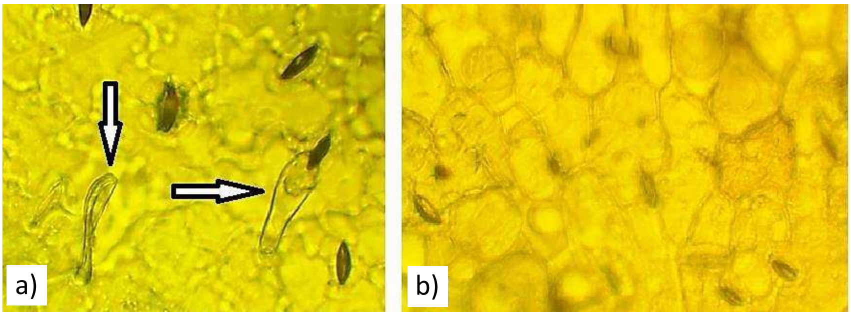

Figure 2. Lower (a) and upper (b) side of the epidermis of the evening primrose's leaf plate *Genoteros* Cultivar. The arrows show papillary unicellular trichomes. Magnification ×280 [Compiled by the authors].

The epidermis of the leaf plate in the *Tverskoj*, *Svetlyachok* and *Fonarik* cultivars are distinguished by large stomata. The stomata of the *Genoteros* cultivar are smaller, their number on the lower epidermis is the lowest: 560.57 ± 39.18 pcs mm^{-2}. The upper epidermis of *Tverskoj* and *Fonarik* cultivars have the lowest stomatal index: 20.45–20.47% (Table 1).

Table 1. Quantifiable and morphometric parameters of the epidermis of the cultivars evening primrose's leaf plate

Cultivars	Side of leaves	Parameters				
		Number of cells, pcs mm^{-2}	Number of stomata, pcs mm^{-2}	S_I, %*	Stomata l ength, microns	Stomata breadth, microns
Tverskoj	upper	1,630.57 ± 144.18	419.88 ± 41.05	20.47	40.22 ± 3.96	31.8 ± 2.54
	Cv%	10.84	19.22		15.09	18.24
	lower	1,704.31 ± 158.96	715.26 ± 63.55	29.56	42.75 ± 3.67	33.85 ± 2.17
	Cv%	10.2	25.94		17.7	11.46
Genoteros	upper	1,579.61 ± 107.22	458.67 ± 38.81	22.5	30.21 ± 2.76	26.34 ± 2.15
	Cv%	21.43	8.56		9.82	10.93
	lower	1,732.48 ± 110.57	560.57 ± 39.18	24.46	31.72 ± 3.38	22.18 ± 2.45
	Cv%	23.22	8.69		6.49	10.12
Svetlyachok	upper	1,477.07 ± 13.86	456.81 ± 41.28	23.62	40.06 ± 4.11	31.79 ± 3.52
	Cv%	26.32	20.71		23.11	19.18
	lower	1,936.13 ± 19.05	613.29 ± 58.73	24	41.08 ± 3.96	34.75 ± 3.66
	Cv%	28.46	26.83		21.13	19.48
Fonarik	upper	1,783.44 ± 16.92	458.59 ± 40.19	20.45	38.16 ± 3.91	32.57 ± 3.08
	Cv%	10.68	19.82		22.24	23.01
	lower	1,885.42 ± 18.09	611.46 ± 54.13	24.48	43.81 ± 4.15	34.63 ± 3.31
	Cv%	13.27	22.07		22.3	23.28

Note: upper – leaf plate upper epidermis; lower – leaf plate lower epidermis; *Stomatal Index, % [Compiled by the authors].

Tverskoj. The walls of the epidermal cells are sinuous. The size of the stomata on the upper epidermis of the leaf is 40.22 ± 3.96 μm long, 31.8 ± 2.54 μm wide. The number of stomata per 1 mm² is 419.88 ± 41.05 pcs. The size of the stomata on the lower epidermis of the leaf has a length of 42.75 ± 3.67 µm and width of 33.85 ± 2.17 µm. The number of stomata per 1 mm² comprises 715.26 ± 63.55 pcs.

Table 2. Morphometric characteristics of simple unicellular hairs of the epidermis of the varieties evening primrose's leaf plate

Cultivars	Side of leaves	Parameters Number of cone-shaped trichomes, pcs mm^{-2}	Length of cone-shaped trichomes, microns	Number of papillary trichomes, pcs mm^{-2}	Length of papillary trichomes, microns
Tverskoj	upper	8.12 ± 0.77	780.23 ± 60.52	Not available	Not available
	Cv%	28.13	41.17		
	lower	9.25 ± 0.83	774.36 ± 47.61	5.87 ± 0.42	57.24 ± 5.82
	Cv%	23.57	38.83	5.88	4.23
Genoteros	upper	11.76 ± 1.71	600.83 ± 41.68	Not available	
	Cv%	44.02	36.21		
	lower	6.55 ± 1.03	398.67 ± 36.89	4.64 ± 0.45	60.27 ± 6.03
	Cv%	32.55	31.81	6.04	4.89
Svetlyachok	upper	Not available	Not available	3.89 ± 0.27	71.02 ± 6.49
	Cv%			5.15	11.03
	lower	Not available	Not available	3.69 ± 0.26	70.93 ± 6.44
	Cv%			6.35	11.5
Fonarik	upper	6.97 ± 0.52	210.02 ± 18.39	4.50 ± 0.48	64.28 ± 6.04
	Cv%	13.7	19.49	6.22	4.72
	lower	6.39 ± 0.48	283.06 ± 27.96	3.61 ± 0.57	63.85 ± 6.24
	Cv%	14.1	20.94	5.38	5.08

Note: upper – leaf plate upper epidermis; lower – leaf plate lower epidermis [Compiled by the authors].

On the upper and lower sides of the leaf, unicellular cover trichomes were found, the length of which varies from 774 µm to 780 µm (mainly along the veins on the lower side of the leaf) (Fig. 3, Tables 1 and 2).

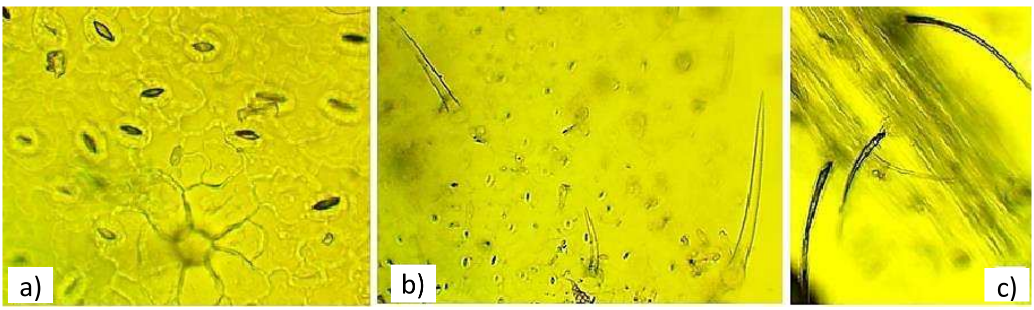

Figure 3. a) – stomata and base of the trichome (magnification ×280). The upper epidermis of the evening primrose's leaf plate the *Tverskoj* cultivar; b) – covering trichomes and stomata. Magnification ×280; c) – The lower epidermis of the leaf of the evening primrose of the *Tverskoj* cultivar, trichomes along the veins (magnification ×70) [Compiled by the authors].

Svetlyachok. The walls of the epidermal cells are sinuous. The size of the stomata on the upper epidermis of the leaf is 40.06 ± 4.11 µm long, 31.79 ± 3.52 µm wide. The number of stomata per 1 mm² is 456.81 ± 41.28 pcs. The size of the stomata on the lower epidermis of the leaf has a length of 41.08 ± 3.96 µm and width of 34.75 ± 3.66 µm. The number of stomata per 1 mm² comprises 613.29 ± 58.73 pcs.

A few papillary unicellular trichomes, 3–4 pcs mm^{-2}, were found on the adaxial side of the leaf (Fig. 4, Table 1 and 2).

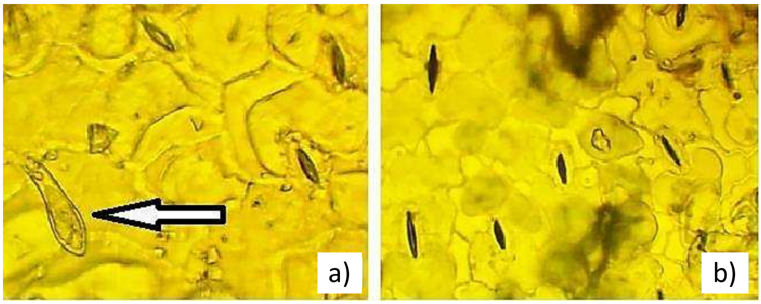

Figure 4. a) – the upper epidermis of the evening primrose's leaf plate, *Svetlyachok* cultivar. The arrow shows a papillary single-celled trichome; b) – the lower epidermis of the leaf of the *Svetlyachok* cultivar. Magnification ×280. [Compiled by the authors].

Fonarik. The walls of the epidermal cells are sinuous. The size of the stomata on the upper epidermis of the leaf is 38.16 ± 3.91 µm long, 32.57 ± 3.08 µm wide. The number of stomata per 1 mm² is 458.59 ± 40.19 pcs. The size of the stomata on the lower epidermis of the leaf has a length of 43.81 ± 4.15 µm and width of 34.63 ± 3.31 µm.

On the upper and lower sides of the leaf, a few (4–6 pcs mm^{-2}) unicellular cover trichomes were found, the length of which is about 200–300 µm. A few papillary unicellular trichomes were revealed on both sides of the leaf (Fig. 5, Tables 1 and 2).

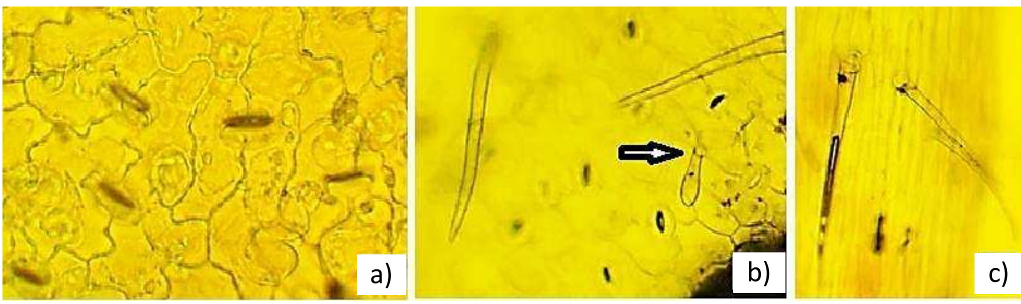

Figure 5. The upper epidermis of the evening primrose's leaf plate, the *Fonarik* cultivar: a) – stomata; b) – cone-shaped and papillary single-celled trichomes (arrow). The lower epidermis of the leaf of the evening primrose *Fonarik*: c) – trichomes along the veins. Magnification ×280 [Compiled by the authors].

The coefficient of variation in the number of cells per 1 mm^2 on the upper and lower epidermis is low in *Tverskoj* and *Fonarik* cultivars, while in *Genoteros* and *Svetlyachok* cultivars this indicator is less variable. The coefficient of variation in the number of stomata per 1 mm^2 on the upper and lower epidermis in *Genoteros* is not very variable. The average length and width of stomata vary on both sides of leaves in *Svetlyachok* and *Fonarik*. The variability of the values of the number of cone-shaped trichomes of the upper epidermis in the *Genoteros* cultivar is quite significant. In the *Tverskoj* cultivar, the indicator of the length of the cone-shaped trichomes of the upper epidermis also has a high coefficient of variation. *Fonarik* is peculiar for low values of the number and length of the cone-shaped trichomes of the upper and lower epidermis. For the remaining characters of the epidermis (the number and length of papillary unicellular trichomes), all studied varieties showed insignificant variability (Tables 1, 2). *Genoteros* and *Tverskoj* cultivars demonstrate medium and *Fonarik* - weak pubescence.

Morpho-anatomical characteristics of the leaves allow assessing the adaptive capabilities of plants: a limited number of stomata and their small size lead to the constant opening of stomata and excessive transpiration, which indicates low adaptability of plants to light and humidity conditions (Vinogradova et al., 2020). An increase in the number of trichomes may be due to the manifestation of the mechanism of the leaves' protection from overheating, lack of moisture, and other stress factors (Ahkubekova & Tamahina, 2020).

Micromorphological differences between cultivars can be explained by the different origins of the original populations on the basis of which the cultivars were created. Field conditions everywhere differ from the places of natural growth of plants: large open spaces, rapidly drying soil-and at the same time-the absence of competition and shading. Leaf pubescence is one of the main signs of the plant's protective reaction to arid conditions.

The walls of the abaxial epidermis cells of the leaf plates of the evening primrose studied varieties are of rounded-sinuous shape. The cells of the adaxial epidermis are rounded-elongated. The stomatal apparatus is of an anomocytic type. Leaf plates of *Genoteros*, *Tverskoj*, *Fonarik* cultivars are covered with agranular trichomes on the lower and upper epidermis. Epidermal cells forming a rosette are located around the attachments of cone-shaped pointed trichomes. This does not contradict early research (Cheryatova, 2014). Trichomes are simple, unicellular, conical in shape, 80 to 190 microns long, located mainly along the veins.

On the evening primrose leaves of the studied cultivars, the covering trichomes are located mainly along the veins. In *Tverskoj* and *Fonarik* cultivars, their number on the upper and lower epidermis is practically equal. In *Genoteros* cultivar, the covering unicellular trichomes are predominantly located on the upper epidermis of the leaf. Covering trichomes were not found in the *Svetlyachok* cultivar. The papillary unicellular trichomes on the leaves of the studied cultivars of the evening primrose are concentrated in the interveinal space, on the upper and lower epidermis, mainly in the middle part of the leaf.

Papillary trichomes have not been previously detected or described. Papillary unicellular trichomes were also found in the interveinal space of the central part of the evening primrose leaves of *Genoteros, Svetlyachok*, and *Fonarik* cultivars.

The leaves of *Svetlyachok* without of trichomes presumably indicate that insect pests may have been absent or rarely encountered in the places of its original growth.

And so we see that a cultivar that is not from European region continues to have a stress despite a long introduction.

The greater number of stomata in the *Tverskoj* cultivar may indicate a significant potential for photosynthetic activity.

CONCLUSIONS

For the first time, the anatomical differences between new cultivars of the evening primrose of the VILAR biocollection have been studied. The analysis showed that the cultivars of the evening primrose differ not only in the habitus of the bush, indicators of productivity, and the direction of economic use but also in a different number of stomata, and quantity of epidermal cells. The degree of variability of quantitative and morphometric indicators of pubescence in the *Svetlyachok* and *Fonarik* cultivars is lower than that in the *Tverskoj* and *Genotero*s. Number of stomata in plants of the *Genoteros* cultivar is low and their size is smaller in comparison with other studied cultivars, while the number of trichomes is much higher. All these features may indicate the weak potentional ecological flexibility of this cultivar. This should be taken into account when cultivating it. The results obtained may be important taxonomic for the correct identification of problematic *Onagraceae* taxa and in further breeding work.

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Predicting farm performance: do indicators of farm economic viability and efficiency signify of probability of bankruptcy?

J. Savickienė* and A. Miceikienė

Vytautas Magnus University Agriculture Academy, Faculty of Bioeconomy Development, Research Institute for Bioeconomy, Universiteto Str. 10-321, LT-53361 Akademija, Kauno r., Lithuania
*Correspondence: jurate.savickiene@vdu.lt

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Abstract. Following the analysis of the indicators characterising the economic viability, efficiency and bankruptcy probability of farms proposed by researchers and employed in practice, the relationships between the indicators and their capacity to predict the prospects of farm activities as well to assess whether or not the indicators are indicative of the same patterns of farm activity, several different researchers' approaches have been identified. Certain researchers have been claiming that all of the indicators provide the same farm performance prospects, while others consider economic viability and efficiency to provide long-term farm performance prospects, while bankruptcy probability-negative profitability in the short term. The methods of convergent and discriminant validation employed allowed for analysis of the risk of potential overlap between the index of economic viability of a family farm and farm economic efficiency coefficient with the already available bankruptcy probability prediction models. For this purpose, categorical regression analysis was employed. This enabled the authors to determine that the index of economic viability of a family farm and coefficient of farm economic efficiency did not repeat the already available and used bankruptcy probability prediction models. Summarizing the results, it could be claimed that the index of economic viability of a family farm and coefficient of farm economic efficiency are not suitable as an alternative for assessment of the bankruptcy probability.

Key words: farm economic viability, farm economic efficiency, bankruptcy prediction models, family farms.

INTRODUCTION

As the global commercial market of agricultural products and competition is becoming more robust, and agricultural manufacturers from previously inactive countries enter the global market, agribusiness entities have been acknowledging the importance of survival in the competition struggle and of the long-term economic prospects (Christensen & Limbach, 2019). Recently, the issues of competitiveness, viability and efficiency have been propelling discussions among farmers, policy makers, and researchers in this regard (Rivza et al., 2017; Dinterman et al., 2018; Kovalova et al., 2020). These issues include the amount and value of the support allocated to agriculture, the moral values of people engaged in farming in terms of preservation of natural resources for future generations, the implications pertaining to the tax systems applied to the entities engaged in agriculture, the prospects of viable farming, and a number of other issues. Two approaches to agribusiness prospects may be identified. Researchers (Gūvenir & Cakir, 2010; Hu & Sathye et al., 2015; Dinterman et al., 2018; Kovalova et al., 2020) predict a decrease in economic viability of farmers and agribusiness companies and an increase in the number of bankruptcies as a result of natural hazards and economic changes taking place worldwide (such as Brexit, etc.). Nonetheless, the public opinion prevailing in the EU is that agribusiness has already been receiving considerable support and gaining substantial profits. This creates polarisation between society and the people working in agriculture, thereby shaping negative views towards farming (Rivza et al., 2017). For these reasons, young people are becoming increasingly reluctant to engage in agricultural activity or establish new farms.

Given the differing views, it is important to explore the actual situation, as it determines the economic prospects of agribusiness and performance results in the long run (Christensen & Limbach, 2019).

Assessment of agribusiness prospects is relevant not only for communities, but for governments as well. Hence, increasing attention has been directed towards the method of assessment of the economic viability, efficiency and long-term prospects of farms, differences between the indicators, identification of the indicators which are more important for economic substantiation of the prospects of farm performance (Scotti et al., 2011; Morel et al., 2017; Savickiene et al., 2017), with the view to both receiving support funds and developing farming activity in the long-term perspective. The guidelines of the EU Common Agricultural Policy 2021–2027 stipulate promotion of the long-term economic viability of farmer's farms, because farmer's farms provide not only for their families, but also for their environment: rural communities and landscape; in other words, social and environmental sustainability. Researchers (Koleda & Lace, 2010; Rivza et al., 2016; Savickiene, 2016; Hosaka, 2019) have different views regarding the indicators which characterise the long-term prospects of farms: some consider economic viability to be the key indicator, others refer to efficiency, while yet others refers to bankruptcy probability. Nonetheless, all of them share the position that similar indicators should be used for assessment of the long-term prospects in agriculture and comparison of individual farms and enterprises.

The present research explores the properties of the indicators characterising the economic viability, efficiency and bankruptcy probability of farms with the view to assessment of positive long-term prospects or bankruptcy of farms.

Research problem: are the indicators of economic viability and efficiency of farms indicative of bankruptcy probability, and what are the methods for verifying this?

Research aim: following identification of the indicators characterising the economic viability, efficiency and bankruptcy of farms, to identify the relationships between these indicators and the ability to predict the prospects of farm performance as well as to assess whether these indicators show the same performance trends of farms.

The family farms engaged in agricultural activities that managed the accounting and provided the information on their production and financial activities were used in the empirical study. The accounting data of Lithuanian family farms for the years 2015 and 2017 were used. The accounting data of family farms were collected with the assistance of Lithuanian Agricultural Advisory Service. Indicators from three groups were analysed as part of the research: the indicators defining economic viability, economic efficiency, and bankruptcy probability of farms. The methods of convergent and discriminant validation (Catreg) and correlation analysis were employed in the research.

MATERIALS AND METHODS

Researchers (Jurkėnaitė 2015; Karas et al., 2017; Spicka et al., 2019) exploring the economic indicators of farms and dynamics thereof have been claiming that the economic viability of farms has been decreasing, while the number of farmer's farms incapable of covering production and general expenses has been increasing as shown by the empirical studies conducted in the recent years; researchers have also noted that the long-term economic attractiveness of agricultural activity has decreased even more significantly. Farming is a very specific and particular industry requiring particular knowledge and specific conditions: the activity is difficult to plan due to unstable natural conditions and imposed stricter environmental, animal welfare and food quality requirements. It is important that the farmer's farms identify the long-term prospects of their own performance not only when planning long-term investments such as purchase of land, buildings, and agricultural equipment, but also when seeking support from the state or EU funds, or applying to lenders for funding.

Assessment of any business prospects is, as a standard, performed by applying the bankruptcy probability prediction models. According to various research findings, assessment of prospects of an agribusiness using these models is a complex task due to its specifics. Although researchers (Václav & David, 2017; Dinterman et al., 2018; Mimra et al., 2018; Hosaka, 2019) and others did apply the discriminant analysis method using the developed Altman (Altman 1, Altman 2, Altman 3), Springate, Liss, Taffler equations for assessment of farm prospects, they have come to the conclusion that these methods have a lot of weaknesses. Hosaka (2019) presented the weaknesses of the Altman, Springate, Liss, Taffler methodology. According to the researcher, where the analysis aims at assessing the probability of the life cycle stage of economic viability of farms, the financial indicators included in the model may differ significantly not only due to the specialisation and specifics of economic activity of the farm, but also due to the accounting methods used. Hence, in the subsequent research, the researchers analysed the indicators of economic viability and efficiency of farms.

By following the Altman, Springate, Liss, Taffler methodologies, (Rajin et al., 2016) performed a comprehensive analysis of the issues encountered by farms in the decline stage of the economic viability life cycle (Rajin et al., 2016). The essential idea of the models is that various areas of farm activity are assessed using financial indicators, which are used to derive the Z-score, a common complex ratio. To substantiate this model, Altman, Springate, Liss, Taffler used the discriminant analysis method involving identification of the linear correlation function parameters. Having explored the reasons behind the decline stage of farm economic viability life cycle, Altman, Springate, Liss, Taffler proposed the system of indicators, a toolkit enabling classification of farms by value as those with high probability of entering the decline stage of the farm life cycle and those with the stability or growth stage of the economic viability life cycle of the farm. Based on this classification, they dealt with the value of the probability of decline of economic viability of farms. However, the findings of this research work do not allow assessment of the current situation of farms and long-term prospects, as only financial indicators are used in the Altman, Springate, Liss, Taffler bankruptcy probability methodologies.

Whereas agribusiness is claimed to be very risky, dependent on a number of different external factors such as natural conditions, agriculture support policy, etc., in their subsequent research work, researchers (Hu & Sathye, 2015; Savickiene 2016; Spicka et al., 2019) have referred to the majority of economic indicators (competitiveness, profitability, solvency, economic viability, negative profitability, etc.) in risk assessment and identification of farm prospects. The analysis of previous studies has shown that, according to the majority of findings, the indicators of economic viability and efficiency are the most frequently used for assessment of the performance prospects. The arguments are the following: both indicators may be calculated for non-trade farms as well; they are referred to as the most appropriate for assessment of the long-term prospects, and cover other indicators mentioned, hence providing a wider context. However, the question is which of the indicators is the most appropriate for assessment of a long-term prospect of farms, and whether or not they can be used to forecast the probability of bankruptcy?

The majority of researchers usually provide a multi-criteria holistic approach towards farm performance prospects (Tisdell, 1996; Scotti et al., 2011; Morel et al., 2017; Jedik & Stalgienė, 2018; Savickienė & Miceikienė, 2018; Spicka et al., 2019).

Research efforts in assessment of farm economic viability can be traced back to over 40 years ago (Savickienė & Miceikienė, 2018), and farm economic viability has become the most relevant field of the studies in viability of agriculture in the recent decades (Savickiene, 2016), and is important both for communities and governments (Rajin et al., 2016). According to Jurkėnaitė (2015), application of the viability theory to practice enables improvement of decision making and provides valuable insights.

According to researchers (Jurkėnaitė, 2015; Christensen & Limbach, 2019), farm viability is determined by three key areas: economic, environmental, and social. Farm viability is often perceived as the dynamics and sustainability covering both current and future generations, taking care of the future generations without defining any future limits. The broad approach enables identification of the key indicators which allow for determination of the lack of resources and issues related to changes in the environment. **It can therefore be claimed that** the concept of viability of farms covers not only the profitable activity of a farm, but also the capacity to differentiate the activity thereby adapting to climate change, the possibilities to maintain family using the farmer's income, stable farm growth, positive return on capital, and investment in farm modernisation. Only farm economic viability has the capacity to show the financial prospects of the farm activity. In the present study, the economic viability of a farm is defined as the capacity of the farm to survive and develop using own and external resources (Savickienė et al., 2017). The purpose of trade family farms is pursuit of farm operations as a business entity, while for other farms, it is satisfaction of the household food needs or expression of an advocated lifestyle.

According to the concept of farm economic viability, the present study employs the index of economic viability of a family farm assessing the economic viability of farms (I_{FEV}) (Savickienė & Miceikienė, 2018) and calculated using the following methodology:

$$I_{FEV} = \frac{GO + A_{CA+}A_{NA}}{INT_{CONS} + D + EXT_{COST} + FFW + D_{ST} + D_{LT}} \quad (1)$$

where GO – gross output (at basic prices); A_{CA} – current farm assets; A_{NA} – fixed farm assets; INT_{CONS} – intermediate consumption; D – depreciation; EXT_{COST} – costs of external resources; FFW – farmer and family members‘ wage; D_{ST} – short-term debt; D_{LT} – long-term debt.

Researchers investigating economic viability and prospects of farms agree that the indicators of economic viability of farms describe long-term economic prospects of farms, but fail to assess the short-term prospects and current financial situation, which are important in assessment of farm performance.

Studies conducted previously (Tisdell, 1996; Savickiene 2016; Morel et al., 2017) have demonstrated that farm economic efficiency is an important indicator in the long-term perspective. In the present study, economic efficiency is considered as the farmer's ability to mobilise capital, labour, and natural resources for the organisation of farm activity with the purpose of receiving income and assuming the associated risks. To perform the comparative analysis of the indicators listed above, complex economic efficiency coefficient has been chosen for assessment of economic efficiency of a farm (Tisdell, 1996; Scotti et al., 2011).

$$FEE = \frac{GO}{INT_{cons} + D + EXT_{COST} + FFW} \quad (2)$$

where FEE – farm economic efficiency; GO – gross output (at basic prices); INT_{CONS} – intermediate consumption; D – depreciation; EXT_{COST} – costs of external resources; FFW – farmer and family members‘ wage.

Comparison of the specific indicators of economic viability and economic efficiency of farms to the conventional indicators used in assessment of bankruptcy probability will help answer the question of which indicators should be calculated and analysed by farmers and agricultural policy makers to assess the prospects of farm performance.

The studies conducted previously have shown that prediction of farm prospects could be applied to farm bankruptcy prevention. Nonetheless, there is lack of research which would allow for assessment of the risk of bankruptcy of farmer's farms. The aspiration is that not only large-sized farmer's farms or agribusiness companies, but also small and mid-sized farmer's farms operate efficiently. Although subsidies and other support are allocated to farms from the European Agricultural Fund for Rural Development and other sources, the losses incurred by farms and bankruptcies have become common, particularly in recent years, when natural risks emerge along with the business risk.

The findings of the analysis of research works on the topic considered have shown contradictory views towards assessment of farm performance prospects; hence, a holistic approach towards assessment of farm performance prospects based on the economic information on the farm is required (Christensen & Limbach, 2019).

Therefore, it is reasonable to compare three groups of complex indicators integrating different financial and economic information on the farms: the groups of indicators of farm economic viability, farm economic efficiency, and bankruptcy probability.

The developed index of economic viability of a family farm (Savickiene, 2016; Savickiene & Miceikienė, 2018) has shown the efforts to earn and accumulate the assets for the family farm to remain sustainable and viable in the future. The numerator is reflected by the accumulation factor (ability to create added value), while the denominator is reflected by the consumption factors (the efforts necessary to create the value). The farm's economic efficiency coefficient is important for all family farms as it shows the condition determined by the farm's current activity (Tisdell, 1996). The size of the assets held reflects the farm's prospects by the types of development of economic viability. The assets held at the family farm may be pledged or unpledged. The pledged share of the assets should ensure the economic growth of the farm, while the unpledged share shows the possibility for the farm to borrow and create added value.

Certain researchers (Koleda & Lace, 2010; Klepac & Hampel, 2017; Václav & David, 2017 and others) claim that economic viability overlaps with other already known bankruptcy prediction models. Hence, the verification of authenticity of the index of economic viability of a family farm and of the coefficient of farm economic efficiency, the bankruptcy probability prediction models as well as the distinctness of the measurement properties has been conducted. Verification of the content authenticity and distinctness of the measurement properties has been conducted on the basis of the convergent and discriminant validation.

Convergent and discriminant validation has been conducted to empirically assess whether or not the index of economic viability of a family farm, created in theory, measures the probability of bankruptcy of family farms, and whether or not the results allow for substantiation of suitability of the index for assessment of economic viability of a family farm. In the dissertation, the convergent and discriminant validation of the index of economic viability of a family farm consists of three stages (Fig. 1).

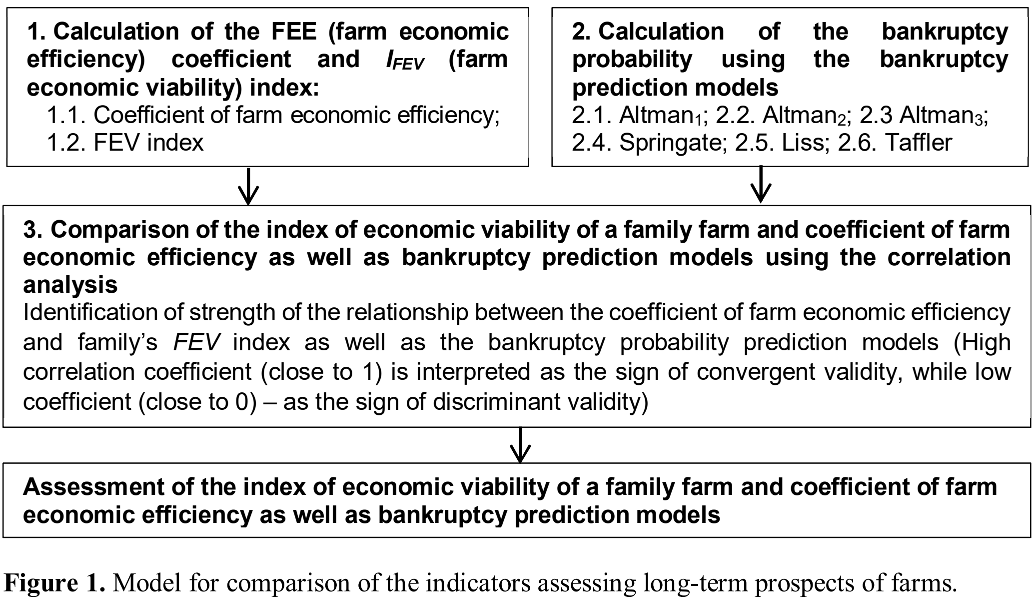

Figure 1. Model for comparison of the indicators assessing long-term prospects of farms.

Convergent Validity describes the degree that the indicators of one formula (in the article: economic viability of a family farm and farm economic efficiency) correlate to the indicators of another formula (in the article: $Altman_1$ (describes different aspects of financial performance), $Altman_2$ (the farms not listed on the stock exchange), $Altman_3$

(intended for individual and service companies), Springate, Liss and Taffler), which has been developed for measurement of the same construct. For example, if the correlation of the index of economic viability of a family farm to the values of $Altman_1$ indicators was equal to 1, the test would be interpreted to have high convergent validity (Vaitkevičius et al., 2013).

According to S. Vaitkevičius et al. (2013), Discriminant Validity is the degree to which the indicators obtained as a result of application of one formula do not correlate to the indicators obtained as a result of another formula, not created for assessment of the same context. Discriminant validity is interpreted as high if the correlation of the index of economic viability of a family farm to the bankruptcy indicators approaches 0. This shows that, at the level of constructs, economic viability of a family farm is not a variety of the bankruptcy probability.

RESULTS AND DISCUSSION

The first stage of convergent and discriminant validation of the index of economic viability of a family farm involves calculation of the index of economic viability of a family farm and coefficient of farm economic efficiency (Fig. 1). To verify the indicators, family farm data for the years 2015–2017 have been used to verify the indicators (3,917 farms). The risk of potential overlap of the index of economic viability of a family farm and coefficient of farm economic efficiency with other already known indicators assessing the financial and economic condition of the farm, has been analysed.

The second stage of empirical formation of convergent and discriminant validation of economic viability of a family farm has involved calculation of the bankruptcy probability using the bankruptcy prediction models: $Altman_1$, $Altman_2$, $Altman_3$, Springate, Liss, Taffler. Farm bankruptcy prediction is the method to assess farm economic efficiency, identify the negative patterns in farm economic efficiency, and the probability of its bankruptcy using quantitative parameters (Garškaitė & Mackevičius, 2010). The aim behind investigation of the bankruptcy probability is to determine whether or not the index of economic viability of a family farm is identical to the indicators of bankruptcy probability. For this purpose, six bankruptcy prediction models have been used. The indicators have been calculated for all the farms studied.

The third stage has involved correlation analysis of the obtained results following the calculation of the index of economic viability of a family farm and coefficient of farm economic efficiency, and bankruptcy probability, in order to determine whether or not the developed index of farm economic viability and coefficient of farm economic efficiency are indicative of the farm bankruptcy probability. The *Pearson* correlation analysis conducted has not shown the presence of any strongly correlating indicators between the economic viability of a family farm and bankruptcy probability. The values of the correlation coefficient varying from 0.038 to 0.041 indicate that the correlation is weak. Hence, this correlation could be interpreted as an indicator of weak convergence and relatively strong indicator of discriminant validity. Therefore, it could be claimed that the developed index of economic viability of a family farm and coefficient of farm economic efficiency do not measure the bankruptcy probability, and thus, are not suitable for use as the indicators in farm bankruptcy prediction (Table 1). Hence, the developed index of economic viability of a family farm potentially measures a different context, i.e. economic viability of family farms, which may be indicative of the farm development prospects.

Table 1. Matrix of the correlation coefficients of the index of economic viability of a family farm and coefficient of farm economic efficiency as well as bankruptcy prediction models

Criteria		I_{FEV}	FEE	$Altman_1$	$Altman_2$	$Altman_3$	Springate	Liss	Taffler
I_{FEV}	Pearson correlation	1	0.383^{**}	0.040^{*}	0.040^{*}	0.040^{*}	0.038^{*}	0.041^{*}	0.038^{*}
	Significance		0.000	0.011	0.011	0.011	0.017	0.011	0.018
FEE	Pearson correlation	0.383^{**}	1	0.012	0.012	0.012	0.016	0.012	0.015
	Significance	0.000		0.460	0.460	0.460	0.313	0.436	0.362
$Altman_1$	Pearson correlation	0.040^{*}	0.012	1	1.000^{**}	1.000^{**}	0.998^{**}	1.000^{**}	0.999^{**}
	Significance	0.011	0.460		0.000	0.000	0.000	0.000	0.000
$Altman_2$	Pearson correlation	0.040^{*}	0.012	1.000^{**}	1	1.000^{**}	0.998^{**}	1.000^{**}	0.999^{**}
	Significance	0.011	0.460	0.000		0.000	0.000	0.000	0.000
$Altman_3$	Pearson correlation	0.040^{*}	0.012	1.000^{**}	1.000^{**}	1	0.998^{**}	1.000^{**}	0.999^{**}
	Significance	0.011	0.460	0.000	0.000		0.000	0.000	0.000
Springate	Pearson correlation	0.038^{*}	0.016	0.998^{**}	0.998^{**}	0.998^{**}	1	0.998^{**}	0.999^{**}
	Significance	0.017	0.313	0.000	0.000	0.000		0.000	0.000
Liss	Pearson correlation	0.041^{*}	0.012	1.000^{**}	1.000^{**}	1.000^{**}	0.998^{**}	1	0.999^{**}
	Significance	0.011	0.436	0.000	0.000	0.000	0.000		0.000
Taffler	Pearson correlation	0.038^{*}	0.015	0.999^{**}	0.999^{**}	0.999^{**}	0.999^{**}	0.999^{**}	1
	Significance	0.018	0.362	0.000	0.000	0.000	0.000	0.000	

The correlation is significant if the significance level: *0.05; **0.01.

Regression analysis (Catreg) as the indicator of convergent and discriminant validity

Catreg analysis has been conducted as an additional indicator of convergent and divergent validation. In contrast to correlation analysis, categorical regression (Catreg) has enabled integrated comparison of the constructs of farm economic viability and economic efficiency to all bankruptcy indicators at the same time. In this case, different from the majority of studies is that poor characteristics of the regression model are the key indicator showing that the hypothesis of convergent validity is rejected, while the hypothesis of discriminant validity is confirmed.

The measurement has been performed by determining the optimal scaling level - spline ordinal, the degree = 2, and the number of interior knots = 2. The categorical regression ranking discretisation method has been applied. Two categorical regression models have been developed: one describes the index of economic viability of a family farm, and the other - the coefficient of farm economic efficiency (see Tables 2–5).

The analysis of characteristics of the index of farm economic viability and coefficient of economic efficiency has shown that the bankruptcy indicators are not very effective (Table 2). This is suggested by the corrected coefficient of determination which, in the case analysed, shows that, when used together, all bankruptcy indicators could explain the index of economic viability of a family farm by only 16.8%, information about variation in the economic viability of a family farm and the coefficient of farm economic efficiency - by 27.9%.

Table 2. Indicators generalising the assessment of economic viability of a family farm

Indicators	Multi-dimensional R	R^2	Corrected R^2	Predictive error probability
I_{FEV}	0.413	0.170	0.168	0.830
FEE	0.529	0.280	0.279	0.720

Dependent variables: coefficient of the index of economic viability of a family farm and farm economic efficiency. Bankruptcy prediction models: $Altman_1$, Z < 2.8; $Altman_2$, Z < 2.9 $Altman_3$, Z < .59; Springate Z < 0.862; Liss Z < 0.037 Taffler Z < 0.2.

Analysis of the developed quality indicators (for FEV index and economic efficiency coefficient) (Table 3) has shown that the sum of squares of the error factor is much higher than the sum of squares of the regression factor. The relationship between the sums of square shows that the regression models is essentially more erroneous than correct. Hence, it is interpreted as failing to explain the relationship between the dependent and independent variables of the model.

Table 3. Description (ANOVA) of the index of economic viability of a family farm and coefficient of farm economic efficiency as well as bankruptcy prediction models

Indicators	Models	Sum of squares	*df*	Mean of squares	*F*	Statistical significance level *(p)*
I_{FEV}	For the regression factor	666.900	12	55.575	66.753	0.000
	For the error factor	3,251.100	3,905	0.833		
	Common factor	3,918.000	3,917			
FEE	For the regression factor	1,096.532	6	182.755	253.328	0.000
	For the error factor	2,821.468	3,911	0.721		
	Common factor	3,918.000	3,911			

Dependent variables: coefficient of the index of economic viability of a family farm and farm economic efficiency. Bankruptcy prediction models: $Altman_1$, Z < 2.8; $Altman_2$, Z < 2.9 $Altman_3$, Z < .59; Springate Z < 0.862; Liss Z < 0.037 Taffler Z < 0.2.

It should also be noted that the properties of the index of economic viability of a family farm and farm economic efficiency are also not too correct on the level of individual variables as well (Table 4). In this case, the analysis of the statistical significance level shows unsuitability of certain variables to the developed index of economic viability of a family farm and coefficient of farm economic efficiency. This is another proof that the bankruptcy prediction models, in general, do not characterise the economic viability of a family farm.

The analysis of the significance of the effect of independent variables on the dependent variable has shown that, in the case of the index of economic viability of a family farm, Liss is relatively more significant. However, the individual Liss correlations and the index of economic viability of a family farm have rejected the hypothesis on the existence of Liss effect in economic viability of a family farm during the earlier analysis; hence, an individual regression model of these variables has not been designed additionally. A similar situation can be observed in relation to the economic efficiency coefficient and relationship under the Springate prediction model, whereas the common model identifies the importance of relationship between them. Nonetheless, the hypothesis of the interaction between them has also been rejected by the analysis of individual relationship (Table 5).

Table 4. Coefficients of the index of economic viability of a family farm and coefficient of farm economic efficiency as well as bankruptcy prediction models

Indicators	Bankruptcy prediction models	Standardised coefficients		*df*	*F*	Statistical significance level *(p)*
		Beta	Bootstrap (1,000) standard error indicator			
I_{FEV}	$Altman_1$	0.095	0.060	1	2.485	0.115
	$Altman_2$	0.100	0.067	2	2.271	0.103
	$Altman_3$	0.121	0.022	2	29.796	0.000
	Springate	0.049	0.022	2	5.185	0.006
	Liss	0.223	0.033	4	46.258	0.000
	Taffler	0.125	0.016	1	61.721	0.000
FEE	$Altman_1$	0.060	0.018	1	11.121	0.001
	$Altman_2$	0.082	0.017	1	21.940	0.000
	$Altman_3$	0.022	0.017	1	1.757	0.185
	Springate	0.350	0.031	1	124.793	0.000
	Liss	0.025	0.040	1	0.405	0.524
	Taffler	0.207	0.067	1	9.655	0.002

Dependent variables: the index of economic viability of a family farm and coefficient of farm economic efficiency.

Table 5. Correlation and tolerance of the index of economic viability of a family farm and coefficient of farm economic efficiency as well as bankruptcy prediction models

Indicators	Bankruptcy prediction models	Correlations			Significance coefficient	Tolerance	
		Zero	Partial	of a part		After transformation	Before transformation
I_{FEV}	$Altman_1$	0.206	0.095	0.087	0.115	0.826	0.785
	$Altman_2$	0.189	0.094	0.086	0.111	0.739	0.745
	$Altman_3$	0.134	0.124	0.114	0.096	0.886	0.974
	Springate	0.187	0.050	0.046	0.054	0.863	0.649
	Liss	0.334	0.215	0.201	*0.438*	0.808	0.731
	Taffler	0.254	0.123	0.113	0.187	0.815	0.609
FEE	$Altman_1$	0.207	0.065	0.055	0.044	0.860	0.785
	$Altman_2$	0.217	0.090	0.076	0.063	0.874	0.745
	$Altman_3$	0.102	0.025	0.022	0.008	0.959	0.974
	Springate	0.465	0.349	0.316	*0.581*	0.817	0.649
	Liss	0.218	0.027	0.023	0.020	0.842	0.731
	Taffler	0.383	0.211	0.183	0.283	0.783	0.609

Dependent variables: the index of economic viability of a family farm and coefficient of farm economic efficiency.

Summarising the convergent and discriminant validation results, it could be claimed that the index of economic viability of a family farm and coefficient of farm economic efficiency are not suitable as an alternative for assessment of the bankruptcy probability. It is therefore concluded that the index of farm economic viability and farm economic efficiency constructs comprising their theoretical basis are of a voluntary nature, measuring the particular context of the farm economic viability, while bankruptcy probability is described by other indicators generated using the respective bankruptcy prediction models.

The researchers (Garškaitė & Mackevičius, 2010; Dinterman et al., 2018; Hosaka, 2019) have discussed the purpose of the indicators of farm economic viability, efficiency, and bankruptcy probability; differing views regarding their purpose have been advocated in their studies. Several scientific approaches could be identified: certain researchers have claimed that all of the indicators provide the same farm performance prospects, while others consider economic viability and efficiency to provide long-term farm performance prospects, while bankruptcy probability - negative profitability in the short term.

Koleda & Lace (2010), Scotti et al. (2011), Christensen & Limbach (2019) accentuate the need of the assessment of the economic viability of farms, since the family farms take decisions related to the preservation of economic viability and determination of activity perspectives. As pointed out by Spicka et al. (2019), recently, the number of measures and methods for the assessment of economic viability of agriculture increases. One of the most frequently used methods for the research of economic viability of farms is based on the indicators of economic viability, but the assessment yet shows that the indicators used are not sufficiently practical and do not reflect the prospects of economic viability of family farms. Rivza et al. (2017) pointed out the assessment of economic viability of family farms is still developing and has not reached the maturity yet. We also should agree to the thoughts of Tisdell (1996), Václav & David (2017) that when forming a comprehensive assessment of the economic viability of the family farm, the aspects related to its feasibility and economic validity are essential.

CONCLUSIONS

The correlation analysis has shown that the indicators of economic viability and economic efficiency of family farms as well as the bankruptcy probability forecasting models do not correlate to each other but measure different phenomena. The *Pearson* correlation analysis has shown that there are no indicators of the economic viability and bankruptcy probability of a family farm that would correlate strongly to each other. The correlation coefficient values vary from 0.038 to 0.041, indicating weak correlation. This correlation may therefore be considered as the identifier of weak convergence and relatively strong indicator of discriminant validity. It can therefore be claimed that the indicators of economic viability and economic efficiency of a family farm do not measure the bankruptcy probability and are not applicable to forecasting of farm bankruptcy. The indicators of economic viability and efficiency of a family farm measure a different context, namely, economic viability of the family farm which may show the development prospects of the farm.

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The productivity of spring barley when using cobalt nanoparticles and liquid-phase biological product

T. Seregina[1], O. Chernikova[1,*], Yu. Mazhaysky[1] and L. Ampleeva[2]

[1]Academy of law and management of the Federal penal service of Russia, Sennaya street 1, RU390036 Ryazan, Russia
[2]Ryazan State Agrotechnological University named after P.A. Kostychev, Kostychev street 1, RU390044 Ryazan, Russia
[*]Correspondence: chernikova_olga@inbox.ru

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Abstract. The purpose of this research is to study the effect of growth activators on the yield and quality of spring barley products. For the most complete disclosure of the potential yield of spring barley against the background of the application of minimal doses of organic fertilizers, cobalt nanoparticles were used, as well as a liquid-phase biological product (LPBP) in various combinations (NPCo, Compost, Compost + NPCo, Compost+ LPBP 1%, Compost+ LPBP 2%, Compost+ LPBP 1%+ NPCo, Compost+ LPBP 2%+ NPCo). The size of cobalt nanoparticles was 40–60 nm, the phase composition - Co - 100%. A suspension of nanoparticles was obtained by dispersing with ultrasound in an aqueous solution in accordance with the requirements of the technical conditions. LPBP is a dark brown liquid with a specific odor, pH = 6.5–7.5, contains N, C, P, K, Ca, Mg, tryptophan, microorganisms. Cobalt nanopowder in solution contained 0.01 g per hectare seeding rate (20 mg L^{-1}), LPBP concentration was 1% and 2%. Compost was used as an organic fertilizer; it consisted of 90% of cattle manure and 10% of poultry manure at a dose of 20 t ha^{-1}. The seeds were soaked 30 minutes before sowing in double distilled water, in a suspension of nanoparticles and LPBP in accordance with the experimental options. The analysis of the results of the research has shown that the studied preparations have a stimulating effect, contribute to an increase in resistance to suboptimal weather conditions, an increase in productivity and product quality. It was noted that the best indicators were obtained with joint pre-sowing treatment of seed material by cobalt nanoparticles with 1% solution of a liquid-phase biological product. Thus, there was an activation of growth processes, the yield increased by 35%, the content of crude protein - by 20.9%, digestible protein - by 10.9%, and crude fat - by 78.8%.

Key words: cobalt nanoparticles, liquid-phase biological product, barley, yield, nutritional value.

INTRODUCTION

Currently, spring barley is grown in almost all regions of Russia. Barley grain is used up to 70% for feed, as well as for food and technical purposes. The fertility of soil of modern agricultural landscapes for the expanded reproduction of their productivity in organic farming presupposes the rejection of the integrated use of various means of chemicalization. One of the promising areas for increasing yields and product quality in agricultural science and production is the use of various physiologically active substances. Pre-sowing seed treatment by biological stimulants in order to protect them from pests, diseases and stimulate germination is considered by scientists as an effective means of eliminating environmental pollution.

Among the numerous methods of increasing the productivity of grain crops, pre-sowing treatment is becoming more and more popular, it contributes to the disinfection of seeds from fungal and bacterial diseases, and also provides an increase in immunity and vigor of plant germination (Polishchuk et al., 2015; Efremova, 2016; Tombuloglu et al., 2020b). There are various methods of seed treatment - chemical, biological, physical impact. All methods are well researched, quite effective and do not require large energy and material costs in comparison with the treatment of crops during the growing season, pre-sowing soil preparation, fertilization, therefore they have found wide application not only among researchers, but also among agricultural producers and in personal subsidiary plots.

One of the safest methods is biological seed treatment - biological products, hormonal preparations, various natural growth regulators, nanomaterials and other growth regulators of multifunctional value, which, along with an increase in crop productivity, increase their resistance to unfavorable environmental factors, disease resistance, and nutrient assimilation and at the same time they are environmentally friendly (Polischuk et al., 2018; Chernikova et al., 2019; Polischuk et al., 2019; Seregina et al., 2020).

The polyfunctional liquid-phase biological product LPBP was developed at the FSBSI All-Russian Research Institute of Reclaimed Lands, its distinctive features are agronomically useful microflora, as well as physiologically active substances and nutrients for plants. LPBP enhances biochemical processes of plant growth and development, photosynthetic reactions, additional assimilation of nutrients, predominant accumulation of nitrogen in generative organs, etc. The effect of LPBP on growth and development is associated with an increase in metabolic processes, in particular, with the transformation of hard-to-reach organic soil compounds (Rabinovich et al., 2009 and 2015).

Experimental data on grain yield demonstrated a good response of plants to the treatment of spring wheat seeds in 1% of LPBP concentration - the increase in actual yield averaged 7% (Rabinovich et al., 2019).

Also, modern nanotechnology and nanomaterials are used to increase food production (Duhan et al., 2017; Ma et al., 2018).

Interest in the use of nanoparticles (NPs) in plant growing and agricultural practice is associated with their unique properties. Long-term studies of dispersed systems and NPs in particular have revealed the following features of the biological action of NPs. NPs have low toxicity, 7–50 times lower than the toxicity of metals in ionic form; have a prolonged and multifunctional effect; stimulate metabolic processes; easily penetrate into all organs and tissues; their biological activity is associated with the peculiarity of the structure of particles and their physicochemical characteristics; NPs exhibit a synergistic effect with natural polysaccharides (Rakhmetova et al., 2015; Olkhovskaya et al., 2019; Tombuloglu et al., 2020a).

A special place is occupied by research related to the pre-sowing treatment of seeds by nanoparticles, which makes it possible to increase the yield up to 30–40% (Churilov et al., 2019, 2020). The close attention of researchers to the use of NPs in the practice of pre-sowing seed treatment is due to the fact that more intensive seed germination contributes to a lower consumption of reserve nutrients by the seed, its productive respiration and growth.

Cobalt occupies an important place among nanoscaled materials. Cobalt participates in the biological processes of plants, animals and humans, being an irreplaceable element, therefore, special attention should be paid to the analysis of the effect of nanosized cobalt on the main indicators of biological processes. The influence of cobalt on the intensity of photosynthesis, as well as an increase in the content of ascorbic acid in plants, was noted. The lack of cobalt can cause disturbances in nitrogen metabolism, a decrease in the amount of chlorophyll, carotenoids, vitamin E in the leaves. For a long time, cobalt was considered a trace element necessary only for animals. But at present, its usefulness or necessity for higher plants has also been proven (Minz et al., 2018).

The main function of cobalt is associated with its participation in fixing atmospheric nitrogen in the nodules of leguminous and non-leguminous plants. The participation of cobalt in the life of higher plants incapable of nitrogen fixation is specific or indirect. The metal stimulates cellular reproduction of leaves by increasing the thickness and volume of the mesophyll in the leaves, the size and number of cells in the columnar and spongy leaf parenchyma. The influence of cobalt on the formation and functioning of the photosynthetic apparatus of plants through the concentration of chloroplasts and pigments in the leaves was established. This is linked with an increase in the volume of the plastid apparatus due to the growth of organelles (Elizareva et al., 2018).

However, at present, a number of basic issues remain unresolved. These issues are related to the mechanisms on that how nanoparticles affect fluid translocation and photosynthesis, what chemical transformations bio-transformations of nanoparticles occur (or do not happen), how are effects electron transport chain, Calvin cycle and other photosynthetic compartments, how nanoparticles affect the soil organisms and plant performances (Rajput et al., 2019).

The purpose of our research was to study the effect of growth activators on the yield and quality of spring barley products. For the most complete disclosure of the potential yield of spring barley against the background of the application of minimal doses of organic fertilizers, cobalt nanoparticles were used, as well as a liquid-phase biological product (LPBP) in various combinations.

MATERIALS AND METHODS

The research was carried out in lysimeters designed by All-Russian Scientific Research Institute of Hydraulic Engineering and Land Reclamation with an undisturbed soil profile. The area of stationary field lysimeters is 1.13 m^2.

Lysimeters are charged with gray forest soil. Samples taken from a depth of 0–25 cm at the beginning of the experiment characterize the soil with a low content of organic matter from 3.8% to 5.4% (on average 4.6% ± 0.6).

The acid-base reaction of the soil is weakly acidic, pH from 5.3 to 6.4 (on average 5.7 ± 0.1). The provision of soil with mobile nutrients on average was as follows: potassium content - 833.5 mg kg^{-1}, total nitrogen - 0.12%, mobile phosphorus - 128 mg kg^{-1}, which characterizes this soil as averagely provided by these elements.

Nanoparticles produced at the Moscow Institute of Steel and Alloys (Dzidziguri et al., 2000). The size of cobalt nanoparticles was 40–60 nm, the phase composition Co - 100%. A suspension of nanoparticles was obtained by dispersing with ultrasound in an aqueous solution. LPBP is a dark brown liquid with a specific smell, pH = 6.5–7.5, contains N, C, P, K, Ca, Mg, trace elements: copper, zinc, manganese, iron, as well as tryptophan and microorganisms. The content of total nitrogen in the composition of LPBP is 0.2–0.5 g L^{-1}, mobile forms of potassium (K_2O) and phosphorus (P_2O_5) - 9.5 and 10 g L^{-1}, respectively. The concentration of the toxic elements: lead, mercury, nickel and arsenic is significantly lower than their maximum permissible concentrations.

Cobalt nanopowder in the solution contained 0.01 g per hectare of seeding rate, which corresponded to 20 mg L^{-1}, the concentration of LPBP was 1% and 2%. The compost was used as an organic fertilizer in all variants of the experiment, it consisted of 90% of cattle manure and 10% of poultry manure at a dose of 20 t ha^{-1}.

The seeds were soaked 30 minutes before sowing in double distilled water, in a suspension of nanoparticles and LPBP in accordance with the experimental variants (Table 1).

Table 1. The scheme of the experiment

No. of variants	Experiment variants	Abbreviations of names in variants in tables
1	Control	Control
2	Cobalt nanoparticles	NPCo
3	Compost (20 t ha^{-1})	Compost
4	Compost + Cobalt nanoparticles	C+ NPCo
5	Compost + Liquid-phase biological product 1%	C+ LPBP 1%
6	Compost + Liquid-phase biological product 2%	C+ LPBP 2%
7	Compost + Liquid-phase biological product 1%+ Cobalt nanoparticles	C+ LPBP 1%+ NPCo
8	Compost + Liquid-phase biological product 2% + Cobalt nanoparticles	C+ LPBP 2%+ NPCo

The spring barley variety 'Kati' was chosen as the object of the research. The mass of 1,000 grains is 46–56 g. It is grain fodder. The protein content is 10.9–14.5%. It is susceptible to root rot.

Sowing was carried out on May 2, 2020. The technology of growing spring barley in lysimeters mimicked the one generally accepted for gray forest soil in this region, but with some peculiarities of lysimetric research. On the plots, grooves were made 3–4 cm deep, at a distance of 12 cm, the seed rate was distributed at the rate of 55 grains per 1 running meter. In the phase, the pips was pulled in and 50 pieces were left per 1 running meter. Agrotechnology of growing crops is generally accepted for this zone. The growing season was 82 days. The barley was harvested in the full ripeness phase. Plants were cut at a height of 1–2 cm from the root collar, placed in bags indicating the variant number and repetition.

In the laboratory, the aboveground mass crop was dried to constant weight. After threshing the grain, the refined grain was weighed. Samples were taken from the crop by repetition, numbered, according to the registration journal, for sending to the laboratory. The mass of 1,000 seeds, grain moisture, nutritional value (crude protein, digestible protein, crude fat) were determined using standard determined by GOST methods.

Statistical processing of the results of the experiments was carried out by the method of variance analysis (Microsoft, Excel 2010). The shown significance is $p < 0.05$.

RESULTS AND DISCUSSION

Of greatest interest for the crop industry is the actual yield indicator - the volume of production per unit of sown area. In the research, the gross product yields for all variants of the experiment exceed the control value by 3.4–35.0%. The highest yield was obtained with pre-sowing treatment of barley seeds in a suspension of cobalt nanoparticles and a liquid-phase biological product at a concentration of 1% and the use of compost (variant 7) - 50.22 c ha^{-1}, which exceeds the indicator for the control variant by 35.0% (Table 2). Moreover, with the separate application of compost with a suspension of cobalt nanoparticles in the 4th variant and compost with a liquid-phase biological product in the same concentration (variant 5), the yield is significantly lower (41.25 c ha^{-1} and 41.42 c ha^{-1}, respectively). The improvement of plant growth and development could be facilitated by the formation of additional channels in seed cells under the influence of metal nanoparticles, which ensured the bioavailability of micro- and macroelements that make up LPBP. It should be noted that with an increase in the doses of the liquid-phase biological product to 2%, a significant decrease in the yield of barley was observed, this is demonstrated by the 8th variant of the experiment, the result is directly related to the inhibition of plant growth and the formation of caryopsis.

Table 2. The effect of cobalt nanoparticles and liquid-phase biological product on barley yield

No.	Experimental variants	Harvest of main products (c ha^{-1})		
		average	change	
			%	c ha^{-1}
1	Control	37.20	-	
2	NPCo	40.76	9.6	3.6
3	Compost	41.25	10.9	4.1
4	C+ NPCo	41.25	10.9	4.1
5	C+ LPBP 1%	41.42	11.3	4.2
6	C+ LPBP 2%	43.87	17.9	6.7
7	C+ LPBP 1%+ NPCo	**50.22**	**35.0**	**13.0**
8	C+ LPBP 2%+ NPCo	38.48	3.4	1.3
	$LSD_{0.95}$	1.96		

The main indicator of the quality of barley received for storage is its moisture content. The presence of liquid in the cereal is determined at the acceptance stage. Water makes changes in the grain, setting in motion a mechanism for activating the growth of microorganisms. The water content in the 'body' of the cereal suggests a lack of nutrients and a predisposition to shorten the shelf life expiration. An unacceptable level of humidity provokes the activation of chemical and physiological activity. The barley structure swells, the movement of enzymes turns on, the breakdown of biopolymer compounds, and the cereal germinates. The grain becomes friable, flowability and protection from machine impact are disturbed and the nature indicator falls. The level of moisture in the structure of the cereal negatively affects the biochemical and physical parameters that determine the food and market value of barley. Without violating the structural composition of carbohydrates, proteins and fats, it is impossible to remove chemically bound moisture. In this state, water is no longer a solvent, being molecularly linked to hydrophiles. Its removal will inevitably affect the change in the barley structure and its technological properties.

The limit value of the moisture content of barley grain established by the standards is 15%. For the presented samples, the parameter is in the range of 10.46–10.78% and characterizes its condition as dry, which will reduce energy costs for processing and storage, however, excessively dried grain is devoid of elasticity, because of which an increase in the ash content of flour is possible (Table 3).

Table 3. Physical and energy indicators of barley grain

No.	Experimental variants	Moisture, %	Exchangeable E, MJ	Feed units, kg
1	Control	10.46	12.0	1.17
2	NPCo	10.68	12.2	1.20
3	Compost	10.74	12.2	1.21
4	C+ NPCo	10.48	12.2	1.19
5	C+ LPBP 1%	10.70	12.2	1.22
6	C+ LPBP 2%	10.57	12.1	1.18
7	C+ LPBP 1%+ NPCo	**10.78**	**12.4**	**1.25**
8	C+ LPBP 2%+ NPCo	10.77	12.1	1.21
	$LSD_{0.95}$	-	-	0.02

Considering that barley is cultivated mainly as a fodder crop, it is important to assess the energy potential of the obtained samples. In all variants of the experiment, the value of the exchange energy indicator was practically at the same level - 12.0–12.2 MJ, slightly higher - in the variant with the use of C + LPBP1% + NPCo. In all experimental samples, the content of feed units in the grain was optimal (with an average standard value of 1.13), the highest indicator was 1.25 kg and was also detected in the 7th variant of the experiment.

Table 4. Nutritional value of barley grain

No.	Experimental variants	Crude protein, %	Crude fat, %	Digestible protein, g
1	Control	11.57	0.80	10.1
2	NPCo	12.81	1.10	10.2
3	Compost	12.16	1.10	10.2
4	C+ NPCo	13.05	1.01	10.4
5	C+ LPBP 1%	13.61	1.34	10.9
6	C+ LPBP 2%	13.39	0.96	10.7
7	C+ LPBP 1%+ NPCo	**13.99**	**1.43**	**11.2**
8	C+ LPBP 2%+ NPCo	12.10	1.03	10.1
	$LSD_{0.95}$	0.52	0.06	0.19

Crude feed protein is the main source of nitrogen for animals, which is necessary for reproduction, milk formation, and deposition in the body. For cereal grains, its content varies from 10% to 15% in 1 kg of feed. A deficit of even 1% of fodder protein in the ration of livestock leads to an overconsumption of 2–3.5% of feed and, ultimately, to an increase in production costs. The grain products obtained as a result of the experiment correspond to the average parameters for the culture, the maximum obtained value of the fraction of crude protein calculated on dry matter is 13.99% and is presented in the experimental version when using C + LPBP 1% + NPCo (Table 4).

Of great importance in the composition of animal feed is the content of crude fat in it, which optimizes the normal functioning of the digestive glands, forms the structure of the protoplasm of all cells and accumulates energy reserves in the body. According to the experimental variants, its share in the dry matter composition was 0.8–1.43% (with an average value for cereal grains of about 2%), the highest indicator was also in the 7th variant of the experiment.

The share of digestible protein in the samples ranged from 10.1 to 11.2% with the highest value in the 7th variant. Probably facilitated by the action of cobalt nanoparticles access to the cells of the macronutrients of magnesium and potassium, which are involved in the synthesis of fats and proteins, activate the work of enzymes that promote the formation of proteins and fats in the grain of the experimental culture in the variant with the use of C + LPBP1% + NPCo proteins and fats are the largest.

CONCLUSIONS

From the obtained data, it can be concluded that the pre-sowing treatment of barley seeds with cobalt nanoparticles and liquid-phase biological product does not have a toxic effect and contributes to an increase in yield. Preliminary soaking of spring barley seeds in a suspension of cobalt nanoparticles at a dose of 0.01 g per hectare of seeding rate and 1% solution of a liquid-phase biological product against the background of organic matter showed the best result, which led to an increase in yield by 13 c ha^{-1} (35%). At the same time, there was no decrease in the quality indicators: exchange energy and feed units. The amount of crude protein increased by 20.9%, digestible protein - by 10.9%, crude fat - by 78.8% compared to the control version of the experiment.

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Assessment of essential oil yield in three mint species in the climatic conditions of Central Russia

O.V. Shelepova[1,*], L.S. Olekhnovich[1], L.N. Konovalova[1,2], T.I. Khusnetdinova[3], A.A. Gulevich[2] and E.N. Baranova[1,2,*]

[1]N.V. Tsitsin Main Botanical Garden of Russian Academy of Sciences, RU127276 Moscow, Russia
[2]All-Russian Scientific Research Institute of Agricultural Biotechnology, RU127550 Moscow, Russia
[3]Faculty of Soil Science, Lomonosov Moscow State University, Leninskie Gory 1/12, RU119234 Moscow, Russia
[*]Correspondence: shov_gbsad@mail.ru; greenpro2007@rambler.ru

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Abstract. The aim of the study was to study the harvest time for the essential oil yield and its qualitative composition in three species of mint *Mentha piperita* L. (Peppermint), *M. spicata* L. (Spearmint) and *M. arvensis var. piperascens* Malinv. ex. Holmes (Sakhalinmint). In 2018, the research was performed with plants of second year of vegetation in the Laboratory of Plant Physiology and Immunity of the NV Tsitsin Main Botanical Garden of the RAS. As a result, it was found that the optimal harvest period for Sakhalinmint and Peppermint should be recommended in a phase of mass flowering: the yield of fresh raw materials was 509–479 g m^{-2}, air-dry raw materials - 110–107 g m^{-2}; the content of essential oil in the aboveground part (a mixture of leaves and inflorescences) of plants - 3.24–4.01%; the proportion of the main component of essential oil (menthol) - 57.3–50.2%. In Spearmint, the optimum time for harvesting is the phase of budding. The content of the main component of essential oil (carvon) was maximum - 67.9%, and the yield of essential oil was 2.6%, while the yield was 381 g m^{-2} of fresh raw materials (81.9 g m^{-2} of air-dry raw materials) at harvesting in this ontogenesis stage. Analysis of the secretory apparatus parameters on a surface of some green tissues in three mint species showed that the maximum density of secretory glands on both sides of the leaf is characteristic of peppermint, which provides a higher yield of essential oil in this type of mint. The study allowed determining the optimal harvesting time for highly productive mint species when they are grown in the conditions of Central Russia. The raw materials of these mint species can be used for the production of essential oils and are of interest for pharmacology and the perfume and cosmetics industry.

Key words: *Mentha piperita* L. (peppermint), *Mentha spicata* L.(spearmint) and *Mentha arvensis var. piperascens* Malinv. ex. Holmes (Sakhalinmint), harvest time, essential oil, secretory glands, chemical composition.

INTRODUCTION

Mint (*Mentha* L.) is one of the most important essential oil crops, widely used in the perfumes, cosmetics, pharmaceuticals, food industries, and medicine (Tucker, 2007; Taneja & Chandra, 2012; Anwar et al., 2019). Mint essential oil has antibacterial, fungicidal, analgesic, neurospasmodic, stimulating and other effects (Sujana et al., 2013; Brahmi et al., 2017; Kalemba & Synowiec, 2020). *Mentha piperita* L. (peppermint), *M. spicata* L. (spearmint) and *M. arvensis* var. *piperascens* Malinv. ex. Holmes (Sakhalinmint) are the most significant members of the *Lamiaceae* family (Lawrence, 2006).

M. piperita is an aromatic herb that is widely used in the cosmetic, confectionery, food and pharmaceutical industries and for the production of essential oils (Mahendran & Rahman, 2020). Peppermint leaves are commonly used in herbal teas and in cooking to add flavor and aroma. The resulting essential oil has astringent, antiseptic, antipyretic, antispasmodic, antimicrobial properties (Sujana et al., 2013; Kalemba & Synowiec, 2020). *M. arvensis* var. *piperascens* (Sakhalinmint) is a valuable medicinal plant that is cultivated in tropical and subtropical regions under irrigation (Singh & Saini, 2008). The characteristic odor and taste of this mint is due to its high menthol content (up to 75–80%) (Singh & Lal, 2020). The essential oil of these mint two species contains the same main components - menthol and menthone. They are among the most produced and sold essential oils in the world. The main producers of peppermint oil are India, United States and China, while the Sakhalinmint is grown in China, India, Brazil and Japan (Jeyakumar et al., 2011; Balakrishnan, 2015). Spearmint (*M. spicata*) is a valuable herb that is used fresh and dried in traditional medicine as a stimulant and adaptogenic agent. It is also widely used for the essential oil production, as the oil has antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties (Singh & Agarwal, 2013; Lukošiūtė et al., 2020). The distinguishing odor of spearmint oil is due to the main major component of the essential oil, carvone. In addition, this herb contains significant amounts of limonene, dihydrocarvone and 1,8-cineole (Hussain et al., 2010).

The production of essential oil by mint plants is mainly due to species characteristics, but it changes significantly under the influence of external environmental factors. To use the mint as an essential oil producer, the sum of the effective temperatures it is believed should be 3,200–3,400 °C for the growing season (Kirichenko, 2008). For the Central Russia region, in particular, for the Moscow region, according to long-term observations this indicator is 2,400–2,600 °C. So, the mint in this region is grown to obtain a pharmacy leaf. But the more frequent weather anomalies in recent years have led to a significant warming of the climate and an increase in the sum of effective temperatures in Central Russia (Pozdnyakov et al., 2019). Thus, if this indicator in the Moscow region exceeded the long-term average value by 1,144 °C in the abnormally hot summer of 2010, while in ordinary years (2015–2019) by 204.8–605.4 °C (Meteorological archive, 2020). Changes in climatic conditions can contribute to an increase in the productivity of mint biomass, and an increase in the yield and quality of essential oil, since air temperature is one of the main regulating factors during the period of intensive biosynthesis of essential oil (Clark & Menary, 1980). In recent years, the growing market demand for essential oil containing natural menthol for various industrial needs has led to an increase in the necessity for mint oil obtaining (Jain, 2017). The production can be increased through the development of high-yielding varieties and by expanding the growing area outside traditional cultivation areas through the development of new cultivation technologies (Rohloff et al., 2005; Aflatuni et al., 2006; Kapp et al., 2020). It is these factors that determine the expansion of the boundaries of the use of highly productive species and hybrids of mint of southern origin to obtain high-quality essential oil in Central Russia (Shelepova et al., 2011).

When grown in warm climatic conditions, a double harvest of green mass is possible, which significantly increases the yield of essential oil. Mint growing in Central Russia allows only one harvest (Shelepova et al., 2016). There is a lot of data on the effect of harvest time on the quality and quantity of mint oil when grown in warm climatic conditions (Singh & Singh, 1997; Santos et al., 2012). There is no information about the effect of the dates of harvesting the green mass of three mint species (Sakhalinmint, Peppermint and Spearmint) on the yield of essential oil in the conditions of the Central Russia in various studies performed. The aim of the study was to determine the dates of the phenological cycle, allowing obtain the maximum biomass (yield) of Sakhalin mint, peppermint and spearmint, as well as a high content of essential oil, to determine the change in the qualitative composition of plant essential oil.

MATERIAL AND METHODS

The three species of mint, mainly grown for the commercial production of mint essential oil: *M. arvensis* var. *piperascens* Malinv. ex. Holmes (Sakhalinmint), *M. piperita* L. (peppermint) and *M. spicata* L. (spearmint) were selected as objects of research. The source of origin and introduction of these species was discussed in detail (Shelepova et al., 2011; Shelepova et al., 2016). The taxonomic affiliation of the samples was carried out by the staff of the Herbarium of the Main Botanical Garden of Russian Academy of Sciences (MHA), later it was clarified with the involvement of herbarium samples of this variety from the SEINet and JSTOR database. The herbarium specimens are stored at the herbarium of the MHA.

The studies were carried out at the experimental site of the Laboratory of Plant Physiology and Immunity of the Main Botanical Garden of Russian Academy of Sciences (55°837′ N – 37°588′ E). Mint plants were propagated by means of runners and rhizomes. Plants were planted at the end of May in furrows at a distance of 10–15 cm from each other with a row distance of 40 cm. Cultivation was performed with additional irrigation during the growing season. The studies were carried out in 2018 on second year plants. Essential oil was obtained from an average sample of aerial parts (a mixture of inflorescences and leaves) of plants by hydrodistillation. A sample (up to 20 g) of air dried aerial parts was ground and then subjected to hydrodistillation for 2 h, according to the standard procedure described in the European Pharmacopoeia V (2004). The essential oil content is in% (v/w). The oils were stored in the dark until analyzed.

Qualitative composition of the oil was determined by gas chromatography at the Center for Collective Use of the Federal Research Center ‘Fundamentals of Biotechnology’, Russian Academy of Sciences, Moscow (RFMEFI62114X0002).

The identification of essential oil components was obtained by GC/MS. Analyses were carried out on a Shimadzu GS 2010 gas chromatograph with a GCMS-QP 2010 mass detector. The gas chromatography column was a using a SPB-1 nonpolar column (solid-stage-bound methyl silicone) (Supelco, Sigma-Aldrich) (30 m × 0.25 mm ID, 0.25 µm film thickness). The carrier gas was helium. The column oven temperature was set at 60 °C for 3 min, increased to 100 °C at a rate of 1.5 °C min^{-1}, increased to 180 °C at a rate of 4 °C min^{-1}, and held at 180 °C for 1 min. Next, it was increased to 200 °C at a rate of 10 °C min^{-1}, increased to 250 °C at a rate of 2.5 °C min^{-1}, and held at 250 °C for 5 min. Injector, interface and detector temperatures were 180 °C, 205 °C and 250 °C, respectively.

The gas chromatography mass analysis was carried out with the same characteristics as used in gas chromatography. A 1.0 μL sample was injected in the split mode, with a split ratio of 1:150. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Column oven temperature program was the same as in GC analysis. Helium was used as a carrier gas at a flow rate of 1.5 mL min^{-1}. Mass range was 30–400 m z^{-1}, while the injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. The percentage composition of the oils was computed by the normalization method from the GC peak areas, which were calculated as mean values of two injections of each oil sample, without using response factors. The identity of the components was assigned by comparison of their retention indices, relative to was assigned by comparison of their retention indices, relative to a mixture of linear paraffins consisting of nonane, decane, tridecane, and pentadecane.

Biometric analysis of glandular trichomes on mint leaves and flowers was carried out on fresh preparations using a Keyence VHX-1000E digital microscope (Itasca, IL, USA). There are mainly two types of secretory glands represented by peltate and capitate glandular trichomes on the surface of leaves and flowers. Identification of secretory glands using a digital microscope does not allow separating these two types of glands, so in our experiment we recorded the sum of peltate and capitate glandular trichomes (Fig. 1).

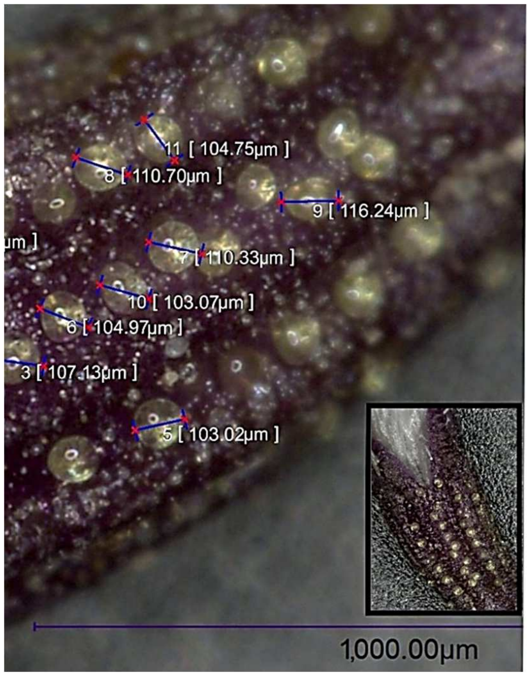

Figure 1. Light microscopy micrographs showing a method for measuring the diameter of the secretory glands of mint plants (bar = 1,000 μm).

Processing of experimental data was done by dispersion analysis using Microsoft Excel, with significance level $p \leq 0.05$.

RESULTS AND DISCUSSION

At the beginning of the plant growing season from late May to early July, all three species developed well-leafy bushes with 2–3 stems. The budding stage was beginning in the last decade of July; the mass flowering of spearmint and peppermint was in the last decade of August, and Sakhalinmint, in the first decade of September.

Studying productivity parameters revealed the lowest yield of raw materials was registered in the budding stage. At the same time, the green mass yield was maximum in Sakhalinmint both in the budding stage and in the mass flowering stage and amounted to 414.9–508.5 g m^{-2}. Respectively, in peppermint the yield was 5–6% lower, and in spearmint - by 8–21% lower than that of Sakhalinmint (Table 1). The productivity of fresh raw materials of Sakhalinmint significantly exceeded the yield of peppermint and spearmint only is due to a high proportion of stems in the total weight.

Table 1. Yield and essential oil content of three *Mentha* species at different stage of ontogenesis

Species	Green crops, g m^{-2}				Essential oil content,% (v/w)		Essential oil production, g m^{-2}	
	Fresh raw materials		Air-dried raw materials					
	Budding stage	Mass flowering stage	Budding stage	Mass flowering stage	Budding stage	Mass flowering stage	Budding stage	Mass flowering stage
Sakhalinmint	414.9	508.5	88.5*	110.3*	2.97	3.24	12.32*	16.48
Peppermint	392.9*	478.9	87.1*	106.5*	3.39	4.01	13.32*	19.20
Spearmint	380.7*	399.5	81.9	86.4	2.58	2.71	9.82	10.83
LSD (0.05)	2.9	3.0	0.9	1.0	0.11	0.12	1.10	1.17

Note: means bearing same * in each column are statistically similar at $p \leq 0.05$.

It should be noted that the yield of raw materials of spearmint did not differ in the budding stage and the mass flowering stage while that of Sakhalinmint and peppermint were the highest in the mass flowering stage. The increase in the yield of green biomass during harvesting in the mass flowering stage in Sakhalinmint and peppermint was by 22.6–21.9%, and in spearmint was by only 4.9%, respectively.

At the same time, there revealed the presence of significant differences between Sakhalinmint and peppermint in terms of yield of fresh materials in both phases of ontogenesis. The samples did not differ in the yield of air-dried raw materials. The total yield of fresh raw materials of spearmint in budding stage did not significantly differ from that of peppermint and was lower than the yield of Sakhalinmint. The productivity of fresh and air-dry raw material of spearmint in the mass flowering stage are significantly lower than those of peppermint and Sakhalinmint.

According to the data obtained and the results of other researchers, the total yield of fresh and air-dry raw material of Sakhalinmint and peppermint increased from budding stage to mass flowering stage. Cutting during mass flowering stage gave the highest biomass and essential oil yield (Rohloff et al., 2005; Zheljazkov et al., 2010). A similar result was also observed in spearmint grown in Northern Finland (Aflatuni et al., 2006). The main reserve of essential oil is formed exactly in the budding and mass flowering stage, so, the maximum yield of essential oil is observed in plants of almost all species of the genus *Mentha* L. in these stages of ontogenesis (Singh & Agarwal, 2013; Straumite et al., 2015; Shelepova et al., 2016; Kapp et al., 2020).

The content of essential oil in the air-dry mass of the above-ground parts of plants of three species of mint was from 2.58 to 3.39% in the budding stage and from 2.71 to 4.04% in the stage of mass flowering (Table 1). The maximum content of essential oil was recorded in the leaves and inflorescences of peppermint, and was 12.4–19.2% lower in Sakhalinmint. Spearmint had the minimum essential oil content, by 23.9–32.4% lower than, that of peppermint.

The yield of essential oil from the aboveground mass of plants in the mass flowering stage due to the higher yield and the maximum content of essential oil was the highest in peppermint (19.20 g m^{-2}), and 16.48 g m^{-2} in Sakhalinmint. Overall, in the mass flowering stage the essential oil yield was increased by 1.44 times in peppermint and by 1.34 times in Sakhalinmint in compared with the budding stage. And only spearmint had the same level of essential oil yield both in the budding stage and in the mass flowering stage - 9.82 and 10.83 g m^{-2}, respectively.

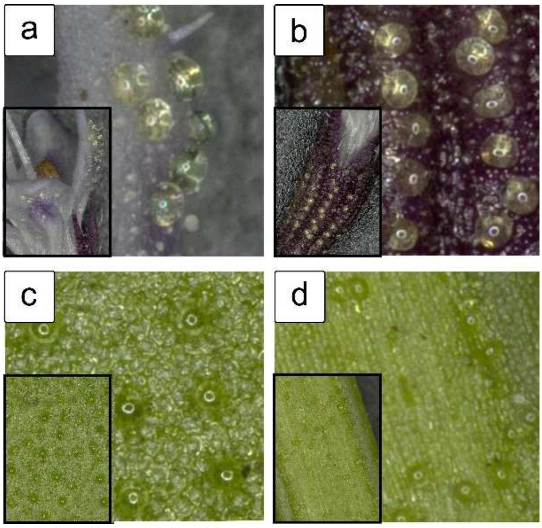

Figure 2. Microscopy micrographs showing the location of glands on the corolla (a), calyx (b), leaf (c) and stem (d) of peppermint plants.

It is known that essential oil, which is a complex mixture of secondary metabolites, is generated and stored in the secretory glands on all organs of the aerial parts of mint plants (stems, leaves, calyx, corolla) (Fig. 2). As the leaves and flowers grow and increase in size, new glands are formed. Moreover, their maximum number is located on flowers, leaves of the middle and upper tiers, and the minimum number is situated on plant stems. Direct observation of glands showed that the gland development is a fairly rapid process, accompanied by the biosynthesis of mono- and sesquiterpenes of essential oil (Tiwari, 2016; Yu et al., 2018).

The glands of the maximum size are recorded on the corolla and calyx of the Sakhalinmint flower - 90.3 ± 7.8 and 93.5 ± 8.8 µm, respectively (Table 2, Fig. 3). Glands of slightly smaller sizes were formed on the leaves, while in all studied species they were generally of the same diameter - 86.8 ± 3.2 - 87.1 ± 2.8 µm on the abaxial side and 86.4 ± 3.5 - 87.0 ± 2.9 µm on the adaxial side (Table 2, Fig. 4).

Table 2. Parameters of the secretory glands of the three *Mentha* species

Species	Diameter of glands on the corolla of a flower, µm	Diameter of glands on the calyx, µm	Diameter of glands on mint leaves, µm		Density of glands on mint leaves, pcs cm^{-2}	
			Abaxial side	Adaxial side	Abaxial side	Adaxial side
Sakhalinmint	90.3 ± 7.8	93.5 ± 8.8	87.1 ± 2.8	87.0 ± 2.9	1,381 ± 72	931 ± 66
Peppermint	87.4 ± 5.5	86.2 ± 6.7	87.0 ± 3.9	86.9 ± 3.2	1,485 ± 54	1,047 ± 65
Spearmint	85.4 ± 3.9	85.0 ± 4.5	86.8 ± 3.2	86.4 ± 3.5	1,032 ± 82	854 ± 61

The density of glands on the abaxial side of the leaves of the middle and upper layers during the mass flowering stage is by 1.21–1.48 times higher than on the adaxial side. The findings are consistent with those of Yu et al. (2018), which demonstrated higher density on the abaximal leaf surface in seven *Mentha* species.

The maximum density of secretory glands on surface with larger size glands recorded in peppermint resulted in a higher yield of essential oil in this species of mint.

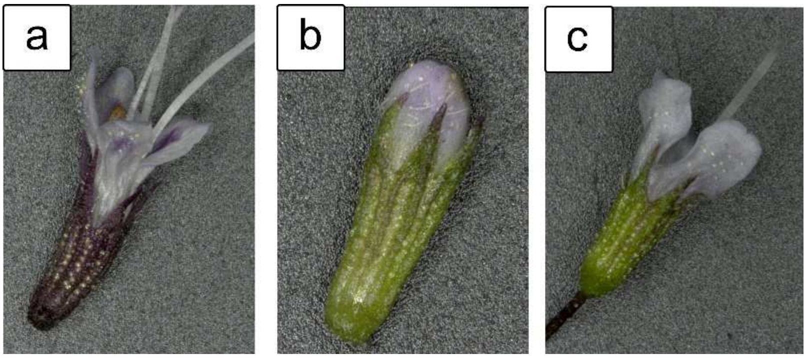

Figure 3. Micrographs showing glands on peppermint flower (a), Sakhalinmint flower (c) and spearmint bud (b).

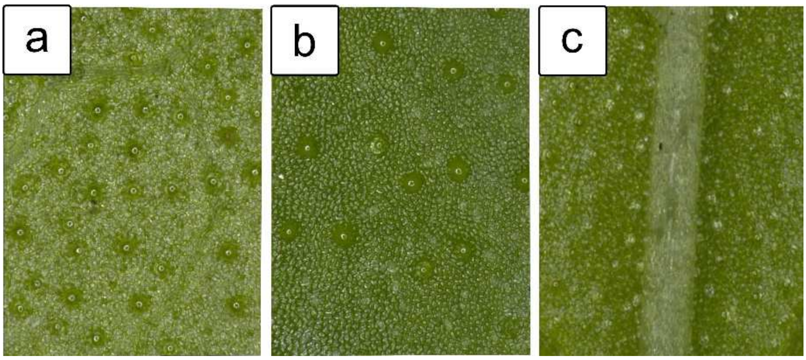

Figure 4. Microscopy micrographs showing glands on the abaxial side of a mature leaf from three species of mint: peppermint (a), spearmint (b), Sakhalinmint (c).

Up to 65 components were found in the composition of the essential oil of three mint species. All components with a content of more than 0.1% of the total amount were easily identified by retention time and mass spectra. According to European Pharmacopoeia (2005), the limits of 10 main components are established in the oil of both species. So, in peppermint oil, determined by gas chromatography, the limits of the content of various compounds were: menthol (30.0–55.0%), menthone (14.0–32.0%), isomenthone (1.5–10.0%), menthyl acetate (2.8–10.0%), menthofuran (1.0–9.0%), 1.8-cineole (3.5–4.0%), limonene (1.0–5.0%), isopulegol (max 0.2%), pulegon (max 4.0%) and carvone (max 1.0%). The limits of the content of these compounds in sakhalinmint oil were similar: menthol (30–50%), menthone (17–35%), isomenthon (5.0–13.0%), menthyl acetate (1.5–7.0%), 1,8-cineole (max. 1.5%), limonene (1.5–7.0%), isopulegol (1–3%), pulegone (max. 2.0%) and carvone (max. 2.0%).

The peppermint and Sakhalinmint essential oils obtained in field trials comply with the recommendations of European Pharmacopoeia (EP 5.0, 2005). It is maximal in the stage of mass flowering in both Sakhalinmint (up to 57%) and peppermint (up to 50%) (Table 3).

Table 3. Composition of essential oil of three *Mentha* species at different stages of ontogenesis

	Sakhalinmint		Peppermint		Spearmint	
	Budding stage	Mass flowering stage	Budding stage	Mass flowering stage	Budding stage	Mass flowering stage
α-pinene	0.20	0.06	0.33	0.29	0.42	0.23
β-pinene	0.46	0.10	0.27	0.32	0.69	0.38
sabinen	0.16	0.08	0.23	0.18	0.20	0.14
β-myrcene	0.64	0.19	0.39	0.25	0.85	0.56
D-limonene	1.83	0.62	1.79	0.95	9.21	6.23
1,8-cineole	0.38	0.29	0.24	0.22	2.37	2.27
menthon	7.80	6.56	10.21	8.97	0.15	0.19
isomenthon	18.92	19.75	19.17	18.51	0.24	0.28
menthyl acetate	8.11	13.20	8.27	18.89	0.70	0.72
isomenthol	1.11	1.17	1.08	1.17	t	t
menthol	52.82	57.30	48.37	50.18	2.15	2.26
pulegone	0.42	0.15	0.16	0.14	t	t
carvone	t	t	t	t	67.89	66.98
dihydrocarvone	t	t	t	t	7.17	10.38
dihydrocarvil	t	t	t	t	3.58	4.51

t – the compound is absent in the oil.

The content of major monoterpenes (menthol, menthone, isomenthone, methyl acetate) in the composition of Sakhalinmint and peppermint essential oils was 90%. But the composition of the essential oil of both mint species was slightly different from each other. Thus, the menthol content was 1.3 times higher in Sakhalinmint than in peppermint. At the same time, the content of menthol and its precursor menthyl acetate in Sakhalinmint and peppermint was higher during the period of mass flowering compared to the budding stage: in Sakhalinmint by 1.1 and 1.6 times, and in peppermint by 1.03 and 2.3 times, respectively. Most studies suggest that the content of menthol and menthyl acetate increases as the Sakhalinmint and peppermint plants develop to the mass flowering stage (Tiwari, 2016; Ostadi et al., 2020). The menthol / menthone ratio in the mass flowering stage increased in both species compared to the budding stage, while in Sakhalinmint it increased more significantly (from 8.05 to 10.26). According to Kalemba & Synowiec (2020), the menthone content of both mint species decreases with increasing menthol content. In peppermint, the maximum menthol content was reached after about 85 days from the start of the growing season to mass flowering. The menthol content in Sakhalinmint was increasing when the harvest was delayed up to 110 days after the start of the growing season.

It should be noted that Sakhalinmint and peppermint have a decrease in the amount of minor monoterpenes and terpenoids (α- and β-pinene, sabinene, D-limonene, 1.8-cineole, and a number of others), which have antifungal and insecticidal properties, during the stage of mass flowering.

Spearmint's major monoterpene are carvone and its derivatives - dihydrocarvone, carviol, dihydrocarvil, which account for up to 80% of the composition. In spearmint, the content of carvone slightly decreases, but the proportion of its derivatives, dihydrocarvone and dihydrocarvine, increases in the mass flowering stage. At the same time, the percentage of minor monoterpenes and terpenoids (α- and β-pinene, sabinene, β-mercene, D-limonene) was also decreased significantly.

CONCLUSION

Thus, the results show that the yield of green mass and the essential oil content depend on the mint species. It should be noted that the amount of oil in the leaves and inflorescences of all mint species studied in late August - early September was significantly higher compared to the beginning of August in the climatic conditions of Central Russia. The optimal harvest period for Sakhalinmint and Peppermint should be recommended in a phase of mass flowering: the yield of fresh raw materials was 509–479 g m^{-2}, air-dry raw materials - 110–107 g m^{-2}; the content of essential oil in the aboveground part (a mixture of leaves and inflorescences) of plants - 3.24–4.01%; the proportion of the main component of essential oil (menthol) - 57.3–50.2%. The yield of fresh raw materials was 509–479 g m^{-2}, air-dry raw materials - 110–107 g m^{-2}; the content of essential oil in the aboveground part (a mixture of leaves and inflorescences) of plants - 3.24–4.01%; the proportion of the main component of essential oil (menthol) - 57.3–50.2%. As for spearmint, carvone content was higher (67.9%) when harvested in the budding stage, while the fresh / air-dry raw materials (381/81.9 g m^{-2}) and essential oil yield (2.6%) were slightly lower than in the mass flowering phase - 400/86.4 g m^{-2} and 2.7%, respectively. Therefore, the optimal harvest time for this mint species should be the end of July - early August.

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Evaluation of morphological traits, genetic diversity and major resistance genes in barley subpopulations cultivated under organic and conventional farming systems

V. Šķipars[1,2,*], D. Ruņģis[2], I. Ločmele[1], S. Seile[1], I. Mežaka[1] and L. Legzdiņa[1]

[1]Institute of Agricultural Resources and Economics, 2 Zinatnes Str., LV-4126 Priekuli, Latvia
[2]Latvian State Forest Research Institute 'Silava', Genetic Resources Centre, 111 Rigas Str., LV-2169 Salaspils, Latvia
[*]Correspondence: vilnis.skipars@silava.lv

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Abstract. Most crop varieties currently grown in organic conditions have been bred for conventional farming, and are not adapted to increased environmental variability under organic farming conditions and unpredictable environmental fluctuations due to climate change. This can be mitigated by the use of heterogeneous material, increasing genetic diversity and enabling adaptation to local conditions. The objective of this study was to determine the effects of several generations of cultivation in parallel under organic and conventional farming systems on the genetic diversity, morphological traits and frequency of major disease resistance genes as indicators of adaptation to the farming system in heterogeneous spring barley populations with differing levels of diversity. Populations in differing generations originating from crosses between two, three, 10 and 15 parental genotypes were cultivated in organic and conventional farming systems for three, four or 10 generations, thus forming subpopulations in each environment. These subpopulations were genotyped, and tested for morphological traits in both farming systems. A significant effect of cultivation environment on tillering capacity ($p < 0.05$) was found for all tested populations and in several cases for plant height, ear length and grain number per spike, indicating some adaptation trends. In the short term, genetic diversity parameters were not decreased in the later generation populations in comparison to the initial populations with the exception of observed heterozygosity, as expected for a self-pollinating species. No clear differences in genetic diversity parameters between populations cultivated under either organic or conventional condition for several generations were identified.

Key words: genetic diversity, genotyping, heterogeneous populations, morphological traits, organic farming, spring barley.

INTRODUCTION

During the previous century, global dynamic changes have significantly affected society - changes in technology, societal structure and interests, food choice and many other aspects. These changes have increased demand for food, and led to the intensification of agriculture and plant breeding, which has had profound consequences for the environment. These include loss of biodiversity, climate change, changes in agricultural practices, diminishment of arable land area. To mitigate these challenges, paradigm shifts in agriculture (Döring et al., 2015), plant breeding (Litrico & Violle, 2015; Raggi et al., 2016), legislation etc. are required to develop new strategies to ensure sustainable agriculture and food security. While increased production volumes of organic farming systems is important within the wider agricultural sector, organic farming can also provide other benefits such as decreased use of chemicals, increased genetic diversity in farmers' fields, and enable more close connections between producers and consumers (Risku-Norja & Mikkola, 2009).

In previous decades, plant breeding has focused on creating high yielding varieties for a limited number of crop species, developed for high-input monocultural agricultural farming systems with highly controlled conditions including a limited number of weeds, high nutrient availability and feasible reduction of pests and diseases (Lammerts Van Bueren et al., 2011; Döring et al., 2015). Most crop varieties currently grown in organic conditions have been bred for conventional farming systems (Lammerts Van Bueren et al., 2011). In most cases, they have not been specifically adapted to the increased environmental variability under organic farming conditions as well as to unpredictable environmental fluctuations due to climate change. Therefore, genotypes selected for high-input farming systems may not have the capacity to buffer lower inputs and respond to novel stress factors, not only in low-input, but also in high-input agricultural systems (Murphy et al., 2007). Within the Baltic states, the most important traits for barley varieties used for organic farming include disease resistance (Bankina & Gaile, 2009), as well as yield and quality (Leistrumaitė et al., 2009). Therefore, Latvian barley breeding for organic farming systems is particularly focused on these traits.

To increase genetic diversity and the resilience of agricultural systems, cultivation of heterogeneous populations of self-pollinating species has been suggested. In contrast to the currently dominating uniform varieties, populations are subjected to natural selection and can respond and adapt to particular environmental conditions (Wolfe et al., 2008). Although cultivation of these heterogeneous populations in a single environment can be expected to decrease genetic diversity due to natural selection eliminating unsuitable genotypes and genetic drift, diversity is sustained by cultivation of populations in different environments, leading to differentiation of populations into subpopulations (Goldringer et al., 2001). Natural selection and competition among plants within a population affects genetic diversity and agro-morphological traits. Agro-morphological traits in populations have been shown to change due to natural selection in particular environments as rapidly as in four generations (Enjalbert et al., 2011). The first traits to respond to natural selection in cereals are earliness (Verhoeven et al., 2008; Rhoné et al., 2010; Raggi et al., 2016) and plant height (Enjalbert et al., 2011; Raggi et al., 2016). In case of disease pressure in the environment, plants with disease resistance prevail in the populations (Enjalbert et al., 2011).

A range of strategies are available for increasing the within-field genetic diversity in agricultural systems. One approach to create genetically diverse populations is by crossing varieties with each other and then optionally to pool the seed from a number of crosses. Previous studies with populations created by crossing as little as two parents (simple populations) (Ločmele et al., 2017; Mežaka et al., 2017), and populations arising from cross combinations using several parents (complex populations) have been reported

(Ločmele et al., 2019). Creation of composite cross populations (CCPs) involving bulks of diallel crosses between a larger number of parents was first described by Harlan & Martini (1929), and they have been used in various studies of genetic and agro-morphological diversity in barley and wheat since then (Harlan & Martini, 1938; Suneson, 1956).

A limited number of studies have investigated population evolution in organic and conventional farming systems. Winter barley populations maintained for 13 generations and screened by molecular markers showed adaptation to particular growing environments (Raggi et al., 2017). In a number of studies of winter wheat populations created in the UK (Döring et al., 2015), different conclusions have been made. In research performed over 11 generations on adaptation of populations to particular farming system, no changes in allele frequencies were reported (Knapp et al., 2013). In a study of changes of weed competitive traits in populations, early vigour was improved after five years of cultivation in organic conditions in comparison to conventionally grown populations (Bertholdsson et al., 2016). Comparison of seedling traits in populations grown in parallel in organic and conventional conditions over ten years found that CCPs cultivated in organic conditions had longer, thicker and deeper root systems, indicating the ability to uptake nutrients from deeper soil layers, whereas in the CCPs cultivated in conventional conditions, almost no changes were observed (Vijaya et al., 2019). Assessment of the populations after five years cultivation in conventional and organic conditions showed that changes in number of tillers, kernel weight and kernel number were not affected by the generation or cultivation conditions (Bertholdsson et al., 2016) and that, to maintain sufficient diversity, the population size should be not less than 12,000–15,000 individuals (Brumlop et al., 2019). Yield stability depends on the genetic background of the populations: in conventional conditions, the population containing a high yielding genetic background was more stable, but in organic conditions the population containing a broader genetic background provided better stability (Weedon & Finckh, 2019). Our previous study on simple populations of spring barley over the course of six generations cultivated in organic and conventional conditions did not detect any differences in genetic diversity or allele frequencies (Mežaka et al., 2017). The effect of cultivation in conventional and organic growing conditions on CCP grain quality and plant morphology has also been studied in wheat and no significant effect of farming system on these traits has been reported (Brumlop et al., 2017).

The objective of this study was to determine the effects of several generations of cultivation in parallel under organic and conventional farming systems on the genetic diversity, morphological traits and frequency of major disease resistance genes and adaptation of the population to the farming system in three types of heterogeneous populations - simple (2 parents), complex (3–4 parents) and composite cross populations (diallel crosses of 10–15 parents).

MATERIALS AND METHODS

Plant material

Three types of spring barley (*Hordeum vulgare* L.) populations (based on number of parents used for crosses and crossing methods) were used in this study: (1) simple populations (SP), created by crossing two parents, (2) complex populations (CP) involved cross combinations consisting of three to four parents and (3) composite cross populations (CCP) involved bulked diallel crosses between either 10 parents (CCP1 and CCP2) or five male sterile parents crossed to 10 pollinators each (CCP3). Two simple populations, two complex populations and three composite cross populations were used in the study (Table 1). Simple and complex populations originated from the breeding program for organic farming and were maintained after the selection of individual plants for breeding purposes was done, but CCPs were created for this study. A more detailed description of the simple populations P/I and A/Dz can be found in Mežaka et al. (2017).

Table 1. Population characteristics

Population denomination	Type of population	Pedigree/parents, year of crossing/ seasons of cultivation[1]	Analyses and generations[2]
P/I	Simple	Primus/Idumeja, **2004/10**	Morphological, SSR (F_{10}, F_{13})
A/Dz	Simple	Anni/Dziugiai, 2004/10	Morphological, SSR (F_{10}, F_{13})
CP-1	Complex	Irbe/PR-5108//PR-5999+PR-6000 (hulless barley), 2011/3	SSR and *mlo11* (F_4, F_6)
CP-2	Complex	KZ14-52/PR-4121//PR-4311, **2011/3**	Morphological and *mlo11, Mla18* (F_3, F_6)
CCP-1	Composite cross	Bulked dialell crosses among group of 10 parents: PR-4814, PR-5105, PR-3605, PR-5135, PR-4871, PR-5279, BZ-14-92, 'Balga', 'Jumara', 'Mik 1', **2013/3**	Morphological, SSR and *mlo11* (F_2, F_4)
CCP-2	Composite cross	Bulked dialell crosses among group of 10 parents:PR-5506, PR-5228, PR-5779, PR-6000, PR-5415, No.51, Irbe, Pirona, CDC Freedom, CDC Fibar (hulless barley), **2013/3**	SSR and *mlo11** (F_2, F_4)
CCP-3	Composite cross	Bulked crosses of 5 male sterile parents each crossed to 10 pollinators: PR-4814, PR-5105, PR-3605, PR-4181, PR-4121, PR-4311, PR-5137, BZ14-12, Golf, Mik-1, **2013/3**	SSR and *mlo11, Mla18* (F_2, F_4)

[1]number of seasons of cultivation in parallel under organic and conventional environments, including 2016; [2]two generations, in which genotyping was performed; * no data available for F_4 conventional subpopulation.

Initial multiplication was done as follows: F1 seeds of simple and complex populations were multiplied under conventional growing conditions, F2 were multiplied in a winter nursery in Chile, and F3 - under organic conditions. For CCPs, F1 seeds from diallel crosses were bulked in equal numbers from each combination and multiplied in a winter nursery in Chile. After initial multiplication, seeds of the F2 generation for CCPs and F4 for the simple and complex populations were divided in two subpopulations and cultivated in parallel under organic and conventional farming systems for a number of generations (3, 4 or 10) (for details about each population, see Table 1), thus forming organic and conventional subpopulations (hereafter indicated with 'O' and 'C'). Subpopulations were then tested in both organic and conventional farming systems to determine if adaptive changes could be identified (Fig. 1).

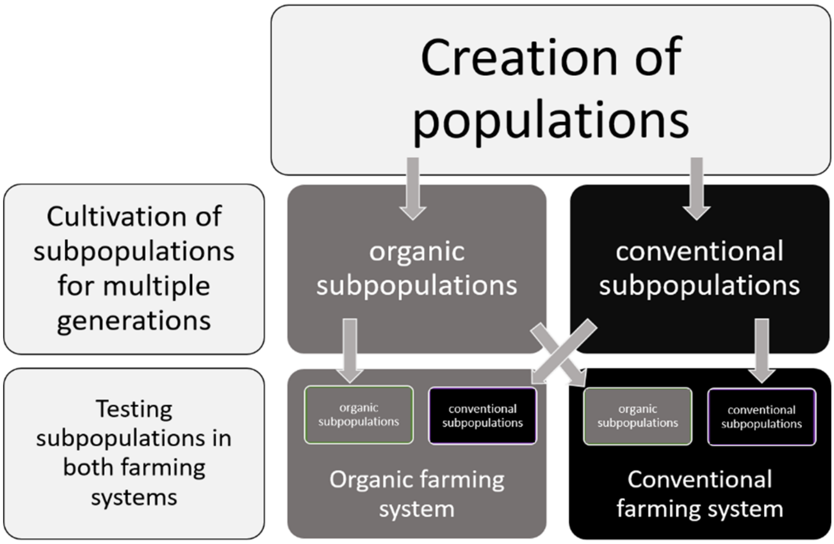

Figure 1. Schematic representation of experimental scheme.

Cultivation conditions

The cultivation of populations was carried out at the Institute of Agricultural Resources and Economics, Priekuli Research Centre (latitude 57°18'53.28" N, longitude 25°20'19.68" E, 120 m a.s.l.) between 2004–2016, under both conventional and organic crop management systems. The soil in all locations was sod-podzolic sand loam (Karklins, 2008). Minimum and maximum values of other soil properties are summarized in Table 2. Pre–crop in all C environments was potatoes, but in O environments - green manure or grain legumes. No fertilizers were used in O sites. In C sites mineral fertilizers were applied before sowing in order to obtain 5 t ha^{-1} grain yield, according to the results of soil property indicators. The amount of pure elements was: nitrogen (N) 80–95, phosphorus pentoxide (P_2O_5) 40–60, and potassium oxide (K_2O) 40–90 kg ha^{-1}. To restrict weeds in C sites, herbicide was applied, but in O sites harrowing at tillering (BBCH 21–29) was performed. No fungicide was applied in any of the cultivation years, but insecticide to restrict aphids (*Sitobion avenae* Fabr.) was applied in some years in C sites.

Table 2. Range of soil agrochemical properties in population cultivation fields during 2004–2016

Properties	Farming system	
	conventional	organic
pH KCL	5.3–6.4	5.1–6.0
Organic matter %	1.7–2.7	1.7–2.4
K_2O mg kg^{-1}	136–240	75–175
P_2O_5 mg kg^{-1}	133–196	90–208

Meteorological conditions are summarized in Fig. 2 and presented according to the periods of cultivation: 2005–2016 for simple populations, 2011–2016 for complex populations and CCPs, and 2016, the year of sampling for genotyping and assessment of plant morphological traits. The weather conditions varied between 10-day periods in 2005, 2008, 2014 and 2015, when after rainy periods there was drought and vice versa. In some growth stages, the temperature delayed or accelerated plant development. Weather conditions were extreme in the year of phenotypic assessment (2016), when, after increased rainfall at the end of April, which delayed sowing, only 18% of the normal precipitation fell in May, delaying the development of plants. There was increased rainfall starting with the middle of June up to the end of August, peaking during the last 10-day period of June, when rainfall was 209% higher than the long-term average for this period. During this period, it was warmer than usual - the average air temperature surpassed the norm by 4.5 °C.

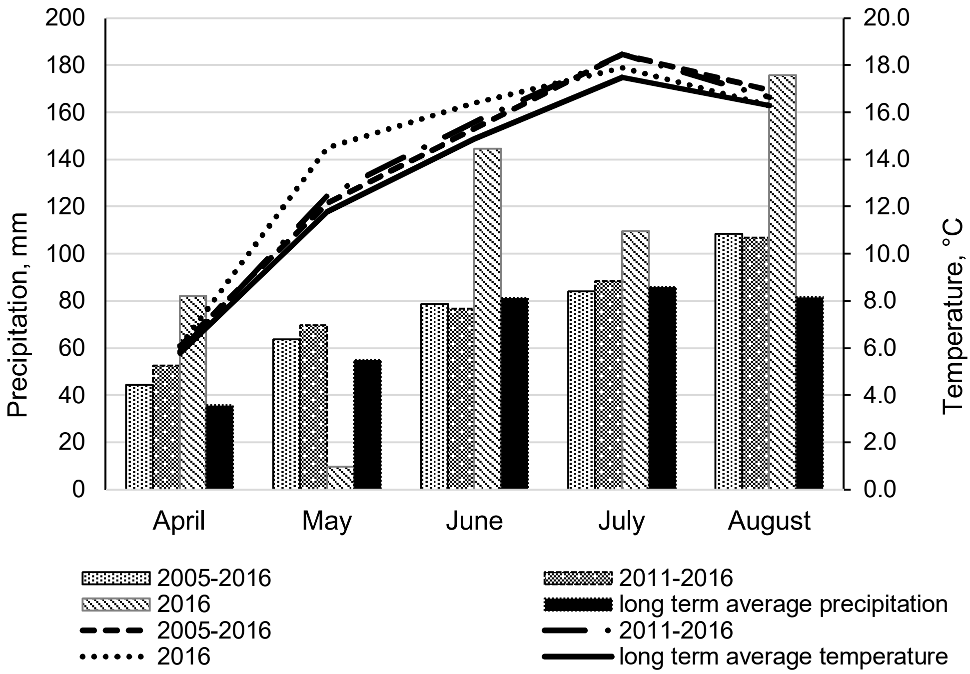

Figure 2. Sum of precipitation (bars) and average air temperature (lines) during barley vegetation periods in 2005–2016, 2011–2016, 2016 and long-term average over 1981–2010.

Phenotyping

Differences in plant morphology due to cultivation environment (O and C) were assessed for subpopulations of P/I, A/Dz, CP-2 and CCP-1. In 2016, both subpopulations (O and C) of each population were sown in both O and C sites next to each other in 12.3 m^2 plots, with a seed rate of 400 untreated seeds per m^2. Plant height, main ear length, number of productive tillers per plant (tillering) and number of grains in the main ear were measured in 100 randomly chosen plants per each subpopulation, collected during the maturity stage (BBCH 90).

SSR genotyping

Plant shoot samples from 96–100 randomly chosen individuals per population/subpopulation were collected for SSR marker genotyping. Each population was genotyped in two different generations: simple populations P/I and A/Dz (F_{10} and F_{13}), complex population CP-1 (F_4 and F_6), and composite cross populations CCP-1, CCP-2 and CCP-3 (F_2 and F_4) (Table 1). The initial generations for CP-1 (F_4) and the CCPs (F_2) were genotyped prior to dividing them into two subpopulations for cultivation under organic and conventional conditions, therefore these initial generations are not split by cultivation conditions. DNA was extracted using the modified protocol of Edwards et al. (1991). Nine simple sequence repeat (SSR) markers were multiplexed in three marker sets: set A (Bmag0125, Bmac0067, Bmac0032), set B (Bmag0135, WMC1E8, Bmag0173) and set C (Bmag0353, Bmac0093, Bmac0156) (Macaulay et al., 2001). The forward primer was synthesized with a 6-FAM, HEX or NED fluorescent label to allow visualisation of amplification products on a genetic analyser. Multiplex polymerase chain reactions (PCRs) were performed in a total reaction volume of 20 µl containing ca. 50 ng DNA, 4 µl 5x HOT FirePol® Blend Master Mix (Solis BioDyne, Estonia, final magnesium chloride ($MgCl_2$) concentration 2 mM), 0.2 µM of forward (labelled) and reverse primers. PCR conditions were as follows: 95 °C for 15 min, 40 cycles of 95 °C – 20 s, 58 °C (set A and B) or 60 °C (set C) - 40 s, 72 °C - 60 s, 72 °C - 10 min. PCR products were separated on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Waltham, USA), and genotyped using GeneMapper 4.0.

Major disease resistance gene genotyping

Markers linked to two major resistance genes (*Mla18, mlo11*), conferring resistance to barley powdery mildew (*Blumeria graminis* (DC.) Speer), were genotyped in populations where they were known to be present in the parental genotypes. The *Mla18* gene was genotyped in two populations, and the *mlo11* gene was genotyped in five populations (Table 1). PCR primers and conditions for *mlo11* and *Mla18* resistance gene analyses have been described previously (Kokina & Rostoks, 2008; Piffanelli et al., 2004). Briefly, PCR was performed in a 20 µl reaction consisting of ca. 50 ng DNA, final concentration of each primer - 0.5 µM, 1 x reaction buffer (Thermo Fisher Scientific, Lithuania), 2.5 mM $MgCl_2$, 0.2 mM dNTPs and 1 U of Hot Start *Taq* polymerase (Thermo Fisher Scientific, Lithuania). PCR products were visualized by agarose gel electrophoresis.

Statistical analysis

The group means and T-tests for plant morphological characters among populations were calculated in R version 4.0.3 (Team), 2013). Statistical differences of means of morphological traits of subpopulations grown at the same testing environment were calculated with Two Sample T-test if variances among groups were equal and Welch Two Sample t-test if variances were not equal ($p < 0.05$). Genetic diversity parameters calculated using GenAlEx 6.5 (Peakall & Smouse, 2012). In summary, total number of alleles and total number of alleles with a frequency over 0.05 were summed over all nine analyzed loci. The observed heterozygosity (Ho) (no. of heterozygous loci/number of samples), expected heterozygosity (He) ($He = 1\text{-}\Sigma p_I^2$, where p_i is the frequency of the

i-th allele), number of effective alleles (Ne) (Ne = 1/(1-He)), mean information index (I) ($I = -\Sigma p_i \ln p_i$) and were calculated for each locus, and subpopulations compared using a T-test. Matching multilocus genotype groups (shared by 2 or more individuals) were identified within each sub-population. Only individuals with exactly matching multilocus genotypes (including missing data) were considered to be included into one group.

RESULTS AND DISCUSSION

Populations respond to selective pressures in different environments by adaptation and evolution (Vijaya et al., 2019; Bocci et al., 2020). However, this is dependent on the presence of genetic and phenotypic diversity, and is influenced by mating system, selective pressure and other factors. This study assessed genetic and morphological changes in CCP, simple and complex barley populations cultivated in both organic and conventional farming systems for multiple generations, thus forming subpopulations in each environment. Subpopulations of four selected populations were then phenotyped in both organic and conventional conditions to assess if O subpopulations were more adapted to organic growing conditions than C subpopulations and vice versa.

Morphological traits

The effect of cultivation system on plant height was significant in four cases. Average plant height was significantly larger for the O subpopulation compared to the C subpopulation in both testing systems for the A/DZ population, as well as in the conventional testing system for the CP-2 population, but was lower in the organic testing system in the CCP-1 population (Fig. 3). Within the organic testing system, significant differences between average ear length in O and C subpopulations were observed for the population CCP-1 and, within the conventional system - for the population P/I (in both cases the average ear length was larger in the conventional subpopulation). Within the organic testing system, tillering differed significantly ($p < 0.05$) between all subpopulations; for CCP-1 the difference was also significant when tested in the conventional system. In the simple populations P/I and A/DZ, more tillers were found in conventional subpopulations, however the CP-2 and CCP-1 subpopulations had an increased number of tillers in organic subpopulations. Number of grains per ear did not significantly differ among subpopulations with the exception of the conventional testing system for the P/I population ($p < 0.05$) (Fig. 3). Coefficients of variation (data not shown) were generally higher for tillering (mean over the populations 38% while tested in O system and 25% in C system) and lower for plant height (12% and 10%, respectively) and with a trend of being higher under O testing system (mean over all traits 22% and 19%, respectively). No constant trends comparing variation of O and C subpopulations were identified.

One of the four measured plant traits, tillering, had significantly different mean values between both subpopulations of all four tested populations (also interpretable as effect of the cultivation environment) when tested in organic conditions. Subpopulations tested in conventional conditions showed no significant differences between number of tillers in simple and complex populations, but were significantly different in the CCP.

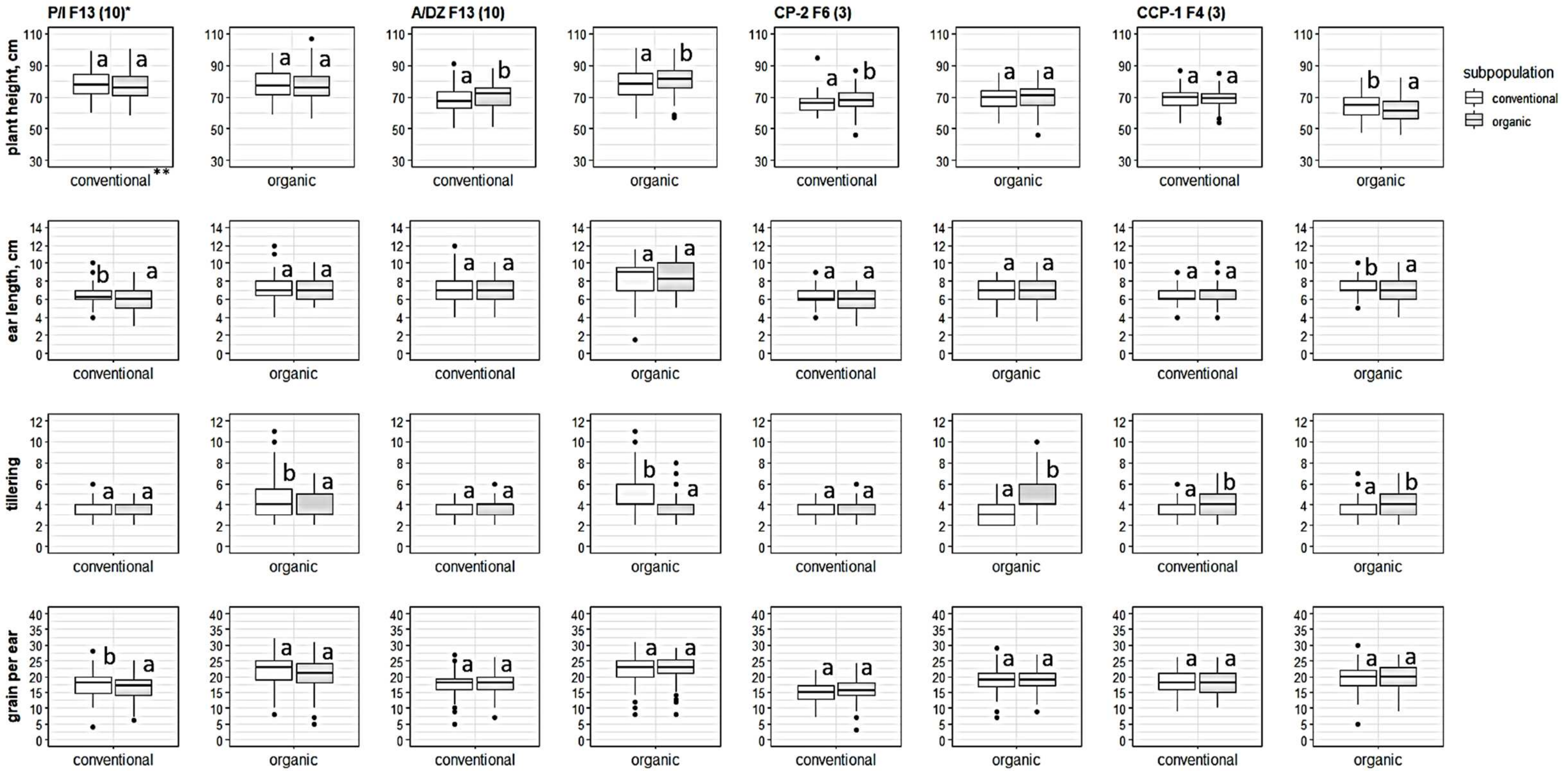

Figure 3. Plant height, ear length, number of tillers per plant, grain number per ear in organic and conventional subpopulations of four barley populations tested in both farming systems. Subpopulations tested in the given testing system indicated with the same letter do not differ according to *t*-test ($p > 0.05$). Horizontal lines within the boxes represent the median values, vertical lines indicate the 95% confidence interval of the median. The boxes represent first and third quartile boundaries. The dots represent outliers. *number in brackets represent number of seasons of cultivation. For description of populations refer to Table 1. ** refers to testing system.

The number of tillers was reduced in the simple organic subpopulations if compared to the conventional ones, while it was increased in the organic subpopulations of complex populations and CCP. It is possible that cultivation of the complex population and the CCP under organic farming conditions over a short term (three seasons) promoted multiplication of plants with better tillering capacity. However, in the case of simple populations, which were cultivated under the respective farming systems for a longer period, the opposite reaction was observed, which might be explained either by the specific reaction of the few genotypes involved (two instead of 10 for CCP) or by the effect of a longer cultivation time. Additional analyses planned for CCPs in F_9 can clarify this. But it should also be taken into account that tillering capacity is a trait with comparatively low heritability, affected to a greater extent by environmental conditions (Mežaka, 2018) which might also cause the observed differences. In contrast to our results, Brumlop et al. (2017) did not identify differences in tillering capacity in three wheat CCPs cultivated for six generations in conventional and organic farming systems.

Plant height is one of the first traits reported to respond in populations cultivated in differing conditions (Enjalbert et al., 2011; Raggi et al., 2016). Natural selection under organic conditions resulted in significantly shorter plants in the CCP-1 subpopulation compared to natural selection under conventional conditions, but resulted in taller plants in the simple population A/Dz cultivated in organic conditions compared to conventional conditions, for a comparatively longer time. The O subpopulation of CP-2 had increased plant height, which was significantly different under conventional testing conditions compared to the C subpopulation. The results indicate that a longer cultivation period (10 seasons) under organic environments can cause an increase in plant height, but over a shorter period (three seasons), plant height can either be increased or decreased. One of the reasons causing natural selection of taller plants could be the weed pressure present in the organic crop management system. With respect to mean ear parameters, shorter ears with less grain per ear were found in the O subpopulation of P/I tested in conventional conditions. However, Bertholdsson et al. (2016) and Brumlop et al. (2017) reported no significant differences in plant height and ear length in three wheat CCPs studied for six generations in conventional and organic farming systems. We can see a trend that differences between the subpopulations are more pronounced when tested in organic farming systems, compared to conventional systems, which can be explained by the smoothing effect of conventional management, ensuring an exogenous supply of nutrients and reducing biotic stress factors. This also confirms that adaptation of populations to specific growing conditions is more relevant for organic farming systems.

Brumlop et al. (2017) have questioned whether contrasting weather conditions over years influence the performance of populations more than differences in farming system. Over the course of our study, weather conditions varied between years and multiple deviations of temperatures and precipitation from long-term averages were observed. However, in this study, cultivation sites were close to each other, and both subpopulations received identical selection pressure in respect to weather conditions, therefore further studies are needed to confirm this.

Genetic analysis

The expected heterozygosity (He) and standard error (*SE*) was calculated over all analysed samples for each marker. The avearage values for each marker were - Bmag0125 (0.494, *SE*: 0.093), Bmac0067 (0.535, *SE*: 0.017), Bmac0032 (0.651, *SE*: 0.071), Bmag0135 (0.350, *SE*: 0.162), WMC1E8 (0.295, *SE*: 0.142), Bmag0173 (0.543, *SE*: 0.231), Bmag0353 (0.551 *SE*: 0.040), Bmac0093 (0.539, *SE*: 0.064), Bmac0156 (0.454, *SE*: 0.209). Expected heterozygosity is equivalent to Polymorphism Information Content (PIC) for inbred lineages (Serrote et al., 2020), and the He/PIC values indicated that the markers were sufficiently informative for analysis of this germplasm. Markers with He/PIC values larger than 0.5 are classified as very informative, and with values between 0.25 and 0.50 as somewhat informative (Botstein et al., 1980).

Changes in genetic diversity between generations within populations

In some cases, the mean number of alleles within each genotyped population increased in advanced generations, compared to the initial generations genotyped. However, this was due to an increase of low frequency alleles, with a frequency of less than 0.05. No significant differences in the number of effective alleles or information index were observed between samples of one population taken from different generations. The observed heterozygosity was significantly ($p < 0.05$) increased in advanced generations in one of the simple (A/Dz) and the complex population CP1 subpopulation grown under organic conditions. A significant ($p < 0.05$) decrease in observed heterozygosity was observed in the advanced generations of all CCP populations as well as in the CP1 subpopulation grown in conventional conditions (Table 3). The number of matching multilocus genotype (shared by two or more individuals) groups is an indicator of the homogenization of the populations. As barley is predominantly self-pollinating, distinct lines sharing matching multilocus genotypes will be formed within populations. A larger number of matching multilocus genotype groups indicates a decrease in diversity within populations, as the number of genetically distinct individuals is decreased. Conversely, if all individuals within a population are genetically distinct, then no matching multilocus genotype groups are formed. In most subpopulations, there was not a substantial change in the number of multilocus genotype groups between initial and advanced generations. The exception was in the A/Dz conventional cultivation subpopulations and the CP1 advanced organic cultivation subpopulation. In both cases, there was a lower number of groups in the advanced generations, which corresponds to the previously reported increase in heterozygosity for these subpopulations. In the CCP populations, where a significant decrease in observed heterozygosity was observed between initial and advanced populations, the number of matching multilocus genotype groups was low, reflecting the increased complexity of the populations, involving crosses between a large number of parental varieties.

Changes in genetic diversity due to cultivation under different farming systems

In general, no significant differences in genetic diversity indices were observed between pairs of subpopulations cultivated in organic and conventional conditions. The observed heterozygosity was significantly ($p < 0.05$) increased in the complex population CP1 cultivated under organic conditions compared to conventional, but other genetic diversity indicators were similar between these two subpopulations (Table 3).

Table 3. Genetic diversity parameters

Population	Sub-population	N (generation)		Total Na		Total Na ($f > 0.05$)		Mean Ne (*SE*)		Mean I (*SE*)		Mean Ho (*SE*)		No. of MMGs	
		I	Ad	I	Ad	I	Ad	I	Ad	I	Ad	I	Ad	I	Ad
P/I	O	95 (F_{10})	100 (F_{13})	27	37	16	17	1.633 (0.173)	1.829 (0.143)	0.525 (0.120)	0.713 (0.092)	0.012 (0.011)a	0.009 (0.002)a	15	14
	C	96 (F_{10})	100 (F_{13})	31	34	15	17	1.731 (0.165)	1.818 (0.149)	0.593 (0.106)	0.676 (0.105)	0.023 (0.014)a	0.002 (0.002)b	21	21
A/Dz	O	95 (F_{10})	100 (F_{13})	32	28	16	17	1.779 (0.192)	1.707 (0.159)	0.598 (0.140)	0.571 (0.114)	0.035 (0.019)a	0.185 (0.072)b	12	8
	C	96 (F_{10})	100 (F_{13})	17	15	26	15	1.649 (0.163)	1.676 (0.166)	0.466 (0.117)	0.508 (0.116)	0.005 (0.005)a	0.309 (0.081)b	25	6
CP-1	O	96 (F_4)	100 (F_6)	31	28	26	23	2.357 (0.333)	2.066 (0.281)	0.887 (0.146)	0.740 (0.140)	0.087 (0.015)a	0.422 (0.110)b	12	2
	C		100 (F_6)		23		18		1.795 (0.235)		0.562 (0.142)		0.010 (0.003)c		16
CCP-1	O	96 (F_2)	100 (F_4)	39	42	34	34	2.929 (0.395)	2.914 (0.330)	1.141 (0.128)	1.164 (0.122)	0.299 (0.044)a	0.075 (0.013)b	1	3
	C		100 (F_4)		39		34		2.941 (0.334)		1.162 (0.119)		0.074 (0.009)b		3
CCP-2	O	96 (F_2)	100 (F_4)	42	45	32	34	2.822 (0.508)	2.757 (0.446)	1.085 (0.154)	1.117 (0.140)	0.262 (0.047)a	0.137 (0.022)b	3	2
	C		100 (F_4)		41		34		2.667 (0.425)		1.080 (0.133)		0.079 (0.016)b		1
CCP-3	O	96 (F_2)	100 (F_4)	44	42	33	33	2.955 (0.405)	2.945 (0.395)	1.155 (0.144)	1.133 (0.141)	0.269 (0.043)a	0.081 (0.021)b	2	4
	C		100 (F_4)		45		34		3.112 (0.417)		1.193 (0.143)		0.079 (0.018)b		1

I – initial; Ad – advanced; O – organic; C – conventional; N – number of analyzed individuals; Na – number of alleles; Ne – number of effective alleles; I – information index; Ho – observed heterozygosity (values with different letters are significantly different ($p < 0.05$)); *SE* – standard error; MMGs - matching multilocus genotype (shared by 2 or more individuals) groups.

The observed heterozygosity was significantly ($p < 0.05$) higher in the in the P/I simple population advanced organic subpopulation compared to the advanced conventional subpopulation, however, average observed heterozygosity over the nine SSR loci was very low in both subpopulations (0.009 vs 0.002, respectively). No significant differences in genetic diversity indices were observed between the CCP subpopulations maintained under organic and conventional conditions for two seasons.

Genetic analysis of the simple populations in the 1st genotyping round (F_{10}) used in this study have been reported previously (Mežaka et al., 2017). The parental varieties of the complex and CCP populations were not genotyped, therefore the initial number of alleles in these types of populations was not known. As expected, overall population genetic diversity increased from the simple, to complex and CCP populations. However, analysis using SSR markers did not detect a proportional increase in genetic or allelic diversity compared to the number of parents used in the creation of these populations. The simple populations were created using two parental varieties, while the CCPs each had 10 parental varieties. Given the larger number of parental varieties, particularly for the CCPs, there was only a modest increase in the total number of alleles. This is probably a reflection of the lack of the genetic diversity of the parental material, where the parental varieties of one population share similar alleles. The total number of alleles slightly increased in most populations genotyped in advanced generations, compared to the initial generations. This increase was due to low frequency alleles, and may be due to stochastic fluctuations, or low levels of outcrossing or other sources of contamination. This reflects the low selection pressure on the populations, and the relatively large population sizes ensured that genetic drift did not affect the major allele composition over the relatively low number of generations. The observed heterozygosities in most of the analyzed populations had decreased after 2–3 generations, which is a result of the self-pollinating nature of barley, and the lack of selective pressure, particularly on heterozygous loci. As barley is a predominantly self-fertilizing species, it is not expected that the genetic diversity of the populations would be considerably increased by cross-pollination. Observed heterozygosity was significantly increased in advanced generations of two populations (A/Dz and CP1). The reason for the increase in observed heterozygosity in the two populations is not clear, and further investigation of these populations is needed (e.g. by genotyping of additional generations of these populations). CCP-3 did not have higher levels of observed heterozygosity or other genetic diversity indicators, despite having five male sterile parents, which would be expected to increase the observed heterozygosity due to increased cross pollination.

The maintenance of heterozygosity and the generation of new allelic combinations by recombination is dependent on outcrossing rates. While barley is a predominantly self-pollinating species, a number of studies have shown that outcrossing rates in barley are usually less than 1% (Kahler et al., 1975; Doll, 1987), but can be up to 5% in winter barley varieties (Doll, 1987). In this study, in most cases, the observed heterozygosity decreased in advanced generations, and no direct estimation of outcrossing rates was done. The rapid decrease in observed heterozygosity observed in the CCPs compared to other population types is due to the initial generations analyzed for each type of population - F_2 for the CCPs compared to F_{10} for the simple populations. However, observed heterozygosity increased in the advanced generations in two subpopulations (A/Dz F_{13} generation cultivated in conventional conditions and CP-1 F_6 cultivated in organic conditions), however the cause of this is not immediately clear. One possible explanation could be differences between genotypes in flower morphology and timing of flowering, but this requires additional investigation to determine the basis of this increase in observed heterozygosity. In general, advanced generations in predominantly self-fertilizing barley, would be expected to have lower levels of observed heterozygosity. In the CCP populations, the number of matching multilocus genotype groups was low, which could be an indication of the increased complexity of the populations, involving crosses between a large number of parental varieties. However, genetic analysis of more advanced generations of the CCP populations is required to reveal if this level of diversity between individuals within populations is maintained.

No significant differences in genetic diversity parameters between the O and C subpopulations were observed, which again, is probably a result of low selective pressure and the small number of generations. Cultivation of subpopulations in organic and conventional conditions for a larger number of generations may increase the genetic diversity differences between them. However, other factors, such as selective pressures and environmental conditions can also affect the rate of differentiation between subpopulations. Subpopulations can also be supplemented with additional germplasm to increase genetic diversity, either from the same source as the original population, or with different germplasm. However, this will also have the effect of diluting any adaptive changes that have occurred in the subpopulations. Further research involving additional genotyping and phenotyping of the CCPs in more advanced generations will provide further data on this. In general, apart from a decrease in observed heterozygosity in advanced generations, as would be expected from a primarily self-pollinating species as barley, no large decrease in genetic diversity was observed in the analyzed populations. In fact, in some subpopulations, an increase in observed heterozygosity occurred, and the factors influencing this should be further investigated.

Comparison of major disease resistance gene frequency

During the course of the cultivation of the populations, powdery mildew (*Blumeria graminis* (DC.) Speer) infection was comparatively low, therefore there was no large selective pressure on these genes. The average powdery mildew score during the three cultivation seasons was 1.1 under O conditions and 2.6 under C conditions (scored from 0 (no infection) to 9 (heavy infection)), reaching 3.3 and 4.4, respectively, in the year 2015 with the highest infection level.

For the *Mla18* gene, there was not a large difference in the proportion of homozygous resistant and susceptible individuals in both populations after cultivation in both organic and conventional conditions. The proportion of heterozygous individuals decreased in the advanced generations in comparison to the initial populations. In contrast, the number of individuals homozygous for the *mlo11* resistance allele decreased in all populations, regardless of cultivation conditions. Results are summarized in Table 4.

Compared to the SSR genotyping results, a larger change in allelic frequencies was observed for the major resistance genes, despite low powdery mildew infection pressure. There was a tendency (in 5 out of 6 cases) that the subpopulations cultivated under conventional conditions had a higher proportion of resistant individuals, which could be a reflection of the higher average powdery mildew score in the conventional cultivation conditions. However, the disease pressure was not high, and the proportion of *mlo11* homozygous resistant individuals decreased in the C subpopulations in comparison to the initial populations. This decrease in the proportion of *mlo11* resistant homozygotes in comparison to the *Mla18* gene may also be due to the pleiotropic effects of the recessive inheritance of the *mlo11* resistance gene, which has been reported to confer a yield penalty because of the necrotic lesions on plant leaves (Wolter et al., 1993). The reduction in the number of individuals homozygous for the *mlo11* resistance allele was more pronounced in the CCP populations, which could be due to the larger number of parental varieties for these populations, and subsequently, a larger amount of *mlo11* susceptibility alleles in the population. Another possibility is that the individuals possessing higher yield potential are *mlo*-susceptible and therefore the proportion of susceptible alleles increased.

Table 4. Percentage of individuals homozygous and heterozygous for resistant and susceptible alleles of the *Mla18* and *mlo11* markers in initial (I) populations and in subpopulations cultivated in organic (O) and conventional (C) conditions

Population	Gene	Subpopulation / Generation	Resistant	Susceptible	Heterozygous
CP-2	*Mla18*	I /F_3	27.3	49.5	23.2
		O /F_6	54.3	40.4	5.3
		C /F_6	61.9	34.8	3.3
CCP-3	*Mla18*	I/F_2	11.1	71.1	17.8
		O/F_4	77.9	17.9	4.2
		C/F_4	80.2	14.6	5.2
CP-2	*mlo11*	I /F_3	82.0	11.0	7.0
		O /F_6	81.2	18.8	0.0
		C /F_6	75.6	17.3	7.1
CP-1	*mlo11*	I/F_4	72.9	14.6	12.5
		O/F_6	21.9	62.2	15.9
		C/F_6	38.1	59.5	2.4
CCP-1	*mlo11*	I/F_2	80.6	10.8	8.6
		O/F_4	10.6	89.4	0.0
		C/F_4	11.0	87.9	1.1
CCP-2	*mlo11*	I/F_2	85.9	10.6	3.5
		O/F_4	18.9	81.1	0.0
CCP-3	*mlo11*	I/F_2	64.2	27.2	8.6
		O/F_4	6.3	92.4	1.3
		C/F_4	16.5	79.1	4.4

CONCLUSIONS

The aim of utilizing heterogeneous populations is to increase the genetic diversity in farmers' fields to buffer potential biotic and abiotic stresses, particularly in more variable organic farming conditions. The results of this study show that in the short term, most genetic diversity parameters are not decreased in the advanced generations in comparison to the initial generations. Observed heterozygosity was decreased in the CCPs between F_2–F_4 but was unexpectedly increased in two other populations between F_4–F_6, and F_{10}–F_{13}, respectively, indicating that in barley, which is predominantly self-pollinating, an increase of observed heterozygosity can occur, and the factors contributing to this should be further investigated. No clear differences in genetic diversity parameters between populations cultivated under either organic or conventional condition for several generations were identified. The lack of clear differentiation between the O and C subpopulations is likely caused by a lack of strong selective pressure and the relatively short time-scale of the study. A significant effect of cultivation environment was found on tillering capacity in organic testing conditions for all tested populations, and in some cases for plant height, ear length and grain number per spike, indicating some adaptation trends.

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Biomass ash as a potential raw material for the production of mineral fertilisers

S. Stankowski[1], E. Chajduk[2], B. Osińska[3] and M. Gibczyńska[4,*]

[1]West Pomeranian University of Technology in Szczecin, Department of Agroengineering, Papieża Pawła VI street 3, PL 71-459 Szczecin, Poland
[2]Institute of Nuclear Chemistry and Technology, Laboratory of Nuclear Analytical Methods, Dorodna street 16, PL 03-195 Warsaw, Poland
[3]Research Institute of Animal Production PIB Kołbacz Sp. z o.o., Warcisława street 1, PL 74-106 Stare Czarnowo, Poland
[4]West Pomeranian University of Technology in Szczecin, Department of Bioengineering, Slowackiego street 17, PL 71-434 Szczecin, Poland
[*]Correspondence: marzena.gibczynska@zut.edu.pl

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Abstract. Ash obtained from biomass combustion could be a valuable product for fertilisation of soil or/and plant. It is connected with high reaction and potassium, calcium, magnesium content as well as low content of heavy metals. The analysed phyto-ash was obtained from Szczecin Power Plant Poland (12.2018–12.2019). The samples of phyto-ash, in the number of 24, were collected on subsequent dates at two-week intervals to determine the total content of the analysed elements (P, K, Mg, Ca, As, Cd, Ni, Cr, Pb, Hg). The differentiated percentage share of macro-elements both in total form as well as available form in ash from wood biomass and Agro-biomass is described as: calcium > potassium > magnesium > phosphorus. Phosphorus is characterised by a very low (10%) and highly variable availability. Ash from biomass is characterised by alkali pH (13.0). Biomass ash can be treated as a mineral fertiliser used for soil deacidification and as a substitute for calcium fertilisers. Biomass ash has a high content of potassium and magnesium, which could qualify this by-product as a source for fertiliser. Mean contents of heavy metals: lead, cadmium, arsenic and mercury in ash do not exceed the limit values for the mineral fertilisers. The variable percentage share of Agro-biomass did not result in significant changes in the amount of available form of macro-elements in ash. The obtained results indicate the pronounced variability, depending on the season in a year, of the content of available macro-elements in biomass ash.

Key words: biomass, macroelements, heavy metals, forest biomass ash, agro-biomass ash.

INTRODUCTION

THE DIRECTIVE 2009/28/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 23 April 2009 defines biomass as a biodegradable fraction of products, waste and biological residues from agriculture (including plant and animal products), forestry and related industries including fishery and aquaculture, as well as a biodegradable fraction of industrial and municipal waste (THE DIRECTIVE 2009/28/EC). The EU Directive does not prevent member states from adopting other definitions of biomass. In Poland, the Act of June 7, 2018 amending the Act on renewable energy sources and certain other Acts, defines biomass as a biodegradable share of products, waste or biological residues, biomass – solid or liquid substances of plant and animal origin, biodegradable, obtained from products, waste and residues of agricultural and forestry production and industry processing these products, as well as cereals grain which do not meet the qualitative requirements for intervention purchase (Journal of Laws of 2018, item 1276). Biomass of plant origin is produced through photosynthesis; $6CO_2 + 6H_2O + light \rightarrow C_6H_{12}O_6 + 6O_2$. The most important chemical compounds are: cellulose, hemicellulose, lignin, pectin and waxes. The elements constituting biomass are mainly carbon, oxygen and hydrogen. In the process of oxidation, more specifically combustion, solid carbon is the main source of energy. The additional energy for the oxidation process is provided by hydrogen, and oxygen sustains oxidation. Utilization of biomass for energy is based on the idea that it is a carbon neutral fuel which will help cut greenhouse gas (Voshell et al., 2018). The chemical composition of biomass is greatly differentiated, to a great extent it is determined by the conditions of the biomass origin. Biomass is used for energy purposes mainly in the form of wood and waste from wood processing, energy crops, agricultural products, organic and municipal waste as well as sewage sludge. As an energy source, biomass can encompass waste from wood processing and cultivation (e.g. bark, sawdust, straw) or intentionally obtained product energy crops (Mierzwa-Hersztek et al., 2019). Burning biomass is conducive not only to obtaining energy, but also to improving environmental protection, (Żelazny & Jarosiński, 2019).

A distinction is made between forest biomass (wood biomass) and non-forest (Agro-biomass). The forest biomass includes wood not meeting standard quality requirements, forestry waste and residue, wood industry waste and residue excluding standard quality wood. The non-forest biomass includes solid and liquid substances of plant or animal origin, waste and residue from agricultural production and industry processing these products, the remaining waste, biodegradable parts, cereals grain not meeting standard quality requirements and energy crops. The most common in Poland are: basket willow, (*Salix viminalis*), sharp-leaf willow (*Salix acutifolia*), poplar (*Populus*), Jerusalem artichoke, (*Helianthus tuberosus* L.), the giant miscanthus (*Miscanthus* x *giganteus*) and multiflora rose (*Rosa multiflora* Thunb.).

The simplest technique of thermal processing is biomass combustion. However, the use of such a heterogeneous fuel results in producing ash which is an environmental challenge. Biomass combustion is universally recognised as being more ecologically friendly than fossil-fuel combustion, owing to a significantly reduced carbon dioxide emission. The basic solid fuel in the form of biomass is forest biomass, however agricultural biomass and the resulting fuels are gaining importance. It is evident that the composition of ash is determined by the type of biomass that is combusted. In comparison to coal, biomass is characterised by a markedly higher content of calcium oxide, potassium and phosphorus as well as variable, yet high at times, content of chlorine. In their analysis of the chemical composition of phyto-ash samples, Diatta & Kowalski (2017) demonstrated that the basic constituent was calcium oxide, with the exception of ash from wheat straw and rapeseed straw where silica was predominant.

Zając et al. (2018) combusted 35 types of biomass (trees and energy crops) and found that the dominant macro-elements in the chemical composition of ash were calcium, potassium, phosphorus and sulphur, which points to the possibility of using ash in agriculture. However, the use of ash from all types of biomass regarding its chemical composition is to be determined on an individual basis depending on the origin of given biomass. The authors provide the qualitative assessment of the share of particular macro-elements in ash depending on the combusted plants and found that the most abundant elements is ash from combustion of hardwood are: calcium > potassium > phosphorus, whereas in ash from combustion of conifers: calcium > silicon > potassium. The most abundant elements in ash from straw are: potassium > calcium > chlorine > phosphorus, and in ash from grass straw: calcium > potassium > calcium > chlorine. In the analysis of ash composition, the following elements are recognised: Si, Al, Ca, K, Na, Mg, Fe, P, Ti, The chemical composition of the mineral matter of ash varied between the samples, however CaO, SiO_2, K_2O, MgO, P_2O_5 and Al_2O_3 are the main compounds of ash (Magdziarz et al., 2018).

In ash from biomass combustion, 229 minerals and phases were identified. Predominantly they are: silicates, oxides and hydroxides, sulphates, phosphates, carbonates, chlorides, nitrates, silica, calcite, anhydrite, periclase and hematite (Vassilev et al., 2013). The content of alkali (sodium, potassium) in biomass is generally high, similarly to the content of chlorine and sulphur (Kassman, 2012). Alkali metals are found in ash predominantly in the form of sulphates, carbonates and chlorides. At high temperature, the decomposition of carbonates, sulphates and silicates is possible, for example in the reaction of $CaCO_3$ (s) → CaO (s) + CO_2 (g) (Johansen et al., 2011). However, Shao et al. (2012) found that the main components of ash from wood biomass combusted at sufficiently low temperature are: potassium carbonate, magnesium carbonate, sodium carbonate and phosphates.

In the composition of biomass, the presence of sulphur, nitrogen and chlorine is not to be overlooked as it may affect the level of harmful emissions due to combustion. The biomass produced from straw shows a high content of chlorine and relatively low content of sulphur. The important concern is the active corrosion in the process of combustion of biomass rich in potassium, chlorine and sulphur. During straw combustion, potassium can react with chlorine and be released in the form of KCl (Boström et al., 2011). At higher temperature, predominant is the sublimation of KCl, formation of which is determined by the amounts of chlorine.

Vassilev et al. (2013) indicate the disadvantages related to the composition and properties of biomass, such as: the presence of alkaline and halogen elements as well some dangerous trace elements, highly variable composition including moisture content (10–60%) determined by the type of biomass and seasoning period. The main environmental, economic and social benefits compensate for technological barriers and other issues resulting from disadvantageous composition and properties of biomass.

The Regulation of the Minister of the Environment of January 20, 2015 on the R10 recovery process classifies ashes from biomass combustion (i.e. phyto-ashes) and codes them as 10 01 03 (fly ash from peat, untreated wood) classifying it as mineral waste, as well as identifying the conditions of them being used as a fertilising material. One of the conditions is the requirement of meeting the limit values for fertilisers (Journal of Laws of 2015, item 132). Phyto-ash is the source of nutrients (Ca, K, P, Mg, Zn, Cu, Fe, Mn) for plants, has deacidifying properties and can be an alternative to mineral fertilisers.

However, the presence of harmful substances, particularly heavy metals, can be problematic. Ash from biomass can also contain trace elements, e.g. arsenic, cadmium, chromium, copper, mercury, lead, nickel, zinc, barium, molybdenum, vanadium, manganese, cobalt and antimony. Trace elements in ash are predominantly heavy metals. Heavy metals, among others Mn, B, Ba, Cu, Sr, Ni, Cr, Zn, Cd, Co, constitute merely 0.1÷0.3% of ash mass, however mercury is found in trace amounts and is usually within the range of 0.01÷1 ppm (Warren & Dudas, 1988).

The chemical composition of ash originating from biomass combustion is varied and determined by a number of factors, such as: the type of biomass, the species or specific parts of the plant, growing conditions, the age of plants, applied fertilisation, dosage of plant protection products and the date of harvest. The said factors can results in an increase or a decrease of element contents in biomass ash. Therefore, owing to the high variability of the quality of ash resulting from the type of the combusted biomass, a detailed analysis of its chemical composition is required prior to determination of the means of its utilisation, particularly as fertiliser (Zając et al., 2018).

Soil fertilisation is applied to maintain the appropriate level of soil parameters and produce high quality yield, therefore the chemical composition of phyto-ash is to be explicitly determined both quantitatively as well as qualitatively (Stankowski et al., 2018).

In summary, due to its chemical composition and alkaline reaction, biomass ashes can be tested for suitability as a mineral fertiliser. Due to the diversity of their origin, a careful analysis of their chemical composition, depending on many factors, is justified.

The aim of the present paper was to analyse the chemical composition of phyto-ash depending on the date of combustion (22.12.2018 – 13.12.2019) and the percentage share of wood biomass or Agro-biomass as well as the assessment of phyto-ash in terms of its usefulness in the production of mineral fertilisers.

MATERIALS AND METHODS

Experiment characteristics

The analysed phyto-ash was obtained from Szczecin Power Plant, Poland. Biomass was combusted in a bubbling fluidised bed (BFB) boiler by the Finnish Metso (https://pl.wikipedia.org/wiki/Elektrownia_Szczecin). The samples of phyto-ash, in the number of 24, were collected on subsequent dates at two-week intervals from 22.12.2018 to 13.12.2019, with a month's interval in the boiler's operation in June 2019.

Chemical analyses

On each subsequent date, a sample of 5 kg of ash was collected. Following the mixing, two samples of approx. 0.5 kg were collected from each sample, and the analytical samples of 250 mg were weighed for the analysis. To determine the total content of the analysed elements (P, K, Mg, Ca, As, Cd, Ni, Cr, Pb) in ash, the samples were mineralised in a sealed microwave system (Anton Paar Multivave 3000). A two-step procedure was employed using a mixture of concentrated nitric(V) and hydrofluoric acids (step one) and the addition of 4% boric acid to eliminate the excess F^- (step two). As the reference material, Polish Certified Reference Material Fine Fly Ash (CTA-FFA-1) was used.

Total content of As, Pb, Cr, Ni and Cd was identified with the method of inductively coupled plasma mass spectrometry (ICPMS); K, Mg, Ca was identified with ion chromatography (IC); P content was determined spectrophotometrically.

Determining the total content of mercury, ash samples were wet mineralised in the mixture of concentrated nitric(V) acid chloric(VII) acids using the Bethe set in CV-AAS method, the pH of ash (pH_{KCl}) was determined potentiometrically with KCl solution of 1M concentration in accordance with (ISO standard 10390 2005).

The available forms of phosphorus and potassium in soil were determined using the Egner-Riehm method based on the extraction of calcium lactate with buffer solution characterised by a pH value of 3.55 (Egner et al., 1960).

The content of the forms of alkali metals (potassium, magnesium, calcium) potentially available for plants were identified following the extraction with 1M HCl at the soil to solution ratio of 1:10 Kabała & Karczewska, 2019). 1M extraction with HCl solution is commonly used in agricultural studies for the purpose of determining bioavailability of metals for plants. The content of metals in the obtained extracts was identified with IC method.

Reagents and materials

In the preparation of all solutions, 18 MΩ cm grade water from Milli-ORG Millipore Co. purification system was used. During the analysis, only the reagents of recognised analytical grade or suprapure were applied. Calibration standards were supplied by Perkin Elmer as stock standard solutions of 10 $\mu g\ mL^{-1}$. Working mixed standard solutions (2–200 $\mu g\ mL^{-1}$) were prepared by dilution stock standard solutions in 0.7% nitric acid. Daily check performance solution (Perkin Elmer) was used for the spectrometer optimization. The following Certified reference materials (CRMs) were used for validation the method: Soil 5 (IAEA) and Fine Fly Ash CTA-FFA-1 (INCT).

Instrumentation

To prepare samples and CRMs, analytical (Sartorius MC5) and micro-analytical (BP221S) balances calibrated using national mass standards traceable to the international standards were used. Philips PU 8625 UV/VIS spectrophotometer was employed for the measurement of absorbance of formed phosphomolybdate complex with the added molybdate followed by the reduction of the complex with hydrazine hydrate in aqueous sulphuric acid medium. Dionex 2000i/SP ion chromatograph was equipped with analytical column Dionex IonPac CS12A, conductivity detector CDM II and the suppression system ASRS 4 mm. The eluent used was 18 mmmmol$\cdot L^{-1}$ methanesulfonic acid, the flow rare flow 1 $mLmin^{-1}$. The ELAN DRC II inductively coupled plasma quadrupole mass spectrometer (PerkinElmer) with crossflow nebulizer with Scott double-pass spray chamber and Ni cones was used for multi elemental analysis. Instrument operation conditions are: RF power - 1,000 W; nebulizer gas flow rate - 0.92 $Lmin^{-1}$; Plasma gas flow rate - 15 $Lmin^{-1}$; auxiliary gas flow rate - 1.2 $Lmin^{-1}$; lens voltage - 6.25 V; detector mode - dual; measurement unit - cps; working mode - standard.

Statistical analysis

The obtained results were statistically developed by calculating the selected characteristics of samples. The data concerning the distribution per individual characteristics is presented in the form of histograms. To compare the means, the Kruskal-Wallis one-way non-parametric analysis of variance was used. The relationships between the variables were calculated using the Spearman rank-order correlation at the level $P > 0.05$

and 0.01 (Dunn & Clark, 1974; Hill & Lewicki, 2021). The calculations were made with the Statistical PL software.

RESULTS

Biomass composition

The main form of the fuel supplied to the boiler was forest biomass (on average 87.7%), Agro-biomass constituted approx. 12.3%. On particular dates of collecting the samples, the percentage share of Agro-biomass varied from 2.10% to 41.28%. Within the range of 0–5% there were 5 samples, 5–10% - 4 samples, 10–15% - 9 samples, 15–20% - 3 samples and over 20% - 3 samples (Table 1, Fig. 1).

Table 1. Characteristics of composition and percentage share of biomass types

No	Sample collection date	Amount of combusted biomass [Mg]				
		Forest	Agro			Sum
			Chips	Pellet	Chips and pellet	
1	22.12.2018	807	114.0	41.4	155.3	1,118
2	7.01.2019	905	119.6	19.4	139.0	1,183
3	21.01.2019	1,321	370.2	49.8	420.0	2,161
4	5.02.2019	1,810	0.0	84.8	84.8	1,980
5	19.02.2019	1,573	0.0	45.4	45.4	1,664
6	5.03.2019	1,331	0.0	81.3	81.3	1,494
7	19.03.2019	1,519	0.0	43.9	43.9	1,607
8	5.04.2019	1,158	125.4	42.6	168.0	1,494
9	19.04.2019	1,290	65.7	77.6	143.3	1,577
10	30.04.2019	1,353	3.0	78.8	81.8	1,517
11	16.05.2019	1,396	139.7	26.3	166.0	1,728
12	29.05.2019	1,370	129.1	0.0	129.1	1,628
13	1.07.2019	489	303.8	40.0	343.8	1,177
14	15.07.2019	1,019	115.8	46.8	162.6	1,344
15	01.08.2019	698	201.8	34.1	235.9	1,170
16	14.08.2019	770	166.9	15.9	182.8	1,136
17	30.08.2019	971	145.8	37.8	183.6	1,338
18	15.09.2019	733	0.0	20.5	20.5	774
19	30.09.2019	1,122	134.4	44.6	179.0	1,480
20	15.10.2019	1,173	135.2	46.3	181.5	1,536
21	30.10.2019	1,186	137.6	64.4	202.0	1,590
22	15.11.2019	1,536	193.7	58.3	252.0	2,040
23	30.11.2019	1,804	0.0	38.7	38.7	1,881
24	13.12.2019	896	0.0	21.9	21.9	940
Sum		28,230	2601.7	1,060.6	3662.2	35,555

The share of Agro-biomass in samples was entirely random, as is demonstrated by the value of rs Spearman's correlation coefficient close to zero. The share of pellets in total biomass was from 0.0 to 84.8 Mg and was by far lower than that determined in the mass of wooden chips. The amount of wooden chips varied from 0.0 to 370.2 Mg (Table 2).

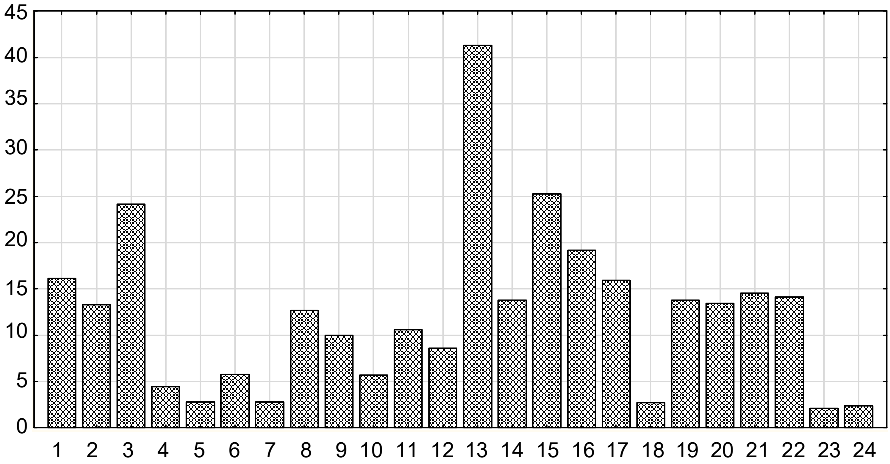

Figure 1. Share of agro biomass (%) in total amount of fuel: 1–24 – number of samples; 0–45 – % value.

Table 2. Statistical analyses of combusted material [Mg]

Type of biomass	Mean	Median	Minimum	Maximum	*V*%
Total amount of biomass fuel	1,481.2	1,371	774	2,161	25.0
Forest biomass	1,176.2	1,180	489	1,810	29.6
Wooden chips	108.4	122	0.0	370.2	91.6
Pellets	44.2	43	0.0	84.8	49.3
Wooden chips+pellets	152.6	159	20.5	420.0	63.7

V% – variability coefficient.

pH and macro-elements content

The analysis conducted on 24 samples of ash indicates that the most stabile feature of ash was pH (pH in KCl). Mean pH value amounted to 13.0, and variability was below 1% (Tables 3, 4). For comparison, the pH of the $CaCO_3$ aqueous solution is lower and amounts to 9 (Martín-Martínez 2002).

Table 3. Characteristics of macro-elements composition and pH of biomass ash (*n* = 24)

Trait	Unit	Mean	Median	Minimum	Maximum	*V*%
pH in KCl	%	13.0	12.9	12.7	13.2	0.82
Total calcium		13.76	14.2	5.33	18.05	22.16
Available calcium		11.9	12.3	4.27	16.90	28.47
Total potassium		5.55	5.37	4.02	7.39	15.05
Available potassium		4.43	4.69	1.65	7.35	32.55
Total magnesium		2.01	1.99	0.77	2.94	28.52
Available magnesium		1.58	1.51	0.49	2.56	34.77
Total phosphorus		1.43	1.36	0.748	2.32	29.8
Available phosphorus		0.136	0.090	0.004	0.401	88.4

V% – variability coefficient.

The ash in the experiment was characterised by a variable content of macro-elements. The largest amounts were identified for both total as well as available calcium; calcium from ash (Table 3). The distribution of data regarding available calcium supports the findings that it is asymmetrical and shows no consistency with the normal distribution (Fig. 2).

Mean potassium content in biomass ash was from 5.5 to 4.4%, respectively for the total and available form. The content of available potassium, showed greater variability than that of total phosphorus, - from 1.65 to 7.35%. The distribution of data regarding available potassium supports the findings that it is asymmetrical and shows no consistency with the normal distribution (Fig. 3).

The content of magnesium, another macro-element vital for plant growth, in ash ranged from2.0 to 2.9%, showed good availability for plants of 77% and relatively slightvariability of the results (*V*% respectively 28 and 35%) (Table 3).

The comparison of ash abundance in magnesium and potassium shows that the content of magnesium was by 50% lower than that of potassium. This relationship is to be taken into consideration when determining soil fertilisation dose.

Table 4. Correlation between time of sampling (x), pH and the content of soluble forms of macro elements in biomass ash

Dependent variability (y)	Spearman test rs value	Significance
pH	0.065	ns
Calcium	0.652	**
Potassium	0.681	**
Magnesium	0.723	**
Phosphorus	0.178	ns

ns – not significant correlation; ** – correlation at $p < 0.01$, very high significant correlation.

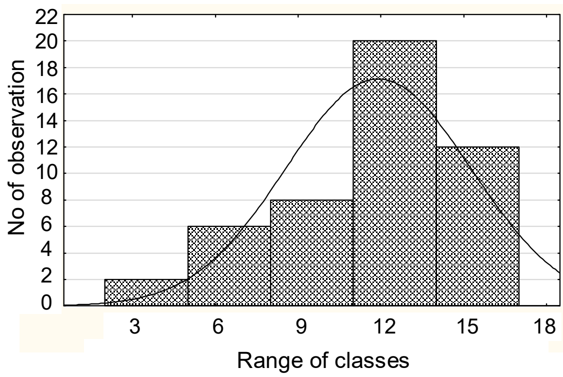

Figure 2. Distribution of results for plant-available calcium in ash from biomass.

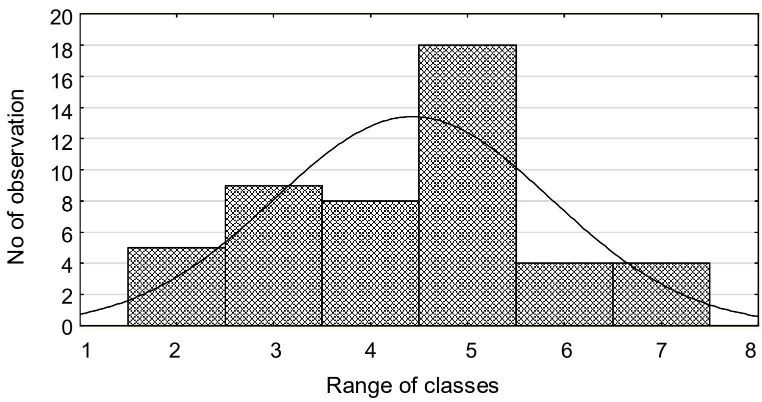

Figure 3. Distribution of results for plant-available potassium in ash from biomass ash.

Yet another issue is the content of phosphors in ash - phosphorus being, next to potassium and nitrogen, the most important constituent of fertilisers. The amount is relatively slight - the total phosphorus is, on average, 1.43% and the available phosphorus only 0.14%. However, the availability is merely at 10% thus it is very low and highly variable. The variability of the results for available form is at 88% (Table 3). The factor which greatly limits the availability of phosphorus is high pH of ash resulting in phosphorus presence in the form of sparingly soluble compounds. Most likely, bioavailability of phosphorus would increase following ash application to soil.

The results of the analyses of variance in which a different share of Agro-biomass was adopted as the factor, practically do not show differentiation of the content of available macro-components and pH in ash (Table 5). The results were found to be lower only for calcium and potassium with the lowest share of Agro-biomass (< 10%) in the combusted material.

Table 5. Effect of share of Agro-biomass (wooden chips + pellets) on chemical composition (available forms in %) of biomass ash

Share of Agro Biomass (%)	pH	Calcium	Potassium	Magnesium	Phosphorus
< 10	12.9^{a}	8.2^{b}	3.02^{b}	1.62^{a}	0.106^{a}
10–15	12.9^{a}	11.6^{a}	4.33^{a}	1.62^{a}	0.117^{a}
15–20	13.0^{a}	12.4^{a}	4.62^{a}	1.85^{a}	0.139
> 20	13.0^{a}	14.2^{a}	5.65^{a}	1.81^{a}	0.202^{a}

In order to obtain the results for individual seasons in a year, mean values were calculated (Table 6). The results show a significant differentiation of the content of available macro-elements in biomass ash. However, the aforementioned relationship does not apply to ash pH level, which is 13. In the winter season, the lowest percentage contents of available calcium, potassium and magnesium, i.e. metals, were identified in ash. As for the autumn season, the identified respective contents were found to be the highest.

Table 6. The effect of seasons in a year on the chemical composition (available forms in %) of biomass ash

Season	pH	Calcium	Potassium	Magnesium	Phosphorus
Winter	12.9^{a}	8.94^{c}	3.69^{b}	1.18^{c}	0.129^{b}
Spring	13.0^{a}	12.18^{b}	4.05^{b}	1.43^{b}	0.081^{c}
Summer	13.1^{a}	12.41^{b}	4.82^{a}	1.57^{b}	0.172^{a}
Autumn	12.9^{a}	14.38^{a}	5.31^{a}	2.09^{a}	0.163^{a}

a, b –mean values described by different letters, indicate significance difference.

Heavy metal content

The aforementioned Ordinance specifies the admissible limit values for pollutants in mineral fertilisers and substances enhancing plant cultivation (Journal of Laws No. 119, item 765 and 2009, item 1804). The values are to be considered when assessing the usability of biomass ash as a mineral fertiliser. Out of the six analysed heavy metals present in biomass ash, the limit values as specified in the Ordinance refer to only four: lead, cadmium, arsenic and mercury. Mean content in ash from wood biomass and Agro-biomass does not exceed the limit values specified in the current Ordinance (Table 7).

Table 7. The content of heavy metals and manganese in biomass ash and the limit content of heavy metals in mineral fertilisers according to Polish regulations

Trait	Units	Mean	Median	Minimum	Maximum	*V*%	Limit content in mineral fertilisers* [mg kg^{-1}]
Lead	mg kg^{-1}	73.3	75.0	25.2	122.6	25.73	140
Chromium		57.5	53.2	33.9	126.1	30.41	-
Nickel		30.9	31.3	15.1	48.2	20.68	-
Cadmium		16.8	15.8	4.2	29.9	45.17	50
Arsenic		7.7	7.2	1.9	14.6	38.14	50
Mercury		0.41	0.45	0.15	0.86	54.30	2

*according to the Ordinance of the Minister of Agriculture and Rural Development of June 18, 2008 on the implementation of same provisions of the Act on fertilisers and fertilisation (Journal of Laws No. 119, item 765 and 2009, item 1804); *V*% – variability coefficient

DISCUSSION

pH and macro-elements content

The deacidifying action of biomass ash results from high contents of potassium, calcium and magnesium compounds. In the process of combustion, in comparison to other elements, alkali metals compounds remain in ash and do not pass into the gaseous form. The problematic of the content of alkali metals in biomass ash is discussed in numerous scientific papers (Shao et al., 2012; Nunes et al., 2016; Kalembkiewicz et al., 2018; Magdziarz et al., 2018; Zając et al., 2018; Zhai et al., 2021). For comparison, Nurmesniemi et al. (2012), while burning biomass in a power plant, obtained a very similar value of ash pH 12.8. Żelazny & Jarosiński, (2019) obtained ash reaction from biomass, highly alkaline, pH in H_2O > 11. Therefore, significant losses of ammonia and phosphorus in the NPK fertiliser should be taken into account (decomposition of ammonia from the nitrate ammonia compound and the formation of phosphorus compounds insoluble in water in these conditions). Because of their properties and influence on soil chemistry, the utilization of wood ashes is particularly suited for the fertility management of tropical acid soils and forest soils (Demeyer et al., 2001).

The ash in the experiment was characterised by a variable content of macro-elements. Most of the inorganic elements assimilated by plants during growth remain in the ash after burning. They include plant macronutrients, for example, K, Ca, Mg, and P, which are the bulk of the materials accumulated in ash (Pasquali et al., 2018). Čepauskienė et al. (2018) differentiates the composition of ash depending on whether ash was obtained from wood biomass or agricultural residue. In the case of ash from wood biomass, the following chemical compounds were identified: $CaCO_3$, K_2SO_4, $K_2Mg(PO_3)_4$, $MnSO_4$. An analogous relationship is given by Jarosz-Krzemińska & Poluszyńska (2020). The total concentrations of macronutrients in the fly ash under study decreased in the descending order of nutritional elements Ca > K > Mg > S > P >. Through their experiment, they confirmed the predominance of calcium in ash found in numerous scientific papers, for example (Diatta & Kowalski, 2017; Magdziarz et al., 2018; Zając et al., 2018).

Similarly to the results of the analyses conducted in the course of the present study, most studies by other authors indicate that the amount of potassium in ash was secondary to calcium (Schiemenz & Eichler-Löbermann, 2010; Füzesi et al., 2015; Magdziarz et al., 2018; Zając et al., 2018; Zhai et al., 2021). Mean potassium content in biomass ash was from 5.5 to 4.4%, respectively for the total and available form. The content of available potassium showed greater variability than that of total phosphorus - from 1.65 to 7.35%. Generally, the amount of total phosphorus is five-fold higher than that identified in manure. For example, Schiemenz et al. (2011) report that the phosphorus concentration in biomass ash ranges from < 1 to 10%. Shi et al. (2017) present the conclusion that the introduction of all tested ashes increased the soil pH, the amount of exchangeable alkaline cations and available phosphorus, but decreased the exchange acidity of the soil. Kramar (2020) specified that about ¾ all the phosphorus contained in the ash is in a form accessible to plants, when interacting with soil, especially acidic. In the performed experiment, an analogous relationship was obtained (80%).

High abundance of total, as well as available potassium, indicates the realistic possibility of using ash for fertilisation purposes.

This could be explained by a lower content of calcium and potassium in stemwood in comparison to branches. According to Gornowicz & Pilarek (2013), the content of calcium in branches is from 0.13 to 0.29% and in wood tree-length from 0.09 to 0.22%, whereas with respect to potassium, the values for branches are from 0.05 to 0.15% and for wood of tree-length from 0.01 to 0.03%. Kramar (2020) reports that wood ash usually contains up to 15% K_2O in the form of K_2CO_3, 7% - P_2O_5 and about 40% CaO. The values for calcium and potassium are higher than those obtained in the performed experiment.

The results obtained in the experiment indicate a significant diversification of the digestible macronutrient content in the biomass ash. The aforementioned dependence is explained by the physiological processes of plants (trees). In spring and summer, trees take up large amounts of calcium, potassium and magnesium from the soil. Consequently, it may lead to the accumulation of elements in trees in the autumn period.

Heavy metal content

One of the factors determining the possibility of using ash for fertilising purposes is the presence of heavy metals. The toxic action of the said elements is connected with their ability to accumulate in the organisms of plants and animals.

When discussing the results of the present study, consideration should be given to a relatively high content of cadmium in ash - from 4.24 to 29.92, mean 16.81 mg Cd kg^{-1}. Other studies on cadmium content in ash from biomass of various origins show a lower content, for example 0.3 and 5.3 mg Cd kg^{-1} (Samaras, 2008). According to Poluszyńska & Ślężak (2015), the combustion of biomass from wood chips and sunflower husk pellets (79 and 21%) produced ash with 10.7 and 19.1 mg Cd kg^{-1}. The comparison of the composition of ash from wood and agricultural residue by Pastircakova (2004) demonstrates a higher content of As, Cd, Pb and Hg in wood ash.

The average and maximum content in ash from wood biomass and Agro-biomass did not exceed the limits set out in the applicable Regulation (Journal of Laws No. 119, item 765 and 2009, item 1804) which justifies the use of these ashes as mineral fertilisers.

CONCLUSIONS

Biomass ash is characterised by alkali pH. Mean pH value was 13.0 and its variability was below 1%. Biomass ash can be treated as a potential mineral fertiliser used for soil deacidification and as a substitute for calcium fertilisers. The ash from the experiment was characterised by a variable macro-element content which was subject to a very high random variability within a one-year long sampling period at two-week intervals. Biomass ash has a high content of potassium and magnesium, which could qualify this by-product as a source for fertiliser. Mean contents of four heavy metals: lead, cadmium, arsenic and mercury in ash from wood biomass and Agro-biomass do not exceed the limit values for the mineral fertilisers. The variable percentage share of Agro-biomass did not result in significant changes in the amount of available form of macro-elements in ash. The obtained results indicate the pronounced variability, depending on the season in a year, of the content of available macro-elements in biomass ash.

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Development of stomatal conductance of maize under moderately hot, dry production conditions

A. Széles[1], É. Horváth[1,*], D. Rácz[1], L. Dúzs[1], Cs. Bojtor[1] and L. Huzsvai[2]

[1]University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute for Land Utilisation, Technology and Regional Development, Böszörményi street 138, H-4032 Debrecen, Hungary
[2] University of Debrecen, Faculty of Economics and Business, Institute of Statistics and Methodology, Böszörményi street 138, H-4032 Debrecen, Hungary
[*]Correspondence: szelesa@agr.unideb.hu

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Abstract. A field trial was conducted in Hungary, in a moderately warm, dry production area to determine the effect of single or split application of N-fertilizer on the stomatal conductance and grain yield of maize. The measurements were performed at on calcareous chernozem soil, in a strip field trial, under different weather conditions (2019 and 2020). In addition to the unfertilized control (A_0) treatment, 60 (A_{60}) and 120 kg N ha^{-1} (A_{120}) applied as spring basal fertilizer were followed by two top-dressing treatments in V6 (A_{90}) and V12 (A_{120}) phenophases with doses of +30 and +30 kg N ha^{-1}, respectively. Stomatal conductance measurements were taken at V6, V12 and R1 phenological phases. It was found that stomatal conductance showed a decreasing trend with advancing phenological phases in both years, with 43.9% and 32.1% ($P < 0.001$; $P < 0.01$) decreases by the R1 phase, respectively. Application of higher doses of N fertilizer increased stomatal conductance, with the exception of the R1 phenological phase, which showed a decrease compared to the A_0 treatment. The decrease in 2019 was caused by a reduction of soil moisture. Although there was sufficient water in the soil in 2020, due to the intermittent water shortages caused by but the large leaf area and rapid transpiration of the plants resulted in the stomata to close. The best fertilisation strategy was A_{60} and A_{120} kg ha^{-1} depending on the crop year. The study showed that the highest yield is obtained when the average stomatal conductance is around 250 mmol m^{-2} s^{-1} during the growing season.

Key words: maize, stomatal conductance, fertilization.

INTRODUCTION

Temperature is one of the most rapidly changing environmental factors, which has been gradually increasing for decades due to climate change, a trend that is expected to continue (Urban et al., 2017). As temperatures rise, even heat-intensive crops such as maize respond with yield declines as the number of days above T_{max} > 30 °C increase. The yield reducing effect of heat stress was demonstrated in a study by Huzsvai et al. (2020). Global warming might exacerbate drought damage by further reducing soil moisture availability (Nicolai-Shaw et al., 2017; Wang et al., 2019). Water stress adversely affects stomatal conductance, causes partial stoma closure (Wang et al., 2018), reduces transpiration (Prado et al., 2018), increases stoma density, and reduces stoma opening, indicating plant adaptation to drought stress (Huang & Xu, 2015; Gamage et al., 2018; Nemeskéri & Helyes, 2019). Furthermore, CO_2 absorption is reduced, which affects photosynthesis, overall plant functionality, and inhibits plant growth and development (Tian et al., 2019; Thruppoyil & Ksiksi, 2020; Yin et al., 2020).

Under climate change conditions, soil moisture deficit and atmospheric drought lead to a decrease in stomatal conductance (Zhang et al., 2021), which has a negative impact on crop yield (El-Sabagh et al., 2017; Faralli et al., 2019). However, the detrimental effect of weather extremes can be mitigated by proper agrotechnology (Széles & Huzsvai, 2020).

Among the agrotechnical elements, fertilisation, especially nitrogen (N), which is an essential plant nutrient, deserves special attention due to its impact on soil and its water balance. It plays an important role in vegetative growth (Zuo et al., 2015) because it is the building block of various substances that regulate plant growth. It plays a key role in photosynthesis (Ghotbi-Ravandi et al., 2015; Guo et al., 2021) and is a central component of the chlorophyll molecule. N deficiency results in reduced photosynthesis (Dwyer et al., 1995; Correia et al., 2005) and slower growth rates (Hammad et al., 2012), leading to significant yield reductions (Berenguer et al., 2009).

In order to overcome yield reduction and to achieve higher grain yield, more than 300 kg ha^{-1} of N fertilizer is applied in production, which is significantly higher than the optimal N rate shown in field experiments (Yang et al., 2017), which is 100–110 kg N ha^{-1} in dry farming (Pepó & Nagy, 1997). Excessive or inappropriate use has negative effects on crops, it greatly reduces N use efficiency (NUE), it is a major problem in air pollution (Han et al., 2017; Fang et al., 2018) and causes significant nitrate leaching losses (more than 50% N in the environment) (McBratney & Field, 2015; Suchy et al., 2018).

Numerous strategies have been developed to reduce nitrogen loss. In addition to the optimal timing of N supply, the frequency of N application is important (Lü et al., 2012; Schoninger et al., 2018; Széles et al., 2019a, 2019b; Davies et al., 2020). Maize uptakes N throughout its entire the growth period. Its N demand is low during the early vegetation period, then increases rapidly and remains high for several weeks. Cassman et al. (2002) consider N reduction in autumn and multiple applications during the growing season a suitable strategy.

The aim of the present work is to investigate (i) how the change of weather affects stomatal conductance and its correlation with N fertilization, (ii) at which stomatal conductance level is the highest yield obtained, and (iii) how N fertilizer rate and timing affect yield.

MATERIALS AND METHODS

The studies were carried out in Hungary at the Látókép Experimental Station of the University of Debrecen (47° 33' N, 21° 26' E, elevation 111 m), on loess-formed, calcareous chernozem soil.

The average pHKCl value of the soil is 6.6 (weakly acidic), in the upper (20 cm) layer Arany's plasticity index is 39, the total amount of water-soluble salts is 0.04% (low salt content). The soil is moderately calcareous (carbonated lime content in the upper

80 cm of the soil is around 0%, from 100 cm to 12%). Its humus content is 2.6–2.8%, P_2O_5 content is medium (133 mg kg^{-1}), its K_2O supply is good (240 mg kg^{-1}).

The experiment is a strip design, two-replicate, small plot field trial, which was set up in 2011, with five maize hybrids and seven fertiliser steps. Each strip includes a certain hybrid with a certain fertilizer treatment.

In both years, 27% Genesis Pétisó fertilizer was applied before sowing. Similar to the experiments by Ritchie et al. (1997) and Berenguer et al. (2009), fertilizer doses were split between basal and top-dressing treatments (Table 1).

Table 1. Fertilizer treatments used in the experiment

Name	Treatment
A_0	Unfertilized control
A_{60}	60 kg N ha^{-1} before sowing
A_{120}	120 kg N ha^{-1} before sowing
$V6_{90}$	60 kg N ha^{-1} before sowing +30 kg N ha^{-1} during the V6 phenophase
$V6_{150}$	120 kg N ha^{-1} before sowing +30 kg N ha^{-1} during the V6 phenophase
$V12_{120}$	60 kg N ha^{-1} before sowing +30 kg N ha^{-1} during the V6 phenophase +30 kg N ha^{-1} during the V12 phenophase
$V12_{180}$	120 kg N ha^{-1} before sowing +30 kg N ha^{-1} during the V6 phenophase + 30 kg N ha^{-1} during the V12 phenophase

Plant density was 73 thousand plants ha^{-1}, the green crop was maize. Maize was sown on 10.04.2019 and 17.04.2020 and harvested on 09.10.2019 and 12.10.2020. The harvested grain yield is corrected to 14% moisture content.

In the present paper, stomatal conductance, soil moisture and yield results of the Fornad (FAO 420) maize hybrid were analyzed, which were measured in 2019 and 2020, in unfertilized control, A_{60}, A_{120}, $V6_{90}$, and $V6_{150}$ treatment combinations. Measurements were taken three times in V6, V12 and R1 growth stages.

Stomatal conductance of the plants, was measured with the Sc-1 Leaf Porometer, exclusively in sunny weather, at noon and early afternoon hours, because then the irradiation and evaporation of the plants are the highest. In each treatment combination, three randomly selected plants were tested, three measurements were taken on each plant, thus the average of nine measurements represented the stomatal conductance of the plot. To obtain a values representing the entire plant, measurements were carried out on the lower, middle and upper leaves of the selected plants, taking into account the different development and shading. Together with the two true replicates, there were 18 measurements in each treatment in each of the three growth stages (V6, V12 and R1), ensuring representativeness.

Soil moisture content was determined by means of a Field Scout TDR 300 soil moisture probe. Two 20 cm measuring rods were used for the instrument. The probe rods were pushed parallel to each other into the soil. After the rods entered the soil, the return time indicated by the instrument was recorded for each measurement, based on which the soil moisture content was determined after calibration. The mean value of three measurements per plot was used to determine the soil moisture content.

The weather was evaluated based on data measured and recorded by an automatic weather station located in the experimental area. The values were compared to the average of the period 1981–2010 (Nagy, 2019). Regarding the phenological stages, the Growing Degree Days (GDD) agrometeorological index was used.

Growing Degree Days (GDD)

$$GDD = \sum\left(\frac{(T_{max}+T_{min})}{2} - T_b\right), \quad (1)$$

where, T_{max} (°C) is the daily maximum temperature, T_{min} (°C) is the daily minimum temperature, T_b (°C) is the base temperature. T_b is the temperature below which the rate of development is considered to be 0. The heat sum was calculated with T_b = 10 °C in accordance with the scientific literature data (Davidson & Campbell, 1983; Gallagher, 1979).

While precipitation total the 2019 growing season (365 mm) was only 19 mm above the multi-year average (346 mm), the precipitation of 2020 was 103 mm above the average. Temperatures in the first year (17.5 °C) were in line with the average (17.6 °C), but due to more precipitation, the 2020 growing season was nearly 1 °C colder (Figs 1–2).

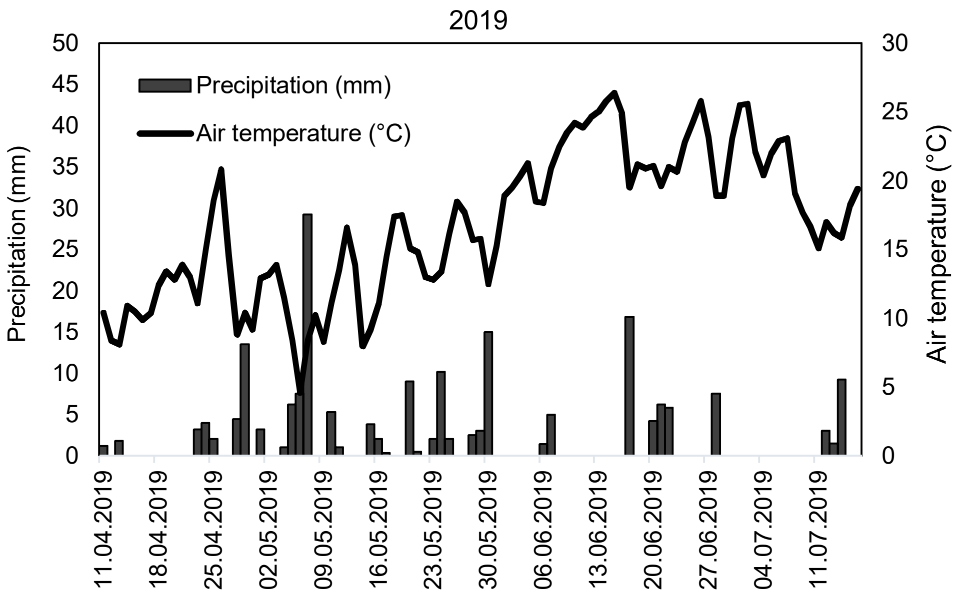

Figure 1. Distribution of temperature and precipitation during the measurement period, 2019.

The effects of fertilization and phenophases on stomatal conductance were evaluated using a repeated measure model. The dependent variable was stomatal conductance. The treatment variable was the fertilizer (N), phenophase was the repeated factor, while the error factor was the plot identifier and the phenophase interaction. The two years were evaluated separately. *Duncan's test* was applied to compare the means of the treatment combinations. The baseline significance level was 5%.

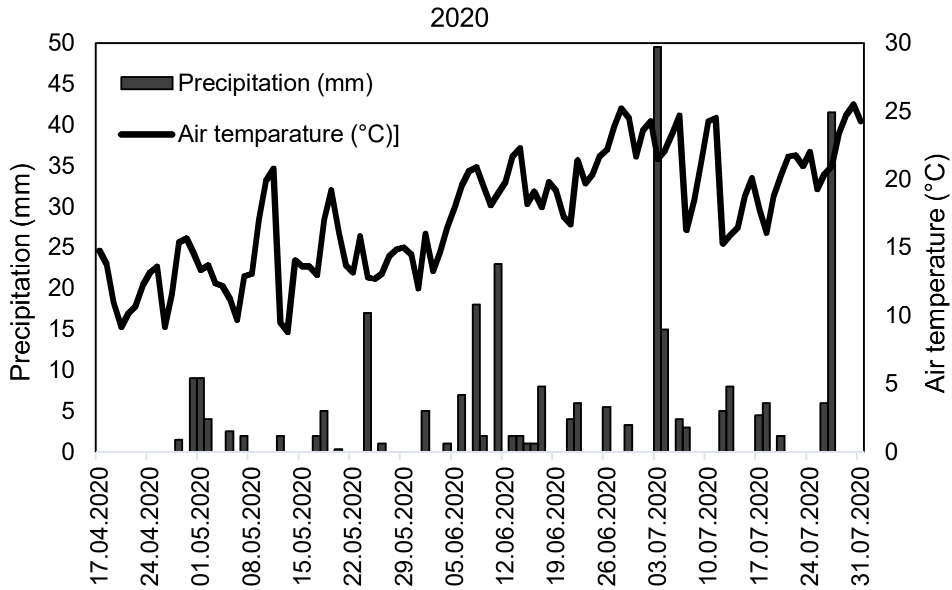

Figure 2. Distribution of temperature and precipitation during the measurement period, 2020.

The correlation between stomatal conductance and soil moisture and between stomatal conductance and yield was analysed by means of correlation analysis. The correlation between yield and stomatal conductance was evaluated separately for the three phenophases.

Data were evaluated using R version 4.1.1 (2021-08-10).

RESULTS AND DISCUSSION

Development of stomatal conductance

The 2019 and 2020 results of the repeated measure model showed that N fertilization and phenological phases and the interaction of the two factors were significant (Table 2–3).

Table 2. Effects and interactions of N fertilization, phenological stages and stomatal conductance of maize, 2019

	Df	Sum Sq	Mean Sq	*F* value	Pr(> s*F*)
N-fertilizer	4	27,538	6,884	38.27	0.000609 ***
Residuals	5	899	180		
Phenological phase	2	253,211	126,606	792.28	9.70e-12 ***
N-fertilizer x Phenological phase	8	81,382	10,173	63.66	1.41e-07 ***
Residuals	10	1,598	160		

Note: *** $P = 0.001\%$.

The stomatal conductance values of the plants between the V6 and R1 phenophases ranged widely. The lowest value of the interval was 208 and the highest value was 589 mmol m^{-2} s^{-1}.

Table 3. Effects and interactions of N fertilization, phenological stages and stomatal conductance of maize, 2020

	Df	Sum Sq	Mean Sq	*F* value	Pr(> *F*)
N-fertilizer	4	20,144	5,036	27.25	0.00137**
Residuals	5	924	185		
Phenological phase	2	88,176	44,088	949.5	3.94e-12***
N-fertilizer x Phenological phase	8	109,384	13,673	294.5	7.48e-11***
Residuals	10	464	46		

Note: *** $P < 0.001\%$; ** $P < 0.01\%$.

Based on the 134 mm precipitation that fell from sowing to the V6 phenological stage in 2019, the GDD value of 236 °C and the stomatal conductance of 513 mmol m^{-2} s^{-1} measured in the average of the treatments, the environmental conditions were suitable for the plants (Fig. 3). During the same period of 2020, a precipitation of 93 mm, a GDD value of 278 °C, and an average stomatal conductance value 22.8% lower than that of 2019 were less favourable conditions for the plants (Fig. 4). Low stomatal conductance values were recorded in 2019 in the V6150 kg in ha^{-1} treatment (412 mmol m^{-2} s^{-1}), while in 2020 in the non-fertilized (339 mmol m^{-2} s^{-1}) treatment. However, the highest stomatal conductivity values were the opposite, as the most suitable environmental condition was provided by the non-fertilized (589 mmol m^{-2} s^{-1}) treatment in 2019, and the $V6_{150}$ kg ha^{-1} (476 mmol m^{-2} s^{-1}) treatment in 2020 (Figs 3, 4). With increasing N-doses, the stomatal conductance value decreased significantly in 2019 (30.1%; $P < 0.01$), but in 2020 the rate of increase was 50.6% ($P < 0.001$). The largest difference between the two years was in the non-fertilized treatment, in 2020 the value of stoma conductance was 46.4% ($P < 0.001$) lower than in 2019.

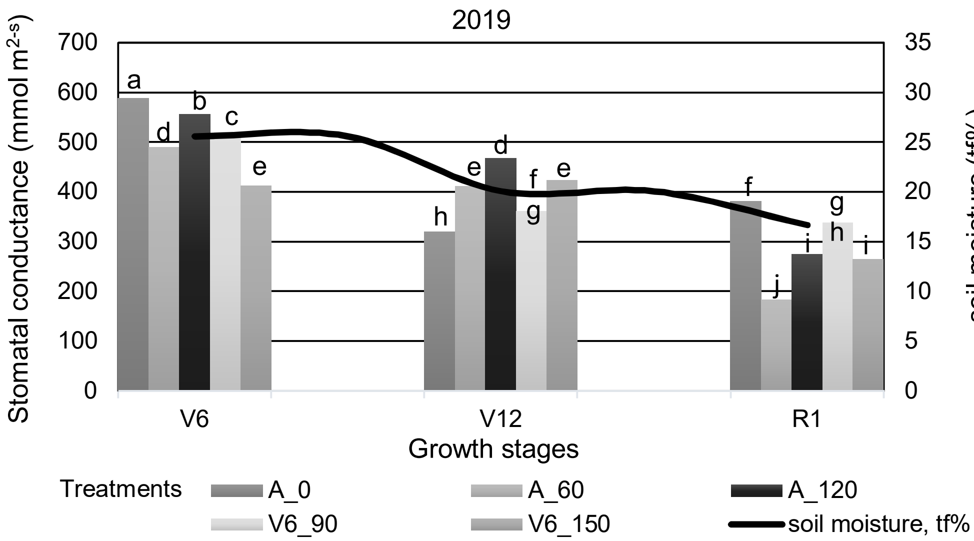

Figure 3. Development of stomatal conductance and soil moisture in 2019.

Note: A_0 = unfertilized control; A_{60} = 60 kg N ha^{-1} before sowing; A_{120} = 120 kg N ha^{-1} before sowing; $V6_{90}$ = 60 kg N ha^{-1} before sowing + 30 kg N ha^{-1} during V6 phenophase; $V6_{150}$ = 120 kg N ha^{-1} before sowing + 30 kg N ha^{-1} during V6 phenophase. The different lower case letters indicate the difference between fertilizer treatments based on *Duncan's test* ($P < 0.05$).

Between the phenological phases V6 and V12, 39 mm of precipitation fell in 2019 with a GDD value of 243 °C. In 2020, there was 10 mm more precipitation (49 mm) and a significantly lower GDD value (177 °C). The stomatal conductance values developed almost similarly in both years (397 and 367 mmol m^{-2} s^{-1}). Increasing the applied amount of N fertilizer can modify the stomatal conductance of leaves, thereby enhancing adaptability (Han et al., 2006), which is confirmed by the obtained results. In the V12 phenological phase, the stomatal conductance value increased in both years compared to the non-fertilized treatment. Stomata of the plants were the most active in the A_{120} kg ha^{-1} treatment in 2019 (468 mmol m^{-2} s^{-1}; 46.3%) ($P < 0.001$), while in the $V6_{150}$ kg ha^{-1} treatment (464 mmol m^{-2} s^{-1}; 39.3%) %) ($P < 0.01$) in 2020.

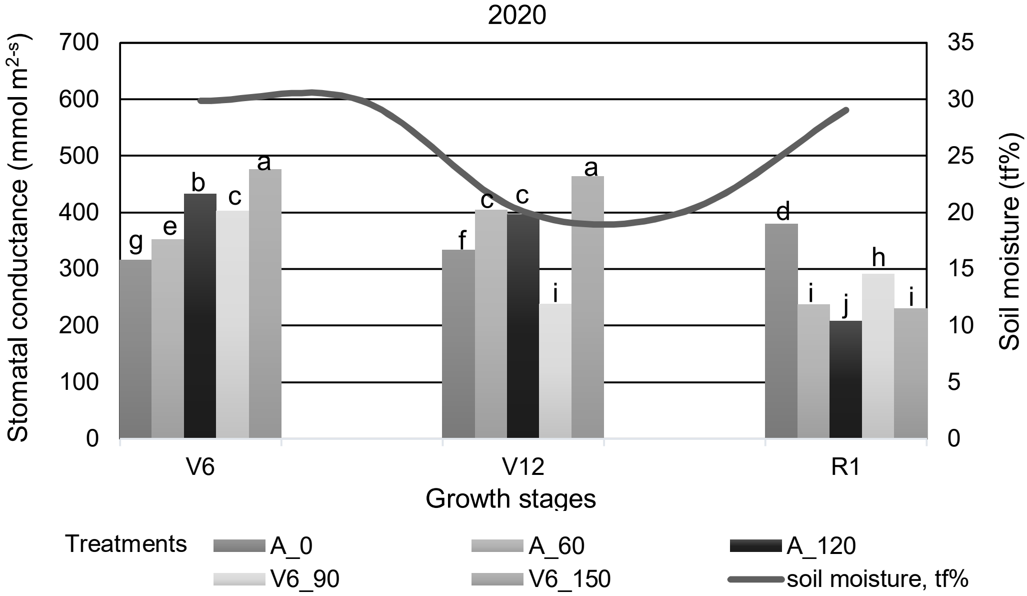

Figure 4. Development of stomatal conductance and soil moisture in 2020.

Note: A_0 = unfertilized control; A_{60} = 60 kg N ha^{-1} before sowing; A_{120} = 120 kg N ha^{-1} before sowing; $V6_{90}$ = 60 kg N ha^{-1} before sowing + 30 kg N ha^{-1} during V6 phenophase; $V6_{150}$ = 120 kg N ha^{-1} before sowing + 30 kg N ha^{-1} during V6 phenophase. The different lower case letters indicate the difference between fertilizer treatments based on *Duncan's test* ($P < 0.05$).

Between the V12 and R1 phenophases, only 8 mm of precipitation fell in 2019 and the GDD value was 182 °C. In 2020, there was also significantly more precipitation (128 mm) and the GDD value was higher as well (256 °C). There was no significant difference between the mean stomatal conductance values of the two years (6.6%). In both years, the plants were under less stress in the control treatment in the R1 developmental phase. Fertilization further reduced the value of stomatal conductance. In 2019, the largest decrease (52%; $P < 0.001$) was caused by the A_{60} kg ha^{-1} treatment, while in 2020, by the A_{120} kg ha^{-1} treatment (45.3%; $P < 0.01$).

The obtained results are in line with the findings of Kron et al. (2008), who stated that the stomata of plants become more closed and stomatal conductance decreases as the phenological phases progress. As the phenological phases progressed, the stomata of the plants became more and more closed and with the exception of the non-fertilized treatment, stomatal conductance decreased. The lowest value was recorded in the R1 phenophase in both years; in 2019 in the A_{60} kg N ha^{-1} treatment (183 mmol m^{-2} s^{-1}), in 2020 in the A_{120} kg N ha^{-1} (208 mmol m^{-2} s^{-1}). The decrease in stomatal conductance between the V12 and R1 phenological phases was 37.7–55.5% in 2019, ($P < 0.05$; $P < 0.001$) and 41.5–50.4% in 2020 ($P < 0.01$; $P < 0.001$). The effect of fertilization increasing stomatal conductance could not be clearly demonstrated. During the V6 phenological phase it varied depending on the crop year, during the V12 phenophase it increased in both years, while during the R1 phase it decreased in both years.

The measurements show that in the V12 phenological phase, the application of irrigation water would have been necessary in all treatments, although at that time the environmental conditions were still suitable for the plants. After that, the stomata began to close due to the decrease in the available water resources. The obtained results confirmed the finding of Anav et al. (2018) according to which the stomatal response to water deficit in soil highlights the need for irrigation.

The effect of soil moisture on stomatal conductance

Soil moisture content is of key importance to stomatal regulation (Anav et al., 2018). In line with the findings of Yu et al. (2015) and Santos et al. (2017), as the phenological phases progressed, soil moisture gradually decreased and became a limiting factor, the plant became water stressed and stomatal conductance decreased. Increased N fertilization increases the size of maize roots (Su et al., 2020; Putra & Ismoyojati, 2021) and leaf area (Amali & Namo, 2015; Hafez & Abdelaal, 2015), consequently it increases transpiration (Zhang et al., 2014) and water demand (Xu et al., 2020). Larger leaf area also means a larger assimilation surface. This leads to higher yields under favourable conditions. In water deficit periods, however, higher LAI can be detrimental. In 2019, by the R1 phenophase soil moisture content significantly decreased, thereby stomatal conductance decreased as well compared to the A_0 treatment (Fig. 3). The rate of decrease from the value of the V6 growth phase to the R1 phenophase was 43.9% ($P < 0.01$) in 2019 while it was 32.1% ($P < 0.05$) in 2020. In 2020, the high soil moisture value and increasing N fertilization measured during the R1 phase despite the decrease did not result in higher stomatal conductance values (Fig. 4). This could be due to the water deficit that occurred intermittently during the day, when the high LAI resulted in such fast plant transpiration that water uptake was unable to keep up with it. This may occur on days with evapotranspiration exceeding 5–6 mm per day during midday and early afternoon. At this time the soil around the root hairs dries out and water uptake ceases, the stomata close. In such cases, there is still sufficient water in the soil, similar to our results (2020, R1 phenophase), but water flow requires time. When the temperature drops, water uptake starts in the evening and during the night and water supply to the plant becomes uninterrupted.

The stomatal conductance value of the plants and soil moisture showed a close correlation ($r = 0.83$ ***) in 2019. As a result of the decrease in soil moisture, the stomata of the leaves of the plant were closed, thereby reducing transpiration and thus stomatal conductance also decreased. Based on the value of the coefficient of determination, soil moisture determined stomatal conductance with 69% ($r^2 = 0.689$). In 2020, the high soil moisture value measured in the R1 growth phase did not result in higher stomatal conductance values, and there was no detectable significant correlation between the two factors.

Yield and stomatal conductance

The yield of maize of the unfertilized control was 8.726 t ha^{-1} in 2019, which shows the good nutrient utilization capacity of the hybrid. Compared to the control, the lowest 60 kg N ha^{-1} base treatment (A_{60}) increased the yield by 38.9% ($P < 0.05$). There was no reliable difference among the rest of the fertilizer treatments. The maximum yield was provided by the $V6_{150}$ treatment (14.023 t ha^{-1}), which was 60.7% higher compared to the control treatment, however, based on the Duncan test, the result of the A_{60} treatment at 12.124 t ha^{-1} can be considered effective. The yield of the maize hybrid of the unfertilized control in 2020 was 6.219 t ha^{-1}. Compared to the control treatment, the A_{60} treatment increased the yield by 3.633 t ha^{-1} ($P < 0.05$). The difference between the A_{60} and A_{120} basic treatments was 4.339 kg ha^{-1}, the higher base dose resulted in a significant ($P < 0.05$) increase. There was no significant difference between the yield of 30 kg N ha^{-1} top dressing ($V6_{90}$) applied in the V6 phenophase after the A_{60} base treatment. There was also a non-significant difference between the yield of the A_{120} and $V6_{150}$ treatment. The highest yield and the statistically confirmed maximum yield were recorded in the A_{120} treatment (14.191 t ha^{-1}).

The natural nutrient utilization capacity of maize was 40.3% ($P < 0.001$) better in 2019 than in 2020. The modifying effect of the crop year was recorded when the 60 kg N ha^{-1} (A_{60}) base treatment was applied (2.272 t ha^{-1}; $P < 0.01$).

There was a moderate linear correlation ($r = -0.69$*, $r = 0.72$*, $r = -0.59$) between stomatal conductance and yield in 2019 in the V6, V12 and R1 growth stages. Based on the value of the coefficient of determination, the yield-modifying effect of stomatal conductance was 55–56% in the V6 and V12 phenophase and 40% in the R1 phenophase. The correlation between the two factors in 2020 in the V6 and R1 growth stages was close ($r = 0.87$***, -0.78*). No reliable correlation could be detected in the V12 phenophase. In this year, the effect of stomatal conductance on yield was higher (V6: 79%; R1: 63%) than in 2019.

CONCLUSIONS

In the present study it was confirmed that stomatal conductance of maize decreases with plant development. N fertilization increases stomatal conductance. However, when soil moisture content decreases, or when there is sufficient moisture in the soil but little time for soil moisture to flow due to the rapid transpiration of higher LAI, the stomata close. When temperature drops, water uptake starts during the evening and night hours and water supply to the plant becomes undisturbed. This demonstrates that the results obtained when measuring stomatal conductance are strongly influenced by the time of day at which the measurement is taken. The measurement of stomatal conductance has proved useful for detecting water deficiency. It was confirmed that the V12 phenological phase would have been suitable for the application of irrigation water (2019 and 2020), as after that stomata started to close due to the reduction of the water available for them. Furthermore, the influence of stomatal conductance on yield in the cooler and wetter year (2020) was higher than in the year of 2019 which was average in this regard.

Finally, the study revealed that the highest yields are obtained when the average stomatal conductance is around 250 mmol m^{-2} s^{-1} during the growing season.

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Product-oriented production of industrial hemp according to climatic conditions

Ē. Teirumnieka[1,*], D. Blumberga[1], E. Teirumnieks[2] and V. Stramkale[3]

[1]Riga Technical University, Institute of Energy Systems and Environment, Azenes Iela 12/1, LV-1048 Riga, Latvia
[2]Rezekne Academy of Technologies, Engineering Institute, Atbrivosanas aleja 115, LV-4601 Rezekne, Latvia
[3]Latvia University of Life Science and Technologies, Institute of Agricultural Resources and Economics, Department of Crop Breeding and Agroecology, Latgale Agricultural Research Centre, Kulturas laukums 1a, Vilani, LV-4650 Vilanu novads, Latvia
[*]Correspondence: erika.teirumnieka@gmail.com

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Abstract. Cultivation area of industrial hemp in Europe has increased since 2012. It is expected that in future its production will increase, because European Union (EU) policy focuses more on the 'green deal' goals. Research into the effects of climate conditions (temperature and rainfall) on growth is important to select the best industrial hemp varieties for hemp products. The objective of the research is identifying industrial hemp varieties suitable for seed, fiber and shives production in varying pedo-climatic conditions in order to obtain products with the highest added value.
Four industrial hemp varieties were used for the research: 'Purini' (Latvia), 'Bialobrzeskie' (Poland), 'USO -31' (France-Ukraine), 'Finola' (Finland). Field trials were carried out in Eastern Latvia in 2010, 2011, 2012, 2013, 2019. Climatic indicators were recorded during the vegetation period from April to September. Yields of seeds, fibre, shives and total biomass were determined during the research. Factor analysis method was used to determine the impact of temperature and rainfall on the yield of seeds, fibres and shives. The study of climatic factors shows that the effect of temperature and rainfall on seed, shives and fiber yields strongly depends on the variety.

Key words: hemp growth, industrial hemp, climate conditions.

INTRODUCTION

The history of use and cultivation of industrial hemp is exceptionally long. Hemp is a multi-purpose crop delivering fibers, shives, seeds and pharmaceuticals. This crop is unique for bioeconomy because the properties of hemp seeds, fiber, shives allow it to be widely used. Traditionally, hemp is used in textiles, bio composites, cosmetics, oil, pharmaceutical industry, building industry as insulation material etc. (Salentijn et al., 2015). Hempcrete is alternative building material which could replace traditional concrete materials and reduce the carbon footprints of buildings and help in saving natural resources. Hempcrete will promote the development of sustainable construction materials and replace also synthetic-based products (Karche & Singh, 2019).

Hemp, adjacent to flax, is currently recognised as a significant source of natural fiber. Replacing fossil raw materials with natural, opens up new opportunities for the use of hemp fiber in the near future. Cultivation area in Europe has increased since 2012. The main cultivation states are France and Netherlands, while in Baltic States it is not so intense (Carus et al., 2013). It is expected that in future its production will increase, because EU policy focuses more on the 'green deal' goals.

Today, in the EU hemp is a niche crop. Because of its unique properties, particularly its environmental benefits and the high yield of natural technical fibres, hemp is a valuable crop for the bio-based economy (Carus et al., 2013).

Climatic conditions are one of the major factors influencing hemp yields (Baldini et al., 2020). The effects of these factors need to be studied at a particular cannabis site to assess the effects of climatic factors on yields (Amaducci et al., 2015). Much of the research has focused on the determination of the cannabidiol (CBD) content of cannabis in relation to climatic conditions, in particular temperature and rainfall (Mazian et al., 2018; Abdollahi et al., 2020a; Abdollahi et al., 2020b). CBD is pharmacologically active substance, very important for use in medical applications (Glivar et al., 2020).

Different meteorological conditions during growth affect cannabis yield (Wei, 2007). The results of many experiments show that yields fluctuate significantly from year to year, even under practically the same agricultural conditions. In Latvia, with very volatile agrometeorological conditions, such fluctuations are inevitable. Yield stability indicators for different crops have been little studied, as even two identical years are not possible under field conditions (Kroģere & Pelēce, 2004). In Eastern Latvia there are large fluctuations of temperature and rainfall during the vegetation period (Baltiņa et al., 2011; Maļceva et al., 2011). The yield of hemp is significantly affected by climatic conditions. Of course, yield is affected not only by climatic conditions, but also by variety, soil, fertilizer and other factors. This study of 4 varieties allows to use the obtained data for the selection of a suitable variety in Eastern Latvia.

MATERIALS AND METHODS

Four industrial hemp (*Cannabis sativa* L.) varieties (Table 1) were used for the research: 'Purini' (Latvia), 'Bialobrzeskie' (Poland), 'USO-31' (France-Ukraine), 'Finola' (Finland).

Table 1. Tested hemp cultivars registered in EU Plant variety database

Cultivar	Grown in research year	Country	Admission	Origin	Climate adaptation	Maturity group
Bialobrzeskie	2010 to 2013	PL	31.12.1967	Poland	Continental	Medium<140 days
Finola	2010 to 2013 and 2019	FI	05.02.2003	Finland	Continental	Early<115 days
USO-31	2011 to 2013 and 2019	CH	07.06.1999	Ukraine	Atlantic	Early<125 days
Pūriņi	2010 to 2013 and 2019	LV	27.02.2020	Latvia	Atlantic	Early<125 days

The hemp variety 'Pūriņi' is a variety selected in Latvia (Common catalogue of varieties of agricultural plant species. 2020 consolidated version; Latvian Plant Varieties Catalogue).

Field trials were carried out in Eastern Latvia in 2010, 2011, 2012, 2013, 2019. The methodology of field trials is presented in Table 2. Characteristics of the test site - Viļāni Parish ((N) 56°34.053′; (E) 26°58.868′), 110 meters above sea level, the terrain is mostly flat and slightly hilly, the climate is moderately continental, moderately warm and humid.

Table 2. The methodology of field trials

Year	2010	2011	2012	2013	2019
Soil type	sod -podzolic gley soil	sod -podzolic gley soil	sod -podzolic gley soil	sod -podzolic gley soil	sod -podzolic gley soil
Organic matter in soil	6.5%	6.5%	6.5%	6.5%	7.4%
Soil pH	7.0	7.0	7.0	7.0	6.6
P_2O_5	145 mg kg^{-1}	145 mg kg^{-1}	145 mg kg^{-1}	145 mg kg^{-1}	152 mg kg^{-1}
K_2O	118 mg kg^{-1}	118 mg kg^{-1}	118 mg kg^{-1}	118 mg kg^{-1}	112 mg kg^{-1}
Soil tillage	field plowed in autumn 2009, cultivated in spring 2010	field plowed in autumn 2010, cultivated in spring 2011	field plowed in autumn 2011, cultivated in spring 2012	field plowed in autumn 2012, cultivated in spring 2013	field plowed in autumn 2018, cultivated in spring 2019
Sowing rate	70 kg·ha^{-1}	70 kg·ha^{-1}	70 kg·ha^{-1}	70 kg·ha^{-1}	60 kg·ha^{-1}
Field area	20 m^2	12 m^2	20 m^2	16 m^2	15 m^2
Repetitions	3	3	3	3	4
Harvesting	For each variety, 1 m^2 of hemp shall be harvested from each variant, tie in the beams and determine yields of seeds, fiber, shives and total biomass after drying				

Hemp samples were taken from each variety (Fig. 1) in 4 replicates. The yield of seeds, shives, fiber and biomass was determined for the samples collected after harvesting.

Pūriņi Finola USO-31

Figure 1. Hemp sample fields.

Hemp sowing dates for each variety are the same in current year but harvesting dates are different for each variety (Table 3) e.g. in 2011 difference is near 1 month.

The plots were not additionally treated with mineral fertilizer, therefore the effect of additional fertilizer on yield is excluded. During the growing season - April to September - there is a fixed temperature and precipitation to assess the impact of climatic conditions on hemp yield depending on the variety and to determine the suitability of the hemp variety for Eastern Latvia or similar climatic conditions. Data analysis was used to determine the most appropriate variety for a particular hemp product - seed, shives, fiber.

Yield and shives content were calculated as the arithmetic mean of three replicates. Analyzes of plant samples were performed in the laboratory (RTA Chemistry Laboratory) using standard methods specified by the State of Latvia.

The shives and fiber content (%) was determined for the average hemp sample of each replicate, which was divided into two portions and dried to 8–10% moisture. 100 g of haulm from each sample were weighed onto the balance (accuracy ± 0.001 g) by grinding with LM-3 and brushing, shaking from the splint fiber to remove the shives.

Table 3. Hemp sowing and harvesting dates

Variety	Year	Sowing data	Harvesting data
Finola	2010	May, 13	September, 1
Purini		May, 13	September, 7
Bialozeberskie		May, 13	September, 16
Finola	2011	May, 6	August, 24
Purini		May, 6	September, 6
Bialozeberskie		May, 6	September, 22
USO 31		May, 6	September, 16
Finola	2012	May, 9	August, 28
Purini		May, 9	September, 11
Bialozeberskie		May, 9	September, 26
USO 31		May, 9	September, 19
Finola	2019	May, 13	September, 12
Purini		May, 13	September, 15
USO 31		May, 13	September, 27

Climatical parameters - temperature and rainfall - of location are shown in Figs 2, 3. Data used from Vilani Meteorological Observation Station. The last ten years have seen a high temperature in relation to the long term mean, more pronounced in the middle of vegetation.

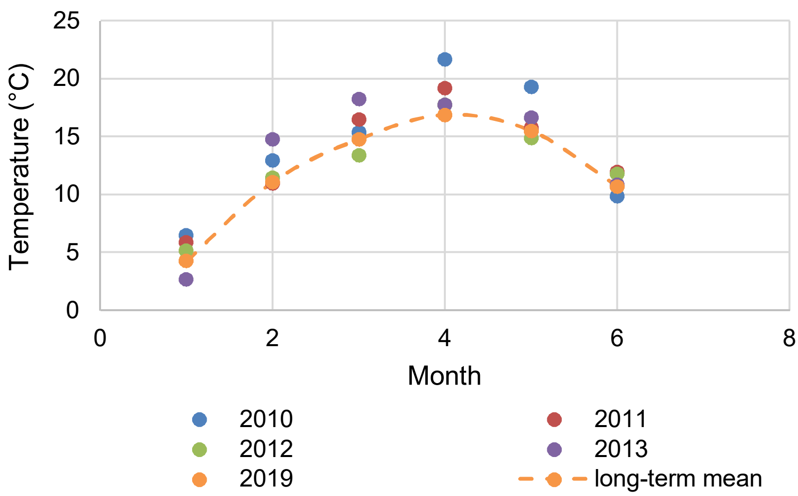

Figure 2. Monthly temperature during the growing season (1-April, 2-May, 3-June, 4-July, 5- August, 6-September) in 2010, 2011, 2012, 2013, 2019 and long-term mean at Vilani (Latvia).

The last ten years have generally seen lower rainfall relative to the long term mean, more pronounced in the middle and at the end of vegetation. An exception during the research was 2012, which was rainy in Latvia.

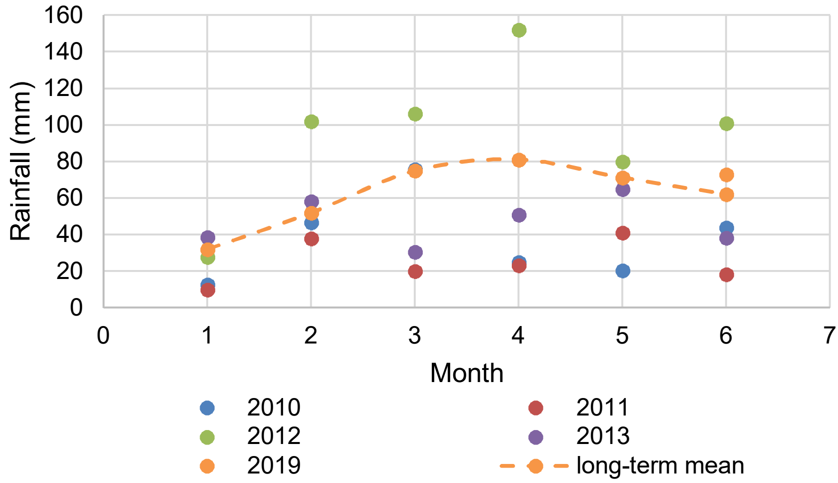

Figure 3. Monthly rainfall during the growing season (1-April, 2-May, 3-June, 4-July, 5-August, 6-September) in 2010, 2011, 2012, 2013, 2019 and long-term mean at Vilani (Latvia).

The results were processed using descriptive and variation statistics, regression and correlation methods. The strength of the mutual link of independent and dependent random variables (correlation) can be assessed by means of a correlation coefficient. In case of a single factor mathematic model, the Pearson's equation is used for its estimations:

$$r = \frac{\sum_{i=1}^{m}(x_i - x)(y_i - y)}{(m-1)S_x * S_y}, \quad (1)$$

where x_i, y_i – independent variables and pairs of corresponding dependent variables; x, y – mean arithmetic values of independent and dependent variables; S_x, S_y – selection dispersions of variables.

RESULTS AND DISCUSSION

Climate data analysis is an important factor influencing hemp yields. The variability of climatic factors in the vegetation period for ruminants from 2010–2013 and 2019 is given in Figs 4, 5.

Unusually low temperatures were observed only in June. So the temperature fluctuations in the summer months become more pronounced year by year.

Rainfall is very variable. In May, rainfall is more frequent than the average for this month, but in June it is higher than the average. In the summer months, there is a marked inequality over the years. The pronounced variability of rainfall during the growing season becomes sharper. To avoid plant stress and obtain viable yields, adequate moisture during active growth is required. Hemp is sensitive to drought conditions and needs an adequate supply of water (Cole & Zurbo, 2008).

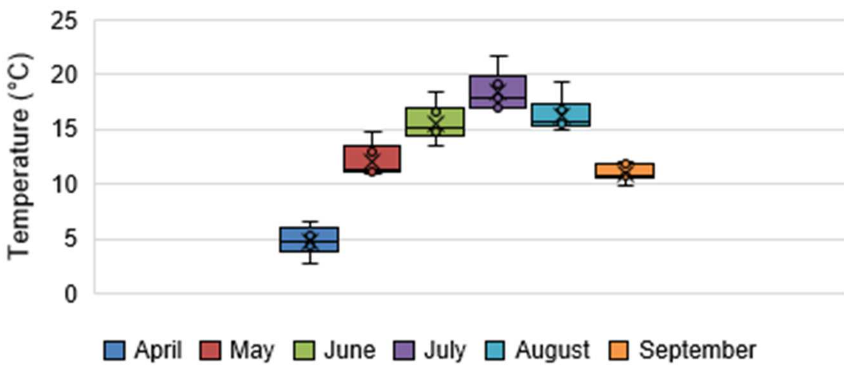

Figure 4. Temperature variability in the vegetation period by months from 2010 to 2013 and 2019.

During sowing - usually April, early May - the average air temperature is 5 °C, which is favourable conditions for seed germination. Precipitation in April is generally lower than in other vegetation months and no sharp changes are observed. As there is sufficient moisture in the soil after the winter period, the conditions for germination are favourable. During the study period, April and May 2010 had low rainfall, which hindered germination. As the dry and hot weather continued in May and June, this had a negative effect on plant development. Assessing the fluctuations of climatic conditions, it can be concluded that higher risk factors for germination may be caused by rising temperatures and low rainfall in May.

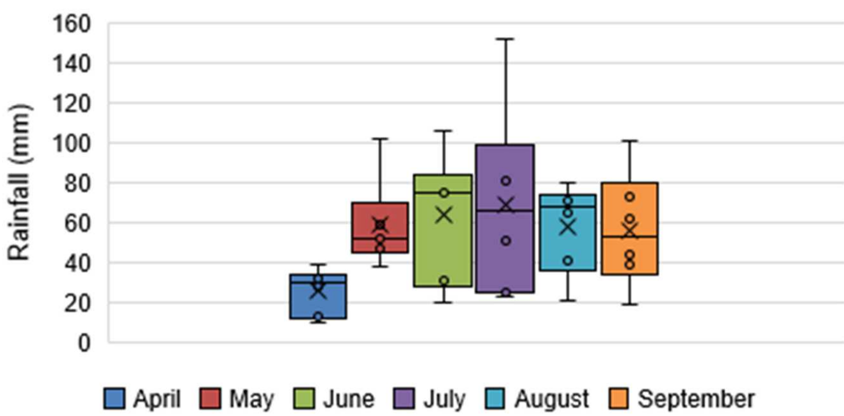

Figure 5. Variability of rainfall during the vegetation period by months from 2010 to 2013 and 2019.

The study observed that weather conditions is quite different in different years. Therefore, varieties that are less sensitive to temperature and humidity fluctuations during sowing and in the early stages of plant development should be selected for cultivation. Relatively warm and wet September can extend the vegetation period, so in the future it will be possible to choose varieties not only with a short, but also with an average vegetation period.

Fiber yield is higher at moderate humidity and lower temperatures Figs 6, 7. With temperature increase there is a significant decrease in the fiber yield for 'Finola' and 'Purini', a slight decrease for 'USO-31', while the fiber yield of 'Bialobrzeskie' is not affected by temperature fluctuations. 'Bialobrzeskie' also has higher absolute numbers of fiber yield, regardless of the year of observation. In turn, the increase in moisture increases the fiber yield for 'Finola' and 'Purini', slightly decreases it for 'USO-31', and does not affect 'Bialobrzeskie'.

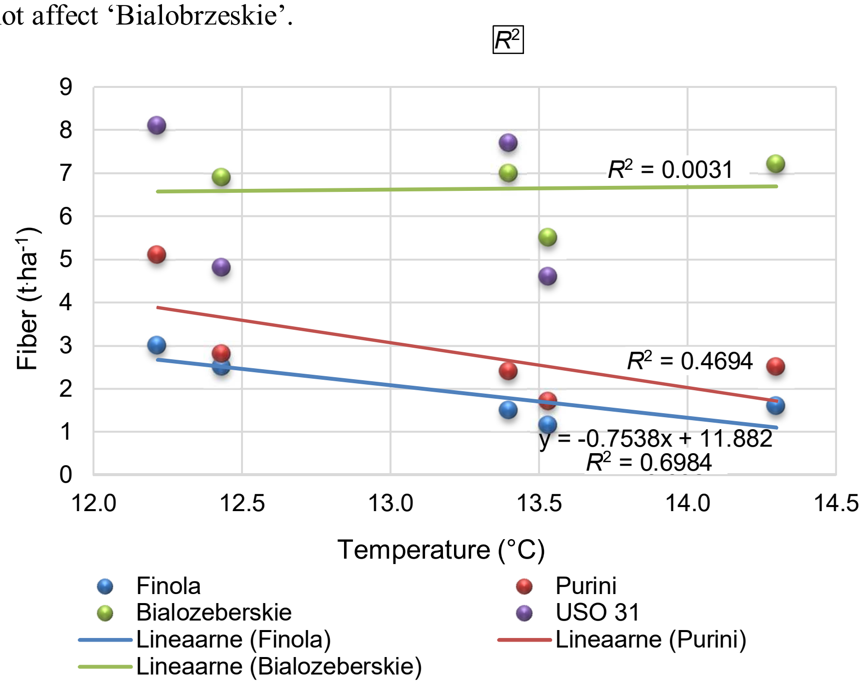

Figure 6. Fiber yield dependence of temperature.

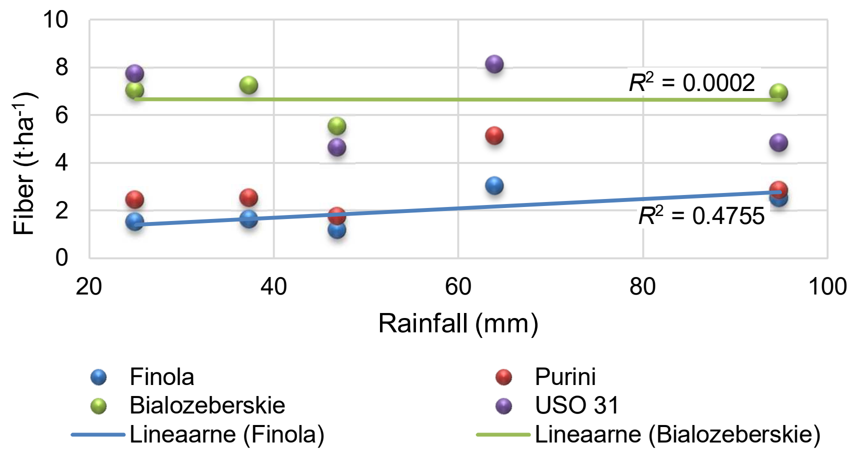

Figure 7. Fiber yield dependence of rainfall.

Similar high level data dispersion and correlations have been observed in the yield of shives (see Figs 8, and 9.), with the exception of 'Bialobrzeskie', where the yield of shives increased due to higher temperature, but slightly decreased due to humidity.

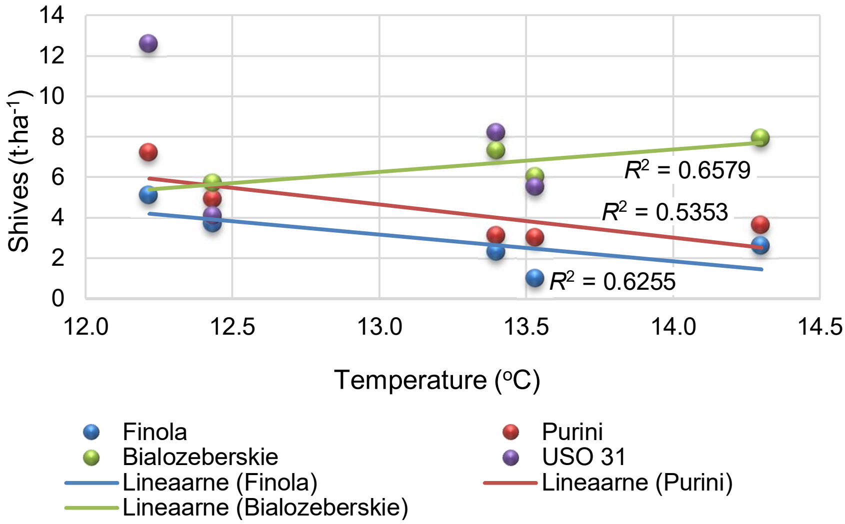

Figure 8. Shives yield dependence of temperature.

At high rainfall (above 80 mm during the growing season) and low temperatures (around 12.5 °C during the growing season) there is the lowest amount of shives.

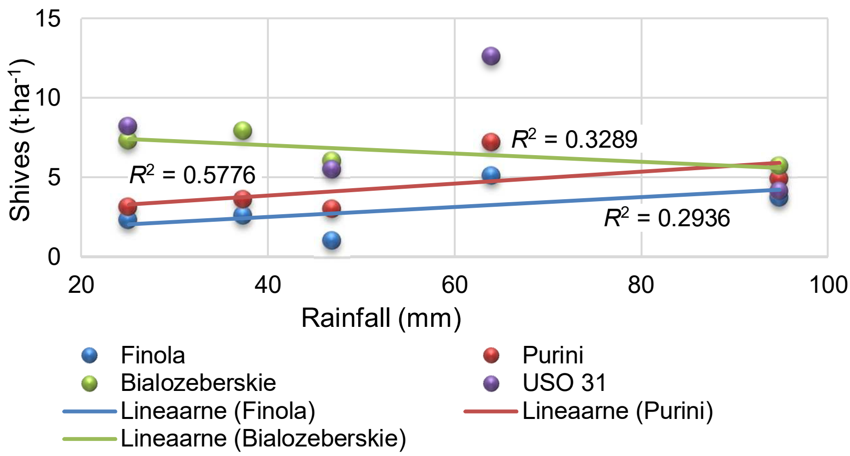

Figure 9. Shives yield dependence of rainfall.

Thus, under the same growing conditions, the variety 'Bialobrzeskie' is more stable and less dependent of climatic conditions in terms of fiber and shives production. 'Bialobrzeskie' also gives a higher total yield per ha.

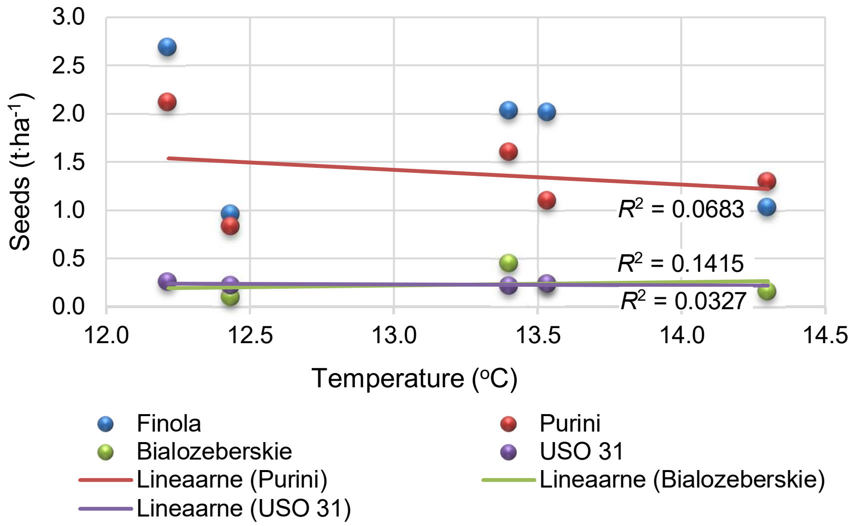

Figure 10. Seeds yield dependence of temperature.

For all varieties, the seed yield decreases at rainfall higher than 80 mm, which is above the long-term averages (Figs 10, 11). 'Finola' gives higher seed yield in changing climatic conditions. The highest yield of green mass was observed at higher humidity, but no conclusions can be drawn about the effect of temperature. Similar high level data dispersion and correlations have been observed in the yield of seeds. Insufficient moisture hinders the development of hemp. The literature (Nelson, 2000) indicates that it is even necessary to irrigate hemp fields to obtain higher yields.

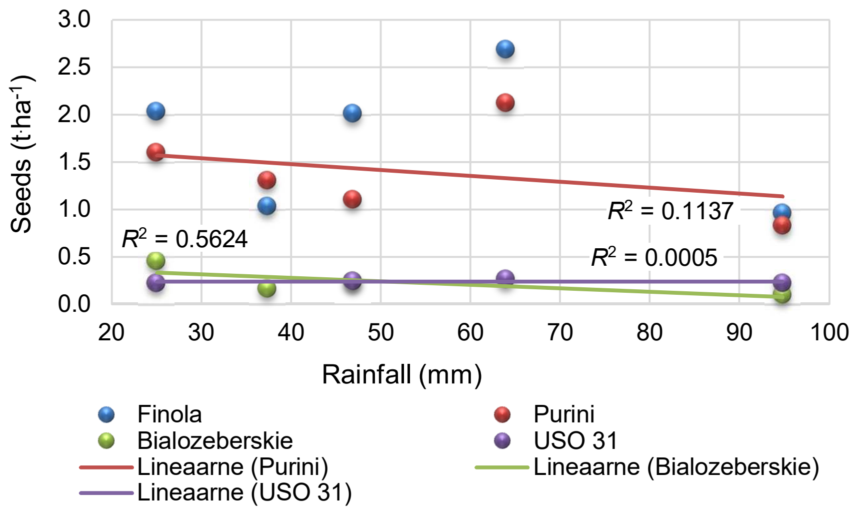

Figure 11. Seeds yield dependence of rainfall.

CONCLUSIONS

The analysis of climatic factors shows that the effect of temperature and rainfall on seed, shives and fiber yields strongly depends on the variety. Thus, it is possible to identify a variety of hemp in order to obtain a specific product containing hemp.

The yield of hemp is more strongly influenced by the amount of rainfall, at high rainfall only the biomass does not decrease. Decreased yields at high humidity and low temperatures are often explained by fungal growth on plants.

The effect of temperature could not be determined in the analysis of some parameters. This can be explained by the fact that in Latvia as a whole the temperature is insufficient for the full development of hemp.

In the changing climatic conditions of Eastern Latvia, it is better to grow early varieties with a short vegetation period primarily for seed production. 'Finola' can be recommended for seed production from the studied varieties. 'Bialobrzeskie' is suitable for fiber and shives. The yields of hemp fibers and shives are variable, so they could only be by-products.

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Relative efficiency of replicated and non-replicated statistical designs in quantifying the variations in maize grain yield

O.E. Zakaria[1], M.M. El-Rouby[1], A.I. Nawar[1], H.E.M. Ibrahim[1] and A.A. Abd El-Salam[2]

[1]Crop Science Department, Faculty of Agriculture, Alexandria University, Aflaton street, El-Shatby, EG21545 Alexandria, Egypt
[2]Soil and Water Science Department, Faculty of Agriculture, Alexandria University, Aflaton street, El-Shatby, EG21545 Alexandria, Egypt
[*]Correspondence: dralinawar@alexu.edu.eg

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Abstract. Two-year field experiment was conducted at the Agricultural Research Station, Faculty of Agriculture, Alexandria University, Egypt, during the two successive summer seasons of 2018 and 2019. The main aim was to evaluate the relative efficiency of two groups of experimental designs in quantifying the variations in maize grain yield as influenced by sowing date (SD), plant density (PD) and phosphorous (P) fertilization, and their interactions. The single hybrid Giza 168 maize (*Zea mays*, L.) cultivar was used during both seasons. The experimental designs under evaluation included replicated (RCBD, SPD, SSPD and 3-DLD), in three replications, and non-replicated (one-rep without and with center points, RCCD and PRCCD) designs. The 3-DLD design was more efficient, within the replicated group, than the RCBD (reference design) with relative efficiency of 3.68. The SPD and SSPD had higher relative efficiencies at the sub-plot and sub sub-plot levels compared to RCBD. Within the non-replicated designs, the one-rep with center points, RCCD and PRCCD were more efficient than one-rep without center points (reference design) in discriminating the more important factors affecting grain yield in maize cultivar Giza 168.

Key words: analysis of variance, experimental designs, experimental error, field replications, statistical relative efficiency.

INTRODUCTION

Maize (*Zea mays* L.) is an important food crop in the world and in Egypt. Increasing the productivity of maize per unit area involves the use of high yielding hybrids and better management of factors affecting that productivity. Sowing date, plant density and phosphorus fertilization are important factors that cause variations in maize productivity (Kadyrov & Kharitonov, 2019; Széles & Huzsvai, 2020 and Ibrahim et al., 2021). Hence, agriculture field experiments to determine the optimal level of each of those factors should be able to elucidate the significant effect of those individual factors, and there interactions, in order to determine their role in affecting the productivity of maize.

Efficient field experiments aim to minimize the experimental error in order to accurately detect the variations in the studied parameters caused by the investigated treatments and their interactions. In order to achieve this goal, it is necessary to control the spatial variations that occur in the field and might be caused by several circumstances such as variations in soil fertility, management practices and other environmental factors. The three principles proposed by Fisher (1935), i.e. randomization, replication and local control, form the basis for controlling the experimental error in any experimental design. Based on these three principles, the randomized complete block design (RCBD) remains the most popular design for many field experiments. However, with the increase in number of treatments, it becomes hard to control the heterogeneity within blocks and, thus, the precision of block analyses decreases (Casler, 2015).

To overcome the disadvantages of RCBD, incomplete block designs were suggested. Those designs permitted the confounding of a factor with the main plots such as the split-plot design (SPD) or confounding an additional factor with the sub sub-plots such as the split split-plot design (SSPD) (Fisher, 1925). Those designs increased the precision for one or two factors and first and/or second order interactions, meanwhile losing information about the confounded factor(s). In addition, the complication of testing the contrasts with more than one experimental error, adds to the disadvantages of those designs.

Thus, Yates (1936), later on proposed the lattice designs to overcome the spatial variation between experimental units in variety trials. These designs proved to be more precise than RCBD in several yield trials carried out by Ma & Harrington (1948) from 1937 to 1946. However, these designs were restricted to a limited number of varieties and the field layout was very stringent (Abd El-Shafi, 2014). Patterson & Williams (1976) introduced the alpha lattice design for unlimited number of varieties, and when spatial variations are high (Müller et al., 2010). However, to our knowledge, none of the lattice designs were used to test quantitative factors in the agricultural experiments, especially those that are difficult to analyze and rigid in field layout such as the three-dimensional (cubic) lattice (Yates, 1939).

In factorial experiments, the number of experimental units increase with increasing the number of factors, number of levels for each factor or both. For example, a 3^2 experiment in three replicates requires 27 experimental units, while a 3^3 experiment will require 81 units. Thus, there is an increased challenge to maintain spatial homogeneity in blocks with the increase in experimental units. To overcome that, Box & Wilson (1951) proposed the rotatable central composite design (RCCD) where each factor is studied at 5 points, i.e. two factorial, 2 star and one central point. That design requires fewer experimental units than the replicated experiments for the same number of studied factors. For example, a 3^3 factorial experiment will require only 20 experimental units in RCCD, (8 factorial points, 6 star points and 6 central points) compared to 81 experimental units required in case of the 3-replication RCBD. However, that design is criticized for estimating error from a few numbers of experimental units that are similarly treated (central points). Therefore, Dykstra (1960) proposed replicating of the factorial and star points in several blocks to obtain the traditionally accepted within-block error component of variations. The partially replicated central composite design (PRCCD) requires more experimental units than RCCD but is expected to give a better estimate of error. Unfortunately, there is no published agricultural field research adopting that design.

The present study was carried out to compare the relative efficiency of two groups of experimental designs, in detecting the importance of the studied factors (sowing date, plant density and phosphorus fertilization), and their interactions, in determining the grain yield of maize cultivar Giza 168. First group contained the replicated designs, i.e;

randomized complete block, split-plot, split-split plot and three-dimensional lattice. The second group included the non-replicated designs, i.e; one-rep without and with center points, rotatable central composite and partially replicated central composite.

MATERIALS AND METHODS

Experimental location

The present investigation was carried out at the Agricultural Research Station, Faculty of Agriculture at 31.2 °N, 29.92 °E, Alexandria University, Egypt, during the two successive summer seasons of 2018 and 2019. Soil samples were taken from the experimental site at 0–30 cm depth. The major physical and chemical characteristics of the experimental soil were determined after Olsen et al. (1954), Richards (1954) and Black et al. (1965) and are presented in Table 1.

Table 1a. Soil physical and chemical properties in first season

Physical properties		Chemical properties	
Sand %	56.40	pH	8.17
Silt %	10.80	EC (dS m^{-1})	1.61
Clay %	32.80	Ca^{+2} (meq L^{-1})	4.22
Texture	Sandy clay loam	Mg^{+2} (meq L^{-1})	3.22
Nutritional properties			
Available N (ppm)	305.43	Na^{+} (meq L^{-1})	10.02
Available P (ppm)	30.10	K^{+} (meq L^{-1})	0.61
Available K (ppm)	465.40	Cl^{-} (meq L^{-1})	6.31
Organic matter (%)	2.02	CO_3^{-2} (meq L^{-1})	1.11
HCO_3^{-} (meq L^{-1})	2.23		
Micro nutrients			
Cu (ppm)	3.42	SO_4^{-} (meq L^{-1})	7.57
Fe (ppm)	4.65	$CaCO_3$ (%)	8.87
Mn (ppm)	4.52	SAR	5.31
Zn (ppm)	1.68		

Table 1b. Soil physical and chemical properties in second season

Physical properties		Chemical properties	
Sand %	57.00	pH	8.09
Silt %	10.70	EC (dS m^{-1})	1.49
Clay %	32.40	Ca^{+2} (meq L^{-1})	4.64
Texture	Sandy clay loam	Mg^{+2} (meq L^{-1})	3.56
Nutritional properties			
Available N (ppm)	308.57	Na^{+} (meq L^{-1})	9.90
Available P (ppm)	31.50	K^{+} (meq L^{-1})	0.55
Available K (ppm)	475.60	Cl^{-} (meq L^{-1})	6.57
Organic matter (%)	2.22	CO_3^{-2} (meq L^{-1})	1.45
HCO_3^{-} (meq L^{-1})	2.61		
Micro nutrients			
Cu (ppm)	3.64	SO_4^{-} (meq L^{-1})	7.93
Fe (ppm)	4.93	$CaCO_3$ (%)	8.23
Mn (ppm)	4.72	SAR	5.45
Zn (ppm)	2.04		

The experimental location is characterized by its Mediterranean climate with its hot and dry summers. Mean minimum and maximum monthly temperatures, average humidity and wind speed during the two growing summer seasons are presented in Table 2, while total monthly precipitation was zero during both summer seasons.

Table 2. Average monthly maximum and minimum temperatures, humidity, and wind speed for the two experimental seasons

Month	Max. Temperature (°C)		Min. Temperature (°C)		Humidity (%)		Wind speed (km hr^{-1})	
	2018	2019	2018	2019	2018	2019	2018	2019
April	25.9	23.1	16	14.1	64.0	65.0	13.7	14.5
May	29.0	28.5	20	18.5	64.7	60.6	15.2	13.5
June	30.5	30.0	22.7	23.3	63.4	67.5	14.3	14.8
July	31.2	31.3	24.8	23.4	68.3	65.9	17.0	15.6
August	31.6	31.0	24.4	24.1	66.4	68.6	14.9	14.8
September	30.6	29.7	24.7	23.3	64.8	64.1	15.2	15.1

Data was compiled from www.wunderground.com

Factors and experimental designs under evaluation

The study investigated the variations of maize grain yield (t ha^{-1}) as influenced by sowing date (SD), plant density (PD) and phosphorous (P) fertilizer levels.

The evaluated designs included two groups:

I - Replicated designs:

1. Randomized complete block with three replications (RCBD), which was considered the reference design for replicated designs.
2. Split plot design with two factors in the main plots and one factor in the sub- plots, with three replications (SPD).
3. Split split plot design with three replications (SSPD).
4. Three-dimensional lattice design with three replications (3-DLD).

II - Non-replicated designs:

1. One-rep design:
   a) without center point using the 2nd order interaction as an error term,
   b) with six center points to estimate the experimental error.
2. Rotatable Central Composite Design with one replication (RCCD).
3. Partially Replicated Central Composite Design (PRCCD).

In the first experimental season (2018), RCBD, SPD and non-replicated designs were performed, whereas in second season (2019), only SSPD and 3-DLD designs were evaluated.

The levels of the studied factors used in experimental designs are presented in Table 3. Levels of factors and number of experimental units employed in each design are shown in Table 4.

Table 3. The applied levels of each factor, according to the different experimental designs

Factor levels	I	II	III	IY	Y
Sowing date (SD)	April 20th	May 1st	May 15th	May 30th	June 9th
Plant density / ha (PD)	15,866	22,758	27,766	32,775	39,666
Phosphorus fertilization (P) (kg P_2O_5/ha)	0	8	25	42	50

Table 4. The levels of the studied factors used in each design, according to experimental design specifications

Design	Levels					No.
	I	II	III	IV	V	of experimental units
I- Replicated designs:						
RCBD	✓		✓		✓	81
SPD	✓		✓		✓	81
SSPD	✓		✓		✓	81
3-DLD	✓		✓		✓	81
II- Non-replicated designs:						
One-rep without center point	✓		✓		✓	27
One-rep with center point	✓		✓		✓	33
RCCD	✓	✓	✓	✓	✓	20
PRCCD	✓	✓	✓	✓	✓	43

As shown in Table 4, all design employed three levels from each factor (I, III and V) except the RCCD and PRCCD which employed all five levels where levels (I) and (V) represent the lowest (S-) and highest (S+) star points, (II) and (IV) represent the lowest (F-) and highest (F+) factorial points, respectively, while level (III) represent the central (C) point for each factor.

The maize (*Zea mays*, L.) cultivar used during both seasons was Giza 168, developed by Maize Research Program, Agriculture Research Center, Giza, Egypt. That cultivar is a single yellow dent hybrid that resulted from crossing Giza 658 and Giza 639. The cultivar required around 105 days from sowing till complete maturity and harvesting.

Sowing and agricultural practices

The experimental site was the same to maintain homogeneity of the experimental units in the two seasons (Idrees & Khan, 2009). The previous winter crop was berseem clover (*Trifolium alexandrinum* L.) in both seasons. After removing the berseem clover, the seedbed was prepared by chisel plowing (to a depth of 20–25 cm), followed by land levelling and ridging. The land was then divided into experimental plots. Each plot contained four ridges (0.7 m width and 2 m long) resulting in a final plot area of 5.6 m^2. Sowing was done in hills on the upper third part of one side of the ridge. The distance between hills varied according to the plant density. Plants were thinned to one plant $hill^{-1}$ at 24 days after sowing (DAS).

Surface irrigation was scheduled every 12 days and applied at the rate of 5,700 m^3 ha^{-1}, as recommended for the region using a pipe system with water meter model TURBO-IR-A DN50-300, 2’12’ manufactured by Bermad Irrigation, and was terminated ten days before harvesting. Nitrogen fertilization (270.4 kg N ha^{-1}) was applied in the form of urea (46.5%N) and split into three doses, the first dose was applied during land preparation (48 kg N ha^{-1}). The remaining amount (222.4 kg N ha^{-1}) was divided into two equal doses that were applied 24 DAS and at the following irrigation. Phosphorus fertilizer was added once with seedbed preparation in the form of monocalcium phosphate (15.5% P_2O_5) according to the levels of the studied phosphorus factor. Potassium fertilization was added at the rate of 110.9 kg K_2O ha^{-1} in the form of potassium sulphate (48% K_2O) at 36 DAS. Experimental units were kept weed-free through hand hoeing at early stages and hand pulling at later stages to eliminate the weeds’ effect.

Measurements

At full-grain maturation, maize was harvested by manually cutting the stalks of the two inner guarded ridges directly above ground level. After that, ears per each plot were separated and shelled to determine the grain yield per plot that was then used to estimate grain yield as ton per hectare.

The parameters used to compare the efficiency of the evaluated experimental designs included; mean square error (MSE), coefficient of variation (C.V.%), coefficient of determination (R^2) from regression analysis, relative efficiency calculated as MSE of reference design divided by MSE for design.

Statistical analyses and modeling

1. Analyses of RCBD, SPD, SSPD and one-rep experiments were performed after Gomez & Gomez (1984) using SAS 9.1 (2002), according to the following statistical models:

- RCBD model: $Yijkl = \mu + Bj + SDi + PDk + Pl + (SD*PD)ik + (SD*P)il + (PD*P)kl + (SD* PD* P)ikl + eijkl$
- SPD model: $Yijkl = \mu + Rk + SDi + PDj + (SD*PD)ij + eijk + Pl + (SD*P)il + (PD*P)jl+ (SD* PD* P)ijl + eijkl$
- SPPD: $Yijkl = \mu + Rk + SDi + eik + PDj + (SD*PD)ij + eijk + Pl + (SD*P)il + (PD*P)jl+ (SD* PD* P)ijl + eijkl$
- One-rep without center point: $Y = \mu + SDi + PDj+ Pk + (SD*PD)ij + (SD*P)ik + (PD*K)jk + (SD*PD*K)ijk$, using the three-factor interaction an error term to test the main effects and first order interaction.
- One-rep with center points: $Y = \mu + SDi + PDj+ Pk + (SD*PD)ij + (SD*P)ik + (PD*K)jk + (SD*PD*K)ijk + eijk$, where experimental error was calculated from the variations between six center points treated similarly with central levels of each factor.

2. Analysis of 3-DLD was performed manually, and in two steps using R software package (agricolae2015) after Yates (1936), according to the model:

3. $Yijkl = \mu + Bj +SDi + PDk + Pl+ (SD*PD)ik + (SD*P)il + (PD*P)kl + (SD* PD* P)ikl + eijkl$

4. Statistical analysis for RCCD and PRCCD was carried out after Dykstra (1960) and Petersen (1985), using the statistical software packages 'STATISTICA 7.0', (StatsSoft, 2012), according to the following models:

$\hat{Y} = \beta 0 + \beta 1X1+ \beta 2X2 + \beta 3X3 + \beta 11X12 + \beta 22X22 + \beta 33X32 + \beta 12X1X2 + \beta 13X1X3 + \beta 23X2X3$

where: X1 = sowing date effect, X2 = Plant density effect, X3 = phosphorus fertilization effect, X1X2; X1X3 and X2X3 = interaction of factors, $\hat{Y}$ = predicted response, β0 = intercept, β1, β2, β3 = linear coefficients, β11, β22, β33 = quadratic coefficients and β12, β13, β23 = linear interaction coefficients.

RESULTS AND DISCUSSION

Analyses of variance of the variations in maize grain yield as affected by the investigated factors, are presented in Table 5, in the replicated experimental designs. Results revealed that, when applying the reference RCBD design, the three studied factors showed highly significant effects ($P \leq 0.01$) on grain yield in addition to a significant ($P \leq 0.05$) influence of the three-way interaction. The two-way interactions were non-significant.

Arranging the studied factors and their levels in incomplete block designs (split-plot and split split-plot) resulted in partitioning the experimental error into errors ‘a’ and ‘b’ in the SP design, and ‘a’, ‘b’ and ‘c’ in the SSP design. The results indicated that this partitioning was successful in decreasing the estimate of error for the sub plots and sub sub-plots compared to the main (whole) plots. Similar to RCBD, both designs indicated significant effects for SD, PD, P and the three-way interaction SD*PD*P on grain yield. Moreover, the SSP design revealed the significant effects of P and the three-way interaction, although they were lower in magnitude compared to the SP design, due to the lower estimate of error ‘c’ compared to error ‘b’. Several researchers reported the advantage of split-plot designs (SP and SSP) over the RCBD in reducing the experimental error especially that of the sub-plots or sub sub-plots (Montogomery, 2001, Kristensen, 2012 and Nishu et al., 2017).

The three-dimensional lattice (3-DLD) design was effective in elucidating the significance of the main effects of the three factors and their first and second order interactions. That design had a lower mean square of error variance compared to the RCBD as a result of minimizing the block size (3 units) hence increasing the homogeneity within blocks (minimizing intra-block error) (Yates, 1939).

Table 5. Mean squares of grain yield as affected by the sowing date (SD), plant density (PD), phosphorous (P) application and their interactions in replicated designs

RCBD				SPD			
S.O.V.	*d.f*	*MS*	*P*	S.O.V.	*d.f*	*MS*	*P*
Rep	2	5.09	0.006	Rep	2	5.09	0.014
SD	2	236.45**	< 0.001	SD	2	236.45**	< 0.001
PD	2	36.16**	< 0.001	PD	2	36.16**	< 0.001
P	2	25.49**	< 0.001	SD*PD	4	0.61	0.400
SD*PD	4	0.61	0.617	Error (a)	16	1.07	0.912
SD*P	4	0.67	0.573	P	2	25.49**	< 0.001
PD*P	4	0.69	0.558	SD*P	4	0.67	0.645
SD*PD*P	8	2.62*	0.010	PD*P	4	0.69	0.632
error	52	0.92		SD*PD*P	8	2.62*	0.031
				Error (b)	36	0.56	
SSPD				**3-DLD**			
S.O.V	*d.f*	*MS*	*P*	S.O.V.	*d.f*	*MS*	*P*
Rep	2	0.044	0.846	Rep	2	0.19	0.616
SD	2	20.36*	0.026	Blocks (adj)	24	0.53	
Error (a)	4	1.99	0.0002	Treatment(unadj.)	26		
PD	2	78.63**	< 0.001	SD	2	1.09*	0.04
SD*PD	4	1.34	0.061	PD	2	86.70**	< 0.001
Error (b)	12	0.44	0.113	P	2	1.73**	< 0.001
P	2	4.45**	< 0.001	SD*PD	4	0.84*	0.021
SD*P	4	0.67	0.055	SD*P	4	0.73*	0.024
PD*P	4	0.49	0.138	PD*P	4	0.83*	0.020
SD*PD*P	8	0.48*	0.021	SD*PD*P	8	1.00**	< 0.001
Error (c)	36	0.27		Error	28	0.25	

*,** significant at 0.05 and 0.01 levels of probability, respectively.

Analyses of variance for non-replicated designs are presented in (Table 6). The one-rep designs (without and with center points) uncovered variations in grain yield as affected by the PD only, even though, the one-rep with center points showed a relatively small MSE. This might be a result of the relatively high mean square for the three-factor interaction (SD*PD*P) used as error term in the one-rep design, in addition to the low d.f. of error (5) in the one-rep design with center points. This finding confirms the adequacy of the one-rep designs in discriminating the importance of the different studied factors and their interactions, which could be included in further replicated experiments. Binet et al. (1955) concluded that the efficiency of these designs is hampered by the loss of information concerning the interactions (for the purpose of obtaining an estimate of error), loss of one experimental unit that will cause failure of the experiment and the occurrence of an outlier values may significantly distort the results. Hence, these types of experiments are not definitive, but are limited for screening which factors, and interactions, are important. EL-Rouby et al. (2021) used a six-factor, i.e. surface irrigation level (I), potassium (K), phosphorus (P) and nitrogen (N) fertilization rates in addition to sowing date (SD) and plant density (PD), in a half replication rotatable central composite design (RCCD) to determine the influence of those factors on grain yield of maize single hybrid Giza 168. They found significant effects for PD, I*K and P*N interactions on grain yield indicating the importance of those input factors in determining the grain yield in maize.

Table 6. Mean squares of grain yield as affected by the sowing date (SD), plant density (PD), phosphorous (P) application and their interactions in non-replicated designs

One-rep without center point				One-rep with center point			
S.O.V.	*d.f*	*MS*	*P*	S.O.V	*d.f*	*MS*	*P*
SD	2	0.06	0.970	SD	2	0.41	0.832
PD	2	31.52**	0.002	PD	2	30.21**	0.002
P	2	5.44	0.157	P	2	3.33	0.277
SD*PD	4	0.95	0.797	SD*PD	4	1.50	0.624
SD*P	4	1.72	0.587	SD*P	4	1.81	0.545
PD*P	4	1.69	0.596	PD*P	4	0.58	0.893
SD*PD*P	8	2.31		SD*PD*P	8	2.20	0.600
				Error	5	0.84	
RCCD				PRCCD			
S.O.V	*d.f*	*MS*	*P*	S.O.V	*d.f*	*MS*	*P*
(1) SD(L)	1	22.42*	0.012	blocks	2	0.29	0.813
SD (Q)	1	12.37*	0.036	(1) SD(L)	1	62.55**	< 0.001
(2) PD (L)	1	6.29	0.100	SD (Q)	1	4.02	0.100
PD (Q)	1	30.59**	0.006	(2) PD (L)	1	34.78**	< 0.001
(3) P (L)	1	3.39	0.204	PD (Q)	1	7.50*	0.027
P (Q)	1	0.09	0.820	(3) P (L)	1	0.002	0.971
SD × PD (L)	1	4.35	0.154	P (Q)	1	0.81	0.452
SD × P (L)	1	0.01	0.935	SD × PD (L)	1	1.65	0.285
PD × P (L)	1	0.10	0.808	SD × P (L)	1	0.26	0.667
Lack of Fit	5	0.70	0.797	PD × P (L)	1	3.31	0.134
Pure Error	5	1.55		Error	31	1.40	

*,** significant at 0.05 and 0.01 levels of probability, respectively.

The central composite design (RCCD), another type of one replication experiments, revealed significant SD variations at the linear and quadratic levels, and PD at quadratic level. The model describing the relationship between the grain yield and the studied factors is: $\hat{Y} = 7.87 - 1.28\ SD + 0.93\ SD^2 + 1.45\ PD^2$. The coefficient of determination (R^2) value for the applied model was high (0.87) indicating the model's adequacy in interpreting the variations in grain yield. The MSE for that design is calculated from central points receiving the same treatment (similar levels from each factor), thus, the variation within these experimental units is expected to be low and increases the ability of that design to elucidate the significance of sources of variation compared to one-rep without center points.

The suggestion of Dykstra (1960) of replicating the factorial and star points to obtain an estimate of experimental error (PRCCD), instead of calculating the error from the central points (RCCD), improved the efficiency of the design over RCCD. That design revealed only the significance of linear main effects of SD and linear and quadratic components of PD, while P effects and first order interactions were non-significant. The model equation that explains the relationship between grain yield and significant components at that stage will be: $\hat{Y} = 8.06 - 1.23\ SD + 0.92\ PD + 1.00\ PD^2$, with $R^2 = 0.73$.

Relative Efficiency of Experimental Design

The RCBD was used as a reference design, thus, the efficiency of the replicated studied designs was compared to RCBD using four parameters, i.e., mean square of error (*MSE*), coefficient of variation (*C.V.* %), coefficient of determination (R^2) and relative efficiency (*R.E.*) calculated as the ratio of *MSE* of the RCBD to *MSE* of the design (Table 7). The split plot designs (SP and SSP) were relatively inefficient compared to RCBD except for error 'a' in both designs. The efficiency of split-plot designs depended mainly on the allocation of studied factors to the main, sub and sub sub-plots (Jones & Nachtsheim, 2009), in addition to smaller block size compared to RCBD. In this study, the main plots of the split plot design included factorial distribution of two highly variable factors, i.e., sowing date and plant density. That resulted in a high *MSE* for the main plots (error 'a'). Same observation was found for the SSP, where sowing date was allocated to the main plots, which resulted in a high *MSE* (error 'a'). The sub-plot error (error 'b') in both designs was lower than error 'a' indicating the efficiency of both designs in minimizing the error for the subplots, and increasing the efficiency of both designs compared to the RCBD. Moreover, in the SSP, the design was efficient in reducing error 'c', thus increasing the efficiency of the sub sub-plots compared to the *MSE* of RCBD. Similar findings were reported by Oladugba et al. (2013) and Nishu et al. (2017).

The three-dimensional (cubic) lattice design showed higher relative efficiency (3.68) compared to the RCBD. That was expressed in lower *MSE* and *C.V.*% values with a high R^2 value of 0.90. This result might be attributed to the minimized block size (three units) which enabled the control of spatial variations of the experimental units within the block. Several researchers reported the higher efficiency of the lattice designs, other than the cubic lattice, over RCBD (Masood et al., 2008, Kashif et al., 2011, Khan et al., 2015 and Masood et al., 2017).

The non-replicated designs included one-rep designs without and with center points, RCCD and PRCCD (Table 7). The one-rep design without center points was used as reference design for that group. The RCCD and PRCCD, in addition to one-rep with center points, were more efficient than one-rep without center points. The low efficiency of the latter design resulted from the high three-way interaction variations resulting in a higher type II error. The higher efficiency of one-rep with center points was an outcome of the relatively small *MSE* (0.84) resulting from center points treated with the same levels of the three studied factors. However, both designs were effective in determining the importance of plant density only as the main factor affecting grain yield of maize. Thus, they could be recommended for determining the relative importance of the studied factors which would be later tested in replicated experiments (Binet et al., 1955).

Table 7. Estimates of efficiency parameters (*MSE*, *C.V.*%, *R.E.* and R^2) of the evaluated experimental designs for grain yield

Design		*MSE*	*C.V.* (%)	*R.E* [(*)]	R^2
I- Replicated designs					
RCBD		0.92	9.83	--	0.93
SPD	error (a)	1.07	6.42	0.86	0.89
	error (b)	0.56		1.64	
SSPD	error a	1.99	4.70	0.46	0.91
	error b	0.44		2.09	
	error c	0.27		3.41	
3-DLD		0.25	5.89	3.68	0.90
II- Non-replicated designs					
One-rep without center points		2.31	22.38	--	0.83
One-rep with center points		0.84	13.16	2.75	0.82
RCCD		1.55	13.02	1.49	0.87
PRCCD		1.40	11.78	1.65	0.73

[(*)] *R.E.* calculated:

- For replicated designs: *MSE* of RCBD / *MSE* for design.
- For non-replicated designs: *MSE* of one-rep without center points / *MSE* for design.

Both RCCD and PRCCD designs were more efficient than one-rep without center points. The RCCD resembles the one-rep with center points in which error is determined from center points treated with same levels of studied factors. However, that design was able to detect significant ($P \leq 0.05$) variations in sowing date in addition to plant density ($P \leq 0.01$). This may be attributed to the higher number of factor levels employed in that design (5 levels) compared to one-rep without center points (3 levels). The PRCCD showed relatively higher efficiency than the RCCD due to lower *MSE* variance, which might be explained by a better error estimate resulting from the replication of both factorial and star points, thus covering a wider space of treatments compared to the RCCD which estimates the error from the center of treatments space. Dykstra (1960) concluded that partial duplication will result in more precision in the estimates of coefficients, a better estimate of experimental error and a more powerful test of the adequacy of the second order model. Ukaegbu & Chigbu (2014, 2015) compared the prediction capabilities of partially replicated central composite designs and concluded replicating the star points of the RCCD resulted in reduction of prediction variance and increase in precision compared to replicating the cuboid points. Several researchers reached the same conclusion (Borkowski, 1995, Borkowski & Lucas, 1997 and Giovannitti-Jensen & Myers, 1989). However, Chigbu & Ohaegbulem (2011) indicated that replicating the cube portion of the RCCD enhances the performance more than the star portion.

CONCLUSION

The results obtained from the present study highlighted the importance of choosing the proper experimental design to investigate the effect of three agricultural factors, i.e., sowing date, plant density and phosphorus application level on grain yield of maize. It could be concluded that the use of three-dimensional lattice would be more appropriate for its higher relative efficiency compared to RCBD. However, if the number of available experimental units is a limiting factor, the partially replicated central composite design would be recommended. The one-rep designs proved inefficient compared to the replicated designs and, thus, their role should be restricted to the determination of the relative importance of the studied factors. The split plot designs (SP and SSP) showed enhanced efficiency for factors and interactions allocated to the sub or sub sub-plots, hence the proper factor allocation to the different types of plots is a major determinant to the design's efficiency. It is, thus, crucial to choose the appropriate design, in relation to the applied treatments, that would reduce the spatial variation between experimental units to minimize the experimental error component of variation and increase the efficiency of the design.

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Nutritive characterization of Musa *spp* and its effects on *in vitro* Rumen fermentation characteristics

S.M.P. Teixeira*, C.S.A.M. Maduro Dias, C.F.M. Vouzela, J.S Madruga and A.E.S. Borba

University of the Azores, FCAA, Institute of Agricultural and Environmental Research and Technology, Rua Capitão João d'Ávila, 9700-042 Angra do Heroísmo, Açores, Portugal
*Correspondence: sofia.mp.teixeira@uac.pt

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Abstract. This research aims to study the effect of nutritive value of *Musa spp* on animal feed. Residues of banana culture, leaves and stems, could be used as a fibre source for animal feeding, especially in Banana producing areas, such as Macaronesia Archipelagos, avoiding wastes and supplementing periods of scarcity of food.
Musa spp were collected and dried at 65 °C in an oven with controlled air circulation. The pseudostems were divided in three different portions and chemical composition, *in vitro* digestibility, and *in vitro* gas production were determined. Regarding dry matter results, they were low (16.54% in leaves and 6.54% DM% in pseudostem), crude protein raging 11.25 DM% in leaves and 7.25% in pseudostem. Concerning fiber values, NDF is higher in leaves (70.07 DM%) than in pseudostems (52.11 DM%) and ADL is higher in leaves (9.90 DM%) comparing with pseudostems (6.21 DM%). *In vitro* DM digestibility is low, (24.42% in leaves and 42.69% in pseudostem), corroborating the NDF values. Cumulative gas production was recorded at 4, 8, 12, 24, 48, 72, and 96 h of incubation. The results showed that the gas production in leaves was lower (11.36 mL 200 mg^{-1} DM) when compared to pseudostem (23.81 mL 200 mg^{-1} DM), being so in accordance with the digestibility results.
The current study suggested that this by-product can be used in animal feed, however, it will be necessary to carry out tests to improve its nutritional value, namely with NaOH and/or with Urea, being a promising strategy for improving ruminant feed efficiency.

Key words: animal science, *in vitro* digestibility, gas production, *Musa spp,* ruminants.

INTRODUCTION

The global increase in demand for food, especially protein, raises the need for more efficient and sustainable animal feeding strategies. The use of the most different food resources to meet nutritional requirements in ruminants is undergoing a wide discussion nowadays. The use of alternative feeds for ruminants is a strategy aimed to reduce feed costs and overcoming the pasturage scarcity problem occurring at critical times.

Livestock production in direct grazing regions often see themselves confronted with periods of pasture production shortage, motivated by normal production curves of pasture or adverse weather conditions strongly influencing grass production. With the current concepts of production, in developing countries, the uses of cereals in diets for animals are rare and expensive due to the direct competition as a source for human food. Thus, there is a need to maximize the use of available resources, which include consuming non-conventional resources (Ramírez-Restrepo & Barry, 2005).

There are several advantages of using non-conventional food in animal production. The main advantage of using alternative products is to reduce the cost of food by using locally available resources. The economic value of these products is often much smaller than conventional feed. Other advantages of using these resources are to reduce competition for food between humans and animals. The uses of food waste, such as fruit, cassava and sweet potatoes are excellent sources of fermentable carbohydrates, being an advantage for ruminants because they possess the ability to use inorganic nitrogen.

The ability of ruminants to efficiently use fibre makes it possible to explore agricultural by-products and wastes from tropical crops, like banana, which may also provide energy and protein.

Banana is one of the most famous and useful plants in the world. Practically all parts of the plant, can be used. Nowadays, people have devoted themselves to forest protection and found reasonable ways to use agricultural and forest residues. This is triggered by the rapid increase in consumption of wood fibre-based products, leading to illegal logging activities due to the reduction in permitted wood resources.

Banana tree is a natural resource, widely produced in tropical and subtropical countries all over the world (Li et al., 2010; Aziz et al., 2011; Sango et al., 2018), and for properly development, it requires constant heat, high humidity and proper water distribution.

These conditions are registered in the range between the 30° parallels of north and south latitude, in the regions where the temperatures are between the limits of 15 °C and 35 °C. However, there is the possibility of cultivation above 30° latitude north and south latitudes, since the temperature and the water system are suitable, with a typical banana plant tropical (Moreira, 1999). Banana is the most consumed fruit in the world, with a production of approximately 113 million tons in 2016. Canary Islands, Azores and Madeira, have climatic conditions to produce subtropical and some tropical crops, where there is work for years, adapting cultural techniques in order to optimize the different cultural systems, allowing to know deeply not only the culture, but also the differences to be taken into due account in the subtropical regions.

According to Batabunde (1992), all parts of banana tree can be all used, except the roots and baby trees. He also states that, around 30–40% of total banana production is available to animal feed as result of being rejected for exportation, suffer damages in the field or simply do not have the dimensions to be sold. FAO (2020) projections for world banana production, are that it should rise 1.5% a year, reaching 135 million of tons in 2028.

A major part of a banana tree is the fruit, so banana stem is rarely used. Generally, after collecting the fruits, the banana trees will be cut directly, since it can only have fruit once. After that, the trees are left there to rot. But it should, because the remaining parts could be used for ruminants feeding, since they contain all kinds of nutrients, and people just waste it.

In the leaves, the highest crude protein is found, followed by fruit peel and pseudostem. Both pseudostem and leaf contain moderate amounts of fibre but are higher than banana peel. The high contents of tannin in the leaf and fruit peel reduced their protein and dry matter digestibility and their value as ruminants. The low crude protein and high moisture contents of pseudostem reduced dry matter intake potential by ruminants.

Since leaves have low digestibility and stem has low dry matter and crude protein, to obtain high animal production from ruminants fed banana wastes, it should be supplemented with protein concentrate feeds.

One way to mitigate the factors caused by climate changes would be the use of alternative foods during scarcity periods. However, in order to optimize the use of these foods in animal feed, it is important to establish a balance between the nutrients in the diet to guarantee efficiency in ruminal fermentation processes and optimization of microbial growth, which will result in the maximization of fiber digestion and in the improvement of productive performance.

However, it is important to know the chemical composition and its nutritional value in the rational use of these foods, considering the level that can be included in the diet, with the objective of obtaining balanced diets that meet the nutritional requirements of the animal, maximizing its consumption (Clementino, 2008).

Therefore, the aim of this study was to study the effect of nutritive value of *Musa spp* on animal feed, to see if it could be an effective way to help in periods of scarcity of food.

MATERIALS AND METHODS

The present study was conducted in the Animal Nutrition Lab '*Prof. Doutor Gourlay Young do Amaral*', Department of Agricultural Sciences, University of the Azores, located in Angra do Heroísmo, Terceira, Azores, Portugal. The plant, manually harvested, in Terceira Island, was chopped and the pseudostem was divided in three portions and were dried in an air oven at 65 °C for 72 h.

Dried samples were then ground through a 1-mm screen using a Retsch mill (GmbH, 5657 HAAN, Germany). These grind samples were analysed for dry matter (DM, method 930.15), crude protein (CP, method 954.01) and total ash method (942.05), according to the standard methods of AOAC (1995). Briefly, the dry matter content o was determined by placing samples in an air oven at 105 °C for 24 h. The ashes were evaluated by igniting samples in a muffle furnace at 500 °C for 12 h. Crude protein was determined by standard micro-Kjeldahl method, using digestion equipment (Kjeldatherm System KT 40, Gerhart Laboratory Instruments, Bonn, Germany) and an automated Kjeltec 2300 Auto-analyser apparatus for distillation and titration (Foss Electric, Copenhagen, Denmark). The acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF), was be determined according to Goering & Van Soest (1970). *In vitro* digestibility was be determined using the Tilley & Terry (1963) method, modified by Alexander & McGowan (1966), and the liquid of the rumen was obtained from the local slaughterhouse (IAMA), as described by Borba et al., (2001).

Regarding *in vitro* gas production (GP) technique, which simulates the rumen fermentation process, and it is used to evaluate the potential of feeds to produce greenhouse gas, each assay was repeated three times (runs). Blanks were used for each inoculum to measure the fraction of total gas production due to substrate in inoculum and these values were subtracted from the total to obtain net GP. All treatments, for each assay, were incubated simultaneously in all runs (Menke et al., 1979).

The preparation of buffer solutions and rumen inoculum was as described by Menke & Steingass (1988).

The initial gas volume was recorded after 4, 8, 12, 24, 48, 72 and 96 hours of incubation.

This gas production represents the kinetic of the rumen apparent GP and is expressed by the McDonald (1981) equation. Gas production profiles were obtained after adjusting the data to the exponential equation of Ørskov & McDonald (1979):

$$p = a + b\ (1 - exp^{-c\,t})$$

where p is the gas production at time t; *the values of a and b represent constant values in the exponential equation*; $a+b$ the total potential gas production (mL g^{-1} DM), and c the rate constant.

The fermentation constants a, b and c were calculated by a suitable curve method using Neway Software Program (Rowett Research Institute, Aberdeen, UK) that was developed by Chen (1997).

For Statistical Analysis, ANOVA was performed, followed with posthoc least significant difference test by IBM SPSS version 24 Statistics Program software (SPSS Inc. Chicago, IL). For all analyses, a P value of < 0.05 was considered statistically different

RESULTS AND DISCUSSION

In the present study, the effect of nutritive value of *Musa spp* for animal feed was analysed and the results are presented at Table 1, as well as chemical composition values.

Table 1. Chemical composition and nutritive value of *Musa spp*

Treatment	DM	100g DM						DMD
	(%)	CP	NDF	ADF	ADL	EE	Ash	(%)
Leaves	16.54^a	11.25^a	70.07^a	42.38^a	9.90^a	2.69^a	17.74^a	22.69^a
	(± 0.58)	(± 1.18)	(± 2.28)	(± 1.63)	(± 0.63)	(± 0.42)	(± 0.94)	(± 1.27)
Pseudostem	6.54^b	7.25^b	52.10^b	30.62^b	6.57^a	1.36^b	24.07^b	40.39^b
Total	(± 0.25)	(± 0.66)	(± 0.51)	(± 1.29)	(± 1.10)	(± 0.09)	(± 1.95)	(± 3.10)

DM – Dry Matter; CP – Crude Protein; NDF – Neutral Detergent Fibre; ADF – Acid Detergent Fiber; ADL – Acid Detergent Lignin; EE – Extract Ether; DMD – In vitro Dry Matter Digestibility. [a,b] different superscript within the same column indicates significant differences, $P \leq 0.05$.

Our results, regarding DM shown significant differences between leaves and pseudostems. For the CP, it shown significant differences between these two parameters ($P < 0.05$). Concerning NDF values, it shown significant differences between leaves and pseudostems, and the higher value was found in leaves (70.07%). These results and the DMD results are in accordance, showing that the pseudostem has a lower NDF value 52.10% vs the 70.07% in leaves and the DMD in leaves is 22.69% but in pseudostem is 40.39%. About ADF, it also shown significant differences between leaves and pseudostems. EE also shown significant differences between them, such in Ash and DMD. ADL shown no statistically significant differences among treatments ($P > 0.05$).

In most parameters, leaves presented the higher values, except for ashes and DMD. This is normal, since leaves has more NDF than pseudostems, so they have lower digestibility. On the other side, pseudostems have less protein, at the limit of ruminal activities and that is way their digestibility is not so high to.

40% of banana trees are considered as waste in the field, producing about 60–80 tons ha^{-1} year^{-1} of banana pseudo stems (Salehizadeh et al., 2017) which as low nutritive value and high-water content, around 93.4%, but based on DM, the nutrient value of CP is 6.5% and the nutrient value of lipid is 1.5% (Tuan, 2004). These values agree with the results of our study, in which the pseudostem values are like that and lower than the ones presented in leaves. According to Cordeiro et al., 2004, pseudostem has high percentage of ash content (14%), but low lignin content (12%), when compared with other plants. Furthermore, the lignocellulose is around 60% up to 85%, 50% of cellulose, over 17% lignin and 4% ash. Regarding leaves, the author states that they have around 26% cellulose, 17% hemicellulose, and 25% lignin (Jayaprabha, et al., 2011; Reddy & Yang, 2014). Another statement regarding the composition of pseudo stem, from Okelana, 2001, is that crude fibre of central portion of pseudo stem is about 20% and 40% in the external portion. Its ash content is from 14–30% and the fat ranges from 18–22%. Nevertheless, the quality of stem can be improved if a silage with other foliage or with yeast is done, as growth promoter, in order to improve protein content, feed intake and live weight gain as well (Ty et al., 2012; Tien et al., 2013; Manivanh & Preston 2015). Also, Chedly, & Lee, stated that, ensiling can also reduce some previously unpalatable products, into useful to livestock, by simply changing the chemical nature of the feed (Chedly & Lee, 1998).

Oliveira et al., (2014) studied the effect of the use of banana pseudostem hay in feeding small and large ruminants and concluded that due to the low protein content, its inclusion in the diet of these animals must be accompanied by supplementation of CP (crude protein).

Cumulative gas production as affected by substitution of ruminant diet with *Musa spp* is shown in Fig. 1 and Table 2.

In vitro gas production technology simulates the rumen fermentation process and has been used to evaluate the potential of food gas production, and it was recorded at 4, 8, 12, 24, 48, 72 and 96 h of incubation. The results showed that compared with the pseudostem (23.81 mL 200 mg^{-1} DM), the gas production in the leaves (11.36 mL 200mg^{-1} DM) was lower, so it was in line with the digestibility results.

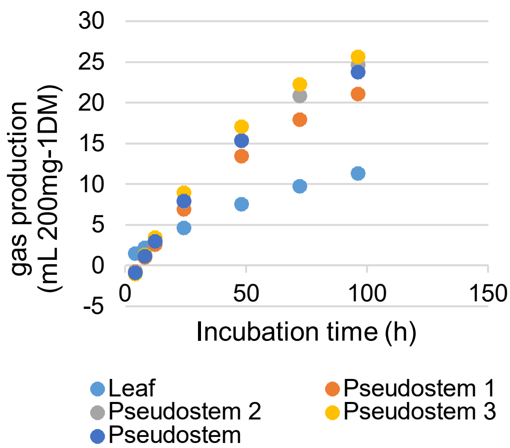

Figure 1. Pattern of in vitro gas production (fitted with exponential model) on incubation of *Musa spp* treated with NaOH, in buffered rumen fluid.

When we observed the Lag time (hr), we can see that pseudostem, is the first to begin his fermentation (1.5 hr after placing the sample to incubate), when compared to the leaves.

The gas production constant rate (c) is also higher in pseudostems, which results in a greater gas production.

Table 2. Effect of *Musa spp* on cumulative gas production (mL 200 mg^{-1} DM) and gas kinetics

Treatment	Incubation Time (hour)							Gas kinetic parameters			
	4	8	12	24	48	72	96	a	b	c	Lag Time (hr)
Leaves	1.49	2.19	2.86	4.67	7.59	9.75	11.36	-1.36	35.21	0.0188	2.1
Pseudostem	1.45	3.64	5.66	10.87	15.33	20.37	23.81	0.91	31.84	0.0192	1.5

a = gas production from the immediately soluble fraction (mL 200 mg^{-1} DM); b = gas production from the insoluble fraction (mL 200 mg^{-1} DM); c = gas production rate constant for the insoluble fraction (mL h^{-1}).

CONCLUSIONS

The study suggested that this by-product can be used in animal feed, however, further research is essential to describe more appropriate techniques to improve the quality of banana pseudo stem, such as to carry out tests to improve its nutritional value, namely with NaOH and/or with Urea.

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