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CONTENTS

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The effect of synbiotic inulin and enterococcus bacteria on digestive health and weight gain in calves

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Abstract. The aim of study was to investigate the effect of a synbiotic containing *Enterococcus* bacteria and 3 different concentrations of inulin on the performance and health status of calves. Forty randomly selected healthy male Holstein crossbreed calves 23 (+/- 5) days old and weighing 50 kg (+/- 5 kg) were randomly allocated to 4 groups: control group (CoG $n = 10$) fed only whole milk, and 3 synbiotic supplemented groups: 1) SynG6 $n = 10$, 2) SynG12 $n = 10$, 3) SynG24 $n = 10$, in which calves received various amounts of prebiotic inulin (artichoke powder 6 g, 12 g, and 24 g) with 0.25 g of the probiotic *Enterococcus faecium* (2×10^9 CFU g^{-1}). At the end of this study all three synbiotic group weight gains were significantly greater than the control group ($p < 0.01$). SynG12 (12 g artichoke powder) group's weight gain was significantly greater than control and the 6g and 24 g synbiotic groups ($p < 0.05$). The average cold carcass weight results were similar to the live weight results: SynG12 was significantly ($p < 0.05$) higher than SynG6 and SynG24. Supplementing feedings with this combination of the synbiotic containing 6 g of inulin (produce in Latvia) mixed with *Enterococcus* (Protexin, UK) bacteria (SynG12) was most effective in achieving the greatest daily weight gain and cold carcass weight.

Key words: calf, *Enterococcus faecium,* feeding, inulin, synbiotic, weight gain.

INTRODUCTION

Carcass quality in ruminants is determined more by dietary components than by the general health status (Blanco-Penedo et al., 2009). Antibiotics are frequently used to achieve this goal. Thus, it is important to identify new and better alternatives to antibiotic use for increasing animal weight gain and general animal health improvement. Probiotics, prebiotics and various combinations of these substances called synbiotics have been studied (Masanetz et al., 2011; Samanta et al., 2013). One of the most researched probiotics for ruminants is *Enterococcus spp*. bacteria (Nocek et al., 2002; Corcionivoschi et al., 2010; Uyeno et al., 2015).

Prebiotics are not digested in the gastrointestinal tract but rather they support the growth and development of beneficial intestinal microflora which limits plaque formation by pathogenic microorganisms such as *Salmonella sp.* or *Escherichia coli* improving the overall health of the animal (Gibson & Roberfroid, 1995; Patel & Goyal, 2012; Kara et al., 2015).

The synbiotic term is used when 2 different substances are combined such as probiotics with prebiotics in order to improve function, digestibility, motility and general health of the intestinal tract and the animal (Uyeno et al., 2015; Markowiak & Śliżewska, 2018). Several studies have shown that inulin, derived from Jerusalem artichoke, improves the health and growth of non-ruminants and chickens (Velasco et al., 2010; Valdovska et al., 2014; Samolińska et al., 2018), but there is no data on feeding synbiotics to ruminants.

In this study, the synbiotic - inulin from artichoke flour (inulin 48.5–50.1%) combined with *Enterococcus faecium* (2×10^9 CFU g^{-1}) was selected in order to test if this supplement could significantly improve calf health by increasing the functionality of the digestive system and thus, enhancing animal live weight gain.

MATERIALS AND METHODS

Study Herd, Housing and Feeding

The research was carried out on a 420 cow dairy farm in Latvia. Forty randomly selected, 23 (± 5) days old healthy male Holstein crossbreed calves, weighing 50 kg (± 5 kg), were randomly allocated to 4 groups. The study started when the animals had reached 4 weeks of age. The calves were kept in groups of 10, under the same conditions and were fed twice a day, ~3.5 liter of whole milk per feeding. The pen for 10 calves was 25 m^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. Composition of the concentrated feed and Jerusalem artichokes flour composition is shown in Table 1.

Table 1. Composition of Concentrated Feed and Jerusalem Artichokes Flour for Study Animals

	Composition (g kg^{-1} Dry Matter basis)					Composition (g mg^{-1} Dry Matter basis)				
	dry matter	CP	NDF	ADF	starch	inulin	free glucose	free fructose	sacc-harose	nucleic acids
Concentrated feed	882	142	481	34	655	-	-	-	-	-
Artichoke powder	950	171	-	-	635	495	8	26	106	21

CP – Crude Protein; NDF – Neutral Detergent Fibre; ADF – Acid Detergent Fibre.

The calves were divided into 4 groups: control group (CoG, $n = 10$) was fed only whole milk, 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)). Jerusalem artichoke powder and Enterococcus faecium were added to the first one of the two daily milk feedings. In this research, the prebiotic we used was artichoke flour concentrate, produced in Latvia at the University of Latvia Institute of Microbiology and Biotechnology, the inulin was 50% DM, thus, each 12 g of artichoke powder contained approximately ~6 g of inulin.

Health monitoring

Throughout the study, daily calf health was evaluated by close visual inspection of the fecal consistency. Animal faeces were evaluated in points, where 0 - points score was for solid faeces without diarrhea sign, 1 - point was for soft faeces with maintained consistency, 2 - points were for liquid feces with lost solidity, and 3 points for watery faeces (Larson et al., 1977).

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). During the control weighing, a general health exam was done and the main physiological indicators were recorded.

The study lasted 8 weeks or 56 days. A similar study framework has been used by other authors (Król, 2011). The planned slaughter of calves occurred at 12 weeks; 57th research day. They were weighed at 12 hours after slaughter to determine their cold carcass weight.

Statistical analyses

MS Excel and the R-Studio were used for the data analysis of live weight gain and cold carcass weights, 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 AND DISCUSSION

During the initial health exam at 4 weeks of age the heart rate, respiratory rate, body temperature were recorded. No significant differences were identified among the control and synbiotic groups and all health indicators were within the normal range.

Throughout the research, the feces were inspected and the consistency recorded in points for each animal. Watery fecal consistency or diarrhea were never observed in any of the calves during the complete study. In all animal groups, softer than normal feces were observed during the 6th week of life. This was most likely due to the addition of fodder to the daily ration resulting in or promoting adaptive changes of digestive juices and microorganisms to the new feed product.

As shown in Fig. 1, after 8 weeks of age, when the calves had become used to the new feed product, calf fecal consistency stabilized in all groups, in a few individual animals softer than normal feces consistency, species-specific, was observed.

The highest fecal consistency number was recorded from the 5th –7th week when feces were softer than normal. This was most likely due to the introduction of synbiotics at the initial stage of the research that coincided with the 6-week life period of the calves.

Throughout the study, the greatest number of calves which had softer than normally soft feces were observed in the control group. In all three synbiotics groups, starting with the 7th week of life, the number of cases with softer than normal feces gradually decreased. The animals, which received 12 g of synbiotics (6 g of inulin), had the most stable fecal consistency among all groups through the study. Control group animals had the most liquid feces with the highest point number in comparison with the other groups.

In the synbiotic group with the lowest intake of inulin, fecal consistency reached 0.55 points (within normal limits) during the 9th week of life, but in the other two synbiotic groups this occurred later, by the 10th week. It may have been easier for calves to adapt to lower doses of inulin.

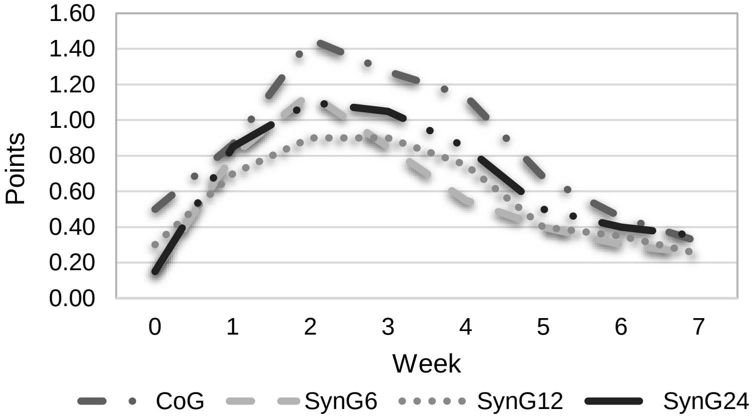

Figure 1. Comparison of the fecal consistency between calf groups from 5 to 12 weeks old (Calculating Values in 0–3 Points by Larson, 1977).

Table 2 displays per group: the average weight at 3 intervals, the total average weight gained during the study, and the average cold carcass weight on the 57th day. The average daily weight gain was also different among all synbiotic groups and the control group ($p < 0.01$) in which the control group had the lowest weight 82.18 kg of all groups.

Table 2. Calf growth dynamics by weight and cold carcass weight

Group	Average weight of calves (kg) at 3 research intervals			Average daily weight gain, kg	Cold carcass weight, kg
	first day	28th day	56th day		
CoG	50.80 ± 1.25	69.09 ± 5.97	82.18 ± 6.53	0.581 ± 0.13	42.6 ± 6.88
SynG6	51.00 ± 1.25	75.40 ± 4.35*	100.10 ± 3.14**	0.876 ± 0.06**	49.7 ± 2.41**
SynG12	51.70 ± 1.0	77.40 ± 1.65**	103.00 ± 2.26**	0.916 ± 0.09**	51.9 ± 1.64**
SynG24	52.13 ± 1.73	74.63 ± 1.19	99.96 ± 3.36**	0.854 ± 0.09**	49.6 ± 1.85**

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

The SynG12 group had the greatest weight gain at 103.00 kg, ($p < 0.05$). This proves that the best inulin effect is achieved with the medium amount (or with 12 grams Jerusalem artichoke powder). This result may be due to firmer fecal masses observed throughout the study when compared to the other groups and due to the optimal combination of probiotics and prebiotics, which provides full digestibility of feed and improved intestinal function. Excessively high doses of prebiotics have been shown to decrease digestive function and the digestibility of feed. Weight gain is insignificantly but consistently higher in group SynG6 (100.10 kg) than in SynG24 (99.96 kg).

Most researchers studying synbiotics report that these supplements improve feed digestibility increasing the bioavailability of nutrients for growth and development. (Hasunuma et al., 2011, Zábranský et al., 2015; Marcondes et al., 2016; Dar et al., 2017; Geigerová et al., 2017).

Average weight gain per day was 0.630 kg in the control group. Daily weight gain higher was in the SynG12 group at 0.916 kg ($p < 0.01$). Daily weight gain was greater in the SynG12 group ($p < 0.05$) than in SynG24 (0.854 kg) and SynG6 (0.876 kg). The average daily weight gain in SynG24 and SynG6 was not significantly different;

however, slightly greater in SynG6. Research by Jatkauskas & Vrotniakiene (2010), showed that calves fed probiotics during the transition period to ruminant status had a 9.4% higher weight gain, than the control animal group which did not receive probiotics.

Throughout the study period, the smallest weight increase was observed in control animals, the greatest - in SynG12 (Fig. 2). The greatest increase in weight was observed during the middle of the study period in 6–8 week old calves when weight increased by an average of 14 kg in two weeks in each synbiotic group. During this period the weight gain of CoG was only 8 kg which was also the average weight gain for CoG throughout the study. The lowest weight increase among the synbiotic groups was observed in the first two study weeks. This is most likely due to the addition of the new synbiotic supplements.

Simon et al. (2001) in their study found that when feeding a probiotic, *Enterococcus faecium*, a significant weight gain was not seen in comparison with the control animals. This study proved that adding inulin with the *Enterococcus faecium* bacteria to the feed, significantly greater weight gain occurred in all the synbiotic groups as compared to the control group (Fig. 2).

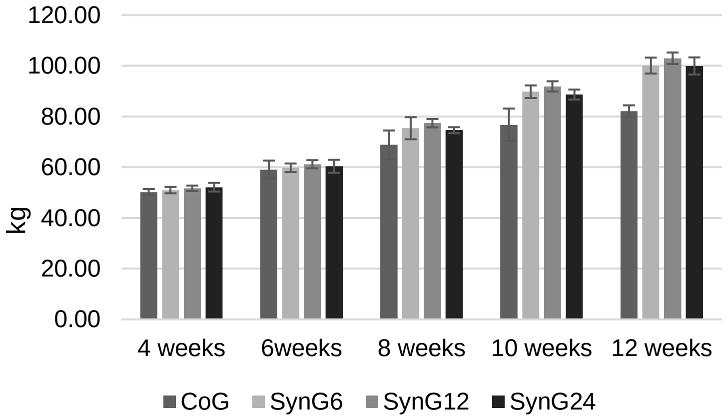

Figure 2. Average weight of calves on different study weeks in different groups.

Roodposhti & Dabiri (2012) found that calves fed with different synbiotics, had the highest food consumption and weight gain when compared to the control group (Marcondes et al., 2016).

Chaucheyras-Durand & Durand (2010), suggest that probiotics as a part of a synbiotic mix, begin colonization of the colon and increase the growth of beneficial bacteria, such as *Enterococcus faecium*, *Lactobacillus* and *acidophilus* which reduce the total number of pathogenic bacteria, thus securing for themselves a stable place in the colon. Prebiotics, under the influence of these beneficial bacteria, get fermented, increasing the total number of beneficial bacteria while reducing the pathogenic bacteria. Moreover, the fermentation process produces volatile fatty acids that improve animal energy usability and change the intestinal morphology (Roodposhti & Dabiri, 2012). In this study feeding beneficial bacteria, *Enterococcus faecium*, together with prebiotics, most likely increased the total number of bacteria. Inulin provides nutrients for microorganisms in the colon. Perhaps, feeding too much inulin (SynG24) changed the intestinal activity which may have resulted in less effective fermentation processes and feedstuff absorption. Therefore, the effect on weight gain was less than in the medium inulin dose (Fig. 3).

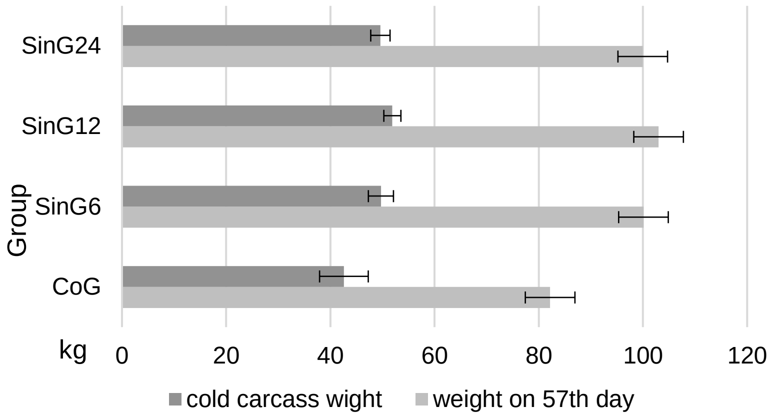

Figure 3. Comparison of mean values of calf live weight and cold carcass weight among control group and synbiotic groups on the 57th study day.

After the planned animal slaughter on the 57th day, the carcasses were chilled and weighed SynG12 carcass weight (52.3 kg) was higher ($p < 0.05$) than SynG6 (49.7 kg) and SynG24 (49.6 kg), although actual weight was on average, only 3 kg more. SynG12 carcass weight outcome was greater ($p < 0.01$) than the CoG, which was 9.3 kg.

The cold carcass weight of the CoG of animals was ($p < 0.01$) lower compared to the weight of other animal groups. Consequently, the average cold carcass weight of synbiotic groups is similar, SynG12 ($p < 0.05$) is higher than SynG6 and SynG24. Group SynG6 and SynG24 weights are similar.

CONCLUSIONS

Feeding synbiotics containing *Enterococcus faecium* and inulin to 1–3 - month old calves can stabilize their fecal consistency, increase average daily weight gain, total weight gain, and cold carcass weight outcome. Optimal inulin dose in this synbiotic mix was 12 g artichoke powder (6 g of inulin) per animal per day. In this case, the most gradual adaptation to the new feed supplement is observed that enables it to provide the most efficient species-specific fecal consistency and stable functioning of the digestive tract. It is possible to achieve the greatest increase in live weight gain and carcass weight outcome by feeding 12 g of Jerusalem artichoke powder together with the genus *Enterococcus* bacteria. Significantly higher weight increase per day (0.916 kg ± 0.09) has been gained by feeding synbiotics (24 g of Jerusalem artichoke powder and *Enterococcus faecium* 0.25 g).

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Effect of zero tillage and different weeding methods on grain yield of durum wheat in semi-arid regions

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Abstract. The high grain yield of wheat is limited by the dominance of weeds, particularly wild oat. Therefore, to improve wheat yield under these conditions, a field experiment was carried out in Maru Agricultural Research Station, Jordan during 2015–2016 and 2016–2017 to investigate yield response of two wheat varieties (*Triticum durum* L.) to different tillage and weeding treatments. The experimental design used was a split-split arrangement in a randomized complete block design with three replicates. Two-tillage treatments (conventional vs. zero tillage) were applied to the main plot, two wheat varieties to sub-plot, and five weeding methods (hand weeding, broadleaf + narrow leaf herbicide, broadleaf herbicide, narrow leaf herbicide, and controls) as a sub-sub-plot. The variety 'Umqais' had higher plant height, biological, grain, and straw yield than the variety 'Sham'. Hand weeding slightly increased grain yield compared with mixed herbicides (the 2,4-D plus Antelope Clodinatop- propagyl). Furthermore, mixed herbicides presented a higher grain yield than using either single herbicide. The interaction between tillage systems and weeding methods was significant in both years. The highest ($P < 0.05$) straw yield (5,990 kg ha^{-1}) was obtained by hand weeding under conventional tillage in the first season while the highest grain yield (2,005 kg ha^{-1}) was obtained by hand weeding under zero tillage in the second season. Under all weed control treatments, the variety 'Umqais' had higher biological, grain, and straw yields than the variety 'Sham' in the second season indicating that variety 'Umqais' performed better under dry conditions. Our results confirmed the superior of zero tillage for increasing the grain yield of the variety 'Umqais', and for increasing the biological and straw yields of the variety 'Sham' under semi-arid rainfed conditions of Jordan.

Key words: biological yield, broadleaf herbicide, conventional tillage, hand weeding, variety x tillage interaction.

INTRODUCTION

Wheat (*Triticum durum*) is the most important field crop grown under rainfed conditions in Jordan and considered essential for food security at the national and the global level. With the increase in world population, there is an increasing demand for wheat. At the global level, the production of wheat has improved intensely from

218.5 million tons in 1961 to 728.4 million tons in 2019 (FAO, 2019a). In 2018, the average area planted with wheat in Jordan was 20 thousand hectares, producing about 25 thousand tons of grains. Jordan imported about 900 thousand tons of wheat (FAO, 2019b).

The primary limitation for the high grain yield of wheat is the low soil moisture and the prevalence of weeds as the main obstacles under rainfed conditions in Jordan. Weeds strongly compete with wheat plants for light, nutrients, and moisture which negatively reduce grain productivity of wheat (Shah et al., 2018). Both broadleaf and narrow leaf weeds result in a decrease in soil moisture and consequently the crop grain yield (Tawaha et al., 2002). Both narrow (grassy) and broad-leaved weeds infest wheat. Among grassy weeds, wild oat (*Avena fatua*) and among broadleaf weeds, fennel (*Foeniculum vulgare*), chicory (*Cichorium spp*.), common groundsel (*Senecio vulgaris*) are of major concern in wheat frield grown rainfed in Jordan. Specifically, wild oat (*Avena spp*.) is a serious problem in wheat fields, particularly in the northern part of Jordan (Turk & Tawaha, 2002).

Hand weeding is a mechanical method used for weed control and management. This weed control method was more effective than herbicide application in suppressing the growth of weed and increasing yield (Tawaha et al., 2002; Sultana et al., 2012; Shah et al., 2018). Usually, the assessment has been done for annual weeds which are propagated by rhizomes and effectively controlled by frequent plowing. However, in wheat-based areas, the most common weeds are annual weeds of the Compositae family and Poaceae family. Nevertheless, hand weeding is not economical nowadays because of the high labor cost and wages. Therefore, chemical control of weeds became potential and caused better weed control and crop yield with the advent of herbicides (Turk & Tawaha, 2003). Several researchers have confirmed the efficiency of herbicides for weed control and increasing grain yield of wheat (Bibi et al., 2008, Mandal et al., 2014).

Many researchers have reported the impact of tillage systems on the grain yield of wheat in recent years (Yang et al., 2018; Xue et al., 2019). In Jordan, conventional tillage (CT) and zero tillage (ZT) systems are being used as described by Al-Issa & Samarah (2006). However, the effect of ZT on crop yield is controversial. Some researchers showed similar yields (Büchi et al., 2017) or yield reductions (Pittelkow et al., 2015) in ZT systems compared to the CT systems. The ZT system may have a positive impact over the CT system with specific soil, climate, and management conditions (Martínez et al., 2008; Taner et al., 2015). The ZT can improve soil structure and reduce the risk of soil erosion, leading to better water infiltration and water-use efficiency (Honsdorf et al., 2020).

The impact of tillage systems and weed control management on the yield of wheat and other crops have been studied previously (Chhokar et al., 2007; Usman et al., 2010; Kumar et al., 2013; Upasani et al., 2014; Ali et al., 2016; Susha et al., 2018; Hofmeijer et al., 2019). Winter wheat under ZT had a higher yield than that under CT when wheat was grown after either pea or spring wheat at intermediate or extensive weed control management (Young et al., 1994). Ali et al. (2016) found that a one tillage practice in a fallow year using moldboard plow resulted in the highest plant height, biological and grain yield, and harvest index of wheat. However, tillage followed by a two-time application of herbicides resulted in a maximum 1,000- grain weight of wheat (Ali et al., 2016). The best chemical method to control grasses and broadleaf weeds and to achieve a higher grain yield of wheat was using affinity herbicides in combination with zero tillage (Usman et al., 2010). Mixed herbicides were the best treatment for controlling weeds and improving wheat yield in ZT (Chhokar et al., 2007).

A few studies have been done in Jordan to identify the best weed control method under different tillage systems. Therefore, two-field experiments were carried out in northern Jordan to study the interaction effect of tillage systems and weeding methods on the growth and grain yield of two-wheat varieties grown under rainfed conditions of Jordan.

MATERIALS AND METHODS

Study site and soil

Field experiments were carried out at Maru Agricultural Research Station (MARS) which belongs to National Agricultural Research Center (NARC), Jordan during the growing years of 2015–2016 and 2016–2017 under dryland farming conditions. MARS is located in Maru, Irbid at 32° 33′N latitude, 35° 51′ E longitude, and 589 m above sea level (Al-Ghzawi et al., 2018). Maru is characterized by hot and dry summer and mild winter with 380 mm annual precipitation and represents an intermediate drought region. Before sowing, samples of soil were collected and analyzed for chemical characteristics from the experimental site (Table 1). The soil at the experimental site is Red Mediterranean Soil (RMS) type. The soil is classified as Cambisol Vertisol (Vertic, Calcic, Chromic, Luvic, Haplic, Cambic) according to World Reference Base (WRB). Soil Texture: clay (Clay 56.2%, Silt 33.8%, Sand 10.00%).

Table 1. Soil characteristic of the experimental site

Parameters	Unit	Value
pH	-	7.75
EC	(ds/m)	0.42
P	(ppm)	0.79
K	(ppm)	194
$CaCO_3$	(%)	1.90
N	(%)	0.097
Clay	(%)	56.2
Silt	(%)	33.8
Sand	(%)	9.95
Organic matter	(%)	0.76
Texture	-	Clay

Weather data at the experimental site in both seasons were shown in Table 2. The total annual precipitation in both growing seasons, 2015–2016 and 2016–2017 was 410.8 and 309.2 mm, respectively.

Table 2. Weather data during the growing seasons of 2015–2016 and 2016–2017

Growing season 2015–2016				Growing season 2016–2017			
Year	Month	T (°C)	Rainfall (mm)	Year	Month	T (°C)	Rainfall (mm)
2015	Oct.	21.73	16	2016	Oct.	21.94	2
	Nov.	15.27	31.5		Nov.	13.70	0.8
	Dec.	9.43	22.9		Dec.	8.58	190
2016	Jan.	8.21	222	2017	Jan.	7.78	63.6
	Feb.	10.19	53.4		Feb.	8.70	23.2
	Mar.	13.77	54		Mar.	12.71	19.2
	Apr.	19.41	11		Apr.	17.53	9.8
	May	21.23	0		May	21.85	0.6
	June	26.3	0		June	26.7	0
Total		-	410.8	Total		-	309.2
Mean		16.2		Mean		15.5	

Treatments

Two tillage systems were applied to the field in the two field experiments: 1) Conventional tillage (CT) using chisel plow (duck-foot) (Italian producer) at a depth of 10–15 cm, and 2) Zero tillage (ZT). Two spring wheat (*Triticum durum*) varieties (Umqais and Sham) were sown in both growing seasons. Five weed control treatments were practiced: 1) Hand weeding (T1), 2) Broadleaf + narrow leaf herbicide (T2), 3) Broadleaf herbicide (T3), 4) Narrow leaf herbicide (T4), and 5) Control (no weeding) (T5). The hand weeding treatment was done two times in each growing season; one time at wheat growth stage of Zadoks 13 and the second time at Zadoks 25 (Zadoks et al., 1974). In the first time, the hand weeding targeted narrow leaf weeds (mainly wild oat) which emerged at the same time of wheat. In the second time, the hand weeding targeted broadleaf (commonly weeds belonging to Compositae family) which must be controlled at the late tillering stage. Therefore, the hand weeding treatment was done to target the two broadleaf and narrow leaf weeds similar to the herbicide treatment. In the herbicide treatment, the broadleaf and narrow leaf weeds were controlled by using 2,4-D [2,4-Dichlorophenoxyacetic acid; 62% (w/v) of 2,4-D Isoocytyle-ester) (Esterdefore, VAPCO, Jordan)) and Antelope [100 g of Clodinafop-propargyl and 25 g of Cloquintocet-methyl (w/v)] herbicides, respectively. The narrow leaf herbicide was applied at a rate of 2.5 mL L^{-1} of water while broadleaf herbicide at a rate of 5 mL L^{-1} water. The herbicide was sprayed at a rate of 20 mL m^{-2}. The herbicides were applied by using a mounted sprayer equipped with a fan-type nozzle. The 2,4 D and Antelope were applied according to the herbicide label at wheat growth stages of Zadoks 24, and 13 (Zadoks et al., 1974), respectively. The most common weed species that present at the experimental site were wild oat (*Avena fatua*)(), wild barley (*Hordeum bulbosum*), wild mustard (*Sinapis arvensis*), and star-thistle (*Centaurea spp*).

Crop management

The main plot size was 2.5 m × 40 m. The size of each sub-sub plot was 2.5 m × 8 m with 2 m apart. Wheat seeds were sown at a rate of 12 g m^{-2} by mechanical planter at 17.5 cm row spacing at a depth of 7–10 cm. Seeds of the two wheat varieties were planted on 30 December, 2015 and 8 January, 2017 during the two growing seasons of 2015–2016 and 2016–2017, respectively. The grains were harvested on 30 June in both growing seasons. In both seasons, wheat was planted after a legume crop. The crop received 10 g m^{-2} of diammonium phosphate (18% N and 46% P_2O_5) at the time of seeding and 5 g m^{-2} of urea $(NH_2)_2CO$ (45% N) at the tillering stage of growth.

Measurements of plant growth and grain yield

In both growing seasons, days to 50% heading (DH) and days to 50% maturity (DM) were recorded. Days to heading (HD) were determined visually by calculating the number of days from seeding to the day when the main spike had emerged from the sheath of the flag leaf. Days to physiological maturity (MD) were determined visually by calculating the number of days from seeding to the day when the plants had reached the physiological maturity stage (the plant spikes turned yellow). Plant height (PH) was measured from the soil surface to the upper part of the spike without the awns. A square quadrate (1 m^2) was placed randomly at the central rows for each treatment to measure biological, grain yield, and straw yield. The wheat moisture content at harvest was 12%.

Experimental design and statistical analysis

A split-split plot arrangement in a completely randomized block design was used for data analysis with three replicates for each treatment. The tillage treatments were considered as the main plot, while wheat varieties as a sub-plot, and weeding treatments as a sub-sub-plot. Data were analyzed using JUMP software. Analysis of variance (ANOVA) was calculated for both main and interaction effects. The differences among means were calculated according to student's t-test at a P value less than 0.05%.

RESULTS

Days to heading, days to maturity, and plant height

Analysis of variance for tillage system, variety, and weeding methods and their interaction effects on several wheat growth and yield parameters was shown for the growing season of 2015–2016 (Table 3) and the growing season of 2016–2017 (Table 4). In the first growing season, the tillage system (T) had no significant effect on days to heading (DH), days to maturity (DM), and plant height (PH) (Table 3). There were significant differences in PH among varieties (V) and weeding methods (W) without interaction among different factors. There was a significant difference in DH among varieties (V). In the second growing season, the tillage system (T) and the variety (V) significantly affected PH of wheat with some interactions among different factors (Table 4).

Table 3. Analysis of variance (F probability values) showing the effect of tillage system, varieties, and weeding methods on days to heading (DH), days to maturity (DM), plant height (PH), biological yield (BY), grain yield (GY), straw yield (SY) and 1,000-grain weight for wheat plants during the growing season of 2015–2016

Source of		F probability values						
variation	DF	DH	DM	PH	BY	GY	SY	1,000-GW
Tillage (T)	1	NS	NS	NS	NS	NS	NS	NS
Block	2	0.0001	0.0001	0.0001	0.0016	0.012	0.0013	0.0001
Error (a)	-	-	-	-	-	-	-	-
Variety (V)	1	0.0096	NS	0.014	NS	NS	NS	0.007
T × V	1	NS	NS	NS	NS	NS	NS	NS
Error (b)	-	-	-	-	-	-	-	-
Weed (W)	4	NS	NS	0.0056	0.0001	0.0001	0.0001	NS
TxW	4	NS	NS	NS	NS	NS	0.035	NS
VxW	4	NS	NS	NS	0.019	NS	0.008	NS
TxVxW	4	NS	NS	NS	NS	NS	NS	NS

NS: Not significant.

The main means of tillage systems and weeding methods for DH, DM, and PH of the two varieties of wheat were shown in both growing seasons (Table 5). There was no significant difference between conventional (CT) and zero tillage (ZT) systems for the three parameters, except for the second season where PH of wheat in ZT (71.5 cm) was higher than those for CT (69.1 cm). Both DH and PH of wheat were significantly affected by the wheat variety in both growing seasons (Table 5). At the first growing season, the variety 'Sham' had significantly earlier DH (79.2) than those for 'Umqais' (81.9). In contrast, the variety 'Sham' showed significantly later DH when compared with

‘Umqais’ in the second growing season. The PH of the variety ‘Sham’ was significantly shorter than those for ‘Umqais’. For weed control methods, the only significant effect was for PH in the first growing season. All weeding methods resulted in significantly longer PH than that of the narrow leaf herbicide treatment (T4).

Table 4. Analysis of variance (F probability values) showing the effect of tillage system, varieties, and weeding methods on days to heading (DH), days to maturity (DM), plant height (PH), biological yield (BY), grain yield (GY), straw yield (SY) and 1,000-grain weight for wheat plants during the growing season of 2016–2017

Source of		F probability values						
variation	DF	DH	DM	PH	BY	GY	SY	1,000-GW
Tillage (T)	1	NS	NS	0.028	NS	0.034	NS	NS
Block	2	0.0001	NS	NS	0.0001	0.0001	0.004	NS
Error (a)	-	-	-	-	-	-	-	-
Variety (V)	1	0.001	NS	0.0001	0.0001	0.0001	0.0005	0.0014
TxV	1	0.043	NS	NS	NS	0.03	0.018	NS
Error (b)	-	-	-	-	-	-	-	-
Weed (W)	4	NS	NS	NS	0.0001	0.0001	0.001	0.0002
TxW	4	NS	0.0005	0.008	0.0002	0.0001	0.035	NS
VxW	4	0.038	0.05	NS	0.024	0.0001	NS	0.026
TxVxW	4	NS	0.021	0.01	0.0015	0.0001	0.049	0.0002

NS: Not significant.

Table 5. Main means for days to heading (DH), days to maturity (DM), and plant height (PH) for two varieties of wheat grown under two tillage systems and five weeding methods during the growing seasons of 2015–2016 and 2016–2017

	2015–2016			2016–2017		
Main Effect	DH	DM	PH	DH	DM	PH
	day		cm	day		cm
Tillage system						
Conventional	81a	112a	68.1a	79a	112a	69.1b
Zero	80a	112a	65.9a	79a	111a	71.5a
$LSD_{(0.05)}$	1.9	1.6	7.1	0.81	2.2	1.7
Variety						
Umqais	82a	113a	71.9a	78b	111a	75.6a
Sham	79b	111a	62.2b	81a	111a	65.1b
$LSD_{(0.05)}$	1.6	1.6	6.4	0.78	0.59	1.8
Weeding methods						
T1	80a	112a	70.9a	79a	112a	71.1a
T2	81a	112a	66.9a	79a	111a	70.8a
T3	80a	112a	67.1a	80a	112a	69.3a
T4	81a	112a	61.8b	80a	111a	70.3a
T5	80a	112a	68.3a	79a	111a	70.1a
$LSD_{(0.05)}$	0.79	0.81	4.5	0.43	0.60	1.5

T1: Hand weeding; T2: Broadleaf and narrow leaf herbicide; T3: Broadleaf herbicide; T4: Narrow leaves herbicide; T5: Control. *LSD*: Least significantly difference at $P < 0.05$. Means followed by the same letters are not significantly different according to Student’s t-test at *P* value of 0.05%.

Effects on biological yield (BY), grain yield (GY), straw yield (SY) and 1,000-grain weight

In the first growing season (2015–2016), the weeding methods (W) had a significant effect on biological yield (BY), grain yield (GY), and straw yield (SY) (Table 3). However, the weeding methods (W), variety (V) and the W × V × T interaction effect had a significant effect on BY in the second growing season (Table 4).

In the first growing season, BY, GY, and SY were not significantly affected by either tillage system or variety (Table 6). However, the variety ‘Sham’ had significantly higher 1,000-grain weight ($P < 0.01$) than the variety ‘Umqais’. For weeding methods, both hand weeding (T1) and Broadleaf + narrow leaf herbicide (T2) gave significantly the highest BY (7,883 and 7,671 kg ha^{-1}, respectively) while the control (T5) gave the lowest BY (5,731 kg ha^{-1}). Grain yield (GY) was not significantly affected by T1, T2, and T4 weeding methods. However, both T1 and T2 had significantly higher SY than other weeding methods (Table 6). The 1,000-grain weight was not significantly affected by weeding methods.

Table 6. Main means for biological yield (BY), grain yield (GY), straw yield (SY), and 1,000-grain weight (1,000-GW) for two varieties of wheat grown under two tillage systems and five weeding methods

	2015–2016				2016–2017			
Main Effect	BY	GY	SY	1,000-GW	BY	GY	SY	1,000-GW
	kg ha^{-1}			g	kg ha^{-1}			g
Tillage system								
Conventional	7,383a	2,184a	5,199a	31.4a	5,051a	1,503b	3,549a	32.3a
Zero	6,486a	2,003a	4,483a	31.8a	5,317a	1,592a	3,725a	31.7a
LSD$_{(0.05)}$	1,516	226	1,303	4.2	309	726	236	1.6
Variety								
Umqais	6,854a	2,136a	4,718a	35.0a	5,744a	1,778a	3,966a	33.6a
Sham	7,015a	2,052a	4,964a	28.2b	4,625b	1,317b	3,308b	30.4b
LSD$_{(0.05)}$	801	219	584	3.7	207	847	173	1.1
Weeding methods								
T1	7,883a	2,435a	5,448a	31.4a	5,542a	1,794a	3,748b	33.1ab
T2	7,671a	2,422a	5,249a	31.6a	5,247a	1,701a	3,546bc	33.6a
T3	6,538b	1,929b	4,609b	31.7a	5,432a	1,408bc	4,024a	31.0c
T4	6,849b	2,244a	4,604b	31.0a	4,842b	1,497b	3,346c	31.8bc
T5	5,731c	1,439c	4,292b	32.4a	4,859b	1,338c	3,521bc	30.5c
LSD(0.05)	659	202	487	1.9	322	105	255	1.4

T1: Hand weeding; T2: Broad and narrow leaf herbicide; T3: Broad leaves herbicide; T4: Narrow leaves herbicides; T5: control. *LSD*, least significant difference at $P < 0.05$. Means followed by the same letters are not significantly different according to Student’s t-test at *P* value of 0.05%.

In the second growing season, ZT had significantly more GY (1,592 kg ha^{-1}) than that of CT (1,503 kg ha^{-1}), but other yield parameters were not affected by tillage systems (Table 6). On the other hand, the variety ‘Umqais’ had significantly higher BY, GY, SY, and 1,000-grain weight than the variety ‘Sham’. Yield components were significantly different among weeding methods (Table 6). Both T1 and T2 had significantly higher BY and GY than the control (T5). The highest and lowest SY were significantly observed in T3 and T4, respectively.

Effect of treatments interaction on wheat yield

In the first growing season (2015–2016), tillage x weeding interaction was significantly ($P < 0.01$) affected by SY (Fig. 1). The CT resulted in a higher straw yield than ZT for the weeding methods of T1, T2, and T5. In both tillage systems, the SY of the T1 and T2 weeding methods was significantly higher than those of T5 weeding treatment (Fig. 1).

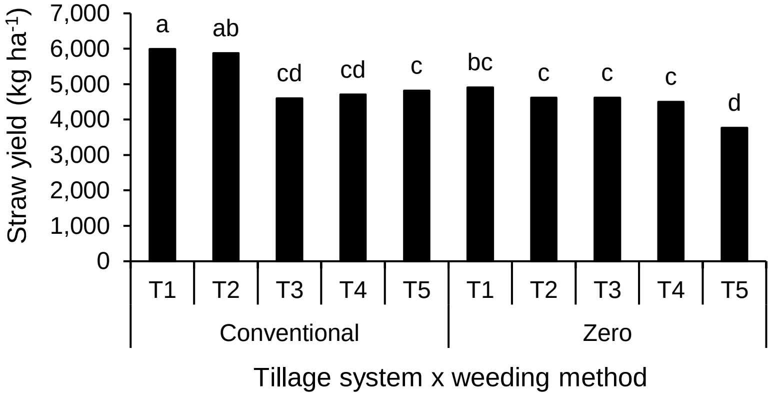

Figure 1. The tillage system x weeding method interaction effect on straw yield (SY) for wheat grown during the growing season 2015–2016. Means followed by the same letters are not significantly different according to Student's t-test at *P* value of 0.05%.

The effect of variety x weeding interaction on BY and SY was also significant (Fig. 2). For the weeding method T1, the variety 'Umqais' had a higher BY than that the variety 'Sham', while the SY was not different between the two varieties. In contrast, the variety 'Umqais' had lower BY and SY than the variety 'Sham' for the T2 weeding method. For the two varieties, the T1 and T2 weeding methods resulted in the highest BY and SY. The T5 weeding method resulted in the lowest BY and SY.

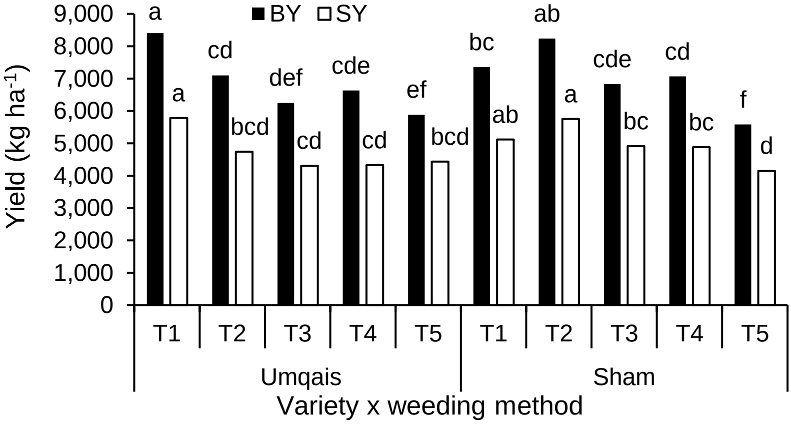

Figure 2. The variety x weeding method interaction effect on biological (BY) and straw yield (SY) for wheat grown during the growing season 2015–2016. Means followed by the same letters are not significantly different according to Student's t-test at *P* value of 0.05%.

For the second growing season, the tillage system x variety (Fig. 3), the tillage system x weeding method (Fig. 4), and the variety x weeding method (Fig. 5) interactions effect on BY, GY, and SY were significant. For the tillage x variety interaction (Fig. 3), ZT resulted in higher BY and SY than CT for the variety ‘Sham’ and higher GY for the variety ‘Umqais’. For both tillage systems, the variety ‘Umqais’ gave significantly higher BY, GY, and SY than the variety ‘Sham’.

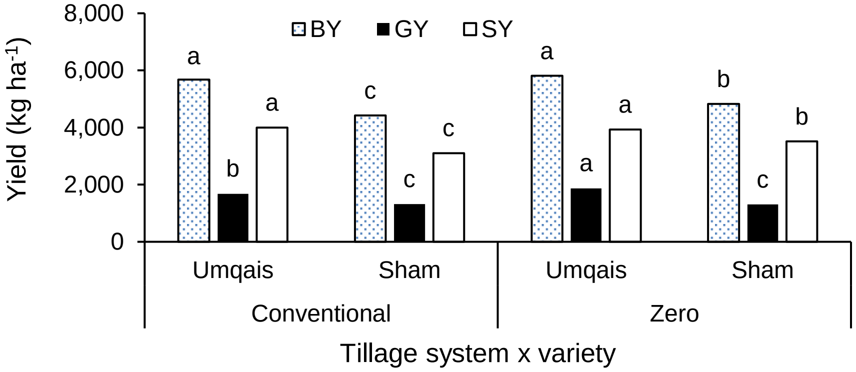

Figure 3. The tillage system x variety interaction effect on biological yield (BY), grain yield (GY), and straw yield (SY) for wheat grown during the growing season 2016–2017. Means followed by the same letters are not significantly different according to Student’s t-test at *P* value of 0.05%.

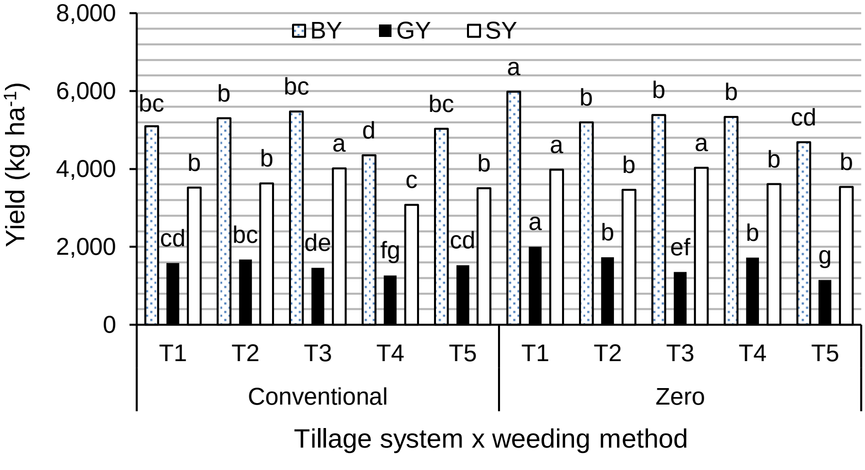

Figure 4. The tillage system x weeding method interaction effect on biological yield (BY), grain yield (GY), and straw yield (SY) for wheat grown during the growing season 2016–2017. Means followed by the same letters are not significantly different according to Student’s t-test at *P* value of 0.05%.

The tillage system x weeding method interaction effect on BY, GY, and SY was shown in Fig. 4. Hand weeding method (T1) and narrow leaf herbicides (T4) resulted in significantly higher BY, GY, and SY in ZT compared with those in CT (Fig. 4). However, the control (T5) gave significantly higher GY in CT system (1,529.8 kg ha^{-1}) than those in ZT (1,146.3 kg ha^{-1}). In CT, T4 weeding treatment gave significantly the lowest yield components. In ZT, the BY and GY were the highest for T1 weeding treatment while SY was the highest in broadleaf herbicide (T3) but the difference was not significant from the T1 weeding treatment (Fig. 4).

For all weeding methods, the variety ‘Umqais’ had significantly higher BY, GY, and SY than the variety ‘Sham’ in the second growing season (Fig. 5). For the variety ‘Umqais’, the effect of broadleaf herbicide (T3) on BY and SY was the greatest (6191.6 and 4,372.5 kg ha^{-1}, respectively), followed by hand weeding (T1) while the control (T5) had the lowest BY (5,275 kg ha^{-1}). For the variety ‘Sham’, the T1 weeding treatment showed the highest BY and GY. However, the T3 treatment gave the highest SY and lowest GY (Fig. 5).

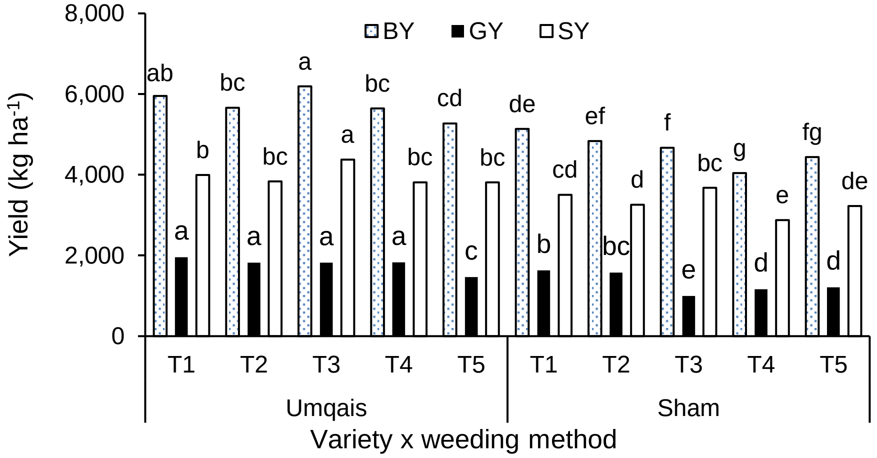

Figure 5. The variety x weeding method interaction on biological yield (BY), grain yield (GY), and straw yield (SY) for wheat grown during the growing season 2016–2017. Means followed by the same letters are not significantly different according to Student’s t-test at *P* value of 0.05%.

DISCUSSION

Main effect of tillage system

This study showed no differences between the conventional (CT) and zero (ZT) tillage systems on yield parameters of wheat except for in the second growing season where plant height and wheat grain yield were significantly greater in ZT treatment. Such results were similar to the outcomes of Al-Issa and Samarah (2006) and Vita et al. (2007). The response of grain yield to tillage systems varied depending upon soil type, crop species, rainfall, and location (Khaledian et al., 2013; Liu et al., 2020).

Wheat yield was not affected by tillage systems in the first growing season which might be due to the higher rainfall in the first season than that in the second season (Table 2). The yield of wheat was lower in the second season than that in the first season. Therefore, the reduction in yield components in the second season might be due to the lower rainfall in the second season. A strong correlation was observed by Vita et al. (2007) between wheat yield and total precipitation throughout the growing season. They found that ZT performed better under limited rainfall as a result of less evaporation of soil water, and thus more water availability to plants (Vita et al., 2007). In addition, water use efficiency (WUE) in ZT has been described to be higher in soils than those in CT (Li et al., 2005; Bryant et al., 2020). In the present study, there was no significant correlation ($r^2 = 0.15$) between plant height and grain yield of wheat (data not shown) for both experiments.

Main effect of wheat variety

Our results showed that the variety 'Umqais' had higher plant height than the variety 'Sham'. This could be associated with larger root systems of the variety 'Umqais'. Moreover, the variety 'Umqais' took slightly longer days to heading (81.9 days) than the variety 'Sham' (79.2 days) in the first growing season and vice versa for the second growing season. The variation in DH among varieties in response to the growing seasons might be due to the variation in rainfall between seasons. Thus, early heading of the variety 'Umqais' in the second season could be associated with lower rainfall because this variety is highly drought-tolerant compared with the variety 'Sham'. However, wheat yield was affected by the variety in the second season only. A higher biological, grain and straw yields for the variety 'Umqais' than those for the variety 'Sham' indicating that the variety 'Umqais' performed better under dry conditions. Other studies have revealed that early heading was positively associated with grain yield under severe drought (Van Oosterom & Acevedo, 1992). In the present study, no correlation between days to heading (DH) and grain yield was found (data not shown) under semi-arid circumstances. A similar result was obtained by van den Boogaard et al. (1996). The variety 'Umqais' was confirmed to be more efficient variety than the variety 'Haurani' due to higher yield under improved growing conditions (Al-Rjoub & Al-Samarrai, 2006).

Main effect of weed control methods

The weeding method had no effect on plant height, days to heading, and days to maturity for the two wheat varieties except for in the first season when PH of wheat was the shortest with the application of narrow leaf herbicide (Table 5). This may be due to a greater broadleaf weeds competition with the phytotoxic effect of this herbicide on wheat crops. However, Tawaha et al. (2002) found the shortest PH in barley were measured with the application of 2,4-D while the longest PH with hand weeding method. Qasem (2007) found the longest PH was under controls (no weeding).

In the present study, weeding methods had a significant effect on yield parameters of durum wheat varieties under tillage systems, although the outcomes were more realistic in the first growing season than in the second season. In both years, hand weeding revealed superior wheat yield when compared with mixed herbicides but the difference was not significant. The increase in grain yield (3%) for both years achieved with hand weed control was primarily due to the efficient control of weeds by a decrease of weed density. Similar results were supported by Turk & Tawaha (2003) and Qasem (2007) who found hand weeding method had the highest wheat grain yield.

In general, mixed herbicides presented a higher efficacy for increasing crop yield than using either single herbicide. However, narrow leaf herbicide treatment had a significantly similar effect on grain yield as mixed herbicides in the first growing season, while broadleaf herbicide had a significantly similar impact on biological yield in the second growing season. Thus, using a single herbicide under certain circumstances had proved efficiency for yield similar to mixed herbicides. For straw yield, the best result in the second year was achieved by 2,4-D herbicide indicating the high performance of this herbicide under lower soil moisture. Results of Qasem (2007) indicated that the maximum straw yield was obtained by ‘imazamethabenz-methyl’ herbicide and the highest biological yield was with imazamethabenz-methyl and weed-free control.

Control or no weeding method reduced grain yield by 41% and 25% in the first and second growing season, respectively in comparison with those for hand weeding (Table 6). The reduction in grain yield was greater in the first season than the second which might be related to higher weed competition under higher soil moisture conditions in the first season. Yield losses of wheat caused by weeds may range between 30% and 80% in Jordan (Turk & Tawaha, 2003).

Tillage system x weed control method interaction

In the present study, wheat yields were significantly affected by the interaction between the tillage system and weeding methods in both seasons. Our results indicated that hand weeding was the best weeding control method under tillage systems. Hand weeding, mixed herbicides and controls (no weeding) methods showed higher straw yield in CT compared with those in ZT at first growing season (Fig. 1). Hand weeding gave the highest straw yield under CT while controls gave the lowest yield under ZT. The increase in straw yield under CT may be due to reduced weed infestation. Similarly, Upasani et al. (2014) found that continuous CT in rice and wheat sequences with application of isoproturon + 2,4- D post-emergence herbicide in wheat, was the most useful practice in direct-seeded rice–wheat system. However, hand weeding and narrow leaf herbicide showed higher biological, grain, and straw yields in ZT than those in CT for the second season (Fig. 4), which could be due to reduced weed competition. The highest and lowest grain yield were obtained from hand weeding and control, respectively under ZT. The observed increase in grain yield of wheat with the use of ZT and herbicides was similar with results of Chhokar et al. (2007). Effective weed control in ZT was based on the use of herbicides (Calado et al., 2010). Conversely, alternative strategies for nonchemical weed control are required to decrease the dependency on herbicides under ZT systems (Kumar et al., 2013).

Wheat variety x weed control method interaction

A significant interaction effect between variety and weeding methods was found on yield parameters of wheat in both years. In the first growing season, hand weeding was the best weed control method for increasing biological and straw yields for the variety ‘Umqais’ while mixed- herbicide method was the best for the variety ‘Sham’ (Fig. 2). Hence, wheat varieties responded differently to weed control methods. In the second season, the variety ‘Umqais’ had higher biological, grain, and straw yields than the variety ‘Sham’ under all weed control methods. Although the maximum grain yield was attained by planting the variety ‘Umqais’ and by using hand weeding treatment. The lowest grain yield was reported for the variety ‘Sham’ using a 2,4-D herbicide treatment (Fig. 5). Sultana et al. (2012) found that weed free treatment resulted in the highest grain yield of the variety ‘Prodip’ while no weeding treatment resulted in the lowest grain yield of the variety ‘Shatabdi’.

CONCLUSIONS

Grain yield of wheat was improved by growing ‘Umqais’ variety under zero tillage and by controlling weeds either by hands or a combination of broadleaf + narrow leaf herbicide, especially in the drier season. Zero tillage resulted in an increase in grain yield of ‘Umqais’variety, and biological and straw yield of ‘Sham’ variety in comparison with conventional tillage in the second growing season (drier season). The grain yield was significantly reduced by 25–41% when weeds were not controlled with greater grain loss in the wetter growing season than the drier one.

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Experimental research into uniformity in spreading mineral fertilizers with fertilizer spreader disc with tilted axis

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Abstract. Improving the efficiency in the work process of mineral fertilizer dressing is a topical problem in today's agricultural industry. The authors have developed a design of the fertilizer spreading tool with a tilted axis and carried out field experiment investigations on it. It has been established by the results of the investigations that the non-uniformity in the spreading of mineral fertilizers along the line of their departure from the fertilizer spreading tool with a tilted axis is most strongly affected by the disc rotation frequency. The obtained results provide for selecting the optimum parameters and modes of operation for the tool under consideration in the situation, when it is installed in fertilizer placing machines. Also, it has been established that increasing the spreading disc rotation frequency in such a tool from 600 to 800 rpm results in the growth of the effective range of mineral fertilizer spreading along the placing line at a level of 10.5 m. Increasing the disc's angle of inclination to the horizontal plane to 20° results in the rise of the effective fertilizer spreading range at a level of the 48th tray (24 m) inclusive as well as the increase of the distance between the tool and the tray that contains the maximum share of the spread fertilizers (5.1%) to 24 trays (12 m). At a constant rotation frequency of the disc in the tilted-axis fertilizer spreading tool, in all its kinematic modes of operation, an increase in the angle of its disc's inclination to the horizontal plane results in the rise of the indices representing the distribution of the mineral fertilizers over the trays along the line of their placing. The width of the mineral fertilizer spreading can be controlled by adjusting the rotation frequency of the disc in the tilted-axis fertilizer spreading tool, when it is set at an angle to the horizontal plane, similar to how it is done in state-of-the-art fertilizer placing machines with horizontally positioned discs in their centrifugal spreading tools. The rotation frequency of the disc in the tilted-axis fertilizer spreading tool has the most significant effect on the coefficient of variation incidental to the distribution of the mineral fertilizers along the line of their placing.

Key words: disc, inclination, mineral fertilizer, uniformity, spreading tool.

INTRODUCTION

It is common knowledge that the dosage provided in the mechanical spreading of mineral fertilizers and the uniformity in their distribution over the soil surface have an effect not only on the yield of the cultivated crops, but also on the quality of the harvest. In particular, the reduction of the non-uniformity in the fertilizer spreading from 30% to 15% results in the crop loss abatement from 8.6–22.4% to 0.6–1.1% (Adamchuk, 2002; 2006). The above-said implies that the lower the non-uniformity, with which the machines spread fertilizers, is, the greater the effect of their application will be. However, the realia of the development of new machines have proved that attempts to reduce the non-uniformity in the fertilizer spreading result in the need to design more sophisticated and more expensive equipment and that creates the economic situation, where the machinery for dressing the soil with mineral fertilisers and chemical soil improvers has low productive capacity and operating and economic efficiency.

Such a situation sets the research and engineering problem of improving the production output of the mineral fertiliser and chemical soil improver placing machines and reducing the costs per unit in the performance of the respective work process.

The issues related to the improvement of efficiency in the work process of dressing the soil with mineral fertilisers have always been and still is a topical problem. The earlier completed research (Yasenetsky & Sheychenko, 2002; Kobets et al., 2017) has established that the efficiency of the mineral fertiliser dressing depends not only on the fertilisers themselves, but on the methods of their placing as well. The primary factor limiting the efficiency of mineral fertiliser dressing is the non-uniformity in the distribution of the fertilisers over the area of the field (Yildirim, 2006; Lawrence et al., 2007; Villette et al., 2007; 2008; Jones et al., 2008; Marinello et al., 2017). It has a material effect on the ripening of the crop, causes variation in the yield and, overall, results in the decrease of the harvest (Adamchuk, 2002).

At the same time, the efficiency of mineral fertiliser application is also affected by the depth of fertiliser placement in the soil. According to the results of the completed investigations, it has been established that the placement of mineral fertilisers in the soil at a depth of 18–24 cm results in their unavailability for the plants in their initial growth period, while during the ripping of the soil with various harrows and cultivators the nutrient substances get stirred and become placed mostly in the upper drying soil layer (0–6 cm), where they are again not fully available for the plants. Many scientists have proved that the drilled fertilizers must become the direct source of nutrients for the plants and they have to be placed in the soil in such a way that they are readily available for the active parts of the agricultural plants’ root systems. Placing the fertilisers close to the plant roots establishes a zone of the increased concentration of nutrients, which facilitates their absorption and improves the efficiency of their application. It has been proved that mineral fertilisers have to be placed in both the upper and deeper soil layers, with the concentration that is commensurate with the plant root system development level.

The theoretical and experimental research into the centrifugal-type spinning-disk fertiliser spreading tools has been undertaken by many scientists (Scheufle & Bolwin, 1991; Dintwa et al., 2004; Villette et al., 2005; Olt & Heinloo, 2009; Šima et al., 2013). They have studied the effect that the structural solution of the disks (Villette et al., 2005; Lü et al., 2016; Liu et al., 2018), vanes and other parts of the fertiliser spreading tool design (Grift et al., 2006; Yildirim, 2008), the parameters and modes of their operation

(Van Liedekerke et al., 2009; Villette et al., 2010; Bulgakov et al., 2020), the physical and mechanical properties of the mineral fertilisers and chemical soil improvers and the conditions (Aphale et al., 2003; Villette et al., 2007; Hijazi et al., 2010; Biocca et al., 2013; Šima et al., 2013; Antille et al., 2015), in which the mineral fertiliser spreading machines operate, have on their effective working width, in particular, on the spreading range, the fertiliser placing non-uniformity etc. However, the schematic models and parameters of centrifugal-type spinning-disk tools discussed in these studies do not take into account the factor of tilting the disc axis in the longitudinal and vertical plane. At the same time, the significance of that factor was proved during the research into the process of spreading mineral fertilisers with the new centrifugal-type fertiliser spreading tool, in which the axes are set with tilt, earlier carried out by the authors.

The aim of this study was to improve the efficiency of the work process of placing mineral fertilisers with centrifugal-type spinning-disk fertiliser spreading tools, in which the disc axes are installed with tilt.

MATERIALS AND METHODS

The authors have designed and produced a mobile version of the experimental unit for research into the mineral fertiliser spreading with the use of centrifugal-type spinning-disk fertiliser spreading tools with tilted rotation axes. The design layout of the experimental unit is presented in Fig. 1, its general appearance is shown in Fig. 2.

On the sliding frame 3 of the experimental unit (Fig. 1), the supply bin 7 (not shown in Fig. 2) is installed, which can be turned in the horizontal plane due to the turning frame 5. In the bottom of the bin 7, there is a seeding hole equipped with the slide valve 8 used for the controlled change of the open area in the said hole. The design of the drive that rotates the tilted-axis fertiliser spreading tool 9 provides for both variation of its rotation frequency and adjustment of the disc inclination angle to the horizontal plane.

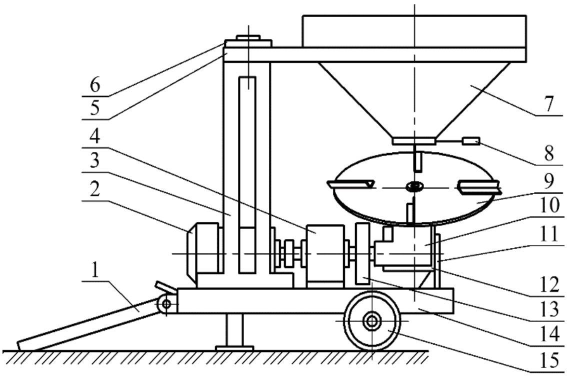

Figure 1. Schematic model of experimental unit for research into mineral fertiliser spreading: 1 – transporting bracket; 2 – electric motor; 3 and 5 – sliding and turning frames, respectively; 4 – chain variable-speed gear; 6 – frame turn swivel; 7 – bin; 8 – slide valve; 9 – fertiliser spreading tool; 10 – bevel-gear speed reducer; 11 and 12 – brackets; 13 – free-wheel clutch; 14 – main frame; 15 – carrier wheel.

The work process of the experimental unit is as follows. The torque of the motor 2 is transmitted via the coupling to the input shaft of the chain variable-speed gear 4 that changes the rotation frequency. The output shaft of the gear drives via the free-wheel clutch the rotary motion of the input shaft in the bevel-gear speed reducer 10. As a result of that process, the rotary motion is imparted to the output shaft of the bevel-gear speed reducer 10, on which the fertiliser spreading tool with a tilted axis is installed. The mineral fertilisers are seeded down from the bin 7 under the action of the gravity force through the seeding hole's open area and arrive in metered amount onto the fertiliser spreading tool 9, the axis of which is set with tilt, where they are captured by the vanes fixed on the disc. When the mineral fertiliser particles get onto the vanes, they accelerate under the action of the centrifugal force, moving along the vanes from the centre of the disc to their peripheral ends. After the mineral fertilisers reach the peripheral ends of the vanes, they depart from the vanes and, on account of the received reserve of kinetic energy, perform free motion in the air along a certain trajectory directed away from the fertiliser spreading tool, the axis of which is set with tilt with respect to the surface of the horizontal site, where the experimental investigations are carried out.

Figure 2. Experimental unit for research into process of mineral fertiliser top dressing with the use of centrifugal-type spinning-disk fertiliser spreading tools with tilted axes, with standard trays placed along the line of spreading.

During the completed experimental investigations, the mineral fertilisers spread with such a fertiliser spreading tool were collected in standard trays sized 0.5×0.5×0.1 m. The trays were placed tightly close to each other, thus preventing any loss of the mineral fertiliser particles falling from above at various angles to the horizontal plane. Each of the trays was placed at a strictly controlled distance from the experimental unit, that is, when the trays were placed along the line of spreading, they were all situated on a straight line with insignificant spacing from each other. Their total number on the line was equal to 80. That corresponded to a mineral fertiliser particle collection distance of more than 40 m.

Similarly, experimental investigations were carried out with the use of the machine equipped with two fertiliser spreading tools of the same design, the axes of which were set at an angle to the horizontal plane. In this case, again, all the trays were placed along the two directions of spreading in the same way, sufficiently close to each other and over a distance of 40 m in the two directions (Fig. 3).

Figure 3. General appearance of the experimental mineral fertiliser top dressing machine with two fertiliser spreading tools, axes of which were set at an angle to horizontal plane, during the field experiment investigations, in which trays were placed along the two directions of spreading.

The field experiment investigations were carried out with the use of the following mineral fertiliser: 'Ammonium saltpetre' (chemical formula NH_4NO_3, nitrogen content 34.7%).

The physical and mechanical properties of it were as follows:

1. Average moisture content – 0.17%;
2. Bulk density – 780 kg m^{-3};
3. Density – 1,500 kg m^{-3};
4. Mean diameter of particles - 1.34 mm;
5. Percentage of size fractions: 0 to 0.5 mm – 13.1%; 1 to 2 mm – 83.5%; 2 to 3 mm – 3.4%;
6. Flight velocities of particles by size: < 1.0 mm – 2.2 to 4.9 m s^{-1}; 1 to 2 mm – 4.1 to 7.9 m s^{-1}; 2 to 3 mm – 5.6 to 8.3 m s^{-1};
7. Coefficient of friction on steel: 0.3 to 0.6.

In order to investigate the effect that the rotation frequency of the spreading tool and the angle of its inclination with respect to the horizontal plane have on the non-uniformity in the distribution of the mineral fertilisers along the line of their placing (i.e. along the line that is perpendicular to the axis of the experimental unit), the two-factor experiment with several levels of the factor variation was designed and carried out. In the experiment, the non-uniformity of mineral fertiliser spreading was measured by the coefficient of variation of the mineral fertiliser distribution over the standard trays.

The mathematical formulation of the processes was performed in the form of generating regression equations. For that purpose, the following factors had been selected:

n – rotation frequency of the disc in the fertiliser spreading tool with a tilted axis, in natural units;

α – disc's angle of inclination to the horizontal plane, in natural units;

X_1 – rotation frequency of the disc, in encoded form;

X_2 – disc's angle of inclination to the horizontal plane, in encoded form.

The experimental investigation of the non-uniformity in the distribution of the mineral fertilisers spread by the fertiliser spreading tool with a tilted axis along the line of their placing was carried out in accordance with the mathematical design of the multiple-factor experiment following the prepared design matrices (Tables 1 and 2).

The data obtained as a result of the experimental investigations were processed with the aim of generating the regression model that would describe the effect of the factors and their interrelations on the non-uniformity in the spreading of mineral fertilisers by the fertiliser spreading tool with a tilted axis into the trays along the line of their placing, with the use of standard techniques of statistical processing.

Table 1. Design matrix of full factorial experiment for research into non-uniformity in spreading of granulated superphosphate by fertiliser spreading tool with a tilted axis along line of its placing

No.	Factor values: in natural (coded) form	
	n [rpm] (X_1)	α [deg] (X_2)
1	600 (–1)	30 (–2)
2	600 (–1)	20 (–1)
3	600 (–1)	10 (0)
4	600 (–1)	0 (+1)
5	800(0)	30 (–2)
6	800(0)	20 (–1)
7	800(0)	10 (0)
8	800(0)	0 (+1)
9	1,000 (+1)	30 (–2)
10	1,000 (+1)	20 (–1)
11	1,000 (+1)	10 (0)
12	1,000 (+1)	0 (+1)

At the first stage of processing the experimental data, after their acquisition, they were checked for repeatability. In order to assess the repeatability, Cochran's test was applied, the value of the criterion was determined with the use of the relation:

$$G = \frac{S^2_{u\,\max}}{\sum\limits_{u=1}^{n} S_u^2} \le G_t \tag{1}$$

where G_t (0.05; n; f_u) – table value of Cochran's criterion at a significance level of 5%, the number of experiments n and the number of degrees of freedom $f_u = m - 1$ with the number of replications m.

Table 2. Design matrix of full factorial experiment for research into non-uniformity in spreading of nitroammophoska and ammonium saltpetre by fertiliser spreading tool with a tilted axis along line of its placing

No.	Factor values: in natural (coded) form	
	n [rpm] (X_1)	α [deg] (X_2)
1	600 (–1)	30 (–2)
2	600 (–1)	20 (–1)
3	600 (–1)	0 (+1)
4	800 (0)	30 (–2)
5	800 (0)	20 (–1)
6	800 (0)	0 (+1)

The variance was determined with the use of the following expression:

$$S_u^2 = \frac{1}{m-1}\sum_{i=1}^{m}\left(y_{uik} - \bar{y}_u\right)^2, \tag{2}$$

where y_{uik} – value of the output variable in the respective replication.

Further, the experimental error was calculated as follows:

$$S_y^2 = \frac{1}{n}\sum_{u=1}^{n} S_u^2 \tag{3}$$

If the checking with the use of the Cochran's test proved that the process was repeatable, the next step in the experimental data processing would be to find the regression coefficients in accordance with the following formulae:

$$\begin{aligned} b_0 &= \frac{1}{n}\sum_{i=1}^{n}\bar{y}_u, \\ b_p &= \frac{1}{n}\sum_{i=1}^{n} X_p\bar{y}, \\ b_{pr} &= \frac{1}{n}\sum_{i=1}^{n} X_p X_r\bar{y}\,. \end{aligned} \tag{4}$$

The adequacy of the regression equation was checked with the use of Fisher's ratio test:

$$F = \frac{S_{ad}^2}{S_y^2} < F_t \tag{5}$$

where $S_{ad}^2 = \dfrac{1}{f_{ad}}\sum\limits_{u=1}^{n}(y - \bar{y}_u)^2$ – adequacy variance; $f_{ad} = n - k - 1$ – number of degrees of freedom of the adequacy variance in case of the number of factors equal to k; $f_y = n(m-1)$ – number of degrees of freedom of the repeatability variance.

The next step was to assess the significance of the coefficients in the regression equation with the use of Student's test. The criterion of the regression equation coefficient's significance was formulated as follows:

$$|b_i| \geq t_t \cdot \frac{S_y}{\sqrt{n}} \tag{6}$$

where t_t – table value of Student's criterion at a significance level of 5%.

The correlation analysis of the data under consideration was aimed at establishing the presence of a relationship between the factors.

The coefficient of the correlation between the values x and y was determined as follows:

$$r_{xy} = \frac{K_{xy}}{S_x S_y} \tag{7}$$

where S_x, S_y – mean deviations of the respective values; K_{xy} – covariance.

If the correlation coefficient is equal to zero, the values are uncorrelated, in case the correlation coefficient is above 0.7, the correlation is strong, at 0.3–0.7 – moderate, at correlation coefficient values below 0.3 – weak.

The root-mean-square error of the correlation coefficient was determined with the use of the following expression:

$$S_r = \sqrt{\frac{1 - r_{xy}^2}{n - 2}} \,. \tag{8}$$

The correlation between the parameters is significant provided that the calculated value of Student’s criterion exceeds its table value. That is:

$$t_r = \frac{r_{xy}}{S_r} \geq t_t \cdot \tag{9}$$

The laboratory investigations were carried out with the following values of the main operating conditions of the experimental unit and the design parameters of the fertiliser spreading tool with a tilted axis:

- rotation frequency of the disc in the fertiliser spreading tool with a tilted axis: 600 rpm, 800 and 1,000 rpm;
- angle of the disc’s inclination with respect to the horizontal plane: 0°, 10°, 20°, 30°;
- diameter of the disc – 650 mm;
- fertiliser seeding radius – 150 mm.

The non-uniformity in the distribution of the mineral fertiliser was measured by the coefficient of variation:

$$v = \frac{100\sigma}{\bar{M}} \,, \tag{10}$$

where σ – mean deviation; M_i – mass of the fertiliser particles in the i^{th} tray; k – number of trays.

RESULTS AND DISCUSSION

In accordance with the results of the experimental investigations, the effect of the rotation frequency of the disc in the fertiliser spreading tool with a tilted axis on the pattern of distribution in the spreading of ammonium saltpetre along the line of its placing has been established and the graphic relations presented in Fig. 4 have been plotted. For that purpose, two fixed disc rotation frequencies have been selected - 600 and 800 rpm. Such a selection is justified by the following facts. Rotation frequencies below 600 rpm cannot provide the mineral fertiliser particles that slip off the vane on the spreading disc with sufficient departure velocities. Consequently, the distances flown by the particles until they touch the ground are automatically shorter in this case. On the other hand, when the spreading disc rotates with a frequency exceeding 800 rpm, the processes of the fertiliser spreading tool vane accelerating the ammonium saltpetre granules and the granules falling on the ground result in the break-up of the granules, which is unacceptable in terms of the agronomical requirements to the top dressing and also, in case the break-up happens on the disc, considerably reduces the distance flown by the broken saltpetre granules. Hence, the experimental investigations have been carried out with the use of the two fixed rotation frequencies of the experimental fertiliser spreading tool with a tilted axis, one of them - the minimum acceptable one and the second - the maximum acceptable one.

In the experimental investigations, it has been established that the horizontal position of the spreading disc rotating with a frequency of 600 rpm stipulates the effective range of spreading ammonium saltpetre along the line of its placing to reach the 30th tray inclusive (15 m), the maximum fraction of the discharged fertiliser (7.2%) being seeded into the 12th tray (6 m). Increasing the rotation frequency of the disc in the fertiliser spreading tool with a tilted axis to 800 rpm results in the increase of the length of the effective ammonium saltpetre spreading area to the 35th tray inclusive (17.5 m). In this case, the maximum fraction of the fertiliser (6.3%) is seeded into the 15th tray (7.5 m).

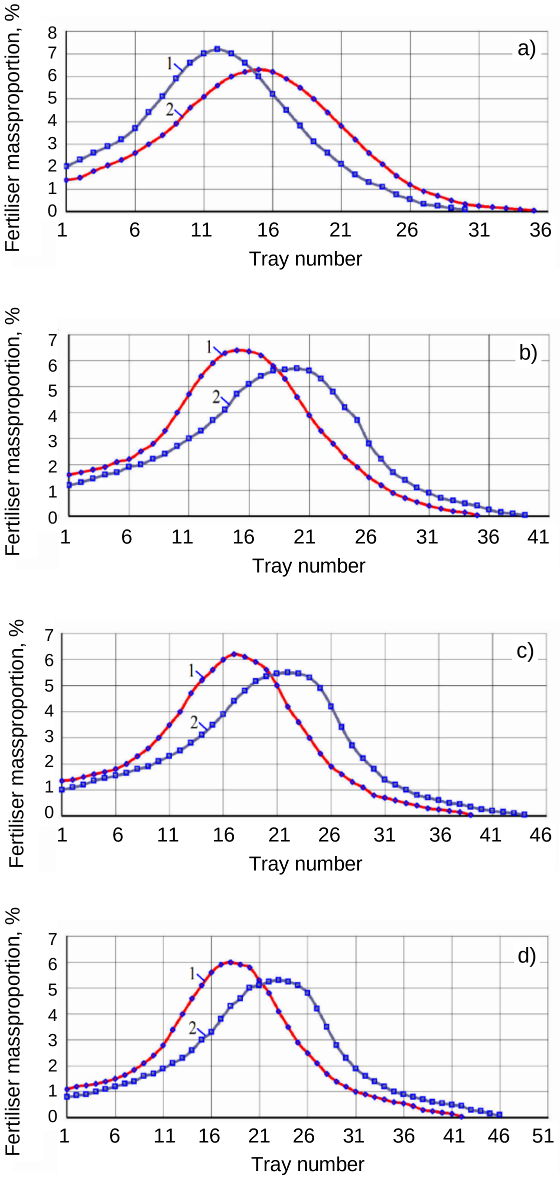

Figure 4. Relation between distribution of ammonium saltpetre spread by fertiliser spreading tool with tilted axis along line of its placing in trays and disc rotation frequency: a, b, c, d – disc’s angles of inclination to horizontal plane, respectively: 0°, 10°, 20°, 30°; 1, 2 – disc’s rotation frequency, respectively: 600 and 800 rpm.

Hence, when the disc in the fertiliser spreading tool is in the horizontal position, increasing its rotation frequency from 600 to 800 rpm results in the rise of the effective ammonium saltpetre spreading range by 16.7%, also the distance from the fertiliser spreading tool to the tray, into which the maximum mass dose of fertiliser is seeded rises by 25%, while the maximum mass fraction of fertiliser seeded into one tray decreases by a factor of 1.14.

Similar patterns with regard to the effect of the rotation frequency of the disc in the fertiliser spreading tool with a tilted axis on the distribution of the spread ammonium saltpetre along the line of its placing are also observed in case of an increase in the disc's angle of inclination with respect to horizontal plane to 30° (Fig. 5). As is seen in the presented graphic relations, the curve 2 representing the ammonium saltpetre distribution pattern at a disc rotation frequency of 800 rpm is significantly shifted to the right as compared to the curve 1 corresponding to the ammonium saltpetre distribution pattern at a disc rotation frequency of 600 rpm. Hence, it is appropriate to analyse the effect of the changes in the angle, at which the disc of the fertiliser spreading tool with a tilted axis is set with respect to the horizontal plane, on the distribution characteristics incidental to the spreading of ammonium saltpetre into the trays along the line of its placing.

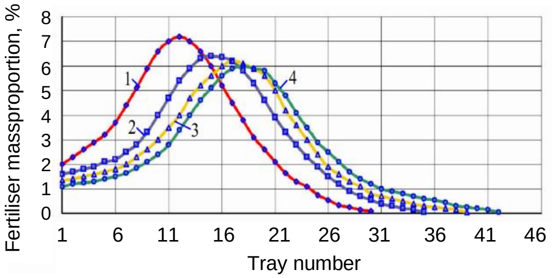

Figure 5. Relation between distribution of ammonium saltpetre spread by fertiliser spreading tool with tilted axis along line of its placing in trays at disc rotation frequency of 600 rpm and angle of disc's inclination with respect to horizontal plane: 1, 2, 3, 4 – disc's angle of inclination with respect to horizontal plane is equal to, respectively: 0°, 10°, 20°, 30°.

When the spreading disc in the fertiliser spreading tool with a tilted axis rotates with a frequency equal to 600 rpm and is installed at an angle of 10° to the horizontal plane (Fig. 5), ammonium saltpetre is effectively spread within the area up to the 35th tray (18.5 m) inclusive, while the maximum fraction of the fertiliser (6.4%) is seeded into the 15th tray (7.5 m). In the case of setting the disc at an angle of 20° to the horizontal plane, the effective spreading of ammonium saltpetre is performed within the area up to the 39th tray (19.5 m) inclusive and the maximum mass fraction of the fertiliser (6.2%) is seeded into the 17th tray (8.5 m). Increasing the angle of the disc's inclination with respect to the horizontal plane to 30° results in the increase of the effective ammonium saltpetre spreading range to the 42nd tray (21 m) inclusive, the maximum mass fraction of the fertiliser (6%) being seeded into the 18th tray (9 m).

Thus, it has been established that, in case the disc in the fertiliser spreading tool with a tilted axis rotates with a frequency of 600 rpm, the change in the setting angle of the disc in the said tool from 0° to:

– 10° results in the increase of the effective ammonium saltpetre spreading range by 16.7% and the distance from the fertiliser spreading tool with a tilted axis to the tray with the maximum mass fraction of the seeded fertiliser - by 25%, while the above-mentioned mass fraction of ammonium saltpetre decreases by a factor of 1.13;

– 20° results in the increase of the effective ammonium saltpetre spreading range by 30% and the distance from the fertiliser spreading tool with a tilted axis to the tray with the maximum mass fraction of the seeded fertiliser - by 41.7%, while the above-mentioned mass fraction of ammonium saltpetre decreases by a factor of 1.16;

– 30° results in the increase of the effective ammonium saltpetre spreading range by 40% and the distance from the fertiliser spreading tool with a tilted axis to the tray with the maximum mass fraction of the seeded fertiliser - by 50%, while the above-mentioned mass fraction of ammonium saltpetre decreases by a factor of 1.2.

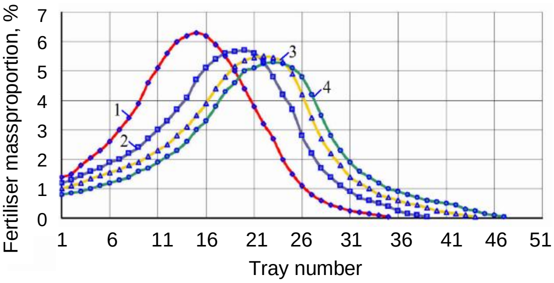

Figure 6. Relation between distribution of ammonium saltpetre spread by fertiliser spreading tool with tilted axis along line of its placing in trays at disc rotation frequency of 800 rpm and angle of disc’s inclination with respect to horizontal plane: 1, 2, 3, 4 – disc’s angle of inclination with respect to horizontal plane, respectively: 0°, 10°, 20°, 30°.

When the disc in the fertiliser spreading tool with a tilted axis rotates with a frequency equal to 800 rpm and is installed at an angle of 10° to the horizontal plane (Fig. 6), ammonium saltpetre is effectively spread within the area up to the 39th tray (19.5 m) inclusive, while the maximum mass fraction of the fertiliser (5.7%) is seeded into the 20th tray (10 m). In the case, when the disc is set at an angle of 20° to the horizontal plane, the effective spreading of ammonium saltpetre is performed within the area up to the 44th tray (22 m) inclusive, the maximum mass fraction of the fertiliser (5.5%) is seeded into the 22nd tray (11 m). Increasing the angle of the disc’s inclination with respect to the horizontal plane to 30° results in the increase of the effective ammonium saltpetre spreading range to the 47th tray (23.5 m) inclusive, the maximum mass fraction of the fertiliser (5.3%) being seeded into the 23rd tray (11.5 m).

It has been established that, in case the disc in the fertiliser spreading tool with a tilted axis rotates with a frequency of 800 rpm, the change in the disc setting angle from 0° to:

– 10° results in the increase of the effective ammonium saltpetre spreading range by 11.4% and the distance from the fertiliser spreading tool with a tilted axis to the tray with the maximum mass fraction of the seeded fertiliser - by 33.3%, while the above-mentioned mass fraction of ammonium saltpetre decreases by a factor of 1.1;

– 20° results in the increase of the effective ammonium saltpetre spreading range by 25.7% and the distance from the fertiliser spreading tool with a tilted axis to the tray with the maximum mass fraction of the seeded fertiliser - by 46.7%, while the above-mentioned mass fraction of ammonium saltpetre decreases by a factor of 1.15;

– 30° results in the increase of the effective ammonium saltpetre spreading range by 34.3% and the distance from the fertiliser spreading tool with a tilted axis to the tray with the maximum mass fraction of the seeded fertiliser - by 53.3%, while the above-mentioned mass fraction of ammonium saltpetre decreases by a factor of 1.19.

The results of the research into the distribution of the ammonium saltpetre spread by the fertiliser spreading tool with a tilted axis along the line of its placing over the trays prove that increasing the disc rotation frequency from 600 to 800 rpm results, within the whole range of values used in the research for the angle, at which the disc in the fertiliser spreading tool with a tilted axis is set with respect to the horizontal plane, in the rise of both the effective ammonium saltpetre spreading range and the distance from the fertiliser spreading tool with a tilted axis to the tray with the maximum mass fraction of the seeded fertiliser. For example, the increase in the effective ammonium saltpetre spreading range is in case of an angle of 0° – 16.7%, 10° – 11.4%, 20° – 12.8%, 30° – 11.9%, while the distance from the fertiliser spreading tool with a tilted axis to the tray with the maximum mass fraction of the seeded fertiliser increases at the following angles of inclination by: 0° – 25.0%, 10° – 33.3%, 20° – 29.4%, 30° – 27.8%.

In case of the disc in the fertiliser spreading tool with a tilted axis rotating at a constant frequency, in all kinematic modes of operation of the tool, increasing the tool disc setting angle to the horizontal plane results in the improved indicators of the distribution of ammonium saltpetre over the trays along the line of its placing. In particular, when the disc in the fertiliser spreading tool with a tilted axis rotates at a frequency of 800 rpm, the rise in the effective ammonium saltpetre spreading range due to the increase in the angle of inclination of the disc in the fertiliser spreading tool with a tilted axis, as compared to its horizontal position, to 10° has a value of 11.4%, 20° – 25.7%, 30° – 34.3%, the respective rise in the distance from the fertiliser spreading tool with a tilted axis to the tray with the maximum mass fraction of the seeded fertiliser is equal to: 10° – 33.3%, 20° – 46.7%, 30° – 53.3%.

The above-stated proves that the parameter of the rotation speed of the disc in the fertiliser spreading tool with a tilted axis can be used for controlling the ammonium saltpetre spreading range also in case, when the disc is set at an angle to the horizontal plane, the same as it is done in state-of-the-art fertiliser placing machines with horizontally set discs in their fertiliser spreading tools.

Increasing the angle of the disc’s inclination with respect to the horizontal plane results in the rise of the indicators representing the ammonium saltpetre spreading range and the working width of the mineral fertiliser placing machine. At the same time, the rate of increase of the above-mentioned indicators is at its peak, when the angle of the disc’s inclination with respect to the horizontal plane rises from 0° to 10°. After that, it declines, as the angle grows up to 30°.

The fertiliser spreading tool with a tilted axis ensures spreading ammonium saltpetre along the line of its placing to greater distances, than in case of the fertiliser spreading tool with a vertical rotation axis. That proves the hypothesis of a possibility to increase the working width of mineral fertiliser placing machines by means of equipping such machines with fertiliser spreading tools with tilted axes.

The regression equations that describe the effect of the disc’s rotation frequency and its angle of inclination with respect to the horizontal plane on the non-uniformity in the distribution of ammonium saltpetre along the line of its placing appear as follows:

$$y = -101.4 - 0.0574n + 4.6034\alpha - 0.0256\alpha^2 \cdot \qquad (11)$$

It has been established by analysing the relation (11) that the non-uniformity in the placing of ammonium saltpetre along the line of its departure from the fertiliser spreading tool with a tilted axis is affected most of all by the value of the disc rotation frequency.

In a similar way, the regression equations that describe the distribution patterns for the spreading of granulated superphosphate along the line of its placing by the fertiliser spreading tool with a tilted axis have been obtained:

$$y = 18.2533 + 0.0992n - 0.3063\alpha - 0.000092n^2 + 0.0037\alpha^2 + 0.00011n\alpha. \quad (12)$$

It has been established by analysing the obtained regression (12) that the most significant effect on the coefficient of variation of the granulated superphosphate distribution along the line of its placing is, again, produced by the rotation frequency of the disc in the fertiliser spreading tool with a tilted axis.

As a result of implementing the two-factor experiment, the following relation between the coefficient of variation of the nitroammophoska distribution along the line of its placing, on the one hand, and the angle of inclination to the horizontal plane of the disc in the fertiliser spreading tool with a tilted axis and its rotation frequency, on the other hand, has been obtained:

$$y = 80.46 - 0.0312n - 0.3825\alpha - 0.000037n^2 + 0.0018\alpha^2 + 0.00046n\alpha. \quad (13)$$

In a similar way to the results of the analysis of the expressions (11) and (12), it has been established that the most important factor with regard to the effect on the non-uniformity in the spreading of nitroammophoska along the line of its departure from the fertiliser spreading tool with a tilted axis is the disc rotation frequency.

The obtained results provide for selecting the optimum parameters and modes of operation for the fertiliser spreading tool with a tilted axis, when it is operated as part of a fertiliser placing machine. However, it is to be taken into consideration that, in case of a machine equipped with two fertiliser spreading tools with tilted axes, where the fertiliser is spread from each of the tools not in the form of a separate strip, but in the form of a fan with an angle at centre of circa 90°, it is necessary to carry out field experiments, in which a tractor with the fertiliser placing machine will pass the control plot, where the trays for the collection of the seeded mineral fertilisers are placed following the standard procedure, in order to obtain the data on the non-uniformity in the spreading of fertilisers within the working width of the machine.

CONCLUSIONS

1. Increasing the frequency of the disc rotation in the fertiliser spreading tool with a tilted axis from 600 to 800 rpm results in the growth of the effective range of spreading mineral fertilisers along the line of their placing at a level of 10.5 m. Increasing the angle of the disc's inclination with respect to the horizontal plane to 20º results in a rise in the effective fertiliser spreading range at a level of the 48th tray (24 m) inclusive as well as an increase in the distance from the fertiliser spreading tool with a tilted axis to the tray with the maximum mass fraction of the seeded fertiliser (5.1%) to the 24th tray (12 m).

2. If the disc in the fertiliser spreading tool with a tilted axis rotates at a constant frequency, in all kinematic modes of the tool operation, increasing the tool disc setting angle with respect to the horizontal plane results in an improvement of the indicators that represent the distribution of the mineral fertilisers over the trays along the line of their placing.

3. The mineral fertiliser spreading range can be controlled by changing the rotation frequency of the disc in the fertiliser spreading tool with a tilted axis, when the disc is set at some angle to the horizontal plane, similarly to how it is done in state-of-the-art fertiliser placing machines, where the disc in the centrifugal tool is in the horizontal position.

4. Increasing the disc's angle of inclination to the horizontal plane results in the growth of the indicators that represent the range of spreading mineral fertilisers and the working width of the machine for their placing. In this growth, the increase in the above-mentioned indicators is most intensive, when the angle of the disc's inclination to the horizontal plane rises from 0° to 10°, but it declines during the further rise of the angle up to 30°.

5. The factor that has the most significant effect on the coefficient of variation incidental to the distribution of mineral fertilisers along the line of their placing is the rotation frequency of the disc in the fertiliser spreading tool with a tilted axis.

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Variation of chickpea nodulation in a Mediterranean agroecosystem: relationship with soil characteristics and thresholds for significant contribution to plant growth

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Abstract. This study was designed to investigate the influence of some environmental constraints on chickpea (*Cicer arietinum* L.) growth and nodulation in a reference agroecosystem. This multi-local field experiment, realized in the agroecosystem of Chlef in northern Algeria, involved 24 sites where the local well-adapted genotype Ain temouchent is grown. Determination of soil properties allowed the identification of three clusters of sites. Plant biomass (SDW) varied significantly among sites from 6.7 to 39.4 g SDW plant^{-1} and was highly correlated with nodule biomass (NDW). The slope of the regression function between NDW and SDW, defined as the efficiency in use of the rhizobial symbiosis (EURS) inside clusters ranged from 67 to 200 g SDW g^{-1} NDW. Analysis of the EURS highlighted the existence of a low nodulation threshold of 0.1 g NDW plant^{-1} below which the contribution of nodulation to the host plant growth was not significant and a high threshold above which the nodule biomass increase was not linked with an increase in shoot biomass. Thus, the significant regression of shoot growth as a function of nodulation ranged from 0.10 to 0.35 g NDW plant^{-1} ($R^2 = 0.54$, $P < 0.001$). Moreover, nodule biomass was positively correlated with soil Olsen-P, more particularly in cluster A ($R^2 = 0.50$, $P < 0.05$) and B ($R^2 = 0.61$, $P < 0.01$). This approach proved to be a rapid and efficient way to identify the major factors affecting nodulation in order to develop strategies to optimize nodule contribution to chickpea growth and yield.

Key words: chickpea, efficiency in use of the rhizobial symbiosis, nodulation, phosphorus, plant-soil interaction, symbiotic nitrogen fixation.

INTRODUCTION

Legumes play a crucial role in Mediterranean farms sustainability through their agro-ecological services. Moreover, in many households they are often the main source of protein and the central core of healthy diet. Chickpea is one of the three major legumes in the world, the second most grown in Algeria after dry faba bean (Abdelguerfi-Laouar et al., 2001b; Rawal & Navarro, 2019) and an important crop for low input rainfed agroecosystems due to its ability to grow on poor and marginal soils. Chickpea is generally cultivated in rotation with cereals for its seeds that have high nutritional value, for its stubbles that provide a good forage and also as break crop and a source of nitrogen to the farming system (Boughrarou, 1992; Herridge et al., 1995; Schwenke et al., 1998; Rawal & Navarro, 2019), nitrogen being the most limiting element of crop growth and yield after water in semiarid areas.

Indeed, chickpea like other legumes have the ability to biologically fix atmospheric nitrogen (SNF) through the establishment of a symbiotic association with specific soil-borne rhizobia species from the genera Mesorhizobium and Ensifer and the formation of root nodules (Dekkiche et al., 2017). Thus, chickpea can fix up to 81% of their requirements from atmospheric nitrogen, a large part of this fixed N is returned to the soil after harvest benefiting the successive crop (Schwenke et al., 1998).

However the success of chickpea cultivation and the expected benefit of its integration into the cropping system depends on an optimal level of nodulation and SNF, otherwise the plant will depend on root uptake, which will negatively affect the pool of N in the soil and lead to poor growth if the soil is already deficient in N (Schwenke et al., 1998; Lazali et al., 2016). Nodule formation and functioning are tightly controlled by the host plant to achieve optimal N nutrition with minimal energy cost (Voisin et al., 2010). However, at field level, nodulation can be affected by several external factors such as the native rhizobial strains in the soil, environmental variables, genotypes differences, farming practices, nutrient availability and soil characteristics (Evans, 1982; Ben Romdhane et al., 2007; Lazali et al., 2016, Dekkiche et al., 2017) and very little is known about what could be considered as an optimal level of nodulation and how to reach this level, moreover, discerning between whether low nodulation is a result or the main cause of low host plant growth remains difficult (Robson et al., 1981; Israel, 1987).

Among these factors, phosphorus deficiency, which could be one of the main causes of low yields of chickpea and legumes in general due to its highly limiting effect on nodule formation and functioning (N_2-fixation) (Robson et al., 1981; Israel, 1987; Ribet & Drevon, 1996; Benlahrech et al., 2018). According to Vance (2001), most of the world's arable land is p-deficient; in Algeria due to the calcareous nature of the soil, soluble P is precipitated (Djili et al., 1999; Tunesi et al., 1999). As a result of this soil deficiency and the high needs of symbiosis, nodule biomass and thereafter SNF are often strongly correlated with soil available P (Lazali et al., 2016).

Nodulation and SNF can be assessed by different methods such as the direct measurement of the nitrogen deriving from biological fixation, nitrogenase activity in the nodule, counting and/or measuring the dry weight of nodules; some of this methods are very costly and difficult to set up for large multilocal field trials. However, the measurement of nodule dry weight (NDW) represent a rapid and efficient way to assess SNF contribution to plant growth (Drevon et al., 2015; Lazali et al., 2016). Several authors proved that NDW augmentation is positively correlated with SNF and plant growth (Evans, 1982; Rao et al., 2002; Voisin et al., 2003; Elias, 2009; Lazali et al., 2016; Benlahrech et al., 2018; Kaci et al., 2018). Low nodulation is often considered as the major limiting factor of chickpea growth and yield in Mediterranean areas (Ben Romdhane et al., 2007). Thus the need arises for a proper analysis of chickpea nodulation in order to determine the major factors affecting nodulation and establish thresholds of optimal nodulation since low NDW was proved to have no significant positive effect on the host plant (Drevon et al., 2015).

In this context, the major objective of this work is to assess the levels of nodulation of winter sown chickpea grown under rainfed conditions in the region of Chlef in the north of Algeria and emphasize the quantitative relationship between plant growth and nodulation in interaction with contrasted soil conditions.

MATERIALS AND METHODS

Study area and diagnostic Sites

The nodular diagnosis was conducted during the 2017–2018 growing season in the semiarid region of Chlef located in the north west of Algeria, in an area of 40 by 30 km extending from 36°26'5"N, 1°6'15"E, altitude 193 m at the northwestern limit, to 36°5'20" N, 1°27'5"E, altitude 517 m at the southeastern limit (Fig. 1). The area was selected for its importance in winter rainfed chickpea production and is characterized by cold wet winter and hot dry summer, with irregular rainfall (603 mm during the growth 2017–2018 season). The 24 sites were chosen on the basis of a previous farm survey to cover a large diversity in agro-ecological conditions. In each location the diagnostic site consisted in a 20 by 20 m homogeneous area.

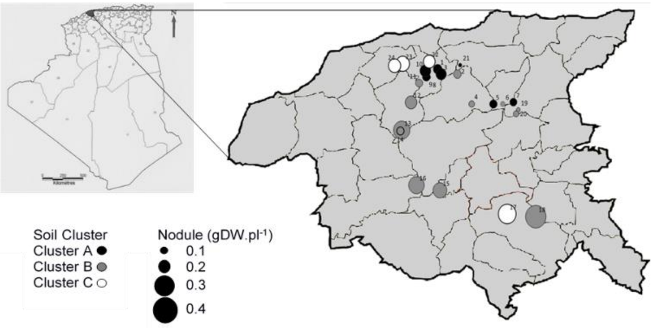

Figure 1. Map of the study area showing the locations of the sampled sites and the spatial variation of nodulation, the size of the dots corresponds to the mean NDW of each site and the color of the dot indicates to which cluster the site belongs.

Plant material and growth condition

In each site chickpea was grown according to conventional agricultural practices, as recommended by local authorities. Weed, pests and diseases were controlled by appropriate pesticides. All sites were rainfed without irrigation, and sown at 30–35 seeds per m^2 without rhizobial inoculation. Before sowing, the topsoil was mechanically prepared using a disc plough and a harrow. Sowing was performed using a seeder followed by a smooth roller, the distances between rows and between plants on the row were 30 and 10 cm, respectively. This experiment was carried out with the well adapted commonly grown cultivar Aïn Témouchent, a vigorous, short-cycle chickpea genotype with erect growth, good culinary and nutritional qualities of grains (Boughrarou, 1992; Abdelguerfi-Laouar et al., 2001a, 2001b).

Soil sampling and analysis

At sowing four random soil samples from each site were collected at 0–30 cm with an auger and conserved at 4 °C before analysis in the laboratory of soil science (ENSA–Algeria). Soil samples were crushed and sieved through a 2 mm sieve to obtain the fine soil fraction before measuring soil pH in distilled water and granulometry by sedimentation with the pipette method (Robinson, 1922), the obtained values were then used to assign each site with a textural class based on the soil textural triangle. Total nitrogen, $CaCO_3$ content, available phosphorus, total organic carbon were measured with the methods of Kjeldahl (1883), Bernard Calcimeter, Olsen (Olsen & Dean, 1965) and Anne (1945), respectively.

Assessment of growth, nodulation and mapping

At full flowering stage which corresponds to period with maximal nodule dry weight (NDW), 20 plants per site were randomly harvested from the middle rows of each site by carefully digging around each plant. Shoots were separated from roots at the cotyledonary node. Nodules, if any, were gently separated from roots and the surrounding soil, washed with water and stored at 4 °C for further measurements. All the samples were oven dried for 48 h at 70 °C before being weighted. Mean NDW for each site were used in QGIS software (3.4.4) to generate the map in Fig. 1. Linear regression models were computed between nodules and shoots dry weights (SDW = a.NDW + b). The slopes of these models (a) were retained as estimators of the efficiency in use of the rhizobial symbiosis (EURS) for shoot biomass productions with the atmospheric N_2 fixed by the nodules i.e the ratio of N_2-dependent growth/nodule mass, while the intercepts (b) are shoot biomass productions without nodules i.e with soil nitrogen only (Zaman-Allah et al., 2007; Drevon et al., 2015).

Statistical analysis

The statistical analyzes were performed with R software version 3.5.2 (R Core team, 2018). Means, standard errors (s.e.) and standard deviations (s.d.) were computed with 20 plants and 4 soil samples by site. Analyzes of variance were performed and means among sites were compared using Tukey's multiple comparison tests at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

Hierarchical clustering of sites was performed using the physico-chemical properties of sites with Ward method. The relationships between nodule biomass, shoot biomass and available soil phosphorus (Olsen-P) were assessed by regression analysis. The relationship between nodulation and shoot biomass was also observed with locally weighted scatterplot smoothing (loess method). Bar graphs and scatter plots were also performed using R v 3.5.2.

RESULTS

Soil physico-chemical properties

Table 1 summarizes some physico-chemical properties of the sites, data show highly significant differences between the sites for all parameters. Overall, the diagnostic sites had a basic pH ranging from 8.09 ± 0.15 to 8.56 ± 0.04 corresponding to site 16 and 19 respectively, total organic matter varied from 1.06 ± 0.01 to 2,47 ± 0.52 corresponding to sites 20 and 13 respectively, moreover the total $CaCO_3$ content ranged from 4.72 ± 0.14 in site 23 to 29.47 ± 6.12 in site 14, the highest values of Olsen-P were observed in sites 24 and 13 with 30.47 ± 2.27 and 32.19 ± 3.03 respectively and the lowest is site 19 with 5.02 ± 0.22, the total nitrogen ranged from 0.93 ± 0.06 in site 05 to 2.40 ± 0.30 in site 11.

Table 1. Physico-chemical properties of the 24 site of the diagnostic. Data are means ± standard deviation of 4 soil samples collected before sowing

Site	Clay	Silt	Sand	OM (%)	Ph	$CaCO_3$ (%)	Olsen-P (mg kg^{-1})	Total N (g kg^{-1})
	***	***	***	***	***	***	***	***
S01	41 ± 0.60	34 ± 1.28	24 ± 1.04	1.4 ± 0.33	8.4 ± 0.32	16.64 ± 0.55	20.32 ± 1.65	1.63 ± 0.15
S02	37 ± 0.49	38 ± 0.95	25 ± 1.08	1.2 ± 0.01	8.3 ± 0.00	21.60 ± 0.96	15.95 ± 1.17	1.43 ± 0.23
S03	43 ± 1.56	36 ± 0.26	20 ± 1.80	1.3 ± 0.04	8.3 ± 0.07	17.91 ± 0.48	23.14 ± 1.10	1.87 ± 0.21
S04	31 ± 0.91	39 ± 0.56	30 ± 1.46	1.1 ± 0.10	8.4 ± 0.15	24.88 ± 0.60	19.28 ± 2.28	1.17 ± 0.25
S05	38 ± 0.70	35 ± 0.85	27 ± 0.95	1.2 ± 0.05	8.4 ± 0.08	16.32 ± 0.42	14.02 ± 0.57	0.93 ± 0.06
S06	30 ± 0.31	32 ± 0.78	38 ± 0.47	1.2 ± 0.03	8.3 ± 0.04	15.60 ± 0.24	6.89 ± 0.71	1.00 ± 0.26
S07	36 ± 0.06	33 ± 0.06	31 ± 0.00	1.3 ± 0.05	8.4 ± 0.03	13.12 ± 0.73	7.80 ± 1.90	1.40 ± 0.30
S08	44 ± 0.40	36 ± 1.25	20 ± 0.87	1.4 ± 0.07	8.3 ± 0.01	12.32 ± 0.37	14.11 ± 3.35	1.53 ± 0.21
S09	40 ± 1.72	37 ± 0.72	22 ± 1.11	1.2 ± 0.03	8.3 ± 0.06	14.56 ± 2.18	13.40 ± 1.37	1.67 ± 0.25
S10	39 ± 1.37	35 ± 0.31	26 ± 1.37	1.1 ± 0.07	8.3 ± 0.01	11.28 ± 2.44	21.17 ± 4.62	1.83 ± 0.06
S11	32 ± 0.26	36 ± 0.83	32 ± 0.85	2.1 ± 0.22	8.4 ± 0.07	12.32 ± 0.14	21.48 ± 0.94	2.40 ± 0.30
S12	36 ± 0.70	41 ± 1.63	23 ± 2.32	1.2 ± 0.05	8.3 ± 0.01	21.12 ± 0.83	18.90 ± 1.77	1.37 ± 0.35
S13	34 ± 0.15	31 ± 0.40	34 ± 0.49	2.5 ± 0.52	8.4 ± 0.12	20.88 ± 0.48	32.19 ± 3.03	2.30 ± 0.10
S14	32 ± 1.12	35 ± 1.57	34 ± 2.68	1.5 ± 0.63	8.4 ± 0.02	29.47 ± 6.12	10.51 ± 3.96	1.27 ± 0.12
S15	31 ± 1.51	40 ± 0.53	29 ± 1.35	1.4 ± 0.09	8.5 ± 0.08	24.80 ± 3.50	26.07 ± 2.59	1.93 ± 0.32
S16	33 ± 0.44	37 ± 1.35	30 ± 0.95	1.3 ± 0.07	8.1 ± 0.15	24.16 ± 0.55	22.62 ± 0.54	2.27 ± 0.31
S17	35 ± 0.67	36 ± 2.57	29 ± 3.12	1.7 ± 0.14	8.4 ± 0.02	8.32 ± 0.28	28.92 ± 2.66	2.30 ± 0.20
S18	30 ± 0.36	34 ± 0.45	35 ± 0.12	1.6 ± 0.06	8.1 ± 0.08	20.16 ± 0.96	24.10 ± 2.19	1.96 ± 0.13
S19	31 ± 1.75	33 ± 0.70	36 ± 1.31	1.1 ± 0.03	8.6 ± 0.04	20.72 ± 0.50	5.02 ± 0.22	1.33 ± 0.50
S20	33 ± 0.29	39 ± 0.95	28 ± 0.86	1.1 ± 0.01	8.2 ± 0.08	26.63 ± 0.39	15.76 ± 2.96	1.47 ± 0.23
S21	42 ± 0.84	38 ± 0.42	19 ± 1.15	1.3 ± 0.09	8.1 ± 0.14	12.64 ± 0.60	6.38 ± 0.65	1.77 ± 0.31
S22	36 ± 0.17	40 ± 2.89	24 ± 2.79	1.4 ± 0.02	8.4 ± 0.13	5.52 ± 0.00	23.13 ± 0.88	2.20 ± 0.26
S23	35 ± 1.13	39 ± 0.55	26 ± 1.64	1.3 ± 0.05	8.3 ± 0.07	4.72 ± 0.14	25.19 ± 3.14	1.93 ± 0.06
S24	37 ± 1.30	37 ± 0.38	26 ± 1.42	1.4 ± 0.06	8.3 ± 0.11	8.41 ± 1.49	30.47 ± 3.27	1.43 ± 0.31

*** indicate significant difference at $p < 0.001$.

The highest values of clay (44%), silt (41%) and sand (36%) were found in sites 8, 12, and 19 respectively. Based on soil particle size, we determined sites textural class of each site according to the USDA texture triangle. Following this textural classes; the sites can be divided into 2 groups: clay sites which regroups sites 1, 3, 8, 9 and 21. This sites exhibit the highest percentage of clay (over 40%), the rest of the sites have a clay loam texture. Fig. 1 shows that all these clay sites are located in the north of the study area.

Clustering analysis allowed regrouping the sites into three clusters gathering between four to twelve sites. Table 2 shows the mean values of soil properties for each cluster. Clusters were significantly different in all parameters except $CaCO_3$ content, pH and total organic matter content. Cluster A regroups 8 sites (1, 3, 5, 7, 8, 9, 10 and 21) and contains all the clay sites. In this cluster mean total N and Olsen-P are also the lowest. Cluster B contains 12 sites (2, 4, 6, 11, 12, 13, 14, 15, 16, 18, 19 and 20) and show the highest $CaCO_3$ content, but medium levels of Olsen P. On the other hand, cluster C which is characterized by a low $CaCO_3$ content and the and highest P content contains 4 sites (17, 22, 23 and 24).

Shoot and nodule biomass

Significant differences in shoot and nodule biomass were observed between sites (Fig. 2), plants in site 8 had no nodules and low shoot biomass (7.9 ± 1.74 g DW plant^{-1}). Site 17 and 18 exhibited the highest nodulation (0.32 ± 0.02 and 0.38 ± 0.03 g DW plant^{-1} respectively). Overall, nodule biomass ranged from 0 to 0.38 g DW plant^{-1} and shoots biomass from 6.7 to 39.4 g DW plant^{-1} corresponding to sites 6 and 17 respectively. Moreover Fig. 1 indicates that all the sites in the north east of the study area suffered from low nodulation, while the sites in the north and south had average to high nodulation.

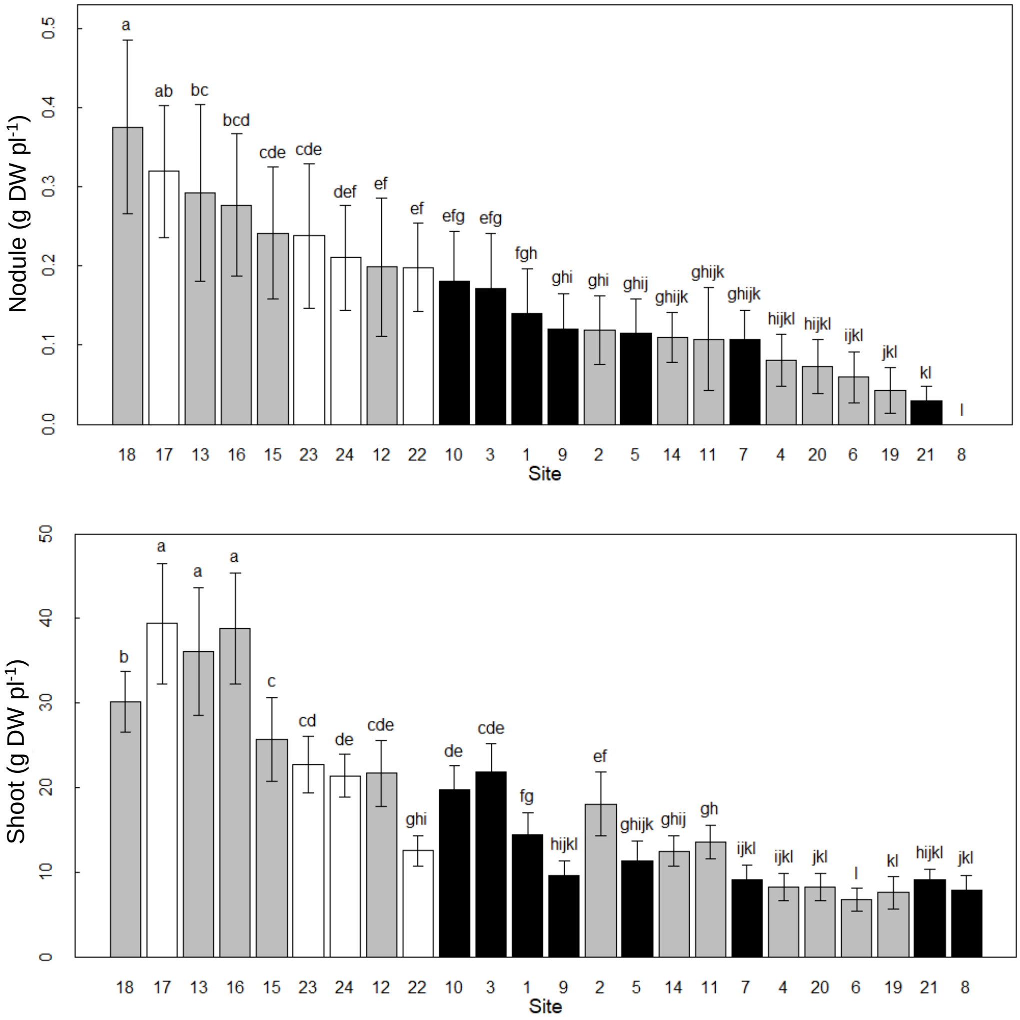

Figure 2. Nodule and shoot biomass of the Ain Témouchent genotype grown in 24 sites of Chlef. Values are means of 20 plants per site harvested at flowering stage. Vertical bars represent standard error. Black, grey and white color corresponds to cluster A, B and C respectively. Different letters indicate significant differences between sites at $P < 0.05$.

Nodulation and growth as a function of soil properties

As shown in Table 2, in cluster A who contains all the clay sites and the lowest values of total N and Olsen-P, shoot and nodule biomass were low. Cluster B who exhibited a high content of $CaCO_3$ and medium content of Olsen-P is characterized by an average shoot and nodule biomass production. On the other hand, cluster C which is characterized by a low $CaCO_3$ content and the highest P content exhibited the highest shoot and nodule biomass.

According to Fig. 3 a positive correlation between P augmentation and NDW was found in cluster A and B ($R^2 = 0.5$ at $P < 0.05$ and $R^2 = 0.61$ at $P < 0.01$ respectively). On the other hand, no significant correlation was found for cluster C which corresponds to the cluster with the highest P content in average (26.93 ± 3.80 mg kg^{-1}).

Table 2. Mean shoot biomass, nodule biomass, physical and chemical properties of the three soil clusters. Data are means ± standard deviation

	A	B	C
Sand (%) *	23.66 ± 3.92^b	31.08 ± 4.55^a	25.96 ± 2.73^b
Silt (%) *	35.75 ± 1.75^c	36.28 ± 3.29^{ab}	38.29 ± 2.38^a
Clay (%) ***	40.59 ± 2.70^a	32.64 ± 2.29^c	35.75 ± 1.19^b
OM (%) (ns)	1.28 ± 0.14	1.41 ± 0.45	1.44 ± 0.19
Total N (g.kg^{-1}) *	1.58 ± 0.34^b	1.66 ± 0.53^{ab}	1.97 ± 0.40^a
Olsen-P (mg kg^{-1})***	15.33 ± 6.13^b	18.23 ± 7.93^{ab}	26.93 ± 3.80^a
$CaCO_3$ (%)(ns)	14.35 ± 2.50	21.86 ± 4.86	6.74 ± 1.84
pH (ns)	8.31 ± 0.14	8.32 ± 0.16	8.37 ± 0.09
SDW (g) ***	12.71 ± 5.32^c	19.17 ± 11.83^b	23.65 ± 10.78^a
NDW (g) ***	0.11 ± 0.07^c	0.17 ± 0.13^b	0.24 ± 0.09^a

*, **, *** indicates significant difference at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively; ns: not significant ($P > 0.05$). Values followed by the same letter are not significantly different at $P < 0.05$.

Efficiency in use of the rhizobial symbiosis

In order to better observe how nodulation links with shoot biomass, the mean SDW of each site was plotted as a function of the mean NDW in each cluster and among all sites.

For the three clusters, as shown in Fig. 4, the SDW and NDW were significantly correlated regardless of cluster N and P contents, with the EURS ranging from 67 to 200 g SDW g^{-1} NDW corresponding to cluster A and C, respectively.

Among all sites (Fig. 5, A), results show a highly significant correlation between shoot and nodule biomasses ($R^2 = 0.81$, $P < 0.001$) with a linear model. On the other hand the loess curve shows three distinct parts, a first part where NDW augmentation is not accompanied by SDW augmentation, a second part that exhibits positive correlation between the two parameters and a third part where SDW is not affected or tends to decrease when NDW increases.

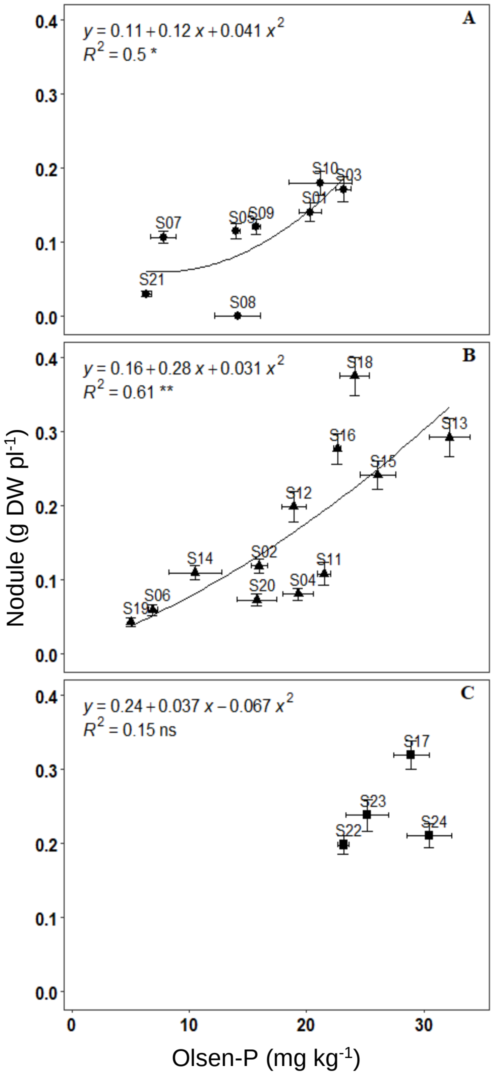

Figure 3. Nodule biomass as related to the concentration of Olsen-P in the sites of the three soil clusters (A, B and C). Each point represents the means calculated on 20 plants per site harvested at flowering stage and 4 soil samples collected before sowing. Vertical and horizontal bars represent standard errors. A curvilinear regression was fitted to the data. *, **, *** indicates significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

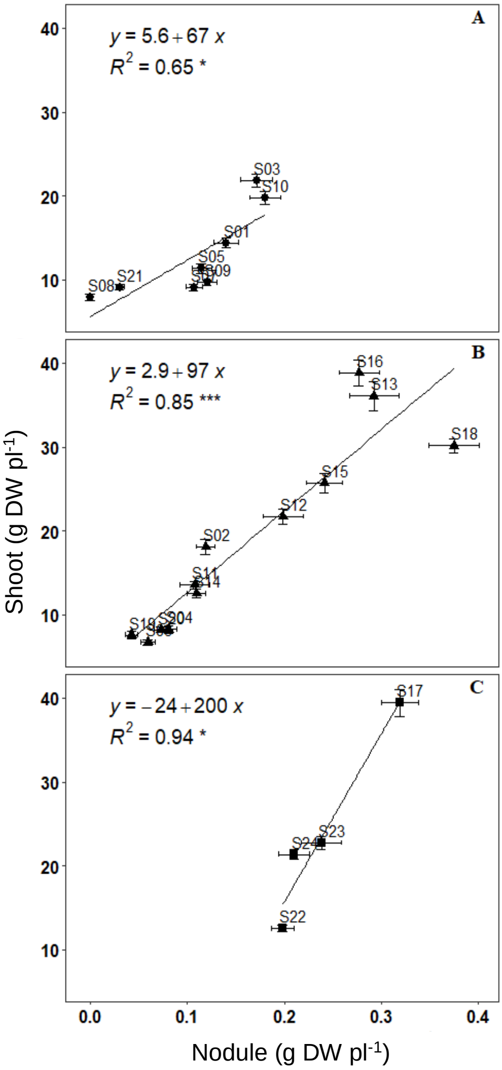

Figure 4. Shoot biomass as related to nodule biomass in the three soil clusters (A, B and C). Each point represents the means calculated on 20 plants per site harvested at flowering stage. Vertical and horizontal bars represent standard errors. A linear regression was fitted to the data. *, **, *** indicates significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

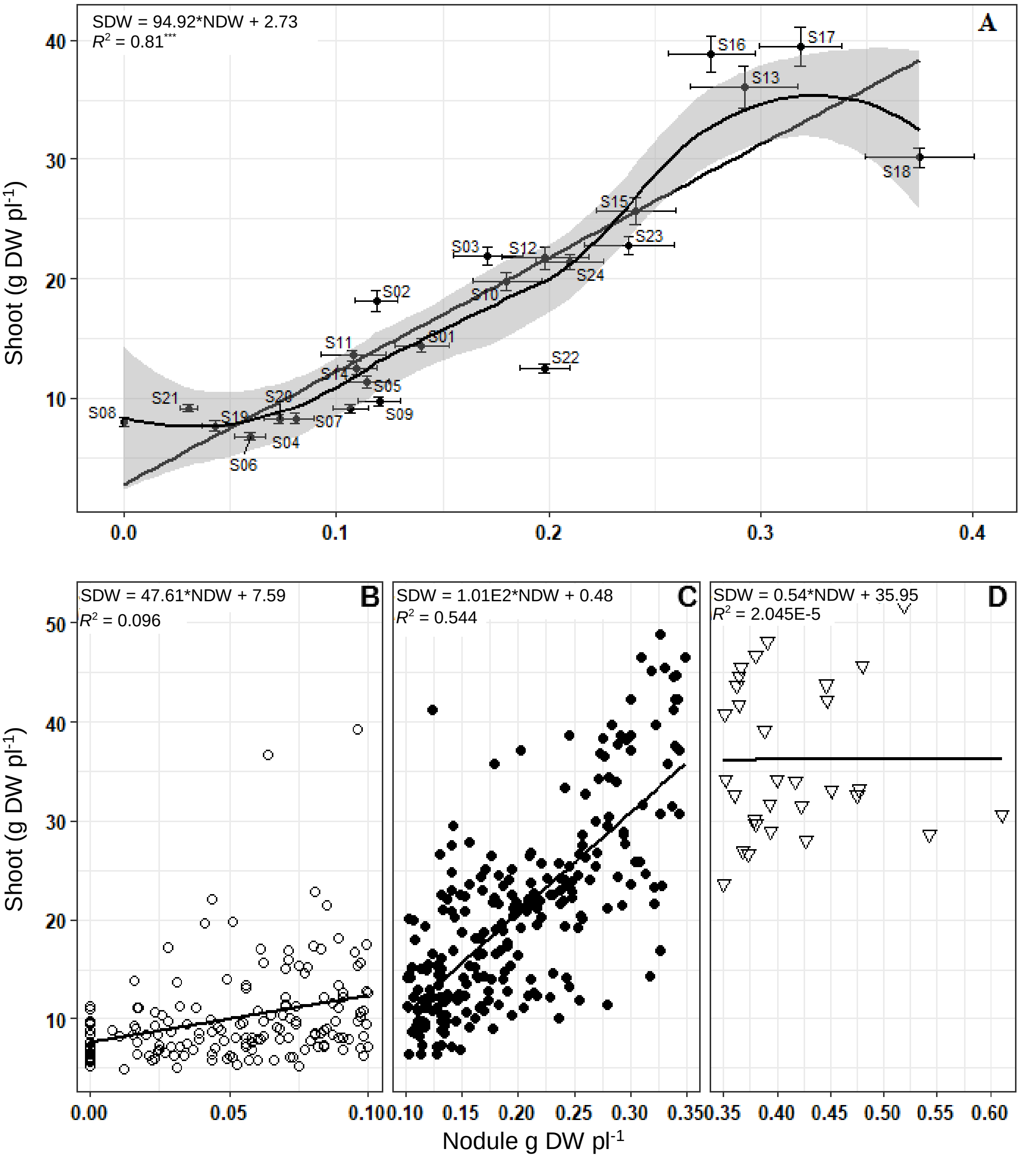

Figure 5. Efficiency in use of rhizobial symbiosis in the 24 site of the study (A); for plants with a NDW less than 0.1 g (B); plants with a NDW between 0.1 and 0.35 g (C) and plants with a NDW above 0.35 g (D). For (A) each point represents the means calculated on 20 plants per site harvested at flowering stage. Vertical and horizontal bars represent standard errors. A linear regression was fitted to the data in (A) and (C) and a loess regression was fitted to the data in (A). *, **, *** indicates significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

To investigate in more details to what extent the NDW was linked to shoot production, all the sampled plants were divided into three groups based on NDW: the first group of plants with nodulation below 0.1 g NDW plant^{-1}; the second with NDW between 0.10 and 0.35 g NDW plant^{-1}; the third with NDW above 0.35 g NDW plant^{-1}

(Fig. 5, B; 5, C & 5, D respectively) based on Drevon et al. (2015) work and the loess regression analysis. Results show no significant correlation between the two parameters for the first and third group (Fig. 5, B & 5, D respectively), on the other hand it shows a highly significant correlation for the second group (Fig. 5, C) ($R^2 = 0.54$ at $P < 0.001$).

DISCUSSION

Our study provided results at several scales: the scale of all the sites, the scale of more homogeneous subsets of sites (soil clusters), and the scale of some particular sites. At all sites scale, we observed a high variability of nodulation and plant growth for chickpea and the correlation between NDW and SDW among all sites was highly significant indicating that growth was dependent on the nitrogen fixed by nodules (except for site 08 which relied entirely on soil nitrogen to grow) in the study area. Indeed Voisin et al. (2003) showed that NDW augmentation was positively correlated with N fixation although Drevon et al. (2015) indicated the existence of a nodulation threshold for significant SNF contribution to the growth of the host plant. Our results in Fig. 2 are in accordance with this author findings because there is no significant differences in SDW for sites 8, 21, 19, 6, 20, 4 which nodulation ranged from 0 to 0.1 g NDW plant^{-1}, showing that below a certain NDW the contribution of nodulation to plant growth is not significant. Moreover our results indicate the existence of a highly significant correlation when NDW ranges from 0.1 g to 0.35 g (Fig. 5, C) proving that below a certain NDW threshold there is no effect of nodules on plant growth and above a certain threshold an increase in NDW does not have positive effect on the host plant. For the upper threshold, it is most likely that plant needs in N were satisfied and growth became limited by other factors which makes NDW augmentation unnecessary especially due to the high energy cost of nodule formation and functioning (Minchin & Pate, 1973; Ryle et al., 1979). As shown by Voisin et al. (2015) the efficiency of the Legume-Rhizobia symbiosis (N benefits for the plant against carbon cost of nodulation) decreases with the augmentation of nodulation, as well as the nodule specific activity (Evans, 1982).

Data also show that among all sites, the increase in nodulation was correlated with P augmentation and no significant inhibitory effect of soil N was observed, in fact the site with the highest SDW and NDW corresponded to site with highest total N. Our results are similar to those of Elias (2009) who indicated that N concentration in 0–30 cm had no significant negative effect on the nodulation of chickpea in Australia. It is well established that nitrogen in the root zone limits nodulation at high levels (Gentili & Huss-Danell, 2002; Namvar et al., 2011; Goh et al., 2016, Bekele et al., 2019), but the presence of adequate levels of N in the soil is proved to not impair nodulation and SNF or even improve it when P levels are optimal (Reinsvold & Pope, 1987; Bekele et al., 2019; Hellsten & Huss-Danell, 2000). It is also possible that the presence of high P content in the soil made the N levels insufficient in absence of other limiting factor which favored nodulation and N fixation. Various authors indicated that having a starter N in the soil before the onset of nodule functioning or at certain stages of plant growth is optimal for nodulation and SNF (Yinbo et al., 1997; Gan et al., 2003, 2004) and Namvar et al. (2011) proved that adding up to 75 kg ha^{-1} N fertilizer to chickpea had a positive effect on growth, yield and nodulation.

The positive effect of P on legumes nodulation and SNF is often explained by either a positive stimulation of the host plant (Robson et al., 1981) or by a direct effect on nodules growth and functioning (Israel, 1987; Hellsten & Huss-Danell, 2000; Gentili & Huss-Danell, 2002), indeed Ribet & Drevon (1996) indicated that NDW was more impaired by P deficiency than SDW even though P concentration in nodules remained unaffected and Drevon & Hartwig (1997) also found similar results for soybean and alfalfa.

At this scale, NDW and SDW were also significantly correlated to organic matter content. Moreover, the site with the highest organic matter was also the site with the highest Olsen-P which could be due to a reducing effect of organic matter on P-insolubilisation in calcareous soil (Braschi et al., 2003) or to the general use of P-rich organic inputs like manure in the study area, highlighting simple relationships between soil properties is often difficult due to their highly intercorrelated character (Ige et al., 2007). Alternatively, it could also be assumed that organic matter promotes the development of symbiotic microorganisms.

Considering that plant growth and nodulation are subjected to different factors, notably to soil characteristics, we chose to use a clustering approach (as previously used by Lazali et al. (2016)) to group sites on the basis of their similarities to obtain homogenous groups (Oumenskou et al., 2018). This approach allowed having a more accurate idea of the effect of some parameters on plant such as the relation between available P and nodulation observed in Fig. 3. Cluster C exhibited the highest nodulation which can be linked to the high P content suggesting that the abundance of P and N promoted biomass production and was not limiting for nodulation in this cluster, the absence of correlation between nodulation and soil available P is mainly due to the high P content in this cluster (26.93 ± 3.80 mg kg^{-1}) compared to the two other clusters which indicate that plant and nodule requirement in that element were covered (Shukla & Yadav, 1982). On the other hand data also show that, site 18 gave the higher NDW per unit of P which can be an indicator of the presence of P efficient rhizobia strains in the site (Zarrin et al., 2006). P deficiency is known to be the major limiting factor of legumes growth and SNF in low input Algerian farming systems because this crops have higher phosphorus needs compared to plant relying on mineral N (Israel, 1987; Ribet & Drevon, 1996) and because of the price of farming inputs no mineral fertilizer is added, in addition to that, P is often precipited due to the high $CaCO_3$ content of soils under alkaline conditions (Tunesi et al., 1999; Mihoub et al., 2016).

Our results also show that clay sites had lesser SDW and NDW compared to clay loam sites, White & Robson (1989) obtained similar results for lupins SDW and NDW on two different soil types and showed that this culture grow poorly on fine-textured soils under alkaline condition, furthermore Msumali & Judith (1998) showed in a jar experiment with inoculated bean that nodulation tends to decline from the sandy through loam to the clay soils and obtained significantly higher shoot weights in the sandy and loam soils than in the clay soil. Our results could be explained by the joined effect of soil type and the native rhizobial population. Indeed Elias (2009) found in a multi-local field trial on chickpea that nodule biomass was largely governed by the size of the rhizobial population in the soil but showed that soil texture and clay content did not affect highly the size of this rhizobial populations.

Soil particle size is known to have a significant effect on plants growth especially legumes which needs adequate soil condition to promote root growth, nodules development and nitrogen fixation, indeed coarse textured soils ensure a better aeration and water infiltration compared to fine-textured soils which tends to puddle and form a hard soil crust after receiving heavy rains. Indeed the effect of soil type on plants and rhizobia is tightly related to water status, Issa & Wood (1995) confirmed that rhizobia survival and multiplication is dependent on the combined effect of soil type and moisture, although fine textured soil improves rhizobia survival (Heijnen et al., 1992). These soils can limit significantly nodulation and growth when water is in excess and the diagnosis region received in 2018 63% more rains than the 35 years average which could cause waterlogging and affect negatively nodulation and growth (Worku, 2016). Chickpea being more sensitive to waterlogging than other legumes such as faba bean which explains the maintaining of spring chickpea in Algeria (Munir, 2016).

In site 8 plants had no nodules despite the fact that the site had average phosphorus content and no high inhibitory nitrogen content, the absence of nodulation in this site can be explained by the absence of rhizobia in the soil or by the presence of other inhibitory factors. Overall, the augmentation of shoot biomass was correlated with the augmentation in nodule biomass (Fig. 5, A) suggesting that plants relied mostly on nitrogen deriving from biological fixation to grow. The low nodulation observed in some site can be explained by a sufficient N supply covering plant needs. Voisin et al. (2002) and (2010) showed that nodule initiation occurs only under situations of N limitation when the uptake by roots appeared to be insufficient to fully satisfy plant N demand. These authors also demonstrated that NDW augmentation is correlated with SDW, which is consistent with our results, indicating that the presence of limiting factors restricting plant growth would restrict nodulation also. The absence of nodulation can also be linked to low population of native rhizobia in the soil or to the ineffectiveness of the indigenous strains of rhizobia (Elias, 2009; Benlahrech et al., 2018).

Finally and although we could not provide a precise quantification of the spatialization of nodulation and plant growth, the map shown in Fig. 1 suggests the existence of a regionalization of nodulation and the associated plant growth, with areas grouping the lowest values and areas grouping the highest values. This regionalization could be due to a regionalization of soil properties, farming practices or also certain environmental factors (climatic or pedoclimatic factors for example) whose impact could be verified in the future.

CONCLUSION

The nodular diagnosis realized in the semiarid region of Chlef emphasized the strong relationship between chickpea growth and nodulation, the latter is mainly controlled by soil Olsen-P as well as by other physical and chemical properties of the soil. The major finding of this work is the highlight of thresholds for optimal nodulation which could be used by farmers as a benchmark to enhance yield and benefits from incorporating chickpea in the farming system. This work proved the necessity to study in more details the factors impairing the symbiotic system of chickpea to profit more from SNF benefits and accomplish a sustainable improvement of production.

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Copper modulates the biochemical and enzymatic activity and growth of tomato cultivars grown *in vitro*

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Abstract. Copper (Cu) is a micronutrient that is neglected for tomato growth. This study sought to identify the effects of exposure to Cu on the growth and biochemical activity of two tomato cultivars. Tomato seeds of 'Carolina' and 'Cereja' cultivars were disinfected and inoculated in MS medium plus copper sulfate concentrations ($CuSO_4$) (default MS, 25, 50, and 100 µm) and had their growth monitored for 30 days. It was estimated that the growth and biomass accumulation of tomato plants 'Carolina' and 'Cereja', both from the aerial part and the roots, were benefited by 25 e 50 µm of $CuSO_4$. However, it was observed that these concentrations were inefficient in controlling hyperhydricity and leaf deformation. There was a reduction of these phenomena in the treatment with 100 µm, in both cultivars. Tomato of 'Carolina' cultivar subjected to 100 µm showed an increase in anthocyanins and superoxide dismutase (SOD) activity in the root system. There was a reduction of catalase (CAT) activity in shoots exposed to Cu. 'Cereja' tomatoes subjected to 100 µm showed an increase in CAT and SOD activity in shoots and roots, respectively. It was concluded that the 'Carolina' and 'Cereja' tomatoes have their growth impaired when exposed to 100 µm $CuSO_4$. Concentrations higher than 50 µm of $CuSO_4$ cause an increase in the antioxidant activity in the shoot of tomato plants from the 'Carolina' cultivar. Concentrations higher than 50 µm $CuSO_4$ increase SOD activity in the root system of tomato plants from the 'Cereja' cultivar.

Key words: *Solanum lycopersicum* L., antioxidant, hyperhydricity, anthocyanins, SOD.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a popular fruit vegetable around the world. Brazil allocated about 57,717 acres to produce this crop and produced 4,004.991 tons of this fruit in the 2019 harvest (IBGE, 2019). However, it has not been quantified how much of this production was directed to grape tomatoes. This fruit class has high added value, as it is used as a culinary ornament and has a large amount of sugar (Maia et al., 2013). The importance of this fruit is mainly due to its nutritional value, which has polyphenols, flavonoids, tannins, vitamin C, and anthocyanins as functional compounds (Butt et al., 2008; Maia et al., 2013). This, combined with the rusticity of the plants, which may show determined or indeterminate growth, makes it a great option for family farming (Brasil, 2018).

In this case, the Cereja and Carolina cultivars have little cultivation information. The Carolina cultivar has indeterminate growth, moderate resistance to diseases, tasty and sweet fruits. The Cereja cultivar, on the other hand, shows vigorous and determined growth, and it is indicated for planting in pots or the garden. Its fruits are sweet. Studies indicate that this fruit consumption can reduce the risk of some diseases whose reactive oxygen species (ROS) are the causative agents. This is due to the high concentration of antioxidant compounds present in this fruit (Arredondo et al., 2016).

Several factors limit the yield of tomatoes; among them, the excessive use of chemical products stands out, with around 36 applications per crop (Dossa & Fuchs, 2017), and a large number of infectious diseases (Lopes & De Ávila, 2005). Another factor is nutritional neglect, especially regarding micronutrients (Da Silva et al., 2006). The absence of a specific fertilization recommendation for tomatoes does not exploit its maximum production potential. Although required in smaller quantities, micronutrients are essential for the proper development of plants, and their imbalance can cause disturbances in plant growth and development (Graham et al., 2001).

Copper (Cu) is an essential micronutrient for cellular processes such as photosynthesis, mitochondrial respiration, and a role in the metabolism of carbon and nitrogen (Hänsch & Mendel, 2009). It is also a protein constituent and is required by more than 30 enzymes, most of which are catalysts for redox reactions (Yruela, 2005; Epstein & Bloom, 2006). The deficiency of this ion can alter the plant architecture, impairing the growth of the plant or giving rise to malformed plants (Yruela, 2005; Yruela, 2009). On the other hand, high concentrations of Cu can also cause reduced growth and impair plant development, as it is a metal with toxic potential (Adrees et al., 2015).

Cupric toxicity causes the formation of reactive oxygen species (ROS) (Nagajyoti et al., 2010), and, consequently, the plant tends to increase the activity of antioxidant enzymes to avoid oxidative damage and maintain cell balance (Wang et al., 2004). Studies that test the tolerance of species to exposure to Cu have been carried out over the years (Drazkiewicz et al., 2004; Xu et al., 2005; Benimeli et al., 2010; İşeri et al., 2011; Azmat & Riaz, 2012; Barbosa et al., 2013), and have found that besides the species, cultivars also have different levels of metal tolerance. Although studies on *in vitro* cultivation have been concerned with defining the interaction between nutrients and plant growth, reports on the nutritional influence on the biochemical activity of tomato plants in this condition are still lacking. Once the functions of Cu are highlighted, it would be interesting to determine the biochemical changes due the exposure to this metal cause in plants.

The determination of biochemical variations caused by the exposure of tomato cultivars to copper will assist in the nutritional process, allowing the plant to reach its maximum growth potential. It is assumed that minimal Cu concentrations will benefit plant growth (Hristozkova et al., 2006). Still, that excess Cu will hinder shoot growth and mass accumulation in general (Adrees et al., 2015). It is also assumed that Cu will cause oxidative stress in the shoots, which will increase the antioxidant activity, either by the activity of enzymes with antioxidant potentials, such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) or by the synthesis of phenolic compounds (Nagajyoti et al., 2010).

Therefore, this study sought to determine the morphological, biochemical, and enzymatic changes in tomato cultivars grown *in vitro* under $CuSO_4$ concentrations.

MATERIAL AND METHODS

Plant material and cultivation conditions

Seeds of the cultivar 'Cereja' originated from the United States of America were purchased commercially (Hortiseres® seeds), lot no. 18000211, with 88% germination and 99% purity. 'Carolina' tomato seeds were purchased commercially (Feltrin® seeds) lot no. 0069701610000280, with 99% germination and 99.9% purity. The seeds were subjected to an asepsis process in 2% sodium hypochlorite solution for 10 minutes, with agitation, and subsequently, washed four times in a row with sterile distilled water.

The Murashige & Skoog (1962) MS culture medium (100%) was supplemented with 30 g L^{-1} of sucrose, 6.5 g L^{-1} of agar (Kasvi®), and pH adjusted to 5.8. Copper sulfate ($CuSO_4$) was added to the medium in three concentrations. The control treatment was composed by the MS medium (default) in its full strength (0.1 µm of $CuSO_4$), and the subsequent treatments were composed by the MS medium in its full strength plus 25, 50, and 100 µm of $CuSO_4$. Subsequently, 50 mL of medium was poured into glass flasks with a capacity of 350 mL. These flasks were autoclaved for 20 minutes at 121 °C.

After asepsis, each flask was inoculated with two seeds of the same cultivar. The flasks were sealed with a plastic cover and polyvinylpyrrolidone film (PVP) and kept in a controlled growth chamber for 30 days, with a 24-hour light period through the use of light emitter diodes (LEDs) Blumenau®, LED T8 10W 6,000K, 100-240V-50/60Hz, power factor ≥ 0.92 (High PF), at a temperature of 25 °C ± 2 °C and light intensity of 72.02 µmol m^{-2} s^{-1}. A completely randomized design was used in the trial; 20 replications per treatment were used, totaling 80 plots per cultivar.

Measurement of plant growth

At 30 days after inoculation, the following variables were measured: length of the shoot (cm) and root (mm), the fresh weight of shoot (g) and roots (g), dry weight of shoot (g) and roots (g), and the number of leaves. Evaluations were made with the aid of a digital caliper and analytical balance to measure the average among all shoots of each treatment.

Physiological disorders observed during *in vitro* culture

During the period in which the plants remained in the growth chamber, three evaluations were carried out at 10-day intervals to measure the percentage of the presence of shoots with deformed leaves and hyperhydric.

Total chlorophylls, flavonoids, and anthocyanins

The total chlorophyll, flavonoid, and anthocyanin indexes were measured in fresh leaves and obtained with the aid of the DUALEX SCIENTIFIC+™ equipment, Quick Start model (FORCE A®, France, Paris) according to the manufacturer's instructions, with four replicates per treatment, on the first expanded leaves.

Total phenolic compounds measured in leaves

Fresh leaves (100 mg) were weighed in falcon tubes to obtain the extract, obtained according to Waterhouse (2002). The determination of the total phenolic content was carried out by the method proposed by Kuskoski et al. (2005). The readings were performed on a 700 plus - Femto spectrophotometer with a wavelength of 750 nm, and the results were expressed in mg of gallic acid equivalents per 100 g of the sample (mg AGE per 100 g). Triplicates were performed with three biological repetitions, and the calculation was performed using the calibration curve of gallic acid $y = 0.0004x + 0.0003$, whose $R^2 = 0.9327$.

Antioxidant activity measured in leaves - DPPH

From the extract obtained to determine total phenolic compounds, antioxidant activity was determined based on the extinction of the absorption of the radical 2.2-diphenyl-1-picryl hydrazine (DPPH 60 µM), proposed by Rufino et al. (2009). The readings were taken on the 700 plus - Femto spectrophotometer with a wavelength of 515 nm. They were monitored every 30 minutes, in a total of three readings, in which the reduction in absorbance was observed until its stabilization. Samples were evaluated in triplicate, with three biological replicates. The results were expressed as a percentage of free radical sequestration (% FRS), according to the equation:

$$\%FRS = \frac{(CA + SA)}{SA} \times 100 \tag{1}$$

where CA = Control Absorbance; SA = Sample Absorbance.

Enzymatic evaluation

The enzymatic extract was obtained (Bonacina et al., 2017) from fresh leaves and roots. All enzymes were evaluated using 96-well flat-bottomed Elisa plates. Biochemical tests were performed in three biological replicates. The equipment used was the UV-VIS spectrophotometer, Spectramax Plus, with SoftMax Pro 6.5.1 software.

SOD (EC 1.15.1.1)

The activity of superoxide dismutase (SOD) was measured by its ability to inhibit nitroblue tetrazolium (NBT) photoreduction, as described by Giannopolitis & Ries (1977). The reading was performed at 560 nm, in which a unit of SOD activity (U) was defined as the amount of enzyme needed to inhibit 50% of reduction in NBT. SOD activity was expressed in U SOD mg^{-1} FM min^{-1}.

CAT (EC 1.11.1.6)

The activity of catalase (CAT) was carried out according to the methodology proposed by Havir & McHale (1987). The degradation of H_2O_2 determined it in 3 minutes at 260 nm. The enzymatic activity was quantified using the molar extinction coefficient of 36 M^{-1} cm^{-1} (Anderson et al., 1995) and expressed in mmol H_2O_2 g^{-1} FM min^{-1}.

APX (EC 1.11.1.11)

The activity of ascorbate peroxidase (APX) was carried out as proposed by Nakano & Asada (1981). The degradation of H_2O_2 determined the activity of this enzyme in 3 minutes at 290 nm. The enzymatic activity was quantified, using the molar extinction coefficient of 2.8 mm^{-1} cm^{-1} (Nakano & Asada, 1981) and expressed in mmol ascorbic acid g^{-1} FM min^{-1}.

Statistical analysis

The data from the growth characteristics were submitted to polynomial regression using the SISVAR 5.6 software (Ferreira, 2011). The maximum values of the cubic equations generated by the polynomial regression were calculated using the equation proposed by Ferreira (2011). When the quadratic model was adopted, the maximum values were obtained through the derivative of the equation.

The data of total chlorophylls, anthocyanins, flavonoids, antioxidant activity, phenolic compounds, and activity of the enzymes SOD, CAT, and APX were submitted to analysis of normality by the Shapiro Wilk test ($p \leq 0.05$) and variance (ANOVA) at $p \leq 0.05$. The means were compared using the *Tukey test* ($p \leq 0.05$), using the SISVAR 5.6 software (Ferreira, 2011). In periodic evaluations, data were plotted in Microsoft Excel, and graphs were constructed with the percentage of variables evaluated at 10, 20, and 30 days of *in vitro* culture. The data were transformed in $ArcoSeno\left[\sqrt{\frac{x}{100}}\right]$.

RESULTS AND DISCUSSION

Copper in initial plant growth

In the 'Carolina' cultivar, the highest average shoot length occurred in the treatment with 25 µm (14.91 cm), which corresponded to a 21.8% increase compared to the control treatment. The lowest average of this variable was observed in the treatment with 100 µm (6.1 cm), where there was a 59.1% reduction compared to the highest average obtained. The averages obtained in the treatment with 50 µm did not differ statistically from the control and 25 µm treatments (Fig. 1, A). There were no significant differences between the control, 25, and 50 µm treatments for the length of the root system, whose average was 102.17 mm. However, in the treatment with 100 µm, there was a reduction of 69.85% in the root length compared to the average of the treatments described previously (Fig. 1, B).

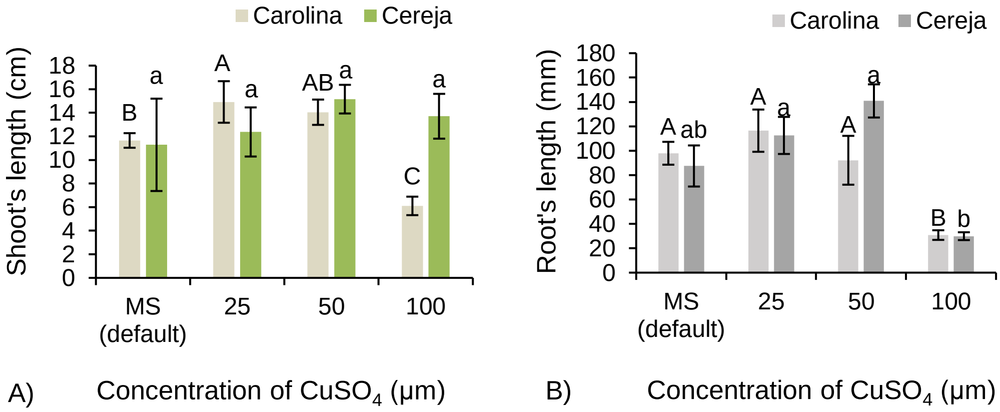


Figure 1. Length of shoots (A) and roots (B) of *Solanum lycopersicum* L. 'Carolina' and 'Cereja' exposed to $CuSO_4$ concentrations; ($n = 20$), *Bar* means ± standard deviation followed by the same uppercase letter and lowercase letter did not differ statistically from each other by the Tukey test ($p \leq 0.05$).

There was no significant difference in the shoot length of the ‘Cereja’ cultivar (Fig. 1, A). There was no significant difference in control, 25, and 50 μm treatments in the root system, whose average was 113.61 mm. There were also no significant differences between the control and 100 μm treatments, with an average of 58.69 mm. However, a 78.7% reduction in root length was observed when the averages obtained at 50 and 100 μm were compared (Fig. 1, B).

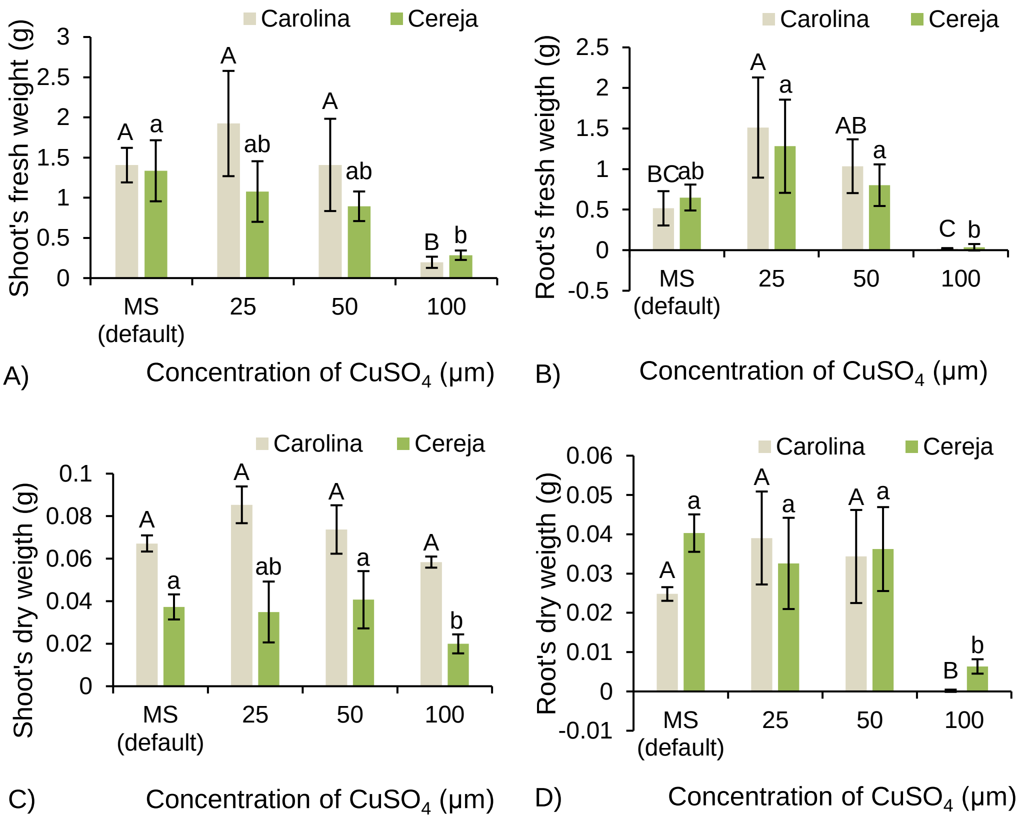
The increase in plant growth variables corroborated the hypothesis that Cu acts as a micronutrient. This ion is a constituent of proteins, required for the normal functioning of more than 30 enzymes (Epstein & Bloom, 2006), such as enzymes that catalyze redox reactions (Yruela, 2005) and enzymes involved in the initial processes of nitrate assimilation (Hristozkova et al., 2006). Its lack can impair the use of the nitrogen (N) absorbed and, consequently, disturb the plant growth (Yruela, 2005; Yruela, 2009). Drazkiewicz et al. (2004) concluded that *Arabidopsis thaliana* tends to accumulate higher amounts of Cu in the root system and lower amounts in the aerial part. Wójcik & Tukiendorf (2003) stated that this phenomenon functioned as a plant defense system, which seeks to delay or even prevent the metal from damaging the shoot. Therefore, the roots suffer more severe damage, both in terms of length and accumulation of mass.

Cu toxicity can reduce the growth of the shoot and roots, preventing cell elongation and/or decreasing the absorption of other minerals (Adrees, 2015). This may result from a variation in the synthesis of some plant hormones, such as abscisic acid (ABA) and jasmonic acid (JA). According to Wilkinson & Davies (2010), the increase in ABA concentration causes stomatal closure, thus reducing the nutrient transition flow. JA, in turn, is considered a molecular sign of the activation of the plant defense apparatus, causing growth retardation (Maksymiec & Krupa, 2007). In their study with hydroponic maize, Reckova et al. (2019) observed an increase in the hormones previously described due to the increase in the concentration of $CuSO_4$ in the nutrient solution.

The comparison of means revealed no significant differences in the fresh weight of ‘Carolina’ shoots among control, 25, and 50 μm treatments. There were also no significant differences among 25, 50, and 100 μm treatments. However, comparing the average obtained in control (1.41 g) with that obtained at 100 μm (0.19 g), there is a reduction of 86.5% in the accumulation of the fresh weight of shoots (Fig. 2, A). In the root system of ‘Carolina’, there were no significant differences between treatments with 25 and 50 μm, whose average was 1.27 g. A reduction of 78.7% was observed in this variable when we compared the average previously described with that obtained in the treatment with 100 μm (0.02 g). The control and 100 μm treatments had no significant differences (Fig. 2, B).

In the shoots of ‘Cereja’, there was a 79.1% reduction in fresh weight comparing the control (1.34 g) and the treatment with 100 μm (0.28 g) (Fig. 2, A). The comparison of means indicated no significant differences between treatments with 25 and 50 μm (1.04 g) for root fresh weight. However, a reduction of 96.2% in the fresh weight of the roots was observed when we compared the average previously described with that obtained in the treatment with 100 μm (0.04 g) (Fig. 2, B).

For the dry weight of shoots, the comparison of means showed no significant differences in the ‘Carolina’ cultivar according to Cu concentration (Fig. 2, C). For the dry weight of the root system, there were no significant differences among control, 25, and 50 μm treatments, whose average was 0.033 g. However, comparing this average to that obtained at 100 µm, there was a reduction of 99.4% in the accumulation of the root dry weight (Fig. 2, D).



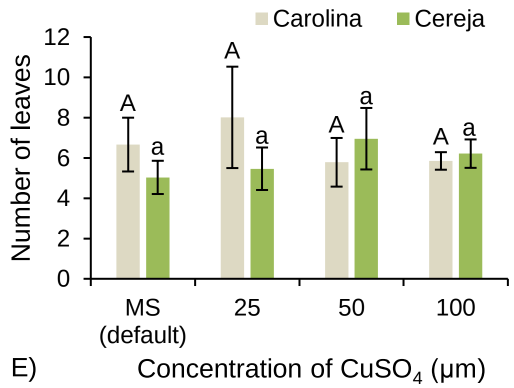


Figure 2. Fresh weight of shoots (A) and roots (B), dry weight of shoot (C) and roots (D), and the number of leaves (E) of *Solanum lycopersicum* L. 'Carolina' and 'Cereja' according to the $CuSO_4$ concentration; (n = 20), *Bar* means ± standard deviation followed by the same uppercase letter and lowercase letter did not differ statistically from each other by the Tukey test ($p \leq 0.05$).

In the 'Cereja' cultivar, the highest average of the dry weight of shoots was observed at 50 µm (0.04 g), not differing statistically from the control and 25 µm. However, this average was 50% higher than the average obtained in 100 µm (Fig. 2, C). There were no statistical differences among control, 25, and 50 µm treatments for the root dry weight, whose average was 0.04 g. The average observed at 100 µm was 85% less than previously described, with 0.006 g (Fig. 2, D).

The reduction of biomass is one of the dominant effects of excess Cu (Adrees, 2015). The negative influence of Cu on the accumulation of dry weight was observed in 'Cereja' tomato, both in the shoot and roots; only the roots of 'Carolina' cultivar suffered this effect.

The data obtained in this work corroborate this statement and the study by Benimeli et al. (2010), in which the application of Cu concentrations greater than 10 µm in the maize nutrient solution (Cargil 350 Hybrid) decreased both fresh and dry mass. Dresler et al. (2014) and Azooz et al. (2012) observed that the excess of this metal decreased the fresh weight of roots and shoots of corn and wheat shoots, respectively. This reduction in biomass accumulation may be related to decreased nutrient absorption caused by cupric stress (Adrees, 2015).

There were no significant changes in the number of leaves of shoots from the 'Carolina' and 'Cereja' tomato cultivars due to the increase in $CuSO_4$ concentration (Fig. 2, E).

Development of deformed leaves during *in vitro* cultivation 'Carolina'

In the first evaluation, carried out at ten days after inoculation, we observed that 10% of shoots in the control treatment (default MS) had twisted and thin leaves, with a translucent aspect (Fig. 3). The treatments with 25 and 50 µm $CuSO_4$ did not show shoots with this disorder, and the treatment with 100 µm $CuSO_4$ had 5% of shoots with deformed leaves (Fig. 3). At 20 days of cultivation, in the control treatment, 15% of shoots had deformed leaves; in treatments with 25 and 50 µm of $CuSO_4$, 25% and 20% of shoots had this disorder, respectively. Treatment with 100 µm continued with 5% of shoots with deformed leaves (Fig. 3).

At 30 days of cultivation, 45% of shoots in the control treatment (MS default) showed deformed leaves. Treatments with 25 and 50 µm $CuSO_4$ had 40% and 35% of the shoots with deformed leaves, respectively, followed by the treatment with 100 µm of $CuSO_4$, in which only 5% of the shoots presented such deformity (Fig. 3).

Based on the Tukey test ($p \leq 0.05$), there were no significant differences in the percentage of shoots with deformed leaves according to the $CuSO_4$ concentration in any of the evaluations performed (Fig. 3).

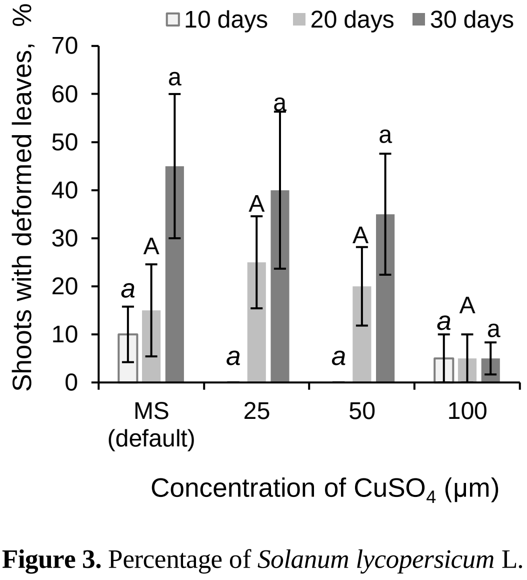


Figure 3. Percentage of *Solanum lycopersicum* L. 'Carolina' with deformed leaves according to the in vitro cultivation days and $CuSO_4$ concentration; (n = 20). *Bar* means ± standard error followed by the same italic letter, uppercase letter, and lowercase letter did not differ statistically from each other by the Tukey test ($p \leq 0.05$).

‘Cereja’

In the first ten days of cultivation, 20% and 30% of the shoots from the control treatment and 50 µm $CuSO_4$, respectively, presented twisted and pointed leaves with and vitreous aspect. Shoots grown in medium with 25 µm of $CuSO_4$ did not present leaf deformation in the first ten days (Fig. 4). However, at 20 days of cultivation, this treatment resulted in 40% of shoots with deformed leaves, and control treatment and 50 µm $CuSO_4$ both presented 60% of the shoots with this disorder (Fig. 4).

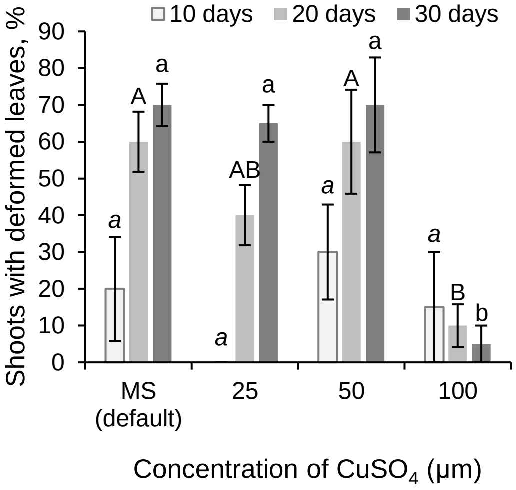


Figure 4. Percentage of *Solanum lycopersicum* L. ‘Cereja’ with deformed leaves at 10, 20, and 30 days of in vitro cultivation; ($n = 20$). Bar means ± standard error followed by the same italic letter, uppercase letter, and lowercase letter did not differ statistically from each other by the Tukey test ($p \leq 0.05$).

At 30 days of cultivation, the control treatment and the concentrations of 25 and 50 µm of $CuSO_4$ presented 70%, 65%, and 70% of the shoots with deformed leaves, respectively (Fig. 4). Shoots exposed to 100 µm $CuSO_4$, in turn, were slow to present this symptom, which occurred in only 5% of them, at 30 days of cultivation (Fig. 4).

According to the comparison of means, there were no significant changes in the percentage of shoots with deformed leaves at 10 days after inoculation. At 20 days, it was found that treatments with 25 and 100 µm did not show significant differences between them. However, the treatment with 100 µm showed significantly fewer seeds with deformed leaves than control and 50 µm treatments. At 30 days after inoculation, it was found that the treatment with 100 µm obtained, statistically, a lower percentage of shoots with deformed leaves (Fig. 4).

According to Broadley (2012), Cu deficiency can alter the leaf construction, giving rise to pointed and twisted leaves, besides reducing the leaf area. Yruela (2005) stated that the Cu deficit impairs leaf construction, possibly due to less lignin biosynthesis, as described by Liu et al. (2018). The results obtained in this work corroborate the statements of Broadley (2012) and Yruela (2005; 2009) since the highest occurrence of deformed leaves occurred in the treatments with the three lowest concentrations of Cu. Lignin synthesis may have been compromised in these treatments. In treatment with 100 µm $CuSO_4$, only 5% of the shoots developed deformed leaves. Our results provide evidence that an increase in lignin synthesis may have occurred, which has reduced the occurrence of deformed leaves.

Hyperhydricity observed during the *in vitro* cultivation ‘Carolina’

In the first ten days of cultivation, the control and 100 µm $CuSO_4$ treatments showed 15% and 5% of hyperhydric shoots, respectively (Fig. 5). This disturbance was only observed at 20 days after inoculation in the concentrations of 25 and 50 µm $CuSO_4$. In this situation, 20% of the shoots had this symptom. Even at 20 days after inoculation, 45% of the control shoots were hyperhydric, and, interestingly, the treatment with 100 µm of Cu did not have shoots with this disorder (Fig. 5).

At 30 days of cultivation, shoots submitted to 100 µm of $CuSO_4$ did not present this disorder. The control treatment showed 55% of hyperhydric shoots. Treatments with 25 and 50 µm $CuSO_4$ had 40% and 35% of hyperhydric shoots, respectively (Fig. 5).

According to the means comparison test, at ten days after inoculation, the treatment with 100 µm obtained a significantly lower percentage of hyperhydric shoots than the control treatment. At 20 and 30 days after inoculation, this same treatment showed a statistically lower percentage of shoots with this disorder than other treatments (Fig. 5).

‘Cereja’

At ten days of cultivation, it was possible to observe in the treatment with 50 µm of $CuSO_4$ that 25% of the shoots had a translucent green stem. However, in other treatments, this phenomenon had not yet occurred (Fig. 6). When the shoots completed 20 days of cultivation, there was a large increase in the number of hyperhydric shoots. It was evaluated that 60% of shoots in the control treatment, 25% of the shoots in the treatment 25 µm, 45% in the treatment 50 µm, and 5% of shoots in the treatment 100 µm presented this disorder (Fig. 6).

At 30 days of cultivation, we observed that the control, 25, and 50 µm of $CuSO_4$ had 70%, 65%, and 75% of the shoots with hyperhydricity, respectively. Treatment with 100 µm of $CuSO_4$ maintained the percentage of hyperhydric shoots; that is, there was no development of this disorder between 20 and 30 days of cultivation (Fig. 6).

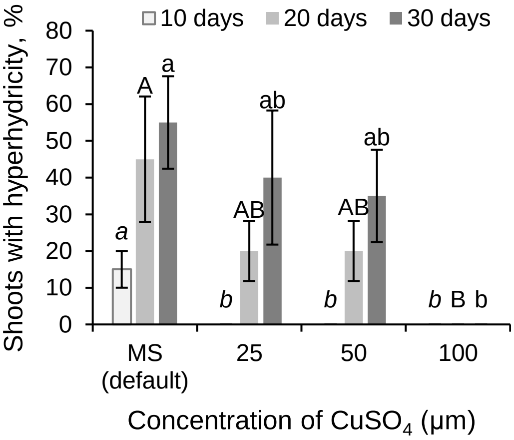


Figure 5. Percentage of hyperhydric shoots of *Solanum lycopersicum* L. ‘Carolina’ over 30 days of in vitro cultivation according to the $CuSO_4$ concentration; (*n* = 20) Bar means ± standard error followed by the same italic letter, uppercase letter, and lowercase letter did not differ statistically from each other by the Tukey test ($p \leq 0.05$).

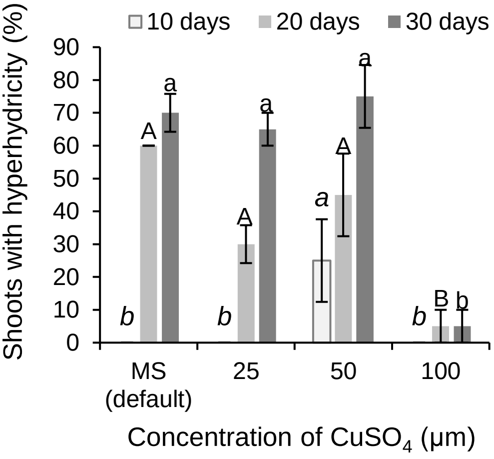


Figure 6. Percentage of hyperhydric shoots of *Solanum lycopersicum* L. ‘Cereja’ over the days of *in vitro* cultivation; (*n* = 20). Bar means ± standard error followed by the same italic letter, uppercase letter, and lowercase letter did not differ statistically from each other by the Tukey test ($p \leq 0.05$).

According to the Tukey test ($p \leq 0.05$), the treatment with 50 µm showed a higher percentage of hyperhydric shoots than the other treatments ten days after inoculation. At 20 and 30 days after inoculation, treatment with 100 µm showed a significantly lower percentage of shoots with such disorder (Fig. 6).

Hyperhydricity is a metabolic disorder common in *in vitro* culture. The conditions of this type of cultivation can trigger this disturbance, such as, for example, the concentration of salts in the medium, the constant temperature, the high relative humidity in the cultivation container, among others (De Vasconcelos et al., 2012). Among the characteristics of hyperhydric plants, there is the excessive accumulation of water in the tissues, the swelling of the tissues of the shoot, such as the stem and leaves, vitreous aspect, low lignin rate in the cell wall, and low accumulation of biomass (Kevers et al., 2004).

In the treatment with 100 µm of $CuSO_4$, smaller shoots with better architecture were observed in both genotypes. According to Zeng et al. (2016), hydrogen peroxide (H_2O_2) formed by cupric stress is involved in the polymerization of lignin and its accumulation in the cell wall. This may have justified the drop in the percentage of hyperhydric shoots due to the increase in the concentration of $CuSO_4$ and leads us to believe that in Cu concentrations below 100 µm, there was not enough lignin synthesis to control hyperhydricity.

Physiological changes due to copper

In ‘Carolina’ plants, the indexes of total chlorophylls (Fig. 7, A) and flavonoids (Fig. 7, B) were constant in all concentrations of $CuSO_4$, with averages of 7.29 and 0.22, respectively. Shoots exposed to 100 µm of $CuSO_4$ showed an increase of anthocyanins of 49.4% compared to the control treatment; 39.28% in the treatment with 25 µm $CuSO_4$ and 46.42% in the treatment with 50 µm $CuSO_4$ (Fig. 7, C).

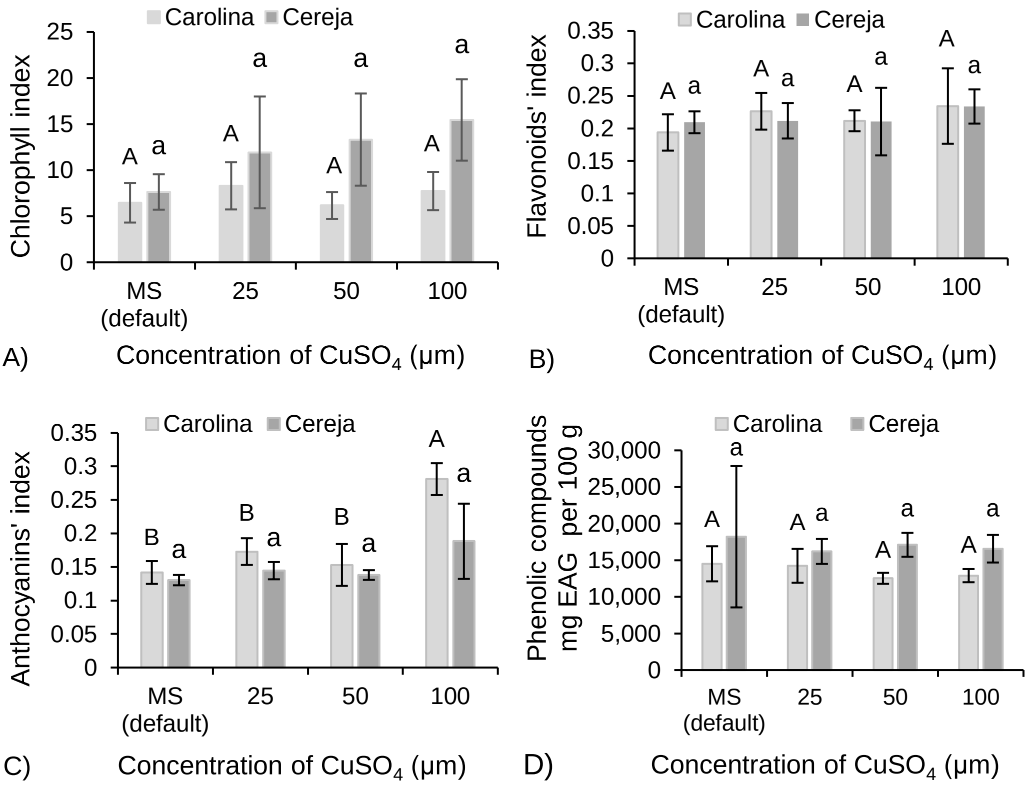
In ‘Cereja’, the total chlorophylls, flavonoids, and anthocyanins were not affected by the $CuSO_4$ concentration. Average levels of 12.75 (Fig. 7, A), 0.226 (Fig. 7, B), and 0.162 (Fig. 7, C) were observed, respectively.

Anthocyanins and total phenolic compounds are part of the metabolism responsible for reducing ROS, mostly serving as a substrate for potential redox enzymes (Juadjur et al., 2015). In the ‘Cereja’ cultivar, there was no change in the concentration of these compounds due to an increase in the Cu concentration to which shoots were exposed against the initial hypothesis that oxidative damage caused by Cu would intensify the antioxidant activity of the plant. Drazkiewicz et al. (2004) stated that, under oxidative stress conditions, not all the antioxidant apparatus is activated. Thus, there may be an overload of a certain metabolism and, consequently, a reduction in the intensity of other metabolic routes. For example, the increase in the intensity of the route responsible for the enzymatic activity can occur simultaneously with reduced synthesis of anthocyanins and phenolic compounds.

The ‘Carolina’ tomato increased the synthesis of anthocyanins when the shoots were exposed to 100 µm of $CuSO_4$. According to Juadjur et al. (2015), anthocyanins have redox potential in *in vitro* conditions. More than any other phenolic compound, they contribute to the degradation of H_2O_2 (Silva et al., 2010), as they are substrates for the peroxidases existing inside the vacuole. Thus, it is assumed that shoots exposed to 100 µm $CuSO_4$ increased the production of anthocyanins to control the H_2O_2 molecules generated by copper toxicity.

Biochemical variations due to copper

'Carolina' lowest average of phenolic compounds was obtained in shoots exposed to 50 µm $CuSO_4$, with 12,534.09 mg AGE per 100 g. The rest of the treatments did not differ statistically, with an average of 14,033.58 mg AGE per 100 g (Fig. 7, D). 'Cereja' synthesis of phenolic compounds did not show sensitivity to the Cu concentrations studied here, maintaining an average of 16,747.35 mg AGE per 100 g (Fig. 7, D).



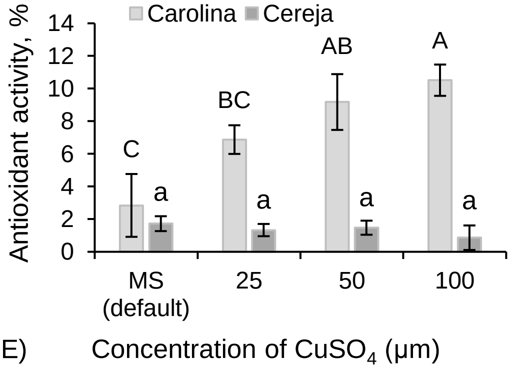


Figure 7. Index of chlorophylls (A), flavonoids (B), anthocyanins (C), phenolic compounds (D) and antioxidant activity (E) of *Solanum lycopersicum* L. 'Carolina' and 'Cereja' according to the concentration of $CuSO_4$; ($n = 20$) * means ± standard deviation followed by the same uppercase letter and lowercase letter did not differ statistically from each other by the *Tukey test* ($p \leq 0.05$).

The antioxidant activity was increased in 'Carolina' shoots exposed to 100 µm $CuSO_4$. When the concentration of 100 µm $CuSO_4$ was compared, 72.97% more activity was observed in the control treatment, and 34.63% and 12.75% concerning the concentrations of 25 and 50 μm $CuSO_4$, respectively (Fig. 7, E). Still, in 'Cereja', the percentage of antioxidant activity did not change in the conditions studied here, maintaining an average of 1.47 (Fig. 7, E).

The activity of the superoxide dismutase enzyme (SOD), when evaluated in the aerial part of the shoot, did not change due to exposure to different concentrations of $CuSO_4$ in either the cultivars (Fig. 8, A), whose mean value was 2,163.982 U SOD mg^{-1} FM min^{-1} in 'Carolina' and 2,252.63 U SOD mg^{-1} FM min^{-1} in 'Cereja' (Fig. 8, A).

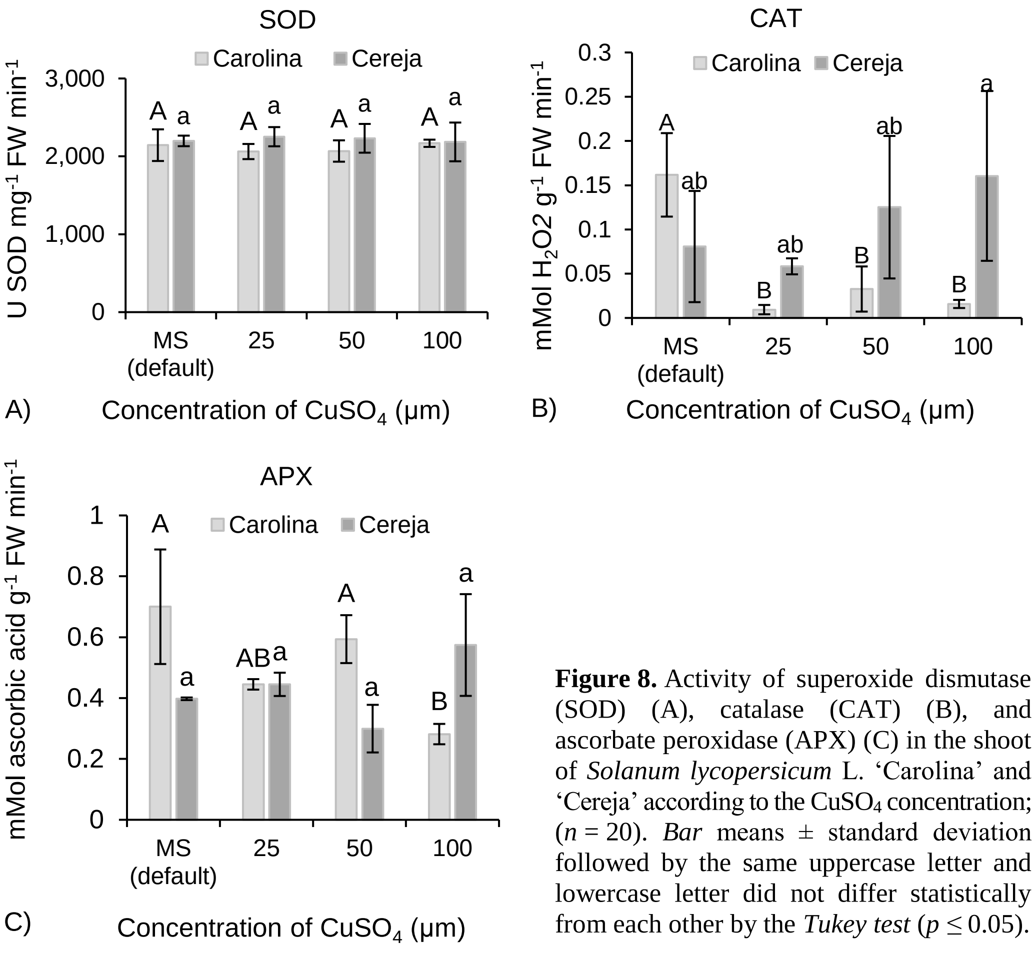


Figure 8. Activity of superoxide dismutase (SOD) (A), catalase (CAT) (B), and ascorbate peroxidase (APX) (C) in the shoot of *Solanum lycopersicum* L. 'Carolina' and 'Cereja' according to the $CuSO_4$ concentration; ($n = 20$). *Bar* means ± standard deviation followed by the same uppercase letter and lowercase letter did not differ statistically from each other by the *Tukey test* ($p \leq 0.05$).

There was a reduction in the catalase activity (CAT) in the shoot of the 'Carolina' cultivar when exposed to Cu. The addition of copper to medium decreased 94.18%, 79.77%, and 90.10% the activity of this enzyme in treatments with 25, 50, and 100 μm of $CuSO_4$, respectively, compared to the control treatment (Fig. 8, B). Drazkiewicz et al. (2004) observed in studies with Arabidopsis thaliana that Cu inhibited CAT activity, while other antioxidant enzymes intensified its activity. This author reported that his results corroborate the hypothesis that high concentrations of O_2^- have a direct effect on CAT inactivation (Salin, 1988). On the other hand, in the 'Cereja' cultivar, there was no significant difference in CAT activity (Fig. 8, B).

Ascorbate peroxidase (APX) in the shoot of the 'Carolina' shoots in the control treatment showed 60% more activity concerning 100 µm $CuSO_4$, not differing statistically from the treatment with 50 µm $CuSO_4$ (Fig. 8, C). In the 'Cereja' shoots, APX activity did not change due to the different Cu concentrations (Fig. 8, C).

When evaluating SOD activity in the root system, it was observed that shoots exposed to 100 µm $CuSO_4$ increased 39.20% in activity compared to 50 µm $CuSO_4$ in the 'Carolina' cultivar. In contrast, in the other treatments, the activity was constant, with an average of 2242.34 U SOD mg^{-1} FM min^{-1} (Fig. 9, A). In the 'Cereja' cultivar, the activity of SOD in the root system of shoots exposed to 100 µm $CuSO_4$ showed an increase of 38.79% compared to that obtained in the control treatment (Fig. 9, A). As previously reported, roots tend to accumulate Cu to protect the shoot (Drazkiewicz et al., 2004; Küpper & Andresen, 2016). The function of SOD is to dismute the anionic superoxide radical (O_2^-) to hydrogen peroxide (H_2O_2) (Bhattacharjee, 2010). Thus, we believe that, in both cultivars, SOD intensified its activity in the root system, dismuting the O_2^- to H_2O_2 to prevent these molecules from damaging the shoot.

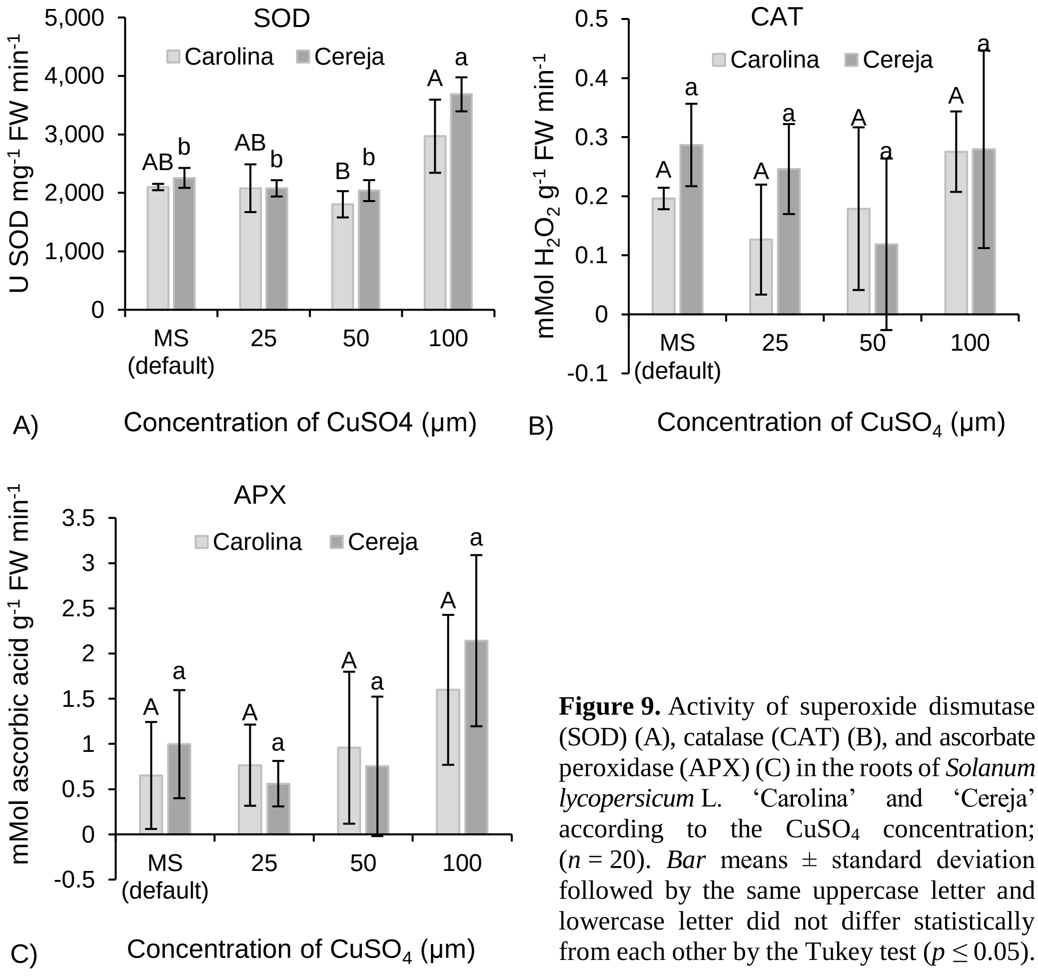


Figure 9. Activity of superoxide dismutase (SOD) (A), catalase (CAT) (B), and ascorbate peroxidase (APX) (C) in the roots of *Solanum lycopersicum* L. 'Carolina' and 'Cereja' according to the $CuSO_4$ concentration; ($n = 20$). *Bar* means ± standard deviation followed by the same uppercase letter and lowercase letter did not differ statistically from each other by the Tukey test ($p \leq 0.05$).

The evaluation of CAT (Fig. 9, B) and APX (Fig. 9, C) activity in the root system demonstrated that the exposure of 'Carolina' and 'Cereja' shoots to different Cu concentrations did not cause statistical differences in those enzymes activity.

The present study provided evidence that the excess of Cu requires the 'Carolina' tomato to redirect its metabolites to combat ROS, which hinders the growth of both shoot and roots. The 'Cereja' tomato, on the other hand, favored SOD activity, especially in the root system, thus controlling the effects of ROS on the shoot. The absence of Cu, in turn, causes an increase in the predisposition of shoots to physiological disorders. Studies still need to be carried out to clarify and control the occurrence of hyperhydricity in 'Carolina' and 'Cereja' tomatoes grown *in vitro* under the $CuSO_4$ concentrations described here.

CONCLUSION

It was concluded that the 'Carolina' and 'Cereja' tomato plants have their growth impaired when exposed to 100 µm de $CuSO_4$. Concentrations higher than 50 µm of $CuSO_4$ cause an increase in the antioxidant activity in the shoot of tomato plants from the 'Carolina' cultivar. Concentrations higher than 50 µm $CuSO_4$ increase SOD activity in the root system of tomato plants from the 'Cereja' cultivar.

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Molecular discovery of new allele associated with loose smut resistance gene *Ut-X* in spring wheat

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Abstract. Genes of resistance to loose smut incited by the fungus *Ustilago tritici* (Pers.) Rostr. are still unknown in the Egyptian spring wheat. Loose smut incidence (LSI) was assessed in ten wheat cultivars through a two-year field trial during 2018–2020. All of the tested cultivars exhibited various percentages of susceptibility (> 10–70% LSI) to the disease except cultivar Misr-3 which exhibited resistance. The most susceptible cultivars were Sakha-93 (60%), Giza-168 (42.1%), and Misr-2 (34.28%). However, the resistant cultivar Misr-3 recorded the least LSI amounting to 5%. The wheat cultivars were screened by the SCAR marker (Xcrc4.2) to identify the presence/absence of loose smut resistance gene *Ut-X*. Molecular data revealed that the SCAR marker (Xcrc4.2) generated two alleles in cultivars with PCR fragments size of 800-bp and ≃ 200-bp. The favorable allele 800-bp was generated only in the resistant Egyptian cultivar 'Misr-3' and the resistant check cultivar 'Biggar', indicating the presence of the gene. Meanwhile, another allele ≃ 200-bp was generated in seven Egyptian cultivars, Giza-168, Giza-171, Misr2, Sakha-93, Gemmeiza-12, N-95, and Shandweel-1, indicating the absence of the resistant gene. This is the first study to report resistance genes to loose smut in Egyptian spring wheat, by detecting *Ut-X* in cultivar Misr-3. In addition, the study documented the first report of another allele ≃ 200-bp associated with SCAR marker (Xcrc4.2). Findings also revealed that the race-specific resistance gene *Ut-X* confers effective resistance to local *U. tritici* races, including race T10 which could be widely incorporated in breeding programs to control the disease.

Key words: SCAR marker, resistance genes, *Triticum aestivum*, *Ustilago tritici*, *Ut-X* alleles.

INTRODUCTION

Wheat is the staple food for approximately one-third of the world population. More than 215 million hectares with an annual production of 700 million tons of wheat were estimated worldwide (FAOSTAT, 2018). In Egypt, wheat is one of the most important winter cereal crops in terms of the planted area and crop production. It provides more than 30% calorie intake of the population. The wheat area grown in Egypt is approximately 1.26 million hectares with a yield of approximately 8.1 million tons, but there is still a big gap, about 50%, between production and consumption (Kishk et al., 2019). Wheat is liable to attack by many important diseases, causing great losses in grain yield and quality. Rusts, mildews, black point, and loose smut are among the most common and widespread diseases of wheat in Egypt (El-Gremi et al., 2017; Gad et al., 2019; Elkot et al., 2020; Draz Abd El-Kreem, 2021; Esmail et al., 2021). Loose smut incited by the basidiomycete fungus *Ustilago tritici* (Pers.) Rostr. commonly occurs in the majority of the wheat-growing countries (Nielsen & Thomas, 1996; Thambugala et al., 2020). The spike produced from infected germinated plants are converted into black powdery spore clusters in which grains are usually not formed, only the rachis remains intact. It is also common for some particles to form on a locally infected head. Contaminated seeds are the only source of perpetuation and loose smut causes yield losses up to 5–7% where farmers plant their harvested infected seeds again (Ramdani et al., 2004). The presence of loose smut infection cannot be predicted until the plant, which is impregnated with the inoculum, produces a spike characteristic symptom i.e., early emergence and blackening of the emerging spike. This seed-borne disease is commonly present in Egypt at different levels of incidence and unfortunately, none of the Egyptian wheat cultivars is known to be resistant against this disease.

The management of loose smut was achieved in wheat with a combination of resistant cultivars, certified seeds, and systemic fungicides applied as seed treatments, yet the absence of an effective control practice resulted in significant yield and economic losses (Nielsen, 1983). Although the use of pesticides to protect the production of crops may have an adverse impact on the environment and the consumers, most farmers still prefer to use chemical control for effective immediate results in disease control. The development of resistant cultivars is an effective eco-friendly approach to eradicate this problematic fungus particularly in organic wheat production and in countries where seed treatment is not readily available (Menzies, 2008; Menzies et al., 2009). Commercial wheat cultivars with effective resistance to loose smut have been developed as a result of the incorporation of loose smut resistance genes of the bread (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* var. *durum*) collected around the world (Nielsen, 1987; Menzies et al., 2003). *U. tritici* races of differing virulence have been reported from both bread and durum wheat worldwide, in which approximately fifty races of *U. tritici* have been identified from various regions of the world growing hexaploid wheat (Menzies, 2016). The virulence *U. tritici* population varies substantially globally (Kaur et al., 2014; Kassa et al., 2015). For instance, the occurrence of races such as T1, T17, T34, and T38 has been reported in Egypt which was virulent on many hexaploid wheat cultivars/lines (Knox & Menzies, 2012). Nowadays distinguishing wheat resistance genes is crucial, since new *U. tritici* races continue to be found in commercial wheat fields in Egypt (Gad et al., 2019).

Previous studies on the genetics or mechanisms of loose smut resistance in wheat have shown that resistance may be inherited as a qualitative or quantitative trait (Knox et al., 2014). Resistance to wheat loose smut is known to be under monogenic control and several resistance genes have been identified and localized in hexaploid wheat (Procunier et al., 1997). To date, at least eleven genes resistant to loose smut were recorded in the catalog of wheat gene symbols, *Ut1–Ut4*, *Ut-X*, *Ut6–Ut11* (Nielsen, 1977, 1982; McIntosh et al., 2013; Kassa et al., 2014; Thambugala et al., 2020). Although the gene originally named *Ut-X* located on chromosome 2BL has been recently identified as *Ut5* (Procunier et al., 1997; Knox et al., 2014). However, the presence of the *Ut5* resistance gene, complementary to the *utv5* virulence gene is debatable (Syukov & Porotkin, 2015). We, therefore, decided to refer the respective resistance gene in the current study to the original named *Ut-X* here on. This gene mapped to the distal end of chromosome arm 2BL and conditioned resistance to *U. tritici* race T10 (Procunier et al., 1997). In wheat, several classes of molecular markers have been successfully used for linkages to resistance genes such as restriction fragment length polymorphisms (RFLP) (Autrique et al., 1995; Schachermayr et al., 1995), random amplified polymorphic DNA (RAPD) (Procunier et al., 1995; Schachermayr et al., 1995), simple sequence repeat (SSR) markers (Abou-Elseoud et al., 2014; Draz, 2017; Shahin et al 2020) and sequence characterized amplified region (SCAR) (Paran & Michelmore, 1993; Procunier et al., 1997; Cao et al., 2001; Gupta et al., 2006; Rai et al., 2017). SCAR markers are derived from a robust PCR and show a visually less complex banding pattern that have an advantage of high reproducibility and locus-specific (Procunier et al., 1997).

Wheat production in Egypt has been improved due to the development of breeding and cultivation techniques to avoid the huge negative impact of loose smut on wheat production. For successful breeding of cultivars resistant to this disease, the breeder and the pathologist, first of all, should have the information on the effective resistance genes for the local area of wheat cultivation. To date, resistance genes to loose smut in Egyptian spring wheat have not been identified yet. Hence, the present study aimed to identify loose smut resistance gene *Ut-X* based on SCAR flanking marker and to report alleles associated with the disease incidence in Egyptian spring wheat.

MATERIALS AND METHODS

Plant material

Seeds of ten Egyptian wheat cultivars were provided by the Wheat Disease Research Department, Plant Pathology Research Institute, ARC, Egypt. Seeds of the wheat material served as susceptible check cultivar 'Diamant' for disease evaluation (Nielsen & Tikhomirov, 1993) and as positive check cultivar 'Biggar' for a molecular assay (Procunier et al., 1997**)** were provided by the International Maize and Wheat Improvement Center (CIMMYT), Mexico. The tested wheat cultivars and their pedigree are provided in Table 1.

Table 1. List of the tested spring wheat cultivars and their pedigree

No.	Cultivar	Pedigree
1	Giza-168	MRL/BUC//Seri-82
2	Giza-171	Sakha-93/Gemmeiza-9
3	Sids-14	SW8488*2/KUKUNA
4	Misr-2	Skauz/Bav-92
5	Sakha-93	Sakha-92/TR810328
6	Beniswef-5	DIPPER-2/BUCHEN-3
7	Misr-3	Rolf-07*2/Kiritati
8	Gemmeiza-12	OTUS/3/SARA/THB//VEE
9	N-95	-
10	Shandweel-1	SITE//MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC
11	Biggar	TOBARI-66/ROMANY-66
12	Diamant	YUBILEI/SADOVO-1

Evaluation of loose smut incidence

In a two-year field trial, loose smut incidence (LSI) was assessed in the tested wheat cultivars (Table 1) during 2018/19–2019/20 growing seasons at Sakha Agricultural Research Station, Agricultural Research Center (ARC), Egypt. During the 2018/19 season, cultivars were grown in three-row plots, each 1.5 m long with 30 cm distance between rows. The plots were arranged in a randomized complete block design (RCBD) with three replicates. All recommended cultural practices for wheat crops in the commercial fields were applied. The wheat cultivars were inoculated with a mixture of local *U. tritici* races including predominant race T10, according to the method described by Nielsen (1987). In which, florets of the plants were inoculated with a teliospore suspension of *U. tritici* races at mid-anthesis (GS 60-65, Zadoks et al., 1974). Independently of each cultivar, ten spikes were inoculated and each spike was tagged. Inoculated spikes were harvested at maturity and the grains from the inoculated spikes were collected in envelopes labeled with wheat cultivar identity. During the 2019/20 season, a minimum of 100 inoculated grains for each cultivar was planted in 1.5 m long with 30 cm between rows. Loose smut incidence (LSI) was assessed in each cultivar according to the method described by Menzies et al. (2009), and was calculated as follows:

$$\text{LSI (\%)} = \frac{\text{Number of smutted plants}}{\text{Total number of plants}} \times 100$$

Molecular assay of loose smut resistance gene *Ut-X*

The molecular assay was carried out in the Biological Laboratory of the Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt. Genomic DNA was extracted using a commercial kit: Thermo Scientific™ GeneJET™ PCR Purification Kit (Thermo Fisher Scientific, Cat. No. K0701) and quantified using a spectrophotometer (MaestroNano, Drop MN-913). The DNA samples were diluted for a final concentration of 100 ng/µL. The SCAR marker (Xcrc4.2) developed by Procunier et al. (1997) was used to detect the loose smut resistance gene *Ut-X* in the ten Egyptian wheat cultivars. Amplification of genomic DNA with SCAR primer pair 5'-TGGGCTCGCTTCATAAATTGGTTC-3’ and 5'-TGGGCTCGCTGCTACCGGGGTGGA-3’ was done in a thermocycler (Techne-Progene, UK). The 25-µL PCR reaction volume was prepared and the PCR program was optimized in the initial study at an annealing temperature of 68 °C according to Procunier et al. (1997). Amplification products were electrophoresed in 1.4% agarose gel with RedSafe™ Nucleic Acid Staining Solution. The tests were repeated twice. The DNA banding patterns were visualized using a UV-transilluminator (Herolab UVT 2020, Kurzwellig) and photographed. The obtained PCR fragments were scored to indicate alleles associated with the gene *Ut-X* and its presence/absence in cultivars.

RESULTS AND DISCUSSION

Loose smut incidence

The twelve wheat cultivars presented in Table 1, consisted of ten Egyptian wheat-tested cultivars and two check cultivars, Biggar as the resistant cultivar, and Diamant as the susceptible cultivar. All cultivars were evaluated for their reactions against wheat loose smut in the field trials during the growing seasons of 2018/19–2019/20. Data illustrated in Fig. 1 revealed the variations in loose smut incidence (LSI) among cultivars ranged from 5 to 70%. The most affected cultivar among the tested Egyptian wheat cultivar was Sakha-93 which recorded 60% LSI, followed by Giza-168 (42.1%), and Misr-2 (34.28%). While Egyptian cultivar Misr-3 was the least affected with a disease incidence of 5%, followed by Gemmeiza-5 and N-95 (12.50% each). The susceptible check cultivar (Diamant) recorded the highest value of LSI with 70%, while the resistant check cultivar (Biggar) recorded only 5% LSI. Diamant is a loose smut differential line (D-6) from the former Soviet Union and susceptible to most races of the loose smut pathogen (Nielsen & Tikhomirov, 1993). It has been used as a susceptible check cultivar for loose smut pathology studies for over 30 years (Thambugala et al., 2020). The Biggar cultivar is a Canada Prairie Spring Red wheat that carries the resistance gene *Ut-X* to the loose smut race T10 (Procunier et al., 1997). Based on the loose smut incidence (%) results, the tested wheat cultivars were classified into resistant and susceptible classes according to (Nielsen, 1987; Kassa et al., 2014), which considered wheat cultivars with 0–10% LSI as resistant and wheat cultivars with > 10% LSI as susceptible. Data showed that all tested Egyptian wheat cultivars were susceptible to the disease with LSI values > 10%, except cultivar Misr-3 which exhibited resistance to the disease with only 5% LSI. Little data are available on the genetic resistance to loose smut of wheat that has not yet been studied in Egypt.

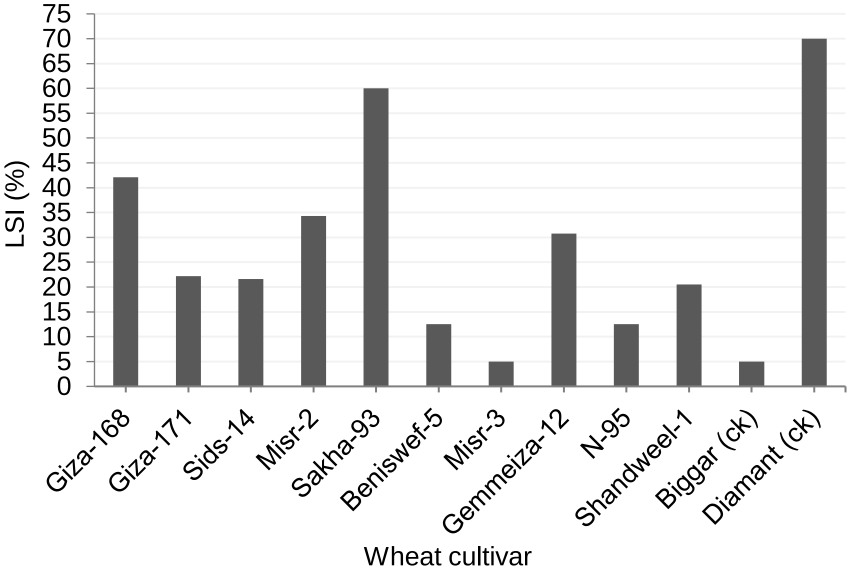

Figure 1. Loose smut incidence (%) in Egyptian spring wheat cultivars and check cultivars, Biggar (resistant) and Diamant (susceptible) in 2019/20 field growing season affected with artificial inoculation with a mixture of *Ustilago tritici* races, including T10.

Molecular detection of *Ut-X* and alleles associated

The tested wheat cultivars were screened by the SCAR marker (Xcrc.4.2) closely linked to the loose smut resistance gene *Ut-X*. The particular SCAR primer at an annealing temperature of 68 °C amplified intense DNA products with two alleles at PCR fragments sizes of ≃ 200 and 800-bp in different cultivars (Fig. 2). A favorable allele with 800-bp was generated in only one Egyptian cultivar ‘Misr-3’ and the resistant check cultivar ‘Biggar’. While, the other allele ≃ 200-bp was generated in seven Egyptian cultivars, Giza-168, Giza-171, Misr-2, Sakha-93, Gemmeiza12, N-95, and Shandweel-1. No amplification products were observed in two cultivars, Sids-14 and Beniswef-5. These findings indicated that the loose smut resistance gene *Ut-X* was present only in resistant Egyptian cultivar Misr-3 (allele 800-bp), while it was absent in nine susceptible Egyptian cultivars (≃ 200-bp or NIL). A loose smut resistance gene *Ut-X* to *U. tritici* race T10 was found to be located on chromosome 2B in "Chinese Spring" wheat using varietal substitution lines (Bernier et al., 1995). The use of longer and specific SCAR primers allows for a more robust PCR reaction and eliminates the multiple banding pattern which increases the advantages of its use over RAPD markers (Cao et al., 2001**)**. The SCAR marker (Xcrc4.2 locus) has been previously reported as a single genetic locus linked (14 cm) to the *Ut-X* locus at 800-bp (Procunier et al., 1997). In the current study, an allele of (≃ 200-bp) was amplified by the SCAR marker (Xcrc4.2) in susceptible cultivars. Also, obtained results revealed that *Ut-X* is an effective resistance gene against local *U. tritici* races, including T10 which should be considered in the breeding program to control the disease. Given the pedigree information, the origin of the loose smut resistance gene *Ut-X* in the cultivar Biggar derived from TOBARI-66/ROMANY-66, is unknown (Procunier et al., 1997**)**. However, the Egyptian cultivar Misr-3 derived from Rolf-07*2/Kiritati which has TOBARI-66 in previous crosses of Kiritati. Therefore, TOBARI-66 may be the origin of the loose smut resistance gene *Ut-X* in both cultivars, Biggar and Misr-3.

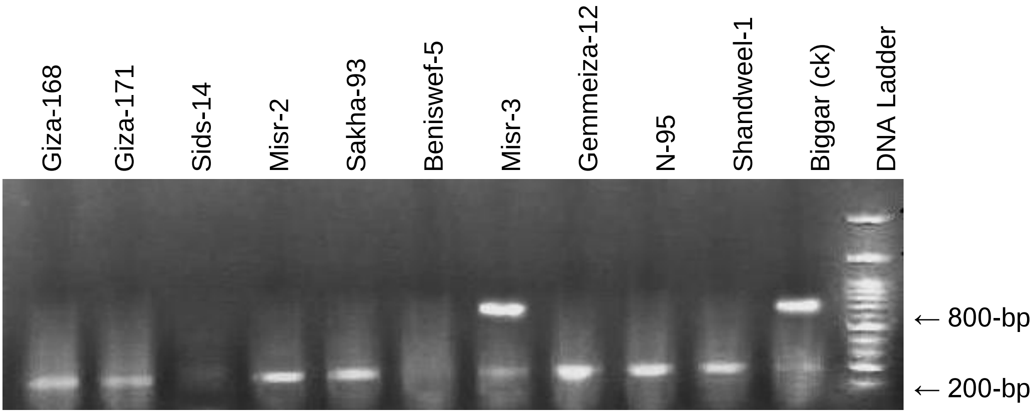

Figure 2. PCR amplification products generated by the SCAR marker (Xcrc4.2) linked to loose smut resistance gene *Ut-X* in Egyptian spring wheat cultivars and resistant check cultivar Biggar carrying *Ut-X*.

The majority of resistance studies carried out so far have also indicated a simple genetic basis for loose smut resistance, with resistance being governed by major genes (Knose et al., 2001; Thambugala et al., 2020). Biggar carries the race-specific resistance gene *Ut-X*. This gene mapped to the distal end of chromosome arm 2BL and conditioned resistance to *U. tritici* race T10 (Procunier et al., 1997). The broad loose smut resistance in the differential wheat line TD-14 (Sonop) is caused by multiple resistance loci (Thambugala et al., 2020**)**. The SCAR flanking marker (Xcrc4.2) linked to a loose smut resistance gene *Ut-X* with resistant allele (800-bp) and susceptible allele (≃ 200-bp) would facilitate the pyramiding of other resistance genes and eliminate the time-consuming progeny testing of individual plants in a breeding program. Also, these markers can be used on seedlings, thus avoiding the lengthy two-generation disease testing time.

CONCLUSIONS

This is the first attempt to determine the genes of resistance to loose smut in Egyptian spring wheat. We identified a major loose smut resistance gene *Ut-X* in Egyptian cultivar Misr-3. *Ut-X* confers resistance to local *U. tritici* races, including T10. In addition, the study documented the first report to characterize the SCAR marker (Xcrc4.2) linked to *Ut-X* with two alleles that have the potential for use in marker-assisted selection in spring wheat breeding programs. Further studies based on quantitative trait locus (QTL) mapping to identify a major QTL controlling LSI in the *Ut-X* gene contributing multiple alleles are in demand.

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Features of bird-cherry that inhibits the breeding of the population *Rhopalosiphum padi* (L.) (Hemiptera: Sternorrhyncha: Aphididae)

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Abstract. Long-term studies (2012–2018) of the formation of the *Rhopalosiphum padi* (L.) heteroecious population (number of remigrants, oviparae and eggs in autumn, number of perished eggs, fundatrices larvae, timing of flight of emigrants in spring) on the two samples of primary host *Padus avium* Mill. and *P. avium* x *P. virginiana* (L.) characterized by various morpho-physiological features have been carried out. By the methods of mathematical statistics, it has been proved that this formation occurs under the significant influence of the year conditions (A), host plant characteristics (B) and these factors interaction (AB). The most visible was their highly significant impact on the number of wintering aphid eggs ($A = 200.42$; $B = 142.6$; $AB = 25.612$). Plant characteristics such as significantly larger leaf area from the middle part of the shoot on *P. avium* x *P. virginiana*, than on *P. avium* ($t = 8.37$; $p_{0.01} = 2.85$) was important for attracting remigrants and the prone buds on *P. avium* x *P. virginiana* - for oviposition and survival of eggs. Correlation between the period of leaf fall and number of aphid eggs on both bird-cherry samples is presented. The effect of weather conditions on *R. padi* and primary hosts relationships is discussed. Correlation ($r = 0.76$; $p = 0.0048$) between average temperature of October and eggs number on *P. avium* is given. *R.* fundatrices.

Key words: gynoparae, oviparae, eggs, fundatrices, emigrants, buds, host-plant, defoliation, year conditions.

INTRODUCTION

Showy flowering, ecological plasticity (widespread, frost resistance, resistance to environmental pollution, drought resistance), medicinal and nutritional value of *Padus* Mill. species attracts the attention of crop producers and breeders (Bezmenova et al., 2010; Simagin & Lokteva, 2012; Kishchenko, 2017). *P. avium* Mill and *P. virginiana* L. are easily interbred and are most widely used for breeding in Russia (Simagin & Lokteva, 2015). *P. maackii* (Ruor.), *P. ssiory* (Fr., Schmidt) C. K. Scneid, *P. serotina* (EhR.) Agardh. are also used in landscaping. In the European part of Russia, *Rhopalosiphum padi* (L.) belongs to the most economically important species of cereal aphids. As heteroecious (host-alternating) species, *R. padi* not only reduces the quantity and quality of grain crops harvest - summer hosts of aphids, but also affects ornamentality and fruiting of bird-cherry, which is its winter host. In Europe, *R. padi* predominantly inhabits *P. avium.*, in North America - *P. virginiana* and *P. pensylvanica* Loisel (Finlay & Luck, 2011). Aphids winter in the egg phase. In spring the fundatrices come out of the eggs, their numerous offspring causes twisting and yellowing of bird-cherry leaves, which persists until the defoliation even after complete aphids emigration to grasses. Despite the large number of works devoted to *R. padi,* the bird-cherry resistance to this pest remains unclarified. Current global climate changes increase the study relevance of trophic phytophages bonds including aphids with high ecological plasticity (Harrington et al., 2007).

The purpose of the study is the search for the bird-cherry characteristics limiting the number of *R. padi*.

MATERIALS AND METHODS

Perennial study (2011–2019) of the settlement terms and the generations number of *R. padi* in autumn (remigrants – gynoparae and males and sexual females - oviparae), as well as development of eggs and fundatrices in spring on agrestic specimen *P. avium* (7 trees) and *P. avium* x *P. virginiana* (5 trees) in conditions of similar pest flight background not far from each other (St. Petersburg-Pushkin; 59°43´N. 30°25´E) was carried out. The bird-cherry specimens differed from one another by morphological and vegetation periods. The trees (not less 25 years old) were the same every year. Eggs and aphid count in spring was carried out on not less than 100 annual shoots from various crown parts on each of the trees. The shoots were randomly selected and marked. The first counts were made before fundatrices hatching on the buds and later on the leaves after the start of their reproduction. The quantity of remigrants and oviparae was monitored in autumn on the leaves of at least 100 annual shoots randomly selected on the same trees. The accounting results were recalculated by 100 record buds or leaves to standardize data. Observations were carried out once a week until the end of leaf fall. Bird-cherry phenophases passage dates were noted, which are associated with periods of seasonal aphids' migration, egg laying and wintering generation release were noted. The lamina area and leaf buds' length from the middle part of the shoot were determined 20 times on each tree. The timing of aphids' emigration on grasses was determined on 15 trees of *P. avium* and *P. avium* x *P. virginiana* growing in Saint-Petersburg and in Leningrad oblast (2012–2018). In autumn 2019 due to technical reasons the accounting was made only for *P. avium* (all trees *P. avium* x *P. virginiana* were chopped down).

Information characterizing the course of environmental conditions during the research period are taken from web-sites http://weatherarchive.ru (temperature) and http://www.pogodaiklimat.ru (precipitation=rainfall). Some meteorological parameters that are particularly important for data analysis are given in Table 1.

The experiments results were processed with ANOVA using Statistica. *R.* The data obtained was described statistically by making a two-factor analyses of variance. The influence of temperature on *R. padi* eggs laying was assessed by Polynomial regression. The F (Fisher's) test was applied. Significance of differences between the mean values was determined using student's t-test.

Table 1. Selected meteorological data characterizing the years of research

Year	Month	Air temperature, °C						Rainfall, mm				
		Decade			Max. (date)	Min. (date)	Average of month	Decade			Max (date)	Total
		I	II	III				I	II	III		
2014	April	-0.3	5.7	8.7	20.0 (21)	-6.0 (2)	4.7	3.9	4.8	1.0	3.0 (6; 14)	9.7
	May	6.4	13.4	16.5	25.8 (20)	-1.0 (3)	12.1	14.0	13.7	64.7	32.0 (28)	92.4
	June	17.7	12.3	12.0	30.0 (6)	4.0 (18)	14.0	21.4	25.6	24.1	20.0 (30)	71.1
2016	April	4.9	5.3	7.4	18.0 (28)	-4.0 (4)	5.9	8.0	26.3	34.6	12.0 (29)	68.9
	May	13.9	11.1	14.6	18.3 (6)	3.0 (12)	13.2	0.0	2.3	28.0	15.0 (27)	30.3
	June	12.5	14.7	18.5	27.0 (26)	4.0 (7)	15.2	11.8	57.4	21.9	28.0 (16)	91.1
2017	April	4.6	-0.2	4.4	15.0 (10)	-9.0 (19)	2.9	13.8	18.1	55.2	15.0 (23)	87.1
	May	5.6	9.8	12.6	24.0 (19)	-2.0 (10)	9.3	1.0	1.1	11.3	7.0 (31)	13.4
	June	11.4	15.2	14.1	24.4 (17)	3.4 (2)	13.6	17.6	26.3	24.6	13.0 (21)	68.5
2011	September	14.6	13.1	11.7	21.2 (6)	6.2 (26)	13.1	28.4	45.0	43	26.2 (20)	116.4
	October	10.0	6.1	6.8	15.2 (1; 7)	-0.8 (15)	7.6	17.4	26.6	1.0	14.1 (12)	15.0
	November	5.8						0.9			0.9 (7)	
2013	September	14.7	13.8	7.8	22.1 (1)	2.0 (30)	12.1	8.0	0.0	47.2	20.0 (23)	55.2
	October	9.0	5.7	7.1	13.9 (5; 9; 10)	-1.5 (22)	7.3	7.6	8.0	53.4	9.0 (27)	69.0
	November	6.7						40.1			16.6 (4)	
2017	September	13.1	13.1	11.2	22.3 (12)	6.2 (28)	12.5	12.1	54.1	0.6	21.0 (13)	66.8
	October	8.6	7.2	1.4	11.7 (10;16;17)	-2.0 (23)	5.7	44.5	35.8	12.4	20.0 (9)	92.7
	November	2.8						3.0			2.0 (7)	
2018	September	18.0	14.9	10.6	27.0 (7)	1.1 (29)	14.5	10.6	38.6	19.8	14.0 (26)	69.0
	October	8.0	10.9	3.4	19.1 (15)	-3.0 (30)	7.4	16.1	0.2	10.5	5.0 (5)	26.8
	November	6.0						0.4				

RESULTS

The characteristics of bird-cherry samples included the duration of vegetation phases, location of buds on the shoot, bud length and leaf area from the middle of the branch and the presence of underbrush (Table 2–3).

Table 2. Characteristics of the bird-cherry samples

Sample	Vegetation times	Location of buds on the shoot	Bud length, mm*	Leaf area (cm^2)**	Presence of underbush
			from the middle of the shoot		
Padus avium x *P. virginiana*	late ripening (4–14 days later)	prone	10.1 ± 0.04 (a)	155.7 ± 7.3 (c)	lack
P. avium	mid ripening	erect	9.2 ± 0.1 (b)	114.9 ± 5.5 (d)	presence

Note: *– significant difference: t = 4.47; $p_{0.01}$ = 2.85 (a–b); **– significant difference: t = 8.37; $p_{0.01}$ = 2.85 (c–d).

We showed that in autumn gynoparae were the first to appear on both bird-cherry specimens, which further produce oviparae larvae. The earliest appearance of gynoparae was noted in the last decade of August, the latest - in early September. Usually males arrive 1–2 weeks later (Table 3) when the first oviparae mature.

Table 3. *Rhopalosiphum padi* (L.) development conjugation with primary hosts (2011–2018)

R. padi	Date *	*p. Padus*	Date	
Indicator		Indicator	*P. avium*	*P. avium* x *P. virginiana*
Fundatrices coming out of eggs	(30.03–15.04) - (14.04–21.04)	The beginning of the bud scale separation - appearance of green cones - budding	28.03–18.04	4.04–25.04
The beginning of the founder's reproductions	18.04–18.05	White buds - beginning of flowering	5.05–10.05	9.05–15.05
Timeline of emigration	(15.05–10.06) - (6.06–28.06)	Completion of flowering	10.05–1.06	17.05–20.06
Gynoparae remigration start	21.08–8.09	Timeline of the defoliation	(9.09–24.09) - (10.10–29.10)	(14.09–12.10) - (16.10–6.11)
The beginning of males' remigration	4.09–18.09			
Egg appearance	15.09–6.11			

* Note: brackets – phenoamplitude of the beginning and the end of the period represented in Indicator during years study.

The peak of remigrants (gynoparae and males) flight is usually observed in the second half of September-early October. The eggs appeared from the beginning to the end of defoliation, sometimes several days longer while the oviparae were alive. The formation of all the identified indicators was highly significantly influenced by the characteristics of the host plant, the years of research (excluding the mortality of the fundatrices larvae I–II age), as well as the interaction of both factors (Tables 4, 5).

Table 4. Two-factor analysis (Fisher's test) of number of remigrants, oviparae, eggs and dead fundatrices larvae I–II age in the years (A) and features of the bird-cherry samples (B)

Factor		Remigrants/100 leaves means ± SD	Oviparae/100 leaves means ± SD	Eggs/100 buds means ± SD	Dead fundatrices larvae I–II age, % means ± SD
A	B				
Factor A (years)					
1		74.8 ± 12.6	176.3 ± 43.9	281.5 ± 34.3	28.6 ± 2.3
2		128.1 ± 19.3	383.6 ± 51.8	65.7 ± 6.3	41.5 ± 4.1
3		29.2 ± 4.9	45.1 ± 4.6	87.4 ± 12.9	39.4 ± 4.5
4		47.4 ± 5.6	185.9 ± 17.9	54.0 ± 4.7	40.2 ± 4.2
5		60.5 ± 6.4	179.0 ± 11.3	64.2 ± 7.7	77.9 ± 3.9
6		70.5 ± 12.9	228.3 ± 16.4	47.2 ± 6.1	24.2 ± 2.3
7		8.3 ± 1.1	32.1 ± 39.0	28.5 ± 5.7	
8				10.9 ± 1.7	
Factor B (bird-cherry samples)					
	1	81.2 ± 9.6	223.9 ± 27.1	105.2 ± 17.4	41,4 ± 4,1
	2	38.5 ± 3.9	127.6 ± 15.4	54.6 ± 8.6	42.5 ± 3.5
Interaction AB					
1	1	110.4 ± 8.6	300.2 ± 30.8	379.8 ± 16.7	24.6 ± 2.3
1	2	39.2 ± 3.7	52.4 ± 6.1	183.2 ± 13.2	32.7 ± 3.3
2	1	184.0 ± 8.7	514.0 ± 56.1	80.8 ± 5.5	36.3 ± 5.8
2	2	72.2 ± 6.7	253.2 ± 20.1	50.6 ± 5.8	46.7 ± 5.2

Table 4 (continued)

3	1	38.2 ± 5.8	54.0 ± 5.0	114.8 ± 14.2	49.1 ± 5.4
3	2	20.2 ± 6.1	36.2 ± 5.5	60.0 ± 13.3	29.5 ± 3.9
4	1	59.2 ± 7.6	232.0 ± 15.3	64.6 ± 5.6	34.6 ± 5.9
4	2	35.6 ± 4.1	139.8 ± 12.4	43.4 ± 3.7	45.7 ± 5.4
5	1	61.2 ± 10.3	184.0 ± 15.1	81.7 ± 6.3	80.8 ± 5.4
5	2	59.8 ± 8.9	174.0 ± 18.4	46.7 ± 8.7	75.0 ± 5.7
6	1	105.0 ± 12.0	242.0 ± 25.1	64.5 ± 3.3	23.1 ± 3.3
6	2	36.0 ± 4.1	214.6 ± 21.9	30.04 ± 2.8	25.1 ± 3.4
7	1	10.2 ± 1.7	41.2 ± 4.4	41.1 ± 7.4	
7	2	6.4 ± 0.9	23.0 ± 2.8	15.8 ± 3.2	
8	1			14.2 ± 2.2	
8	2			7.7 ± 1.5	

Note: A (1 – 2012, 2 – 2013, 3 – 2014, 4 – 2015, 5 – 2016, 6 – 2017, 7 – 2018, 8 – 2019 years); B (1 – *Padus avium* x *P. virginiana*, 2 – *P. avium*).

Table 5. Results of two-factor analysis (Fisher's test) of number of remigrants, oviparae, eggs and dead fundatrices larvae I–II age in the years (A) and features of the bird-cherry samples (B)

Factor	Remigrants/100 leafs		Oviparae/100 leafs		Eggs/100 buds		Dead fundatrices larvae I–II age	
	F	p	F	p	F	p	F	p
A	58.149	0	58.505	0	200.42	0	32.072	0
B	127.18	0	68.016	0	142.46	0	0.1505	0.69977
AB	17.405	0	13.003	0	25.612	0	3.1948	0.01436

At the same time, the influence of the host plant on the remigrants and oviparae number during its peak was higher than the dependence on the year. It was noted that the number of *R. padi* eggs were most influenced by both factors and their interaction. The influence of the bird-cherry sample on the remigrants number (Table 4–5) may be caused by leaf area. The lager leaves of the hybrid (Table 2) attracted aphids more strongly than the leaves of *P. avium*. It was shown that the number of eggs was determined by the timing of the leaf fall end. Late leaf fall was more favorable for oviparae oviposition on both bird-cherry samples (Fig. 1–2). On *P. avium* x *P. virginiana* buds, oviparae have always laid more eggs than on *P. avium*.

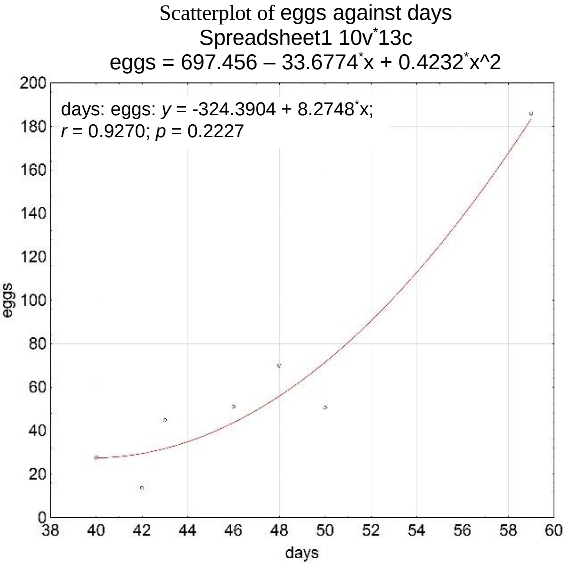

Figure 1. Correlation between the duration of leaf fall on *P. avium* and *R. padi* laid eggs.

In general, later leaf fall on *P. avium* x *P. virginiana* (16.10–6.11) than on *P. avium* (10–25.10) was leading to lager eggs on first sample (Table 4–5). In particular, autumn 2011 was characterized by a very late leaf fall (up to 6.11), which resulted in the maximum of eggs in spring 2012. Egg numbers declined in subsequent years. We determined the dependence between the average daily temperatures for October and the number of eggs (Fig. 3).

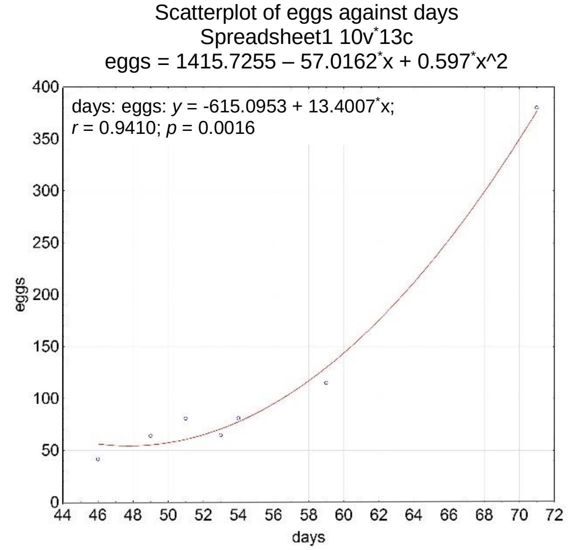

Figure 2. Correlation between the duration of leaf fall on *P. avium* x *P. virginiana* and *R. padi* laid eggs.

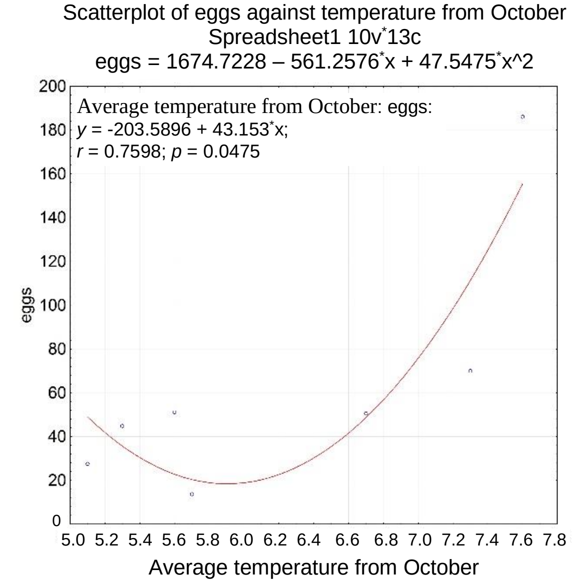

Figure 3. Correlation between the average temperature from October and *R. padi* eggs number on *P. avium.*

In 2012–2017, the number of laid eggs was significantly higher near each populated prone and longer *P. avium* x *P. virginiana* buds (Table 2), than near erected and shorter *P. avium* ones (4.3 ± 0.91 and 2.05 ± 0.44 eggs/populated bud, respectively: $t = 2.47$; $p_{0.05} = 2.45$). After 2012, the number of aphid eggs decreased and was the lowest in 2017–2018 (Table 4). Possible reasons could include a lot of spiders (up to 20 individuals/100 shoots in autumn 2013–2014), as well as urbanization. Over the research years in spring, eggs generally died to a greater extent near buds erect from shoots than those prone (14.5 ± 1.4 and 7.8 ± 1.2 respectively $t = 3.72$; $p_{0.01} = 3.50$).

However, in 2017, when the amount of April precipitation was 87.1 mm - 278% of the norm (Reference and information portal ‘Weather and Climate’), the number of dead eggs along prone buds was higher (7.9 ± 0.3 and 0.61 ± 0.13 respectively $t = 12.42$; $p_{0.001} = 5.96$) due to their soaking. The fundatricesfundatrices hatch from eggs and their future fate were largely determined by the conjugation of insect development with the host plant phenology (Table 3). Vegetation of *P. avium* is known to begin at temperatures above 0 °C, and *P. virginiana* - at +5.2 °C (Kishchenko, 2017). In hybrids, the threshold temperature can vary, but is always higher than that of *P. avium.* The threshold temperature for the development of *R. padi* is considered +4.5°

(Bokina, 2009). The first fundatricesfundatrices’ larvae may massively die if they hatch from eggs before the appearance of green cones and budding on *P. avium* x *P. virginiana*, as happened in 2014. (Fig. 4).

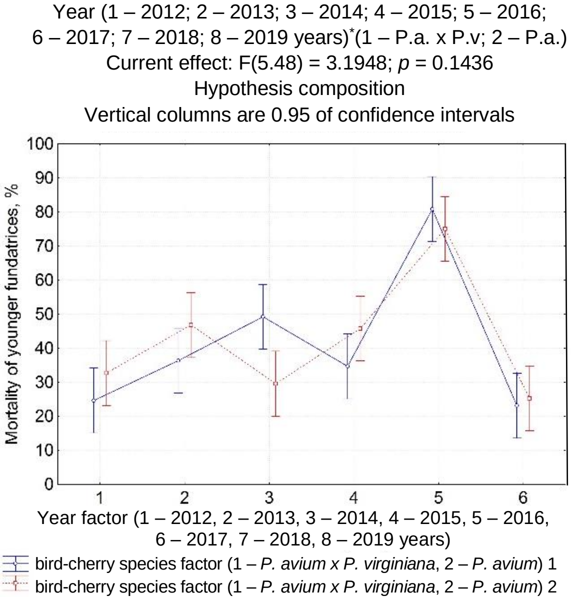

Figure 4. Number (%) of dead fundatrices larvae I–II age *Rhopalosiphum padi* (L.).

High mortality of younger fundatrices larvae on *P. avium* x *P. virginiana* in 2017–2018 was associated with low temperatures, excessive precipitation in April and delayed budding, which is essential not only for feeding, but also for the fundatrices larvae shelter. In 2015–2016, fundatrices larvae on *P. avium* survived to a lesser extent. fundatrices.

The ambiguous influence of the bird-cherry sample on the death of fundatrices larvae in different years became the reason for its statistical insignificancy (Table 5).

The fundatrices reproduction beginning and the increase in the colonies’ population coincides with the phase of ‘white buds’ and the beginning of bird-cherry flowering. The duration and intensity of emigration is largely determined by the timing of the host's flowering (Table 3). As a rule, the emigrants flight began in the second half of May and ended in the first decade of June. The deadline for the end of emigration was marked in 2017 and was associated with weather conditions (see above). The duration of emigrants’ flight in different years was 17–39 days. With the end of flowering, aphids leave bird-cherry, although they can reproduce throught the season on the underbrush.

DISCUSSION

Heterecia (seasonal change of host) in aphids life cycles developed in the process of long-term joint phylogenesis with plants. In the typical case, trees or shrubs were more ancient (primary), secondary - grasses (Shaposhnikov, 1987; Williams & Dixon, 2007) - very systematically distant from each other plants. According to the modern classification, they are often included not only in different orders, but also classes (APG III, 2009). Heterecia includes spring aphids’ emigration to grasses and autumn remigration to the primary host - the only one on which the first overwintered generation (fundatrices) can survive. It can be assumed that the ability of woody plants to survive from year to year in mass aphids breeding conditions is the result of prolonged strict selection accompanied by their self-protection formation (Aphids on the world’s plants. An online identification and information guide). The basis for selection was trophic relationships of hosts and phytophages. Aphids are characterized by feeding in areas of inflow or outflow of assimilates, i.e. on growing or aging plants (Dixon, 1998). Thus, the very emergence of heterecia is associated with the onset of a period unfavorable for aphids feeding, when active seasonal growth of the woody host ends. On the contrary, aphids' autumn remigration coincides with the period of leaf life end. To have time to lay their eggs, aphids begin to leave the grasses long before the defoliation start. The limited range of primary hosts, their large size, distance from grasses, clonal differences in the timing of remigrations in aphids contribute to sexes meeting and prevent inbreeding. Often, remigrants guided by the smell of plant leaves. It was no coincidence that the large leaves of *P. avium* x *P. virginiana* were more attractive to gynoparae than the smaller ones - *P. avium*. Males appeared later. It is assumed that they are also guided byoviparae pheromones and volatile substances associated with aphid aggregation (Pickett & Glinwood, 2007). Other authors note variants where *R. padi* males appear earlier than gynoparae and may be attracted only by the plant (Austin et al., 1996). However, early appearance of males is not biologically justified, as the preimaginal period in oviparae is 7–11 days, and life expectancy from 19 to 47 days is enough for the sexes to meet (Vereschagina & Gandrabur, 2016). Oviparae have been shown to be able to anticipate defoliation and migrate in time (Glinwood et al., 2003), but they cannot control windy weather and massively fall with leaves, especially on *P. avium* with early growing terms.

Long prone buds on hybrid shoots are also more favorable for laying eggs as eggs are laid along the buds. The overall decline in egg numbers over the years of research may have occurred not only due to rapid defoliation due to cold and windy weather, but also the abundance of entomophages, especially spiders, elimination of habitats of aphids agrestic hosts as a result of large-scale construction, increase of the power lines length, placement of cell towers, as well as other external and intra-population factors.

In the spring, the fundatricesfundatrices hatch from eggs before budding leads to high larvae mortality of younger larvaeas we observed on the late ripe *P. avium* x *P. virginiana*. Hence, it is necessary to clarify the forecast of the pest population based on the number of eggs without considering the death of the overwintered generation. The fundatrices begin reproduction with the appearance of white buds on bird-cherry, by mass flowering the colonies population rapidly increases, the first emigrants appear. Simultaneously with the end of flowering, bird-cherry ends active growth of leaves and inflorescences (Kishchenko, 2017), the decrease in the flow of assimilates to available feeding places combined with the high number of aphids cause them to complete emigration. It is possible that the damaged bird-cherry may release substances that contribute to aphids' emigration (Pickett & Glinwood, 2007), but insects did not move to intact leaves or between trees during the flowering phase. Late and prolonged bird-cherry flowering increases the duration of the aphids' flight, as at this time aphids feed mainly on inflorescences as we observed on both bird-cherry specimens. Due to different reactivity of plants and insects on global climatic changes there are significant disturbances in the conjugation of their phenologies. The likely adverse effects of such disturbances can be reduced by revising the pest monitoring methods and the use of resistant plants.

CONCLUSION

Based on long-term comparative study on the development of *R. padi* on *P. avium* and *P. avium* x *P. virginiana*, characterized by different morpho-physiological features (statistically established), it was shown that later vegetation onset, rapid passage of vulnerable development phases (before flowering end), early leaf fall, as well as lack of underbrush, buds erection from the shoot, small laminas deter reproduction of aphids on the bird-cherry.

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Evaluation of soil properties, irrigation and solid waste application levels on Cu and Zn uptake by industrial hemp

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Abstract. A three-year experiment was performed to study the alteration of copper and zinc levels in industrial hemp grown in different soils using elevated sewage slurry solid waste applications. Two soil samples, an acidic and an alkaline one, with different soil properties, such as percentage of $CaCO_3$ and cation exchange capacity values, were used. Three treatments of waste solid with provided elevated concentrations of Cu and Zn were combined with two irrigation levels. The application of high doses of the solid residue as well as high irrigation level lead to an increase of the mobility of metals in hemp leaves in acidic soil in contrast to alkaline. On the contrary, in alkaline soil along with a reduced irrigation level, there is a decrease in the mobility of Cu and therefore its accumulation in the roots or stems was observed. Concluding, hemp seem to be a promising plant remediator, after the application of the proper irrigation level and taking into account the physico-chemical soil properties of moderately contaminated (with copper and zinc) soils.

Key words: Heavy metals, *Cannabis sativa*, sewage sludge, phytoremediation.

INTRODUCTION

Industrial hemp (*Cannabis sativa subsp. Sativa)* is an annual herb cultivated for versatile industrial purposes such as the production of natural constructive and insulating materials from the fibres or making paints, varnishes and soaps from the oil contained in seeds. Industrial hemp seeds, high in protein and oil, are also edible and are considered a healthy food supplement (Wogiatzi et al., 2019). Industrial hemp has also outstanding features like high biomass yield in relatively short time, biomass of high calorific value and low input requirements making it suitable as an energy crop (Kolarikova et al., 2015).

During the last decades heavy metal contamination of agricultural soils is one of the most important environmental problems (Kabata-Pendias, 2011; Alloway, 2013; Murtic et al., 2019). Heavy metal present and persistance nature may have dangerous effects on wild life and human life as well (Craven et al., 2019). On the other hand, phytoremediation technologies (such as phytostabilization, phytodegradation, phytoextraction and phytovolatilization) use plants and wild trees to ameliorate contaminated soils (Citterio et al., 2003; Murtic et al., 2019). Industrial hemp could be a potential soil phytoremediation agent, as it is a tall plant with about one-meter-deep roots that grows fast and easily in dense stands, with high biomass production potential and extracts heavy metals (Moghaddam et al., 2020).

Sewage sludge is a solid waste by-product obtained from urban and industrial wastewater treatment plants. Traditionally it is disposed on landfills either as raw material or after incineration. Its high nutrient concentration (N, P, S, Fe, Mg and Ca) however, evoked the challenge to use it as an agricultural amendment to improve soil fertility and soil structure (Breda et al., 2020). Sewage sludge however, contains also potentially toxic compounds, such as heavy metals and persistent organic pollutants. Long term use as a soil amendment may impose serious risks of heavy metal soil contamination (Singh & Agrawal, 2008; Praspaliauskasa et al., 2020). The application of sewage sludge, produced by the municipality of Volos and Almyros, in industrial hemp plants cultivation, may be a good and cheap alternative to the fertilizer’s application improving soil quality (Lazdina et al., 2011; Zielonka et al., 2020).

Moreover, there is a lack of knowledge regarding industrial hemp mechanisms for accumulation and tolerance to high or moderate contaminant concentrations in the soil (Safari Singani & Ahmadi, 2012; Zerihun et al., 2015). Futhermore, it is well known that industrial hemp is a crop with high water demands and irrigation is essential in low rainfall environments such as southern Europe (Amaducci et al., 2015). Irrigation regimes play an important role in heavy metals mobility into the soil enhancing their leaching to grounwaters (Haykiri-Acma et al., 2011; Ahmad et al., 2018; Azouzi et al., 2019). Soil moisture levels along with pH and organic matter values are crucial in accumulation of heavy metals (Stafford et al., 2018). Enhanced heavy metal absorption was noticed under alternate irrigation (wetting and drying) by Song et al., 2021, while metal absorption by plants is increased under ample soil water regimes (Angle et al., 2003; Marchiol et al., 2007; Wogiatzi et al., 2019).

The aim of the present study was to evaluate the ability of industrial hemp to extract copper and zinc from different soil types, dressed with different doses of manucipalty sewage sludge and therefore, achieving different concentrations of the heavy metals. The effect of the soil water regime was also considered by introducing two alternative irrigation treatments.

MATERIALS AND METHODS

The experiment was conducted at the farm of University of Thessaly from 2018–2020. The farm is located near the Athens-Thessaloniki National Road, in the area of Velestino, Magnesia (39° 23′ 44″ N, 22° 45′ 26″ E), at an altitude of 70 m above sea level and covers an area of 15 hectares. First seeding took place in April 2018 applying industrial hemp (*Cannabis sativa subsp. sativa*) seed cultivar ‘Futura 75’. Due to the non-uniform emergence in few experimental plots and in order to achieve the same population in all plots, the plants were diluted to a population of approximately 90 plants m^{-2}. The mature plants were harvested about 16 weeks later, with an average stem height of 125 cm. Soil 1 (S1) is the native of the farm, while for soil 2 (S2) a mixture of farm soil with soil collected from cultivated fields at the immediate vicinity of the industrial area of Volos was used in 1:3 ratios. The waste solid residue is part of the sludge obtained by the local Biological Treatment Industry (Municipality of Volos and Almyros). The plant operates around 30,000 m^3 per day of raw sewage which undergoes primary physical, biological and chemical treatments including screening, thickening, dewatering, anaerobic and aerobic digestion alkaline stabilization and composting that led to a stabilized solid waste with around 90% of solids (Gudulas et al., 2007). The solid waste analyzed for its chemical, physical properties and the results are presented in Table 1. The solid waste was incorporated into the experimental plots by means of a rotary cultivator.

A uniform sprinkler irrigation was applied to all the plots immediately after sowing to facilitate crop emergence. Thereafter, a drip irrigation system was established. Drip pipes with drips at 40 cm and a capacity of 2 L h^{-1} were placed every second crop row. The pipe network was divided into two subnetworks to fulfil the needs of the experiment. The evapotranspiration (ET_f) was measured using Thornthwaite method, while field capacity, permanent wilting point, available soil moisture and critical soil moisture was calculated (Zhang et al., 2020). The above data were used to calculate the irrigation dose, while the field soil moisture was measured using TDR. When soil moisture reached critical soil moisture, an irrigation event was planned. Mean ET_f value in the farm was 435 mm. Six to eight irrigation events were performed each year, depending on the weather conditions. Totally, for the 100% ET_f treatment, 320 mm of irrigation water were applied in 2018, 450 mm in 2019 and 450 mm in 2020, while total seasonal precipitation was 262.2 mm, 108.2 mm and 108 mm respectively. Half of these doses were applied on the 50% ET_f treatment.

Three treatments of solid waste disposal (W_1 = 3.5 kg m^{-2}, W_2 = 7 kg m^{-2} and W_3 = 10 kg m^{-2} incorporated 21.8, 43.8 and 62.5 kg per plot in each treatment, respectively) providing elevated concentrations of Cu and Zn were combined with two irrigation levels ((I_1 = 50% ET_f and I_2 = 100% ET_f) over two soil types (S_1 = alkaline, S_2 = acidic). Two more treatments, one for each soil type, received 100% ET_f irrigation but no solid waste disposal and used as a control (C). As a result, a total of 3×2×2+2 = 14 treatments were founded and arranged in a complete randomized block design with three side by side replications. The plot size was 6.25 m^2 (2.5×52.5 m wide; 10 rows per plot and 56 plants per row) with a total area of the experiment including corridors and margins, of 0.04 ha. All the treatments were conducted in three replicates.

During harvest period (16 weeks after emergence), 20 plants were uprooted from the inner plot in case to avoid the border effect. Thereafter to separate the root system from the soil, the root was placed in aqueous sodium polymethosphate solution for five days for structure dissolving. Then the soil was rinsed with plenty of water and finally only the root system remained. After harvest, a partitioning of the plants took place and hemp parts were placed in a dry chamber at 50 °C until a constant weight was reached. Soil samples were collected, dried and both soil and hemp parts were analysed for Cu and Zn. Soil samples were analysed (Page et al., 1982) for: pH and electrical conductivity (EC) (1:2.5 H_2O), organic matter (Walkley and Black method), and cation exchange capacity (CEC, 1M CH_3COONH_4, pH7). The $CaCO_3$ (%) was determined using the Bernard method. Soil and hemp samples were mineralised with 3 mL of concentrated HNO_3, 3 mL of HF and 1 mL of HCl at 750 Watt, 6 MPa, in a microwave digestion system (JAOAC 1984). The analysis of the extractants prepared from the soil, roots, stems and leaves samples were performed using an ICP-OES (Inductively Coupled

Plasma - Optical Emission Spectrometry) (Thermo Scientific). Determinations in each of the analysed samples were carried out in three replications. For the data acquisition of the samples, a quantitative analysis mode was used. Certified material to test the analytical method (NIST 1573a, tomato leaves) was used.

A One-way Anova was applied to analyze the data using the SPSS-v26 suite. The LSD and Tuckey's test ($p < 0.05$) were applied for post-hoc analysis and mean separation. Graphs were built on Microsoft Excel.

RESULTS AND DISCUSSION

Table 1 shows the physico-chemical composition of the two soil samples as well as the waste solid used in the study. The soil sample of the university farm characterized as clayloam and has a pH value 7.6 (Soil 1), while soil 2 has a pH value of 5.9. The content of organic matter is rather low in both soils, a characteristic property of Greek soils that have undergone long-term tillage. Soil 1 has a low $CaCO_3$ content, while in soil 2 there was no $CaCO_3$ detected. The Cation Exchange Capacity (CEC) and clay content is higher in the 1st soil sample.

Table 1. Chemical, physical properties and Cu and Zn concentrations of control soil samples and waste solid

Treatments	Soil 1	Soil 2	Waste solid
pH (1:2.5)	7.6 ± 0.2	5.9 ± 0.3	8.4 ± 0.1
Organic matter (OM) (%)	1.6 ± 0.1	1.7 ± 0.2	24 ± 1.1
Bulk specific gravity (g cm^{-3})	1.3 ± 0.3	1.2 ± 0.4	2.3 ± 0.2
$CaCO_3$ (%)	4.8 ± 0.4	ND	9.3 ± 0.3
CEC ($cmol_c kg^{-1}$ soil)	29 ± 0.5	21 ± 0.2	52 ± 0.5
Total Cu (mg kg^{-1} dry soil-dry matter*)	4.3 ± 0.5	3.5 ± 0.3	67.4 ± 1.2*
Total Zn (mg kg^{-1} dry soil-dry matter*)	5.2 ± 0.7	4.5 ± 0.4	75.8 ± 2.1*

(*n* = 9; mean of the three years of the study ± RSD is reported; ND: not detected).

The analysis of variance showed no signifficant differences among the years so the average values are presented. Waste solid application dose has a significant ($p < 0.05$) effect on the heavy metals concentrations in the soil, as metal concentrations in the soil increased along with waste solid application dose. The highest increase in heavy metals concentrations after waste solid application was for Cu, while the effect on the Zn concentrations was less pronounced (Table 2).

Table 2. Cu and Zn composition of soils after treatments with waste solid (mean of the three years of the study). (Waste solid doses: 1–3.5, 2–7, 3–10 kg m^{-2})

		Cu	Zn
Treatments		mg kg^{-1} dry soil	
W1: 3.5 kg m^{-2}	Soil 1	8.6 ± 0.9	10.2 ± 1.1
W2: 7 kg m^{-2}	Soil 1	19.9 ± 1.3	22.4 ± 1.4
W3:10 kg m^{-2}	Soil 1	31.9 ± 2.4	35.2 ± 2.1
W1: 3.5 kg m^{-2}	Soil 2	8.3 ± 1.2	9.1 ± 2.1
W2: 7 kg m^{-2}	Soil 2	17.3 ± 0.9	19.3 ± 0.8
W3:10 kg m^{-2}	Soil 2	29.2 ± 1.4	32.4 ± 1.3
EU limits (86/278/EEC Directive) (Council European Communities, 1986)		70–140	300

Copper and Zinc concentrations range at trace levels lower than the Maximum Allowable Concentrations defined in the European Council Directive 86/278/EEC. Cummulative Cu and Zn uptake by hemp plants during the growth period (four months) is presented in Figs 1 and 2. The increase of Cu reaches at 746.4% while Zn increase is up to 613.7% in regarding control soil 2. Cu and Zn in hemp parts were higher than the initial concentrations (control soils), due to the higher content of these metals in waste solid applied in the soils. It is apparent that industrial hemp absorbs Cu and Zn in roots, stems and leaves as well, in different amounts, with respect to different irrigation levels and waste solid treatments (Wogiatzi et al., 2019). The largest amounts of Cu were accumulated in the roots and stems, while Zn was accumulated in all parts of the industrial hemp and is higher than Cu in all tissues (Moghaddam et al., 2020). In alkaline soil (S_1) (Fig. 1) the retention of both copper and zinc by the solid phase of soil is stronger. This is probably due to the high percentage of clay content and the high value of the Cation Exchange Capacity (CEC) as well. As a consequence, copper concentrations were higher in the roots than in stems or leaves of the plants. On the other hand, zinc seems to be more mobile as higher zinc concentrations were measured both in stems and leaves. Accumulated Zn in hemp was rather equally distributed among the roots, stems and leaves. The highest increase in Zn concentration was detected in leaves, both for $I_2W_3S_1$ (in soil 1, Fig. 1) and $I_2W_3S_2$ (in soil 2, Fig. 2) treatments, 269.6% and 277.6% respectively.

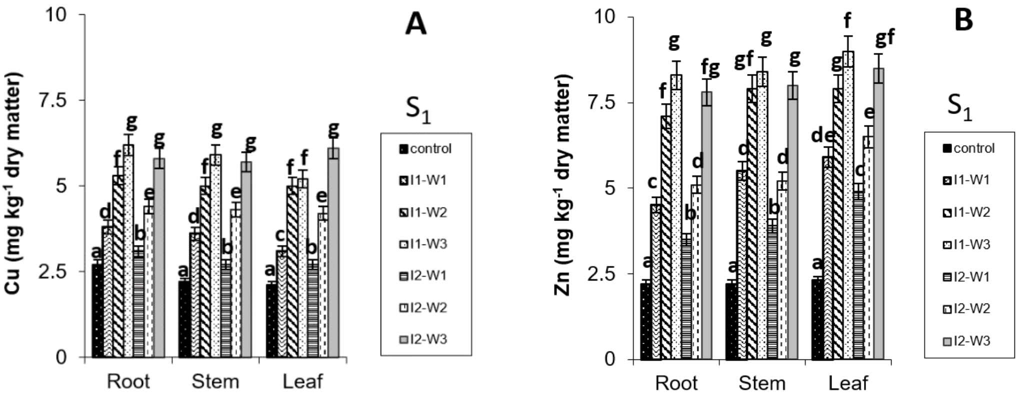

Figure 1. Mean concentrations of Cu (A) and Zn (B) in roots, stems and leaves of industrial hemp after irrigation and solid waste treatments application in alkaline soil S_1 (three years' average values) I_1: 50% ET_f, I_2: 100% ET_f, W_1: solid waste 3.5 kg m^{-2}, W_2: solid waste 7 kg m^{-2}, W_3: solid waste10 kg m^{-2}. Vertical lines at top of the bars denote LSD at $p < 0.05$, different letters 'a', 'b' 'c' e.t.c. denote a significant difference according to the post-hoc Tukey's test at $p < 0.05$.

In acid soil (S_2) the bioavailability of metals increases. This is shown in Fig. 2, where the concentrations of copper and zinc transferred from soil to plant are higher than in soil 1, despite the fact that the initial metal concentrations do not differ statistically significantly between soil 1 and soil 2 (Table 1 & 2). The increase in Cu concentration in industrial hemp was correlated to waste solid application and irrigation rates as well. The copper concentration gradually increased from 40.7% to 129.6% in the roots compared with the control (Fig. 1). Regardless of Cu low mobility, there was an increase in Cu concentration in the leaves in I_2W_3 treatment, which reached 190.5% up to control soil 1. The elevated irrigation level (I_2) enchase copper solubility and translocation of copper from soil to the leaves of the plant. Irrigation regimes influences metal mobility, accelerating cations' movement to aerial tissues (Ahmad et al., 2018). The increase of Cu concentration in the stem of the plant is also quite important, regardless the fertilization intensity. Relatively high stem Cu concentrations could be explained by metals translocation via stems (Kabata-Pendias, 2011; Alloway, 2013; Murtic et al., 2019).

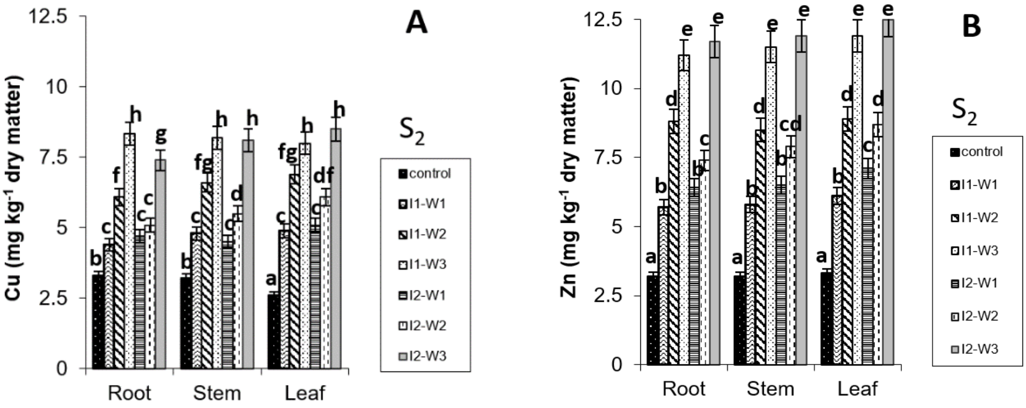

Figure 2. Mean concentrations of Cu (A) and Zn (B) in roots, stems and leaves of industrial hemp after irrigation and solid waste treatments application in acidic soil S_2 (three years' average values) I_1: 50% ET_f, I_2: 100% ET_f, W_1: solid waste 3.5 kg m^{-2}, W_2: solid waste 7 kg m^{-2}, W_3: solid waste10 kg m^{-2}. Vertical lines at top of the bars denote LSD at $p < 0.05$, different letters 'a', 'b' 'c' e.t.c. denote a significant difference according to the post-hoc Tukey's test at $p < 0.05$.

In soil 1 Cu concentation in I_1 treatment follows the order roots>stems>leaves, while in high irrigation treatment (I_2) the order changes to leaves>stem>roots. For Zn the order is leaves>stem>roots, both in acid and alkaline soil regardless the irrigation level applied. The comparison of metals accumulation from waste solids application to soils by energy plants has revealed that Zn accumulation was the highest one (Breda et al., 2020; Praspaliauskasa et al., 2020). The availability of metals in soil is influenced by soil properties, as well as water content (Azouzi et al., 2019). The chemical form of heavy metals in soil significantly affects their translocation in plant parts (Alloway, 2013; Ahmad et al., 2018). Both copper and zinc cations are soluble in soil solution and their mobility increases as the irrigation level rises. The amount of water added in combination with fertilization practices seem to be the key parameter for Zn availability and transportation from soil to plant (leaves) (Lazdina et al., 2011; Wogiatzi et al., 2019). Soil pH along with clay content are considered the most important soil factors determining the concentration of metals in the soil solution, and thus their mobility and availability to plants.

CONCLUSIONS

Concentrations of Cu and Zn in industrial hemp parts (roots, stems and leaves) are significantly affected by soil physico-chemical parameters and especially by soil pH. In the acidic soil and, after the maximum irrigation level application, Zn has sufficiently moved from the soil to the industrial hemp leaves, while when the amount of water was reduced at 50%, higher concentrations of Cu were observed in the roots and stems of the plants. In alkaline soil the mobility was lower and the movement towards the leaves was due to the increase of irrigation water level. The present 3-year study assessed that industrial hemp could be used for phytoextraction purposes of moderately contaminated soils, after controlling the amount of irrigation water added, along with the solid waste levels application.

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Introduced assessment of agrestic legumes in the middle Urals

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Abstract. Currently, in fodder production industry, there is an acute problem of finding legumes that are well adapted to the natural and climatic conditions of the Middle Urals, possessing great longevity, as well as high fodder values. In the Middle Urals, as well as in the Russian Federation, as a whole, legumes valuable for forage from wild vicia and peavine species are still not used in culture. Both of these genuses are of great practical interest for their introduction into culture. They significantly differ from legumes traditionally used in fodder production in a number of parameters: long longevity (7–10 years in natural phytocenoses), resistance to a complex of natural and climatic conditions (rather high winter hardiness), early regrowth in spring. The **purpose of the experiment** is to reveal the characteristics of the growth and development of perennial wild legumes under conditions of introduction. The **tasks of the study** included the determination of the height and average daily growth dynamics, the timing of the passage of phenological phases, and the productivity of aboveground biomass.

Research methods. The study on the introduction of agrestic legumes was carried out in the ‘Uralets’ educational and experimental farm (2005–2012), located in the Beloyarsky district of the Sverdlovsk region on the educational and experimental field of the crop production and breeding department. The experimental design includes 3 options: 1 opt. - *Vicia sylvatica* L. (forest vicia - control); 2 opt. - *Lathyrus pisiformis* L. (pea-shaped peavine); 3 opt. - *Lathyrus pratensis* L. (meadow peavine). The use of grass stand is single-cut, mowing carried out in the phase of mass flowering of the plants studied. To identify the floristic composition dynamics, a complete geobotanical description of vegetation was carried out. The following botanical composition was distinguished: introduced (cenose-forming) species: *Vicia sylvatica* L., *Lathyrus pisiformis* L., *Lathyrus pratensis* L.; non-seeded species - this group includes all wild-growing species (grasses and forbs) that have invaded the vegetation cover.

Results. During the study it was established that the earliest regrowth is characteristic of pea-shaped peavine, it significantly outpaced other types of legumes in its development. The different phyto-cenotic stability of the cenose-forming species in the grass stand was discovered, the highest observed in the meadow peavine.

The productivity of aboveground biomass in meadow peavine (by year of study) was significantly higher than in other studied species; on average for 2006–2012 it amounted to 15.3 t ha^{-1}, which is 1.9 t ha^{-1} higher than forest vicia and 4.6 t ha^{-1} more than pea-shaped peavine. Scientific novelty. For the first time, in the conditions of the Middle Urals, the growth and development features of wild perennial species from the family *Fabaceae*: *Vicia sylvatica* L., *Lathyrus pisiformis* L., *Lathyrus pratensis* L., were studied.

Key words: introduction, characteristics of growth and development, forest vicia, pea-shaped peavine, meadow peavine.

Target setting

A balanced fodder base remains the main factor in the development of animal husbandry and the efficiency of agricultural enterprises. Under the conditions of the non-chernozem zone of the Russian Federation, the greatest economic effect in the production of feed is obtained from perennial legumes cultivation, as their energy efficiency coefficient is 2.5–3 times higher than that of cereals and grain forage crops (Belyak, 2008). In addition, perennial legumes are an essential element in the creation of highly productive agrocenosis (hayfields and pastures) (Lazarev et al., 2018; Korsakova et al., 2019). They are excellent precursors for almost all field crops; enrich the soil with organic matter and biological nitrogen; they provide a fairly high productivity yield per area unit and contain native protein (Kalashnikov, 2003; Korsakova et al., 2019).

In the grassland feed industry, the range of legumes used is extremely limited. Mainly in the structure of sowing acreage, clovers (meadow clover, hybrid and their varieties) are common, which differ in a small life expectancy (Kutuzova et al., 2018; Lazarev et al., 2018). Natural and climatic conditions of the Middle Urals are very peculiar. The main factors influencing the climate of the Middle Urals are the large length of the territory from North to South, the meridional direction of the chain of mountains and ridges, the complex rugged terrain adjacent to the mountains, as well as the West-East transport of air masses prevailing over the Urals (Gafurov, 2008). Therefore, in the Middle Urals, in connection with difficult meteorological conditions, as well as in conditions of limited resource provision for the agro-industrial complex, the role of legumes in the feed industry is especially increasing. Most of the newly released varieties have high potential productivity, but under production conditions, due to poor adaptation to natural and climatic conditions, it is less than half realized (Nagibin & Tormozin, 2011; Nagibin et al., 2018). Therefore, the problem of finding legumes with great longevity and high foraging advantages is acute, well adapted to local natural and climatic conditions in the Middle Urals, as well as in the Russian Federation, in whole.

Legumes from wild species of vicia and peavine high-valued in terms of forage are still not used in culture. Both of these species are of great practical interest for introducing them into culture (Abramchuk, 2009; Abramchuk, 2013; Abramchuk, 2014a; Korsakova et al., 2019). Unlike legumes used in forage production, they have a great longevity (7–10 years in natural phytocenoses), resistance to a complex of natural and climatic conditions (rather high winter hardiness), early regrowth in the spring.

In the flora of the Middle Urals, the following perennial agrestic species are common: vicia - sepium, forest, tuberiferous, leptophyllous; peavine - pea-shaped, meadow, Gmelina, etc. The Department of Plant Production and Breeding of the Ural State Agrarian University conducts a multiple-year research on the introduction of agrestic legumes (Belyak, 2008; Abramchuk, 2009; Abramchuk, 2013; Abramchuk, 2014a; Abramchuk, 2014b).

RESEARCH METHODOLOGY AND METHODS

The study on the topic ‘Introductory assessment of agrestic legumes in the Middle Urals’ was carried out in the educational and experimental farm ‘Uralets’, located in the Beloyarsky district, Sverdlovsk region. In 2005 (September) we've conducted an experiment establishment, three wild legumes served as the objects of the study: *Vicia* *sylvatica* L. (forest vicia); *Lathyrus pisiformis* L. (pea-shaped peavine); *Lathyrus pratensis* L. (meadow peavine), widely distributed on the natural anthropogenic landscapes of the Middle Urals. Seeds from local populations collected in similar climatic conditions were used for sowing, in close proximity to the site of the study. Bare fallow was used as a precursor. Before sowing the soil was cultivated and rolled. Depth of seed sowing - 2–3 cm. The area of the plots was 10 m^2 (2 m × 5 m), the repetition is three times.

The object of the study is to identify the features of the growth and development of perennial wild legumes in crop conditions. **Research tasks:** determination of the dynamics of height and average daily growth (regularly, once a week, measurements of plant height were carried out); timing of the phenological phases was established visually. To reveal the dynamics of the botanical composition in the studied phytocenoses, a complete geobotanical description of vegetation was carried out, the following botanical composition was distinguished: introduced *(cenose-forming)* species: *Vicia sylvatica* L.; *Lathyrus pisiformis* L., *Lathyrus pratensis* L.; non-seeded species - this group includes all wild-growing species: cereals and forbs that have penetrated into the vegetation cover. The experiment included three variants distinguished by the cenolate-forming species: 1 opt. - *Vicia sylvatica* L. (forest vicia) - taken for control; 2 opt. - *Lathyrus pisiformis* L. (pea-shaped peavine); 3 opt. - *Lathyrus pratensis* L. (meadow peavine). During all years of study, grass stand was mown once per vegetation, in the mass flowering phase of studied species (2nd decade of July). The care of grass stand in the first four years (2005–2008) after sowing, was reduced to the tillage of space between rows and weeding of undesirable plants, of which the most onerous were: common dandelion, quitch-grass. Since 2009 weeding has been excluded, with only one harrowing performed annually in the phase of spring plant regrowth.

To identify the dynamics of floristic composition in the last year of the experiment (2012), a complete botanical description of vegetation was carried out with indication of abundance on the scale of Drude: sol. - plants are single; sp. - plants are rare; var. $_1$ - plants are pretty plentiful; var. $_2$. - plants are abundant; var. $_3$. - plants are very plentiful. Mathematical processing was carried out according to Dospekhov B.A. (Dospekhov, 2014).

STUDY RESULTS

In the introduction of plants into cultivation, such indicators as dynamics of height and average daily growth, which characterize the growth and development of plants during the growing period, make it possible to predict future harvest. The studied species differed quite significantly in height. At the time of harvesting (the second decade of July) the average height of plants was: forest vicia 88 cm - the highest value obtained in the experiment; meadow peavine - 70 cm; plant height of pea-shaped peavine - 59 cm. The average daily increase varied by accounting dates from 0.4 to 2.1 cm.

The earliest regrowth is noted in pea-shaped peavine, in its development it was significantly ahead of other legume species. For forest vicia, beginning of vegetation coincided with the pea-shaped peavine, but was less active; transition to the generative cycle of development (budding phase) was observed 6–8 days later than in pea-shaped peavine. The meadow peavine is characterized by slower and later regrowth from spring. The budding phase occured 4–6 days later than the forest vicia and 10–13 days later than the pea-shaped peavine. In general, the development of the studied plants went with different intensity: the earliest growth was observed in pea-shaped peavine, the maximum in its development was noted at the end of the first and beginning of the second decade of June.

One of the tasks facing the experiment was to study the dynamics of floristic composition in phytocenoses of introduced species. During the long period of observation, in the absence of weeding (weeding stopped in 2009), agrestic (non-seeded) species of plants were introduced in the grass stand. In 2012 we carried out a complete description of the vegetation, the following botanical composition was identified: introduced (*cenose-forming)* species*: Lathyrus pisiformis* L., *Lathyrus pratensis* L., *Vicia sylvatica* L.; non-seeded species- all agrestic species are included in this group: cereals introduced into the vegetation cover, such as: *Elytrigia repens* (L.) Nevski., *Poa pratensis* L., *Festuca pratensis* L., et al.; legumes: *Trifolium pratense* L., *Trifolium repens* L., *Vicia cracca* L.; the following species are the most widely distributed in the grass stand: *Achillea millefolium* L., *Alchemilla vulgare* L., *Galium boreale* L., *Galium mollugo* L., *Taraxacum officinale* Wigg et al. (Table 1).

Table 1. Floristic composition of studied phytocenoses, 2012

	Experiment options (legume types)		
Species and groups of plants	1. opt. - *Vicia sylvatica* L. (forest vicia) - control	2.opt. - *Lathyrus pisiformis* L. (pea-shaped peavine)	3.opt. - *Lathyrus pratensis* L. (meadow peavine)
Introduced species:			
	Legumes:		
Lathyrus pratensis L.	sp.	sp.	var.$_2$ - var.$_3$
Lathyrus pisiformis L.	-.	var.$_1$ - var.$_2$	-
Vicia sylvatica L.	var.$_2$	-	-
Non-seeded species:			
	Legumes:		
Trifolium pratense L.	sp.	-	sp.
Trifolium repens L.	sp.	sp.	sol.
Vicia cracca L.	sp.	sp.	sp.
	Cereals:		
Elytrigia repens (L.) Nevski.	var.$_1$. - var.$_2$	var.$_2$	var.$_1$
Festuca pratensis L.	var.$_1$	var.$_1$	var.$_1$
Festuca rubra L.	sp.	sp. - var.$_1$	sp.
Poa pratensis L.	sp. - var.$_1$	var.$_1$	sp. - var.$_1$
Phleum pretense L.	sp. - var.$_1$	sp.	sol.
	Herbs:		
Achillea millefolium L.	sp.	sp. - var.$_1$	sp.
Alchemilla vulgare L.	sp.	sp.	sp.
Carum carvi L.	sp.	sp.	sol. - sp
Galium boreale L.	sol.	-	sol. - sp
Galium mollugo L.	sol.	sol.	sol.
Glechoma hederacea L.	sol.	-	-
Linaria vulgaris Mill.	sol.	sol.- sp	-
Plantago major L.	sol.- sp.	sol.- sp	-
Plantago media L.	sol.	sol.	-
Taraxacum officinale Wigg.	sp. - var.$_1$	sp. - var.$_1$	sol.- sp
Total species (pcs.):	20	17	15

The different phyto-cenotic stability of the cenose-forming species is revealed. During the years of research, *Lathyrus pisiformis* L. was the most vulnerable, instead of 90–95% of the plant cover it had in the first years of observation, in 2012 its participation in the addition of the grass stand did not exceed 27% (var. $_1$ - var. $_2$). This option is characterized by active introduction in phytocenosis of non-seeded agrestic plants: cereals began to take a leading position, accounting for 50.5% of all aboveground phytomass; the role of the dominant moved to quitch-grass (var. $_2$). The admixture of different herbs is significant, the proportion of which was 20.5%, especially active introduction in grass stand was noted in *Achillea millefolium* L. and *Taraxacum officinale* Wigg (abundance - sp. - var. $_1$).

The most tolerant species in the experiment turned out to be meadow peavine (*Lathyrus pratensis* L), for it is characterized by high abundance - var. $_2$ -var. $_3$; in the structure of grass stand, peavine accounts for 54%. This variant has significantly lower participation of non-seeded species: cereals- 31.5%, of which the dominants are *Elytrigia repens* (L.) Nevski. - var. $_1$ and *Festuca pratensis* L. - var. $_1$; the lowest participation in grass stand of different herbs is 11.5%; the proportion of non-seeded legumes does not exceed 2.0%, they are dispersed in the grass stand. Meadow peavine better resists to introduction in grass stand of agrestic plants, has high adaptive potential, is quite frost and drought resistant.

The forest vicia (*Vicia sylvatica* L.) in terms of sustainability in the grass stand has proved itself better than the pea-shaped peavine (*Lathyrus pisiformis* L.) but is significantly inferior in terms of this indicator to meadow peavine (*Lathyrus pratensis* L.). In 2012, the participation of forest vicia in grass stand was 35%, which is 8% higher than *Lathyrus pisiformis* L., but 19.0% lower than *Lathyrus pratensis L.* Quite a high abundance of cereals - 37.5%, of them the following predominate: *Elytrigia repens* (L.) Nevski. - var. $_1$. - var. $_2$, *Festuca pratensis* L. - var. $_1$. Significant admixture of different herbs - 16.5%.

During the harvest period, in the studied species the layer distribution of aboveground biomass on the vertical profile was considered. This indicator allows to determine the structure of the aboveground biomass; uniformity of leaves, inflorescences and fruits arrangement in height; ascertain the proportion of biomass that is concentrated to the height of mowing (0–7) cm) and remains in the meadow after harvest in the form of stubble (the amount of crop losses during mowing).

It is found that pea-shaped peavine has the lowest vertical profile, it includes 5 horizons (Table 2). The largest concentration of biomass is observed in three horizons: 15–30 cm - 30.2%; 30–45 cm - 28.1%; 45–60 cm - 22.2%. They account for 80.5%. The largest mass of leaves is also concentrated in these three horizons, with a maximum of leaves marked in the horizon of 30–45 cm - 14.3%. Inflorescences are located at a height of 45–60 cm, their share accounts for - 1.2%. This species is characterized by a low inflorescence content in biomass - 1.2% and the highest fruit mass - 20.3%.

For meadow peavine, the largest concentration of biomass is concentrated at a height of 15–70 cm, accounting for 82.9%. The participation of leaves in the structure of aboveground biomass was 54.3%, the largest mass of leaves is concentrated in two horizons: 30–45 cm - 14.1%; 45–60 cm - 11.9%. In the lower horizon (0–7 cm) there are only stems - 5.0%.

The highest vertical profile is noted for forest vicia, it includes seven horizons, the top one ends at 75–90 cm.

Table 2. Distribution of aboveground biomass of vicia studied species by vertical profile (%), 2012

Experiment options (legume types)	Aboveground biomass							
	vertical profile (horizons, cm)							
	plant organs	0–7	7–15	15–30	30–45	45–60	60–75	75–90
1. opt. -	leaves	-	5.9	9.7	10.5	10.6	8.8	5.3
Vicia sylvatica L.	stems	3.7	4.5	4.6	4.1	3.5	3.2	2.3
(forest vicia) - control	inflorescences	-	-	-	-	-	5.9	6.7
	fruits	-	-	-	4.6	3.1	1.9	1.1
2.opt. -	leaves	-	7.2	13.9	14.3	12.2	-	-
Lathyrus pisiformis L.	stems	5.5	6.8	7.7	5.8	5.1	-	-
(pea-shaped peavine)	inflorescences	-	-	-	-	1.2	-	-
	fruits	-	-	8.6	8.0	3.7	-	-
3.opt. -	leaves	-	7.2	10.9	14.1	11.9	10.2	-
Lathyrus pratensis L.	stems	5.0	4.9	5.1	5.0	3.8	3.1	-
(meadow peavine)	inflorescences	-	-	2.5	4.0	5.4	3.3	-
	fruits	-	-	2.3	1.3	-	-	-

The distribution of aboveground biomass is more uniform. Leaves mass was 50.8%; fruits - 10.7%; inflorescences - 12.6%. Only stems are in the horizon up to mowing height - 3.7%. The forest vicia by all indicators occupies an intermediate position, there is a high participation of leaves and inflorescences in the structure of aboveground biomass.

The analysis showed that at the time of grass stand mowing, the lowest loss of biomass was observed in forest vicia, which amounted to 3.7%; the highest - in pea-shaped peavine - 5.5%.

When introduced, the productivity of the plant introduced into the cultivation is a priority. From the data shown in Table 3 it is evident that higher productivity was obtained in the first years of the study (2006–2008), there was a decrease in aboveground biomass in subsequent years, which is associated with grass stand being introduced with non-seeded low-productive species, such as: *Achillea millefolium* L., *Alchemilla vulgare* L., *Galium boreale* L., *Galium mollugo* L., *Taraxacum officinale* Wigg etc.

Table 3. Productivity of aboveground biomass of perennial legumes (2006–2012)

Options of the experiment (legume types)	Green mass								
	average for 2006–2008			average for 2009–2012			average for 2006–2012		
	productivity, t ha^{-1}	deviation from control, (+, -)		productivity, t ha^{-1}	deviation from control, (+, -)		productivity, t ha^{-1}	deviation from control, (+, -)	
		t ha^{-1}	%		t ha^{-1}	%		t ha^{-1}	%
1. opt. - forest vicia (control)	14.9	-	-	11.9	-	-	13.4	-	-
2.var. - pea-shaped peavine	12.3	-2.6	17.4	9.2	-2.7	22.7	10.7	-2.7	20.1
3.opt. - meadow peavine	16.7	+1.8	12.1	13.8	+1.9	16.0	15.3	+1.9	14.2
LSD_{05}:	2006	0.95	-	2009	0.68	-	-	-	-
	2007	1.05		2010	0.73				
	2008	1.09		2011	0.81				
				2012	0.75				

Quite low productivity was obtained in the pea-shaped peavine (10.7 t ha^{-1}); by the years of research it was significantly lower than in the forest vicia and the pea-shaped peavine. Among the studied species, the best results were provided by meadow peavine, it formed consistently high productivity of aboveground biomass during all years of observations. On average for 2006–2012 productivity amounted to 15.3 t ha^{-1}, which is 1.9 t ha^{-1} higher than that of forest vicia and 4.6 t ha^{-1} more than that of pea-shaped peavine. Mathematical processing of the obtained results gives grounds to assert that by the years of research, the productivity of the meadow peavine is significantly higher than that of the forest vicia and the pea-shaped peavine, the deviation from the control significantly exceeds the value of the LSD_{05}.

CONCLUSION

The study showed that the studied species vary significantly in the intensity of regrowth - early regrowth from spring is typical of pea-shaped peavine (*Lathyrus pisiformis* L.), later - for meadow peavine (*Lathyrus pratensis* L). Inclusion of these species in the ‘green conveyor’ (constant feed provision) will provide animals with green feed throughout the whole vegetative season.

Phyto-cenotic stability of studied species has been revealed, the forest vicia (*Vicia sylvatica* L.) in terms of resistance in the herbage has proven itself better than the pea-shaped peavine (*Lathyrus pisiformis* L.), but it is way below in this indicator to the meadow peavine (*Lathyrus pratensis* L.). The most stable species in the experiment is the meadow peavine (*Lathyrus pratensis* L), it accounts for 54% in the structure of herbage, it resists the introduction of wild plants into the herbage much better, has a high adaptive potential, is sufficiently winter-hardy and drought-resistant.

Thus, under the same cultivation conditions, the best results were provided by the meadow peavine, which retained its dominance in the phytocenoses and formed a significantly higher productivity during all the years of observation. On average for 2006–2012 productivity was 15.3 t ha^{-1}, which is 1.9 t ha^{-1} (14.0%) higher than that of the forest vicia and 4.6 t ha^{-1} (34.3%) more than that of the pea-shaped peavine.

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Morphological and morphometric characteristics of Cornelian Cherry (*Cornus mas* L.) in natural conditions of the Crimean Peninsula

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Abstract. Cornelian cherry (*Cornus mas* L.) is a valuable fruit and ornamental plant in the Cornaceae family. This is a promising frost- and drought-resistant crop, undemanding to growing conditions. Fruits and leaves of plants are rich with biologically active substances which allows them to be used in pharmaceutical, food, liquor, and other industries. In the natural non-cultivated communities on the Crimean Peninsula the species is represented by a wide variety of forms that are promising for study from the point of view of botanical and breeding research. There is practically no information in the scientific literature about the characteristics of cornelian cherry for the most common places of its growth on the Crimean Peninsula, regarding the morpho-biological features and patterns of development of cornelian cherry plants, which determines the relevance of research. The purpose of this work was to study the intraspecific variability of cornelian cherry plants within natural coenopopulations under varying ecological conditions in the foothill, mountain, and South Coast of the Crimea in order to identify the most promising forms for further breeding research. The results of a three-year study (2017 to 2019) revealed differences in the life form of plants, in the timing of fruit maturation, in the shape and color of the leaf and fruit, and in their metric parameters. Promising forms of plants with the largest fruits (an average weight from 1.65 g to 1.81 g) and a low percentage of endocarp - from 10% to15% (Form 1 - CP I, CP II, CP V, Form 2 a - CP IV) can be recommended for further breeding research.

Key words: *Cornus mas* L., coenopopulation, leaf, fruit, metric parameters.

INTRODUCTION

The gene pool of economically valuable plants of scientific and practical interest is enriched by selecting the best forms from natural coenopopulations for use in breeding and applications (Mratinić et al., 2015). Flora of Crimea, according to many authors, numbers from 2,532 to 2775 species of higher plants growing across the ecological zones of the Crimean Peninsula. Many of them have economic and cultural significance, providing people with food, medicines, fuel, clothing, building materials, and raw materials for production (Yena, 2012; Czerwińska & Melzig 2018). The study of wild plants is a promising direction for understanding the characteristics of the species and the laws of evolution, and is also the basis for conducting selection research (Tarko et al., 2014; Jaćimović & Božović, 2017; Salejdaet al., 2018; Khoury et al., 2019).

In recent years, the problem of extinction of certain species of plants has exacerbated owing to shrinking of their native habitat areas, in connection with changes in the environment due to several factors: deforestation, mowing of herbaceous cover, uncontrolled cattle grazing, invasion by alien species, destruction of ecosystems, climate change, depletion of soil, etc. Therefore, it is important to study wild plants in order to preserve the extant intraspecific diversity and the species as a whole and further to study the most promising forms for the selection purposes (Klimenko, 2007; Koblyakov, 2008; Bijelić, 2015; Szot et al., 2020).

Among the multitude of wild fruit plants, cornelian cherry is very popular and perspective crop - a culture that is little studied across the Crimean Peninsula. It is undemanding to growing conditions, tolerant to low negative temperatures (low as -35 °C) and drought, because it is able to regulate transpiration by folding leaves into a tube, and is very rarely damaged by pests and pathogens (Cornescu & Cosmulescu, 2017; De Biaggi et al., 2018; Kazimierski et al., 2019). The fruits and leaves of cornelian cherry contain physiologically active substances: organic acids, glucose, fructose, vitamins C, B3, and P, flavonoids, micro-and macronutrients, essential oils, phytoncides, which allows them to be used in pharmaceutical, food, liquor, and other industries (Tarko et al., 2014; Kucharska et al., 2015; Dinda et al., 2016; Hosseinpour-Jaghdani et al., 2017; Adamenko et al., 2018; Salejda et al., 2018; Dumitrașcu et al., 2019). Due to its decorative properties (resistant to pruning, winter-hardy, resistance to industrial pollution, early-flowering), cornel can be used in landscaping and in home gardens alike, in soil-protecting plantings, as well as a medium-forming phytoncide plant (De Biaggi et al., 2018; Dumitrașcu et al., 2019; Yarılgaç et al., 2019).

Cornelian cherry trees are native in Southern Europe and South west Asia, being spread in many countries such as Turkey, Bulgaria, Romania, Italy as wild fruit trees and are crop due to its resistance in harsh environmental conditions (Klimenko, 2007; Dumitrașcu et al., 2019; Ochmian et al., 2019). It cultivated mainly in Central and Southern Europe, in Asia Minor, and in the Caucasus (Cetkovská et al., 2015). In the Ukraine, Bulgaria, Slovakia, Austria, France, Germany, Poland and Turkey a systematic collecting, selecting and breeding program has started in the last years (Klimenko, 2004, 2007). Large plantings are found in Moldova, Rostov, Krasnodar, Stavropol, the Crimea, and the Caucasus (Klimenko et al., 2003; Klimenko, 2007). The Caucasus is one of the world's richest hotbeds of various forms of this plant (Ujukhu, 2006; Tigieva, 2007). In the native of the Crimea cornelian cherry is part of the forest undergrowth, rising to a nelevation of up to 1,200 m above sea level. On the southern macroslope of the Main ridge, it is present in oak and oak-hornbeam forests and forms a belt of juniper-oak forests with separate bushes. A wide variety was observed on the territory of the villages of Sokolinoe, Golubinka, Schastlivoye, Shelkovichnoye, Maloe Sadovoe (Bakhchisarai district), Perevalnoye, Petrovka, Novo-Pavlovka (the Simferopol district), Kizilovka, Sinekamenka (the Belogork district) (Klimenko et al., 2003, Klimenko, 2007).

Cornelian cherry is 2–8 m high shrub and tree with a spherical, pyramidal, oval, umbrella-shaped, or spreading crown (Klimenko, 2007; Cetkovská et al., 2015; Czerwińska & Melzig, 2018; Szczepaniak et al., 2019). The plants develop yellow flowers, small, actinomorphic, that are collected in paniculiform, umbel-shaped, or head-shaped inflorescences. The species is also grown as an ornamental plants for its late winter yellow flowers. The fruit is a lower syncarpous drupe with a juicy and fleshy exocarp and a hard endocarp. The color of the fruit varies from bright red to dark red, almost black, oval shaped, 10–30 mm long drupes with a weight of 2–5 g. As a result of selection, varieties with yellow and pink fruits were released (Klimenko, 2007; Cetkovská et al., 2015; Jaćimović et al., 2015; Cornescu & Cosmulescu, 2017; Popović et al., 2017; Adamenko et al., 2018).

The study of the natural gene pool and selection of the most promising forms of cornelian cherry in Russia is carried out in the South of the country, in the Caucasus - in the Republics of North Ossetia-Alania and Adygea, in the Krasnodar Krai (Ujukhu, 2006; Tigieva, 2007; Koblyakov et al., 2008). In the scientific literature there is practically no information about the characteristics of the places of growth and morpho-biological features of the development of cornelian cherry plants on the Crimean Peninsula. Rational use of the species is difficult without knowledge of the features of intraspecific variability or the biology of development and fruiting of plants. Therefore, the study of the degree and nature of the form variability of cornelian cherry in natural conditions of growth on the Crimean Peninsula is an urgent and promising direction.

The **purpose of the research** is the study of intraspecific variability of cornelian cherry plants within the coenopopulations of the species in different ecological condition of the Crimea and identification of promising forms for selection research.

MATERIALS AND METHODS

Studies on the diversity of cornelian cherry plants in native on the territory of the Crimea in various ecological conditions were conducted in 2017 to 2019: in the Western and Eastern parts of the mountainous and foothill zones on the Northern macroslope of the Main ridge of the Crimean Mountains and on the Southern coast.

The study of coenopopulations was carried out according to standard methods of geobotanical research. The type of forest vegetation was determined according to the typological classification of Crimean forests developed by Posokhov (1971) and supplemented by Plugatar` (2015). The species of plants was determined according to the Keys to Higher Plants of Crimea (Rubtzov, 1972), the names of taxa are given according to the database ‘The Plant List’ (2013), and ‘International Plant Names Index’ (2020). Analyzing intraspecific variability, we paid attention to the life form of plants, the variety of fruits and leaves forms. Morphological analysis of fruits and their classification were carried out according to Klimenko (2007).

The sample size depended on the number of plants in the coenopopulation and ranged from 15 to 26 individuals. For morphological analysis, 35 leaves and fruits per tree were selected from the lower, middle, and upper parts of the plant crown in each coenopopulation to remove metric parameters. The biometric data of eight quantitative traits (leaf length, leaf width, fruit weight, fruit length, fruit diameter, endocarp weight, endocarp length, endocarp diameter) were subjected to statistical analyses using the Microsoft Office Excel 2010 and Statistica 10 software package using standard indicators (arithmetic mean value, standard deviation (SD) and coefficient of variation (Cv). To assess the influence of the interaction of factors, a two-way analysis of variance (ANOVA) was used (Lakin, 1990; Lotova, 2001; Dospekhov, 2012).

The first studied coenopopulation of cornelian cherry (CP 1) is located on the Southern Coast of the Crimea in the Ulu-Uzen river valley (near Generalskoe village, near Alushta), on a slope with a gentle slope of 25 to 30° of the Southern right-bank exposure, at an altitude of 250 to 300 m above sea level. The coenopopulation inhabits an area of 0.10 ha, represented by 18 specimens of woody life forms. According to the typological classification of Crimean forests (Posokhov, 1971; Plugatar`, 2015) this coenopopulation is a part of the dry oriental hornbeam sudubrava (C1-ohOd), where the type-forming rock is *Quercus pubescens* Willd. IV growth class, with a tall of no more than 10 m. The community forest stand is composed (0.65). The composition of the shrub layer, in addition to *Cornus mas*, contains *Carpinus orientalis* Mill., *Paliurus spina-christi* Mill., *Juniperus excelsa* M. Bieb., and *Cotinus coqqyqria* Scop. occurs occasionally. The grass cover is dominated by steppe perennial grasses with a projective cover of no more than 25%.

The second coenopopulation of cornelian cherry (CP 2), is located in the western part of Mountainous Crimea, on the northern macroslope eat an altitude of 600 to 650 above sea level in the vicinity of the Nauchny village (Bakhchisaray district). This coenopopulation is represented by 26 plants, with a total area inhabited of 0.25 ha, and is a part of dry hornbeam oakery with *Quercus pubescens* (D_1- ohOd), along the South-Eastern slope of one of the unnamed spurs of Sel-Bukhra mountain, gently descends into a deep gill. The community forest stand is composed (0.7). Both shrubs and tree life forms of *Cornus mas* are found here. The dominant role belongs to *Quercus pubescens* IV–III growth class. The plants does not exceed 12 m. tall of in the first tier, *Fraxinus excelsior subsp. coriariifolia* (Scheele) A.E. Murrey is separately found. In the undergrowth, *Cornus mas*, *Carpinus orientalis*, and *Euonymus europaeus* L. create a weakly expressed shrub layer. The herbaceous cover is sparse, poor in floristic terms, with a projective cover of no more than 20% and is represented by xerophilous grasses and dicotyledonous species.

The third coenopopulation of cornelian cherry (CP 3) is located in the eastern part of Mountainous Crimea, on the northern macroslope of the Main ridge, grows in the area of the Sheitan-Kapu gorge of the Tanasu river valley (the vicinity of Krasnoselovka village (Belogorsky district) at an altitude of 570 m above sea level. The gorge is formed by four tower-like rocks located in pairs on each side of the river up to 50 m high. This coenopopulation, inhabiting an area of about 0.30 ha, is represented by shrub life forms in the number of 22 plants, and is part of fresh hornbeam oakery with *Quercus petraea* (Matt.) Liebl. (D2-hOf), where it forms a section of oak-cornel forest, with a slope of 10 with *Quercus petraea*. The forest stand is closed (0.75 to 0.85). The trees does not exceed 20 m tall. In the community, *Fagus sylvatica* L. is found separately. The second tier contains *Cornus mas* (dominate), *Carpinus betulus* L., *Acer campestre* L. The herbaceous layer is strongly sparse, with a projective cover of no more than 8%, formed by mesoxerophytic dicotyledonous plants.

The fourth coenopopulation of cornelian cherry (CP 4) is located in the eastern part of the foothill zone on the northern macroslope, in the vicinity of the village of Topolevka (Belogorsky district), at an altitude of 480 m above sea level. To the South is Tau-Bashi mountain (772 m), to the North - Kulyaba mountain (521 m), to the

West - Kubalach mountain area (768 m). The coenopopulation inhabiting an area of about 0.30 ha, is represented by shrub and tree life forms in the number of 23 plants of cornel, and is part of dry oakery (D1-Od-Of). The forest stand is closed (0.8), clearly defined, formed by two types of oak *Quercus pubescens* and *Quercus petraea* up to 18 m tall. *Cornus mas* along with *Euonymus europaeus, Ligustrum vulgare* L. and few single plants of *Carpinus betulus* form a dense shrubby undergrowth. The herbaceous layer changes its projection coverage from 90% in spring (April) to 15% in summer (July) and is represented by xeromesophytic grasses.

The fifth coenopopulation of cornelian cherry (CP 5) is located in the eastern part of the foothill zone on the northern macroslope of the Crimean Mountains in the vicinity of the city of Stary Krym (Kirovskyi district) at an altitude of 350 to 400 m above sea level. On a total area of 0.20 ha, it is represented by 15 plants of shrub life forms, grows on the edge of an oak-cornel forest, is a part of dry cornel oakery with *Quercus pubescens* (D1-COd). The dominant tree species is *Quercus pubescens*, up to 12 m tall. The forest stand is closed (0.6). The undergrowth is formed by *Cornus mas* (projective cover up to 60%), *Corulus avellana* (L.) H. Karst., *Cornus sanguinea* L., *Ligustrum vulgare*, *Carpinus orientalis*, sporadically - *Rosa canina* L. and *Acer campestre*. The herbaceous layer is represented by xeromesophytic grasses with a projective cover of up to 40%.

RESULTS AND DISCUSSION

Weather conditions in the natural zones of the Crimean Peninsula are quite contrasting, due to the influence of the Black and Azov seas and the presence of mountains. The presence of seas softens the climate during the cold season and provides coolness during the warm period; mountains serve as a barrier that holds back cold air flows (Bagrova et al., 2001). Weather conditions in the locations of coenopopulations (foothill and mountain zones, Southern Coast) were different and unstable during the growing seasons of 2017 through 2019 (Table 1). These weather conditions were obtained from weather stations located near the study areas: Belogorsk, Vladislavovka, Pochtovoye, and Generalskoe (https://rp5.ru).

The Southern Coast of Crimea (Generalskoe village, CP 1) is characterized by a Mediterranean climate. The duration of the frost-free period is 220 to 240 days, and the summer period is 120 to 140 days (Bagrova et al., 2001). The average annual temperature is 14.2 °C. Winter is quite warm and humid, the average temperature of the coldest month (January) - +4.4 °C, and the warmest (August) - 27.7 °C (Table 1). The maximum summer temperature for the three years of the study was 37.5 °C (https://rp5.ru). The temperature regime in 2017 was within the limits of the average long-term indicators, only in February, March, and June there was an excess of the norm by 2; 3.6, and 2.2 °C, respectively. In 2018, the indicators exceeded the long-term average during the entire vegetation period of plants by 2.4 to 5.4 °C. In 2019, an excess of average annual indicators was noted in all months of the growing season. The maximum excess was recorded in March (+2.4 °C to normal), June (+5.6 °C to normal), August (+3.7 °C to normal).

Precipitation in 2017 was below the long-term average, with the exception of a slight excess in May and August. Conditions in 2018 were characterized by very low amount of precipitation during the active fruit formation phase (June to August). The average annual precipitation varied from 280 to 400 mm (Table 1).

Table 1. Average amount of precipitation and air temperature during the active vegetation period Cornelian cherry, 2017 through 2019

Coeno-population (CP)	Years	Months								
		January	February	March	April	May	June	July	August	September
Amount of precipitation, mm										
CP 1	2017	49.0	10.0	17.0	23.0	48.0	19.0	37.0	52.0	9.0
	2018	61.0	73.0	35.0	5.0	40.0	8.0	17.0	1.0	45.0
	2019	81.0	45.0	9.0	49.0	9.0	7.0	15.0	12.0	15.0
	Normal*	63.2	49.4	44.0	39.0	46.0	55.0	45.0	45.0	40.0
CP 2	2017	15.0	43.0	24.0	15.0	41.0	28.0	18.0	44.0	0.7
	2018	19.0	45.0	19.0	12.0	24.0	19.0	119.0	2.0	71.0
	2019	40.0	30.0	15.0	34.0	28.0	100.0	58.0	22.0	21.0
	Normal	49.1	36.5	36.0	33.0	37.0	51.0	44.0	39.0	39.0
CP 3	2017	46.0	37.0	27.0	73.0	72.0	57.0	55.0	19.0	1.0
	2018	24.0	19.0	18.0	4.0	34.0	23.0	80.0	3.0	43.0
	2019	20.0	18.0	8.0	18.0	20.0	66.0	61.0	11.0	24.0
	Normal	46.6	37.6	42.0	39.0	47.0	69.0	53.0	48.0	37.0
CP 4	2017	47.0	29.0	34.0	10.0	49.0	54.0	44.0	32.0	4.0
	2018	43.0	24.0	31.0	7.0	40.0	25.0	52.0	12.0	43.0
	2019	49.0	28.0	10.0	35.0	48.0	77.0	40.0	22.0	31.0
	Normal	41.5	33.1	42.0	39.0	47.0	69.0	53.0	48.0	37.0
CP 5	2017	35.0	23.0	29.0	25.0	17.0	44.0	34.0	29.0	16.0
	2018	30.0	19.0	25.0	0.3	12.0	20.0	46.0	1.0	45.0
	2019	81.0	29.0	13.0	16.0	44.0	27.0	44.0	15.0	6.0
	Normal	51.0	42.0	39.0	38.0	43.0	48.0	41.0	47.0	40.0
Average monthly temperature, °C										
CP 1	2017	3.6	4.5	8.9	9.7	15.7	21.5	24.8	17.7	22.9
	2018	4.6	5.4	7.4	14.5	16.6	23.4	25.3	27.7	21.9
	2019	4.9	4.9	7.7	11.3	17.1	25.9	24.0	26.0	21.2
	Normal	2.1	2.5	5.3	10.9	16.2	20.3	22.9	22.3	18.0
CP 2	2017	1.4	2.5	5.5	12.1	13.8	19.7	22.4	24.7	20.7
	2018	2.1	3.7	6.8	13.1	17.8	21.6	22.6	23.4	18.7
	2019	2.5	3.1	5.8	9.2	16.5	23.0	21.2	22.9	17.4
	Normal	0.7	1.5	4.1	10.4	15.4	19.3	21.8	21.2	16.9
CP 3	2017	-1.7	1.2	7.9	9.4	15.2	20.1	22.9	24.1	16.8
	2018	0.8	1.7	5.5	13.8	18.0	21.3	23.1	23.6	17.9
	2019	2.3	1.8	5.4	9.5	17.3	22.6	21.8	22.3	17.7
	Normal	-0.8	-0.5	3.2	10.0	14.9	18.5	22.3	20.2	15.2
CP 4	2017	0.8	2.7	5.6	10.8	13.0	19.4	21.5	23.8	21.0
	2018	1.0	3.1	6.1	12.7	17.7	21.1	23.1	22.7	18.6
	2019	1.5	1.8	5.8	8.7	16.5	22.5	21.4	22.2	17.0
	Normal	-0.8	-0.5	3.2	10.0	14.9	18.5	22.3	20.2	15.2
CP 5	2017	1.0	1.6	6.3	14.1	14.1	20.9	23.3	26.1	22.1
	2018	1.3	2.1	5.7	12.5	18.0	22.7	25.4	25.2	19.6
	2019	4.3	4.2	6.4	9.4	16.5	24.3	22.8	23.6	18.9
	Normal	-0.5	-0.3	2.6	9.0	14.6	18.9	21.7	21.3	16.6

Note: * average annual indicator over 20 years (Bagrova et al., 2001).

The climate of eastern part the Crimean foothill zone (Topolevka village, CP 2, Staryi Krym, CP 5) is moderately continental. The average annual temperature is 12.5 °C. The temperature of the coldest month (January) - +0.8 °C, and the warmest (August) -25.4 °C (Table 1). The maximum temperature in summer during the three years of the study is 34.4 °C (https://rp5.ru). The temperature regime in 2017 was characterized by increased indicators in winter and spring (1.2 to 2.1 °C above normal). In the summer, the indicators were at the level of the long-time average. In 2018, there was an excess of the average values from May to August by 1.5 to 2.6 °C. The warm period lasted, on average, 290 days, and the cold period lasted up to three months. The duration of the frost-free period, on average, was 190 to 200 days (https://rp5.ru). In 2019, in all months of research, an excess of the average long-term of the temperature regime by 0.7 to 5.4 °C was noted. The maximum excess was recorded in March (+3.8 °C to normal), June (+5.4 °C to the long-term average). In 2017, the average amount of precipitation in the summer period was slightly lower than the average annual values, creating good conditions for plant moisture supply (Table 1). The maximum amount of precipitation fell in June (54 mm with a norm of 68 mm). In 2018, there was an increased amount of precipitation in winter compared to the norm. However, in the phase of active fruit formation (March to August), this indicator was lower than the long-term average. The average annual precipitation was 360 to 440 mm. In 2019, the amount of precipitation was lower than the average long-term data, with the exception of January (81 mm at a rate of 51 mm in the Staryi Crimea) and June (77 mm at a rate of 69 mm in Topolevka). The climate of the Mountainous Crimea (Nauchny village, CP 3, Krasnoselovka, CP 4) is moderately continental. The amount of precipitation falling here increases, and temperatures decrease with increasing altitude of the area above sea level. The average annual air temperature is +11.7 °C. The temperature of the coldest month (January) is -1.7 °C, and the warmest (July) is 23.1 °C (Table 1). The maximum air temperature in the summer period 2017 to 2019, (July to August) - 37 to 40 °C. There was an excess of the norm during the entire growing season of plants by 1.3 to 5 °C (April, August). The warm period lasted 260 to 280 days, and the cold period lasted up to three months. The average duration of the frost-free period is 170 days.

In 2017, over the entire period of the study, precipitation was at the level or slightly lower than the long-time average annual (Table 1). In 2018, extremely dry conditions were noted, with the exception of July (80 mm with a norm of 53 mm in the village of Krasnoselovka and 119 mm with a norm of 44 mm in Nauchny village). In some months, the amount of precipitation was 1.5 to 3 times below normal. In 2019, the amount of precipitation fell significantly from the average long-term data. In June, the largest amount of precipitation fell - 100 mm at a rate of 51 mm in Nauchny village; in July in Krasnoselovka village by 8 mm, and in Nauchny village by 14 mm above the norm). The average annual precipitation was 360 to 520 mm.

The highest values for the weather indicators of the analyzed characteristics were noted in 2017. This is associated with the optimal temperature regime and sufficient precipitation during the flowering period, formation, and development of leaves and fruits. On the contrary, 2018 was characterized by arid conditions and high temperatures, which led to accelerated fruit maturation and shedding before the due date. In general, the analysis of data from two years of research showed minor differences in the main morphometric parameters, which is obviously due to the high adaptive properties of the species. In 2019, the temperature and water regime were intermediate compared to the two previous years.

The vegetation period of the plants of the studied cornelian cherry coenopopulations began from the second decade of February, which was facilitated by the accumulation of average temperatures in the range of 5 to 12 °C for 5 to 7 days. Flowering lasted about 20 to 25 days, after which the formation of fruits began. Growth and development of plants, fruiting, and quality of fruits are influenced by the growing conditions, including the exposure of the slope, the terrain. It was noted that the Southern, South-Western and South-Eastern slopes are more favorable for the growth and development of plants and the early onset of all phenological phases of development and the intensity of fruiting.

Analysis of plants in the studied coenopopulations revealed differences in the metric and morphological characteristics of leaf and fruits.

The leaf is a soft organ that plays an important role in the study of morphometric indicators of plant variability. Providing many functions of the plant organism, it is exposed to external factors and adapts to them through long-term selection. Often within the same species there are leaf of the same shape, but significantly different in size, and vice versa - leaves of different shapes, but similar in size parameters. Such differences may be related to the peculiarities of plant ontogenesis (Klimenko, 2007). Analysis of the morphological characteristic of the cornelian cherry leaf in various coenopopulations revealed the following shape: oval, ovoid, and lanceolate with a wedge-shaped base and a pointed tip (Fig. 1).

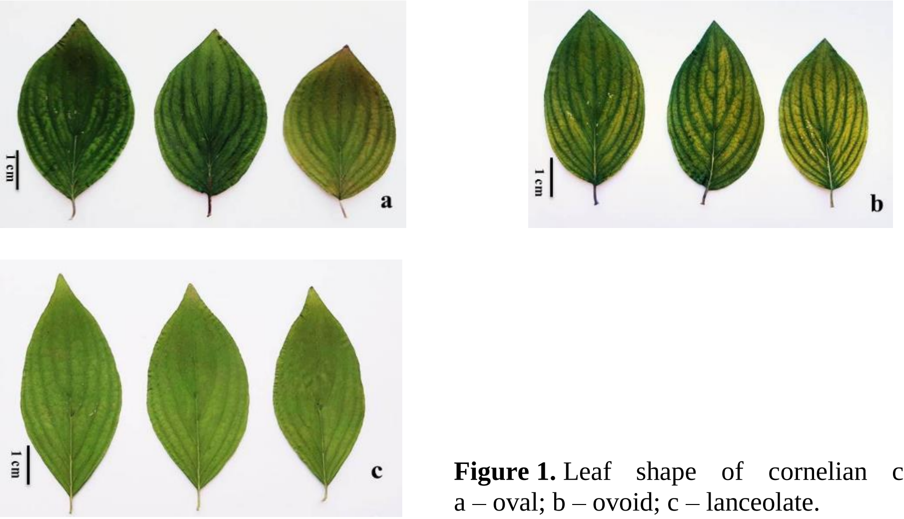

Figure 1. Leaf shape of cornelian cherry: a – oval; b – ovoid; c – lanceolate.

The average leaf length (L_{cp}) varied from 58.6 mm to 81.8 mm, and the width (B_{cp}) - from 23.4 mm to 47.4 mm (Table 2). The longest leaf is noted in CP 4 with an ovoid shape, and the smallest in CP 1, CP 3, and CP 5 with an oval shape. Lanceolate leaves in CP 2 and CP 4 had intermediate values for this parameter.

In terms of the leaf width, the smallest values are noted in CP 2 and CP 4 with a lanceolate shape, and the largest - in CP 4 with an ovoid shape. CP 1, CP 3, and CP 5 with an oval shape had intermediate values for this indicator. Analyzing the data obtained for 2017 to 2019, it should be noted that the maximum values of the length and width of the cornelian cherry leaf for all coenopopulations were in 2017, with the exception of CP 4, and the lowest values were in 2018. In CP 4 with a lanceolate leaf shape, the maximum values were recorded in 2019. The values of the coefficient of variation (Cv - from 3.5% to 19.7%) made it possible to conclude that the indices of the parameters of the cornelian cherry leaf over the entire period of the study are insignificant (Table 2). Although in literature there is little information about the shape diversity and metric parameters of cornelian cherry leaves, our data confirm the information previously provided by other authors (Ujdukhu, 2006; Klimenko, 2007; Tigieva, 2007).

Table 2. Morphological and metric characteristics of cornelian cherry leaf in various coenopopulation

Coenopopulations (Factor B)	Leaf shape	Year (Factor A)	Metric parameters of the leaf			
			length (L_{cp}), mm	Cv, %	width (B_{cp}), mm	Cv, %
CP 1	Oval	2017	67.5 ± 0.09	8.2	40.3 ± 0.12	17.1
		2018	60.2 ± 0.09	9.5	34.4 ± 0.06	10.8
		2019	64.8 ± 0.11	8.9	36.2 ± 0.07	11.3
		Average	63.9 ± 0.10	8.9	37.7 ± 0.08	13.1
CP 2	Lanceolate	2017	79.8 ± 0.16	12.1	29.6 ± 0.05	10.8
		2018	72.6 ± 0.08	6.3	23.4 ± 0.02	4.7
		2019	76.8 ± 0.08	5.9	26.2 ± 0.02	4.2
		Average	76.2 ± 0.11	8.1	26.5 ± 0.03	19.7
CP 3	Oval	2017	64.6 ± 0.06	5.8	36.2 ± 0.04	6.7
		2018	58.6 ± 0.07	7.7	29.4 ± 0.03	5.1
		2019	60.3 ± 0.07	7.3	36.2 ± 0.05	8.1
		Average	61.6 ± 0.07	6.9	32.8 ± 0.04	6.6
CP 4	Ovoid	2017	81.8 ± 0.08	5.6	47.4 ± 0.02	3.0
		2018	76.3 ± 0.08	5.7	39.8 ± 0.10	13.5
		2019	75.4 ± 0.08	6.0	43.3 ± 0.06	8.1
		Average	79.1 ± 0.08	5.8	43.6 ± 0.06	8.2
	Lanceolate	2017	75.4 ± 0.12	8.9	28.9 ± 0.12	15.4
		2018	70.5 ± 0.05	3.5	24.6 ± 0.05	7.2
		2019	79.3 ± 0.06	4.5	35.1 ± 0.04	5.3
		Average	73.0 ± 0.08	5.6	29.5 ± 0.07	9.3
CP 5	Oval	2017	74.4 ± 0.06	4.6	38.7 ± 0.04	6.2
		2018	66.7 ± 0.06	5.5	31.8 ± 0.03	5.4
		2019	71.1 ± 0.06	4.5	44.0 ± 0.06	7.5
		Average	70.6 ± 0.06	4.9	35.2 ± 0.04	6.4
LSD_{05}(Factor A)			0.09		0.07	
LSD_{05}(Factor B)			0.14		0.10	

Cv – the coefficient of variation.

The data of two-way analysis of variance (ANOVA) showed that the greatest influence on the biometric parameters of leaf is exerted by genetic characteristics of studied plants (Factor A). The share of influence in terms of leaf length was 82.9%, and in width - 77.3%. The share of influence of factor B was insignificant and amounted to 10.20% in terms of leaf length and 12.30% in terms of leaf width (Table 3).

Table 3. Data of two-way analysis of variance of cornelian cherry leaf in various coenopopulations

Factor	SS	dF	MS	F	*p*-value	Shape of influence, %
Leaf length						
A	38.41	2	19.21	74.1	0.000000	10.2
B	313.27	5	62.65	241.7	0.000000	82.9
AB	10.04	10	1.00	3.9	0.000041	2.7
Error	15.86	612	0.26			4.2
Leaf width						
A	38.97	2	19.485	161.11	0.00	12.3
B	246.61	5	49.323	407.81	0.00	77.3
AB	21.31	10	2.132	17.63	0.00	6.7
Error	12.01	612	0.121			3.7

Fruits are an important generative structure in the plant body, so the study of their diversity by morphological and anatomical features gives an idea of the degree of intraspecific polymorphism in coenopopulations. Such research is also very relevant in connection with the search for desirable natural forms of cornelian cherry in order to create varieties with a high content of mesocarp (pulp) and a low percentage of endocarp.

The conducted research allowed to identify the diversity in shape, color, ripening period, and metric parameters of fruits in studied coenopopulation (Table 4).

Table 4. Morphological characteristics of cornelian cherry fruit in various coenopopulations

Coenopopulation	Form	Fruit color	Fruit shape	Ripening period
CP 1	1*	Bright-red - dark-red	Oval	Average
CP 2	1*	Bright-red - dark-red	Oval	Late
CP 3	3	Dark-red, almost black	Barrel-shaped	Early
CP 4	4 a	Red	Oval-cylindrical	Average
	4 b	Dark-red	Broad-pear	Late
CP 5	1*	Bright-red - dark-red	Oval	Average

Note: * plant shapes are distinguished by the morphological structure of fruits.

Fruits, in CP 1, 2, 5 are of oval shape with color from bright-red to dark-red, medium (August 20 - through September 10) and late ripening (September 10 through 25), are combined into a single Form 1 due to similar morphological and morphometric characteristics. In CP 3, growing in the area of the Sheitan-Kapu gorge, the plants had small fruits of a dark-red, almost black color, ripening early (August 5 through 20), barrel-shaped, separated into a distinct Form 3. In CP4 two distinct fruit shape forms were identified: Form 4a - with a oval-cylindrical shape, bright-red color, average ripening time (August 20 - through September 10) and Form 4b - with an broad-pear shape, dark-red color, late maturity (September 10 through 25) (Fig. 2). In CP 2, individuals with shrub and tree life forms were found, but the relationship between the shape of the fruit and their life form was not established.

The shape and color variety of cornelian cherry fruits in native is noted by other authors. The color of the fruit varies from bright red to dark red, almost black, Most often, fruits are oval, spherical, barrel-shaped, cylindrical shape, less common are pear-shaped fruits. Fruit ripening was noted in July - September (Demir & Kalyoncu, 2003; Klimenko, 2004, Ujukhu, 2006; Klimenko, 2007; Tigieva, 2007; Prokaj et al., 2009; Mratinić et al., 2015; Dinda et al., 2016; Cornescu & Cosmulescu, 2017). As a result of selection, varieties with yellow and pink fruits were released (Klimenko, 2004, 2007; Cetkovská et al., 2015; Jaćimović et al., 2015; Cornescu & Cosmulescu, 2017; Popović et al., 2017; Adamenko et al., 2018).

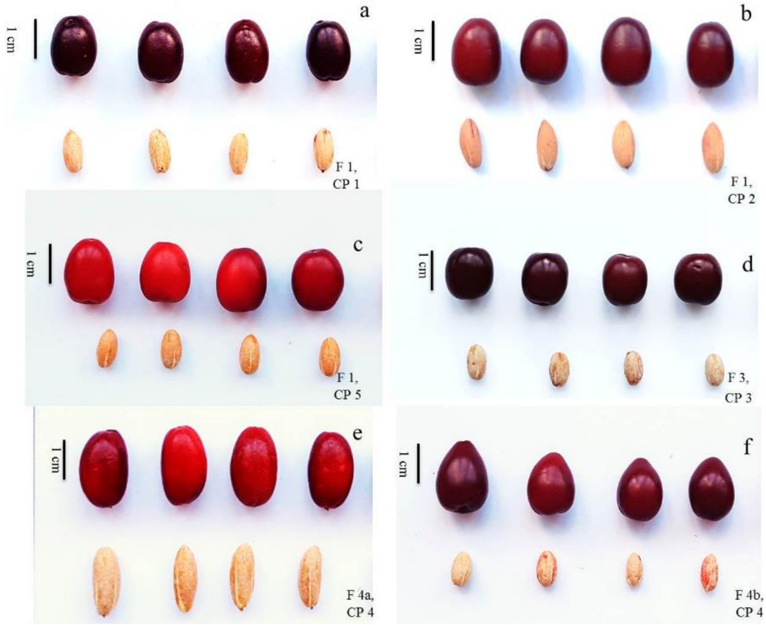

Figure 2. Fruit shape of cornelian cherry plants: a, b, c – oval (CP 1, CP 2, CP 5); d – barrel-shaped (CP 3); e – oval-cylindrical (CP 4); f – broad-pear (CP 4).

As a result of studying the morphometric characteristics, it was found that fruits in various coenopopulations differed in length - from 12.1 mm to 17.2 mm, in diameter - from 8.0 mm to 12.8 mm, and in weight - from 0.56 g to 1.77 g. The greatest length and diameter were found in plants with oval-cylindrical fruits in CP 2 (Lcp = 17.2 mm, Bp = 12.8 mm), and the smallest - in plants with barrel-shaped fruits in CP 3 (Lcp = 12.1 mm, Bp = 8.0 mm).Fruits with the greatest weight are noted in CP 5, and with the smallest - in CP 3. The maximum values of the fruit diameter are noted in CP 4, the minimum - in CP 3. Endocarp length varied from 6.7 mm to 13.5 mm, diameter - from 3.1 mm to 5.1 mm, and weight - from 0.148 g to 0.267 g. The longest endocarp was noted in CP 5, and the shortest - in CP 3. High endocarp weigth was noted in CP 5, low - in CP 3. The share of endocarp to pulp varied from 10.75 to 26.28%. The maximal values for this indicator were in CP 3, the minimal - in CP 2 (Table 5).

Table 5. Morphometric characteristics of cornelian cherry fruit in various coenopopulations

Coenopopulations (CP) (Factor B)	Form (F)	Year (Factor A)	Metric parameters ($X_{cp} \pm m_{cp}$)						Share of endocarp, %
			Fruit			Endocarp			
			Length (L_{cp}), mm	Diameter (D_{cp}), mm	Weight(m_{cp}), g	Length (L_{cp}), mm	Diameter (D_{cp}), mm	Weight (m_{cp}), g	
CP 1	F 1	2017	16.2 ± 0.02	12.1 ± 0.01	1.74 ± 0.01	11.7 ± 0.02	5.0 ± 00.1	0.210 ± 0.003	12.07 ± 0.02
		2018	15.5 ± 0.02	11.2 ± 0.01	1.65 ± 0.02	10.1 ± 0.02	4.3 ± 0.01	0.197 ± 0.002	11.94 ± 0.02
		2019	16.0 ± 0.01	11.8 ± 0.01	1.70 ± 0.01	11.3 ± 0.01	4.6 ± 0.01	0.202 ± 0.002	11.88 ± 0.02
		Average	15.9 ± 0.02	11.7 ± 0.01	1.70 ± 0.01	11.03 ± 0.02	4.6 ± 0.01	0.203 ± 0.002	11.96 ± 0.02
CP 2	F 1	2017	17.7 ± 0.02	13.1 ± 0.01	1.67 ± 0.04	13.0 ± 0.03	4.0 ± 0.01	0.171 ± 0.004	10.24 ± 0.04
		2018	16.8 ± 0.01	12.5 ± 0.01	1.54 ± 0.03	12.2 ± 0.01	3.2 ± 0.01	0.178 ± 0.003	11.56 ± 0.03
		2019	17.0 ± 0.01	12.8 ± 0.01	1.71 ± 0.01	12.7 ± 0.01	3.5 ± 0.01	0.179 ± 0.003	10.46 ± 0.02
		Average	17.2 ± 0.01	12.8 ± 0.01	1.64 ± 0.03	12.6 ± 0.023	3.6 ± 0.01	0.159 ± 0.004	10.75 ± 0.02
CP 3	F 3	2017	12.5 ± 0.01	8.5 ± 0.01	0.60 ± 0.02	8.1 ± 0.01	4.9 ± 0.01	0.154 ± 0.003	25.67 ± 0.02
		2018	11.6 ± 0.02	7.9 ± 0.02	0.53 ± 0.02	7.7 ± 0.01	4.2 ± 0.01	0.148 ± 0.004	27.92 ± 0.02
		2019	12.2 ± 0.01	7.6 ± 0.01	0.57 ± 0.01	6.7 ± 0.02	4.1 ± 0.01	0.144 ± 0.002	25.26 ± 0.01
		Average	12.1 ± 0.01	8.0 ± 0.01	0.56 ± 0.01	7.5 ± 0.01	4.4 ± 0.01	0.148 ± 0.003	26.28 ± 0.02
CP 4	F 4a	2017	16.6 ± 0.01	11.1 ± 0.01	1.12 ± 0.02	13.2 ± 0.01	3.9 ± 0.01	0.213 ± 0.006	19.01 ± 0.01
		2018	15.6 ± 0.01	10.3 ± 0.01	1.03 ± 0.03	12.6 ± 0.02	3.1 ± 0.01	0.198 ± 0.002	19.22 ± 0.02
		2019	15.9 ± 0.01	10.9 ± 0.02	1.09 ± 0.01	12.9 ± 0.01	3.4 ± 0.01	0.191 ± 0.003	17.52 ± 0.01
		Average	16.0 ± 0.01	10.8 ± 0.01	1.08 ± 0.01	12.9 ± 0.01	3.5 ± 0.01	0.207 ± 0.005	18.58 ± 0.01
	F 4b	2017	15.9 ± 0.02	8.9 ± 0.02	0.79 ± 0.01	13.1 ± 0.02	3.8 ± 0.01	0.179 ± 0.004	22.65 ± 0.01
		2018	15.0 ± 0.02	7.9 ± 0.02	0.70 ± 0.01	12.5 ± 0.02	3.2 ± 0.01	0.171 ± 0.005	24.44 ± 0.01
		2019	15.4 ± 0.01	8.5 ± 0.01	0.76 ± 0.01	12.9 ± 0.01	3.7 ± 0.01	0.174 ± 0.002	22.89 ± 0.01
		Average	15.4 ± 0.02	8.4 ± 0.02	0.70 ± 0.02	12.8 ± 0.01	3.6 ± 0.01	0.175 ± 0.004	23.33 ± 0.01
CP 5	F 1	2017	16.9 ± 0.01	12.4 ± 0.01	1.81 ± 0.03	13.5 ± 0.02	5.1 ± 0.01	0.271 ± 0.003	14.97 ± 0.02
		2018	15.5 ± 0.01	11.6 ± 0.01	1.73 ± 0.03	12.6 ± 0.01	4.4 ± 0.01	0.260 ± 0.005	15.03 ± 0.02
		2019	16.2 ± 0.01	12.2 ± 0.01	1.77 ± 0.01	13.2 ± 0.01	5.0 ± 0.01	0.269 ± 0.003	15.19 ± 0.01
		Average	16.2 ± 0.3	12.1 ± 0.01	1.77 ± 0.02	13.1 ± 0.01	4.8 ± 0.01	0.267 ± 0.004	15.00 ± 0.02
LSD_{05} (Factor A)			$F_f < F_{tabl}$	0.02	0.013	$Ff < F_{tabl}$	$Ff < F_{tabl}$	0.004	$Ff < F_{tabl}$
LSD_{05} (Factor B)			$Ff < F_{tabl}$	0.02	0.023	$Ff < F_{tabl}$	$Ff < F_{tabl}$	0.005	$Ff < F_{tabl}$

Our data confirm the information previously provided by other authors about the phenotypic diversity of cornelian cherry fruit. Evaluation of cornelian cherry fruit, even in a small area studied coenopopulation of the Crimean Peninsula, revealed phenotypic diversity among accessions for all the characters studied. Similar level of variability and similar intervals of variation for fruit were found with different cornelian cherry growth in natural population in Serbia (Mratinić et al., 2015), Adydea (Ujukhu, 2006), Slovakia (Brindza et al., 2009), Romania (Cornescu & Cosmulescu, 2017), Hungary (Prokaj et al., 2009), Turkey (Demir & Kalyoncu, 2003; Ercisli et al., 2006), Iran (Hassanpour et al., 2012) (Table 6). According to the data of references, the majority cornel populations in other region have a fruit weight from 1.2 to 3.3 g, fruit length - from 15.0 to 22.0 mm, fruit diameter - from 9/0 to 15.0 mm. The lowest parameters of fruit weghts were found in natural population in Serbia, Adygea and Crimean Peninsula.

Table 6. Average metric parameters of cornelian cherry fruit in various region

Metric parameters	Region							
	Crimean Peninsula	Serbia	Adygea	Slovakia	Hungary	Romania	Turkey	Iran
Average fruit length (Lcp), mm	11.6–17.7	14.9–18.6	13.5–21.0	12.0–19.5	15.4–20.0	12.0–21.0	16.0–20.8	15.2–22.3
Average fruit diameter (Dcp), mm	7.6–13.1	10.7–12.8	8.0–15.0	7.4–15.2	10.4–14.7	9.0–12.0	10.9–16.4	10.3–16.3
Shape index, (Lcp/Dcp)	1.33–1.89	1.40–1.48	1.40–1.69	1.28–1.62	1.36–1.48	1.05–1.55	–	1.19–1.51
Average fruit weight, g	0.53–1.81	1.25–1.91	1.40–1.70	0.50–3.40	1.20–2.85	1.33–2.31	1.49–4.12	1.49–3.29
Average endocarp (stone) lengths (Lcp), mm	6.7–13.5	12.0–13.4	9.0–16.0	9.5–15.9	–	–	–	11.6–14.2
Average endocarp diameter (Dcp), mm	3.1–5.1	5.5–6.1	4.0–7.0	4.5–8.9	–	–	–	6.1–7.5
Average endocarp weight, g	0.144–0.271	–	0.155–0.210	–	0.270–0.410	0.340–0.590	–	0.249–0.425
Endocarp ratio, %	10.5–27.9	–	7.0–18.0	–	–	20.7–27.9	–	10.5–21.8
Mesocarp ratio, %	72.1–89.5	–	82.0–93.0	–	72.9–86.7	72.1–79.3	–	78.2–89.5

The analysis of the data obtained showed that the maximum values of the biometric parameters of the fruits were noted in 2017, the minimum - in 2018, and the 2019 values were intermediate. At the same time, low values of the coefficient of variation (Cv < 10%) were set for most parameters. Only for the endocarp diameter in almost all coenopopulations in all years of studied, the average values of the variation of the trait were established (Cv up to 17.3%) (Table 7).

The data of two-way analysis of variance showed that the greatest influence on the biometric parameters of fruits is exerted by the diversity form and genetic characteristics of studied plants (fruit length - 72.2%, fruit diameter - 81.8%, fruit weight - 96.4%, endocarp length - 80.2%, endocarp diameter - 70.1%, endocarp weight - 79.4%, $p < 0.00001$). The share of the influence of weather conditions of the year (Factor A) was insignificant and amounted from 0.6% to 9.8% (Table 8).

Table 7. Coefficients of variation of morphometric parameters of cornelian cherry fruit in various coenopopulation

Coenopopulation (CP)	Form (F)	Year	Coefficient of variation (Cv), %					
			Fruit			Endocarp		
			Length (Lcp)	Diameter (Dcp)	Weight	Length (Lcp)	Diameter (Dcp)	Weight
CP 1	F 1	2017	7.4	5.8	4.6	9.5	16.0	5.0
		2018	7.8	8.0	7.3	7.9	10.0	5.3
		2019	4.4	6.8	2.9	6.3	10.9	4.9
		Average	6.5	6.7	4.9	7.9	12.3	5.1
CP 2	F 1	2017	5.1	6.2	3.0	11.5	14.7	5.9
		2018	12.5	5.6	10.4	5.7	15.6	6.7
		2019	4.8	3.1	4.7	5.5	14.8	5.9
		Average	7.5	5.0	6.0	7.6	15.0	6.2
CP 3	F 3	2017	7.2	5.9	15.2	8.8	14.6	6.7
		2018	10.3	11.4	10.4	7.9	17.1	7.1
		2019	4.9	6.6	7.0	13.4	12.2	7.6
		Average	7.5	7.8	10.8	10.0	14.6	7.1
CP 4	F 4a	2017	3.1	6.3	12.0	5.3	17.3	14.3
		2018	4.5	7.6	4.7	8.1	14.2	5.1
		2019	4.4	8.3	8.6	5.8	14.3	9.1
		Average	4.0	7.4	8.4	6.4	15.3	9.5
	F 4b	2017	5.7	11.1	6.3	8.1	13.2	13.4
		2018	7.1	13.8	5.4	8.1	15.2	5.9
		2019	4.5	5.9	7.8	8.5	10.8	5.8
		Average	5.8	10.3	6.5	8.2	13.1	8.4
CP 5	F 1	2017	4.7	6.5	3.7	8.1	14.0	5.9
		2018	3.6	5.9	3.5	4.6	11.3	11.6
		2019	4.9	7.7	3.2	3.1	8.9	7.8
		Average	4.4	6.7	3.5	5.3	11.4	8.4

Table 8. Data of two-way analysis of variance of cornelian cherry fruit in various coenopopulations

Factor	SS	dF	MS	F	*p*-value	Shape of influence, %
Fruit length						
A	0.86	2	0.434	52.5	0.000000	2.9
B	22.84	5	3.139	379.5	0.000000	79.2
AB	0.09	10	0.009	1.1	0.347701	0.3
Error	5.06	612	0.008			17.6
Fruit diameter						
A	0.56	2	0.2839	44.4	0.000000	2.2
B	20.34	5	4.0698	636.0	0.000000	81.8
AB	0.12	10	0.0124	1.9	0.037854	0.4
Error	3.91	612	0.0064			13.5
Fruit weight						
A	0.76	2	0.3822	55.3	0.000000	0.6
B	142.92	5	28.5843	4133.4	0.000000	96.4
AB	0.23	10	0.0237	3.4	0.000223	0.2
Error	4.23	612	0.0069			2.8

Table 8 (continued)

Endocarp length						
A	0.69	2	0.3460	42.0	0.000000	2.2
B	25.03	5	5.0074	608.3	0.000000	80.2
AB	0.48	10	0.0488	5.9	0.000000	1.6
Error	5.03	612	0.0082			16.0
Endocarp diameter						
A	0.44	2	0.2218	61.69	0.000000	9.8
B	1.80	5	0.3605	100.28	0.000000	70.1
AB	0.08	10	0.0084	2.34	0.010389	1.8
Error	2.20	612	0.0036			18.3
Endocarp weight						
A	0.007	2	000380	11.38	0.000014	6.5
B	0.852	5	017048	510.09	0.000000	79.4
AB	0.009	10	000091	2.72	0.002788	0.8
Error	0.204	612	000033			13.3

Plants with a largest fruits (an average weight from 1.65 g to 1.81 g) and a low endocarp ratio - from 10% to15% (Form 1 - CP I, CP 2, CP 5, Form 4a - CP 4) are interest for breeding research, since there is continued search for new forms with a high of contents mesocarp.

CONCLUSIONS

Studies conducted have shown that cornelian cherry on the territory of the Crimean Peninsula has a wide range of variability in a number of morphological features, which indicates a high form diversity of the species within its natural habitat. The greatest variety of cornelian cherry fruits was noted in the foothill zone and mountain zones, and our data confirm the information previously provided by other authors (Klimenko et al., 2003; Klimenko, 2004 and 2007) about the significant intraspecific diversity of cornel forms on the Crimean Peninsula.

The results of the test of differences in means using ANOVA tests indicates that the cornelian cherry plants differ significantly with respect to most metric characteristics in various coenopopulations. Analysis showed that the hydrothermal indicators of the year practically did not affect on the metric parameters of cornelian cherry plants. The genetic characteristics of plants within coenopopulations significantly influenced the studied biometric parameter.

The results have shown that there was a high diversity in cornelian cherry populations within different ecological areas of the Crimean Peninsula. For most of the studied morphometric parameters of leaves and fruits in 2017 to 2019, a low value of the coefficient of variation was revealed, which made it possible to conclude that these characteristics are not very variable. Differences were exhibited in terms of fruit size and shape. The results obtained can be useful for botanical research as additional information about this species, as well as in selection research when searching for natural forms of desirable characteristics for creating cultural varieties of cornelian cherry. The plants with largest fruits and the lowest proportion of endocarp in the total mass of the fruit can be recommended for further breeding research.

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Experimental research into new harrowing unit based on gantry agricultural implement carrier

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Abstract. From the point of view of energy saving, research into agricultural gantry units equipped with new implements specially adapted for operation in such systems and capable of efficiently functioning in controlled traffic (permanent lane) and wide span (gantry) systems is an important and topical issue in soil tillage science. The range of wide span systems includes harrowing units for fallow land tillage. This paper describes the experimental determination of the operating characteristics of the harrowing unit, in which a gantry implement carrier propels harrows for fallow land tillage, for the purpose of establishing the compliance of their parameters with the fundamental principles of the effective implementation of the controlled traffic and wide span soil management. The experimental investigations were done using strain-gauge and control-and-measuring equipment with analogue-to-digital conversion of the signals received from the sensing elements. An agricultural wide span unit with a track width of 3.5 m and harrows for fallow land tillage were investigated. The results show the harrowing unit is well adapted to operation in controlled traffic and wide span soil tillage systems and delivers high quality performance of the harrowing process. The latter effect is also due to the fact that the gantry travels on the compacted tracks which have a few surface irregularities compared to cultivated soil. The coefficient of variation of the draught force applied to the hook of the gantry during harrowing operations did not exceed 10%. This is evidence of the high stability (low variability) of the harrowing process, which has a positive effect on the motion stability of the harrowing unit based on the gantry tractor.

Key words: controlled traffic farming, harrowing unit, wide span system.

INTRODUCTION

The highly efficient development of controlled traffic and wide span soil management throughout the world is possible subject to wide implementation of the achievements by the science and technology progress (Rohde & Yule, 2003; Raper, 2005; McHugh et al., 2009; Tullberg, 2009; Kingwell & Fuchsbichler, 2011; Gasso et al., 2013 and 2014; Chamen, 2015; Antille et al., 2015 and 2019; Nadykto, 2019). In this context, the topic of searching for new soil tillage implements, machines and tools capable of efficiently operating in controlled traffic and wide span soil management systems becomes a top priority (Nadykto, 2012; Bulgakov et al., 2020b). Agricultural implements for soil surface tillage are among the representatives of the family of the soil tillage machines designed for operation in controlled traffic and wide span soil management systems. The harrows for fallow land tillage are one of the types of such implements. The functional capabilities of tillage implements, when used as part of agricultural wide span units for minimum soil cultivation, provide for the ripping of the soil surface layer within the agronomic zone of the field without carrying over the moisture to the surface, the stable operation of the tools with regard to the depth of their movement, the high quality of soil tillage etc. However, while the controlled traffic and wide span soil management is now widely accepted as a promising development trend, there is lack of attention to the experimental research into the new dedicated machines and implements designed in this area. The quality of the performed soil management operations depends on the design features of the employed agricultural implement, the mode of operation of the unit, the properties of the soil etc. In case of soil harrowing that includes also such factors, as the evenness of the surface in the farmed piece of land, the uniformity of the tilling depth and some other. Currently, in view of the introduction of new tilling implements operated as part of non-conventional (wide span) units, the industry faces a lack of the data required for validating the permissible variation of individual performance quality indices, and those data can be obtained only by means of special research. Alongside with that, one of the aims targeted by the introduction of wide span units into the controlled traffic soil management system is reducing the energy intensity of soil cultivation processes in view of the fact that the track width of such units (working span) can be very large. That said, in contrast to the conventional harrowing units that operate at acceptable travelling speeds of 8–15 km h^{-1}, the reduction of the energy data in case of wide span units can be achieved also through the lowering of their operational travel rates to values below 5 km h^{-1}. Thus, the research into the discussed gantry units has to aim at both finding out their main operation and process characteristics and developing the new implements (in particular, harrows) that provide for the required agronomic performance quality of the work process.

The detailed analysis of the results obtained during the experimental investigations of agricultural machines and implements as parts of controlled traffic and wide span soil management systems as well as the accumulated practical knowledge are of invaluable importance for science.

Many scientists Chamen et al. (1992, 1994), Taylor (1994), Bochtis et al. (2010), Onal (2012), Pedersen et al. (2013, 2016), Bulgakov et al. (2017, 2018, 2019), Chamen (2000) have made a great input into the development and promotion of the controlled traffic and wide span soil management. At the same time, the analysis of the research results published by them has revealed that their papers still feature insufficient attention to the issue of fundamental experimental research into the characteristics of wide span soil tillage units based on gantry agricultural implement carriers.

As regards the use of the latter with implements adapted for operation with them, a design of interest is the harrow with tines equipped with subsurface segment blades (Adamchuk et al., 2020). In all the rows of tines the blades are installed in the horizontal plane at the same angle of inclination. But such a harrow design does not provide for the efficient extirpation of weeds (weed stalks and roots become wound on the tools). The difficulty in the penetration of these harrowing tools into the soil, especially in case of the latter's increased compactness, which results, overall, in the insufficient quality of the soil tillage with regard to the depth of tillage, is another problem faced, when using the said type of harrows.

Another design of harrow for soil tillage (Bulgakov et al., 2020a) has been developed for arid soil management conditions. In this design, the subsurface segment blades in the first row are set in the longitudinal vertical plane, the blades in the second row are set at a greater inclination to the horizontal plane than the blades in the last rows. According to the authors, such a harrow design solution provides for the high quality of soil tillage and is appropriate for use in the agricultural wide span gantry units that travel on permanent traffic lanes.

The aim of the research described in this paper was to establish the conditions required for the efficient use of harrows in fallow land tillage and to determine the optimum parameters and characteristics for the harrowing unit based on a gantry agricultural implement carrier operating in a controlled traffic and wide span soil management system.

MATERIALS AND METHODS

The experimental investigations were carried out standard techniques and the ones specially developed by the authors, with the use of up-to-date strain-gauge and control-and-measuring equipment with analogue-to-digital conversion of the signals received from the installed strain-gauge and other slide-wire transducers. The obtained test experiment data were processed on the PC with the use of the probability calculus, the regression analysis as well as the correlation spectrum analysis.

During the experiments, the following tasks were pursued: to determine the longitudinal irregularity profiles of the permanent process lane and of the cultivated soil before and after harrowing, the moisture content and density of the soil, and the soil tillage depth.

The equipment used was a wide span gantry agricultural implement carrier with a track width of 3.5 m, the design of which had been developed by the authors (Fig. 1), and harrows designed for fallow land tillage (Adamchuk et al., 2020).

Figure 1. Harrowing unit based on gantry agricultural implement carrier.

The structural layout of the tools installed in the harrow for fallow land tillage is presented in Fig. 2. In this harrow, the front row tines feature flat segment blades installed at their ends in the longitudinal vertical plane. The tines in the second and all the remaining rows have at their ends flat segment blades installed in the horizontal plane at an angle of α. Starting from the third row and further, the angle α, at which the flat segment blades are installed, decreases.

The area of the flat segment blades is determined by their linear dimensions $a \times b$. The depth, at which the tines of the harrow under consideration travel in the soil, is designated h.

The use of the tines that have flat segment blades fixed at their ends to facilitating the efficient cutting of weeds was a distinctive feature of the harrow used in the experimental investigations. The dimensions of the flat segment blades were selected so they would not obstruct the penetration of the tines into the soil to the required depth h, and would also efficiently rip the soil to the pre-set depth without carryover of its lower moisture-laden layer to the field surface, thus achieving the necessary quality of soil tillage.

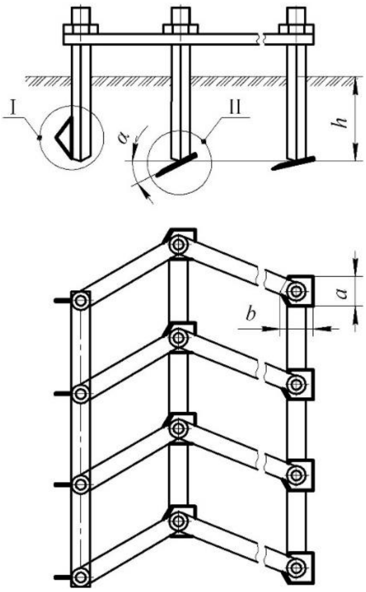

Figure 2. Design of harrow for fallow land tillage: I – first row tines; II – second row tines.

The harrowing unit used in the field experiments was attached to the gantry agricultural implement carrier, which was fitted with 9.5R32 tyres. The experiments were carried out with the use of a specially equipped field laboratory.

During the experiments, the irregularities of the longitudinal profile of the permanent process lane and the cultivated soil were recorded with a high accuracy automated profile recorder developed by the authors (Fig. 3).

Figure 3. Automated field surface profile recorder.

The draught resistance of the harrows was determined with the use of a traction strain-gauge link attached to the SA-type dynamometric automatic hitch specially adapted for this purpose (Fig. 4).

The cultivated soil profile irregularities and the draught resistance of the harrowing tools, were recorded with the use of an integrated measuring and recording unit based on an analogue-digital converter and a personal computer (PC) loaded with the respective software programmes (Fig. 5).

Figure 4. SA-type dynamometric automatic hitch with traction strain-gauge link.

Figure 5. Integrated measuring and recording unit.

The records obtained in the process of the experiments were transferred in the form of digitised data into the Mathcad Prime 5.0 environment, where the calculations were performed. Mathcad was used to calculate the following statistical characteristics: mean value; root-mean-square (standard) deviation; variance; coefficient of variation; sample mean accuracy; normalised correlation function; normalised spectral density. The above-mentioned statistical parameters were determined with the use of techniques described by Brandt (2014). The error of direct parameter measurement did not exceed 1%.

The moisture content of the soil was determined in accordance with the standard thermostat-oven weighing method. The soil tillage depth was with a specially developed depth gauge at 10 locations on the diagonal of the cultivated land lot.

The experimental research was carried out on dark chestnut (*Kastanozem*) lightly alkaline (*Solonetz*) type of soil. The share of the fractions with soil clod diameters of up to 2.0 cm was at least 80%. The soil cloddiness (presence of lumps with diameters greater than 4 cm) was maximum 2–3 lumps per square metre. The cultivated soil used in the described experiments was a field prepared for sowing.

During the experiments, the mean value of the soil moisture content within the 0–10 cm layer was 26.8%, of the soil dry bulk density - 1.23 Mg m^{-3}.

The experiments were carried out in a 50 m long test plot at speeds ranging from to 3.6, 4.1 and 4.5 km h^{-1}. At each of these travelling speeds, the experiments with data recording were performed in 3 replications, with the harrowing unit moving in both directions. Hence, the total number of experiments exceeded 30.

RESULTS AND DISCUSSION

Analysis of the experimental data shows that the profile of the permanent process lane is smoother than the profile of the cultivated soil after its tillage. For example, the root mean square deviation of the harrowed soil profile was ±1.36 cm, while for the profile of the lane it was ± 0.84 cm, which is 1.6 times less (Fig. 6).

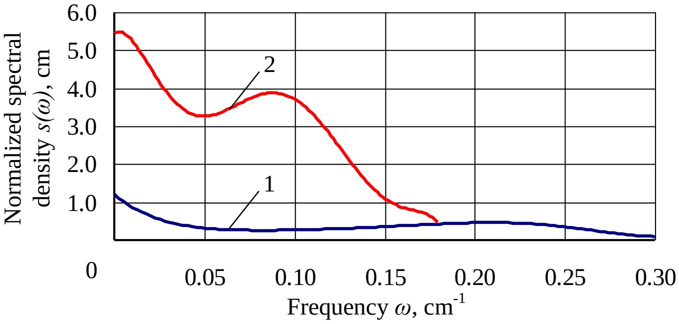

Figure 6. Diagrams of normalised spectral density of irregularities in longitudinal profiles of permanent process lane track (1) and harrowed soil (2).

As regards their inherent structure, the irregularities in the profile of the permanent process lane are characterized by the function that together with random components contains also harmonic ones represented by damped periodic oscillations of the normalised correlation function (Fig. 7). According to it, the correlation length between the ordinates of the irregularities in the profile of the permanent process lane was circa 0.18 m, which corresponds to the spacing of the lugs on the tyres of the gantry agricultural implement carrier, the latter being equal to 0.175 m.

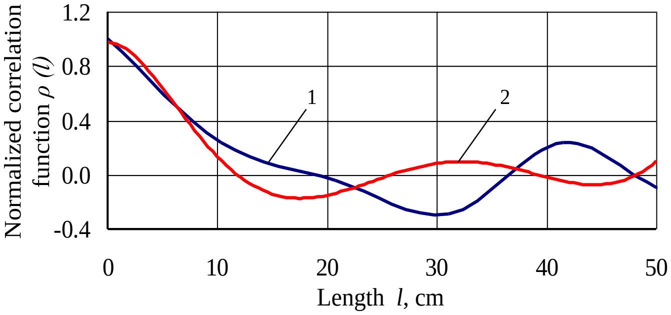

Figure 7. Diagrams of normalised correlation function of irregularities in longitudinal profiles of permanent process lane track (1) and harrowed soil (2).

The spectrum of the frequencies representing the random function of the irregularities in the profile of the permanent process lane is specified by the normalised spectral density of the ordinates of the said irregularities (Fig. 6). It has been established by analysing the normalised spectral density of irregularities in the profile of the permanent process lane that the cut-off frequency for this process was approximately 0.3 cm^{-1}. The main part of the variance of the track profile irregularity ordinate oscillations is concentrated within the range of frequencies 0–0.3 cm^{-1}. Their root mean square deviation corresponds to the height of the lugs on the tyres of the gantry agricultural implement carrier, which was 0.03 m.

The above analysis of the characteristics observed in the irregularities of the profile of the permanent process lane, on which the gantry agricultural implement carrier travels repeatedly many times, gives evidence of the fact that these irregularities are generated by the parameters of the lugs on its tyres.

The inherent structure of the irregularities in the profile of the cultivated lot of cultivated soil is to some extent different from that of the irregularities in the profile of the permanent process lane (Fig. 7). The correlation distance between the ordinates of the irregularities in the profile of the harrowed cultivated soil was about 12 cm. Such a profile of the agronomic background is typical of the fields cultivated for the purpose of sowing many agricultural crops.

As distinct from the irregularities generated by the tyres on the wheels of the gantry agricultural implement carrier in the profile of the permanent process lane tracks, the profile of the harrowed lot of agronomic background has a lower-frequency pattern (Fig. 7). The correlation length between the ordinates of the irregularities of the permanent process lane tracks is equal to about 0.175 cm.

The oscillations of the draught resistance offered by the harrowing implement represent a random function, in which harmonic components are absent (Fig. 8). The main variance spectrum of the tractive resistance oscillations is concentrated within the range of frequencies 0–3.5 s^{-1}.

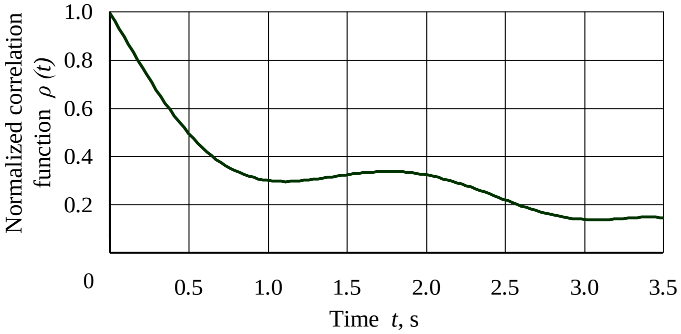

Figure 8. Diagram of normalised correlation function of oscillations in draught resistance of harrowing implement.

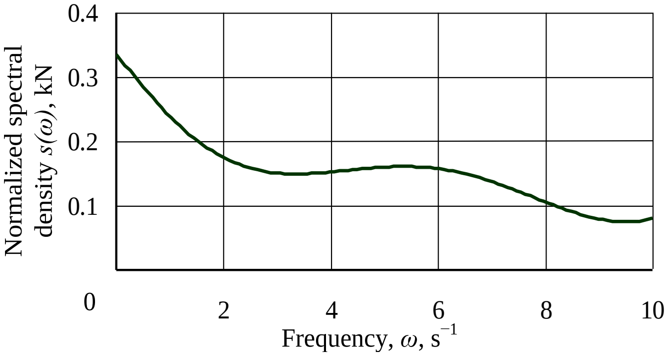

Figure 9. Diagram of normalised spectral density of oscillations in draught resistance of harrowing implement.

The energy of the oscillation variance of the draught resistance offered by the harrow was equal to 0.027 kN^2, and the root mean square deviation σ_{Ph} was equal to 0.166 kN (Fig. 9). If the mean value of the draught resistance P_h produced by the three harrows in the gantry unit is equal to 1.71 kN, the coefficient of variation k of its oscillations in the process of harrowing is equal to 9.76% [$k = (\sigma_{Ph}\ 100)\ (P_h)^{-1} = (0.166 \cdot 100)\ (1.71)^{-1} = 9.76\%$]. Such a result is a good sign of high stability (low variability) of the process of harrowing using the gantry agricultural implement carrier.

The quality indices of the soil harrowing performed with the use of the gantry agricultural implement carrier equipped with harrows for fallow land tillage comply with the agrotechnical requirements applied to this process operation (Bulgakov et al., 2020a). In particular, the deviation of the actual cultivation depth h from the target value does not exceed ± 1 cm, while the height of the ridges on the agronomic background does not exceed 2 cm.

CONCLUSIONS

1. As a result of the experimental investigations carried out on a gantry agricultural implement carrier and a harrow for fallow land tillage, it has been proved that the implement is well adapted to the operation in controlled traffic and wide span soil management systems and delivers a high quality tillage result. The latter fact is also due to the gantry agricultural implement carrier travelling on the compacted tracks of the permanent process lane, the irregularity profile of which has a lower-frequency pattern as compared with the longitudinal profile of the harrowed soil.
2. The design of the harrow for fallow land tillage has a positive effect on the variation of its draught resistance. It has been established that the oscillations of the draught resistance produced by the harrowing implement represent a random function, in which harmonic components are absent. The coefficient of variation of the oscillations in the resistance force applied to the hook of the gantry agricultural implement carrier during harrowing work does not exceed 10%. That is indicative of the high stability (low variability) of the soil harrowing process. This characteristic, in its turn, provides for lower variation of the moment of resistance applied to the running gear of the gantry tractor, which, finally, has a positive effect on the stability of motion of the gantry agricultural implement carrier-based harrowing unit under consideration.
3. The operation of the agricultural gantry-carrier harrowing unit at a low rate of travel (less than 5 km h^{-1}) does not impair the quality indices specified for the work process under consideration by the agronomic standards. That renders possible significantly reducing in the future the energy intensity of the soil cultivation processes based on the use of gantry-carrier units travelling on the tracks of the permanent process lane, by means of reducing the operational travel rates for units with increased working widths.

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Zinc content in different muesli samples

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Abstract. There is no specialized zinc storage system in the body, therefore there must be a daily intake of zinc to achieve a steady state. Long-term zinc deficiency due to inappropriate nutrition may result in immunological or autoimmune diseases. The aim of this study was to develop muesli with naturally high zinc content. Zinc is found in various plant-based foods as grain flakes and seeds which often are one of the raw materials for muesli. Muesli is one of the grain-based food trends nowadays as people`s life habits are changing due to fast-paced life, still, it is important to obtain all nutrients. According to zinc content in raw materials five different muesli samples were prepared, from all samples, muesli with the highest zinc content with 3.80 mg 100 mg^{-1} was chosen for further analysis. Zinc bioavailability is assessed through the determination of absorbability. The kinetic *in vitro* intestinal digestion suggests how much zinc is released during digestion and could be absorbed in the small intestine. The results show that during digestion approximately 22% of zinc was absorbed in the small intestine. Such characteristics of *in vitro* digestion test shows that by one meal (50 g of muesli) it is possible to replenish our body zinc level by 0.42 mg. To check additional nutrient content in selected muesli samples different mineral and vitamin analyses were done. Mineral and vitamin content in the muesli sample was calculated according to their content in raw materials. Per portion, muesli is source of iron, magnesium, phosphorus, zinc, vitamin B1, vitamin B2, vitamin B3, vitamin B6 and vitamin E.

Key words: absorption, digestion, in vitro, muesli, zinc.

INTRODUCTION

Recent years plant-based product purchasing is growing since vegetarian and vegan diet is getting more popular. Vegetarians and vegans may potentially be at risk in zinc deficiency (Saunders et al., 2013) well planned diets can provide amounts from plant sources (Rose & Strombom, 2019), yet it is important to consume more products with naturally high zinc content. The role of breakfast cereals in balanced diet has been recognized for many years. Breakfast cereals are important source of vitamins and minerals, also, potentially important source of antioxidants and phytoestrogens, and wholegrains (Williams, 2014). Zinc is found in various plant-based foods as grain flakes and seeds which often are one of the raw materials for muesli.

Zinc is second most abundantly distributed trace element after iron. Zinc functions in biology are numerous but can be separated into three main categories: catalytic, regulatory, and structural roles (Bhattacharya et al., 2016). It catalyses enzyme activity, contributes to protein structure, and regulates gene expression (Saper & Rash, 2009; Kambe et al., 2015). Zinc is cofactor for over 1,000 enzymatic reactions and is necessary for over 2000 transcription factors. Zn-fingers protein function for DNA interaction, RNA packaging, activation of transcription, regulation of apoptosis, folding and assembly of protein, and lipid binding. Also, around 10% of human proteins bind to zinc. This is the reason why zinc is associated with a wide variety of organic activities such development, differentiation, and cell growth (Ogawa et al., 2017).

Zinc absorption occurs throughout the small intestine. Approximately 30% of zinc in the diet is absorbed in the small intestine. Of the absorbed zinc, 80% and 20% are bound to blood albumin and alpha-2-macroglobulin. However, this protein-bound zinc comprises only 0.1% of the total body zinc, indicating that only this amount is replenished daily. This serum zinc is delivered and stored in peripheral tissues including skeletal muscle (60%), bones (30%), skin (5%) and liver (5%). Moreover, the skin is the third among tissues with the most abundance of zinc in the body. By strict regulation, zinc is stably maintained in the human body in a weight 2–3 g (Ogawa et al., 2016). It is generally agreed that most of the dietary zinc is absorbed in the proximal small intestine, through a transcellular saturable carrier-mediated mechanism. Even though the jejunum appears to have the highest rate of zinc absorption, the duodenum is first exposed to zinc during the postprandial period and is likely to also contribute to zinc absorption (Gopalsamy et al., 2015).

There is no specialized zinc storage system in the body, therefore there must be a daily intake of zinc to achieve a steady state. Long-term zinc deficiency due to inappropriate nutrition may result in immunological or autoimmune diseases. During zinc deficiency different immune functions are decreased. All these impaired functions are completely restored by zinc supplementation (Rink & Gabriel, 2000). Zinc bioavailability should also be considered which is determined in three basic stages - absorbability, mucosal transfer into systemic circulation and utilization within the body. As for human dietary studies, bioavailability is assessed through the determination of absorbability (Bel-Serrat et al., 2014). Phytate have considerable impact on percentage of zinc accessible for absorption. It can bind zinc in the intestinal lumen and form an insoluble complex that cannot be digested or absorbed because humans lack the intestinal phytase enzyme. Inhibitory effect on zinc absorption in adults can be substantial as there is no evidence of an adaptive response to habitual high-phytate intakes (King, 2016). Still some studies show that the total amount of zinc in a meal may have greater effect on zinc absorption than the presence of phytate (Saunders et al., 2013).

The aim of this study was to develop muesli with naturally high zinc content. Zinc is found in various plant-based foods as grain flakes and seeds which often are one of the raw materials for muesli. As previously mentioned, Bel-Serrat et al. (2014) reported that in human dietary studies, zinc bioavailability is assessed through the determination of absorbability. The kinetic *in vitro* intestinal digestion suggests how much zinc is released during digestion and could be absorbed in small intestine. *In vitro* digestion could be helpful after different high nutrition product development to formulate product which would have effect on consumer health. There are not many studies about zinc *in vitro* bioavailability from muesli or other grain products. This study will show the amount of zinc absorbed after one muesli portion. To see additional nutrient content in selected muesli sample, different mineral and vitamin analysis were done.

MATERIALS AND METHODS

Muesli development experiments were carried out at the laboratory of Felici LLC. Three repeated *in vitro* gastrointestinal digestion tests were carried out at the laboratory of Kauna Technical University (KTU). All ingredients used for muesli sample preparation and analysis were supplied by Felici LLC.

Raw material and product analysis

To check zinc content six different whole grain samples and two seed samples were tested. Muesli samples were prepared according to zinc content in selected raw materials, also, according to raw material price to develop market price relevant muesli. The main requirement was 70% flakes, 25% seeds, 5% additional raw materials as freeze-dried fruits. Freeze-dried fruits are needed to achieve a more pleasant taste. According to previously mentioned points, five different muesli samples were prepared. Raw material and muesli sample list and their codes tested for zinc content are listed in Table 1.

Table 1. Sample codes used in research

Raw materials	Sample code
Toasted wheat flakes	F1
Toasted triticale flakes	F2
Toasted oat flakes	F3
Toasted barley flakes	F4
Rolled oat flakes	F5
Extruded buckwheat flakes (without sugar)	F6
Pumpkin seeds	S1
Sunflower seeds	S2
Muesli samples	Sample code
Muesli with F1, F6, S1, S2	M1
Muesli with F1, F5, F6, S1, S2	M2
Muesli with F4, F6, S1, S2	M3
Muesli with F4, F5, F6, S1, S2	M4
Muesli with F2, F5, S1, S2	M5

Raw material and muesli sample zinc concentration was determined at food quality testing laboratory by ICP-MS (inductively coupled plasma mass spectrometry) according to PB-223/ICP, ed. II of 12.01.2015. method according to EN 15763:2010 standard.

In vitro analysis

Static *in vitro* simulation operated under adult conditions. Muesli was digested *in vitro* according to INFOGEST 2.0 protocol (Minekus et al., 2014) INFOGEST static in vitro simulation of gastrointestinal food digestion protocol (Brodkorb et al., 2019). Muesli sample was grinded (Braun GmbH, Australia) into homogenous mix, particle size from 0.5 to 2.0 mm. To form a bolus grinded sample was taken in a beaker and same amount of distilled water (1:1) was added. 5 ± 0.01 g of sample was weighted (KERN&SOHN GmbH, Germany) in beaker. To initiate oral phase simulated saliva fluids (SSF) with salivary alpha-amylase from porcine pancreas (75 U mL^{-1} of digest) to a final volume of 10 mL were added. Oral step lasted 2 minutes, pH 7 ± 0.1. Gastric phase was initiated by adding simulated gastric fluids (SGF) containing pepsin (2,000 U mL^{-1} of digest) till 20 mL ± 1 and incubated for 120 min, under pH between 2 ± 0.1 and 3 ± 0.1. After incubation intestinal phase was initiated by adding simulated intestinal fluids (SIF) containing pancreatin (100 U mL^{-1} of digest), lipase (2,000 U mL^{-1} of digest), and bile salts (10 mM of digest) till 40 mL ± 1 and incubated for 120 min, under pH 7 ± 0.1. Temperature controlled water bath at 37 ± 0.1 °C with continuous shaking at 150 rmp (Thermolab, Germany, GFL 1092) was used.

The digestion was stopped during gastric (G) phase after 0, 60, 120 minutes and duodenal (D) phase at 5, 60, 120 minutes. To neutralize sample to a pH of 7.0 ± 0.1 and stop digestion process the samples were cooled to 0–4 ± 0.1 °C in ice water. Sample pH was controlled by MW102-FOOD (Milwaukee Instruments, USA). After digestion samples were centrifuged at 4,000 rmp, at +4 °C temperature (MPW-260RH, MPW Med. Instruments, Warsaw, Poland) and filtered. The soluble fraction was collected and freeze-dried before analysis. Digestion procedure was performed thrice.

Zinc concentration was determined by atomic absorption spectroscopy method, which is based on selective absorption of electromagnetic waves by non-exited atoms of analyte. Samples were analysed with Perkin Elmer AAnalyst 400 (Perkin Elmer, USA) spectrometer utilizing flame as atomizer and zinc hollow cathode lamp as the source of electromagnetic radiation. Atomizer was produced by burning acetylene with air with oxidizing lean blue flame and absorption. Specific to zinc, it was measured at 213.9 nm, this wavelength provides linear range for measurements up to 1.0 mg L^{-1} with characteristic concentration of 0.018 mg L^{-1}. Using method of calibration curve using series of zinc standards with concentration ranging up to 1.0 mg L^{-1} quantification was performed. After each digestion step zinc content was determined, digestion procedure was performed twice.

Data processing

To analyse obtained data MS Excel 2016 was used. ANOVA analysis were performed to determine the difference between the samples. Factors were defined as significant if *p*-value was below 0.05.

RESULTS AND DISCUSSION

Zinc content in different raw materials and muesli samples

As base for muesli production Felici LLC usually is using two types of wholegrain flakes, toasted, and rolled. Both used for their different structure, but the main reason is their nutrition. To check zinc content different grain flakes as toasted triticale, barely, oat, wheat flakes, rolled oat flakes and extruded buckwheat flakes were chosen and tested, as for seeds pumpkin and sunflower seeds were tested. Table 2 shows zinc content in different grain flakes and seeds. Zinc amount between grain flakes ranged from 1.21 to 2.83 mg 100 g^{-1}. Lowest zinc content was in extruded buckwheat flakes (without sugar), highest 2.23 and 2.82 mg 100 g^{-1} were in toasted triticale flakes and rolled oat flakes. As for seeds it was 5.00 mg 100 g^{-1} for sunflower seeds and 7.50 mg 100 g^{-1} for pumpkin seeds. To decide on the most appropriate raw materials, a review for zinc content in different grain flakes and seeds before analysis was done. Different studies emphasize zinc content in grains or rolled flakes, there are not many studies about zinc content in toasted and extruded flakes. Frolich et al. (2013) reported that 100 grams barley has 1.4 mg, rye 2.4 mg, wheat 2.6 mg and oats 2.9 mg of zinc, highest content was in whole grain oats. Biel et al. (2020) reported results showed that per 100 g oat grains has the lowest zinc content of 2.1 mg, as for other flakes, barley 2.3 mg, triticale 2.5 mg, and wheat have the highest content around 3.2 mg of zinc per 100 grams. In other study, Kruma et al. (2018) compared zinc content in triticale grains and flakes.

Zinc content per 100 g triticale grains was 3.12 mg, rolled flakes 2.84 mg, but content in toasted flakes prepared by different technological parameters varied from 2.49 till 2.68 mg. Study reported that loses of minerals were mainly influenced by rolling thickness. The major losses could occur due to the solubilisation of minerals during the steaming process. This explains lower zinc content in toasted grain flakes. In whole obtained results showed that zinc content in grains and flakes in each study is different. Studies does not mention grain variety or flakes thickness what could affect zinc content in crops.

Table 2. Zinc content in analysed raw materials and muesli samples

Raw material sample code	Zinc content, mg 100 g^{-1}
F1	1.44 ± 0.01
F2	2.23 ± 0.01
F3	1.72 ± 0.01
F4	1.35 ± 0.01
F5	2.82 ± 0.01
F6	1.21 ± 0.01
S1	7.50 ± 0.01
S2	5.00 ± 0.01
Muesli sample code	**Zinc content, mg 100 g^{-1}**
M1	1.89 ± 0.01
M2	2.44 ± 0.01
M3	2.09 ± 0.01
M4	2.54 ± 0.01
M5	3.80 ± 0.01

As for seeds, Saunders et al. (2013) reports that pumpkin seeds contain 7.5 mg, sunflower seeds 5.8 mg, sesame seeds 5.5 mg, and flaxseed 4.3 mg of zinc per 100 grams. Other studies report that zinc content in pumpkin seeds is 7.8 mg 100 g^{-1} (Syed et al., 2019), sunflower seeds is 5.0 mg 100 g^{-1} (Anjum et al., 2012), sesame seeds is 3.6 mg 100 g^{-1} (Alyemeni et al., 2010), flaxseeds is 4.0 mg 100 g^{-1} (Bernacchia et al., 2014). All studies reported that pumpkin seeds and sunflower seeds has the highest zinc content. Obtained results showed similar zinc content in pumpkin and sunflower seeds as in previously mentioned studies.

After results five different muesli samples were prepared and analysed, results shown in Table 4. Zinc content in muesli samples varied from 1.89 till 3.80 mg 100 g^{-1}. Looking at the results, it was decided to use M5 muesli sample with 3.80 mg 100 g^{-1} zinc content for *in vitro* digestion test and for additional mineral and vitamin analysis. M5 sample contained following raw materials - toasted triticale flakes, rolled oat flakes, pumpkin seeds, and sunflower seeds, in total 96%. To improve muesli, taste freeze-dried pomegranate seeds, freeze-dried mango, banana, passiflora smoothie pieces and safflower petals were used.

According to regulation (EU) No 1169/2011 of the European Parliament and the council of 25 October 2011 and to regulation (EC) No 1924/2006 of the European Parliament and the council of 20 December 2006 daily reference intake for zinc is 10 mg so 3.80 mg 100 g^{-1} in developed muesli sample is high zinc content. By one portion, 50 g, it is possible to consume 1.90 mg of zinc, what will be 19% of daily reference intake, what would be source of zinc.

After European Commission request European Food Safety Authority (EFSA) has written scientific opinion on dietary reference values for zinc (EFSA panel on dietic products, nutrition, allergies (NDA)) which includes zinc average requirements and population reference intake for four different amounts of dietary phytate. This information is shown on Table 3.

As for EFSA (2014) scientific opinion on dietary reference values for zinc, for women varies from 7.5 mg day^{-1} till 12.7 mg day $^{-1}$ for but for men from 9.4 mg day^{-1} till 16.3 mg day^{-1}. Reference intake is based on level of phytate intake, from 300 till 1,200 mg day^{-1}. Estimations show that starting from phytate intake of 600 mg day^{-1} zinc reference intake is higher than 10 mg day^{-1}. Further analysis for phytate content in muesli should be done.

Table 3. Estimations of Average Requirement (AR) and Population Reference Intake (PRI) for zinc according to phytate intake and body weight (adults) (EFSA, 2014)

Level of phytate intake, mg day $^{-1}$	Body weight, kg	AR	PRI [(c)]
300	58.5 (a)	6.2	7.5
	68.1 (b)	7.5	9.4
600	58.5 (a)	7.6	9.3
	68.1 (b)	9.3	11.7
900	58.5 (a)	8.9	11.0
	68.1 (b)	11.0	14.0
1,200	58.5 (a)	10.2	12.7
	68.1 (b)	12.7	16.3

(a) Median body weight of 18 to 79 year old women based on measured body weight of 19,969 women in 13 EU Member States assuming a BMI of 22 kg m^{-2} (see Appendix 11 in EFSA NDA Panel (2013)). At this body weight the psychological zinc requirements are 2.9 mg day^{-1} (EFSA, 2014);

(b) Median body weight of 18 to 79 year old men based on measured body weight of 16,500 men in 13 EU Member States assuming a BMI of 22 kg m^{-2} (see Appendix 11 in EFSA NDA Panel (2013)). At this body weight the psychological zinc requirements are 3.2 mg day^{-1} (EFSA, 2014);

(c) Dietary zinc intake of subjects with a body weight at the 97.5 percentile of the reference body weights (i.e., 79.4 kg for men, 68.1 kg for women) (EFSA, 2014).

Mineral and vitamin content in selected muesli sample

Nutritional quality of breakfast cereals is very important, as they can contribute significantly to daily intake of energy, carbohydrate, protein, dietary fibre, vitamin and mineral (Jones & Poutanen, 2020). To check additional nutrient content in selected muesli samples different mineral and vitamin analysis were done. Mineral and vitamin content in muesli sample were calculated according to their content in raw materials. As for minerals - iron, calcium, magnesium, phosphorus, potassium, and zinc and for vitamins - vitamin B1, vitamin B2, vitamin B3, vitamin B6 and vitamin E. Content and percent of daily reference intake is shown in Table 4.

Table 4. Mineral and vitamin content in selected muesli sample

Minerals and vitamins	Content, mg 100 g^{-1}	Daily reference intake*, %
Iron	5.98 ± 0.01	42.71 ± 0.01
Calcium	49.80 ± 0.01	6.23 ± 0.01
Magnesium	220.73 ± 0.01	58.86 ± 0.01
Phosphorus	519.62 ± 0.01	74.23 ± 0.01
Potassium	460.85 ± 0.01	23.04 ± 0.01
Zinc	3.80 ± 0.01	38.00 ± 0.01
Vitamin B1	1.07 ± 0.01	97.27 ± 0.01
Vitamin B2	0.89 ± 0.01	63.57 ± 0.01
Vitamin B3	11.33 ± 0.01	70.81 ± 0.01
Vitamin B6	1.17 ± 0.01	83.57 ± 0.01
Vitamin E	8.04 ± 0.01	67.00 ± 0.01

* According to regulation (EU) No 1169/2011 of the European Parliament and the council of 25 October 2011.s

Results show that muesli sample contains is 5.98 mg 100 g^{-1} of iron, per portion it would be 21.36% of daily reference intake. Iron, as zinc, is most abundant trace mineral in human body, with 3–4 g of iron present in average adult. Iron and zinc are frequently assessed together as these minerals share common dietary sources, the absorption of both nutrients from food is believed to be enhanced and inhibited by similar compounds, and consequently, deficiency of both nutrients occur simultaneously (Lim et al., 2013). Results show that muesli sample contains 49.80 mg 100 g^{-1} of calcium, per portion it would be 3.11% of daily reference intake. Winiarska-Mieczan et al. (2016) reported similar average results in muesli and crunchy, around 39.22 mg 100 g^{-1}. Calcium is abundant element in human body, it contains around 1 kg of calcium with more than 99% deposit in the bone in the form of calcium phosphate. Through interacting with numerous proteins distributed in different cellular compartments, calcium is involved in large amounts of aspects of life, such as muscle contraction, enzyme activation, cell differentiation, immune response, programmed cell death and neuronal activity (Pu et al., 2016). Results show 220.73 mg 100 g^{-1} of magnesium, per portion it would be 29.43% of daily reference intake. Winiarska-Mieczan et al. (2016) reported average result of 84.33 mg 100 g^{-1} what is 2.6 times less than in tested muesli sample. Magnesium is the fourth most common mineral in the human body after calcium, sodium and potassium and is the second most common intracellular cation after potassium. Within the frame of 70 kg individual, there is an average of 25 g of magnesium in reserve with 53% in bone, 27% in muscle, 19% in soft tissues and less than 1% in the serum (Schwalfenberg & Genius, 2017). It plays an important role in molecular, biochemical, physiological, and pharmacological functions in the body (Faheemuddin & Abdul, 2019). After analysis it is possible to see that muesli sample contains 519.62 mg 100 g^{-1} of phosphorus, per portion it would be 37.12% of daily reference intake. Phosphorus is one of the most abundant minerals in the body, majority stored in bone and teeth. Maintenance of extracellular and intracellular phosphate levels within a narrow range is important for many biological processes, including energy metabolism, cell signalling, regulation of protein synthesis, skeletal development, and bone integrity. The recommended dietary allowance, 700 mg day^{-1}, for healthy adults, is meant to maintain serum phosphorus concentrations within the physiologic range of 2.5 to 4.5 mg dL^{-1} (Kiela et al., 2017). Results show that muesli sample contains 460.85 mg 100 g^{-1} of potassium, per portion it would be 11.52% of daily reference intake. Winiarska-Mieczan et al. (2016) reported average result of 182.9 mg 100 g^{-1} what is 2.5 times less than in tested muesli sample. Potassium is the main intracellular cation in the body and is principally involved in membrane potential and electrical excitation of both nerve and muscle cells and acid-base regulation. On average, the potassium content of an adult human is estimated to be around 1.6 to 2.0 g kg^{-1} body weight (Lanham-New et al., 2012). Results show 1.07, 0.89, 11.33 and 1.17 mg 100 g^{-1} of vitamin B1, B2, B3 and B6 in the product. After eating one portion it is possible to admit 48.63% of daily reference intake for vitamin B1, 31.78% of daily reference intake for vitamin B2, 35.40% of daily reference intake for vitamin B3 and 41.79% of daily reference intake for vitamin B6. Vitamins B comprises a class of water-soluble complexes. It has very important molecular function in the human body. Vitamin B1 (thiamine) strengthen the immune system, helps neuronal communication, maintains processes in cells and tissues. Vitamin B2 (riboflavin) metabolize fats, protect from ischemia reperfusion. Vitamin B3 (niacin) works in DNA proliferation, produces energy (Karunaratne et al., 2017). Vitamin B6 participates in more than one hundred transamination, decarboxylation, and other types of reactions, including the initial step of porphyrin synthesis, glycogen mobilization, amino acid transsulfuration, and neurotransmitter synthesis (Kohlmeier, 2003). Results show that product contain 8.04 mg 100 g^{-1} of vitamin E, for one portion it is 33.5% of daily reference intake. Vitamin E mostly recognized for its antioxidant function that terminates the self-perpetuating cycle of lipid peroxidation (Bruno & Mah, 2014).

Daily reference intake was calculated according to regulation (EU) No 1169/2011 of the European Parliament and the council of 25 October 2011. Per portion muesli is source of iron, magnesium, phosphorus, zinc, vitamin B1, vitamin B2, vitamin B3, vitamin B6 and vitamin E.

In vitro analysis

Zinc digestion results are presented in Fig. 1 as average release. Average results show that in small intestine in total 22% of zinc was absorbed, it means that by one muesli portion it is possible to replenish our body zinc level approximately by 0.42 mg, maximum 0.63 mg of zinc. In the end of gastric (G) phase 67% of zinc was released. At the beginning of duodenal (D) phase 77% of zinc released, after 60 minutes 84% of zinc were released, but in the end of duodenal (D) phase, after 120 minutes, 89% of zinc were released from loaded muesli sample. In one of three measurements in the end of duodenal phase 100% of zinc was absorbed, what shows that maximum 33% of zinc could be absorbed in small intestine. Values indicates significant difference between *in vitro* digestion stages ($p < 0.05$).

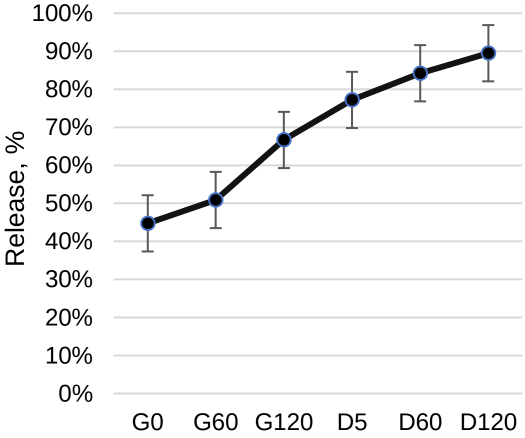

Figure 1. Release of zinc during gastric (G) and intestinal (D) *in vitro* digestion: G0 – gastric phase at 0 min digestion; G60 – gastric phase at 60 min digestion; G120 – gastric phase at 120 min digestion; D5 – duodenal phase at 5 min digestion; D60 – duodenal phase at 60 min digestion; D120 – duodenal phase at 120 min digestion.

As reported by Ogawa et al. (2016) approximately 30% of zinc in the diet is absorbed in the small intestine, obtained results are similar. Looking at other zinc digestion tests, Silva et al. (2020) in their study, where they compared organic and inorganic forms of zinc in salmonid diet, reported that solubility was similar in both diets tested. The amount of soluble zinc was low in the acidic hydrolysis (3–8%) and lower in the alkaline hydrolysis (0.4–2%). Results are significantly lower than in this study, yet they reported that solubility is impacted, also, by the pH of the gastrointestinal environment. In other study, Martinez et al. (1998) reported that the solubility of zinc of green beans evaluated by an *in vitro* method was from 14.7 till 55.1%. Observed results showed wide range. Results can be different in salmonoid diet as it contains both animal and plant ingredients, and it is lipid rich sample (Silva et al., 2020). It is possible to see that zinc solubility during digestion is impacted by different factors as mineral chemical form, pH of the gastrointestinal environment, phytate intake, fibres such as cellulose, etc. (Maares & Haase, 2020).

CONCLUSIONS

This study shows that zinc content in grain flakes varies according to grain variety and used flake type. As mentioned in other studies the major losses could occur due to the solubilisation of minerals during the steaming process. This shows why zinc content in toasted flakes will be lower than in rolled flakes. Also, as different grain varieties during production are used it is needed to follow grain variety and it zinc content each season. *In vitro* digestion test average results showed that by one meal (50 g of muesli) it is possible to replenish our body zinc level by 0.42 mg. Overall results show that this muesli can be used for consumers with different diets to replenish their body zinc level each day. Of course, zinc solubility during digestion is impacted by different factors as mineral chemical form, pH of the gastrointestinal environment, phytate intake, fibres such as cellulose, etc. Different raw material processing methods as thermal processing, malting (followed by germination), fermentation, soaking in water, hydrothermal treatment can reduce dietary phytate content (Gibson et al., 2018). Developed muesli contains raw materials which have had thermal processing and hydrothermal treatment what potentially leads to reduced dietary phytate content in muesli. Further investigation on dietary phytate content, it impacts on digestion should be done. In this study, other mineral and vitamin content in muesli sample were calculated according to their content in raw materials. Per portion, muesli is source of iron, magnesium, phosphorus, zinc, vitamin B1, vitamin B2, vitamin B3, vitamin B6 and vitamin E. Further laboratory tests on total mineral and vitamin content in developed muesli sample for more precise results should be applied. Results show that by one muesli portion it is possible to receive different naturally present minerals and vitamins.

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Comprehensive study on wheat flour quality attributes as influence by different agrotechnical factors

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Abstract. The present polyfactorial long-term experiment was conducted to determine the role of different agrotechnical factors, like fertilization, forecrop, year and cultivar on the quality and yield attributes of a classical and a modern winter wheat genotypes. The research gives a complex view of the alteration of the most quality parameters (32) that are rarely done together in a single experiment ($n = 96$). All the studied factors had a significant effect on some tested properties. First growing season with fair water supply was beneficial for yield (+21.7%) however, the 2nd one with mild weather in spring and average annual precipitation was favourable for quality parameters (HFN: +3%, GI: +19%, GS: -51%, DDT: +22.4%, ST: +24.6%, DS: -14%, PDR: +37.6%, PD: +6%, PMR: +51.5%, PE: +52.7%, W: +25.8%, LV: +16.3%, HI: +13.3%). The optimal fertilizer demand of the different parameters varied to realize the potential of the varieties ($N_{90}P_{68}K_{80}$ for Y, VWA, PWA, PMR, PE, LV, Mavg, KW and HI; meanwhile $N_{150}P_{113}K_{133}$ for CP, WGC, ZI, VQN, ST, W and P/L). In the case of suboptimal nutrient supply, the effect of fore crops was significant (Y, CP, DGC, ZI, FE, VQN, DDT, ST, DS, PDR, PMR, PE, Mavg and all alveographic values). Comparing the varieties, GK Öthalom had better fertilizer response attribute (NUE_{CP}) and quality parameters, till then Mv Ispán possessed significantly better natural nutrient utilizing property (+22.9%), NUE_{Y} and yield (+31.7% in 2019). Summarizing the results, there is a need to put great emphasis on the selection of adapted cultivar and variety-specific agrotechnology practices, also these data contribute to a better understanding of the relationships between the quality parameters.

Key words: wheat flour quality, agrotechnical factors, yield, baking quality, fertilization.

Used abbreviations: Yr: year; Cv: cultivar; Fc: forecrop; Tr: treatment; FE: flour extraction rate; VWA: valorigraphic water adsorption; VQN: valorigraphic quality number; VQG: valorigraphic quality group; DDT: dough development time; ST: dough stability; DS: dough softening; GI: gluten index; WGC: wet gluten content; DGC: dry gluten content; GS: gluten spread; HFN: Hagberg falling number; LFR: loaf form ratio; LV: loaf volume; PWA: promilographic water adsorption; PDR: promilographic ductility resistance; PD: promilographic ductility; PMR: promilographic maximum resistance; PE: promilographic energy; P: alveographic tenacity; L: alveographic extensibility; P/L: ratio of tenacity and extensibility; W: alveographic deformation work; SD: starch damage; ZI: Zeleny index; Mavg: Malvern average (particle size distribution); CP: crude protein; HI: hardness index; KD: kernel diameter; KW: kernel weight;

NIRP: NIR crude protein; NIRWG: NIR wet gluten content; PSD: particle size distribution; VU: valorigraphic unit; PU: promilographic unit.

INTRODUCTION

Wheat flour is playing an unquestionably important role in our daily diet (Shewry & Hey, 2015), since it is the basic material of many industries, like baking, confectionery and pasta. The continuously increasing demands have led to the current challenge of agriculture, namely improving yield sustainably without exploiting the environment (Hawkesford et al., 2014). Another urgent problem is the frequency of abiotic stresses (Guzman et al., 2016), like temperature over 40 °C during grain filling period and the distribution and the amount of precipitation (Kong et al., 2013), which indicates that modern wheat cultivars have to possess good stress and disease tolerance ability next to proper yielding and quality attributes (Tayyar, 2010). In 2019, 24% of global wheat production was sold in international trade (OECD, 2019). The sales price of the exported wheat is determined by quality parameters of the given batch (Budai & Fükő, 1996). Hungary is traditionally a high-quality wheat growing country long ago (Bedő et al., 2018), which is also presented in the complexity of the national wheat standard (Table 1). In the case of growing wheat to export, every country has its quality standard that has to be taken into account. For instance, in Hungary, farinograph and valorigraph (QN) are one of the most substantial methods, in England starch damage, meanwhile in Italy P/L value, in France, South America, Portugal, Spain and Bosnia W value. In the past, the opinion of the researches changed a lot about which parameter or method is the most informative: VQN and HFN (Pollhamer, 1981); baking test (Markovics, 2001); VQN (Diósi et al., 2015); HFN, VWA, W and dough ductility (Huen et al., 2018). Wheat quality is excessively complex (Massaux et al., 2008), also it is impossible to define it with only one parameter. The quality parameters of wheat can be divided into two major groups: 1) chemical properties, like protein and wet gluten content, sedimentation value (Pasha et al., 2010); 2) physical ones, like colour, shape, weight and kernel hardness (Szabó et al., 2014), these attributes determine together the milling and baking value.

Table 1. Hungarian wheat standard (MSZ 6368:2017)

Parameters	Premium	I. class	II. class	
Crude protein	14.0	12.5	11.5	%
Wet gluten content	34	30	26	%
Hagberg falling number (min)	300	250	220	s
Zeleny index	45	35	30	mL
Valorigraph quality group	A	B	B	
Valorigraph water absorption	60	55	55	%
Stability	10	6	4	min
W	280	200	150	10^{-4} J
P/L (max)	1.0	1.5	1.5	

The quality of wheat is a genetically coded characteristic, although it is primordial to choose the right agronomy practice for realizing its yield and quality potential. These attributes can be greatly affected by forecrop, which is favourable if it does not exploit the nutrient and water supplies of the soil (Ragasits, 1998). Forecrop can influence significantly the yield in the case of control nutritional treatment and drier growing seasons, but fertilizing can mitigate these differences (Pepó, 2010c). The more unfavourable ecological conditions are left behind by a preceding crop, the greater economic investments are needed to be performed to create the adequate, basic conditions for growing good quality crop (Hajdu, 1977). In the 3-year experiment of Stoeva & Ivanova (2009), next to average fertilization, there was no observable difference between sunflower and maize as a forecrop considering WGC, LV, quality number and sedimentation value. Maize is an acceptable forecrop, but the earliness of its harvest is a substantial factor because of the appropriate preparatory works (Koltay & Balla, 1982). In Hungary, the growing area of sweet corn increased four-fold in the last 3 decades (HCSO, 2020). In addition to this, Hungary gives the one-fifth of European sunflower production (HNCAE, 2017).

Nagy & Pepó (2015) summarized the results of their 10 years long experiment, the yield surplus could be attributed to 50% fertilizing, 28% preceding crop, 16% pest control and 4% cropping year. In addition to this, Pepó & Sárvári (2011) concluded that the quality of wheat was influenced by 27% cultivar, 22% year-effect, 8% forecrop and 20% fertilizing. The 480–550 mm optimal water demand of wheat is considered moderate in accordance with other crops (Uthayakumaran & Wrigley, 2017). The utilization of fertilizers is influenced by water supply. In the experiment of Pepó (2010a) the yield surplus was 0.9–2.1 tonne ha^{-1} in droughty seasons, till then this value was 3.6–4.3 tonne ha^{-1} in average seasons. Lack of available water in autumn and spring can suppress early growth, stem elongation and fertilization (Pepó & Sárvári, 2011). In the study of Pepó (2010b), 1 mm precipitation induced 13.2 kg in the case of control treatment, meanwhile 20.7 kg next to optimal fertilization. In rainy seasons the grain filling period can be lengthened, which has a positive effect on yield (Gooding et al., 2017). In spring, rapid warming has a detrimental effect on tillering, until then the high temperature can worsen the grain filling period (Tayyar, 2010; Pepó & Sárvári, 2011; Szabó et al., 2017).

One of the most vital agrotechnics is proper nutritional supply, which can be achieved by fertilization (Győri & Győriné, 1998). Discussed by Nagy & Pepó (2015), in a given season the difference between two varieties can be 3 tonne ha^{-1} next to the same agronomic practice. The usage of fertilizers is influenced by nutrient reactionary properties (Pepó, 2011). Cultivar-effect significantly affected VQN, VWA, DDT, DS, WGC, CP, GI, ZI, LV, HFN, FE and SD parameters (Lukow & Vetty, 1991; Kovács, 1992; Panozzo & Eagles, 2000; Tanács & Gerő, 2003; Masauskiene & Ceseviciene, 2005). According to Borghi et al. (1995), nitrogen fertilizer has a decisive influence on the baking quality of wheat. The crude protein content is determined fundamentally by the genetically potential of the grown wheat genotype and the available nitrogen supply in the soil (Fowler, 2003). Above a certain threshold, increasing fertilizer dosage does not improve statistically yield and quality of winter wheat. This threshold was 210 kg ha^{-1} in Ying et al. (2017), 180 kg ha^{-1} N in Walsh et al. (2018) and Kovács (1992) and 168 kg ha^{-1} N in Shi et al. (2007) researches. The optimal N fertilizer dosage is between 120–150 kg ha^{-1}, recommended by many researchers (Kovács, 1992; Montemurro et al., 2007; Horváth et al., 2014; Asthir et al., 2017). In practice, a smaller amount of fertilizer is needed to maximize yield than realizing quality potential (Pepó, 2010c), for instance, in the research of Garrido-Lestache et al. (2004) the optimal N fertilizer dosage was 100 kg ha^{-1} for yield and 150 kg ha^{-1} for crude protein. Calculating optimal fertilizer dosage: soil type, preceding crop, variety and nutrient supply of soil have to be taken into account (Bicskei, 2008). According to Gugava & Korokhashvili (2018), 40–50% of the applied fertilizer is actually taken up by crops, the rest is leached out, evaporated or mineralized, this is why the timing of fertilizing is so substantial. Nitrogen fertilization increased significantly CP (Rao et al., 1993), WGC (Litke et al., 2018), water absorption (Kovács, 1992), DDT (Linina et al., 2014), quality number (Pollhamer, 1973) LV, yield, GI (Massoudifar et al., 2014), sedimentation value (Linina & Ruza, 2012), W, P/L (Garrido-Lestache et al., 2004), kernel hardness (Guarda et al., 2004), starch damage, DGC, ST, PSD (Cho et al., 2018) and P (Matuz et al., 2007), decreased DS and extensographic extensibility (Wooding et al., 2000), and worsened gluten spread and LFR (Pollhamer, 1965), but did not affect flour extraction rate (Cho et al., 2018). It must be emphasized that high wet gluten content is not equal with good gluten quality (Curic et al., 2001). Fertilizer dosage was in tight positive correlation with crude protein and wet gluten content (Masauskiene & Ceseviciene, 2005; Eser et al., 2017). Nitrogen fertilization can affect significantly the ratio and the number of gluten proteins (Wieser & Seilmeier, 1998), therefore the baking test volume and the gluten spreading as well (Pollhamer, 1973).

According to Chantret et al. (2005) kernel hardness is an inherited characteristic, and it can determinate damaged starch content, flour extraction rate, PSD and water absorption (Eliasson & Larsson, 1993; Manley, 1995). Particle size distribution is influenced by kernel hardness and milling technology, moreover the harder the wheat is, the greater the PSD will be after milling. Also, smaller PSD results in better water absorption, because of the increased surface (Preston and Williams, 2003). HFN can be significantly modified by the effect of cultivar and year (Gerő & Tanács, 2003).

Gabriel et al. (2017) pointed out that, defining crude protein is not enough to determine the quality potential of a flour batch, because over 12% CP content, the correlation between loaf volume and crude protein was not significant in their experiment, so high CP does not mean loaf with big volume in every case. The real quality value of wheat flour is expressed during processing (Pollhamer, 1981), which can be predicted by testing samples with different rheological methods, like alveograph, valorigraph or farinograph, extensograph or promylograph. Using these techniques, kneading properties, water absorption, strength and extensibility of dough can be prognosticated. Discussed by Preston et al. (1987), P was in a significant relationship with water absorption and starch damage. Sipos et al. (2007) concluded that L was in tight correlation with VQN, WGC, CP and extensibility; meanwhile, W was in a strong relationship with CP and WGC. Loaf volume was in a significant relationship with CP, DDT and ST (Cho et al., 2018).

Next to the same agronomy practice and cultivar, the yield can differ even more than 4–5 tonne ha^{-1} because of the ecological factors (Pepó, 2010b; Mohammed et al., 2013). Furthermore, Fuertes-Mendizábal et al. (2010) submitted that, next to the selection of proper variety, ecological factors can significantly influence CP content. Year-effect significantly affected WGC (Zecevic et al., 2013), HFN (Johansson, 2002), KW, FE, sedimentation value, water absorption (Muchová, 2003), LV, ST (Koppel & Ingver, 2010), CP, gluten index (Masauskiene & Ceseviciene, 2005), Hardness index, KW (Guarda et al., 2004). This opportunity brings up the most critical question for the farmers every year: sow the same variety like the last season, or experimentalize a new one in the hope for better yield and quality. Year and fertilizing significantly affected W in the study of Tóth et al. (2005).

It is estimated that, in the next 20–30 years, the demand for wheat will double due to the rapidly increasing population. This fact can cause serious problems in terms of the utilization of soils, therefore the knowledge of currently used agrotechnics, like water productivity and fertilizing has to be widened, recommended by Spiertz & Ewert (2009). In Hungary, the number of growable cultivars exceeded 150, thus the average lifetime of varieties are maximum 5–7 years (Pepó & Sárvári, 2011). These facts valorise the value of the long-term experiments and the continuous testing of the recently registered cultivars. Thus, the object of this paper was to study the effect of different agrotechnical factors such as fertilization, forecrop, year and cultivar on the quality and yield attributes of a classical, widely-used and a modern, currently available winter wheat genotype. In addition, the comprehensiveness of the experiment can help to understand the relationship between the parameters of the common and infrequently-used methods.

MATERIALS AND METHOD

The experiment was done at Látókép Experimental Farm (University of Debrecen) in two consecutive growing seasons (2017–2018, 2018–2019) in split-split plot design. The long-term experiment was set up in 1983, 15 km from Debrecen (NL 47°33’, EL 21°27’). The area belongs to calcareous chernozem and loam type and has medium humus content: 2.7–2.8% (humus layer: 80–100 cm), medium P (0–25 cm: 133.4 ppm; 25–50 cm: 48.0 ppm; 50–75 cm: 40.4 ppm; 75–100 cm: 39.8 ppm) and K (0–25 cm: 239.8 ppm; 25–50 cm: 173.6 ppm; 50–75 cm: 123.0; 75–100 cm: 93.6 ppm) supply and neutral pH (6.46). The forecrops were sweet corn and sunflower because of their substantial role in Hungarian agriculture. The effect of three fertilizer levels (control, $N_{90}P_{67,5}K_{79,5}$; $N_{150}P_{112,5}K_{132,5}$) was tested in 10 m^2 plots in 4 repetitions to understand the influence of medium and over-fertilization (Table 2).

Table 2. Fertilizing treatments of long-term experiment

Treatments	Time of application	N	P	K
		(kg ha^{-1})		
Ø (Control)	-	-	-	-
3 (N_{90}PK)	autumn (basic fertilizing)	45	67.5	79.5
	spring (top dressing)	45	-	-
5 (N_{150}PK)	autumn (basic fertilizing)	75	112.5	132.5
	spring (top dressing)	75	-	-

The 50% of N and the whole amount of the P and K were applied in autumn (02.10.2017; 20.09.2018, respectively), the remaining 50% of the N fertilizer was applied in spring as top dressing (12.04.2018; 20.03.2019, respectively). Following winter wheat genotypes were tested: GK Öthalom, a classic Hungarian wheat cultivar (year of registration: 1985, awnless head and early maturity type, improve quality, A_1–A_2 class, breeder: GabonaKutató Kft) and Mv Ispán, a modern cultivar (year of registration: 2015, awned head and medium maturity type, good baking quality, A_1–B_1 class, breeder: Marton Genetics).

First, the samples were treated by SLN Pfeuffer sample cleaner, then conditioned to 15.5% moisture content, lastly milled into flour with Brabender Quadrumat Senior laboratory mill (MSZ 6367/9-1989). Crude protein (Kjeldahl method), wet gluten content (ISO 21415-2:2015), Zeleny index (MSZ EN ISO 5529), dry gluten content (ISO 21415-4:2006), gluten index (ISO 21415-2:2015), falling number (ISO 3093:2009), starch damage (ISO 17715:2013), baking test (MSZ 6369/8-1988), valorigraph (MSZ ISO 5530-3:1995), promylograph and alveograph (ISO 27971:2015) parameters were defined at the Institute of Food Engineering, University of Szeged. The method of Promylograph is very similar to extensograph, where a 500-consistency dough is made, and after 45–90–135’ resting time the moulded doughs are torn by a metal hook, till then the load on the dough is recorded. With this method dough stretchability can be tested.

Calculating nitrogen use efficiency (NUE) values, the following equitation’s were used:

$$\frac{Yield_N - Yield_C}{A_N} = NUE_Y \qquad \frac{CP_N - CP_C}{A_N} = NUE_{CP}$$

where $Yield_N$ = fertilized yield; $Yield_C$ = control yield; A_N = amount of *N*; CP_N = fertilized *CP*; CP_C = control *CP*.

For processing the results of the measurements, R studio 3.6.1 version was used. For arranging and filtering the data, dplyer package (Wickham et al., 2019) was utilized. One-way ANOVA with least significant difference (*LSD*) post-hoc tests on $P > 0.05$ significance level of agricolae package (Mendiburu, 2019) and Pearson’s correlation analysis of SPSS Statistics 25 were performed. According to Tóthné (2011) there are very tight, tight, medium and loose correlations if the correlation coefficient is between 0.9–1, 0.75–0.9, 0.5–0.75 and 0.25–0.5, respectively. For graphical representation Seaborn 0.9.0 library (boxplot chart) of Python 3.7 version was used.

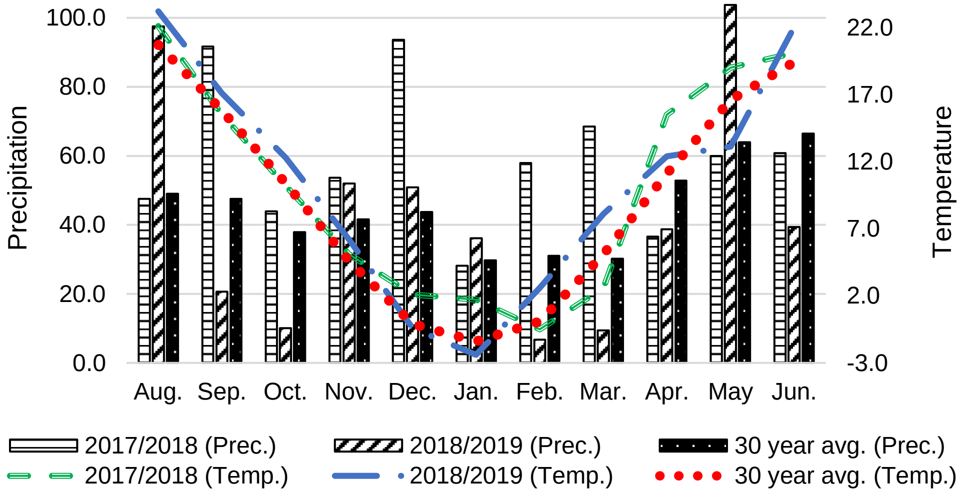

Figure 1. Agrometeorological parameters of the two growing seasons.
Season 1: Sowing date: 04.10.2017 – Harvest date: 05.07.2018;
Season 2: Sowing date: 05.10.2018 – Harvest date: 09.07.2019.

The first season was rainy (total precipitation: 642.4 mm), till then the second was normal (465.1 mm), but in both seasons (2017–2018: 10.3 °C; 2018–2019: 10.4 °C) the average temperature was higher with 1 °C, compared to 30-year average (9.3 °C) (Fig. 1), which correlates well with the global warming trends, like rising temperature, extreme conditions and less precipitation. To summarize, the weather of both growing seasons was unfavourable for the vegetative and generative development of wheat plants.

RESULTS AND DISCUSSION

According to our results, all the 4 factors had significant effect on some studied parameters. Main attributes varied between: 7.2–13.5% (CP); 14.87–32.46 (WGC); 287–420 s (HFN); 19.4 (C2)-70.7 (A2) (VQN); 105.9–329.9 (W); 680–1,030 cm^3 (LV), 2,714–8,871 kg ha^{-1} (Y), which are presented in the Table 3. and Table 6.

Table 3. Effect of the studied factors on classical parameters (Debrecen, Hungary)

Yr	Cv	Fc	Tr	Y (kg ha^{-1})	CP (%)	ZI (cm^3)	WGC (%)	DGC (%)	GS (mm)	HFN (s)	NIRP (%)	NIRWG (%)
2018	GK Öthalom	Sweet corn	Ø	5,795	8.79	20.4	16.65	5.91	0.4	374	8.44	16.86
			$N_{90}P_{68}K_{80}$	7,216	12.04	32.4	25.19	8.90	1.0	356	12.65	27.14
			$N_{150}P_{113}K_{133}$	6,425	13.14	36.7	28.26	9.84	0.8	343	13.74	29.75
		Sunflower	Ø	3,610	7.47	15.6	16.65	5.79	0.6	365	7.07	12.86
			$N_{90}P_{68}K_{80}$	6,046	11.29	29.5	24.72	8.63	1.8	402	11.91	24.93
			$N_{150}P_{113}K_{133}$	7,821	12.45	32.5	29.24	10.25	2.9	390	12.91	27.48
	Mv Ispán	Sweet corn	Ø	6,806	9.55	25.9	20.68	7.10	1.4	382	10.38	19.91
			$N_{90}P_{68}K_{80}$	8,871	12.03	35.1	27.08	9.31	1.5	390	13.15	26.93
			$N_{150}P_{113}K_{133}$	8,505	12.84	38.2	28.82	9.91	1.1	387	13.81	28.60
		Sunflower	Ø	2,714	8.25	20.7	16.06	5.61	0.8	370	9.59	17.05
			$N_{90}P_{68}K_{80}$	8,710	10.90	31.5	24.80	8.45	1.4	377	12.28	24.61
			$N_{150}P_{113}K_{133}$	8,708	12.03	35.0	27.59	9.45	1.9	394	13.36	27.83
2019	GK Öthalom	Sweet corn	Ø	3,276	10.65	26.7	21.10	7.62	0.0	390	10.93	23.02
			$N_{90}P_{68}K_{80}$	5,202	12.61	34.3	30.36	10.94	0.7	396	13.23	28.73
			$N_{150}P_{113}K_{133}$	6,298	13.00	34.9	28.65	10.26	0.3	391	13.56	29.45
		Sunflower	Ø	3,285	11.07	31.4	22.59	8.11	0.3	378	11.90	25.84
			$N_{90}P_{68}K_{80}$	5,083	12.00	35.5	25.48	9.24	0.6	403	12.96	28.06
			$N_{150}P_{113}K_{133}$	5,650	12.94	40.4	27.83	10.05	0.7	391	13.87	29.98
	Mv Ispán	Sweet corn	Ø	5,335	9.66	25.8	20.21	7.01	0.3	384	10.77	21.30
			$N_{90}P_{68}K_{80}$	7,408	11.32	30.0	25.06	8.51	0.4	388	12.53	25.69
			$N_{150}P_{113}K_{133}$	7,154	11.80	32.2	27.29	9.35	0.7	393	13.11	27.26
		Sunflower	Ø	4,772	9.93	27.0	21.14	7.38	0.7	389	11.20	22.96
			$N_{90}P_{68}K_{80}$	6,380	11.04	30.1	24.82	8.60	0.9	380	12.40	25.68
			$N_{150}P_{113}K_{133}$	6,880	11.90	33.0	27.53	9.55	1.1	380	13.22	28.08
			$LSD_{5\%}$ (Yr):	1,451.1	0.64	2.41	1.79	0.63	0.26	7.86	0.71	1.80
			$LSD_{5\%}$ (Cv):	1,421.3	0.64	2.45	1.81	0.64	0.30	8.17	0.73	1.87
			$LSD_{5\%}$ (Fc):	1,513.3	0.64	2.45	1.80	0.64	0.29	8.16	0.73	1.87
			$LSD_{5\%}$ (Tr):	1,413.0	0.47	1.89	1.24	0.47	0.35	9.98	0.54	1.42

Fertilizing ($N_{90}P_{68}K_{80}$: 6,865 kg ha^{-1}; $N_{150}P_{113}K_{133}$: 7,180 kg ha^{-1}) increased (+53% and +61%, respectively) significantly the yield, compared to control samples (4,449 kg ha^{-1}), however in 2018 yields were higher (2018: 6,769 kg ha^{-1}; 2019: 5,560 kg ha^{-1}) with 21.7%, but this amount was not significant (Fig. 2), which was also reported by Pepó (2010a). The explanations were already concluded by Gooding et al.

(2017), namely precipitation during grain filling can observably improve yield. In 2018, considering only the control samples, sweet corn (6,301 kg ha^{-1}) gave significantly ($LSD_{5\%}$: 2,906.2 kg ha^{-1}) higher yield, compared to sunflower (3,162 kg ha^{-1}). In 2019, Mv Ispán (6,321.5 kg ha^{-1}) had significantly ($LSD_{5\%}$: 1,488.5 kg ha^{-1}) higher (+31.7%) yield in comparison to GK Öthalom (4,799 kg ha^{-1}). These results are in compliance with the findings of Pepó (2010c), who stated that the effect of forecrops can be significant in the case of control treatments, but these differences disappeared with optimal nutrient supply. In general, only $N_{90}P_{68}K_{80}$ dosage could improve significantly the yield, further increment of fertilizer had no observable effect, which confirms the findings of Kovács (1992), Garrido-Lestache et al. (2004), Shi et al. (2007), Ying et al. (2017) and Walsh et al. (2018). First growing season was a rainy one, this amended the yield compared to the 2nd one, which had average precipitation, thereby our results affirmed the observations of Tayyar (2010) and Gooding et al. (2017). Also, our results underlined the importance of variety selection, since the difference between cultivars can be significant next to the same agrotechnics.

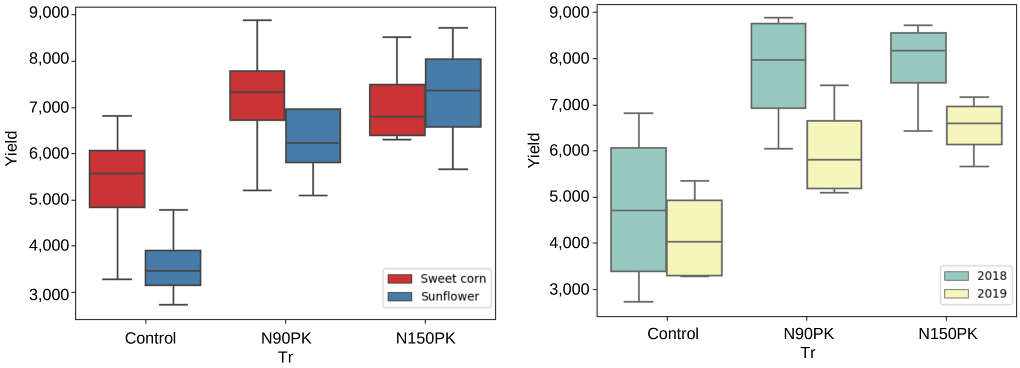

Figure 2. Effect of forecrops (left), year (right) and treatment on yield.

Table 4. shows the effect of different fertilizer doses on nitrogen use efficiency (NUE) in the case of the two most important post-harvest indicators, namely yield and CP. Several studies evaluated these parameters. Yield NUE was 8.2 kg ha^{-1} in the research of Lollato et al. (2019), meanwhile protein NUE was 0.025% in the study of Miceli et al. (1992) and 0.004% in the article of Lollato et al. (2019). Examining GK Öthalom, it can be said that, yield NUE decreased slightly with the increment of fertilizer, however studying Mv Ispán, it was significantly higher with the first dose ($N_{90}P_{68}K_{80}$: 32.62 kg ha^{-1}), but this difference disappeared in the case of $N_{150}P_{113}K_{133}$ dosage (19.37 kg ha^{-1}). Considering the crude protein NUE, GK Öthalom performed better in the case of both dosages ($N_{90}P_{68}K_{80}$: 0.028%; $N_{150}P_{113}K_{133}$: 0.023%), comparing to Mv Ispán ($N_{90}P_{68}K_{80}$: 0.022%; $N_{150}P_{113}K_{133}$: 0.019%). Comparing the varieties, GK Öthalom had better fertilizer response attribute (NUE_{CP}) and CP, till then Mv Ispán possessed considerably better natural nutrient utilizing property (+22.9%) and NUE_{Y}.

Table 4. Nitrogen use efficiency considering yield and crude protein

Cv	Tr	Yield		Crude protein	
		avg. (kg ha^{-1})	NUE	avg. (%)	NUE
GK Öthalom	Ø	3,992	-	9.50	-
	$N_{90}P_{68}K_{80}$	5,887	21.06	11.99	0.028
	$N_{150}P_{113}K_{133}$	6,549	17.05	12.88	0.023
Mv Ispán	Ø	4,907	-	9.35	-
	$N_{90}P_{68}K_{80}$	7,842	32.62	11.32	0.022
	$N_{150}P_{113}K_{133}$	7,812	19.37	12.14	0.019

Before performing the standard methods, the NIR measurements were executed to have a basic view of moisture, crude protein and wet gluten content. Both fertilizer doses (C: 10.03%; $N_{90}P_{68}K_{80}$: 12.64%; $N_{150}P_{113}K_{133}$: 13.45%) improved significantly NIR Protein. 2019 (12.47%) gave observably higher NIRP than 2018 (11.61%), but forecrops and cultivars did not differ. Studying only control samples in 2018, Mv Ispán (9.98%) had significantly ($LSD_{5\%}$: 1.02%) greater NIRP, than GK Öthalom (7.76%), but in 2019 GK Öthalom (13.72%) possessed higher ($LSD_{5\%}$: 0.21%) NIRP compared to Mv Ispán (13.16%) with $N_{150}P_{113}K_{133}$ treatment. This meant that, GK Öthalom had better fertilizer reactionary attribute, till then Mv Ispán possessed significantly better natural nutrient utilizing property. NIRWG showed similar trends like NIRP, 2019 (26.34%) gave significantly greater NIRWG values than 2018 (23.66%), also both nutrient treatments (C: 19.98%; $N_{90}P_{68}K_{80}$: 26.47%; $N_{150}P_{113}K_{133}$: 28.55%) increased significantly NIRWG, but forecrops and cultivars did not differ in general. Considering control samples in 2018, sweet corn (18.39%) improved observably ($LSD_{5\%}$: 2.8%) NIRWG values in contrast with sunflower (14.96%). In 2019, GK Öthalom (27.51%) produced significantly ($LSD_{5\%}$: 1.68%) better NIRWG than Mv Ispán (22.13%).

Scrutinizing the standard methods, there were no considerable differences between the years, forecrops and cultivars, but both fertilizer (C: 9.42%; $N_{90}P_{68}K_{80}$: 11.65%; $N_{150}P_{113}K_{133}$: 12.51%) doses increased significantly the crude protein. In 2018, studying the control samples, sweet corn (9.17%) gave observably higher CP, than sunflower (7.86%). In 2019, GK Öthalom (12.05%) owned significantly greater CP values (Fig. 3), compared to Mv Ispán (10.94%), as well as in the experiments of Rao et al. (1993), Masauskiene & Ceseviciene (2005) and Fuertes-Mendizábal et al. (2010).

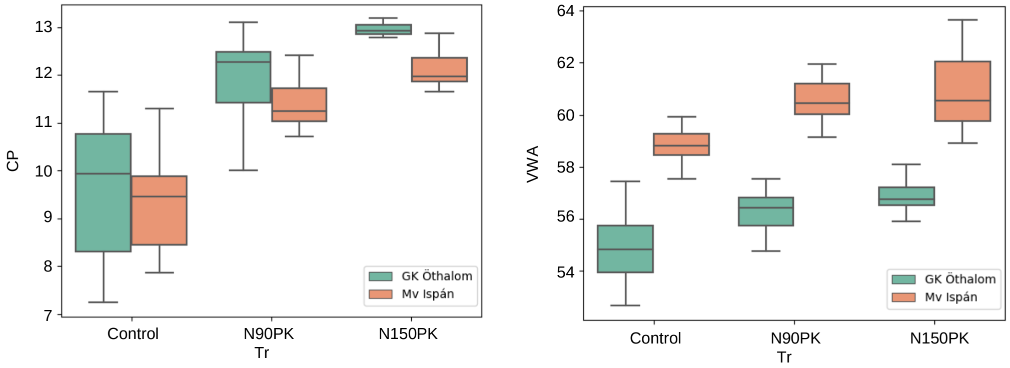

Figure 3. Effect of cultivars, treatment on crude protein (left) and water absorption (right).

Wet gluten content was significantly improved by fertilizing (C: 19.38%; $N_{90}P_{68}K_{80}$: 25.94%; $N_{150}P_{113}K_{133}$: 28.15%), like in the study of Litke et al. (2018), at the same time forecrop, year and cultivar did not affected WGC in general. Considering the effect of preceding crops, Stoeva and Ivanova (2009) got the same results. In 2019, in the case of $N_{90}P_{68}K_{80}$ dose, GK Öthalom (27.92%) had significantly ($LSD_{5\%}$: 2.59%) higher WGC, compared to Mv Ispán (24.94%). Dry gluten content showed similar results like wet gluten content in general, namely the fertilizing significantly affected the studied parameter (C: 6.82%; $N_{90}P_{68}K_{80}$: 9.07%; $N_{150}P_{113}K_{133}$: 9.83%). In 2019, in the case of control treatment, sweet corn (7.87%) gave observably greater DGC than sunflower (7.19%), also studying the results of $N_{90}P_{68}K_{80}$ and $N_{150}P_{113}K_{133}$ dose, GK Öthalom ($N_{90}P_{68}K_{80}$: 10.09%; $N_{150}P_{113}K_{133}$: 10.16%) owned considerably ($LSD_{5\%}$: 0.85%, 0.43%, respectively) higher values, than Mv Ispán ($N_{90}P_{68}K_{80}$: 8.56%; $N_{150}P_{113}K_{133}$: 9.45%). These results indicate that GK Öthalom had a better fertilizer reactionary property.

Both fertilizer dose (C: 24.19 cm^3; $N_{90}P_{68}K_{80}$: 32.29 cm^3; $N_{150}P_{113}K_{133}$: 35.37 cm^3) significantly improved Zeleny index, however there was no serious difference between the studied years, cultivars and forecrops. A similar conclusion was done by Linina & Ruza (2012), Stoeva and Ivanova (2009). In 2018 with control treatment, sweet corn (23.15 cm^3) as a forecrop gave significantly ($LSD_{5\%}$: 3.78 cm^3) higher ZI values than sunflower (18.15 cm^3). In 2019, GK Öthalom (33.86 cm^3) had significantly ($LSD_{5\%}$: 2.14 cm^3) higher ZI, compared to Mv Ispán (29.66 cm^3), also the samples with control treatment, grown after sunflower (29.21 cm^3) had significantly ($LSD_{5\%}$: 2.39 cm^3) greater Zeleny index, than sweet corn (26.24 cm^3).

Effect of fertilizing on gluten quality was reflected in change in the gluten index and gluten spread, where increasing fertilizer dosage (C: 95.5%; $N_{90}P_{68}K_{80}$: 83.42%; $N_{150}P_{113}K_{133}$: 80.24%) significantly weakened the gluten strength (GI), like in the study of Massoudifar et al. (2014). Also, the difference of the studied cultivars (GK Öthalom: 90.03%; Mv Ispán: 82.74%) was observable, like in the research of Masauskiene & Ceseviciene (2005). Interestingly, the findings of Masauskiene & Ceseviciene (2005) were affirmed, because year-effect significantly influenced the gluten index, namely in 2019 the GI was 93.91%, in contrast with 2018 it was just 78.86%, but forecrops had no visible effect. Scrutinizing the results of gluten spread, that can be observed, there was no difference between the cultivars, however 2018 (1.3 mm) gave significantly higher values, than 2019 (0.55 mm). Samples grown after sunflower (1.13 mm) had observably greater GS, compared to sweet corn (0.72 mm), also increment of fertilizer (C: 0.56 mm; $N_{90}P_{68}K_{80}$: 1.04 mm; $N_{150}P_{113}K_{133}$: 1.17 mm) significantly augmented gluten spread, just like in the study of Pollhamer (1965). In 2019, Mv Ispán (0.68 mm) gave observably ($LSD_{5\%}$: 0.25 mm) higher GS values, than GK Öthalom (0.42 mm). To compile, increment of fertilizer, high temperature in April and May, large amount of annual precipitation and sunflower (exploitive) as preceding crop deteriorate significantly the gluten strength.

Studying the flour extraction rates, it can be seen that GK Öthalom (70.99%) had significantly higher FE values compared to Mv Ispán (69.16%), meanwhile fertilizing and forecrop had no considerable effect. Our results were in compliance with Chantret et al. (2005) and Cho et al. (2018). In 2018, sweet corn (72.86%) gave significantly ($LSD_{5\%}$: 0.89%) higher FE values, than sunflower (71.76%), also this difference was even greater in the case of control samples (sweet corn: 73.24%; sunflower: 71.02%).

Fertilizing, forecrop and cultivar did not affect the Hagberg falling number, however year exerted influence on the studied parameter, namely 2019 (388.5 s) gave significantly higher (+3%) HFN, compared to 2018 (377.3 s), which affirmed the statements of Johansson (2002) and Gerő & Tanács (2003).

Table 5. Effect of the studied factors on rheological parameters (Debrecen, Hungary)

				Valorigraph					Promylograph					Alveograph			
Yr	Cv	Fc	Tr	VWA (%)	VQN	DDT (min)	ST (min)	DS (VU)	PWA (%)	PDR (PU)	PD (mm)	PMR (PU)	PE (cm^2)	P (mm)	L (mm)	P/L	W (x10^{-4} J)
2018	GK Öthalom	Sweet corn	Ø	53.7	33.1	1.4	2.6	155	48.6	286	94	295	40	63.6	51.4	1.3	120
			$N_{90}P_{68}K_{80}$	56.4	44.8	2.0	7.1	130	49.5	374	118	455	70	64.5	94.3	0.7	208
			$N_{150}P_{113}K_{133}$	57.1	49.5	2.3	8.5	118	50.1	382	119	478	72	67.7	108.6	0.6	241
		Sunflower	Ø	53.8	22.7	1.0	2.3	195	47.8	211	103	214	32	73.9	36.6	2.0	108
			$N_{90}P_{68}K_{80}$	55.4	41.7	1.8	6.3	139	49.0	298	111	326	50	62.5	87.4	0.7	176
			$N_{150}P_{113}K_{133}$	56.4	45.9	2.0	7.1	134	48.8	286	113	315	49	62.0	101.2	0.6	201
	Mv Ispán	Sweet corn	Ø	58.2	34.8	1.1	3.1	145	51.1	370	98	395	52	89.7	52.4	1.8	167
			$N_{90}P_{68}K_{80}$	61.3	49.6	2.3	8.0	123	53.2	332	117	409	62	92.2	83.8	1.1	249
			$N_{150}P_{113}K_{133}$	61.8	54.8	2.5	8.8	108	53.7	361	124	448	73	95.9	87.1	1.1	272
		Sunflower	Ø	58.4	25.5	1.0	1.9	171	50.2	463	82	469	51	116.8	34.4	3.4	132
			$N_{90}P_{68}K_{80}$	61.1	44.2	1.6	6.4	131	52.5	401	108	458	64	109.5	60.6	1.9	238
			$N_{150}P_{113}K_{133}$	62.5	50.6	2.0	7.9	110	53.6	436	120	557	83	118.0	75.3	1.6	313
2019	GK Öthalom	Sweet corn	Ø	55.7	37.3	1.5	4.5	147	49.1	341	112	427	62	70.1	74.1	1.0	194
			$N_{90}P_{68}K_{80}$	57.1	59.6	2.6	11.3	88	49.4	482	127	658	104	82.9	97.2	0.9	283
			$N_{150}P_{113}K_{133}$	57.1	64.1	3.0	11.9	80	49.7	414	130	600	95	85.1	99.3	0.9	297
		Sunflower	Ø	56.1	47.9	2.1	7.1	116	49.0	555	107	669	91	91.2	71.5	1.3	227
			$N_{90}P_{68}K_{80}$	56.2	57.1	2.9	9.9	91	49.6	508	117	687	96	91.1	92.4	1.0	295
			$N_{150}P_{113}K_{133}$	56.7	64.9	3.8	11.8	75	49.6	546	133	785	128	81.7	102.9	0.8	311
	Mv Ispán	Sweet corn	Ø	59.1	32.7	1.4	3.0	160	50.6	481	97	519	66	93.0	47.3	2.0	169
			$N_{90}P_{68}K_{80}$	60.2	41.1	1.5	5.6	145	51.3	411	116	503	74	90.0	63.5	1.4	239
			$N_{150}P_{113}K_{133}$	59.7	47.4	2.0	7.0	123	51.1	397	125	529	83	97.1	86.2	1.1	285
		Sunflower	Ø	59.4	35.3	1.5	3.6	153	51.8	573	99	620	80	108.6	51.1	2.2	207
			$N_{90}P_{68}K_{80}$	59.7	41.8	1.5	4.7	130	51.6	546	103	630	84	107.7	67.2	1.6	257
			$N_{150}P_{113}K_{133}$	59.5	46.0	1.8	6.6	120	51.6	527	121	671	102	101.0	81.8	1.2	286
			$LSD_{5\%}$ (Yr):	1.00	4.44	0.29	1.21	11.84	0.69	34.3	5.49	43.9	7.33	7.17	9.23	0.27	23.45
			$LSD_{5\%}$ (Cv):	0.54	4.50	0.29	1.18	12.21	0.42	42.3	5.51	60.8	9.64	4.80	8.45	0.23	25.63
			$LSD_{5\%}$ (Fc):	1.00	4.61	0.30	1.24	12.45	0.69	41.9	5.57	59.8	9.59	6.95	9.18	0.26	25.76
			$LSD_{5\%}$ (Tr):	0.47	3.99	0.30	1.07	11.72	0.80	53.4	4.84	71.9	10.43	8.95	7.61	0.27	21.58

The relevant data given in Table 5, which subsume the results of the rheological methods. Valorigraphic water absorption was significantly increased by fertilizing compared to control samples (C: 56.79%; $N_{90}P_{68}K_{80}$: 58.42%; $N_{150}P_{113}K_{133}$: 58.85%), but forecrop and year had no influence on VWA. Moreover, Mv Ispán (60.07%) gave observably greater VWA (Fig. 3), compared to GK Öthalom (55.97%), which is in compliance with the results of Tanács & Gerő (2003). The findings of Tanács & Gerő

(2003) were affirmed, because GK Öthalom (47.39) had significantly better valorigraphic quality number than Mv Ispán (41.97). In addition to this, 2019 (47.93) gave observably higher VQN compared to 2018 (41.44), also increment of fertilizers (C: 33.67; $N_{90}P_{68}K_{80}$: 47.49; $N_{150}P_{113}K_{133}$: 52.89) increased significantly the quality number, but there was no difference between the studied preceding crops. In 2018, sweet corn (44.43) gave significantly ($LSD_{5\%}$: 5.89) better VQN than sunflower (38.45). Interestingly, in the case of $N_{150}P_{113}K_{133}$ dosage, in 2018, Mv Ispán (52.68) gave significantly ($LSD_{5\%}$: 3.36) better VQN than GK Öthalom (47.71), but in 2019, GK Öthalom (64.5) gave considerably ($LSD_{5\%}$: 3.49) higher VQN, compared to Mv Ispán (46.69). Increasing fertilizer doses (C: 1.38 min; $N_{90}P_{68}K_{80}$: 2.02 min; $N_{150}P_{113}K_{133}$: 2.42 min) significantly improved DDT, also 2019 (2.13 min) gave observably (+22.4%) greater DDT values compared to 2018 (1.74 min). GK Öthalom (2.19 min) had noticeably higher DDT than Mv Ispán (1.68 min). In 2018, sweet corn (1.92 min) gave significantly ($LSD_{5\%}$: 0.32 min) higher DDT than sunflower (1.56 min). Our results corroborated the statements of Tanács & Gerő (2003) and Linina et al. (2014). Valorigraphic dough stability was significantly increased by both fertilizer dosage (C: 3.52 min; $N_{90}P_{68}K_{80}$: 7.4 min; $N_{150}P_{113}K_{133}$: 8.7 min), also 2019 (7.25 min) gave appreciably (+24.6%) greater values than 2018 (5.82 min). The difference between years also reappeared in the research of Koppel & Ingver (2010). Moreover, GK Öthalom (7.53 min) had significantly higher ST values, compared to Mv Ispán (5.55 min), however forecrops did not affect results in general. In 2018, in the case of control samples, sweet corn (2.88 min) gave significantly ($LSD_{5\%}$: 0.65 min) higher ST, than sunflower (2.06 min). In 2019, scrutinizing the $N_{90}P_{68}K_{80}$ treatment samples, GK Öthalom (10.56 min) had observably ($LSD_{5\%}$: 1.55 min), two times greater ST, compared to Mv Ispán (5.15 min). Dough softening was significantly improved by both fertilizer doses (C: 155.21 VU; $N_{90}P_{68}K_{80}$: 122.03 VU; $N_{150}P_{113}K_{133}$: 108.28 VU). In addition to this, GK Öthalom (122.22 VU) had significantly lower DS values than Mv Ispán (134.79 VU), also 2019 (118.89 VU) gave observably (-14%) less DS than 2018 (138.13 VU). In 2018, sweet corn (146.67 VU) gave significantly ($LSD_{5\%}$: 15.39) better DS, compared to sunflower (129.58 VU). In 2019, GK Öthalom (99.44 VU) had better ($LSD_{5\%}$: 13.9) DS, compared to Mv Ispán (138.33 VU). These findings were in compliance with Wooding et al. (2000) and Tanács & Gerő (2003).

Studying the promilographic water absorption, it can be concluded that, Mv Ispán (51.84%) had significantly higher PWA, than GK Öthalom (49.17%), but there was no difference between years and forecrops, however fertilizing ($N_{90}P_{68}K_{80}$:50.73%; $N_{150}P_{113}K_{133}$: 51.02%) increased PWA compared to control samples (49.77%). In 2018, Mv Ispán (53.66%) had significantly ($LSD_{5\%}$: 1.03%) better PWA, than GK Öthalom (49.43%) with $N_{150}P_{113}K_{133}$: treatment, but this difference was smaller ($LSD_{5\%}$: 0.28%) in 2019, where Mv Ispán owned 51.34%, till then GK Öthalom 49.63% PWA. Fertilizing did not affect promilographic ductility resistance, but 2019 (481.7) gave significantly higher (+37.6%) PDR, than 2018 (349.96). Also, Mv Ispán (441.47) owned observably greater PDR, compared to GK Öthalom (390.19), and sunflower (445.8) improved significantly values than sweet corn (385.86). In 2019, the difference was even higher between forecrops, where sunflower (542.51) gave significantly (32.57) greater PDR, than sweet corn (420.89). Both fertilizer dosage (C: 98.92; $N_{90}P_{68}K_{80}$: 114.45; $N_{150}P_{113}K_{133}$: 122.83) increased significantly the promilographic ductility, just like in the research of Wooding et al. (2000), but forecrops caused no change. GK Öthalom

(115.23) owned significantly greater PD values, than Mv Ispán (108.9). Besides this, 2019 (115.44) gave appreciably (*LSD*$_{5\%}$: 5.49) better (+6%) PD, compared to 2018 (108.69). $N_{150}P_{113}K_{133}$ dosage (547.85) significantly (*LSD*$_{5\%}$: 71.9) augmented promilographic maximum resistance compared with control samples (450.84). 2019 (608.17) gave significantly (*LSD*$_{5\%}$: 43.89) greater (+51.5%) PMR values than 2018 (401.46), however forecrops and cultivars did not differ perceptibly in general. In 2018, Mv Ispán (455.71) had significantly (*LSD*$_{5\%}$: 56.82) higher PMR, than GK Öthalom (347.21), this difference was even higher in the case of control treatment, where Mv Ispán had 431.75, till then GK Öthalom had 254.38 PMR. In 2019, sunflower (677.06) gave significantly (*LSD*$_{5\%}$: 46.4) greater PMR, than sweet corn (539.29). In contrast with 2018, in 2019 GK Öthalom (672.5) gave considerably (*LSD*$_{5\%}$: 73.14) higher PMR, than Mv Ispán (566.5) with $N_{90}P_{68}K_{80}$ dosage. In general, both fertilizer dosages ($N_{90}P_{68}K_{80}$: 75.44; $N_{150}P_{113}K_{133}$: 85.7) increased significantly promilographic energy, compared to control samples (58.9). 2019 (88.65) gave appreciably higher (+52.7%) PE than 2018 (58.04), nevertheless cultivars and forecrops made no difference. In 2018, there was observable difference between the studied varieties, namely Mv Ispán (63.96) had significantly (*LSD*$_{5\%}$: 9.35) higher PE, than GK Öthalom (52.13), but in 2019 GK Öthalom (96.0) possessed higher (*LSD*$_{5\%}$: 10.34) values, than Mv Ispán (81.29). Also, sunflower (96.81) gave observably (*LSD*$_{5\%}$: 10.12) greater PE, than sweet corn (80.49).

Sunflower (93.66) gave significantly greater alveographic P, than sweet corn (82.64), also Mv Ispán (101.61) owned higher P compared to GK Öthalom (74.69), but year and fertilizing made no difference in general. This phenomenon was in contrast with Matuz et al. (2007), who stated that fertilizing significantly increased P. Studying alveographic L, it can be concluded, that both fertilizer doses (C: 52.36; $N_{90}P_{68}K_{80}$: 80.79; $N_{150}P_{113}K_{133}$: 92.81) increased significantly the values, also GK Öthalom (84.74) owned observably higher L, than Mv Ispán (65.9). Year and preceding crop made no difference in general. In the case of control samples, in 2018 sweet corn (51.9) gave significantly (*LSD*$_{5\%}$: 7.98) greater L compared to sunflower (35.5). Generally, there was no difference between the P/L values of the studied years, however Mv Ispán (1.71) had significantly higher P/L than GK Öthalom (0.98). In addition, sunflower (1.53) gave significantly greater P/L, than sweet corn (1.16). $N_{150}P_{113}K_{133}$ treatment (1.87) increased appreciably (*LSD*$_{5\%}$: 0.27) P/L, compared to control (0.99) and $N_{90}P_{68}K_{80}$ dose (1.16). Both fertilizer dosages (C: 165.51; $N_{90}P_{68}K_{80}$: 243.13; $N_{150}P_{113}K_{133}$: 275.65) increased significantly the alveographic W, also 2019 (254.18) gave greater (+25.8%) W, than 2018 (202.02), but forecrop and cultivar did not affect W. In 2018, Mv Ispán (228.63) possessed significantly (*LSD*$_{5\%}$: 35.11) greater W than GK Öthalom (175.4). In 2019, sunflower (217.03) gave significantly (*LSD*$_{5\%}$: 30.62) higher W compared to sweet corn (181.52). The effect of fertilizing on P/L and W were in agreement with Garrido-Lestache et al. (2004).

Comparing to control samples (822.55 cm^3), fertilizing ($N_{90}P_{68}K_{80}$: 883.7 cm^3; $N_{150}P_{113}K_{133}$: 897.87 cm^3) augmented significantly loaf volume, also GK Öthalom (896.11 cm^3) had appreciably greater LV, than Mv Ispán (839.97 cm^3). Loaf volume (Fig. 4) results were significantly higher (+16.3%) in 2019 (933.47 cm^3), compared to 2018 (802.6 cm^3), but there was no difference between studied preceding crops in general. However, in 2018 sweet corn (823.54 cm^3) gave significantly (*LSD*$_{5\%}$: 32.49 cm^3) better LV, than sunflower (781.67 cm^3). In 2019, GK Öthalom (983.13 cm3) gave significantly (*LSD*$_{5\%}$: 42.48 cm^3) better LV, than Mv Ispán (904.17 cm^3) with

$N_{90}P_{68}K_{80}$ treatment. The effect of fertilizer and year were in compliance with Koppel & Ingver (2003) and Massoudifar et al. (2014). 2018 (2.07) gave significantly higher loaf form ratio, than 2019 (1.93). Studying cultivars, Mv Ispán (2.07) owned greater, than GK Öthalom (1.92). Both fertilizer dosages (C: 1.84; $N_{90}P_{68}K_{80}$: 2.0; $N_{150}P_{113}K_{133}$: 2.15) increased significantly LFR, just like in the experiment of Pollhamer (1965). In 2019, in the case of control samples, sunflower (1.85) gave significantly ($LSD_{5\%}$: 0.08) higher LFR, than sweet corn (1.76).

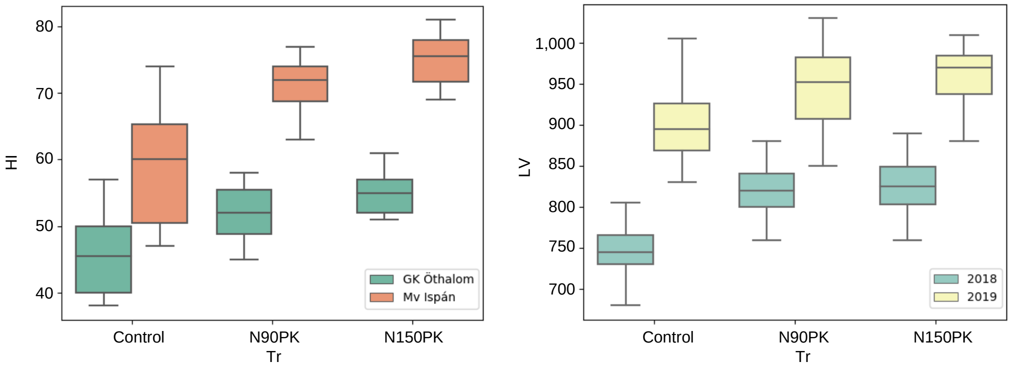

Figure 4. Effect of cultivars, year, treatment on Hardness index (left) and loaf volume (right).

Scrutinizing starch damage (UCD), only cultivars differed significantly, namely Mv Ispán (22.58) had greater values than GK Öthalom (18.52), which results affirmed the findings of Lukow & McVetty (1991).

Fertilizing ($N_{90}P_{68}K_{80}$: 82.59; $N_{150}P_{113}K_{133}$: 83.45) significantly increased average particle size compared to control treatments (76.02), but years did not differ in general. A similar conclusion was done by Cho et al. (2018). Mv Ispán (82.59) possessed observably greater PSD, than GK Öthalom (78.78). Sweet corn (81.7) gave significantly higher PSD than sunflower (79.67).

2018 (41.74) gave significantly higher kernel weight, than 2019 (40.68), which confirmed the findings of Muchová (2003) and Guarda et al. (2004). In addition, GK Öthalom (42.16) owned significantly greater KW, compared to Mv Ispán (40.26). Fertilizing ($N_{90}P_{68}K_{80}$: 41.42; $N_{150}P_{113}K_{133}$: 42.12) increased observably KW in contrast with control samples (40.1). In 2018, in the case of control treatment, sweet corn (41.91) gave significantly ($LSD_{5\%}$: 2.24) greater KW, than sunflower (37.48).

Generally fertilizing and preceding crop had no effect on kernel diameter, however 2018 (3.02) gave significantly greater KD, than 2019 (2.97), also GK Öthalom (3.05) possessed appreciably higher KD in contrast with Mv Ispán (2.95).

Except forecrop, all the other 3 factors had statistically provable effect on Hardness Index. Mv Ispán (68.3) owned significantly higher HI (Fig. 4), than GK Öthalom (50.96). Also, 2019 (63.34) gave greater (+13.3%) HI compared to 2018 (55.92). Fertilizing ($N_{90}P_{68}K_{80}$: 61.54; $N_{150}P_{113}K_{133}$: 65.04) increased significantly HI in contrast with control treatment (52.3), as well as in the research of Guarda et al. (2004).

Table 6. Effect of the studied factors on the remaining parameters (Debrecen, Hungary)

Yr	Cv	Fc	Tr	FE (%)	GI (%)	LFR	LV (cm^3)	SD	Mavg (µm)	KW (mg)	KD (mm)	HI (%)
2018	GK Öthalom	Sweet corn	Ø	73.1	98.7	1.86	791	19.23	75.05	41.55	3.07	41
			$N_{90}P_{68}K_{80}$	74.5	87.0	1.87	873	18.23	83.05	43.50	3.08	51
			$N_{150}P_{113}K_{133}$	73.2	81.2	1.96	900	17.90	84.07	42.65	3.06	53
		Sunflower	Ø	71.0	98.3	1.85	743	18.45	67.09	35.38	2.94	40
			$N_{90}P_{68}K_{80}$	73.7	71.2	2.06	795	17.80	81.13	44.68	3.14	49
			$N_{150}P_{113}K_{133}$	73.5	62.9	2.14	838	17.98	82.87	44.90	3.13	53
	Mv Ispán	Sweet corn	Ø	73.4	83.5	1.85	756	22.08	79.71	42.28	3.00	57
			$N_{90}P_{68}K_{80}$	71.8	69.4	2.26	820	22.30	86.55	41.30	2.96	69
			$N_{150}P_{113}K_{133}$	71.0	66.3	2.51	801	22.55	86.89	40.58	2.92	71
		Sunflower	Ø	71.0	92.2	1.95	705	21.40	74.74	39.58	3.03	48
			$N_{90}P_{68}K_{80}$	71.1	63.7	2.16	808	22.48	85.42	42.03	2.99	68
			$N_{150}P_{113}K_{133}$	70.3	71.8	2.31	803	23.35	88.31	42.45	2.99	73
2019	GK Öthalom	Sweet corn	Ø	68.5	98.5	1.75	932	19.23	78.10	41.33	3.01	50
			$N_{90}P_{68}K_{80}$	68.7	94.8	1.90	969	18.53	81.08	43.08	3.04	57
			$N_{150}P_{113}K_{133}$	69.4	94.4	1.92	983	18.95	81.93	42.00	3.00	58
		Sunflower	Ø	68.9	98.6	1.86	938	19.43	74.25	40.65	2.98	53
			$N_{90}P_{68}K_{80}$	68.5	97.2	1.87	998	18.55	78.65	42.98	3.09	53
			$N_{150}P_{113}K_{133}$	68.8	97.5	2.04	997	18.03	78.10	43.20	3.06	56
	Mv Ispán	Sweet corn	Ø	67.8	97.1	1.76	886	23.35	78.98	38.60	2.93	65
			$N_{90}P_{68}K_{80}$	66.9	90.0	1.95	935	23.05	81.93	40.00	2.93	75
			$N_{150}P_{113}K_{133}$	65.5	82.6	2.10	955	22.55	83.10	38.05	2.86	79
		Sunflower	Ø	67.8	97.0	1.85	830	22.63	80.28	41.40	2.99	65
			$N_{90}P_{68}K_{80}$	66.6	94.1	1.95	873	22.80	82.90	39.37	2.90	72
			$N_{150}P_{113}K_{133}$	66.6	85.1	2.21	908	22.45	82.33	37.55	2.84	77
			LSD$_{5\%}$ (Yr):	0.64	4.37	0.08	23.86	0.88	1.94	1.03	0.03	4.46
			LSD$_{5\%}$ (Cv):	1.06	5.14	0.08	33.99	0.31	1.79	0.98	0.03	3.10
			LSD$_{5\%}$ (Fc):	1.12	5.35	0.09	35.33	0.89	1.91	1.06	0.04	4.70
			LSD$_{5\%}$ (Tr):	1.37	5.70	0.09	40.95	1.09	1.79	1.26	0.04	5.12

Pearson's correlation analysis:

The results of the Pearson correlation analysis can be seen in Table 7, where only those values are shown, which was at least ± 0.5. Fertilizing was in tight correlation with CP (0.812**), ZI (0.776**) and WGC (0.828**); in medium with VQN (0.704**), DDT (0.571**), ST (0.711**), PD (0.721**), W (0.726**), L (0.746**), LFR (0.588**) and Mavg (0.675**); however fertilizing was in negative medium correlation with DS (-0.634**) and P/L (-0.573**). Similar results were got by Masauskiene & Ceseviciene (2005) and Eser et al. (2017). These results suggest that, proper nutrient supply is essential for growing wheat with good rheologic and protein properties.

Table 7. Pearson's correlation analysis

/	F	CP	ZI	WGC	GI	GS	FE	VWA	VQN	DDT	ST	DS	PWA	PDR	PD	PMR	PE	P	L	P/L	W	LFR	LV	SD	Mavg	KD	HI
F	1																										
CP	0.812**	1																									
ZI	0.776**	0.958**	1																								
WGC	0.828**	0.941**	0.898**	1																							
GI	-.504**				1																						
GS					-.721**	1																					
FE							1																				
VWA								1																			
VQN	0.704**	0.866**	0.869**	0.808**					1																		
DDT	0.571**	0.739**	0.755**	0.668**					0.874**	1																	
ST	0.711**	0.856**	0.842**	0.812**					0.968**	0.884**	1																
DS	-.634**	-.796**	-.818**	-.732**					-.967**	-.790**	-.910**	1															
PWA								0.924**					1														
PDR							-.550**							1													
PD	0.721**	0.794**	0.750**	0.767**					0.744**	0.694**	0.768**	-.642**			1												
PMR			0.538**				-.581**		0.554**		0.507**	-.626**		0.926**		1											
PE		0.628**	0.694**	0.588**			-.526**		0.719**	0.633**	0.688**	-.745**		0.763**	0.608**	0.934**	1										
P								.712**					0.652**	0.540**				1									
L	0.746**	0.906**	0.831**	0.843**					0.799**	0.727**	0.810**	-.732**			0.781**		0.510**		1								
P/L	-.573**	-.746**	-.632**	-.674**					-.643**	-.589**	-.651**	0.561**			-.701**			0.632**	-.867**	1							
W	0.726**	0.831**	0.871**	0.818**					0.842**	0.694**	0.805**	-.821**			0.743**	0.680**	0.806**		0.689**		1						
LFR	0.588**				-.678**			0.569**					0.597**									1					
LV		0.579**	0.539**						0.622**	0.596**	0.603**	-.590**			0.594**	0.595**	0.693**		0.542**	-.520**	0.608**		1				
SD								0.818**					0.792**					0.724**						1			
Mavg	0.675**	0.694**	0.711**	0.690**	-.629**			0.703**	0.511**				0.679**								0.618**	0.567**			1		
KD							0.570**																	-.620**		1	
HI							-.524**	0.854**					0.790**					0.591**			0.631**			0.774**	0.658**	-.627**	1

The importance of good crude protein content has to be emphasized, because 26/15 (58%) of the studied parameters in PCC were at least in medium correlation with CP, namely positive, very tight with WGC (0.941**), ZI (0.958**) and L (0.906**); tight with VWA (0.866**), ST (0.856**), PD (0.794**) and W (0.831**); in medium with DDT (0.739**), PE (0.628**), LV (0.579**) and Mavg (0.694**); but in negative, tight with DS (-0.796**) and negative, medium with P/L (-0.650**). Practically, the higher the CP is, the more the WGC is, and it develops slower, but it stays more stable after kneading, and the dough made out of it will be more ductile, which attributes are essential for getting loaves with good volume.

The other decisive attribute was wet gluten content, since 26/14 (54%) of the studied properties were at least in medium correlation with WGC. This parameter was in very tight relationship with CP (0.941**), ZI (0.898**), VQN (0.808**), ST (0.812**), PD (0.767**), W (0.818**) and L (0.843**); in medium, positive with DDT (0.668**), PE (0.588**) and Mavg (0.690**); meanwhile in negative, medium with DS (-0.732**) and P/L (-0.674**).

Studying gluten quality, Zeleny index was in very tight, positive correlation with CP (0.958**); in tight with WGC (0.898**), VQN (0.869**), DDT (0.755**), ST (0.842**), PD (0.750**), W (0.871**) and L (0.831**); in medium with PMR (0.538**), Mavg (0.711**), PE (0.694**) and LV (0.539**); but in negative, tight with P/L (-0.632**) and DS (-0.818**). The relationship of GS (-0.721**), LV (-0.678**) and Mavg (-0.629**) with gluten index was negative, medium.

Valorigraphic water absorption was in very tight correlation with PWA (0.924**); in tight with starch damage (0.818**) and Hardness index (0.854**); in medium with Mavg (0.703**), LV (0.569**) and P (0.712**). These results suggest that, the harder the grain is, higher the starch damage and average particle size are, which results in better water absorption because of the increased surface. VQN was in very tight relationship with ST (0.968**), in tight with ZI (0.869**), CP (0.866**), DDT (0.874**), W (0.842**), L (0.799**) and WGC (0.808**); in medium with PD (0.744**), PMR (0.554**), PE (0.719**), LV (0.622**) and Mavg (0.511**) so it can be concluded that, the VQN can predict well some rheological parameters. DDT and ST showed almost the same correlations like VQN. Dough softening was in negative, very tight relationship with VQN (-0.967**) and ST (-0.910**); in tight with CP (-0.796**), ZI (-0.818**), DDT (-0.790**) and W (-0.821**); in medium with WGC (-0.732**), PD (-0.642**), PMR (-0.626**), PE (-0.745**), L (-0.732**) and LV (-0.590**); however in positive, medium with P/L (0.561**).

Promilographic water absorption was in very tight relationship with VWA (0.924**); in tight with HI (0.790**) and SD (0.792**); in medium with P (0.652**), LV (0.597**) and Mavg (0.679**). PDR was in very tight correlation with PMR (0.926**); in tight with PE (0.763**); in medium with P (0.540**); meanwhile in negative, medium with FE (-0.550**). Promilographic ductility was tight relationship with CP (0.794**), ZI (0.750**), WGC (0.767**), ST (0.768**) and L (0.781**); in medium with VQN (0.744**), DDT (0.694**), PE (0.608**), LV (0.594**) and W (0.743**); but in negative, medium with DS (-0.642**) and P/L (-0.701**). PMR was in very tight, positive correlation with PDR (0.926**) and PE (0.934**); in medium with VQN (0.554**), ST (0.507**), W (0.680**) and LV (0.595**); in negative, medium with FE (-0.581**) and DS (-0.626**). Studying promilographic energy, it can be summed that, PE was in medium, positive relationship with CP (0.628**),

ZI (0.694**), WGC (0.588**), VQN (0.719**), DDT (0.633**), ST (0.688**), PD (0.608**), L (0.510**) and LV (0.693**), in tight with PDR (0.763**) and W (0.806**); in very tight with PMR (0.763**); but in negative, medium with FE (-0.522**) and DS (-0.745**). Comparing alveographic and promilographic results, it can be seen that, W and PE; L and PD correlated significantly.

Alveographic P was in medium, positive correlation with VWA (0.712**), PWA (0.652**), PDR (0.540**), HI (0.591**), P/L (0.632**) and SD (0.724**). The correlation of P with VWA and SD was also observed by Preston et al. (1987). L was in medium, negative correlation with DS (-0.732**); but in very tight, positive with CP (0.906**); in tight with ZI (0.831**), WGC (0.843**), VQN (0.799**), ST (0.810**) and PD (0.781**); in medium with DDT (0.727**), PE (0.510**), W (0.689**) and LV (0.542**). Our results affirmed the findings of Sipos et al. (2007), where they found tight correlation between L, VQN, WGC, CP and extensibility. P/L was in medium, negative relationship with CP (-0.746**), WGC (-0.674**), ZI (-0.632**), GS (-0.506**), VQN (-0.643**), DDT (-0.589**), ST (-0.651**), PD (-0.701**) and LV (-0.520**); in tight with L (-0.867**); meanwhile in positive, medium with DS (0.561**) and P (0.632**). One of the most important parameter of alveograph is W, which was in tight, positive correlation with CP (0.831**), ZI (0.871**), WGC (0.818**), VQN (0.842**), ST (0.805**), PE (0.806**); in medium with DDT (0.694**), PD (0.743**), PMR (0.680**), L (0.689**), LV (0.608**), Mavg (0.618**) and HI (0.631**); but in negative, tight with DS (-0.821**). The correlation of CP and WGC with W have been previously reported also by Sipos et al. (2007).

Considering parameters of baking test, LFR was in medium, positive relationship with Mavg (0.567**), VWA (0.569**) and PWA (0.597**); but in negative, medium with GI (-0.678**). In addition, loaf volume was in medium, positive correlation with CP (0.579**), ZI (0.539**), VQN (0.622**), DDT (0.596**), ST (0.603**), PD (0.594**), L (0.542**), PMR (0.595**), PE (0.693**) and W (0.608**); but in negative, medium with DS (-0.590**) and P/L (-0.520**). These findings are connecting to the statement of Gabriel et al. (2017), because the correlation between LV and CP was only medium positive, meanwhile W and PE, the main parameters of alveograph and promylograph were in tighter relationship with LV. Cho et al. (2018) reported similar alterations in the case of CP, DDT and ST with LV. These values suggest that, good volume loaf can only be baked from flours with proper protein and ductility parameters.

Starch damage was in tight, positive relationship with VWA (0.818**), PWA (0.792**) and HI (0.774**); in medium with P (0.724**); but in negative, medium with KD (-0.620**). Mavg was in medium, positive relationship with CP (0.694**), ZI (0.711**), WGC (0.690**), VWA (0.703**), PWA (0.679**), VQN (0.511**), W (0.618**), LV (0.567**) and HI (0.658**); but in negative, medium with GI (-0.629**). The relationship of water absorption and PSD is in compliance with the findings of Preston & Williams (2003). Kernel diameter was in medium, positive correlation with flour extraction (0.570**), meanwhile in negative, medium with HI (-0.627**).

Studying the relationship of Hardness index with other attributes: HI was in tight, positive correlation with SD (0.774**), VWA (0.854**) and PWA (0.790**); in medium with P (0.591**), W (0.631**) and Mavg (0.658**); but in negative, medium with FE (-0.524**) and KD (-0.627**). These results affirmed the findings of Eliasson and

Larsson (1993) and Manley (1995), namely the kernel hardness can determine starch damage, water absorption, PSD and flour extraction as well.

CONCLUSIONS

The outstanding yields of the 1st growing season can be explained by the fair water supply (+149.5 mm compared to 30-year avg.), in addition, lack of precipitation and the detrimental effect of the rapid warming in February (+2.5 °C compared to 30-year avg.) and March (+3 °C compared to 30-year avg.) considering the 2nd growing season. Nevertheless, the quality parameters of the studied samples were appreciably better in the 2nd growing season, due to the ecological parameters of 2019 (mild weather of April and May and average annual precipitation). This phenomena was also discussed by Pepó (2010a), Tayyar (2010), Pepó & Sárvári (2011), Gooding et al. (2017) and Szabó et al. (2017). The ecological conditions of the 2nd growing season also significantly affected the quality parameters of the classic winter wheat cultivar, GK Öthalom, which are reflected mainly in the improvement of all protein linked attributes (CP, WGC, ZI, GS, VQN, ST, DS, PMR, PE and LV).

Addition of fertilizer significantly augmented the quality parameters, however in the case of some properties (Y, VWA, PWA, PMR, PE, LV, Mavg, KW, HI) increasing the amount of fertilizer above $N_{90}P_{68}K_{80}$ dosage, had no further, statistically provable improver effect. Thinking over these results, like yield, water absorption and loaf volume, which are one of the most determinative parameters for the baking industry, it can be summed that, $N_{90}P_{68}K_{80}$ dosage was enough to realize the quality and yield potential of the studied genotypes. Moreover, the measured data indicates that fertilizing plays a decisive role in determining the main post-harvest quality indicators considering the Hungarian wheat standard, like crude protein, wet gluten content, Zeleny index, VQN, stability, W and P/L, because even the $N_{150}P_{113}K_{133}$ dosage significantly increased them, which is substantial since more than half of the Hungarian wheat production is exported.

The results in the 1st growing season suggest that, in the case of suboptimal nutrient supply, choosing of the right preceding crop can considerably improve the yield and quality parameters (Y, CP, DGC, ZI, FE, VQN, DDT, ST, DS, PDR, PMR, PE, Mavg and all alveographic values) of winter wheat, which has been already partly reported by Pepó (2010c). In our case, sweet corn performed better because the deep root system of sunflower exploits the nutrient and water supply of the soil.

In the perspective of the baking industry, one of the most eloquent quality indicators is the baking test, more precisely loaf volume, which was in medium, positive correlation with the main parameters of all the used methods: VQN (0.622**), PE (0.693**) and W (0.608**) in the Pearson’s correlation analysis. Also, the importance of Hardness Index has to be underlined, because it was in a tight relationship with starch damage (0.774**) and water absorption (0.854**); medium with Mavg (0.658**) and W (0.631**).

Effect of studied agrotechnical factors on gluten quality can be observed in the change of gluten index and gluten spread. GI was considerably affected by year, cultivar and fertilization, meanwhile GS was influenced observably by year, preceding crop and fertilization. To compile, increment of fertilizer, high temperature in April and May, large amount of annual precipitation and sunflower (exploitive) as preceding crop deteriorate the gluten strength.

Variety-specific agronomy practice has to be pointed out, due to there were significant differences between the studied varieties in general, considering GI, FE, VWA, DDT, ST, DS, PDR, PD, P, L, P/L, LV, LFR, SD, Mavg, KW, KD and HI values. Only one sample, GK Öthalom (2019, sweet corn and $N_{90}P_{68}K_{80}$) met the requirements of the Hungarian I. class except one parameter, the Zeleny index, all the other studied samples met maximum the requirements of II. class, which shows well that none of the examined growing seasons was optimal for the generative development of winter wheat. Besides this, without fertilizing, none of the samples met the minimal specifications of II. class, which means that they could be only sold for feed purposes. It has been previously reported that fertilizing exerts a strong influence on the baking quality of wheat (Borghi et al., 1995).

Summarizing, these data contribute to a better understanding of the effect of agrotechnical factors on the quality and yielding of winter wheat, and the relationships between the parameters of the common and infrequently-used methods.

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Use of soil enzyme activity in assessing the effect of No-Till in the South of Russia

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Abstract. The activity of 11 enzymes (catalase, dehydrogenases, peroxydases, polyphenoloxidases, ascorbateoxidase, ferrireductase, β-fructofuranosidase, amylase, urease, phosphatase and protease) was assessed under conditions of steppe zone in the south of Russia when using different tillages. Winter wheat and sunflower are main crops in these soils. Moreover, chickpea, coriander, lint, barley, corn and some other crop are cultivated here in the crop rotation duration 6 years. Enzyme activity was compared in soils of 15 fields with long-term no-till (NT) versus to 15 fields with conventional tillage (CT). The researches were held along the whole Haplic Chernozem Loamic at a depth of 0–65 cm. Special attention was paid to top soil (0–10 cm), which is directly subject to the mechanical effect. The carbon cycle enzyme (β-fructofuranosidase) activity was the most sensitive indication for NT use. In top soil the enzyme activity was greater by 16–35% at NT versus to CT. Activity of this enzyme reduces by 28–293% when soil depth increasing in both the tillages. Enzymes of different classes had different behaviours in soils depending on season, crops and tillage thanks to biochemical nature. Hydrolases and oxidoreductases were assessed by the indices characterizing soil condition and health. For this purpose geometric mean by hydrolase activity (GME_{hd}) and geometric mean by oxidoreductase (GME_{ox}), as well as integral index of biological soil condition (IIBC) were used. Index GME_{ox} in soil under sunflower reduced by 16% in summer versus to spring. Thereby, hydrolase index GME_{hd} reduced by 60%. At NT activity of oxidoreductase was lower by 10 and 13%, and activity of hydrolase was increased by 12 and 14% versus to CT. Soil IIBS values at NT increased by 18–35% at average within three years (2016–2018). The use of NT technology contributes to an increase in the activity of hydrolases and soil quality due to the conservation of moisture in the soil.

Key words: bioindicator, biological activity, soil enzymes, No-Till, soil health indexes.

INTRODUCTION

Biological indicators are often used as sensitive indicators of soil fertility under different tillages and the degree of its degradation under anthropogenic impacts (Trasar-Cepeda et al., 2008; Burns et al., 2013; Minnikova et al. (2019a, 2019b, 2019c)). The soil enzyme activity is an important diagnostic criterion during assessment of soil quality at different types of man-caused impact on soils (Trushkov et al., 2019; Kazeev et al., 2020). The study of the enzymatic activity of soils is important: characterization of the metabolic potential of soils, quality and fertility, assessment of resistance to various natural and anthropogenic influences (Shukla & Varma, 2011; Akimenko et al., 2014; Loeppmann et al., 2016). Enzymes serve as indicators of the cycle of carbon (β-fructofuranosidase, amylase), nitrogen (urease, protease), phosphorus (phosphatase). These cycles play a key role in assessing soil quality and maintaining soil health. The activity of soil enzymes is important in assessing the quality of soils and diagnosing their ecological state (Hugh, 2012; Burns et al., 2013; Kazeev et al., 2015; Kolesnikov et al., 2019).

During conventional tillage (CT) fruitful soils obtain new properties, the morphogenetic statuses and soil-formation factors are changed (Azarenko et al., 2020). One of the processes resulting in reformation of tillable chernozem is tillage practices (Minnikova et al., 2017, Minnikova et al., 2018; Mokrikov et al., 2019). The nutrient cycle without the appropriate fertilizer element returning in the form of fertilizers is violated due to change in soil microbiota that affects soil organic matter pool. Therefore, mouldboard plowing causes decrease of humus level and reduction of soil enzyme activity (Dadenko et al., 2014; Garbuz et al., 2016; Sharkov et al., 2016; Zhelezova et al., 2017). In case of no-till (NT) as resource-saving analogy of the conventional tillage, soil dysfunction is significantly reduced that protects soil from degradation towards resistivity in combination with the crop rotation. The number of macroaggregates is an important microbiological process for control mechanism and organic substance maintenance (Beare et al., 1994; Dick et al., 1984; Gupta & Germida, 1988). When using various tillages with deep moldboard plowing, nitrogen and carbon cycles are violated in soil that causes change in microbial biomass and soil enzyme activity (Franchini et al., 2007; Paz-Ferreiro & Fu, 2016; Hlisnikovský et al., 2020). Due to maintenance or destruction of organic matters content the enzyme activity can be a powerful indicator of soil agrogenic transformation and remediation processes (Lagomarsino et al., 2009; Mancinelli et al., 2013; Marinari et al., 2015; Vilny, 2015; Shirokikh et al., 2017; Bai et al., 2018). The soil biochemical activity is a good index for assessment of tillage effect on the structure and microbiological activity of the soils. Under influence of second crop residues organic-matter degradation processes are intensified and carbon cycle enzyme (dehydrogenases, fluorescein diacetate hydrolysis, β-glycosidase, alkaline phosphatase) activity is increased. In case of no-till the soil structure is improved that causes increase in content of organic matter and microbial biomass (Garbuz et al., 2016; Sharkov et al., 2016; Mokrikov et al., 2020).

This work gives brief results for the comparative assessment of long-term use of NT under conditions of the steppe zone of Russia in the Rostov Region within the last 5 years. This resource-saving and soil protective technology is widely used in the world, but it is poorly known in Russia. It allows gathering steadily heavy yields of agricultural crops even under conditions of unstable watering within the risks farming in agriculture zone. Particularly under such conditions NT shows its advantages versus to conventional moldboard plowing (CT) and other tillage with soil turbating (Riley et al., 1994; Soane et al., 2012; Gristina et al., 2018). The CT with plowing result in dehumification and reduction of the biological activity for chernozem in the south of Russia (Dadenko et al., 2014; Azarenko et al., 2020). On the contrary, NT has a positive effect on the reserve of fertilizer elements and soil physical properties (Minnikova et al., 2018; Trofimova et al., 2018; Kravtsova et al., 2019; Mokrikov et al., 2019).

The research gap of our study to the assessment of soil enzyme activity in assessing the effect of NT in the South of Russia. NT technology is reserved moisture versus CT and increases the activity of soil enzymes of different classes. Analyze the change in the activity of hydrolases and oxidoreductases under different processing technologies. Assess soil quality using condition indices and IIBC.

MATERIALS AND METHODS

Site Description. The research objects were fields with NT and CT. The experimental fields have been processing within 10 years by NT and are located in the south of the steppe zone in Russia. The researched area exceeds 5.5×10^3 ha. Within the researched area ordinary chernozems are commonly spread, which genesis, properties and fertility are well-known from the literature source (Valkov et al., 2008, 2012). In total pair wise comparison of 30 fields with different tillages (15 fields with NT and 15 fields with CT) were researched. The areas with NT were compared with CT areas at a distance of 50–100 m from each other. The major part of the crop production areas are occupied with grain crops (winter wheat and barley) - 49%, grain maize - 10%, sunflower and permanent legume grasses (melilot, lucerne, sainfoin, tare) - 10%, grain legume (chickpea, lentil, pea) - 8%, winter crucifers (false flax, rape, mustard) - 6.5%, coriander - 6%, lint - 4%, false saffron - 2.5%, buckwheat - 2.0%, permanent grasses - 2%.

The agricultural crops were seeded during NT by the following machines: tractor Buhler Versatile 2375 + Great Plains NTA 3510 (10.7 m) and Case Magnum 315 + Great Plains NTA 3510 (10.7 m), all crops were seeded with row width of 19.1 cm. As per the articles when using NT fuel consumption was 26 L ha^{-1}, during conventional tillage with moldboard plowing - 74.1 L ha^{-1} (Mokrikov et al., 2017, Minnikova et al., 2018; Mokrikov et al., 2019).

Experiment Methodology. As a result of complex environmental researches 45 parameters were determined characterizing ecological state of soils in 30 fields with NT and CT. Soils were researched 3–5 times per a season in 2017–2020. Samples were taken from different depths 0–10, 10–20, 20–30, and 50–60 cm. The mixed samples for the surface soil layer were obtained from 5 individual samples taken at a distance of 3–5 m from each other along the diagonal. The soil was dried out in the shade, free from roots and organic residues, sieved through 1 mm screen and analysed within several days after sampling in 3–9-fold analytical replication. The work represents the results of the enzyme activity analysis for 2016–2020.

In 2016 the large-scale comparative diagnostics of soil ecological and biological condition by activity of 11 enzymes of oxidoreductase and hydrolase classes were performed (Table 1). Within the next 4 years quantity of researched enzymes were restricted with the most informative indices well proven within the first year of the our research (2016). Comparison of the activity for separate enzymes is not always informative. Thus, for assessment of soil quality and condition by the indices of soil enzyme activity of each class the geometric means were calculated for enzymes of each class in absolute units (Hinojosa et al., 2004). The geometric means (GME_{ox}, GME_{hd}) were used as a single index number for integration of all soil enzyme activity indices being similar by nature, but having different ranges of variation.

Table 1. List of determined enzymes and methods of determination

No.	List of enzymes (EC)	Method of determination
	Class of Oxidoreductases	
1.	H_2O_2: H_2O_2-oxidoreductase, EC 1.11.1.6.	by the volume of decomposed oxygen during the decomposition of hydrogen peroxide
2.	TPP : NAD (F) - oxidoreductase, EC 1.1.1	for the reduction of tetrazolium salts to formazan
3.	NAD (F) • H_2: Fe_2O_3 – oxidoreductases, EC 1.6.99	by the amount of reduced ferric iron
4.	O-diphenol: oxygen – oxidoreductase, EC 1.10.3.1	on the oxidation of hydroquinone to quinones
5.	quinone: H_2O_2 – oxidoreductase, EC 1.11.1.7	on the oxidation of hydroquinone to quinones
6.	L - ascorbate: oxygen oxidoreductase, EC 1.10.3.3	by the difference between the amount of residual ascorbic acid and the amount of formed dehydroascorbic acid (DHAA)
	Class of Hydrolases	
7.	invertase, sucrose, EC 3.2.1.26	by the amount of glucose during the hydrolysis of sucrose, colorimetrically using Felling's reagent
8.	β-amylase: 1,4 - α - glucan - maltohydrolase, EC 3.2.1.2	by the amount of glucose during start hydrolysis
9.	urea – amidohydrolase, EC 3.5.1.5.	by the amount of ammonia with Nessler's reagent, with hydrolysis of carbamide
10.	phosphohydrolases of monoesters of orthophosphoric acid, EC 3.1.3.1-2	by the amount of inorganic phosphorus during hydrolysis of sodium phenolphthalein phosphate
11.	peptide – hydrolases, EC 3.4.4	by the number of amino acids during proteolysis of casein

The more index value is the better soil capability to function and maintain the plant performance.

The geometric mean of the oxidoreductase activity by formula 1:

$$GME_{ox} = \sqrt[6]{CAT \times DHG \times ASC \times PER \times PHEN \times FER} \qquad (1)$$

CAT – activity of catalase, 1 mL O_2 × 1 g soil^{-1} × 1 min^{-1}; DHG – activity of dehydrogenases, mg TPP × 10 g soil^{-1} × 24 hours^{-1}; ASC – activity of ascorbatoxidases, mg DHAA × 1 g soil^{-1} × 24 hours^{-1}; PER – activity of peroxidases, mg 1,4 benzoquinone × 1 g soil^{-1} × 30 min^{-1}; $PHEN$ – activity of polyphenoloxidases, mg 1,4 benzoquinone × 1 g soil^{-1} × 30 min^{-1}; FER – activity of ferrioreductase, mg Fe_2O_3 × 100 g soil^{-1} × 48 hours^{-1}.

The geometric mean of the hydrolase activity by formula 2:

$$GME_{hd} = \sqrt[5]{INV \times UR \times PHOS \times AM \times PRL} \qquad (2)$$

INV – activity of β-fructofuranosidase (invertase), mg of glucose × 1 g soil^{-1} × 24 hours^{-1}; UR – activity of urease, mg NH_3 × 1 g soil^{-1} × 24 hours^{-1}; $PHOS$ – activity of phosphatase, mg P_2O_5 × 100 g soil^{-1} 1 hour; AM – activity of amylase, mg glucose × 1 g soil^{-1} × 24 hours^{-1}; PR – activity of protease, mg glycine × 1 g soil^{-1} × 24 hours^{-1}.

Moreover, for assessment of enzyme activity the previously proven integral index of biological soil condition ($IIBC$) was used (Kazeev et al., 2016). This index allows combining a great amount of soil enzyme activity indices.

Specific values of each NT soil enzyme were calculated versus to values of CT soils by formula 3:

$$B_1 = \frac{B_{NT}}{B_{CT}} \times 100\% \tag{3}$$

Then values of the integral index of soil biological activity were calculated by formula 4:

$$IIBC = \frac{B}{B_{max}} \times 100\% \tag{4}$$

where B – average score point for activity of all enzymes; B_{max} – maximum score point for activity of all enzymes.

For assessment of high correlation ratio between enzyme activity indices and soil moisture content correlation factors were calculated.

Data were statistically processed using Python 3.6.5 program, Matpolib package.

RESULTS AND DISCUSSION

Climate and moisture reserve in the soil with different tillages: Within the researched period the climatic conditions differed from average long-term ones by total amount of precipitations (Fig. 1).

Precipitations laid down within the vegetation season for 2016–2018 exceeded the climatic rate by 66, 35 and 21%. Based on the precipitation amount 2016 and 2017 were humid due to precipitations in spring.

In 2018 since April till June there was less amount of precipitation by 79–83%, than within previous years (Mokrikov et al., 2019). The temperature conditions were closer to the climatic norm; the average annual air temperature was from 12.1 °C in 2016 to 11.6 °C in 2018. The soil temperature was determined by the air temperature and according to the researches it was changed by loggers in soil within a year. For understanding features of thermal conditions within the researched area accumulating temperature sensors - loggers Thermochron DS1921 were positioned. The sensors were located at three depths 10, 20 and 30 cm. The reading frequency is every 6 hours. This allowed assessing the temperature features in top mostly biogenic layer with maximum root expansion of agricultural plants.

As a result it was detected that the surface layer (0–10 cm) has significantly higher temperature fluctuations than at depths of 20 and 30 cm from 21.3 to 31.5 °C. While depth increasing daily temperature fluctuations were less intensive. Temperatures of 0 °C and only by 3–4 °C lower in the soil at a depth of 10 cm prevail since November till the beginning of March. At a depth of 20 cm negative temperatures are settled for 3 winter months. At a depth of 30 cm low temperatures occur within the restricted time since the end of January till the end of February.

Temperature below 10 °C, which is taken as a temperature of active biological processes, at a depth of 10 cm was since the middle of October till the beginning of April. Moreover, achievement of this level was noted in the middle of November during temporary warming. At a depth of 20 cm the border below 10 °C was overcome since the first half of October till the beginning of April. At a depth of 30 cm temperature of

10 °C being optimum for the biological processes was settled only by the beginning of May due to long-term and chilly spring.

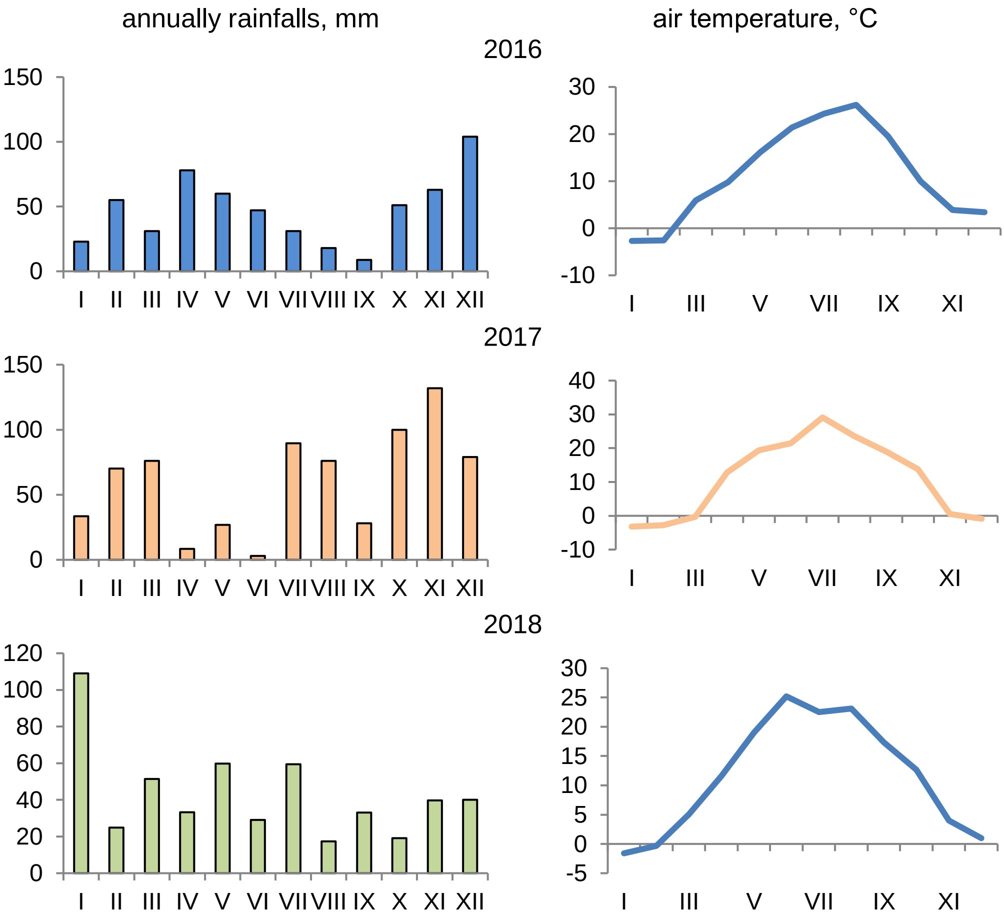


Figure 1. Changes in annually rainfalls and air temperature (2016–2018).

As Fig. 2 shows, at NT since September till November moisture conservation by 19–92% was detected versus to CT.

In general within 5 years of observations the soil moisture content with NT was higher by 22% versus to CT. This is conditioned by fewer amounts of process operations and availability of after harvesting residues at the soil level. They keep the snow cover in winter and contribute to moisture accumulation.

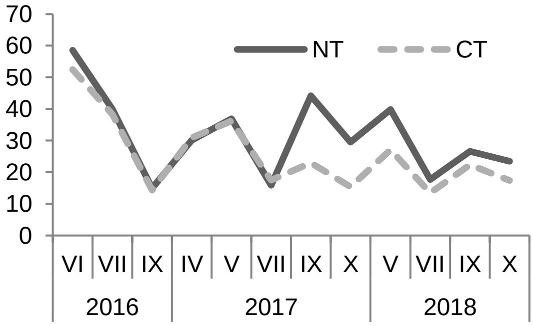
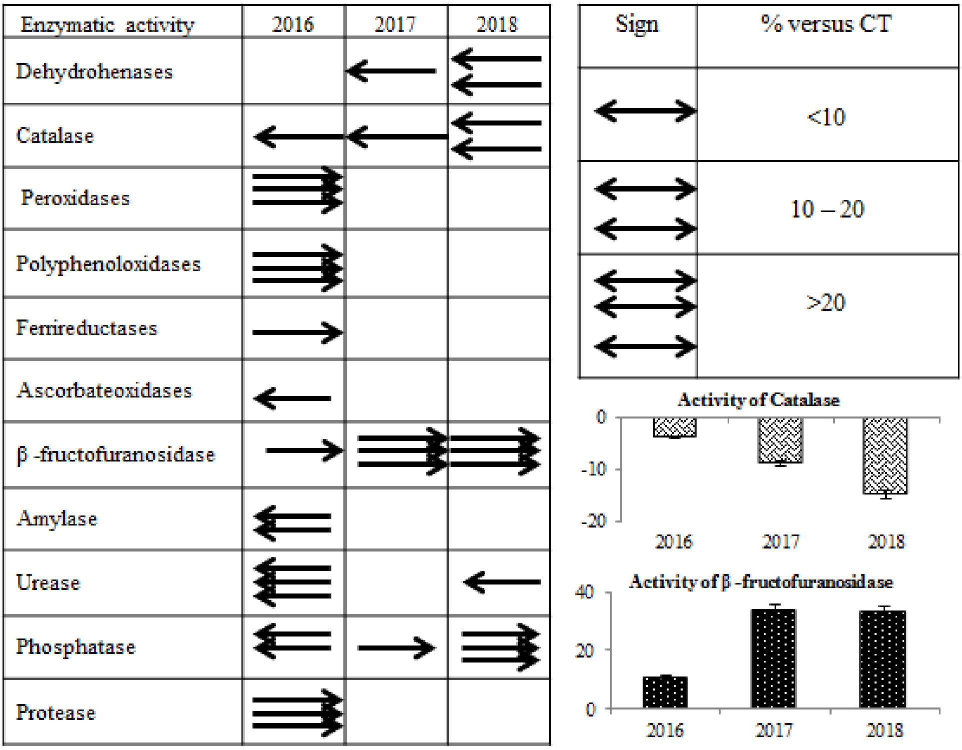


Figure 2. Dynamics of soil moisture NT for the period from June 2016 to November 2018 in the top soil, % versus CT.

Change in the enzyme activity of soils NT versus to CT. Change in the activity for enzymes of oxidoreductase and hydrolase classes at NT versus to CT is given in Fig. 3. The activity of oxidoreductase (peroxydases, polyphenoloxidases and ferrireductase) changes versus to CT both towards stimulation by 8–44% and inhibiting (dehydrogenases, catalase and ascorbateoxidase) by 8–15% versus to CT.



Enzymatic activity	2016	2017	2018
Dehydrohenases		←	← ←
Catalase	←	←	← ←
Peroxidases	→ → →		
Polyphenoloxidases	→ → →		
Ferrireductases	→		
Ascorbateoxidases	←		
β -fructofuranosidase	→	→ → →	→ → →
Amylase	← ←		
Urease	← ← ←		←
Phosphatase	← ←	→	→ → →
Protease	→ → →		

Sign	% versus CT
↔	<10
↔ ↔	10 – 20
↔ ↔ ↔	>20

Figure 3. Estimation of the enzymatic activity of soils under NT, % versus to CT.

The activity of hydrolase class enzymes was changed by other principle. Thus, the activity of carbon cycle enzymes (β-fructofuranosidase and amylase) was compared in 2016 (1 year of research). It is determined that NT reduces the amylase activity by over 30%, but stimulates the activity of β-fructofuranosidase by 10%. Thereby, in 2017 and 2018 during the research more stimulation of β-fructofuranosidase activity was obtained by 33–34% versus to CT. Such an effect is conditioned by increase in concentration of organic matters in top soil due to agricultural crop organic residues digestion.

Multistage changes β-fructofuranosidase activity of soil under different tillages. The specialized change in the activity of the major part of oxidoreductase (catalase and dehydrogenases) at NT didn’t differ from CT versus to hydrolase. Among hydrolases β-fructofuranosidase is the most sensitive to tillage tehnologies. The activity of β-fructofuranosidase as a carbon cycle enzyme depends on the carbon content in the soil and the accessibility of its forms (Fig. 4). The enzyme activity reduces at lower levels during all tillage types by 81–293% at NT, by 28–152% at CT. In 0–10 cm layer the enzyme activity was higher by 16–27% at NT (in April, May, September and

October). In July within the period of maximum moisture lack in the soil and in the absence of precipitations the actual difference of the β-fructofuranosidase activity from CT was not detected. Within spring months in the beginning of the intensive vegetation different results are obtained.

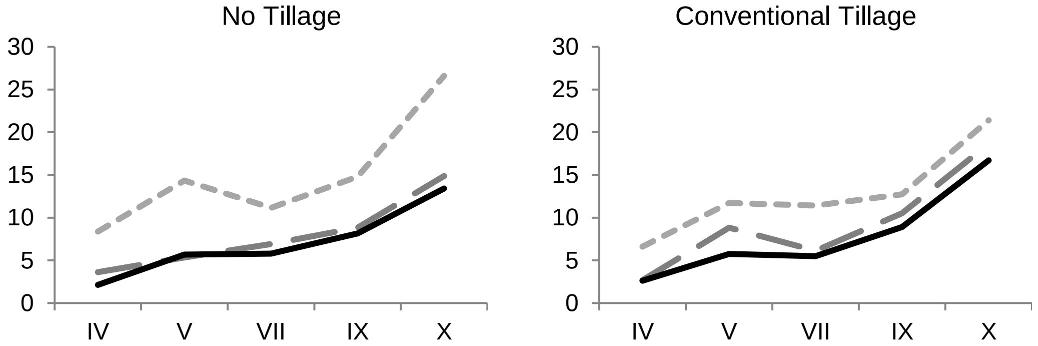


Figure 4. Change in β-fructofuranosidase activity under different tillage, mg of glucose × 1 g soil^{-1} × 24 hours^{-1}.

In April at a depth of 25–35 cm increase in the enzyme activity by 35% was increased versus to CT. On the contrary in May inhibiting of the enzyme activity by 40% was observed. In September and October at this depth difference from CT varied from 16 to 20%. This is conditioned by the fact that at NT the surface root layer is kept. Thus, the β-fructofuranosidase activity was stimulated by moisture content increase. In underlying layers the activity was increased to 40%. At a depth of 55–65 cm differences from CT were detected only in April and October by 19–20% higher than within similar months at CT.

Assessment of soil condition using soil health quality indexes. For assessment of the soil condition by all enzyme activity indices the indices based on geometric mean by each enzyme class (oxidoreductases and hydrolases) were used. As a result of soil enzyme activity determination the soil quality index was calculated. According to data of the each class enzyme activity the geometric mean was calculated for all hydrolases (GME_{hd}) and all oxidoreductases (GME_{ox}). Table 2 shows change in the geometric mean for 11 enzymes of hydrolase class (β-fructofuranosidase, amylase, urease and phosphatase and protease) and oxidoreductase class (dehydrogenases and catalase, peroxydases, polyphenoloxidases, ascorbateoxidase) during the research to 2016.

It was detected that in July versus to May reduction in GME_{ox} values by 16% and for hydrolases reduction in GME_{hd} values by 60% were observed. Moreover, the index values for oxidoreductase is less by 2–4 times versus to hydrolase. Reduction in the hydrolase activity is directly associated with change in the soil moisture content due to less amount of precipitation in

Table 2. Geometric mean for hydrolases (GME_{hd}) and oxidoreductases (GME_{ox}) of soils with binary crops to 2016

month	May	July	September
GME_{ox}	4.8	4.0	3.9
GME_{hd}	16.7	6.2	9.0

Note: GME_{ox} – geometric mean activity of oxidoreductases (n = 6); GME_{hd} – geometric mean of hydrolases activity (n = 6).

July and August. In September, the hydrolase activity became higher, than in July by 46%, but less, than at the beginning of vegetation. This is associated with autumn precipitations. For oxidoreductase activity this dependence is less expressed.

When comparing the tillages for 2016–2018 the following results were obtained at average per each season. Table 3 shows the results of the geometric mean calculation for hydrolases (β-fructofuranosidase, urease and phosphatase) and oxidoreductases (dehydrogenases and catalase) 2016–2018.

Table 3. Geometric mean for hydrolases (GME_{hd}) and oxidoreductases (GME_{ox}) of soils with different tillage during the vegetation seasons of 2016–2018

(numerator – NT, denominator – CT)

year	2016	2017	2018
GME_{ox}	14.8	4.7	14.1
	15.1	5.1	16.2
GME_{hd}	10.1	2.2	5.1
	10.6	1.9	4.5

Note: GME_{ox} – geometric mean activity of oxidoreductases ($n = 6$); GME_{hd} – geometric mean of hydrolases activity ($n = 6$).

In comparison with sunflower seeding NT of different agricultural crops had stimulating impact or didn’t have impact. Only within 2017–2018 the oxidoreductase activity at NT was decreased by 10 and 13% versus to CT. On the contrary for hydrolases GME_{hd} was higher by 12 and 14% in 2017 and 2018 accordingly. Such common trends of the activity as oxidoreductase and hydrolase were conditioned increased moistening at the beginning of vegetation and subsequent non uniform amount of precipitations within the season of 2016. In 2017 and 2018 according to the data of Table 3 amount of precipitations (in mm) was less, than in 2016 that determined optimum conditions and favourably effected the activity of the soil enzymes, and, thus, the soil ecological condition.

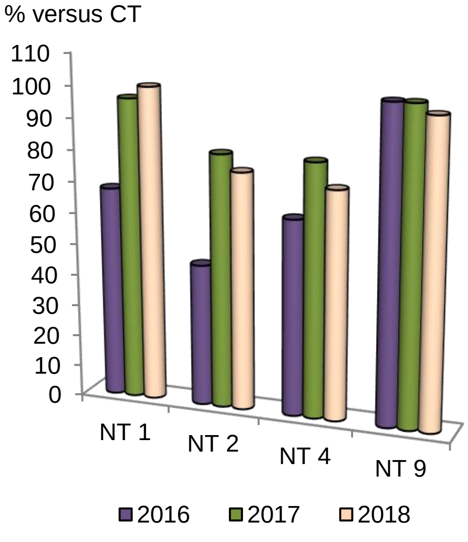


Figure 5. Change in the integral indicator of the biological state of soils by fields with NT for 2016–2019, % versus CT.

Within 2016–2018 in NT fields the integral index of the soil biological condition (IIBC) increased by 18–35% (Fig. 5). Only in one field IIBC remained unchanged: its level was close to CT within the whole observation period.

Within 2016–2018 during the investigation maximum increase of IIBC values was observed in NT 1 and NT 2 fields - 33 and 30% versus to CT. In NT 4 field the index value growth was 10%. Such an increase is used as indicator of soil condition improvement during NT use.

We conducted an experiment in the fields with the same crop - winter wheat, which showed a high sensitivity of enzymes of the hydrolase class, the values of which were generally higher on soils with NT versus to CT. The physical properties of soils such as moisture, temperature, density, penetration resistance have an indirect effect on the change in the enzymatic activity of soils. The activity of oxidoreductases, in comparison with hydrolases, changes to a lesser extent during agricultural use of arable land (Mokrikov et al., 2017; Azarenko et al., 2020). However, the possibility of using the activity of catalase and dehydrogenases in assessing the state of soils and the relationship with hydrothermal conditions and enzymatic activity in dynamics, taking into account crop rotation (Demina et al., 2016; Franke et al., 2018). The activity of enzymes from the class of oxidoreductases was less dependent on soil treatment than the activity of hydrolases (Roldan et al., 2005; Acosta-Martínez et al., 2008; Melero et al., 2009; Sinsabaugh, 2010; Papp et al., 2018). The activity of β-fructofuranosidase as an enzyme of the class of hydrolases is associated not only with root exudates, crop residues, but also with density, resistance to penetration, the amount of moisture in the soil, and pH (Vazquez et al., 2017). Similar results on the stimulation of hydrolase activity in different types of soil cultivation were obtained earlier by other authors. This is due to the fact that hydrolytic enzymes are involved in the metabolism of organic substances, an increased amount of which is formed on the soil surface during NT. As a result, the activity of these enzymes is associated with the organic carbon content in soils (Katsalirou et al., 2010; Gong et al., 2018). In the studied soils using the NT technology, an increase in the degree of organic matter humification and the content of permangate oxidized organic matter was found (Mangalassery et al., 2015; Hok et al., 2018). Along with an increase in soil moisture when using NT technology, an increase in the activity of hydrolases associated with hydrolytic processes of transformation of organic matter in soils is explained. The major indication of soil restoration at NT is soil organic matter accumulation and an increase of soil fertility caused by soil microbiota (Kudeyarov, 2015; Mokrikov et al., 2020).

CONCLUSIONS

It was found that the activity of enzymes in the chernozems of southern Russia depends on the season, soil moisture and depth of sampling, soil different tillage and the class of enzyme. The maximum change in enzymatic activity was noted in the top soil horizon. A decrease in soil moisture leads to inactivation of enzymes, regardless of the method of soil cultivation. The activity of hydrolase enzymes is recovered to a greater extent by soil moisture, and the activity of oxidoreductase enzymes depends more on soil temperature. Long-term use of NT technology leads to the increase of the activity of soil enzymes, especially the enzyme of the carbon cycle - β-fructofuranosidase.

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Dynamics of tuber weight in early potato varieties in the contrasting weather conditions of the Northwestern Russia

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Abstract. Under climate changes, it is particularly important to search for characteristics of varieties that are steady in contrasting weather conditions. The aim of the present research was to study the relationship between the growth of tubers and haulms in early potato varieties in the Northwestern Russia. Fourteen early potato varieties were studied in the field experiments conducted in 2017–2019 in the vicinities of St. Petersburg. The results of three intermediate digs on the 45th, 60th and 75th day from the planting date were analyzed. The correlation analysis, ANOVA, and regression were used. The average haulm and tubers weight significantly differed in the years of the study, while the number of tubers per plant and the haulm to tubers weight ratio did not differ. The relative growth rate of tubers weight from day 45 to 60 and from 60 to 75 did not differ significantly between 2018 and 2019, the years contrasting in terms of weather conditions. On the average, the tubers weight increased 2.6–2.8 times from day 45 to 60, and 1.4 times from day 60 to 75. It has been established that the early prediction of productivity in early potato varieties can be based on the haulm weight on the 60th day after planting; its coefficient of correlation with productivity was 0.72–0.79. Every 100 grams of the haulm weight on day 60 ensures a 100–200 g increment in the tubers weight by the time of harvest on day 80. The obtained results can be used in the development of a morphometric indicator-based automated system for monitoring the growth of potato plants.

Key words: biometric indicators, dynamics, potato, productivity, relative growth rate.

INTRODUCTION

Potato is the third most important food crop in the world after wheat and rice. It is characterized by the ability to grow in various ecological and geographical regions, to yield in unfavorable conditions and on soils with low fertility. In the modern world, where more than two billion people (26% of the world's population) lack a regular food supply (FAO, 2019), potato is regarded as the main crop for combating poverty and malnutrition in developing and underdeveloped countries. The nutritional value of potato and the ability to produce yield in a relatively short growing season have contributed to the development of potato production in most countries in Asia, Africa and Latin America (Chandrasekara & Kumar, 2016; Devaux et al., 2020). Climatic changes and instability of weather factors have a noticeable impact on the potato industry and require adaptation measures (Hijmans, 2003; Menzel et al., 2005; Kaukoranta & Hakala, 2008; Iglesias et al., 2009; Olesen et al., 2011; IPCC, 2014). Depending on the region and climate model, the predicted declines in yield by 2050 range from 2.5% to 23% (Pradel et al., 2019). The climate changes observed in recent decades in the European part of Russia have significantly affected the manifestation of economically important traits of potatoes, including productivity and starch content (Novikova et al., 2017; Maho et al., 2019). For the successful functioning of the industry in conditions with unstable weather factors, there is a need in abiotic stress tolerant varieties, and in appropriate technologies for their cultivation (Hijmans, 2003; Olesen et al., 2011; Dahal et al., 2019; Devaux et al., 2020).

In terms of acreage and gross harvest, Russia's share in world potato production is about 10% (Korshunov et al., 2018). According to the leading Russian breeders, there is a shortage of highly productive table varieties with improved quality characteristics, such as early-maturing, late blight and nematode resistance (Simakov et al., 2016). Testing of new domestic varieties is annually carried out in experimental fields of the 'Pushkin and Pavlovsk Laboratories' Scientific and Production Base of the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (P&PL SPB of VIR) located near St. Petersburg (59.7 N, 30.4 E), in the Northwest Federal District of Russia. Conditions in the Northwestern District are favorable for the production of seed potatoes, therefore, testing of new domestic potato varieties in this region is of particular interest for all the parties involved in potato production, including commodity producers and seed producers. However, insufficient knowledge of the mechanisms of potato plants resistance to stress factors, and of their ability to respond to the changing environmental conditions during ontogenesis, complicate the development of a methodology for screening varieties with a high adaptability potential (Eremeev et al., 2003; Dahal et al., 2019).

In breeding of potatoes and other root tubers, the dynamics of growth of roots and tubers, as well as of vegetative mass, is important (Guzhov et al., 2003, p. 179). At the beginning of vegetation, biomass is consumed for the leaf surface formation, and then the biomass produced by the plant is spent on tuberization. To ensure high yields, it is necessary to provide sufficient leaf area by the onset of tuberization. This information is valuable for assessing performance and adaptation particularly in areas with short growing seasons, where harvesting has to be carried out during the bulking period, that is, before leaf senescence. Assessment and documentation of tuber bulking maturity of potential varieties can be useful in the selection of early bulking maturity (Mihovilovich et al., 2014).

Despite the large number of varieties currently available, there is still a need in new early-maturing varieties (Eremeev et al., 2015; Kawar et al., 2018; Ierna & Mauromicale, 2020). Early ripening allows avoiding biotic and abiotic stresses that occur in later periods of the year. Early maturing varieties also ensure an even distribution of potato production over a season (Kawar et al., 2018). Early tuber initiation and growth are necessary for acceptable production in areas where potatoes are often harvested prior to physiological maturity (Mihovilovich et al., 2014).

The main parameters of an early potato variety are as follows: 60–70 days from planting to tuber bulking, productivity of 12–15 t ha^{-1} on day 60, 6–8 marketable tubers per plant, and 100–120 g average weight of a tuber (Simakov et al., 2016). From the agricultural point of view, an early maturing potato variety implies an ability to produce a high yield of tubers early, however, many varieties that are attributed to early ones, continue increasing the weight of tubers after 75, 80, and even 90 days of growth, which indicates that they have not reached technological ripeness by the time of harvesting (Kiru et al., 2016). Earlier (Kiru et al., 2016), a criterion for true early maturity has been proposed, that is, a decrease in the tuber weight relative growth rate after 60 days from the planting. Growth rate is a characteristic of a time series; it expresses changes in values of a variable between two periods of time (Eliseeva et al., 2006). In our case, it is the relative growth rate in weight of tubers or haulm between two digs (Kiru et al., 2016).

Plant breeders are compelled to screen large numbers of genotypes for their yield potential under field conditions using the time- and cost-intensive methods. The use of easy phenotyping techniques could improve the understanding of potato yield-related mechanisms and allow low-cost predictive assessments (Prey & Schmidhalter, 2020). Knowledge of the biological regularities of plant growth and development allows one to make early forecasts of yield using mathematical, in particular regression models (Guide to agrometeorological forecasting, 1984).

The aim of the present research was to study the relationship between the growth of tubers and haulms in early potato varieties in the Northwestern Russia.

MATERIALS AND METHODS

Site description and materials

Fourteen potato varieties were studied in 2017–2019 in the experimental field of P&PL SPB of VIR, St. Petersburg (Table 1). The soils of the experimental field were medium-loamy, slightly acidic (pH 5.7), and the humus content was 3.4%.

The set represents varieties of different geographical origin, from Germany, the Netherlands, European and Asian parts of Russia; old cultivars and new hybrids (Table 1). The varieties Gala, Red Scarlett and Udacha were used as references, being the most popular early varieties in Russian potato industry (Korshunov et al., 2018).

Each variety was represented by 20 plants planted in three replications on randomized plots. During each growing season, such biometric indicators of plant growth and development as the number of stems, the stems length, the weight of haulm (g), the total weight of tubers (g), the number of tubers (pieces), the weight of marketable tubers (g) and the number of marketable tubers (pcs) were recorded. The records were made on day 45, 60 and 75 after planting by evaluating two plants of each variety in three replications. At the final harvesting on day 80, the total weight and number of all tubers, and the weight and number of marketable tubers were recorded. Tubers weighing 30 g on day 45 and 40 g on day 60 and subsequent days after planting were considered as marketable ones. Marketability was calculated as the marketable tubers weight to the total tubers weight ratio as a percentage.

Table 1. Origin of 14 potato varieties studied in 2017–2019 at P&PL SPB of VIR

Potato variety, hybrid name	Originator (research institute, company)	Year included in the State Register for Selection Achievements
Antonina	Siberian Research Institute of Agriculture and Peat, Russia	2005
Bravo	Urals Research Institute of Agriculture, Russia	2015
Gala	NORIKA GMBH, Germany	2008
Gulliver	A.G. Lorkh All-Russian Research Institute of Potato Farming, Russia	2018
Hybrid S 112-03	Siberian Research Institute of Plant Industry and Breeding, Russia	Not included
Hybrid 3-43-2	Polar Experiment Station, a branch of VIR, Russia	Not included
Zhigulyovsky	A.G. Lorkh All-Russian Research Institute of Potato Farming and N.M. Tulaykov Samara Agricultural Research Institute, Russia	2006
Lyubava	A.G. Lorkh All-Russian Research Institute of Potato Farming and Kemerovo Agricultural Research Institute, Russia	2003
Lux	Urals Research Institute of Agriculture, Russia	2016
Red Scarlett	HZPC HOLLAND B.V., the Netherlands	2000
Sarovsky	Siberian Research Institute of Plant Industry and Breeding, Russia	2014
Start	Smolensk Agricultural Research Institute, Russia	Not included
Udacha	A.G. Lorkh All-Russian Research Institute of Potato Farming, Russia	1994
Yuna	Siberian Research Institute of Plant Industry and Breeding, Russia	2013

Data analysis

The two-way ANOVA was used to analyze the dependence of the final tuber indicators, recorded on day 80 after planting, on the variety and year.

For two years, 2018 and 2019, a more detailed study of the dynamics of haulm and tubers weight increase was carried out using the data of intermediate digs. A comparison of dynamics indicators was carried out using the Student *t-test* for dependent samples, which has greater power than unpaired tests because the random intra-group variation is eliminated (Fradette et al., 2003; Khalafyan, 2010).

Indicators of the dynamics of haulm and tubers weight increase were calculated according to formula (1) for the periods from day 45 to 60 and from day 60 to 75, that is, the 15-day growth rates were obtained.

$$K_{n/m} = \frac{Y_n}{Y_m} \tag{1}$$

where Y_n, Y_m – the weight on days *n* and *m* after planting, respectively.

A comparison of dynamics indicators in two contrasting years was carried out using the Wilcoxon matched pairs test.

The dependence of the weight of tubers on day 80 on that of haulm and tubers during intermediate digs was investigated using the correlation and regression analysis.

The statistical analysis was performed using the software package Statistica 13.3 (TIBCO Software Inc., Palo Alto, CA, USA).

Weather conditions

The daily data from the Pushkin meteorological station of VIR were used. During the three years of research, 2018 was the warmest, and 2019 was the coldest year (Fig. 1, a). The average temperature over 80 days of the growing season (from planting to digging up) was 16.5 °C in 2017, 17.5 °C in 2018, and 15.9 °C in 2019. The year 2017 was the wettest, with the total precipitation of 360.4 mm during the growing season (Fig. 1, b). In 2018, it equaled 87.6 mm, and 126.8 mm in 2019.

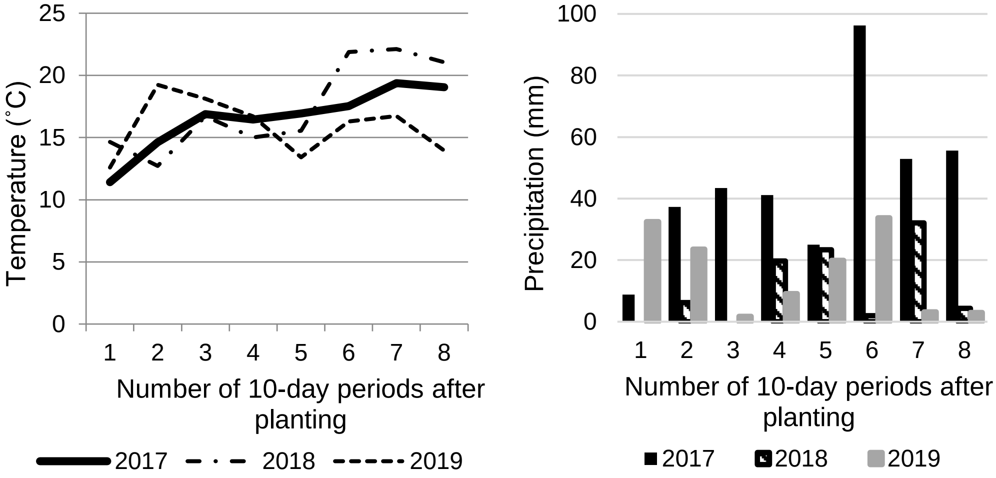

Figure 1. Heat and moisture conditions during potato vegetation per 10-day periods from the planting date: a) average temperature; b) total precipitation.

RESULTSS

Comparison of productivity in three years of research

The two-way ANOVA showed that tuber productivity significantly depends on both the variety (p < 0.001) and year (p < 0.001). The highest productivity (recorded on day 80 after planting, which corresponds to day 60–65 after emergence) was observed in 2018 (Fig. 2); the average for 14 varieties was 994.7 g plant^{-1}, the lowest of 791.6 g plant^{-1} was recorded in 2019, and in 2017 it averaged 832.3 g plant^{-1}. The productivity amounting to 1 kg of tubers plant^{-1} with 27 thousand plants ha^{-1} and tubers marketability of 75–80%, provides a yield of 20–21 t ha^{-1}, which corresponds to the parameters of an early potato variety.

The number of tubers plant^{-1} did not differ over the years (p = 0.092) and amounted to 11.5–12.4 pcs. The number of marketable tubers averaged 6.7–7.1 pcs. plant^{-1} (p = 0.396), which corresponds to characteristics of an early variety. Noticeable differences were found in the average tuber weight: the largest (81.9 g) for the studied set of varieties was recored in 2018, the smallest (70.2 g) in 2019, and in 2017 it was 71.2 g ($p < 0.001$). The number of stems plant^{-1} was minimal in 2018 (2.6 pcs.), it was maximal in 2019 (4.3 pcs.), and medium in 2017 (4.1 pcs, $p < 0.001$). The indicator of marketability was the lowest in 2017 (75.5%) compared to 2018–2019 (90, 91%, respectively; $p < 0.001$).

So, the number of tubers was relatively constant, while the growing season conditions significantly influenced the average tuber weight. The indicators of productivity, tuber weight and stem number were the most contrasting in 2018 and 2019.

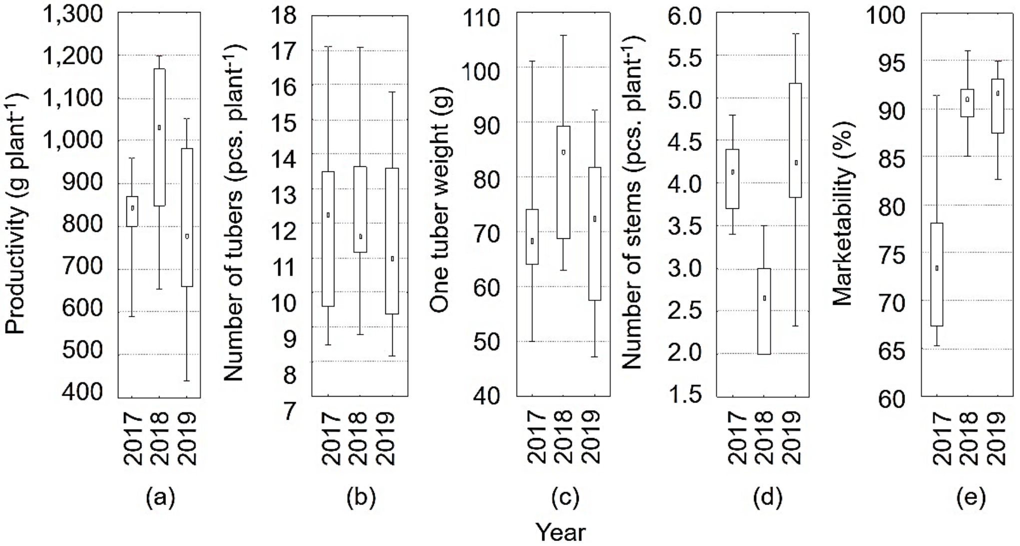

Figure 2. Productivity and its components for a set of 14 early potato varieties within three years of research: a) productivity; b) number of tubers; c) one tuber weight; d) number of stems; e) marketability. The median, qsuartiles, minimum and maximum values are presented.

Comparison of the haulm and tubers weight from day 45 to 75 in contrasting 2018 and 2019

To analyze the dynamics of haulm and tubers weight increase during the growing season, 2018 and 2019 were chosen as the most contrasting years (Fig. 3).

Haulm. The small size of seed tubers determined formation of a smaller number of stems on plants in 2018 compared to 2019 (Fig. 3, a), 2.6 pcs. *vs*. 4.3 pcs. on an average, respectively. The differences between the years were significant, with $p = 0.004$ on day 45; $p < 0.001$ on day 60, and $p < 0.001$ on day 75, according to the Student *t-test* for dependent samples. The stem length did not vary significantly between different years (Fig. 3, b) on day 45 and 60, and amounted to 44.0 and 46.2 cm on day 45 ($p = 0.137$), and to 49.6 and 48.7 cm on day 60 ($p = 0.713$). On day 75, the stem length was significantly lower in 2018 than in 2019 due to the faster development, and amounted to 40.9 cm and 49.0 cm, respectively ($p = 0.004$). The haulm weight (Fig. 3, c) was higher in 2018 than in 2019 on day 45 (349.0 g *vs*. 269.2 g, $p = 0.047$) and on day 60 (376.1 g *vs*. 298.3 g, $p = 0.026$), while on day 75 there were no significant differences (301.8 g *vs*. 276.4 g, $p = 0.376$).

Tubers. The dynamics of the number of tubers did not depend on the year. The final number of tubers that had formed by the first dig (Fig. 3, d) was 10.9 *vs*. 11.8 tubers plant^{-1} on an average on day 45 in 2018 and in 2019, respectively (p = 0.341), 12.1 *vs*. 11.7 on day 60 (p = 0.494); and 12.8 *vs*. 12.3 on day 75 (p = 0.603).

Despite the different temperature conditions till day 45 after planting in 2018 and 2019, the total weight of tubers per plant (Fig. 3, e) on day 45 did not differ significantly and amounted to 241.0 g in 2018 and 214.7 g in 2019 (p = 0.193). On other control dates, the total weight of tubers was higher in 2018 than in 2019, and amounted to 608.2 and 536.3 (p = 0.024) on day 60; and to 874.2 g and 754.2 g on day 75 (p = 0.010).

The haulm to tubers weight ratio (Fig. 3, f) decreased in the seasonal course of development. The differences in years were not significant: the ratio was 1.8 on day 45 in 2018 and 1.3 in 2019 (p = 0.193), 0.6 on day 60 (p = 0.168), and 0.3 and 0.4 on day 75 (p = 0.241).

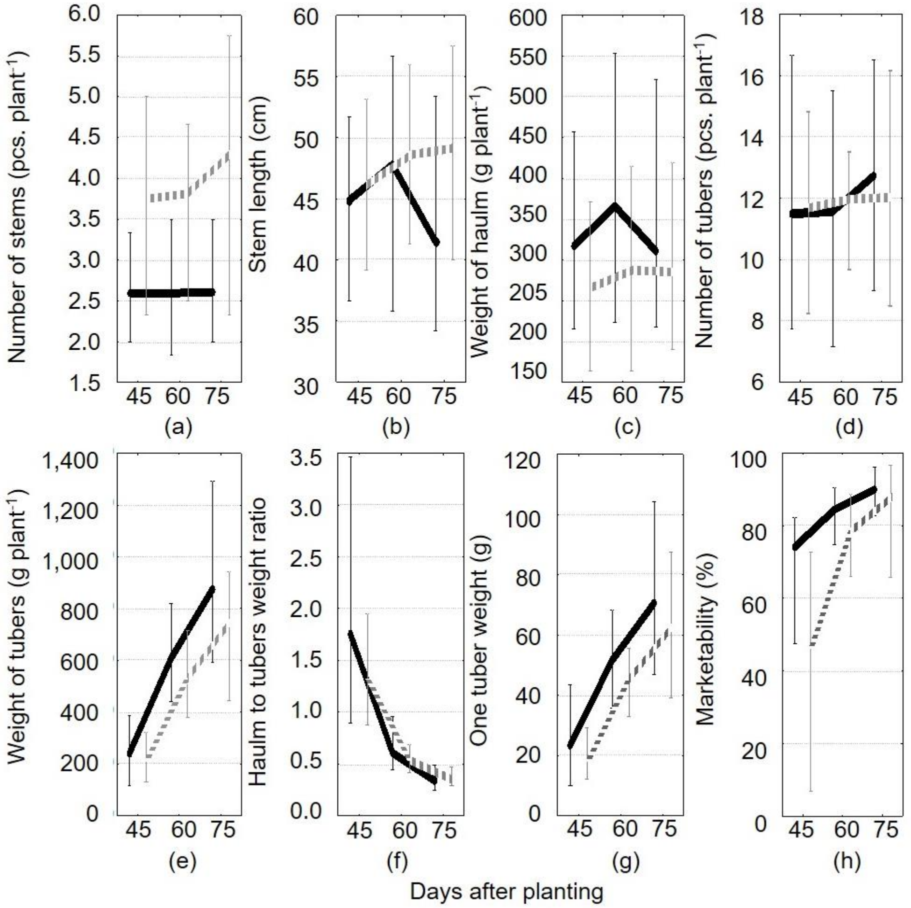

Figure 3. Dynamics of potato growth in 2018 and 2019: a) number of stems; b) stem length; c) weight of haulm; d) number of tubers; e) weight of tubers; f) haulm to tubers weight ratio; g) one tuber weight; h) marketability. Designations: 2018 – solid line, 2019 – dotted line. The average, minimal and maximal values for 14 varieties are presented.

The average weight of one tuber (Fig. 3, g) in all three digs was higher in 2018, but the differences were significant only on day 45: 23.4 g in 2018 and 18.4 g in 2019 ($p = 0.043$), and insignificant on the later dates: 51.6 g *vs*. 45.7 g ($p = 0.066$) on day 60, and 70.6 g *vs*. 63.1 g ($p = 0.247$) on day 75.

The marketability was significantly higher in 2018 (Fig. 3, h), that is 75.1% *vs*. 46.6% in 2019 on day 45 ($p = 0.000$), and 84.4% *vs*. 78.8% on day 60 ($p = 0.009$). On day 75, marketability reached 89.9% in 2018 and 88.1% in 2019, and the difference between the years ceased to exist ($p = 0.527$).

Thus, the number of tubers per plant and the haulm to tubers weight ratio on the control dates turned out to be less sensitive to changes in the growing season conditions than the number of stems, the weight of haulm, and the total weight of tubers.

Indicators of the dynamics of the haulm and tubers weight growth from day 45 to 75 in 2018 and 2019

Visually, the lines of the dynamics of tubers weight increase (Fig. 3, e) in 2018 and 2019 are in parallel, despite the differences in the absolute values of the weight on different dates. For a quantitative analysis of the dynamics of the haulm and tubers weight increase, the dynamics indicator was calculated as the 15-day relative growth rate between intermediate digs. The average, minimum and maximum indicators of the dynamics of the haulm and tubers weight increase in the studied group of 14 early varieties in different periods are shown in Table 2.

Table 2. Potato haulm and tubers weight relative growth rates

15-day relative growth rate	Year	Days 45–60			Days 60–75		
		Average	Min	Max	Average	Min	Max
Haulm	2018	1.105	0.825	1.631	0.818	0.586	0.996
	2019	1.143	0.818	1.573	0.941	0.744	1.177
	*p**	0.594			0.035		
Tubers	2018	2.750	1.541	5.621	1.443	1.168	1.948
	2019	2.621	1.724	3.665	1.417	1.163	1.745
	p	0.593			0.594		

**p* is the significance level of differences between years according to the Wilcoxon test.

Haulm. The relative increment rate of the haulm weight from day 45 to 60 did not depend on the conditions of the year and was 1.105–1.143, i.e. the increment rate was 10.5–14.3% over 15 days ($p = 0.594$). From day 60 to 75, the haulm weight began to decrease. The process was more intense in 2018 (-18.2% over 15 days) than in 2019 (-5.9% over 15 days; $p = 0.035$). This was probably due to a higher temperature in this period in 2018 and the faster development of plants.

Tubers. Indicators of the dynamics of tuber weight increase did not differ over the years. On an average, the weight of tubers increased for the studied varieties by 2.621–2.750 times, i.e., by 162.1–175.0% ($p = 0.594$) over 15 days from day 45 to 60; and by 1.417–1.443 times, i.e., by 41.7–44.3%, from day 60 to 75 ($p = 0.594$). Thus, the dynamics of tuber weight increase in early varieties was less dependent on the year than that of the haulm weight.

On the 2-year average, the weight of tubers in 14 varieties increased 2.0–3.9 times from day 45 to 60, and 1.3–1.7 times from day 60 to 75.

The tubers – haulm weight relationship

The correlation and regression analysis of the varieties productivity in two contrasting years was carried out.

2018. In 2018, the final productivity of the variety strongly correlated with the characteristics of a variety on day 60 (by which conditions for active growth had formed), that is, with the stem length ($r = 0.83$), the weight of haulm ($r = 0.72$), and with the weight of haulm on day 75 ($r = 0.77$).

2019. In 2019, the varieties could be differentiated by the size of haulm earlier than in 2018, and the final productivity was actually determined by the weight of haulm on day 45 ($r = 0.83$). Productivity was found to strongly correlate with the stem length on day 45 ($r = 0.76$) and the weight of tubers ($r = 0.72$). The final productivity correlated with such variety characteristics on day 60, as the total weight of tubers ($r = 0.88$), the weight of haulm ($r = 0.79$), and the total number of tubers ($r = 0.80$), as well as with such characteristics on day 75 as the total weight of tubers ($r = 0.78$) and the weight of marketable tubers ($r = 0.74$).

In both years with the contrasting temperature profiles, the weight of haulm on day 60 strongly correlated with productivity on day 80 with a coefficient of 0.72–0.79 (Fig. 4).

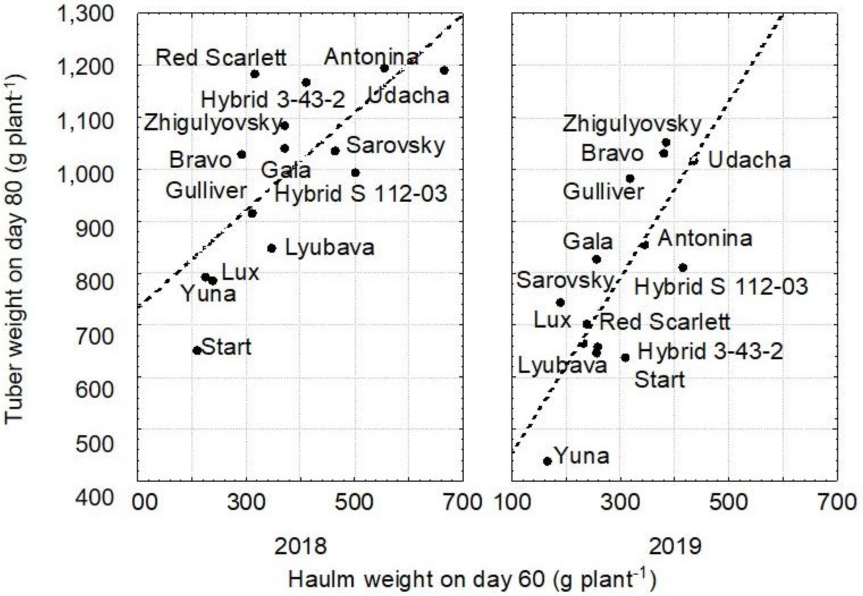

Figure 4. The dependence of the weight of tubers on day 80 after planting on the weight of haulm on day 60.

The final productivity (Y) dependence on the haulm weight on day 60 (V):

$$2018:\ Y = 640.25 + 0.94V \qquad R^2 = 0.52 \tag{2}$$

$$2019:\ Y = 284.92 + 1.70V \qquad R^2 = 0.62 \tag{3}$$

where R^2 – the coefficient of determination. It follows from the above that every 100 grams of the weight of potato haulm recorded on day 60 ensures a 100–200 g increment in the weight of tubers by the time of digging up on day 80.

For the average values of two years:

$$Y = 477.75 + 1.23V \qquad R^2 = 0.57 \tag{4}$$

The equation can be improved if the number of tubers per plant on day 60 (N) is known:

$$Y = 236.15 + 0.93V + 28{,}84N \qquad R^2 = 0.69 \tag{5}$$

In the present study, only Udacha variety out of the 14 studied ones, was distinguished by high and stable productivity, regardless of the weather conditions in the growing season (Fig. 4). It is the most common commercial variety in the Russian Federation recommended for cultivation in 8 out of the 12 agro-climatic regions of the country. The rest of the varieties and hybrids have genotypes adapted to different climatic zones (Siberia, the Volga region, the Northern or Central regions of the European part of Russia) and differ in their response to changes in growing regimes.

DISCUSSION

The conditions for potato growth were the best in 2018 and the worst in 2019. The beginning of the growing season in 2018 was cool; the average temperature within 45 days after planting (14.8 °C) was lower than in 2019 (16.3 °C), and medium in 2017 (14.9 °C). The amount of precipitation over 45 days was sufficient: 140.6 mm in 2017, 49.3 mm in 2018 and 86.5 mm in 2019. The period of tuber weight increase from day 45 to 80 was characterized in 2018 by a favorable average temperature (20.9 °C), by the lowest in 2019 (15.3 °C), and medium in 2017 (18.6 °C). The amounts of precipitation in 2018–19 were sufficient (38.3 mm and 40.3 mm, respectively), and excessive in 2017 (219.8 mm). As a result, the highest yield was obtained in 2018; it was 994.7 g plant^{-1} on an average for 14 varieties. The lowest yield was obtained in 2019 (791.6 g plant^{-1}), and it was medium in 2017 (832.3 g plant^{-1}). The productivity amounting to 1 kg of tubers plant^{-1} with 27 thousand plants ha^{-1} and tubers marketability of 75–80%, provides a yield of 20–21 t ha^{-1}, which corresponds to the parameters of an early potato variety.

In 2017, the conditions of the growing season favored early infestation of plants with late blight. Moderate temperatures and the abundance of precipitation, especially their significant increase in the last three 10-day periods (Fig. 1, b), caused a late blight outbreak and a need in early harvesting to prevent losses. Small tubers prevailed in the harvested potato, which is reflected in the marketability indicator, the lowest in 2017. Small tubers developed a smaller number of eyes capable of germinating the next year (Alsmik et al., 1979; Dimante & Gaile, 2018), which led to formation of only 2.6 stems per plant. The tubers harvested in 2018 produced an average of 4.3 stems per plant in 2019.

The two-way ANOVA revealed that the number tubers did not differ significantly in three years, i.e., turned out to be not very sensitive to changes in the growing regime. Low temperatures increase the number of tubers per plant (Levy & Veilleux 2007), however, in our case, differences in the average temperature of 45 days after planting in 0.5 °C (14.8 °C in 2018 and 16.3 °C in 2019) had less effect on the number of tubers, which was 12 pcs plant^{-1} on an average, but had an effect on the tuber weight, which varied from 70.2 to 81.9 g. The number of tubers is known to be a more stable character of variety than one tuber weight and yield (Petr et al., 1984).

An analysis of dynamics of biometric parameters of the haulm and tubers in potato varieties in 2018 and 2019 showed that on day 60 and 75 they were higher in 2018 than in 2019. Potato is well adapted to a mean temperature of 17 °C: haulm growth optimum is 20–25 °C, tuber growth about 15–19 °C (Tadesse et al., 2001; Levy & Veilleux 2007; Çalişkan, 2016). At a lower temperature (15 °C) and at a higher temperature (25 °C) tuberization is delayed; high temperature above optimum delays tuber initiation and reduces harvest indices (Tadesse et al., 2001; Levy & Veilleux 2007; Çalişkan, 2016). In 2018, favorable growing conditions compensated for the small number of stems, relatively low temperatures at the first part of growing led to more haulm weight. A gradual increase in temperature and precipitation in 2018 ensured the vigorous growth and development of plants. A decrease in temperature that began in the second 10-day period after planting in 2019, and a lack of heat that continued until the end of the growing season negatively affected the biomass increment. Our data are consistent with the previously established differences in optimal temperatures for the growth of the potato haulm and tuberization (Alsmik et al., 1979; Kim & Lee, 2019). The relative growth of various organs in comparable periods of time is similar, i.e., the percentage of dry matter attributable to different organs at certain periods of development is approximately the same in varieties of the same ripening period (Petr et al., 1984).

The use of dynamics indicators made it possible to reveal the relative independence of the tuber weight increase in early varieties from the conditions of the year, in contrast to the haulm weight growth. The haulm to tubers weight ratio (Fig. 3, f) decreased in the seasonal course of development. The differences in years were not significant. The average relative increment rate for a set of 14 varieties did not differ significantly in the contrasting years. The weight of tubers increased on an average 2.6–2.8 times from day 45 to 60, and 1.4 times from day 60 to 75. In studies of a set of 63 early and mid-early varieties at the Polar Branch of VIR (Murmansk Province), the weight of tubers increased from 1.4 to 3.7 times from day 60 to 75, that is, potato varieties of different maturity groups significantly vary in the rate of tubers weight increase in the same conditions (Kiru et al., 2016). In our study of 14 varieties, the average growth rate was 1.3–1.7 from day 60 to 75. Thus, the 14 studied varieties had relative growth rates close to the minimum. The constancy of relative indicators for early varieties is stated in work of Särekanno et al. (2012).

High productivity of potato requires a good development of the haulm. The works of Alsmik (1979) revealed the influence of the potato plant morphobiological type on the productivity and longevity of a variety. The grouping of plants proposed by him according to the shape of the bush, the emergence of stems from soil and the number of stems, has been used by Belarusian breeders to select promising high-yielding varieties (Makhanko & Kolyadko, 1997). A strong relationship between the manifestation of morphological and agronomic characteristics has been established for the early potato varieties developed in Ukraine: it exists between productivity and the leaf weight with the correlation coefficient of 0.83, leaf area size (0.82), total haulm weight (0.84), marketable tubers weight (0.93), and the marketable tubers number (0.73) (Podgaetsky & Kupriyanova, 2008). A conclusion about the possibility of predicting potato productivity on the basis of physiological and morphological parameters of plants was made by Polish researchers based on the results of three-year experiments with early and mid-early varieties. A comparative analysis employed such predictors of potato productivity as the leaf surface index, total chlorophyll content and chlorophyll fluorescence, while the greatest correlation was found between the tuber yield and leaf surface index (Zarzynska & Pietraszko, 2017). Our research revealed, that for the early potato varieties the final productivity of potato plants in contrasting years depended on the weight of haulm on day 60 after planting, and the correlation coefficient was 0.72–0.79. Every 100 grams of the weight of haulm recorded on day 60 ensures a 100–200 g increment in the weight of tubers by the time of digging up on day 80.

CONCLUSIONS

The contrast weather conditions leads to the significant differences in the early potato varieties productivity. But the number of tubers and haulm to tubers weight ratio turned out to be less sensitive to changes in the growing regime.

The use of dynamics indicators made it possible to reveal the independence of the tuber relative growth rate in early varieties (in contrast to the haulm growth) from the conditions of the year.

The revealed relationship between the final productivity of early potato varieties and the weight of haulm on day 60 after planting can be used to advance forecasting the yield. The obtained results can be used for the development of automated systems for recording the morphometric parameters of potato plants.

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Detection of sour and sweet cherry viruses in Ukraine

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Abstract. One of the main obstacles on the way to successful cultivation of orchards and planting material of sour and sweet cherry is infection with viral pathogens. They can adversely affect yields, reduce plant resistance to abiotic factors and cause losses in the nursery. Therefore, it is important to determine the spread of viruses in plantations and the selection of virus-free plants for the establishment of nuclear stock collections. In order to study phytovirological state of the orchards, tests for the presence of five viral pathogens were performed: Tomato black ring nepovirus (TBRV), Cherry leaf roll nepovirus (CLRV), Apple chlorotic leaf spot virus (ACLSV), Petunia asteroid mosaic tombusvirus (PeAMV), Plum pox virus (PPV). The level of infection with these pathogens reaches 19.2% for sour cherry, 5.8% - for sweet cherry, and 7.4% - for rootstocks. PPV prevailed in sour cherry material - 7%, in sweet cherry material - ACLSV (3.6%), while the rootstock samples were most infected by PeAMV - 5.6%. Until now, this virus has not been reported in Ukraine. TBRV and ACLSV viruses have been known in other crops so far, while not yet been detected in sour and sweet cherry. It was determined that PPV isolates (Mahaleb 1, Mahaleb 2) which were detected in sour cherry, belong to strain D.

Key words: ELISA, TBRV, CLRV, ACLSV, PeAMV, PPV, phylogenetic.

INTRODUCTION

The modern horticultural industry involves the use of planting material that is free of viral pathogens and meets current standards. Growing planting material of cherry in Ukraine today is guided by EPPO PM 4/29 (1) standard, which includes 15 viral pathogens. The distribution of many viruses in domestic plantations is still unclear. Survey of propagation stock orchards of sour cherry, sweet cherry and their clonal rootstocks, carried out in 2018, has not detected the presence of TBRV, CLRV, ACLSV, ApMV and PPV (Pavliuk et al., 2019). Although it is previously known that all these viruses, except for PeAMV, were found in Ukraine in other types of sour and sweet cherry plantations, or in other crops (Gospodaryk et al., 2005; Tryapitsyna & Vasiuta, 2010; Tryapitsyna et al., 2013; Kutsenko et al., 2019).

In addition, new Plum pox virus (PPV) strains have recently been reported in neighboring countries, infecting sour and sweet cherries, including PPV-CR (Cherry Russian) and PPV-CV (Cherry Volga) strains (Chirkov et al., 2017, 2018). Therefore, there is a need to test plant material for this virus. Typical symptoms of PPV are appearance of chlorotic spots, or slightly pigmented yellow rings or lines. The fruits deform and abscise prematurely (Dehkordi et al., 2017). Virus of the C strain can also affect yield and fruit quality. The fruits do not ripen, and necrosis is observed on sweet cherry branches (Nemchinov et al., 1996). In general, PPV is considered to be one of the most destructive pathogens of *Prunus* species, crop losses can reach 30–40%, and in some years on sensitive varieties - 70–90% (Yusko, 2009). In 2006, losses caused by PPV worldwide were estimated at around € 10 billion in total over the last 30 years (Cambra et al., 2006).

Therefore, we included PPV to the list of tested pathogens. As of today, it has been identified in 10 regions of Ukraine (Odessa, Cherkasy, Kyiv, Kharkiv, Ivano-Frankivsk, Vinnytsia, Lviv, Mykolaiv, Ternopil, Chernivtsi regions) and the Autonomous Republic of Crimea. Private, collection, selection, and propagation stock orchards of certain stone fruit crops were checked for this pathogen. Crops such as plum, myrobalan plum, apricot, and peach were mainly tested. Sour and sweet cherry were tested selectively (Kondratenko & Udovichenko, 2006; Tryapitsyna, 2016; Kutsenko et al., 2019).

Thus, the aim of our research is to determine the spread of uncommon viral pathogens in sour and sweet cherry and their rootstocks.

MATERIALS AND METHODS

During the vegetation period of 2018–2020, 291 samples were tested, including 138 - of sweet cherry, 99 - of sour cherry, 54 - of clonal rootstocks. Samples were collected from 10 regions of Ukraine (Ivano-Frankivsk, Kyiv, Zaporizhzhya, Ternopil, Donetsk, Kharkiv, Kherson, Transcarpathian, Dnepropetrovsk, Cherkasy regions). A total of 17 sweet cherry cultivars, 13 sour cherry cultivars and 4 types of rootstocks were tested. Testing was performed by Enzyme-linked immunosorbent assay (ELISA) method using Loewe Biochemica GmbH (Germany) and Bioreba AG (Switzerland) commercial test kits. ELISA was performed according to standard methods of M. Clark and A. Adams (1977). Test tissue was taken from the basal part of the leaf, then it was homogenized with the addition of conjugate buffer with 1:20 ratio. Test results were read on a microplate spectrometer ImmunoChem-2100 Microplate Reader (USA), at a wavelength of 405 nm. Analysis of variance of the research data was performed using STATISTICA software, at confidence level $p < 0.05$. Positive PPV samples were further tested by reverse transcription polymerase chain reaction (RT-PCR) method to confirm the presence of the virus in the plant and to establish its strain affiliation. Fresh plant tissues were used for total RNA extraction, and isolation was performed using a commercial RNeasy Plant Mini kit (Qiagen, UK) according to the manufacturer's instructions. A commercial Verso 1-Step RT-PCR Kit ReddyMix (Thermo Scientific, USA) was used according to the manufacturer's recommendations to set up the reaction. The following components were used to carry out one reaction with a volume of 20 μl: 2X 1 Step PCR ReddyMix - 10 μl, Verso Enzyme Mix - 0.4 μl, RT Enhancer - 1 μl, Primer forward - 0.4 μl (10 mmol), Primer reverse - 0.4 μl (10 mmol), H_2O - 6.6 μl, RNA - 50 ng per reaction. The following primers were used for molecular identification - P1: 5’-ACC GAG ACC ACT ACA CTC CC-3’ and P2: 5’-CAG ACT ACA GCC TCG CCA GA-3’, proposed by Wetzel et al. (1991). The expected amplification product is 243 bp. Primers to the *Nad-5* mitochondrial dehydrogenase gene, with an expected 181 bp fragment, were used to control reverse transcription (Menzel et al., 2002).

Amplification was performed in a programming thermostat ‘Ependorf Mastercycler Personal’ (Ependorf AG, Germany) according to the following parameters: 55 °C - 15 min, 95 °C - 2 min, 40 amplification (95 °C - 20 s, 60 °C - 30 s, 72 °C - 1 min), final elongation at 72 °C - 5 min.

The presence of amplification fragments was checked by separating the PCR products in 2% agarose gel, with TBE buffer with the addition of ethidium bromide.

The resulting amplicons were sequenced. Nucleotide sequences were compared with those deposited in the Genbank (www.ncbi.nlm.nih.gov) PPV isolates using BLAST software. Sequence alignment was performed using CLUSTAL W option in MEGA X software (Kumar et al., 2018). The construction of the phylodendrogram was performed by Neighbour-Joining method (NJ) (Saitou & Nei, 1987), using bootstrap 500 analysis (Felsenstein, 1985).

RESULTS AND DISCUSSION

Enzyme-linked immunosorbent assay

Serological testing of the material of sour cherry, sweet cherry, and rootstocks showed that the general level of infection of sour cherry was 19.2% (± 0.08), while sweet cherry and rootstocks were infected only by 5.8% (± 0.04) and 7.4% (± 0.07), respectively.

In general, all viruses for which plant material was tested were detected in sour and sweet cherry cultivar samples (Table 1). In sour cherry samples, PPV prevailed - 7%, while in sweet cherry it was 1.4%, and in rootstock samples - 1.9%. Monitoring data from foreign colleagues indicates the absence of PPV in the collection plantations of sour and sweet cherry in Serbia (Mandic et al., 2007) and in stone crop orchards of Isparta province (Turkey) (Çevik et al., 2011). In Bosnia and Herzegovina, on the other hand, in the orchards of the same type of stone crops (including sour and sweet cherry), the virus was present in 47% of the tested material (Matić et al., 2008).

Table 1. Distribution of infection to different viruses, % (2018–2020)

Tested plants	Number of samples tested (n)	Viruses, %				
		PPV	PeAMV	ACLSV	TBRV	CLRV
Prunus avium	138	1.4 ± 0.02	1.4 ± 0.02	3.6 ± 0.03	0.7 ± 0.01	0.7 ± 0.01
Prunus cerasus	99	7 ± 0.05	1 ± 0.02	3 ± 0.03	5 ± 0.05	2 ± 0.03
Rootstocks	54	1.9 ± 0.04	5.6 ± 0.06	1.9 ± 0.04	-	-

We detected PeAMV for the first time in Ukraine. Until now, there were no reports of its discovery, and it was considered absent in sour and sweet cherry orchards. The highest level of infection was observed in rootstock samples - 5.6%, in sweet and sour cherry samples - 1.4 and 1%, respectively. In general, this virus has been reported in stone fruit crops in Canada, Germany, Switzerland, Latvia, and Serbia (Koenig & Kunze, 1982; Mandic et al., 2007; Gospodaryk et al., 2013), however, there is no monitoring data on the level of infection of the orchards with this pathogen. In Latvia, infection with this virus was found on ‘Kubanskaya Kometa’ and ‘Skoroplidna’ plum cultivars. These varieties are popular in Ukraine and are used in home gardens and industrial orchards.

Here it occurred as a complex of infections togsether with ArMV, TBRV and SLRV (Gospodaryk et al., 2013). In our research, PeAMV was found on rootstock samples, ‘Bigarreau Burlat’ sweet cherry cultivar and ‘Shalun’ia’ sour cherry cultivar.

ACLSV is characterized by cosmopolitan distribution, making it one of the most economically important pathogens. In addition to severe deformation of leaves and fruits, cracking of the bark, incompatibility of scion-rootstock combinations (Desvignes & Boye, 1989; Pasquini et al., 1998), infected plants become more susceptible to bacterial and fungal diseases (Zawadzka, 1989). The level of ACLSV infection in investigated plantations of sweet cherry (3.6%), sour cherry (3%) and rootstocks (1.9%) was significantly lower than in fruiting orchards of sweet cherry in Spain, where the infection with this virus reached 16% (Sánchez et al., 2015). In the Mediterranean region, the rate of ACLSV infection for sour and sweet cherry was 14% (Myrta & Savino, 2008), in Bosnia and Herzegovina in plantations of stone fruit crops, virus was present in 4% of the tested material (Matić et al., 2008), the same result (4%) was obtained when testing sweet and sour cherry cultivars in Serbia (Mandic et al., 2007). The lowest level of infection with this pathogen was found in Isparta province (Turkey), where only 1% of samples of sour and sweet cherry were infected with this virus (Çevik et al., 2011). Despite the wide spread of ACLSV, it was not detected in sour cherry orchards in Algeria (Rouag et al., 2008). In sour cherry orchards of Palestine, the infection rate reached 25%, but PPV, TBRV and CLRV viruses were not detected there (Jarrar et al., 2001).

Tomato black ring nepovirus was identified only in sweet (0.7%) and sour cherry (5%) orchards. Tested rootstock samples were not infected with this pathogen. In other types of sour and sweet cherry plantations, the virus was not previously identified. However, it is widespread in Ukraine in raspberry plantations - 29.6% (Tryapitsyna et al., 2013). Typical symptoms may include chlorotic spots, mosaic, necrotic lesions, ring spots or patterns, and deformation of the fruit (Šneideris & Staniulis, 2014). In Poland, this pathogen has been identified on elderberry (*Sambucus nigra)* (Pospieszny et al., 2004), in Lithuania - on tomato (*Lycopersicon esculentum*), rhubarb (*Rheum rhabarbarum* L), strawberry (*Fragaria vesca* L) (Šneideris & Staniulis, 2014). As one can see, the range of host plants of this virus is quite wide, so many plants can be reservoirs for its accumulation, which in turn increases the likelihood of infection of the main host plants.

According to the preliminary data, the collection plantations of sweet cherry in Ukraine are infected with CLRV by 21.63%, while sour cherry - by 16.19% (Tryapitsyna & Vasiuta, 2010). In Russia, the virus was detected in plantations of sour, sweet cherry and their rootstocks in 8.9-28.6% of the samples (Upadyshev et al., 2017). Data from our research indicate the presence of the pathogen in 0.7% of sweet cherry and 2% of sour cherry. On infected cherry trees, virus causes deformation and twisting of leaves, mosaic, as well as growth retardation and death of individual branches or the whole tree (Tryapitsyna & Vasiuta, 2010).

Complex pathogen infection was also identified: PPV + ACLSV was detected in samples of sour cherry (1% ± 0.02) and rootstocks (1.9% ± 0.03), while sweet cherry had a complex infection with PPV + TBRV (0.5% ± 0.01). As these viral pathogens do not have common vectors of carriers, it is obvious that infection occurred due to non-compliance with the requirements of care and agronomic measures.

Territorial distribution of pathogens

Analysing the geographical distribution of viral pathogens, one see that all studied viruses were found in Donetsk region, except for TBRV. According to the State Statistics Service, sour and sweet cherry plantations in this region occupy about 1,300 ha. This region is one of the largest regions for growing sour and sweet cherry.

The spread of some viruses may depend on the variety of strains and isolates that may infect plants selectively, focusing on the host plant rather than the range (Rebenstorf et al., 2006). For example, the CLRV strain that infects walnut (*Juglans regia* L.) is not transmitted to sour, sweet cherry and birch (Langer et al., 2009). According to some studies, in Ukraine the Polissya (the north-western part of the country) area is the most vulnerable to the spread of this pathogen, as it hosts the largest number of plants that can be reservoirs of pathogen accumulation and source of its spread (Tryapitsina & Vasiuta, 2010). However, in our research, CLRV was identified only in Donetsk and Kharkiv regions, thus refuting previous assumptions.

When analysing the distribution by region, one can observe a sporadic spread of viral pathogens, rather than some specific pattern. Most likely, this distribution is a consequence of the human factor - non-compliance with sanitary requirements for agronomic measures and the spread of infected planting material.

Infection of tested cultivars and types of rootstocks

In total, 17 cultivars of sweet cherry, 13 cultivars of sour cherry, and four types of rootstocks were tested. Among the sweet cherry material, ‘Bigarreau Burlat’, ‘Donetskyi Uhol`ok’, ‘Donetska Krasavitsa’, ‘Nizhnist’ and ‘Krupnoplidna’ cultivars were infected (Table 2). The most infected samples were found in Bigarreau Burlat - 11.8%. This cultivar is of French origin, dating from 1915. Since then, planting material has spread to Central Europe, and later the cultivar became popular in Ukraine. Apparently, over the years of its spread there was an uncontrolled reproduction of planting material, which caused a high level of infection.

Table 2. Infection of cultivars of the tested material

Cultivar	Number of samples tested (*n*)	Infected samples, %
Bigarreau Burlat	17	11.8 ± 0.15
Donetskyi Uhol'ok	11	9 ± 0.17
Donetska Krasavitsa	13	7.7 ± 0.14
Nizhnist'	17	5.9 ± 0.11
Krupnoplidna	21	4.8 ± 0.09
Shalun'ia	14	28.6 ± 0.24
Bohuslavka	15	20 ± 0.20
Kseniia	31	3.2 ± 0.06
P.mahaleb	18	22.2 ± 0.19

‘Donetskyi Uhol`ok’ and ‘Donetska Krasavitsa’ cultivars, infection level of which was 9 and 7.7%, respectively, are cultivars of selection of Bakhmut Resarch Station of Horticulture, in the vicinity of which the largest localization of pathogens was detected. ‘Valery Chkalov’, ‘Kitaivska Chorna’, ‘Melitopolska Chorna’, ‘Talisman’ and ‘Udivitelna’ cultivars were free from the tested pathogens.

In contrast to sweet cherry, in tested sour cherry samples three infected cultivars were found - ‘Shalun’ia’ (28.6%), ‘Boguslavka’ (20%) and ‘Kseniia’ (3.2%), while ‘Vstrecha’ was virus-free. CLRV predominated in the samples of ‘Shalun’ia’ cultivar, while PPV prevailed in ‘Boguslavka’ samples.

Today, both seedling (*P. mahaleb*) and clonal (VSL-2, Colt, Studenykivska) rootstocks are used in industrial sweet and sour cherry orchards in Europe (Jänes & Pae, 2004; Maas et al., 2014; Vasyuta & Ryabyi, 2017; Csihon et al., 2018). Analysed Colt, VSL-2 and Studenykivska rootstock samples were free of the tested pathogens, while *P. mahaleb* smaples were infected. These samples were dominated by PeAMV. The ability of these viruses to be transmitted by seeds has not been reliably confirmed, but their particles were isolated from the seeds of sour and sweet cherry plants (Pfeilstetter et al., 1992).

Strain identification of Plum pox virus

Strain affiliation of PPV, which circulates in Ukraine and infects sour and sweet cherries, has not been previously studied. Of the nine known strains, only C, CR and CV are thought to be able to infect sour and sweet cherry (Chirkov et al., 2017, 2018; James et al., 2013; García et al., 2014). CV strain can cause typical chlorotic spots on sour cherry (Chirkov et al., 2017, 2018). In Germany, the same symptoms were observed on leaves of sour cherry infected with C strain, as well as leaf deformation and dark rings on the fruits (Jelkmann et al., 2018).

A potential vector of the pathogen is aphids. It is believed that virus can be transmitted by roughly 20 species of aphids, but the most effective vectors are *Aphis craccivora*, *A. spiraecola*, *A. cardui*, *Brachycaudus helichrysi*, *B. cardui, Hyalopterus pruni*, *Myzus persicae*, *Phorodon humuli* (Gaborjanyi & Basky 1995; Labbone et al., 2001). It is known that some of them are common in Ukraine and other European countries (Yusko et al., 2008; Latinović et al., 2017; Musa et al., 2020). These insects can easily be the carriers of the pathogen, because virus particles together with the sap of infected trees can be stored for about 4 hours in the food apparatus of the insect, while the insect can change the plant it feeds on during that period, thus spreading infection in the orchards (Jordovic, 1968). However, the effectiveness of this way of virus transmission depends on the strain, cultivar and species of the host, aphid species, and season (Levy et al., 2000).

After ELISA testing, samples positive with PPV were further tested by RT-PCR. The analysis confirmed the presence of this virus in all tested samples which was confirmed by the presence of the expected fragment of amplification. Two samples of *P. mahaleb* rootstocks from Zaporizhzhia region were chosen for sequencing.

After sequencing, the resulting fragments of Mahaleb 1 (MW055900) and Mahaleb 2 isolates were equal to 243 bp, which reffered the position 9313-9555 in the viral genome. According to the results of comparison of our isolates with known ones, its affiliation to D strain was established. To date, it has been investigated that D strain of PPV can cause epidemics in peach, apricot and plum orchards (Gottwald et al., 1995; Dallot et al., 1998; Polák & Komínek, 2009). In contrast to the samples affected by cherry strains, which were described by other authors (Chirkov et al., 2017, Jelkmann et al., 2018), our infected samples did not show symptoms of virus infection on the leaves.

Direct analysis of all Ukrainian isolates of this virus present in the GenBank (www.ncbi.nlm.nih.gov) has indicated that 83% of them belong to D strain. The other 17% of isolates belong to strains W, M and Rec. The host plants are mainly peach, apricot and plum. Mahaleb 1 and Mahaleb 2 isolates are the first PPV isolates of D strain isolated from *P. mahaleb* in Ukraine.

For phylogenetic analysis, all known isolates of PPV circulating in Ukraine, as well as representatives of cherry strains C, CR, CV were selected.

Since this segment is highly conservative, at least 272 known isolates from different countries and crops have been found to be 100% identical to our isolates. Mahaleb 1 and Mahaleb 2 isolates showed a high level of identity between themselves - 100%. When comparing all Ukrainian isolates, variability within the group was found, identity ranged between 89.5–100%, and the overall mean identity was 97%. Since some isolates were distant by nucleotide sequences, they were included in a separate group (IV) on the phylogenetic tree (Fig. 1). Thus, MK209075 (Kharkiv region, apricot) and MK209074 (Odessa region, plum) isolates turned out to be distant. By nucleotide sequences, the identity of our isolates between them was 94 and 89.5%, respectively.

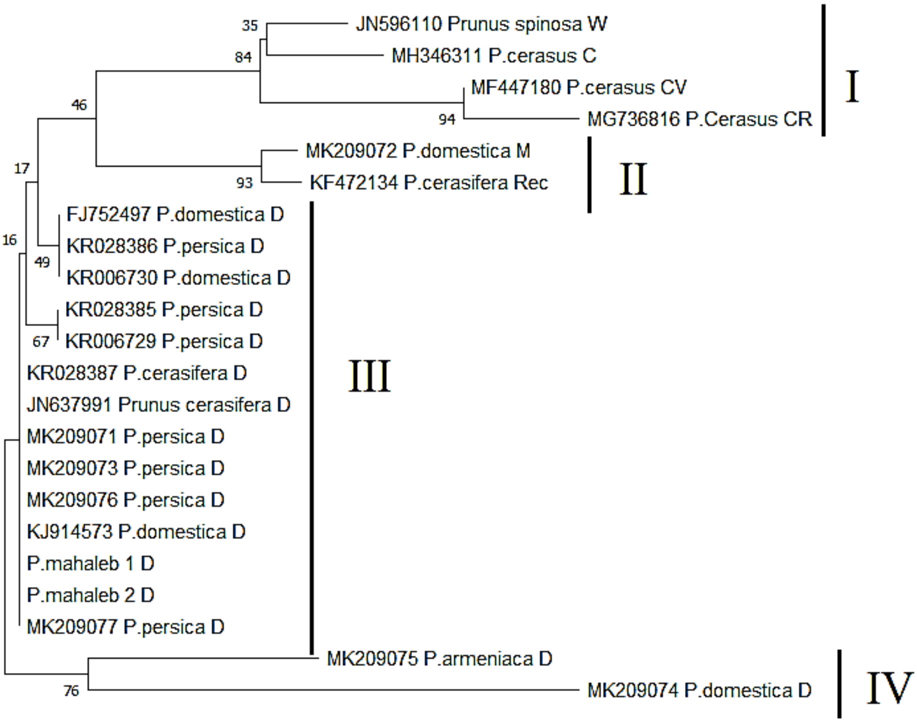

Figure 1. Phylogenetic tree of Plum pox virus isolates reconstructed using a 149-bp nucleotide fragment polyprotein gene (position of 9369-9517) constructed by Neighbour-Joining method.

Mahaleb isolates were classified in group III, together with the most identical (100%) isolates of this strain: plum isolate KJ914573, peach isolates MK209077, MK209076, MK209073, MK209071, and myrobalan plum isolates JN637991 and KR028386.

Representatives of cherry strains combined a separate group, together with isolate JN596110, which belongs to strain W. Mahaleb isolates are identical by 90% to isolate MG736816 (CR), by 92.5% to isolate MF447180 (CV), and by 93.3% to MH346311 (C).

Isolates of M and Rec strains also formed a separate group. When comparing Mahaleb isolates with isolates belonging to these strains, an identity level of 95% was observed.

CONCLUSIONS

This research demonstrates the presence of previously undetected sour and sweet cherry viruses in Ukraine. The presence of a panel of viral pathogens identified by EPPO PM 4/29 (1) indicates the need to test plant material and comply with sanitary requirements when maintaining orchards and when propagating material to prevent the spread of pathogens with planting material. Detection of viruses that have not been previously reported in Ukraine (PeAMV) and in sour cherry orchards (TBRV, ACLSV) once again confirms the need for testing of plant material and further investigation of their molecular characteristics.

Since PPV isolates identified by us belong to D strain, it is necessary to further determine its ability to be transmitted to plants that are more susceptible to the virus, as well as to investigate its harmfulness in sour and sweet cherry orchards.

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Study of influence of heat stress on some physiological and productive traits in Holstein-Friesian dairy cows

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Abstract. The aim of the research was to study the effect of heat stress (HS) on some physiological and productive traits in Holstein-Friesian dairy cows. The study included 22 cows on different parities. In the building where the cows were housed, the temperature-humidity index (THI) was reported at 10:00 and 15:00 h, at the same time the rectal temperature (RT) and respiratory rate (RR) were reported for each of the examined cows. The daily rumen activity was taken from the SCR system by Allflex. The average THI values in May were 71, in June - 75, in July - 74, and in August - 77, from which it follows that in the summer months the cows were in conditions of mild to moderate heat stress throughout the day. The average daily milk yield of the cows increased from May to June and reached 41.44 kg day^{-1}, then decreased in July and August to 37.2 and 32.48 kg day^{-1}, respectively. With an increase in the THI values, an increase in the RR and RT was registered, as in THI above 79 the RR was 56.54 per min, and the RT was 39.33 °C. With increasing the THI values, the rumination of the cows decreased from 563 per day at THI < 72 to 542.5 at THI > 79. In cows with high daily milk yield, a higher RT was registered, and in cows with more than 50 kg per day, the RT was 39.09 °C. A more intense rumination was found in cows with higher daily milk yield. In cows with an average daily milk yield of 33.26 kg, an average of 450 ruminations per day were reported, and in those with an average milk yield of up to 42.89 kg - 650 ruminations per day. From the research conducted it was found that the studied physiological traits - rectal temperature, respiration rate and rumination are influenced by HS and the intensity of this effect depend on the daily milk yield of cows and THI levels.

Key words: dairy cows, heat stress, rectal temperature, respiratory rate, temperature-humidity index.

INTRODUCTION

Reports from international and government research centers show alarming trends for systematic global warming (Herbut et al., 2018). Some studies suggest that by 2050 the planet's temperature will rise by 2 °C (Trnka et al., 2011) this warming is expected to have a significant impact on the welfare and productivity of farm animals and in particular dairy cows in many climatic zones around the world (Cook et al., 2005;

De Palo et al., 2006; Herbut et al., 2018). Due to the continuous increase in productivity in dairy cows, there is an increased sensitivity to high ambient temperatures and it is very likely that they will fall into a state of heat stress at lower temperatures (Herbut et al., 2019). Physiological traits such as rectal temperature, skin temperature, vaginal temperature), respiration rate, and pulse rate are widely used as indicators for HS in dairy cattle (e.g. Silanikove, 2000). According to Lee (1965), stress can be caused by all external interventions that affect the body and provoke a series of reactions to maintain its homeostasis. Respiration rate is the first visible response of cattle to heat stress and oscillates with thermal environment (Milan et al., 2016). Brown-Brandl et al. (2005) confirmes that physiological parameters respond earlier to HS than production-related parameters (feed intake and feeding behavior) or behavior changes (shade usage). According to the authors Respiration rate is considered as the most appropriate indicator to monitor HS, i.e. RR is the most valuabe indicator among all physiological, productive, and behavioral indicators. Physiological traits (RT and RR) were also used as predictors of the decline in milk production traits and feed intake during moderate stress in multiparous US Holstein (Spiers et al., 2004). There are many studies in the literature on the effect of HS on dairy cows in subtropical and tropical climates, but according to Roth (2017), heat stress in cows is no longer a characteristic only of the hot regions of the planet. Studies on the negative effects of high temperatures on animals in temperate and Mediterranean regions of Europe are also beginning to appear in the literature (Bernabucci et al., 2014). Some studies by climatologists and meteorologists indicate that global warming poses a threat to the whole of Europe (Peltonen-Sainio et al., 2010).

The aim of the study was to determined the effect of heat stress on some physiological and productive traits in Holstein-Friesian dairy cows under conditions of the temperate continental climate.

MATERIAL AND METHODS

The study was conducted in the period between May and August 2018 on a dairy cattle farm with Holstein-Friesian cows in the region of Karnobat, Southeastern Bulgaria (42°38'46.2"N 26°47'14.0"E). Cows were housed in a semi-open free-stall dairy barn, fed year-round ad libitum with a total mixed ration. On the farm, the cows were cooled by water sprinklers in the holding area of the milking parlor and all a day ventilation in the barn during the warm months of the year. The study included the cows calved between 1st of April and 10th of May 2018, thus excluding the effect of the lactation stage. The included cows were at first (9), second (6), third and more parity (7) in a total of 22 cows. The daily milk yields of the cows included in the studywere taken from their official monthly milk performance records (May and August). To measure the heat stress, a temperature-humidity index was estimated using a ‘Kestrel’ automatic measuring instrument (https://kestrelmeters.com/products/kestrel-5200-professional-environmental-meter). THI was measured in the cows housing premises twice a day, at 10 am and 3 pm, as at the same time the physiological traits RT and RR per min of each cow were also recorded once at 10 am and once at 3 pm. RT was measured by a digital thermometer in degrees Celsius. RR was reported by visual observation and recording of the movement of the chest for a period of one min according to the method of Zimbelman et al. (2009). The data on the rumen activity, expressed by the number of ruminations for 24 hours, were taken from the farm management software. This activity was reported by belts placed on the neck and fixed to them microphones of the SCR system by Allflex, with which each rumination of the cows was registered. The daily milk yield, as well as the content fat and protein percentage of the cows included in the study, were taken from their official monthly milk performance records (May and August).

For a better approximation, the factors subject to the study were presented in classes as follows:

THI is presented in three classes according to the THI scale proposed by Armstrong (1994), respectively: Class 1 - THI up to 72 (optimal thermal conditions); Class 2 - 72 to 79 (mild heat stress conditions) and Class 3 - THI above 79 (moderate heat stress conditions).

Daily milk yield: Class 1 - up to 30 kg day^{-1}; Class 2 - from 30 to 40 kg day^{-1}; Class 3 - from 40 to 50 kg day^{-1} and Class 4 over 50 kg day^{-1}.

Rectal temperature: Class 1 - up to 38.5 °C; Class 2 - from 38.5 to 39 °C; Class 3 to 39 to 39.5 °C and Class 4 - above 39.5 °C.

Respiratory rate per min: Class 1 - up to 40 per min; Class 2 - 40 to 45 per min; Class 3 - 45 to 55 per min and Class 4 - over 55 movements per min.

The rumination: Class 1 - up to 450; Class 2 - from 450 to 550; Class 3 - from 550 to 650; Class 4 - over 650 ruminations in 24 hours.

For basic statistical processing of the data a package MS Excel was used, and for obtaining the average values, errors, and analysis of variance, the corresponding modules of STATISTICA of StatSoft (Copyright 1990–1995 Microsoft Corp.)

The following model was used to assess the influence of controlled factors on the THI values:

$$Y_{ijkl} = \mu + M_i + H_j + M \cdot H_k + e_{ijkl} \qquad (1)$$

where Y_{ijkl} – is the dependent variable (THI values); μ is the mean effect; M_i – is the effect of the month of reporting; H_j is the effect of the hour of reporting; $M \cdot H_k$ is the associated effect of the month and hour of reporting and e_{ijkl} is the random residual effect.

The following model was used to assess the influence of controlled factors on physiological traits:

$$Y_{ijk} = \mu + THI_i + L_j + e_{ijk} \qquad (2)$$

Y_{ijk} – is the dependent variable (each of the studied physiological traits); μ is the mean effect; THI_i – is the effect of the *THI* (in classes); L_j– is the effect of the daily milk yield, and $\mathbf{e_{ijk}}$ is the random residual effect.

By analysis of variance for the model were obtained by classes of fixed factors the means of least squares (*LSM*).

RESULTS AND DISCUSSION

Table 1 presents the average values and the variation of THI by months of reporting. Only in May the average THI values were below 72. Tapkı & Șahin (2006) found that in the conditions of the eastern Mediterranean region of Turkey during the months from June to August, dairy cows were also under conditions of heat stress, as we found in temperate continental climate in our study.

Table 1. Average values and variation of *THI* by months of reporting

Month of reporting	THI		
	x ± SE	min	max
May	71.72 ± 0.30^{a}	69.3	74.2
June	75.06 ± 0.26^{b}	71.8	81.5
July	74.32 ± 0.16^{c}	71.6	77.6
August	77.35 ± 0.33^{d}	74.5	81.5
Average	74.62 ± 0.16	69.3	81.5

a, b, c, d – the differences are significant at $P < 0.05$.

Table 2. Analysis of variance for the influence of the month and hour of reporting on the values of THI

Sources of variation	Degrees of freedom (*n*–1)	THI		
		MS	F	P
Total for the model	7	148.34	56.45	**
Month of reporting	3	225	85.8	***
Hour of reporting	1	354	134.8	**
Month* Hour of reporting	3	16	6.0	***
Error	242	3		

*** – the differences are significant at $P < 0.001$.

Table 2 presents an analysis of variance for the influence of the month and the hour of reporting on the values of THI, the data showed that each of the studied factors, as well as the combination between them, significantly ($P < 0.001$) influenced the THI values. The effects of hour of reporting and month of reporting well represented the thermal conditions in the premises for the animals during the day.

Fig. 1 shows the LS mean values of THI by month and hour of reporting in the period May–August 2018. The study found that only in May, the values of THI, reported at 10:00 am were in the range of temperature comfort, THI < 72. According to Armstrong (1994), at these values of THI cows are in optimal thermal conditions of the environment. In June and July, both morning and afternoon THI values showed that the cows were under conditions of mild HS almost all day. The highest values of THI were reported in August when the morning values were 75.5 and the afternoon 79.2. These values showed that in August the cows on the studied farm were under conditions of moderate to severe heat stress almost all day. Although during the summer fans were operating in the barn, the reported THI values showed that this did not contribute to the betterthermal comfort of the cows. Our results confirm the study of Dimov et al. (2017), who found under the conditions of Southern Bulgaria in buildings for dairy cows of the same type that the values of THI in the summer vary from 74.13 in the building to 76.12 outside it. In their study, the same authors found that the actions taken to cool the cows in the buildings can reduce the values of THI by only up to 2.5 units in the spring and by about 2 units in the summer, which is not enough to achieve conditions of thermal comfort for the animals.

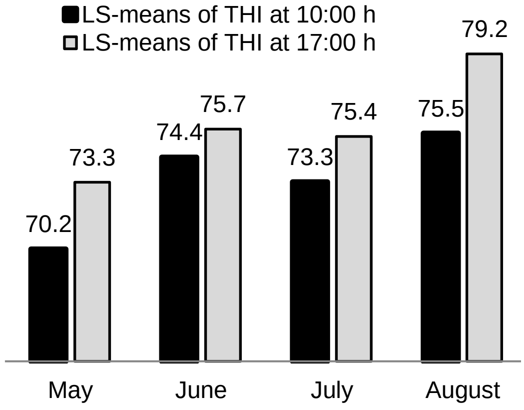

Figure 1. LS-mean values of THI by months and hour of reporting during the period May-August.

Table 3 presents the average values for the productive and physiological traits by months of reporting. The highest daily milk yield in cows included in the study was reported in June - 41.44 kg and the lowest in August 32.48 kg. Regarding the rectal temperature, there was a tendency to increase the average measured rectal temperature from May to August from 38.49 to 38.95 °C. In the RR per min, the lowest values were reported in May - 38.28, and the highest in August - 47.9. In the trait, rumination was found that the highest values were in May - 570.95, and the lowest in June - 521.5 per day, after which their number increased and reached the values for May. This decline in ruminations in June was probably due to the rapid onset of heat stress from May to June, which did not allow cows to adapt to new environmental conditions. According to Bernabucci et al. (1999) at the onset of HS, as an initial reaction a decrease in the movement of the rumen is observed, and in the rumination, respectively. The authors found that with prolonged exposure to HS, the animals acclimatize, whereby the movement of the rumen is restored to the values reported before HS, which was reported as a trend in our data for July and August also.

Table 3. Average values for productive and physiological traits by months of reporting

Month of reporting	No	Daily milk yield, kg	Ruminations number, 24h	No	Rectal temperature, °C	Respiratory rate, min
	n	x ± SE	x ± SE	n	x ± SE	x ± SE
May	21	41.17 ± 2.53	570.95 ± 13.81	42	38.49 ± 0.06^{a}	38.28 ± 1.32^{a}
June	42	41.44 ± 1.72	521.50 ± 11.64	84	38.85 ± 0.05^{b}	45.57 ± 1.09^{bc}
July	42	37.20 ± 1.22	572.90 ± 9.47	84	38.90 ± 0.04^{b}	43.47 ± 1.05^{b}
August	20	32.48 ± 1.59	563.40 ± 11.72	40	38.95 ± 0.07^{b}	47.90 ± 1.64^{bd}
Average	125	38.54 ± 0.90	573.78 ± 6.16	250	38.83 ± 0.03	44.02 ± 0.64

a, b, c, d – the differences between the months are significant at $P < 0.05$.

The data in Table 3 show that the average values of rectal temperature and respiration rate increased from May to August. Body temperature is an important physiological indicator that is indicative of the health of dairy cows (Hicks et al., 2001). A number of studies have shown that high ambient temperatures lead to a significant increase in body temperature and respiration rate in dairy cows (Brown-Brandl et al., 2005; Collier et al., 2006). Table 3 shows that rectal temperature and respiration rate were the physiological traits that increased during the warmest months of the study (June - August). It should be noted that in the respiration rate a certain decrease in July compared to June was observed. This decrease in respiratory rate fully corresponds to the THI data presented in Table 1. In July the average values for THI were 74.32, and in June 75.06. In July, lower morning and afternoon values of THI were reported (Fig. 1). According to Atkins et al. (2018) the intensity of respiration in dairy cows is the most accurate physiological indicator of the effect of HS on them. The results of our study showed that the respiration rate was the physiological trait indicating a direct dependence on environmental conditions - the values of THI. According to Bohmanova et al. (2007) the influence of HS varies depending on the characteristics of the climate, as in a humid climate, such as in our study, the influence of air humidity is stronger than the temperature of the environment. According to Hansen (2007), with increasing daily milk yield, cows produce more metabolic heat, which makes them even more sensitive to HS.

In our study, the daily milk yield of cows was relatively high. In addition, cows were in the first part of lactation, when their productivity is highest, respectively, and their sensitivity to HS.

Table 4 presents an analysis of variance for influence of THI and daily milk yield on the studied physiological traits in dairy cows it was found that THI had effect with high significance ($P < 0.001$) on the values of rectal temperature and respiratory rate, but no significant effect was observed on cow rumination, the level of daily milk yield had a high significant effect on the three studied physiological traits ($P < 0.001$). Studies by other authors West et al. (2003); Gaworski & Rocha, (2016); Pilatti et al. (2018) found that HS leads to a number of physiological and behavioral disorders.

Table 4. Analysis of variance for influence of THI and daily milk yield on the studied physiological traits

Sources of variation	Degrees of freedom (*n*–1)	Respiratory rate F P	Rectal temperature F P	Ruminations F P
Total for the model	5	9.85***	14.48***	13.92***
THI	2	21.17***	26.1***	0.15 n.s
Milk yield	3	5.61***	12.7***	13.58***
Error	244			

* – significant at $P < 0.05$; ** – significant at $P < 0.01$; *** – significant at $P < 0.001$; *n.s.* – no significant effect.

Fig. 2 shows the LS-mean values for respiratory rate depending on THI. The data showed that with increasing THI the respiratory rate increased, the differences between conditions out of HS (THI < 72) and in mild HS (THI 72-79) were less than 4 breaths per min. With the onset of moderate HS at THI > 79, the LS-mean values for respiratory rate reached 56.54 breaths per min or 13 breaths min^{-1} more compared to mild HS (THI 72–79) and 17 compared to temperature comfort conditions (THI < 72). Lemerle & Goddard (1986) found that respiratory rate began to increase before THI values reached 73, with a sharp increase observed at THI > 80. Our results completely confirm these studies. In the acute phase of HS, the respiratory rate can reach 60–70 breaths min^{-1} (Collier et al., 2012). According to Dalcin et al. (2016) the number of respiratory movements per min is the best physiological indicator that reflects the effect of HS on dairy cows.

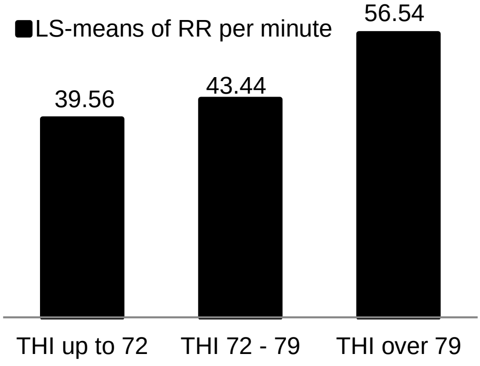

Figure 2. LS-mean values for respiratory rate depending on the values of THI.

Fig. 3 shows the respiratory rate depending on the daily milk yield of the cows. A slight increase in respiration rate in cows with higher daily milk yield was observed, this increase in RR was not so linear in accordance to the level of daily milk yield, as observed in relation with the THI level, it can be said that the intensity of RR increased in cows with daily milk yield over 40 kg compared to those with lower daily milk yield, all this showed that the respiratory rate in cows was more strongly influenced by the level of HS than by milk yield level. According to Pinto et al. (2019) for each kilogram of milk production an increase of RR with 0.23 breaths per min was reported. Our results showed an increase in respiratory rate of 6.7 breaths per min in cows with daily milk yields over 40 kg compared to those with daily milk yields up to 30 kg. This effect of milk performance level on the respiration rate in dairy cows was a consequence of the increased metabolism in the body of high producing animals.

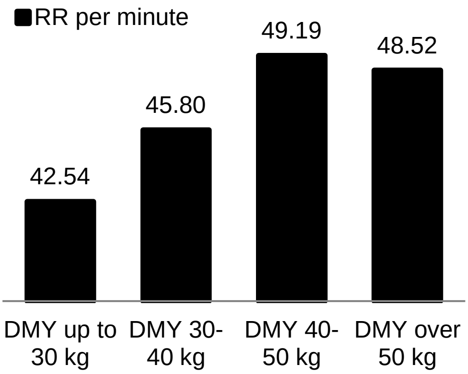

Figure 3. Respiratory rate depending on daily milk yield.

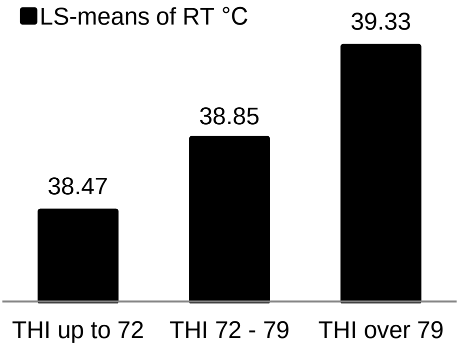

Figure 4. LS-mean values of rectal temperature depending on THI.

Fig. 4 shows the LS-mean values for rectal temperature depending on THI, it was found that with the increase of the values of THI the values of the rectal temperature also increase. The difference in the reported rectal temperature under optimal thermal conditions (THI < 72) and mild HS conditions (THI from 72 to 79) was minimal - less than 0.5 °C. With the onset of moderate HS conditions (THI > 79), the rectal temperature reached 39.33 °C. Although the differences in rectal temperature values between the different classes of THI were not as large as those observed in the respiratory rate, the reported increase in the values was significant from a physiological point of view. According to Ammer et al. (2016) the normal range of rectal temperature in dairy cows ranged from 38 to 39.2 °C. Our results showed that under conditions of moderate heat stress (THI > 79) the values of the rectal temperature in dairy cows exceeded the physiological range. The study of Zimbelman et al. (2009) is consistent with our results. According to the authors, the effect of THI on dairy cows also depends on their daily milk yield. Zimbelmann et al. (2009) found that in cows with a daily milk yield of 35 kg day^{-1}, the values of THI, which have a negative impact on their body, are lower - THI 68, compared to the lower producing ones.

Fig. 5 presents the LS-mean values of rectal temperature depending on daily milk yield. The mean values of the rectal temperature increased in cows with higher daily milk yield, as in cows with daily milk yield up to 30 kg day^{-1} it was 38.61 °C, and in those with more than 50 kg day^{-1} - 39.09 °C. The higher rectal temperature of cows with higher daily milk yield was due to the fact that they digested more feed, produced more metabolic heat, which in turn makes them more sensitive to HS (Kadzere et al., 2002). For this reason, the regulation of body temperature in highly producing dairy cows under HS conditions is more difficult (Berman et al., 1985; Umphrey et al., 2001; Berman, 2005).

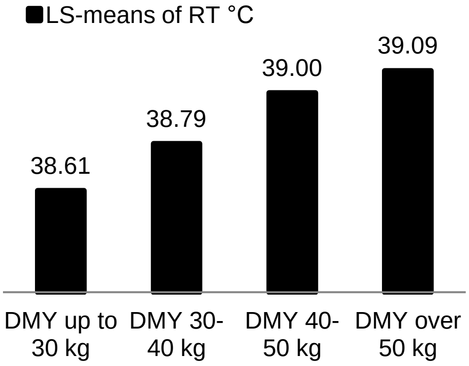

Figure 5. LS-mean values of rectal temperature depending on daily milk yield.

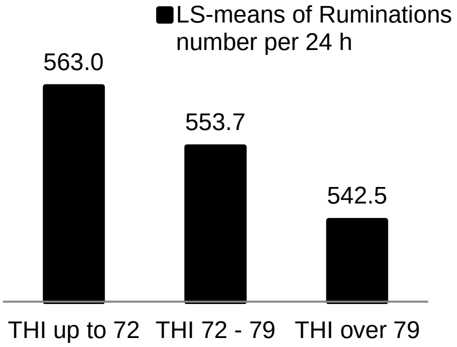

Figure 6. LS-mean values of ruminations depending on THI.

Fig. 6 shows the LS mean rumination values depending on the THI values, with the highest LS mean values of ruminations were cows under thermal comfort conditions (THI up to 72), and the lowest in cows under conditions of moderate HS (THI over 79), 563 and 542.5 ruminations in 24 hours, respectively. Our results, confirmed the studies of Tapkı & Şahin (2006), which also found a decrease in cow rumination with increasing ambient temperature, this decrease may be due to a reduction in roughage intake at the expense of concentrated feed under HS (Coppock & West, 1986), this behavior is considered to be the adaptation of cows to reduce the heat generated during fermentation in the rumen (Beede & Shearer, 1991). On the other hand, under HS, ruminants drink more water (Roger & Davis 1982; Muna & Abdelatif, 1992), which makes the contents of the rumen more fluid and thus reduces the number of ruminations and belching, respectively. Another probable reason for the decrease in the number of ruminations in dairy cows is that under HS the thermoregulatory center in the hypothalamus is activated, which, through the vagus nerve, reduces the motor activity of the stomach compartments (Varlyakov et al., 2012), as a result of the reduced motor activity of the rumen, the speed of the feed passing through the stomach compartments is slowed down (Bernabucci et al., 1999).

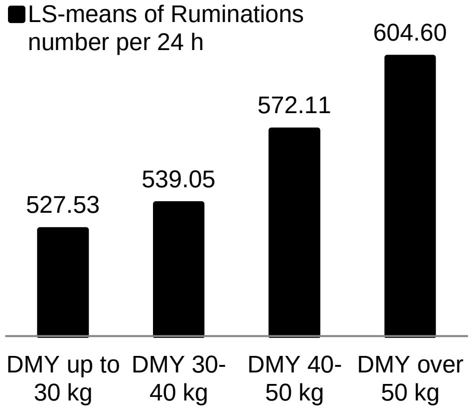

Figure 7. LS-mean values of ruminations depending on daily milk yield.

Bernabucci et al. (1999) found that with short-term exposure to HS, the rate of nutrient degradation in forestomachs of ruminants decreased, but with long-term exposure, it remained close to those reported under temperature comfort conditions. According to the authors, this was an adaptation of the animals to the conditions of HS. The speed, at which feed passes through the stomach compartments, as well as the degree of nutrient degradation, is of great importance for the productivity of cows. From the data presented in Fig. 7 it can be seen that with the increase of the daily milk yield the number of ruminations for 24hours also increased, in a study by Tapkı & Şahin (2006), higher-yielding cows in the morning at lower ambient temperatures spent more time ruminating than lower-yielding cows.

Table 5. Analysis of variance for the influence of THI, respiratory rate, rectal temperature and ruminations on Test day milk yield

Sources of variation	Degrees of freedom (*n*–1)	Test day milk yield, kg MS	Test day milk yield, kg F P
Total for the model	11	768.01	10.92 ***
THI	2	1,440.28	20.47 ***
Respiratory rate	3	478.93	6.81 ***
Rectal temperature	3	599.90	8.53 ***
Ruminations	3	414.41	5.89 ***
Error	238	70.36	

*** – significant at $P < 0.001$.

The relationship between thermal conditions, physiological indicators and productivity in dairy cows is not unidirectional, on the one hand, high temperatures negatively affect a number of physiological indicators and productive traits in dairy cows, and on the other hand the level of milk productivity of animals is a prerequisite for higher values of a number of physiological indicators and higher sensitivity to HS. Table 5 presents an analysis of the variance for the effect of THI, respiration rate, rectal temperature and ruminations number on the daily milk yield of the cows included in the study, the presented data show that all controlled factors had an impact on the daily milk yield of cows. The effect of the individual factors on milk yield is presented in more detail in graphical form in Figs 8–11, respectively.

Fig. 8 shows the LS means for daily milk yield depending on THI values. The highest daily milk yield of cows was reported under optimal thermal conditions (THI < 72), 45.62 kg day^{-1}, respectively. With increasing values of THI, the daily milk yield of cows decreased, as under conditions of mild HS (THI from 72 to 79) the average milk yield was 38.90 kg day^{-1}, and under conditions of moderate HS (THI over 79) it was 28.95 kg day^{-1}. However, the data in Table 3 show that the highest average daily milk yield was reached in June, when high values of THI were registered during the morning and afternoon measurements (Fig. 1) - 74.4 and 75.7, respectively, and at the same time, the ruminations number of the cows was the lowest (Table 3). All this confirms the significant influence of THI on the daily milk yield of cows. The decrease in productivity under HS may be due to a decrease in the amount of dry matter intake. Baumgard et al. (2011) believe that reduced dry matter intake can explain only 35–50% of reduced milk yield. According to Slimen et al. (2016) HS causes a reorganization in the use of body resources such as fat, protein and energy, which is the other reason leading to reduced productivity.

Fig. 9 shows the LS means of daily milk yield depending on the respiratory rate. A linear nature of the dependence of respiratory rate on milk productivity of cows was not reported. The highest respiratory rate (over 55) had cows with 38.35 kg day^{-1}, while those with the highest milk yield - 40.97 kg day^{-1} had a respiratory rate of 40–45. The influence of HS on cows depends on many factors, such as breed, age, productivity, stage of lactation, as well as on earlier influence of HS (Zimbelman et al., 2009). According to the authors, more productive cows are more sensitive to HS, but physiological responses such as respiratory rate do not correspond directly to the heat load of the animals. It was found that there is some delay in the physiological response of animals to HS. Gaughan et al. (2000) found a 2-hour delay in increasing respiratory rate after an increase in THI values. According to Atkins et al. (2018) the presence of cooling systems, as there were in the farm we studied, can also affect the respiratory rate of cows. All this is probably the reason for the observed variations in the presented figure.

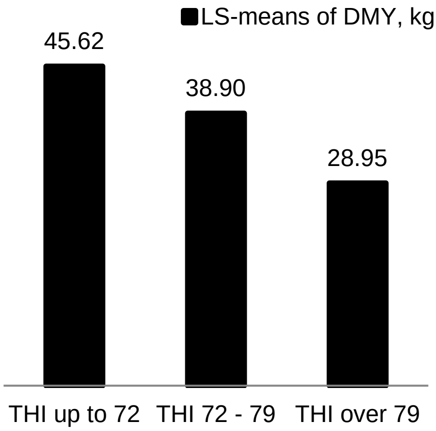

Figure 8. LS-mean values of daily milk yield depending on THI.

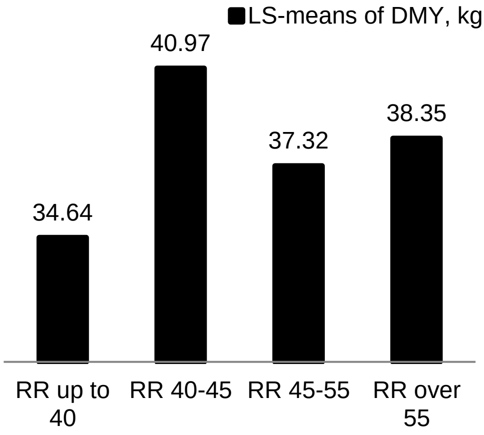

Figure 9. LS-mean values of daily milk yield depending on the respiratory rate.

Fig. 10 shows the LS means for daily milk yield depending on rectal temperature. The cows with a rectal temperature of up to 38.5 °C had the lowest daily milk yield of 32.45 kg day^{-1}, and the cows with a rectal temperature of 39 to 39.5 °C had the highest daily milk yield of 41.01 kg per day. In cows with a rectal temperature above 39.5 °C, the daily milk yield was just over 40 kg day^{-1}. There was a tendency to increase the rectal temperature in cows with high milk yield. According to Dikmen & Hansen (2009), the regulation of body temperature in cows in conditions of hyperthermia can be expected to be ineffective with an increase in daily milk yield due to the metabolic release of more heat. However, in their study, the authors did not find such a relationship. Berman et al. (1985) found a higher rectal temperature in cows with higher milk yields. According to Dikmen & Hansen (2009), the lack of relationship between rectal temperature and milk yield of cows may be due to the fact that daily milk yield of cows was not recorded on the same day as rectal temperature was measured.

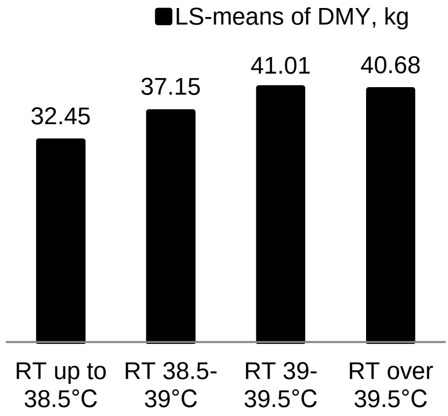

Figure 10. LS-mean values of daily milk yield depending on rectal temperature.

Fig. 11 shows the LS means of daily milk yield depending on ruminations. With the lowest milk yield - 33.26 kg per day were the cows with the lowest number of ruminations - up to 450 per day. In cows with high milk yield an increase in rumination was reported, and in cows with 42.89 kg day^{-1} the ruminations number reached over 650 per day. All this shows that the daily milk yield of dairy cows on the studied farm depended on the activity of the forestomachs, respectively on the number of ruminations and belchings of the cows. Our results were also confirmed by the studies of Antanaitis et al. (2018), who found a higher number of ruminations in cows with higher daily milk yield. The data presented in Table 3 show that with the onset of HS in June, rumination in cows decreased, but in July and August it returned to levels measured in May, when the cows were under conditions of temperature comfort. According to Bernabucci et al. (1999) digestive tract of ruminants can adapt when the exposure to HS is in a long-term.

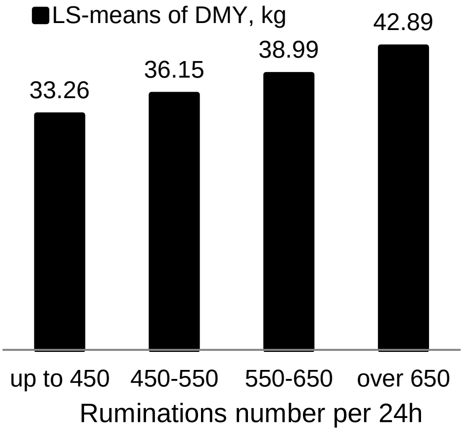

Figure 11. LS- mean values of daily milk yield depending on ruminations.

CONCLUSION

Based on the study, it was found that under conditions of mild to moderate heat stress, rectal temperature and respiratory rate increased. With the onset of HS, the rumen activity decreased, and subsequently, when the cows adapted, the activity of the rumen was returned to the values registered in the thermo neutral months. Changes under the influence of HS in the studied physiological traits - rectal temperature, respiratory rate and rumination number depend also on the milk performance of cows. In cows with higher daily milk yield under HS conditions, a more pronounced increase in rectal temperature and respiratory rate was observed. With an increase in HS expressed by the values of THI, a decrease in the rumen activity was reported, as well as a decrease in the daily milk yield of cows. The study proved that in cows with higher daily milk yield changes in physiological parameters - rectal temperature, respiratory rate and rumination number are more strongly expressed under the influence of HS.

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Evaluation of interspecific potato breeding material with a complex of genes of immunity to Potato virus Y using molecular markers

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Abstract. The article presents the results of research on potato culture, the presence of Ry_{adg}, Ry_{sto}, and Ry_{chc} genes in complex interspecific potato hybrids, and based on the use of the DNA markers for selection of resistant forms to Potato virus Y (PVY). These genes are derived from different genetic sources of the species *Solanum andigenum, S. stoloniferum and S. chacoense*, respectively. The selected potato formswith a complex of agronomic characters are recommended for inclusion in the selection process, for creation of new varieties, as well as as a valuable source material for interspecific hybridization.

Key words: variety, source material, R-genes, assisted selection (MAS), stability.

INTRODUCTION

Development of potato varieties with extreme genetic resistance to pathogens, especially the Potato virus Y (PVY), is associated with a number of difficulties. In particular, during the hybridization of tetraploid forms in hybrid populations, a wide variation and a complex nature of economically valuable characteristics splitting is observed, which ultimately complicates the selection of genotypes with the desired characteristics due to the need to develop sufficiently large amount of hybrid potato seedlings.

Many methods are labor-intensive enough, long (they take up the entire growing season), and not accurate enough. The solution of this problem in improving the potato breeding efficiency is the use of the method of molecular marker of genes (*R*-genes) of resistance to pests and diseases (Barone, 2004; Kilchevsky & Khotyleva, 2014; Ermishin et al., 2016).

The study of potato DNA markers is associated with the application and introduction into the breeding process of a wide range of genetic material of wild and cultivated forms of potatoes, with genes of resistance to pathogens (Hawkes & Jackson, 1992; Jansky, 2000; Gebhardt & Valkonen, 2001).

An important area of molecular markers application is marker assisted selection (MAS), which is widely introduced into breeding practice. DNA markers are successfully used both at the stage of initial sources selection for hybridization, and at the subsequent analysis of the hybrid material and the resulting variety. DNA-markerof economically valuable characteristics allows them to be involved in MAS, which is designed to provide higher efficiency, lower cost and shorter duration of obtaining new varieties and hybrids compared to traditional methods of selection (Watanabe & Peloquin, 1991; Shamshin & Porotnikova, 2014).

The availability of specific information about certain genes present in potato allows optimizing the selection of parental forms and predicting the probability of seedling selection in hybrid populations with economically valuable characteristics (Ermishin et al., 2016). The use of molecular technologies makes it possible to significantly simplify the pyramiding of genes for resistance to one pathogen, but originating from different sources (Solomon-Blackburn & Barker, 2001; Vales, 2010; Szajko et al., 2014). Currently, there are more than 10 known genes that provide resistance to PVY, some of which are cloned or mapped (Solomon-Blackburn & Barker, 2001; Barone, 2004; Flis, 2005; Sato, 2006; Szajko et al., 2014; Grech-Baran et al., 2020). Some of them are hypersensitive resistance (HR) genes involving programmed cell death (HR), which are isolated in some wild species and in various varieties of *Solanum tuberosum*. These genes provide resistance to only some of the currently known PVY strains (Tian & Vakonen, 2013). The genes of extreme resistance (ER) - are of the greatest interest and value for breeding. It is known that the ER genes - Ry_{adg}, and Ry_{chc} were mapped on chromosomes XI, XII, respectively. The recent study found that Ry_{sto} is associated with the TIR-NLR immune receptor (Grech-Baran, 2020).

Potato is one of the crops with the most fully mapped genome (350 markers that characterize about 90% of the genome) (Gebhardt & Valkonen, 2001; Gebchardt, & Vreugdenhil, 2007). According to a number of authors, it is possible to increase the potato resistance to PVY and ensure its resistance to a wide range of strains by using ER genes in breeding and combining them in a single genotype (Solomon-Blackburn & Barker, 2001; Vales, 2010; Tian & Vakonen, 2013).

Thus, the use of DNA markers makes it possible to simplify the selection of valuable samples. The efficiency of selection increases due to a decrease in the sample size (based on the presence of economically valuable characteristic), therefore, only samples with identified loci that are characteristic of this species and are of interest for selection.

The purpose of this research was to identify the genes originating from different types of potatoes in complex interspecific potato hybrids, to isolate genotypes with a complex of different genes that provide resistance to PVY with a complex of agronomic characters.

CONDITIONS, MATERIALS AND METHODS

Experimental work was carried out in the Selection and Seed Center of Potato Growing of the Ural SRIA− Branch of the FSBSU UrFARC UrB RAS, city of Yekaterinburg, within the framework of the scientific and technical program on the assignment of Exploratory research carried out by the contractor-organizations within the framework of the CPSR Development of potato breeding and seed growing. The object of the study was a complex interspecific hybrids of collection nursery potato and collection nurseries variety samples, created in the Selection and Seed Center of Potato Growing of the Ural SRIA-Branch of the FSBSU UrFARC UrB RAS, in total 189 samples were analyzed. Field experiments were laid in the second half of May on the experimental field of selective crop rotation. The predecessor is a cropped fallow. The soil is soddy-medium podzolic with the following agrochemical characteristics: humus content (GOST 26213-91) - 4.86%; salt pH (GOST 26483-85) - 5.68; total absorbed bases ‒20.0 m–eq. 100 g^{-1}; easily hydrolyzed nitrogen– 124 mg kg^{-1}; mobile phosphorus (GOST 26207-91) - 360 mg kg^{-1} and potassium exchange (GOST 26207-91) - 136 mg kg^{-1} of soil (Vasiliev & Gorbunov, 2020) nutrient status - $N_{60}P_{60}K_{90}$.

The evaluation of selection and source material for the presence of DNA markers were carried out in the PCR laboratory of the Selection and Seed Center of Potato Growing of the Ural SRIA - Branch of the FSBSU UrFARC UrB RAS. In our research, we used genetic material from potato species: *Solanum tuberosum* sbsp. *andigenum*, *S. stoloniferum and S. chacoense.* Based on reference data (Kasai et al., 2000; Flis, 2005; Song & Schwarzfischer, 2008; Mori et al., 2011), to evaluate the source material of potato, intended for creating varieties with ER to PVY, DNA markers of resistance genes were selected, which are most reliable and technological in use: RYSC3$_{321}$, YES3-3A$_{341}$, Ry186 (Table 1).

Table 1. Markers characteristics of *R*-genes of potato resistance to PVY used in the work

Gene	Character	Marker	Primers (5’ – 3’)	Source
Ry_{adg}	Resistance to PVY	RYSC3$_{321}$	F – ATACACTCATCTAAATTTGATGG R – AGGATATACGGCATCATTTTTCCGA	Kasai, 2000
Ry_{sto}	ER to PVY	YES3-3A$_{341}$	F – TAACTCAAGCGGAATAACCC R – AATTCACCTGTTTACATGCTTCTTGTG	Song & Schwarzfischer, 2008
Ry_{chc}	Resistance to PVY	Ry186	F –TGGTAGGGATATTTTCCTTAGA R –GCAAATCCTAGGTTATCAACTCA	Mori, 2011

F – forward primer; R – rewers primer.

A set of Synthol reagents was used to isolate plant DNA. DNA extraction protocol is standard for Syntols set. The crushed potato leaves are transferred into microcentrifuge 2 mL tubes. Added 800 µl of lysis solution and 15 µl of proteinase-K to each tube and then mixed the solution. The tubes were incubated for 30 min. at 60 °C, occasionally mixed on a shaker. After that, the tubes were cooled for 5 min. at room temperature (RT) and centrifuged for 5 min. at 13,000 rpm. During that, a precipitation reagent was prepared in new tubes, 200 µl of sorbent buffer and 40 µl of sorbent. 200 µl of supernatant was gently transferred to new tubes with precipitation reagent. Incubated for 10 min. at RT. Centrifuged for 1 min. at 7,000 rpm. The supernatant was removed. Added 300 µl of washing solution A to the precipitate and mixed. Centrifuged for 30 sec. at 7,000 rpm. The supernatant was removed. Added 500 µl of wash solution B to the precipitate, mixed and centrifuged for 30 sec. at 7,000 rpm. The supernatant was removed. This step was repeated two times. Opened tubes were placed in a thermostat at a 60 °C until the liquid had completely evaporated. Added 200 µl of TE-buffer to the dry precipitate, mixed on a shaker. Incubated for 5 min. at 60 °C. The suspension was centrifuged for 2 min. at 13,000 rpm. 150 µl supernatant with DNA was transferred into new 0.2 µl tubes.

The protocol for DNA isolation is standard. Under the same amplification conditions, the reaction was set up separately for each marker. The reaction mixture for PCR with 12 mL primers contained: 2 µl of DNA, 1 µl of standard buffer for Taq-polymerase, 0.3 µl of dNTP, 0.4 µl of $MgCl_2$, 0.25 µl of each primer, 0.1 µl of Taq-polymerase, and 8.3 µl of H_2O. Amplification was performed in the mode: 94 °C - 3 min.; 35 cycles: 92 °C - 45 sec., 60 °C - 45 sec., 72 °C - 1 min; 72 °C - 10 min.

Based on the results of amplification and subsequent electrophoresis in 1.5% agarose gel, all results are displayed on PC screen.

RESEARCH RESULT

In the field conditions of 2018–2019, an assessment of complex interspecific genotypes by a complex of characteristics was made. During the growing season, interspecific potato hybrids were evaluated for resistance to viral diseases, in particular to PVY against a natural infectious background. During the budding-flowering phase, leaf samples were collected for testing for the presence of viruses and for the presence of DNA markers of resistance to PVY. When diagnosing the disease pathogen by PCR analysis, it was found that in the studied samples, PVY was present in only nine hybrids, this is 4.8%. A high occurrence was noted for the spread of the Potato virus M (PVM) - 32.1%. The number of samples with Potato virus X (PVX), Potato virus A (PVA), and Potato Leafroll virus (PLRV) viruses was low - 0.2–1.3%.

The screening of the potato gene pool and breeding material was performed using three (RYSC3321, YES3-3A341, Ry186) practically applicable markers of PVY resistance genes. The selective value of hybrids and varieties with resistance to PVY increases if several resistance genes from different potato species are present. Dominant alleles of the Ryadg, Rysto, and Rychc genes in complex interspecific hybrids were identified by DNA markers. According to research data, 32% of hybrids with DNA markers for three PVY resistance genes were isolated from the total number of studied samples (Table 2).

Table 2. Characteristic of the best potato samples with a complex of agronomic characters, in the genotype of which percense all three molecular markers of resistance to Potato virus Y, 2018–2019

Variety sample	Yield (t ha^{-1})	Starch content (%)	Resistance to leaf blight, points
05-15-15	32.2	16.5	8.0
05-15-40	34.7	15.2	7.0
05-15-7	42.9	18.7	8.5
06-11-1	32.9	11.2	5.0
08-10-1	31.2	16.0	5.0
08-20-2	29.3	15.8	7.0
08-41-5	35.5	11.0	7.0
08-41-7	32.0	10.9	5.0
10-11-24	34.4	14.8	7.0
10-22-21	32.1	14.2	8.0
10-22-7	36.7	10.0	7.0
10-54-5	29.8	14.7	8.0
10-54-9	30.0	14.8	8.0
10-9-3	33.7	15.4	5.0
12-12-8	31.7	13.2	5.0
12-22-47	31.2	12.2	5.0
12-22-66	30.2	12.0	5.0
13-41-13	36.9	16.5	8.5
12-29-12	32.6	12.8	7.5
12-24-14	32.8	13.5	7.0
12-47-13	35.7	12.8	8.5
15-2-4	30.8	14.4	5.0
15-22-4	40.5	10.9	7.0
12-32-8	38.9	17.8	7.0

High-yielding varieties were identified: 05-15-7 (42.9 t ha^{-1}), 15-22-4 (40.5 t ha^{-1}); with increased starch content in tubers: 05-15-7 (18.7%), 12-32-8 (17.8%), 05-15-5 (16.5%), 13-41-13 (16.5%); with better relative resistance to leaf blight: 05-15-7 (8.5 points), 13-41-13 (8.5 points), 12-47-13 (8.5 points). According to the results of the analysis, as the most valuable for inclusion in hybridization, in order to pyramid resistance genes to PVY from various sources, varieties are recommended that simultaneously carry markers to three resistance genes that are listed in Table 2.

A group of potato varieties from this list has been selected, which have now passed the state test and are included in the Register of Selection Achievements of the Russian Federation and are allowed to be used: 05-15-7 - Alaska; 05-15-40 - Gornyak; 08-10-1 - Mishka; 08-41-7 - Terra; 10-9-3 - Carmen; 10-22-7 - Prime; 12-32-8 - Flamingo.

The Alaska variety. Mid-ripening variety of table potato, tubers are oblong-oval, red, white color of flesh. Starch content is 13–20%. Average weight of commercial tuber is 110–140 g. Number of tubers in the bush is 12–16 pcs. Potential yield is 82 t ha^{-1}. Consumer qualities: good taste, culinary type BC. The variety is resistant to potato wart disease, potato cyst nematode, wrinkled and streak mosaic, curlytop virus. Preservation capacity when stored is good. Note: intensive type of variety, characterized by a consistently high yield, resistance to late blight. Included in the State Register of the Russian Federation and approved for use since 2020 for 4 and 12 regions.

The Gornyak variety. Early-ripening table variety. Tuber skin color is yellow, flesh color is light yellow, tuber shape is rounded. Potential yield is 60.0 t ha^{-1}, number of tubers per bush is 12–18 pcs., average weight of commercial tuber is 90–140 g., starch content is 14.0–19.0%. Medium-resistant to heat and drought. The variety is resistant to potato wart disease and late blight, poorly susceptible to potato cyst nematode. Preservation capacity when stored is good. It is recommended to cultivate on light and medium types of soil, it is flexible, highly resistant to scab. It is included in the State Register of the Russian Federation since 2015 for 4 and 9 regions.

The Mishka variety. Early, for table purposes. Tubers are round-oval, skin color is red, flesh is white. Potential yield is 50.0 t ha^{-1}, number of tubers per bush is 10–14 pcs., average weight of commercial tuber is 100–130 g., starch content is 13.0–17.7%. Resistance to disease: variety is resistant to potato wart disease, potato cyst nematode, medium-resistant to late blight and scab. Preservation capacity when stored is good. A distinctive feature is the early accumulation of commercial yield. It is included in the State Register of the Russian Federation since 2018 for 4 and 10 regions.

The Terra variety. Very early variety, for table purposes. Tuber skin color is yellow, flesh color is light yellow, tuber shape is oval. Potential yield is 55.0 t ha^{-1}, number of tubers per bush is 10–12 pcs., average weight of commercial tuber is 150–180 g., starch content is 10.0–14.0%. Taste is good, overcooking is weak (type B). Resistant to potato wart disease, potato cyst nematode; medium-resistant to late blight. Keeping capacity when stored is good. Note: early accumulation of commercial yield, high marketability. It is included in the State Register of the Russian Federation since 2020 for 4 and 10 cultivation regions.

A group of potato varieties with two resistance genes was identified - 39% of the total analyzed number, which also deserve close attention as genetic sources of resistance to PVY. The absence of all three studied genes was recorded in 8% of the studied genotypes. It is indicative that these samples also lack economically valuable characteristics - they are characterized by low yield, starchiness and low resistance to late blight.

As a result of a comprehensive assessment of the studied material by economically valuable characteristics (field experiments), evaluation by PCR analysis for the presence of pathogens and molecular genetic labeling, a number of promising samples were selected for use in selection programs for the creation of virus-resistant potato varieties. The value of these samples as genetic sources is complex resistance to PVY, although they belong to different groups of ripeness, but the breeding significance is higher in the samples of the early-ripening group: 06-11-1 and 08-41-5. Characteristics of samples that are of interest in terms of saturation with various PVY resistance genes from several sources, with a complex of economic and useful features, are presented in Table 3.

Table 3. Characteristic of valuable potato genotypes that combine a comprehensive assessment of field resistance and the presence of PVY resistance markers, 2018–2019

Variety sample	Ripeness	Field resistance to Potato virus Y, point	Identified presence of markers of Potato virus Y resistance genes
05-15-15	mid	8.0	RYSC3$_{321}$ - gene Ry_{adg}; YES3-3A$_{341}$ - gene Ry_{sto}; Ry186 - gene Ry_{chc}
05-15-7	mid	8.0	RYSC3$_{321}$ - gene Ry_{adg}; YES3-3A$_{341}$ - gene Ry_{sto}; Ry186 - gene Ry_{chc}
05-22-35	mid-early	7.5	RYSC3$_{321}$ - gene Ry_{adg}; YES3-3A$_{341}$ - gene Ry_{sto}
06-11-1	early	9.0	RYSC3$_{321}$ - gene Ry_{adg}; YES3-3A$_{341}$ - gene Ry_{sto}; Ry186 - gene Ry_{chc}
08-41-5	early	7.0	RYSC3$_{321}$ - gene Ry_{adg}; YES3-3A$_{341}$ - gene Ry_{sto}; Ry186 - gene Ry_{chc}
10-11-24	mid	7.5	RYSC3$_{321}$ - gene Ry_{adg}; YES3-3A$_{341}$ - gene Ry_{sto}; Ry186 - gene Ry_{chc}
10-22-7	mid	7.0	RYSC3$_{321}$ - gene Ry_{adg}; YES3-3A$_{341}$ - gene Ry_{sto}; Ry186 - gene Ry_{chc}
10-22-23	mid	7.5	RYSC3$_{321}$ - gene Ry_{adg}; Ry186 - gene Ry_{chc}
10-54-5	mid-early	8.0	RYSC3$_{321}$ - gene Ry_{adg}; YES3-3A$_{341}$ - gene Ry_{sto}; Ry186 - gene Ry_{chc}
10-54-9	mid-early	8.0	RYSC3$_{321}$ - gene Ry_{adg}; YES3-3A$_{341}$ - gene Ry_{sto}; Ry186 - gene Ry_{chc}
12-32-8	mid-early	7.0	RYSC3$_{321}$ - gene Ry_{adg}; YES3-3A$_{341}$ - gene Ry_{sto}; Ry186 - gene Ry_{chc}
12-47-10	mid-early	7.0	RYSC3$_{321}$ - gene Ry_{adg}; YES3-3A$_{341}$ - gene Ry_{sto}
12-47-13	mid-early	9.0	RYSC3$_{321}$ - gene Ry_{adg}; YES3-3A$_{341}$ - gene Ry_{sto}; Ry186 - gene Ry_{chc}
13-41-13	mid-early	8.5	RYSC3$_{321}$ - gene Ry_{adg}; YES3-3A$_{341}$ - gene Ry_{sto}; Ry186 - gene Ry_{chc}
13-41-53	mid-early	8.0	RYSC3$_{321}$ - gene Ry_{adg}; Ry186 - gene Ry_{chc}
14-9-24	mid-early	7.0	RYSC3$_{321}$ - gene Ry_{adg}; Ry186 - gene Ry_{chc}
15-22-4	mid-early	7.0	RYSC3$_{321}$ - gene Ry_{adg}; YES3-3A$_{341}$ - gene Ry_{sto}; Ry186 - gene Ry_{chc}

The sample 06-11-1 (the Start variety) presented in the table deserves special attention, it has a maximum value of the characteristic of the field resistance to PVY (9 points), according to the results of PCR analysis on average over the years, the pathogen of the potato virus Ywas not detected in any sample. This variety is included in the Register of Selection Achievements, but is not allowed to be used because of the average indicators of economic valuable characteristics. With the presence of three studied markers in the genotype RYSC3$_{321}$, YES3-3A$_{341}$, Ry186, field resistance to PVY, this variety is recommended as a genetic source for targeted selection, the fertility of this variety is 52.0%, which allows to include both the maternal and paternal forms in hybridization.

CONCLUSION

As a result of a comprehensive assessment of potato selection material in 2018–2019 for agronomic characteristics, field resistance and genotyping for the presence of DNA markers of the corresponding PVY resistance genes, 17 promising selective samples with a complex of markers of ER to PVY were identified, which are recommended for use as initial parent forms for hybridization in the direction of creating virus-resistant forms, as well as a valuable material in breeding: 05-15-15, 05-15-7, 05-22-35, 06-11-1, 08-41-5, 10-11-24, 10-22-7, 10-22-23, 10-54-5, 10-54-9, 12-32-8, 12-47-10, 12-47-13, 13-41-13, 13-41-53, 14-9-24, 15-22-4.

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Biochemical contents of highbush blueberry fruits grown in the Western Forest-Steppe of Ukraine

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Abstract. The study of the physical and consumption qualitative indices of the highbush blueberries (the cultivars 'Reca', 'Elizabeth' and 'Bluegold') in the Western Lisosteppe of Ukraine has shown that the biggest fruit mass was accumulated by the berries of two last mentioned vs (1.54–1.50 g respectively). 'Bluegold' has this indicator as the least variable ($V = 10.6\%$). The accumulation of fruit mass of all studied cultivars was facilitated by a moderately humid period of their development in 2018 with a hydrothermal coefficient of 1.1–1.2. The favorable year for the intensive synthesis of the dry matter and soluble solids was 2017 when the precipitation amount did not exceed 44 mm. The biggest number of the mentioned substances during the research period was accumulated by fruits of 'Reca' - 17.48 and 13.24%, respectively. It was established that the dry matter content had high level of stability ($V = 8.2\%$), and middle was for soluble solids ($V = 19.5\%$). The amount of monosaccharides from which glucose and fructose were investigated in fruits varied from 6.11 ('Bluegold') to 7.85 ('Reca'), it was slightly lower in 'Elizabeth' berries - 7.78%. 'Bluegold' fruits were characterized by high stability ($B = 9.6\%$) of the biggest content of titrated acids among the studied cultivars (2.42%). The dry weather with low number of precipitation in 2017 (hydrothermal coefficient 0.3–0.4) was favourable for the accumulation of both the mentioned acids and vitamin C in fruits of highbush blueberries in the period of their formation and growth. The amount of ascorbic acid in fruits in the specified year varied from 20.00 mg 100 g^{-1} WM ('Reca') to 27.00 mg 100 g^{-1} WM ('Elizabeth') with an intermediate value of 22.50 mg 100 g^{-1} WM ('Bluegold'). The latest of the mentioned varieties had the most constant index ($V = 7.0\%$). The content of polyphenolic substances was slightly dependent on weather conditions during the period of fruit growth, the coefficients of variation were 6.2% ('Reca'), 7.0% ('Elizabeth') and 5.8% ('Bluegold'). The fruits of the last mentioned cultivars were characterized with the biggest anthocyanins and chalcones content (68 and 13 mg 100 g^{-1} WM, respectively). The substantial indirect dependence of the content of the nutritive substances and anthocyanins on the berry mass was revealed in the 'Reca' fruits.

Key words: *Vaccinium corymbosum* L., average mass, nutritional and bioactive substances, correlation.

INTRODUCTION

The popularity of highbush blueberries has increased over the last ten years, and now these are grown in many countries around the world. In 1990, *V. corymbosum L.* was cultivated in only 10 countries (Statistics Division, 2015), while in 2011 its commercial cultivation was introduced in 27 ones (Evans & Ballen, 2014). World production of highbush blueberry fruit increased from 262 t in 2006 to 556 t in 2016 (Aliman et al., 2020). According to the FAOSTAT data, the leaders of world blueberry production are the United States (269 tons) and Canada (179 tons). In Ukraine, over the past 10 years, tall blueberries from a little-known niche culture have become one of the main berry species. In Ukraine, during the last 10 years, *V. corymbosum* L. from a little-known niche crop have turned in to one of the main small fruit crops. Since 2017, Ukraine has become among five biggest blueberry fruit producers in Europe. In a period from 2007 to 2017 the total area of highbush blueberry plantations in Ukraine has dramatically increased from 130 ha to more than 1,500 ha (end of 2018 year) and it is expected to be 2,000 ha circa now. The rapid increase in the area under *V. corymbosum L.* was due to the high demand of the population for its fruit, which was formed due to significant consumer, dietary and medicinal properties, which is confirmed in the research by well-known scientists (Basu et al., 2010; Krikorian et al., 2010; Carey et al., 2014; Nil & Park, 2014; Singh, 2018).

Blueberry fruits contain large amount of organic and inorganic substances, which is mostly determined by the variety genotype, growing conditions, including ecological and technological, as well as the state of berry ripeness (Gündüz et al., 2015). Polish scientists established that content of *V. corymbosum L.* fruits is, %: water - 84, carbohydrates - 9.7, albumins - 0.6, fats - 0.4 (Michalska & Łysiak, 2015); other components are polyphenols, antioxidants, vitamins, minerals and fiber (Ścibisz & Mitek, 2007). The energetic value of a 100 g fresh berry portion is estimated at 192 kilojoules. Fruits of blueberry are also a good source of nutritive fibre accounting for 3–3.5% of their mass. In addition to good taste, the main interest in these berries is due to the moderate content of vitamin C, as 100 g of blueberries provide, on average, 10 mg of ascorbic acid, equal to 1/3 of the recommended daily norm (Prior et al., 1998; NHMRC, 2015). According to many scientists, polyphenolic and antioxidant compounds of blueberries provide positive effect on human health; reduce the risk of various diseases (Ramata-Stunda, 2020). These substances can also prevent neurodegenerative disorders (Ramassamy, 2006; Correa-Betanzo et al., 2014; Giacalone et al., 2015; Diaconeasa et al., 2015; Alibabić et al., 2019). Among berry species, highbush blueberries are distinguished by the presence of various types of anthocyanins (Gao & Mazza, 1994), in particular, such as: malvidin, delphinidin, petunidine, cyanidin and peonidine with sugar fragments of glucose, galactose and arabinose. According to some researchers, malvidin and delfinidine are the main components and amount to nearly 75% of all detected anthocyanins (Scibisz & Mitek, 2007).

The main world originators of *V. Corymbosum* L. varieties are the United States (Lyrene, 2007; Brevis et al., 2008), however breeding programs that aim to create new varieties adapted to the conditions of the region are the task of breeders from other countries (Scalzo et al., 2013), including Ukraine. Creation of varieties with high standards of marketable and consumer qualities, stable to the growing conditions of Ukraine, can be realized only in a case of selection of appropriate parental forms.

The aim of our research was to study the biochemical contents of highbush blueberry small fruits grown in the climatic conditions of the Western Forest-Steppe of Ukraine, however it is very important to find out the nutritional value of the introduced varieties. Knowledge of the degree of correlation between the biochemical components themselves and with physical ones will allow breeders to select parent pairs for conducting the breeding process and creating varieties with a stable content of consumer and biologically active substances. Our research will be the basis for the breeding process aimed at creating varieties of *V. corymbosum* L. with a significant polyphenolic complex, and in particular its anthocyanin component, which makes the fruits of this crop a trendy food product.

MATERIALS AND METHODS

The research was conducted during 2017–2019. The experiment included cultivars with different ripening time: ‘Reca’ - early season (origin - New Zeland), ‘Elizabeth’ and ‘Bluegold’ - mid season (origin - the USA). Fruit samples were taken on the plots of the Institute of Horticulture of NAAS which is situated in the Western Forest-Steppe of Ukraine, altitude is 100–140 m. Climate of the region is moderate continental the minimum of average air temperature is 3.2 °C, maximum - 21.3 °C, average year temperature is 10 °C, annual precipitation is 619 mm. Plot`s soil is grey forest slightly loam. The plantation was created in 2016; the distance between the bushes in a row was 1 meter, and 3 meters between the rows. Rows were mulched with sawdust and there was turf between rows.

Analytical research was performed in the laboratory on the storage and processing of fruits and berries of the IH NAAS. Fruits of highbush blueberries with a characteristic shape and color were selected at the stage of consumer ripeness in the amount of at least one kilogram, according to the ‘Methods for assessing the quality of fruit and berry products’ (Kondratenko et al., 2008). The average berries mass was determined by weighing of 50 fruits on laboratory scales. Crushed analytical samples of fruits to determine the content of nutrients and biologically active substances were prepared using a homogenizer. The sample was weighed on analytical scales. The biochemical components such as: the content of total dry matter (drying samples method by the temperature of 98–100 °C - due to the national standard 7804:2015), soluble solids (Brix) - refractometrically (NS 8402:2015), titrated acids and vitamin C content by titration (NS 7803:2015), sugar and polyphenolic substances content - spectrophotometrically (NS ISO 4954:2008) were analyzed. Anthocyanins and chalcones were determined by the method of Kryventsov (Kryventsov, 1982). The sugar-acid index was calculated by dividing the total amount of sugars by the content of titrated acids. The experiments were conducted in 3-time replications.

Meteorological data for the trial evaluation years were obtained at the Vantage Pro2 Plus weather station. The hydrothermal coefficient (SCC Selyanova) was calculated by dividing the amount of precipitation in mm by the sum of active temperatures of 10 °C and above for the period of growth and development of fruits. The obtained data were decreased 10 times.

Statistical analysis

Statistical data processing was carried out with the use of the program STATISTICA 13/1 (StatSoft, Inc., USA). The results are presented as mean values with their standard errors, as average ± standard error (x ± SE). The differences between repetitions, as well as average inter-varietal value were determined using ANOVA. The research results were presented at the level of $P < 0.05$. Two-factor analysis of variance of the significant impact of variety genetic and climatic conditions into the content of nutritional and biologically active substances of highbush blueberry fruits and the correlation analysis were conducted in Excel.

RESULTS AND DISCUSSION

Weather conditions for three-year research in the period of plants and fruits development (varieties - 'Reca', 'Elizabet', 'Bluegold') were different, both in temperatures and precipitations. 2018 turned out the wettest, the amount of precipitation for this period was higher than 125 mm, the least fell in 2017 (43.9 mm). The average daily temperature varied from 18.0 °C in 2017 to 23.6 °C in 2019. The sum of active temperatures above 10 °C during the period varied from 1,396 to 1,424 °C. The ratio of precipitation and the sum of active temperatures of 10 °C and above was in the range from 0.3 in 2017 to 1.2 in 2018 (Table 1).

Table 1. Weather indices during the period of *V. corymbosum* L. fruits growth and development (2017–2019)

Cultivars		Amount of days from flowering to ripening	Average daily temperature, °C	Sum of effective temperature > 10 °C	Precipitation, mm	Humidity, %	HTC
'Reca'	2017	62	18.0	1,095	39.2	48.6	0.4
	2018	60	20.0	1,180	125.1	49.7	1.1
	2019	65	22.2	1,424	94.1	55.7	0.7
	Average for 3 years	62	20.1	1,233	86.1	51.3	0.7
'Elisabeth'	2017	62	20.2	1,277	36.9	49.0	0.3
	2018	54	20.0	1,082	126.8	49.9	1.2
	2019	57	23.6	1,348	73.1	51.1	0.5
	Average for 3 years	58	21.3	1,236	78.9	50.6	0.7
'Bluegold'	2017	70	18.2	1,257	43.9	48.3	0.3
	2018	58	19.6	1,139	136.0	50.0	1.2
	2019	62	22.8	1,396	87.6	55.2	0.6
	Average for 3 years	63	20.2	1,264	89.2	51.2	0.7

According to Spanish scientists, mass of highbush blueberry fruits must have more than 0.75 g in order to be acceptable for market (Molina et al., 2008). The average berry weight grown in Bosnia ranged from 1.12 to 2.11 g (Aliman et al., 2020), and Portuguese berries weighed 1.4–2.4 g (Correia et al., 2016). The fruit mass from Macedonia was approximately the same (Arsov et al., 2010), from Serbia it was 1.86−1.94 g

(Zorenc et al., 2016), and from Korea - 1.83–2.21 g (Kim et al., 2013). The weight of fruits studied in our laboratory was: 1.29 g for ‘Reca’, 1.50 - ‘Bluegold’, 1.54 - ‘Elizabeth’. The heaviest mass fruits had in 2018, in particular at ‘Reca’ - 1.60; ‘Bluegold’ - 1.70 and ‘Elizabeth’ - 1.80 g. ‘Bluegold’ was characterized as the variety with the most stable fruit weight over the years of observation with the coefficient of variation of 10.6% (Table 2).

Table 2. Physical and consumption indicators of the *V. corymbosum* L. fruits quality (2017–2019) ($n = 9$)

		2017	2018	2019	Average for 3 years	Coefficient variation, %
Average mass, g	‘Reca’	1.13 ± 0.07	1.60 ± 0.08	1.13 ± 0.08	1.29 ± 0.27	18.6
	‘Elizabeth’	1.62 ± 0.09	1.80 ± 0.06	1.20 ± 0.10	1.54 ± 0.31	17.8
	‘Bluegold’	1.40 ± 0.06	1.70 ± 0.05	1.40 ± 0.09	1.50 ± 0.18^{a}	10.6
	SE				0.17	
	x				1.44	
Dry matter, %	‘Reca’	19.10 ± 0.45	16.00 ± 0.96	17.33 ± 0.38	17.48 ± 1.63	8.2
	‘Elizabeth’	18.40 ± 0.45	16.00 ± 0.84	17.33 ± 0.51	17.24 ± 1.30	6.7
	‘Bluegold’	16.80 ± 0.91	14.83 ± 0.49	15.07 ± 0.16	15.57 ±1.17^{b}	6.7
	SE				0.94	
	x				16.76	
Soluble solids, %	‘Reca’	16.64 ± 0.44	11.80 ± 0.57	11.28 ± 0.37	13.24 ± 2.92	19.5
	‘Elizabeth’	16.24 ± 0.30	11.20 ± 0.29	11.48 ± 0.59	12.97 ± 2.80	19.1
	‘Bluegold’	12.63 ± 0.63	11.00 ± 0.24	11.48 ± 0.31	11.70 ±0.90^{b}	6.8
	SE				1.44	
	x				12.64	
Sugars, %	‘Reca’	13.45	9.44	9.02	10.64 ± 2.77	23.0
	‘Elizabeth’	13.10	9.10	9.40	10.53 ± 2.52	21.0
	‘Bluegold’	10.14	9.10	9.30	9.51 ± 0.62	5.80
	SE				0.70	
	x				10.23	
Glucose and fructose, %	‘Reca’	9.52 ± 0.54	6.97 ± 0.20	7.06 ± 0.51	7.85 ± 1.47	16.6
	‘Elizabeth’	8.54 ± 0.59	6.02 ± 0.30	8.78 ± 0.66	7.78 ± 1.57	17.9
	‘Bluegold’	5.89 ± 0.44	5.56 ± 0.18	6.89 ± 0.84	6.11 ± 0.75^{b}	10.9
	SE				0.91	
	x				7.25	
Titrated acids, %	‘Reca’	1.68 ± 0.11	1.23 ± 0.11	1.63 ± 0.07	1.51 ± 0.26	15.0
	‘Elizabeth’	2.65 ± 0.18	1.23 ± 0.15	1.98 ± 0.23	1.95 ± 0.71	32.2
	‘Bluegold’	2.60 ± 0.12	2.20 ± 0.22	2.46 ± 0.25	2.42 ± 0.26	9.6
	SE				0.36	
	x				1.96	

Note: $^{a;\ b}$ are values of indicators that differ significantly from the average (x) for the studied group at $P < 0.05$.

The dry matter content in different fruits and small fruit species, according to our data, ranges from 7 to 26%, however content of it in lowbush blueberries, according to Canadian scientists, varies from 14.03 to 16.23% (Kalt & McDonald, 1996). In our research the biggest amount of dry matters in blueberry fruits was acquired in 2017, when the precipitation sum did not exceed 44 mm, and it was 16.80, 18.40 and 19.10% for varieties ‘Bluegold’, ‘Elizabeth’ and ‘Reca’ respectively. The lowest content of these substances, for varieties ‘Reca’, ‘Elizabeth’ (16.00) and ‘Bluegold’ (14.83%), was observed in 2018. That year the precipitation index was more than 1.1. Bigger content of DM comparing with average data for the studied varieties was observed for ‘Reca’ (17.48%) and ‘Elizabeth’ (17.24%). The stability of the DM content in highbush blueberry fruits obtained high; the variation coefficient (V) did not exceed 8.2% (Table 2).

The amount of soluble solids (SS), according to Colombian scientists, determines fruit sweetness (Cortés-Rojas et al., 2016). The content of soluble solids was bigger in fruits of highbush вlueberries grown in the highland, what is explained by the fact that this area is characterised by higher level of sunlight than elsewhere (Fischer et al., 2012). Hot and rainless weather of temperate climates (Naumann & Wittenburg, 1980) accelerates the rate of photosynthesis (Taiz & Zeiger, 2010), this leads to an increase of the soluble solids concentration (Jifon & Syversten, 2001). This fact was confirmed by our research. The biggest SS amount of high-bush blueberry fruits (12.63–16.64%) was in 2019, when hydro-thermal coefficient was 0.3–0.4. The least level of SS was in 2018 from 11.00% (Bluegold variety) to 11.80% (Reca variety), when the precipitation amount was less than average, by 89.3 mm than in the analogous period of 2017 and by 65.8 mm than in 2019. The *V. corymbosum* L. fruits cultivated in Belorussia contained 13–15.3% of SS (Zenkova & Pinchykova, 2019), those from the Northern-eastern Turkey - 13.3% (Celik et al., 2018). The highbush blueberry fruits cultivated in the Western Lisosteppe of Ukraine accumulated from 11.70% (‘Bluegold’ variety) to 13.24% (‘Reca’ variety) (Table 2), which corresponded to the data obtained by scientists from neighbouring countries. The most stable SS amount among the researched varieties was determined for ‘Bluegold’ (V = 6.8%), for ‘Reca’ and ‘Elizabeth’ the coefficients of variation corresponded to the average value of 19.5 and 19.1%, respectively (Table 2).

In highbush blueberry fruit, sugars determine their organoleptic quality (Li et al., 2020), in particular, taste (Okan et al., 2018). It was reported by Gündoğdu (2019) that environmental factors and cultural practices (rootstock, irrigation, etc.) were effective on biochemical compounds such as sugar, phenolics and organic acids in fruits. The content of the mentioned substances in *V. corymbosum* L. fruits grown in Bosnia and Herzegovina was 9.73–9.94% (Aliman et al., 2020), in Russia it was at a level of 10.15–14.8% (Kirina et al., 2020). The biggest sugar content of high-bush blueberry fruits cultivated in the Western Lisosteppe of Ukraine was determined for ‘Reca’ variety (6.97–9.52%). The varieties Elizabeth and Bluegold were characterized by having lower sugar amount 6.02–8.78% and 5.56–6.89% respectively. The tendency of sugars accumulation in highbush blueberry fruits of the studied varieties was identical with the dynamics of dry matter and soluble solids accumulation. None of the studied varieties was determined as stable according to the sugar content; the coefficients of variation were higher than 10.9%, but lower than 20.0% (Table 2). Two-factor analysis of variance established the significance of the influence of weather and climatic conditions, at the level of 72.7%, on the sugar content in the fruits of the studied varieties, while genetic features determined their sugar content by only 9.3% (Fig. 1).

Besides the taste quality, sugars determine the calorific value of fruit, which is usually insignificant. Their dietetic properties are based on a high content of simple carbohydrates, namely glucose and fructose (Skrovankova et al., 2015), which are the main highbush blueberry sugars (Kalt & McDonald, 1996). Turkish researchers state that the ratio of glucose and fructose in high-bush blueberry is almost the same (Ayaz, 2001), and range from 2.9 to 7.1% - fructose and from 2.7 to 6.9% - glucose, and their total number is from 9.1 to 9.9% (Hirvi & Honkanen, 1983). Other researchers emphasize that the amount of suitable substances is from 2.64 to 4.65% to 2.29–4.31%, respectively, and the total amount is 6.95–8.96% (Aksic, 2019).

Our research proved the presence of simple sugars in fruits of the high-bush blueberry at the level of 7.25% with the average minimum 6.11% and maximum 7.85%. The amount of glucose and fructose in fruits differed depending on the conditions of the year. The level of those substances ranged from 9.52% in 2017 at Reca variety, when the precipitation number was about 44 mm, to 5.65% in 2018 at Bluegold, with the precipitation over 125 mm in a fruits growing period. These facts prove the influence of the weather conditions to the accumulation and content of sugars (Table 2). The two- factor disperse analysis found the dependence of the content of these substances on varietal characteristics at 37.1%, and on weather and climatic factors - 34%.

According to Estonian scientists, the content of titrated acids (TA) in V. *corymbosum* L. fruits is genetically fixed and to some extent adjusted by growing conditions (Starast et al., 2007), that is confirmed by our research data. Thus, the two-factor analysis of variance has shown that variety by 72.2% determines the acidity of berries and only by 35.8% it depends on weather and climatic factors (Fig. 1). Although other researchers emphasize the variability of titrated acids from growing conditions (Gerçekçioğlu & Esmek, 2005). In our research the highest amount of titrated acids was accumulated by Bluegold berries 2.20–2.60%), slightly less (in Elizabeth (1.23–2.65%) and Reca (1.23–1.68%), which corresponds with the data obtained by Belarusian researchers, the content of these substances was 1.10–2.05% (Zenkova & Pinchykova, 2019). The comparison of weather conditions with titrated acids of fruits showed the increase of the mentioned substances in ‘Elizabeth’ variety to 2.65% in the year of 2017, when the number of precipitation was low, with HTC - 0.3. In the other two varieties in the same year, there was also an increased amount of organic acids, in particular 2.60% in ‘Bluegold’ and 1.68 in ‘Reca’. The most stable number of TA was in fruits of ‘Bluegold’ variety - with a coefficient variation 9.6%, whereas the most unstable with high dependence of the TA amount from the weather conditions was ‘Elizabeth’ variety (32.2%) (Table 2).

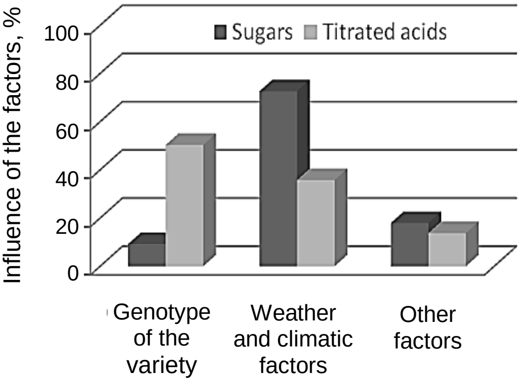

Figure 1. Particles of factors of significant influence that determine the content of sugars and titrated acids in the *V. corymbosum* L. fruits.

Organoleptic of berries depends on the number of titated acids and sugars (Ehlenfeldt et al., 1994). In our research we have established that high index of titrated acids and sugars correlation determines better taste of blueberry fruits. The sugar-acid index (SAI) of fruits of studied varieties was: ‘Bluegold’ - 3.9, ‘Elizabeth’ - 5.7, ‘Reca’ - 7.1 (Fig. 1). The fruits of the researched varieties were the sweetest according to the sugar-acid index in 2018, when the hydro-thermal coefficient was 1.1–1.2. In particular, SAI of vr ‘Reca’ fruits was 7.7, ‘Elizabeth’ - 7.4, ‘Bluegold’ - 4.1. Higher than in other years of research, the average daily temperatures in the period of fruit formation and ripening in 2019 (Table 1) negatively affected the taste of blueberries. The lowest taste coefficient (3.8) appeared in ‘Bluegold’ variety, ‘Elizabeth’ had it not much higher (4.7), the fruits of ‘Reca’ had it 5.5 (Fig. 2).

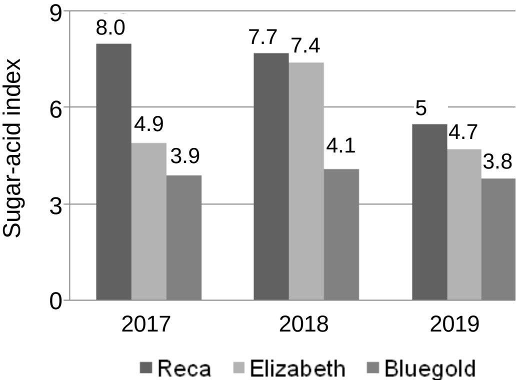

Figure 2. Sugar-acid index of the highbush blueberries (2017–2019).

Ascorbic acid relates refers to bioactive compounds that function in the human body as antioxidants (Celik et al., 2018). The amount of vitamin C in blueberry fruits was not the same in research of foreign scientists, for example data from the Baltics was 11.8 mg 100 g^{-1} (Rupasova et al., 2009), Belarus (60.5–72.2 mg 100 g^{-1}) (Zenkova & Pinchykova, 2019) from Northeast Turkey - 39 mg 100 g^{-1}. Portuguese scientists claim that the different content of vitamin C in blueberries is a varietal trait and can be adjusted to the conditions of the year and range from 6 to 162 mg 100 g^{-1} (Correia, et al., 2016). The amount of vitamin C in blueberry fruits grown in the Western Lisosteppe of Ukraine is little higher than in those from Baltic countries, but lower than in Belarusian ones, however, it is within the vitamin potency declared by Portuguese sciences. In our research the biggest vitamin C number was accumulated in fruits of the highbush blueberries in 2017, in particular, by ‘Reca’ - 20.00 mg 100 g^{-1}, ‘Bluegold’ - 22.5 and ‘Elizabeth’ 27.0 mg 100 g^{-1}. The fruits of such varieties as ‘Elizabeth’ and ‘Bluegold’ contained 20.17 and 20.90 mg 100 g^{-1} of the ascorbic acid respectively that was higher than the average content for the studied group of varieties (19.46 mg 100 g^{-1}). The content of vitamin C in the fruits of ‘Bluegold’ and ‘Reca’ varieties (7.0 and 12.3%, respectively) can be considered stable in terms of the coefficient of variation, and ‘Elizabeth’ (25.5%) can be considered unstable (Table 3).

The phytochemical composition of blueberries, according to many researchers, has a significant positive effect on the health of the human body (Stevenson & Scalzo, 2012). Turkish scientists claim that the content of polyphenols in highbush blueberries is a genetically fixed indicator, as much as the number of varieties grown under the same conditions differed significantly (Celik et al., 2018). The total polyphenol content in *V. corymbosum* L. fruits grown in the United States ranged from 48 (Ehlenfeldt & Prior, 2001) to 304 mg 100 g^{-1} by mass (Moyer et al., 2002) and was strictly dependent on the variety (Taruscio et al., 2004) was adjusted growing conditions and the state of their maturity (Zadernowski et al., 2005; Castrejón et al., 2008). The fruits of highbush blueberries, which were analyzed by Baltic scientists, accumulated these substances at the level of 228.63 mg 100 g^{-1} (Ozola & Dūma, 2020). Using two-factor analysis of variance, we confirmed the results of Turkish and American colleagues. We found a significant relationship on the amount of polyphenolic substances and anthocyanins on the genotype of the variety at the level of 98.4 and 92.9%, respectively. The content of chalcones was more adjusted by weather and climatic conditions of the growing year (65.5%), and the influence of other factors was higher by 2.55 than varietal peculiarities (Fig. 3).

Table 3. Content of the biologically active substances in the *V. corymbosum* L. fruits (2017–2019) ($n = 9$)

		2017	2018	2019	Average for 3 years	Coefficient variation, %
Ascorbic acid, mg 100 g^{-1} WM	‘Reca’	20.00 ± 1.13	15.70 ± 0.55	16.20 ± 0.57	17.30 ± 2.41	12.3
	‘Elizabeth’	27.00 ± 1.33	16.70 ± 0.40	16.80 ± 0.45	20.17 ± 5.82	25.5
	‘Bluegold’	22.50 ± 5.00	19.60 ± 0.62	20.60 ± 0.68	20.90 ± 1.65	7.0
SE					2.39	
x					19.46	
Polyphenolic substances, mg 100 g^{-1} WM	‘Reca’	375.33 ± 12.46	348.00 ± 18.11	350.67 ± 33.95	358.00 ± 25.04	6.2
	‘Elizabeth’	297.33 ± 14.15	268.00 ± 12.45	276.00 ± 27.16	280.44 ± 22.23	7.0
	‘Bluegold’	504.67 ± 33.95	492.67 ± 31.69	485.33 ± 40.17	494.22 ± 32.16^{a}	5.8
SE					61.58	
x					377.33	
Anthocyanins, mg 100 g^{-1} WM	‘Reca’	39.00 ± 2.26	30.13 ± 1.41	35.33 ± 1.73	34.82 ± 4.86^{b}	11.8
	‘Elizabeth’	55.00 ± 3.39	48.00 ± 0.45	44.10 ± 2.38	47.37 ± 3.60	6.7
	‘Bluegold’	71.00 ± 3.39	64.00 ± 5.66	68.20 ± 4.70	67.73 ± 5.32^{a}	6.9
SE					9.73	
x					50.50	
Chalcones, mg 100 g^{-1} WM	‘Reca’ (c)	12.03 ± 0.62	11.80 ± 0.23	10.21 ± 0.24	11.35 ± 1.04	8.1
	‘Elizabeth’	15.10 ± 1.24	10.00 ± 0.23	10.07 ± 0.07	11.72 ± 2.93	22.1
	‘Bluegold’	16.00 ± 1.13	112.60 ± 0.11	11.13 ± 0.17	13.24 ± 2.51	16.8
SE					1.42	
x					12.10	

Note: $^{a;\ b}$ is the notion of the indices which differ substantially from the average one (x) for the studied group of varieties ($p < 0.05$).

The amount of polyphenolic substances in highbush blueberry fruits of the studied cultivars on average in variety 'Bluegold' was 494 mg 100 g^{-1} with a minimum of 485 and a maximum of 504 mg 100 g^{-1} of raw mass, 358 - 'Reca' (min - 348, max - 376 mg 100 g^{-1}) and 280 - 'Elizabeth' (min - 268, max - 297 mg 100 g^{-1}). In the fruits of such varieties as 'Bluecrop' and 'Duke' from Romania, the amount of polyphenolic substances was 424.84–952.27 mg 100 g^{-1} (Bunea et al., 2011), which is significantly higher than our data. In fruits cultivated in Turkey, the amount of polyphenolic substances ranged from 111.60 to 438.9 mg 100 g^{-1} (Gündoğdu, 2016), this data is comparable with the data obtained by us and confirm the results regarding the significance of the genotype of the variety. The coefficient of variation from 5.8 ('Reca') to 7.0% ('Elizabeth') proves the low correlation among the amount of polyphenols in the fruits of the studied varieties and weather and climatic conditions of the year of cultivation (Table 3).

The content of anthocyanins in blueberry fruits according to Mazza & Miniati (1993) varies from 25 to 495 mg 100 g^{-1} of raw weight, and it depends on the variety, fruit size, stage of ripening, as well as climatic and weather conditions of the growing region. The fruits of highbush blueberry of the studied varieties contained from 35 to 68 mg 100 g^{-1} of anthocyanins. In our research, the biggest content of the mentioned substances was accumulated in fruits of Bluegold from 64 to 71 mg 100 g^{-1}, in Elizabeth berries there were a little less (44–55 mg 100 g^{-1}) and 'Reca' (30–39 mg 100 g^{-1}) had the least amount. The variability of this indicator over the years of research in all varieties was insignificant, the coefficients of variation was at the level and less than 11.8%. The most favourable year for the accumulation of anthocyanins in the fruits of all studied varieties of the highbush blueberries was 2017, when the HTC was 0.3–0.4 (Table 3).

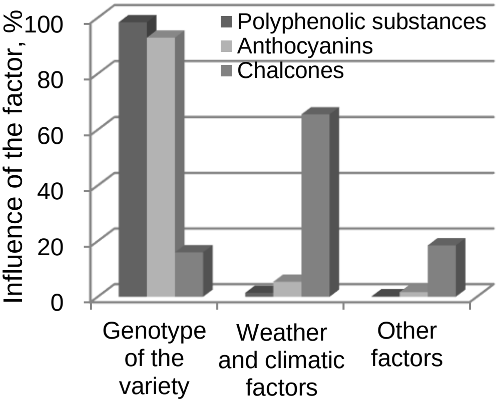

Figure 3. Effect of the factors established the amount of the polyphenolic substances, anthocyanins and chalcones in the highbush blueberries.

The chalcone component in fruits of the studied highbush blueberry varieties was insignificant - 11–13 mg 100 g^{-1}. Their biggest content was accumulated in fruits of all studied varieties in 2017, in particular 'Reca' - 12 mg 100 g^{-1}, 'Elizabeth' - 12 and 'Bluegold' - 16 mg 100 g^{-1}; the average content for the years of research was 11, 12 and 13 mg 100 g^{-1}, respectively. The content of chalcones was stable in terms of variation in the variety 'Reca' (V = 9%), the average value of variability was in the varieties 'Bluegold' (V = 19%) and 'Elizabeth' (V = 25%) (Table 3).

By correlation analysis, we established the relationship between the berry mass and biochemical parameters of the fruits of highbush blueberries. It was determined, the increase in fruit mass of 'Reca' has a strong indirect effect on the content of dry matter, titrated acids and anthocyanins, (r = -0.822; -0.995 and -0.911 respectively) and on that of the soluble solids, sugars, vitamin C and polyphenols (r = -0.442; -0.424; -0.575

respectively). The amount of dry matter and soluble solids, which was contained in ‘Reca’ fruits are strongly positively correlated with most of the studied substances, the level of correlation is medium and strong. However, the correlation of DM with chalcones and sugar-acid index was not detected (*r* = 0.197 and 0.082). The content of glucose and fructose in the highbush blueberry fruits significantly depends on the amount of total sugars (*r* = 0.996), and also determines the content of polyphenols and anthocyanins (*r* = 0.998 and 0.830 respectively). The latest two mentioned substances had positive substantial direct correlation with sugars (*r* = 0.985 and 0.759 respectively). The correlation coefficient at a level of 0.948 proved the considerable connection of the titrated acids and anthocyanins contained in the ‘Reca’ fruits. A strong and medium correlation was established between the content of active substances of Reca, a correlation coefficient above 0.508; however, no correlation of the content of anthocyanins with the number of chalcones was detected (Table 4).

The content of monosaccharides (glucose and fructose) in berries of the ‘Elizabeth’ variety has a significant indirect correlation with fruit weight (the correlation coefficient is - 0.783). The correlation of medium strength was established between the weight of the fruit and the amount of anthocyanins (*r* = 0.555). There existed a close direct dependence between the last of the mentioned substances and soluble solids (*r* = 0.917). The positive connection between dry matter and SS, as well as the amount of sugars, titrated acids, ascorbic acid, polyphenols and chalcones was determined at the level of correlation coefficients *r* = 0.859; 0.999; 0.998; 0.976; 0.917 and 0.995, respectively. The soluble solids have a strong positive connection with most of the studied substances, medium – with glucose and fructose (*r* = 0.475) and indirect with a taste index (SAI) (*r* = 0.976). Titrated acids strongly correlated with the content of ascorbic acid, polyphenols and chalcones (*r* = 0.854, 0958 and 0.856 respectively) and had reverse correlation with the sugar-acid index (r = -0.959). SAI of ‘Elizabeth’ fruits indirectly, but significantly depends on the amount of vitamin C, polyphenolic substances, anthocyanins and chalcones (*r* = -0.966; -0.995; -0.808; -0.967 respectively). The biologically active substances of ‘Elizabeth’ berries were in a close direct correlation connection, with coefficients above 0.808 (Table 5).

The content of soluble solids, titrated acids, ascorbic acid and anthocyanins of the ‘Bluegold’ variety had an indirect strong correlation with the weight of fruits (the coefficients *r* = -0.727; -0.939; 0.764 and -0.918 respectively). The medium-strength connection was established between fruit weight and the amount of dry matter, glucose and fructose (*r* = -0.594; 0.692 respectively). The increase of the content of sugars, titrated acids and biologically active substances significantly depended on the increase of the amount of dry matter and soluble solids. There was no established dependence between the content of DM and SS with the amount of glucose and fructose (*r* = -0.170; 0.008 respectively). The correlation of glucose and fructose content with the sugar-acid index was positive (*r* = 0.729). However, the total amount of sugars had a reverse connection with the taste indicator (SAI) of the highbush blueberry variety ‘Bluegold’ (*r* = -0.754). At the same time a direct significant correlation was established with the content of the titrated acids, ascorbic acid, polyphenolic substances, anthocyanins, chalcones, the corresponding coefficient: *r* = 0.871; 0.987; 0.843; 0.898; 0.887.

Table 4. Dependence of the consumption quality indicators of *V. corymbosum* L. fruits ‘Reca’ variety

	Average mass	Dry matter	Soluble solids	Glucose and fructose	Sugar	Titrated acids	Sugar-acid index	Ascorbic acid	Poly-phenolic substances	Antho-cyanins	Chalcones
Average mass	1										
Dry matter	-0.822	1									
Soluble solids	-0.422	0.863	1								
Glucose and fructose	-0.527	0.917	0.993	1							
Tonal sugar	-0.424	0.864	0.998	0.993	1						
Titrated acids	-0.995	0.876	0.512	0.610	0.513	1					
Sugar-acid index	0.500	0.082	0.574	0.473	0.573	-0.410	1				
Ascorbic acid	-0.589	0.944	0.981	0.997	0.982	0.668	0.405	1			
Polyphenolic	-0.575	0.938	0.984	0.998	0.985	0.655	0.421	0.998	1		
Anthocyanins	-0.911	0.984	0.758	0.830	0.759	0.948	-0.099	0.870	0.861	1	
Chalcones	0.396	0.197	0.665	0.572	0.664	-0.301	0.993	0.508	0.524	0.017	1

Table 5. Correlation dependence of consumption quality indicators of *V. corymbosum* L. fruits ‘Elizabeth’ variety

	Average mass	Dry matter	Soluble solids	Glucose and fructose	Sugar	Titrated acids	Sugar-acid index	Ascorbic acid	Poly-phenolic substances	Antho-cyanins	Chalcones
Average mass	1										
Dry matter	-0.351	1									
Soluble solids	0.177	0.859	1								
Glucose and fructose	-0.783	0.858	0.475	1							
Tonal sugar	0.159	0.868	0.999	0.490	1						
Titrated acids	-0.323	0.998	0.874	0.842	0.883	1					
Sugar-acid index	0.043	-0.950	-0.976	-0.656	-0.980	-0.959	1				
Ascorbic acid	0.217	0.838	0.998	0.438	0.998	0.854	-0.966	1			
Polyphenolic	-0.040	0.950	0.976	0.653	0.980	0.958	-0.995	0.967	1		
Anthocyanins	0.555	0.584	0.917	0.084	0.910	0.608	-0.808	0.933	0.809	1	
Chalcones	0.213	0.840	0.995	0.441	0.998	0.856	-0.967	0.996	0.968	0.931	1

Table 6. Correlation dependence of indicators of consumption quality of *V. corymbosum* L. fruits ‘Bluegold’ variety

	Average mass	Dry matter	Soluble solids	Glucose and fructose	Sugar	Titrated acids	Sugar-acid index	Ascorbic acid	Poly-phenolic substances	Antho-cyanins	Chalcones
Average mass	1										
Dry matter	-0.594	1									
Soluble solids	-0.727	0.984	1								
Glucose and fructose	-0.692	-0.170	0.008	1							
Tonal sugar	-0.649	0.998	0.994	-0.101	1						
Titrated acids	-0.939	0.835	0.919	0.401	0.871	1					
Sugar-acid index	-0.011	-0.798	-0.678	0.729	-0.754	-0.335	1				
Ascorbic acid	-0.764	0.973	0.998	0.063	0.987	0.940	-0.637	1			
Polyphenolic	-0.138	0.879	0.780	-0.620	0.843	0.471	-0.989	0.744	1		
Anthocyanins	-0.918	0.864	0.940	0.348	0.898	0.998	-0.387	0.958	0.520	1	
Chalcones	-0.223	0.917	0.831	-0.549	0.887	0.546	-0.972	0.799	0.996	0.592	1

The titrated acids correlated quite strongly with the content of ascorbic acid and anthocyanins, the corresponding coefficients were 0.940 and 0.998. The amount of ascorbic acid significantly depends on the increase of the content of both polyphenols and anthocyanins and chalcones ($r = 0.744$; 0.958; 0.799, respectively). The total amount of polyphenolic substances had a significant direct connection with content of chalcones ($r = 0.996$) and the middle-strength of the anthocyanins ($r = 0.520$). The established correlation between the last two mentioned substances was on the same level ($r = 0.592$) (Table 6).

The research of different scientists (Mazza & Miniati, 1993; Prior et al., 1998; Starast et al., 2007; Correia et al., 2016) revealed the dependence of physical and biochemical quality indicators of highbush blueberries fruits, not only on the genotype of the variety, but also on weather conditions. Thus, the accumulation of sugars in berries was influenced considerably by the climatic conditions (Correia et al., 2016), and especially the light intensity, as fruit metabolism depends on photosynthesis (Mikulic-Petkovsek et al., 2014).

Using mathematical analysis, namely data approximation, we detected significant effect of one of the weather factors (precipitation during fruit development) on the content of dry matter and soluble solids in highbush blueberries (approximation coefficients - 0.565 and 0.586, respectively) (Fig. 4).

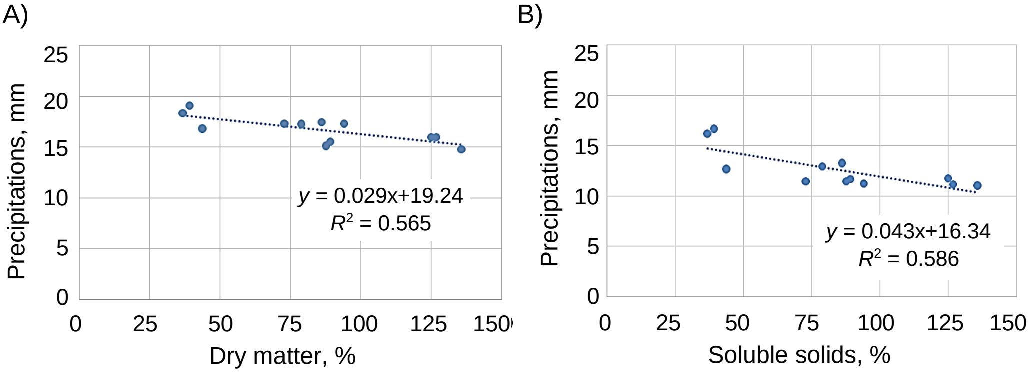

Figure 4. Correlation dependence of dry matter (A) and soluble solids (B) related to the precipitation amount.

CONCLUSIONS

It was established that the content variation of organic substances in highbush blueberry fruits of the studied varieties such as Reca, Bluegold, Elizabeth is an evidence of their genetic diversity from one side and the influence of weather conditions from the other side. Fruits of Bluegold variety according to coefficient of variation have a high stability of dry matter soluble solids, titrated acids and biochemically active substances, which is important for the breeding process.

We found a significant dependence of the content of titrated acids, polyphenols and anthocyanins on the genetic characteristics of the variety. A high correlation was found among the biochemical parameters of highbush blueberries. There was established a significant indirect effect of increasing fruit weight on the content of nutrients in fruits of Reca variety.

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Anatomical traits and structural components of peduncle associated with lodging in *Avena sativa* L.

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Abstract. Lodging dramatically reduces the yield of cereals and increases the difficulty of mechanical harvesting. Because it is a complex phenomenon, new cultivars with genetic resistance to lodging is a sustainable alternative in agricultural production systems. This resistance is associated with a combination of factors, such as stem thickness and stiffness, being closely linked to anatomical traits and structural carbohydrates present in the stem. In the present study we compared, under field conditions, eight contrasting oat cultivars in terms of lodging resistance. Our aim in this study was to investigate the association of anatomical traits and structural components of the peduncle with resistance to lodging, aiming to assist in the plant selection process. In addition, a second objective was to understand the genetic dissimilarity among oat cultivars according to the characters studied. Some characteristics for potential indirect selection were studied in this work and if correlated with lodging can be used to identify superior genotypes. From the anatomical point of view, the correlation obtained between the internal vascular bundle and the lodging resistance factor allowed us to confirm that this trait can be used in indirect selection to lodging resistance. The structural components of peduncle, in the two ways explored in the present study, comparison of mean and correlation, did not demonstrate the potential to be used exclusively as plant selection characters traits for lodging resistance. There is noticeable variability in oat cultivars for most stem traits.

Key words: cereal, indirect selection, genetic variability, oat.

INTRODUCTION

In agricultural production of cereals, lodging is a serious problem, and can drastically reduce yield, cause decreased qualitative parameters and increase difficulty of mechanical grain harvesting (Packa et al., 2015). Lodging is defined as the displacement of the stem from its vertical position (Pinthus, 1974).

The effects of lodging are increased by environment and mannagement, such as rain, strong winds, high levels of fertilization (mainly nitrogen) (Kangor et al., 2010), population densities of plants above the recommendation, intrinsic characteristics of the genotypes, as well the stage of development of the plant when subjected to lodging stress (Khobra et al., 2019). The lodging generates unfavorable conditions for plant growth, limiting the plant's access to light and air, obstructing photoassimilation and respiration, and contributing to the proliferation of pathogens (Ingver et al., 2010; Packa et al., 2015). In severe cases, lodging can block the transport of water, minerals and photoassimilates, leading to declines in grain yield (Berry & Spink, 2012; Mulsanti et al., 2018). Furthermore, lodging reduces quality of cereals produced (Berry et al., 2004; Shah et al., 2017).

Lodging resistance is associated with a combination of thickness and stiffness of stem (Ookawa et al., 2010). In this sense, morphological, anatomical traits and structural components of the stem, with emphasis on weight and diameter, concomitantly with short stature, thick walls and a high number of vascular bundles, are characteristics of cultivars with more resistance to lodging (Zuber et al., 1999). Nevertheless, it has been reported that stem stiffness is related to the main cellular components, such as cellulose, hemicellulose, lignin, silica and starch (Sato, 1957; Li et al., 2008). The lack of general associations of anatomical characters of the stem with lodging, confirms the complex nature of this phenomenon (Kelbert et al., 2004). Production of oats as well as production of other cereal grains such as wheat and barley serving as human food and animal feed are affected by lodging.

In the present study, conducted under field conditions, we compared the anatomical and structural of the stem of eight oat cultivars differing in resistance to lodging. Our objective in this study was to investigate the association of anatomical characters and structural components of the peduncle with resistance to lodging, aiming to assist in the process of plant selection.

MATERIALS AND METHODS

Plant material

Eight oats (*Avena sativa* L.) cultivars were evaluated for lodging resistance (Table 1). The data presented were obtained from the Brazilian Commission for Oat Research (2017), on average from 18 sites in the states of Rio Grande do Sul, Santa Catarina and Paraná (Brazil).

Table 1. Agronomy characters of cultivars of oats used in the study of lodging resistance

Cultivar	Height (cm)	Cycle[2] (days)	LO[3] (%)
FAEM 5 Chiarasul	117	128	42
UPFA Gaudéria	117	129	39
FAEM 4 Carlasul	117	129	39
Brisasul	110	133	33
URS Brava	127	130	31
IPR Afrodite	114	133	25
URS Altiva	120	128	15
URS Taura	101	128	15

Fonte: Lângaro et al. (2017); [2]Time from emergence to maturation; [3]Lodging occurence.

Experiment site

The experiment was conducted in the experimental field of the Faculty of Agronomy and Veterinary Medicine

(FAMV) of the University of Passo Fundo (UPF), in the municipality of Passo Fundo / RS (28º 15 '46 ”S, 52º 24' 24” W), from May to November 2018. The soil of the region is classified as humic dystrophic Red Latosol (Santos et al., 2018). The soil samples from the study site were collected from 0–20 cm depth and have the following chemical characteristics: clay = 45%; pH (H_2O) = 5.2; pH SMP = 5.6; P (mg dm^{-3}) = 29.8; K (mg dm^{-3}) = 182; organic matter = 2.9%.

The climate is humid subtropical according to the Köppen-Geiger classification (Moreno, 1961), with an average annual rainfall of 1,746 mm and temperature of 20 °C. Precipitation and wind speed for the period of the experiment were obtained from the experimental station of the Brazilian Agricultural Research Corporation (Embrapa), National Wheat Research Center, Passo Fundo, Rio Grande do Sul, Brazil (Fig. 1).

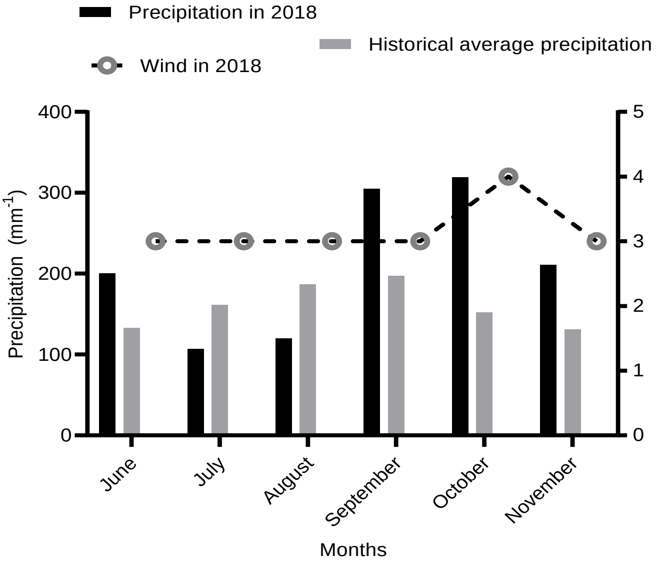

Figure 1. Monthly rainfall and average wind speed in the experimental period and climatological normals. Passo Fundo, Rio Grande do Sul, Brazil, 2020.

Source: Embrapa Wheat.

Experimental design

The treatments were eight cultivars of oats (Table 1), arranged in a randomized complete block design, with three replications. The experimental units consisted of plots of five rows and five meters in length, with a spacing of 0.17 m between rows and 0.40 m between plots.

Procedure

The site was desiccated fifteen days before the sowing of the experiment with 2 L ha^{-1} of glyphosate (Roundup Original®) + 1.5 L ha^{-1} of 2,4-D (Aminol®) + 0.5 L ha^{-1} of clethodim (Select 240®) and two days before sowing 2 L ha^{-1} of paraquat (Gramoxone). The experiment was conducted in a field with soybean [*Glycine max* (L.) Merr.] as the previous crop. The sowing rate was 300 seeds m^{-2}. Cultural treatments were carried out with reference to the phenological stages of oats (Zadoks et al., 1974). The fertilization used at sowing was 10 kg ha^{-1} N, 40 kg ha^{-1} P_2O_5, and 40 kg ha^{-1} K_2O. Nitrogen (N) was applied in the form of urea at growth stage 25 (30 kg N ha^{-1}) and at the beginning of stage 31 (15 kg N ha^{-1}). At the end of growth stage 25, 1.5 L ha^{-1} of 2,4-D (Aminol®) was used for weed control. The plots were treated with 0.8 L ha^{-1} of epoxiconazole + fluxapyroxad + pyraclostrobin (Ativum®), at growth stages 41, 61 and 70 (Zadoks et al., 1974) to control *Puccinia coronata* var. *avenae*, *Drechslera avenae* and *Puccinia graminis* f. sp. *Avenae*.

Anatomical characters and structural components of peduncle

The evaluations of peduncle characteristics occurred at the phenological stage 70 (Zadoks et al., 1974). For the evaluations, ten main stems of each cultivar cultivar were evaluated from each replication. Six anatomical traits and four structural components of peduncle and resistance to lodging were evaluated: a) neutral detergent fiber; b) acid detergent fiber; c) hemicellulose; d) lignin; e) length of the sclerenchyma over the internal vascular bundle; f) length of the sclerenchyma over the external vascular bundle; g) external vascular bundle; h) parenchyma; i) internal vascular bundle; j) sclerenchyma up to the medullary cavity.

For the evaluation of the structural components of the peduncle wall, an analysis of the neutral detergent fiber (NDF) and acid detergent fiber (ADF) content was obtained by the methods of Van Soest et al. (1991). Hemicellulose (HEM) was obtained through a difference between the levels of NDF and ADF. The determination of the lignin content (LIG) was determined by the sequential method of Van Soest (1991), in ten stems of the main stem for each cultivar.

To observe the anatomical structure inside the peduncle (Fig. 2), the middle portions of the stem tissue were used. After collecting the peduncles, they were immediately fixed in FAA (formaldehyde = 5 mL; acetic acid = 5 mL; ethyl alcohol = 90 mL). 70 for 48 h and, subsequently, preserved in 70° GL ethanol (Johansen, 1940). Anatomical cuts were made freehand, transversely from the median portion of the peduncle, later stained with a combination of fuchsin and alcian blue (Luque et al., 1996) and assembled with 50% glycerin.

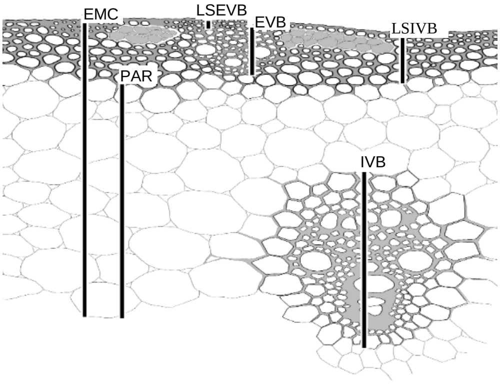

Figure 2. Scheme of the cross section of a oat stem and measured anatomical traits. Passo Fundo, Rio Grande do Sul, Brazil, 2020.

*LSIVB: length of sclerenchyma over the internal vascular bundle; LSEVB: length of sclerenchyma over the external vascular bundle; EVB: external vascular bundle; PAR: parenchyma; IVB: internal vascular bundle; EMC: sclerenchyma up to the medullary cavity.

Resistance of stem to lodging

To evaluate the stem resistance, the main stem was evaluated for lodging resistance factor (cLr): it was determined by the formula proposed by Grafius & Brown (1954):

$$\text{cLr} = \frac{F}{b}$$

where ‘F’ is the weight in grams, of the part that was suspended, of a chain with 0.5 g per link, with two links per centimeter, attached at the base of the panicle and ‘b’ is the height of the stem (cm), measured from the base of the plant to the base of the panicle.

The number of links was defined as the point at which the stem interrupted its downward movement, thus establishing a balance between resistance to torque and the torque that tended to cause lodging. In order to reach the value of the coefficient of stem resistance to lodging, the number of links in the chain was counted and multiplied by the weight of the links. Subsequently, the stem length was measured. In this index, the weight of the stem, leaves and panicle are excluded by deposition, since the plant under field conditions is fully capable of supporting its own weight.

Statistical analysis

Analysis of variance (ANOVA) was used, and in case of significant difference, the Tukey test was applied at 5% probability of error for comparisons between means. The normality and homogeneity of variances of the data were verified by the Shapiro-Wilk and the Bartlett test, with no need to transform the data for the variables under study. The data were submitted to Pearson's correlation analysis. The magnitudes of correlation coefficients were classified as $r = 0$ was considered null; $r = 0$ to 0.30 was considered weak; $r = 0.30$ to 0.60 was considered average; $r = 0.60$ to 0.90 was considered strong; $r = 0.90$ to 1 was considered very strong and $r = 1$ was considered perfect.

The cluster analyses used to assess genetic variability were performed using the hierarchical clustering methods. The analysis was performed for the anatomical traits and structural components of the peduncle, by generating the Mahalanobis distance matrix (D^2). The illustration of the (dis)similarity among cultivars was performed using a dendrogram. The number of groups was defined by the Mojena (1977) procedure, based on the relative size of distances in the dendrogram. For the variables, the relative contribution of the characters to genetic divergence was obtained, by the method of Singh (1981). For data analysis, the statistical program GENES (Cruz, 2016) was used.

RESULTS AND DISCUSSION

There was a significant difference ($p < 0.05$) among cultivars for 10 of 11 characters. The variation coefficients were low, showing an acceptable experimental error (Table 2).

The lodging resistance factor was the index used to determine the resistance among cultivars (Figs 3, 4 and 5). This index is a predictor of the strength that the stem can support until the moment of flexion (Grafius & Brown, 1954; Kashiwagi et al., 2007). Over the years, studies with oats, wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) have demonstrated the reliability of the stem resistance coefficient as a means of indirect assessment of resistance to lodging (Norden & Frey, 1959; Cruz et al., 2001; Cruz et al., 2004; Hirano et al., 2017). For this index, the highest lodging resistance factor was demonstrated by cultivar URS Taura (0.34 g cm^{-1}); and, the lowest resistance was observed in the FAEM 4 Carlasul (0.18 g cm^{-1}), FAEM 5 Chiarasul (0.18 g cm^{-1}) and URS Brava (0.17 g cm^{-1}).

Table 2. Analysis of variance for the characters studied in eight cultivars of oats (*Avena sativa* L.). Passo Fundo, Rio Grande do Sul, Brazil, 2020

Source	DF	Mean square						
		NDF	ADF	HEM	LIG	LSIVB	LSEVB	EVB
Block	2	24.54	65.86	10.18	0.01	1,298.73	234.97	568.90
Cultivar	7	38.47*	104.18*	28.25*	2.58*	8,682.24*	559.38^{ns}	4,141.19*
Error	14	8.16	6.73	4.07	0.18	1,156.55	275.06	984.78
Average	-	69.23	40.42	28.81	6.38	284.02	158.21	468.95
CV (%)	-	4.13	6.42	7.00	6.59	11.97	10.48	6.69

Source	DF	Mean square			
		PAR	IVB	EMC	cLr
Block	2	116,805.62	4,435.91	100,026.47	0.00
Cultivar	7	1,308,605.02*	162,244.90*	1,418,488.03*	0.01*
Error	14	60,842.66	4714.18	49,891.42	0.00
Average	-	1,786.95	1,244.90	2,278.00	0.23
CV (%)	-	13.80	5.52	9.81	13.27

*significant at 5% probability by the F test. ns = not significant. NDF: neutral detergent fiber; ADF: acid detergent fiber; HEM: hemicellulose; LIG: lignin; LSIVB: length of sclerenchyma over the internal vascular bundle; LSEVB: length of sclerenchyma over the external vascular bundle; EVB: external vascular bundle; PAR: parenchyma; IVB: internal vascular bundle; EMC: sclerenchyma up to the medullary cavity; cLr: lodging resistance factor.

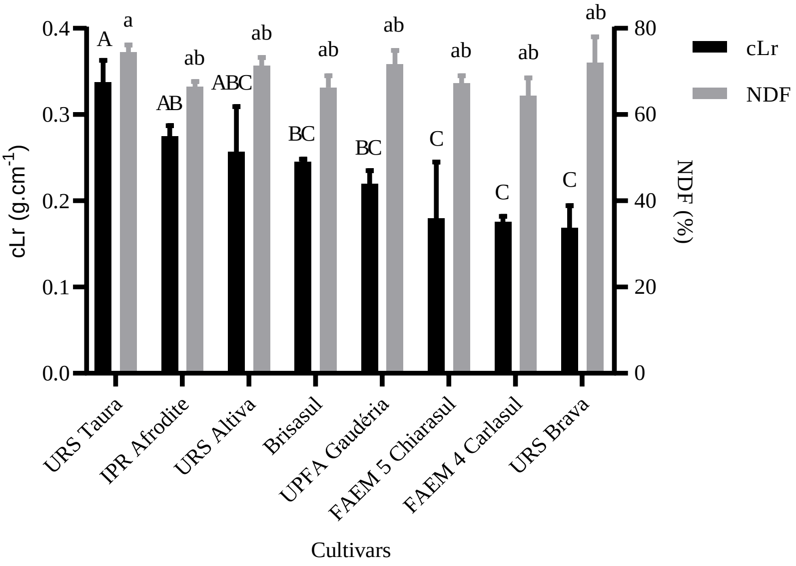

Figure 3. Structural components (cellulose + hemicellulose + lignin) through neutral detergent fiber of peduncle and lodging resistance factor. Passo Fundo, Rio Grande do Sul, Brazil, 2020.
*cLr: lodging resistance factor; NDF: neutral detergent fiber.

There are many analytical methods for measuring specific components of the cell wall (Jung, 1997). The structural components are made up of neutral detergent fiber (NDF) and acid detergent fiber (ADF). The NDF comprises the fraction composed of cellulose + hemicellulose + lignin (Fig. 3); the ADF is composed of cellulose + lignin (Fig. 4) (Van Soest, 1991). Hemicellulose, on the other hand, is obtained from the difference between the NDF and ADF fractions. In this sense, URS Taura presented greater resistance in relation to the other cultivars, as well as higher content of NDF and ADF, with 74.45 and 46.41% (Figs 3 and 4), respectively. For these structural components, the lowest levels were obtained for FAEM 4 Carlasul and FAEM 5 Chiarasul, 64.33% and 31.10%, respectively. Both cultivars showed low resistance to lodging (0.18 g cm^{-1}). The structural components of the peduncle do not prove to be parameters for identifying genotypes in terms of lodging resistance.

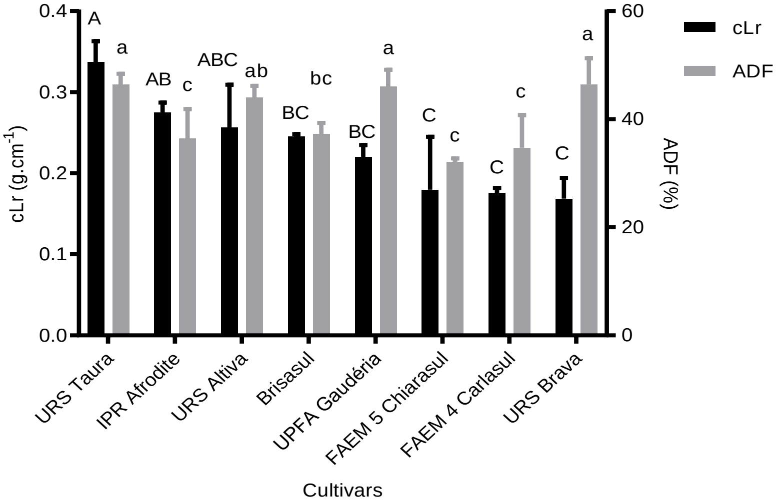

Figure 4. Structural components (cellulose + lignin) through the acid detergent fiber in the peduncle and lodging resistance factor. Passo Fundo, Rio Grande do Sul, Brazil, 2020.

*cLr: lodging resistance factor; ADF: acid detergent fiber.

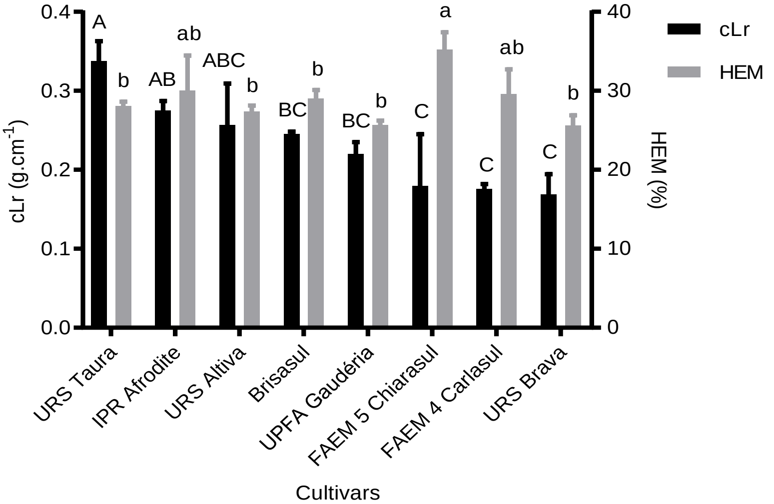

Figure 5. Peduncle hemicellulose and lodging resistance factor. Passo Fundo, Rio Grande do Sul, Brazil, 2020.

*cLr: lodging resistance factor; HEM: hemicellulose.

For the hemicellulose character the cultivar URS Taura show a distinct tendency (Fig. 5) than for ADF and NDF. The cultivar FAEM 5 Chiarasul showed higher levels for this character (35.20%), in addition to low lodging resistance. The lowest content of this character was obtained with URS Brava (25.58%), concomitant with the lowest lodging resistance factor (0.17 g cm^{-1}). The structural components of the stem are formed by cellulose, hemicellulose and lignin that play a significant role in stem resistance to lodging, as they constitute the cell wall of vegetables (Wang et al., 2006; Kong et al., 2013). In wheat, higher levels of lignin and hemicellulose increased stem resistance and reducing lodging (Berry et al., 2003). In contrast, in this study, the levels of hemicellulose (Fig. 5) and lignin (Fig. 6) were higher for cultivar FAEM 5 Carlasul and its lodging resistance factor was lower than other cultivars.

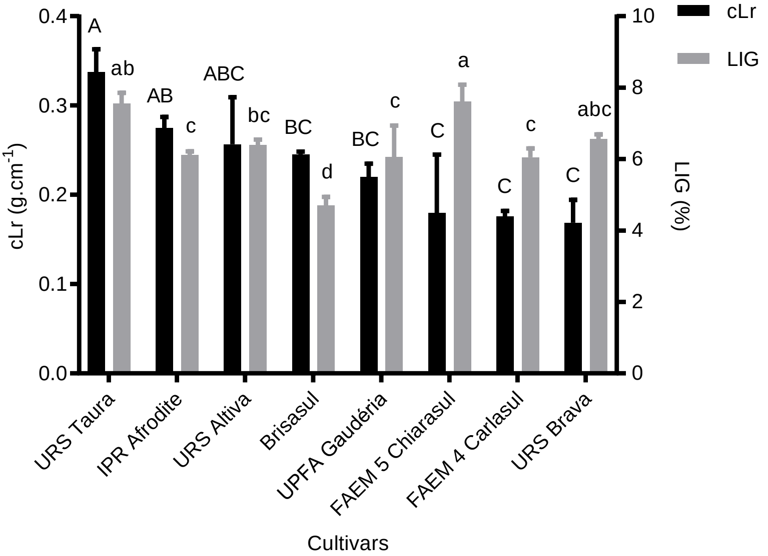

Figure 6. Peduncle lignin content and lodging resistance factor. Passo Fundo, Rio Grande do Sul, Brazil, 2020.

*cLr: lodging resistance factor; LIG: lignin.

For lignin (Fig. 6), cultivars FAEM 5 Chiarasul, URS Taura and URS Brava presented the highest levels with values of 7.61%, 7.55% and 6.56%, respectively; already, the lowest value was obtained for the cultivar Brisasul (4.70%). The lignin content showed a negative correlation with the sclerenchyma length over the internal vascular bundle (Table 4). In wheat, lignin was strongly correlated with stem diameter, wall thickness and stem lodging resistance index (Kamran et al., 2018). This reinforces the idea that the lignin content is correlated with the resistance to lodging, as it provides rigidity and mechanical support determining the stem physical strength; low levels of these components weaken it (Berry et al., 2003; Ookawa et al., 2014; Dorairaj & Ismail, 2017). The correlation between stem strength and lignin suggests that genotypes with greater lignin accumulation can be used in breeding programs in order to develop cultivars that are more resistant to lodging (Peng et al., 2014; Shah et al., 2017). However, in the present study, the highest levels of lignin did not show an association with the lodging resistance factor (Fig. 6). The results obtained corroborate a study conducted on rice, in which the highest lodging resistance factor was not always obtained in cultivars with a higher lignin content (Okuno et al., 2014). Furthermore, in another study, with barley, it was shown that lignin and hemicellulose may also not be associated with resistance to lodging (Stanca et al., 1979). According to Ma (2009) the role of lignin and cellulose in resistance to lodging is not yet consistent for use in indirect selection for resistance to lodging.

The correlations between the evaluated characteristics are shown in Table 3. Among the evaluated traits, only the internal vascular bundle showed a significant correlation coefficient with the coefficient of stiff resistance to lodging (Table 3). In work with barley and wheat, there was no evidence of a correlation in the number of vascular bundles of basal internodes and resistance to lodging (Stanca et al., 1979; Zuber et al., 1999; Kong et al., 2013). Also in wheat, it was shown that large proportions of sclerenchymatic tissue are correlated with resistance to lodging (Wang et al., 2006). More recently, in rice, there was a positive correlation between internal and external vascular bundles and resistance to lodging (Zhang et al., 2016). Differences in correlations among characters can be attributed to the variability between cultivars used. Therefore, for each set of cultivars, an appropriate selection strategy must be chosen (Lúcio et al., 2013).

Table 3. Correlation coefficient among anatomical traits, structural components of peduncle and lodging resistance factor. Passo Fundo, Rio Grande do Sul, Brazil, 2020

	NDF	ADF	HEM	LIG	LSIVB	EVB	IVB	PAR	EMC
NDF	1								
ADF	0.90**	1							
HEM	-0.57	-0.87**	1						
LIG	0.46	0.11	0.33	1					
LSIVB	-0.21	0.00	-0.25	-0.78**	1				
EVB	0.01	-0.19	0.37	0.53	-0.11	1			
IVB	0.22	0.19	-0.11	0.05	0.53	0.68	1		
PAR	0.06	0.25	-0.41	-0.59	0.88**	-0.04	0.59	1	
EMC	0.12	0.30	-0.44	-0.55	0.87**	0.00	0.63	1.00**	1
cLr	0.42	0.34	-0.16	0.10	0.43	0.30	0.76*	0.35	0.40

** and * significant at 1 and 5% probability by t test.

*ADF: acid detergent fiber; HEM: hemicellulose; LIG: lignin; LSIVB: length of sclerenchyma over the internal vascular bundle; EVB: external vascular bundle; PAR: parenchyma; IVB: internal vascular bundle; EMC: sclerenchyma up to the medullary cavity; cLr: lodging resistance factor.

Based on the correlations obtained (Table 3), the internal vascular bundles of the peduncle are indicated for indirect selection aiming at lodging resistance. In addition, cultivars that are more resistant to lodging presented greater internal vascular bundle length compared to the most susceptible cultivars (Fig. 7). For this character, the cultivars URS Taura, IPR Afrodite and UPFA Gaudéria showed the highest thickness with values of 1,617.74 μm, 1,443.94 μm and 1,425.65 μm, respectively; already, the smaller thickness of the internal vascular bundles was found in URS Brava (954.92 μm).

Estimates of the relative contribution of the ten anatomical characters and structural components of peduncle to the genetic dissimilarity among the eight cultivars of oats were evaluated by the method of Singh (1981) and are shown in Table 4. The traits that contributed most to genetic divergence were neutral detergent fiber and hemicellulose totaling 89.37% of the genetic divergence among oats cultivars. Otherwise, the characters with the least contribution to genetic dissimilarity were sclerenchyma, lignin, length of sclerenchyma over the internal vascular bundle, internal vascular bundle, and lodging resistance factor, acid detergent fiber, external vascular bundle and parenchyma. The selection of genotypes for breeding based only on genetic divergence, without considering their traits of interest, can be an inefficient strategy in a breeding program (Carpentieri-Pípolo et al., 2000). Thus, in addition to the genetic dissimilarity for identifying parents, their performance *per see*, in specific environments, deserves greater attention (Nardino et al., 2017).

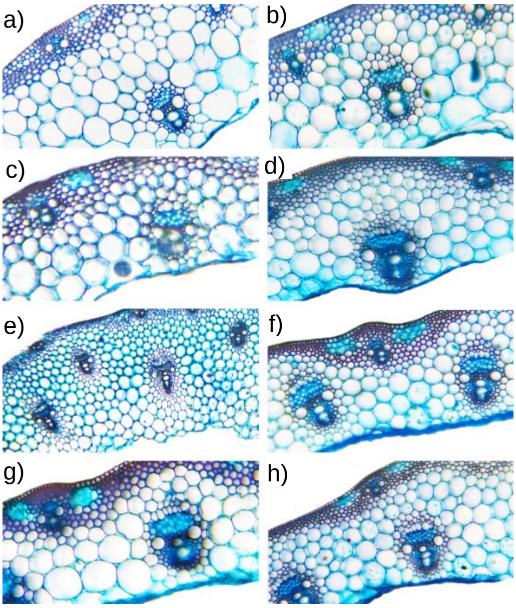

Figure 7. Cross section of the stem of eight cultivars of oats. Passo Fundo, Rio Grande do Sul, Brazil, 2020.

*a) Brisasul; b) FAEM 4 Carlasul; c) FAEM 5 Chiarasul; d) IPR Afrodite; e) UPFA Gaudéria; f) URS Altiva; g) URS Brava; h) URS Taura.

Table 4. Relative contribution of structural and anatomical components of peduncle to genetic dissimilarity (RCGD) among eight cultivars of oats, in decreasing order of importance by the method of Singh (1981), based on the values of Sj. Passo Fundo, Rio Grande do Sul, Brazil, 2020

Characte[1]	Sj	RCGD (%)
NDF	50,984.69	65.22
HEM	18,881.30	24.15
EMC	3,190.62	4.08
LIG	2,601.77	3.33
LSIVB	1,819.55	2.3275
IVB	560.47	0.72
cLr	136.89	0.18
ADF	0	0
EVB	0	0
PAR	0	0

*NDF: neutral detergent fiber; HEM: hemicellulose; EMC: sclerenchyma up to the medullary cavity; LIG: lignin; LSIVB: length of sclerenchyma over the internal vascular bundle; IVB: internal vascular bundle; cLr: lodging resistance factor; ADF: acid detergent fiber; EVB: external vascular bundle; PAR: parenchyma.

Through hierarchical methods, it is possible to illustrate the formation of groups by means of dendrograms (Fig. 8). The Mahalanobis distance matrix (data not shown) revealed high amplitude in this measure of dissimilarity, between 37.17 to 949.37. The cultivars Brisasul and URS Taura were the most dissimilar ($D^2 = 949.37$), whereas FAEM 4 Carlasul and IPR Aphrodite were the most similar ($D^2 = 37.17$). The cofenetic correlation coefficient, which estimates the representativeness of data from the dendrogram dissimilarity matrix, revealed a magnitude of 0.72, which indicates that the the data of matrix showed a satisfactory adjustment in the graphical representation presented by the dendrogram (Fig. 8). The cofenetic correlation index, when greater than 0.70, indicates the adequacy of the method to the adopted dissimilarity matrix (Streck et al., 2017).

The UPGMA grouping method, using the averages of the distances between all pairs of cultivars for the formation of each group, allowed the formation of two distinct groups, with a higher concentration of cultivars in group I (75%), which were: group 1 (FAEM 4 Carlasul; IPR Aphrodite; URS Brava; URS Altiva; FAEM 5 Chiarasul; URS Taura); group 2 (UPFA Gaudéria; Brisasul). This type of distribution portrays the high similarity between genotypes (Kaur et al., 2018).

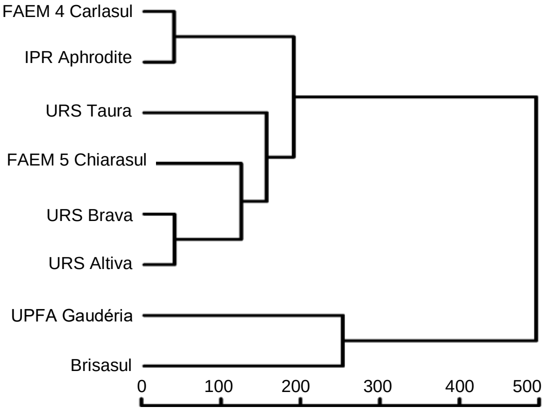

Figure 8. Dendrogram obtained by the hierarchical grouping method UPGMA, based on the Mahalanobis distance (D²) from the characters studied in eight cultivars of oats. Passo Fundo, Rio Grande do Sul, Brazil, 2020.

In the dendrogram (Fig. 8), group 1 was formed by six cultivars, due to the higher levels of neutral detergent fiber (average = 69.32%) and hemicellulose (average = 29.30%) in relation to group 2. Regarding the characters of lower relative contribution (Table 4), group 1 had a higher lignin content (mean = 6.71%), shorter sclerenchyma length over the internal vascular bundle (mean = 261.47 µm) and internal vascular bundle (mean = 1,213.51 µm) in relation to group 2. The resistance to lodging was measured through the lodging resistance factor, considered as the main parameter to predict the physical strength that the stem can support. Despite presenting a low relative contribution to genetic divergence (Table 4), among the eight cultivars of oats studied, the groups had the same average value of 0.23 g cm^{-1}.

Rapid investigations based on morphological, anatomical and structural components of plants are frequently considered in the literature. It was expected that cultivars with higher stem resistance to lodging had higher levels of structural components, such as neutral detergent fiber, acid detergent fiber, hemicellulose and lignin. This was not observed for all cultivars studied. The anatomical components, for cultivars with greater stem resistance to lodging, presented higher sclerenchyma values over the internal and external vascular bundle, internal and external vascular bundle and sclerenchyma compared to the cultivars most susceptible to lodging. In this sense, the number of vascular bundles in combination with the lignin content can confer resistance to lodging (Stanca et al., 1979; Kong et al., 2013). However, some authors report that, in anatomical terms, no feature was found associated with lodging and, therefore, not useful for indirect selection in breeding programs (Kelbert et al., 2004). Thus, in cereals, the selection of plants with resistance to lodging, should not be made based only on anatomical characters and structural components of stem. In addition, traits used to select superior genotypes in terms of lodging resistance should be: 1) fast, easy and stable to measure; 2) strongly inheritable; and 3) be strongly correlated with resistance to lodging (Shah et al., 2017).

Some characteristics, possible for selection were studied in this work and can be used to identify of superior genotypes in terms of lodging resistance. There is noticeable variability in oat cultivars for most stem traits. In addition to visual assessments of lodging, the lodging resistance factor is the most used test, as it is a predictor of the strength that the stem can withstand. Finally, the peduncle structural components, in the two ways explored in the present study, did not demonstrate a concise result to be used exclusively as plant selection characters to lodging resistance. The correlation obtained between the internal vascular bundle and the lodging resistance factor allowed us to confirm that this character can be used in indirect selection aiming to lodging resistance.

CONCLUSIONS

Variability in stem characters was observed among the eight whit oat cultivars studied. Of the anatomical characters, the internal vascular bundles were associated with resistance to lodging, allowing to identify it as a possible character of selection of plants. For the structural components of the stem, there was no significant association with resistance to lodging. The lodging resistance factor is an important tool for assessing stem resistance to lodging in oats.

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Effects of retained fetal membranes treatments and dry period length on the subsequent lactation in cows - milk yield and somatic cell count

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Abstract. Different treatment strategies in cows with retained fetal membranes (RFM) may affect subsequent lactation in various ways. Also, excessively short or long dry periods (DP) can decrease milk yield (MY), increase the risk of poor udder health, and increase the risk of RFM. This study aimed to evaluate how different treatment strategies of RFM affect subsequent lactation in cows, i.e., MY and udder health determined on the somatic cell count (SCC) bases in milk, expressed as somatic cell score (SCS). A secondary but equally important objective was to analyse the dry period length (DPL) effect on the subsequent lactation in cows. The cows from two herds were divided into three groups: group 1–healthy control; group 2–cows with RFM, removed manually and treated with intrauterine (IU) antibiotics (AB); group 3–cows with RFM, not removed, but treated IU with AB. The DP of cows was divided *post factum* into shortened (up to 46 days), traditional (47 to 70 days), or prolonged (over 70 days). Statistical analyses were performed using linear multiple regression and multivariate analyses. Differences were statistically significant when $P < 0.05$. The effect of different RFM treatment strategies on MY and SCS was evaluated. There were no statistically significant effects of RFM treatments on the MY in the subsequent lactation. However, there was a tendency ($P = 0.07$) for SCS in standard lactation to be higher in cows in group 2. The highest economic losses, calculated from the decrease in MY, were detected in the same group. Significantly lower MY was observed in cows with a shortened DPL during the first 30 days PP ($P < 0.05$). The DPL did not affect the SCS.

Key words: cows, retained fetal membranes, treatment strategies, dry period length, milk yield, somatic cell count.

INTRODUCTION

Fetal membranes in cows are considered to be retained if they have not been expelled during the first 24 hours after a calf delivery (Guard, 1999; Kimura et al., 2002; Risco & Hernandez, 2003; Maas, 2004; Han & Kim, 2005; Mordak, 2006; LeBlanc, 2007; Sheldon et al., 2008; Könyves et al., 2009; Dubuc et al., 2011; Zobel & Tkalčić, 2013; Gilbert, 2014; Opsomer, 2015). Tucho & Ahmed (2017) indicated that RFM causes significant economic losses, mainly reduced milk yield, worse reproductive performance, and infertility. Therefore, it is important to choose the right treatment strategy to improve animal health and future productivity and avoid animals' early culling. Melendez et al. (2006) pointed out that RFM decreases milk yield, but cow treatment may counteract this decrease. Their study demonstrated that applied monensin capsule before expected parturition decreased the incidence of RFM and improved milk yield in multiparous cows. Goshen & Shpigel (2006) wrote that a 2-week treatment using intrauterine tetracycline for RFM did not affect milk production. However, Bayril et al. (2015) demonstrated that supplementation in feed with Se and vitamin E during the dry period increases Se level in serum, milk yield, and improves udder health (decreasing SCC) in subsequent lactation, but did not decrease the number of RFM cases.

Excessively short or long dry periods (DP) have multiple adverse effects. The DP is considered as the cow's resting period, about 60 days long before calving. Numerous scientific studies have indicated that shortening or omitting the DP may result in improvements in animal health and energy balance, as well as provide economic and management benefits (Grummer & Rastani, 2004; Rastani et al., 2005; van Knegsel et al., 2013; Heeren et al., 2014), and possibly even improve cow fertility in general (Gümen et al., 2005; Pezeshki et al., 2008; Watters et al., 2009). However, this strategy may cause an increase in RFM (Hamidreza et al., 2017). Bachman (2002) and Annen et al. (2004) demonstrated that milk yield is not affected by shortening or omitting the DP, while other studies show that a shorter DP results in lower MY during the first 100 days PP (Rastani et al., 2005; Steeneveld et al., 2014; Hamidreza et al., 2017; O`Hara et al., 2017) and a higher SCC (O`Hara et al., 2019). Van Knegsel et al. (2014) research demonstrated that shortening or omitting the DP reduced the total MY, protein yield, fat and lactose percentage ($P < 0.05$). However, omitting the DP increases the percentage of milk fat and protein, and SCC in milk, compared to a traditional DP (60 days). O`Hara et al. (2020) described that a shortened DP, as well as a prolonged dry period from 80 to 89 days, both reduce milk yield and increase SCC in milk ($P < 0.001$).

In order to accurately assess SCC during a study, these values should be expressed as somatic cell scores (SCS). The Western Canadian Dairy Herd Improvement Services DHI explains that linear scores, or SCS, are used to determine the udder health status of a cow or herd. A very high SCC for at least one cow in a herd strongly affects the herd's average SCC, but the average SCS of the herd is less affected. Thus, the mean lactation SCS is more representative of real milk loss than the average SCC. Dadpasand et al. (2013) described in their study that high SCS is associated with udder health and milk loss and negatively affects dairy cows' fertility and longevity.

The present study hypothesised that different RFM treatment strategies and different dry period length (DPL) could significantly affect MY and SCS in cows during the subsequent lactation. Therefore, the present study aimed to evaluate how different treatment strategies of RFM affect subsequent lactation in cows, i.e., MY and udder health determined on the somatic cell count (SCC) bases in milk expressed as somatic cell score (SCS). A secondary but equally important objective was to analyse the DPL effect on the MY and SCS in the subsequent lactation.

MATERIALS AND METHODS

Animals and Management

The present research was performed in two Holstein's black-and-white breed dairy herds with 650 and 300 cows. Thirty multiparous cows were included in this study: 9 apparently healthy cows with normal expulsion of fetal membranes were randomly selected from both herds, and 21 RFM cows, apparently healthy at the moment when cow grouping was done, 3–8 years old were selected according to the report from a farm veterinarian. RFM cows were examined clinically.

During this study, the average milk yield per cow in a standard lactation was 7,975 kg per year on the first farm and 6500 kg on the second farm, but the average SCC in milk was 197,000 cells mL^{-1} and 248,000 cells mL^{-1}, respectively. On both farms, all management procedures were similar. The cows were fed twice a day, and water was freely available. During the winter, the animals on both farms were head-haltered in a tie-stall system barn, fed silage of maize, alfalfa, clover and grass, prepared in film wrapped rolls, in silage pits and mixed in a feed mixer. Before the silage, the cows were fed concentrates (barley+wheat) and trace elements, rapeseed flour, and salt. During summer, the cows were allowed to graze for twenty-four hours, while during milking, they were fed concentrates with trace elements, salt and rapeseed flour. In their study, Leso et al. (2019) indicated that the keeping system directly affects dairy cows' longevity and udder health status.

The dry period on both farms was planned to be 60 days.

RFM treatment strategies

All study animals (*n* = 30) were divided into 3 groups depending on the expulsion of fetal membranes and the applied treatment strategy (Table 1). Group 1 was the control. Group 2 was RFM treatment group where fetal membranes were manually easily separable, and group 3 was RFM treatment group where RFM not easily separable. For all treatment group cows, the cotyledon-caruncle junction was gently tested manually for the first two caruncles. Group 2 and group 3 RFM cows were treated with antibacterial preparation-neomycin sulfate 350000 IU and oxytetracycline hydrochloride 500 mg (Gynobiotic bolus; Novartis Animal Health, Slovenia), 3 boluses were inserted into the uterus.

Table 1. Allocation criteria for cows into the study groups during the deliberate grouping

Group of cows	Fetal membranes expelled during 24 h PP	RFM removed manually	Cows treated IU with antibiotics
Group 1 (*n* = 9)	YES	-	NO
Group 2 (*n* = 13)	NO	YES	YES
Group 3 (*n* = 8)	NO	NO	YES

All cows involved in the present investigation were clinically examined in the first 14 days postpartum (PP). RFM cows which increased a rectal temperature ≥ 39.5 °C, showed signs of PP metritis, but still had appetite received systemic antibiotic treatment with ceftiofur hydrochloride (1.1 mg kg^{-1} subcutaneously per day) for 3 consecutive days. In most severe case (cow did not eat, became apathetic), animals received procaine benzylpenicillin (10 mg kg^{-1} intramuscularly per day) for 5 consecutive days. PP metritis was diagnosed when the uterus becomes filled with a large amount of reddish-brown foul-smelling uterine discharge, mostly containing necrotic tissue debris (putrid discharge that corresponds with Sheldon et al. (2006) definitions of uterine diseases).

The reproductive performance of the multiparous cows involved in the study is shown in Table 2.

Table 2. The summary of cows' reproductive performance

The reproduction indices	Group 1 ($n = 9$)	Group 2 ($n = 13$)	Group 3 ($n = 8$)
Days open to the first service, days	79 ± 9.7^{A}	100 ± 14.2	121 ± 28.3^{A}
Services per pregnancy	3.22 ± 0.66	2.25 ± 0.46	1.83 ± 0.47
First service conception, %	$11^{B,C}$	$33^{B,D}$	$50^{D,C}$
Days open, days	187 ± 25.3	132 ± 19.7	163 ± 22.4

^{A,B,C,D} $P < 0.05$; Group 1 – control group; group 2 – cows with RFM, manually removed and IU antibiotics; group 3 – cows with RFM, not removed and IU antibiotics.

Dry period length (DPL)

The DPL of cows ($n = 30$) was divided *post factum* into the shortened DPL up to 46 days, the traditional DPL from 47 to 70 days, and the prolonged DPL over 70 days. The influence of DPL on MY and SCC was analysed in the control group cows ($n = 9$), where fetal membranes expelled during 24 h PP and cows with RFM ($n = 21$). The DPL for each animal involved in the study was obtained from the Agricultural Data Centre Republic of Latvia*. Agricultural Data Centre Republic of Latvia is a State Institution under the supervision of the Ministry of Agriculture that was established in 1997 to aggregate, process and analyse zootechnical, veterinarian and agricultural information in the Latvian Republic with the goal to create a whole state animal and herd register, pedigree information system in compliance with international requirements.

In both herds, dry cow treatment was done using Cloxacillin benzathine 600 mg together with ampicillin trihydrate 300 mg in a long-acting base (Bovaclox DC, Norbrook Laboratories Ltd, Ireland). The cows were clinically healthy before dried off.

Milk yield (MY) and Somatic cell count (SCC)

MY and SCC for the cows in current and previous lactation were obtained from Latvia's Agricultural Data Centre. Both parameters were evaluated for 30 days PP (MY_{30}; SCC_{30}), 100 days PP (MY_{100}; SCC_{100}) and 305 days PP (MY_{305}; SCC_{305}). The difference in MY and SCC were calculated subtracting the previous lactation from the current lactation.

Somatic cell count in milk indicates not only udder health but also the health of the entire cow (Sematovica et al., 2020). In the present study, the optimal threshold for cow SCC was defined as 200,000 cells mL^{-1} based on previous research by numerous scientists (DeGraves & Fetrow, 1993; Harmon, 1994; Hillerton, 1999; Madouasse et al., 2008; Lusis et al., 2010; Petzer et al., 2017; Lusis et al., 2019). All SCC data were transformed into the logarithmic [$\log2 (SCC \times 10^{-5}) + 3$] somatic cell scores (SCS) before statistical comparison. The lactation average SCS was the arithmetic mean of the

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*www.ldc.gov.lv

monthly test day SCC from 7 to 305 days after calving. SCC was expressed on a SCS scale from 0 to 9, where (0) ranged from 0 to 24,000 cells mL^{-1}; (1) 25,000–49,000 cells mL^{-1}; (2) 50,000–99,000 cells mL^{-1}; (3) 100,000–199,000 cells mL^{-1}; (4) 200,000–399,000 cells mL^{-1}; (5) 400,000–799,000 cells mL^{-1}; (6) 800,000–1599,000 cells mL^{-1}; (7) 1600,000–3199,000 cells mL^{-1}; (8) 3200,000–6399,000 cells mL^{-1} and (9) 6400,000–12799,000 cells mL^{-1}.

Statistical analysis

Data were analysed using software Stata IC 12.1 (StataCorp LP, 4905 Lakeway Drive, College Station TX77845, USA, version Stata IC 12.1 for Windows) and displayed for each group as a mean ± SEM. Differences between current and previous lactation MY_{30}, MY_{100}, $MY_{305,}$ SCS_{30}, $SCS_{100,}$ and SCS_{305} were analysed using a Wilcoxon matched-pairs signed-rank test. Linear multiple regression (Multivariable analyses) were performed to assess the RFM treatment's impact on the all above mentioned MY and SCS. Multivariate regression analyses of variance (MANOVA) were performed to assess the impact of DPL on the all above mentioned MY and SCS. All effects were corrected for herd influence. Distribution of DPL between herds was compared by Fisher`s exact test. Differences in results were evaluated as statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

The analyses of RFM treatment strategies' effects on MY_{30} and SCS_{30} showed no significant differences between the study groups ($P > 0.05$). Comparing the current lactation to the previous of each individual cow, MY_{30} in group 2 cows (RFM, manually removed, AB treated) was decreased on average by 98.74 ± 91.19 kg (corrected for herd effect) as compared to the control group as a reference category. In group 3 (RFM, not removed, AB treated) the decrease was on average by 52.89 ± 99.06 kg. Despite a lack of a significant difference in MY, there were considerable losses noticed from an economic point of view (Table 3). For 1L of milk, the average production cost was 0.24 euro in those herds, and the average purchase price was 0.30 euro, resulting in a total loss of 0.54 euro.

Table 3. Milk yield decrease and economic losses in cows with retained fetal membranes during the first 30 days PP

Group of cows	Milk yield decrease per cow, L	Milk yield decrease per cow group, L	Economic losses per cow, euro	Economic losses per group, euro
Group 1 (*n* = 9)	Reference category	Reference category	Reference category	Reference category
Group 2 (*n* = 13)	98.74	1,283.62	53.32	693.15
Group 3 (*n* = 8)	52.89	423.12	28.56	228.48

Group 1 – control group; group 2 – cows with RFM, manually removed and IU antibiotics; group 3 – cows with RFM, not removed and IU antibiotics.

Comparing the current to the previous lactation of each individual cow, SCS_{30} in cows group 2 and 3 was on average 1.42 ± 1.25 and 1.38 ± 1.36 (corrected for herd effect) higher than in the control group cows, respectively ($P > 0.05$).

Continuing to analyse milk yield parameters and udder health in study cows in the first 100 days PP, there were no significant differences between them ($P > 0.05$). Comparing the current to the previous lactation of each individual cow, MY_{100} in group 2 cows was decreased by 328.13 ± 254.04 kg (corrected for herd effect) compared to the control group as a reference category. In cows of group 3, this tendency was weak. Basically, SCS_{100} was increasing between days 30 and 100 PP in both herds; from 3.03 ± 0.56 to 3.74 ± 0.38 in the herd 1 and from 4.01 ± 0.45 to 4.38 ± 0.37 in the herd 2. Comparing cows' groups, the SCS_{100} in groups 2 and 3 were less than one unit higher than the SCS_{100} of the control group. The economic losses for MY_{100} are shown in Table 4.

Table 4. Milk yield decrease and economic losses in cows with retained fetal membranes during the first 100 days PP

Group of cows	Milk yield decrease per cow, L	Milk yield decrease per cow group, L	Economic losses per cow, euro	Economic losses per group, euro
Group 1 (*n* = 8)	Reference category	Reference category	Reference category	Reference category
Group 2 (*n* = 12)	328.13	3,937.56	177.19	2,126.28
Group 3 (*n* = 7)	21.50	150.50	11.61	81.27

Group 1 – control group; group 2 – cows with RFM, manually removed and IU antibiotics; group 3 – cows with RFM, not removed and IU antibiotics.

Further evaluating the effect of RFM treatment methods on MY during the standard lactation period (305-days milk yield), there were no significant differences between the study groups ($P > 0.05$). In contrast, SCS_{305} differences in the standard lactation period in group 2 by 1.51 ± 0.78 ($P = 0.07$), in group 3 by 1.09 ± 0.98 ($P > 0.05$), respectively, were higher than in the control. The variance of MY_{305} results was too high to compare economic losses.

Tucho & Ahmed (2017) indicated that RFM causes considerable economic losses, mainly due to reduced MY and infertility. Furthermore, because there are many different causes of fetal membrane retention, commonly used RFM treatment strategies do not show any significant effects. In principle, management systems must be designed to decrease the occurrence of RFM. This could be achieved by genetic selection, proper feeding during the dry period, and increased activity such as walking (Tucho & Ahmed, 2017).

The dry period is an important phase of a dairy cow's life cycle. During this phase, the cow and its udder are preparing for the next lactation; hence any abnormalities during the dry period will have a negative effect on the cow's health and milk production after parturition. In the present research, data was collected and analysed to understand the effect of the DPL on MY and SCC in subsequent lactations of cows with and without RFM.

In the control group, cows with fetal membranes expelled during 24 h PP, the average DPL was 66 ± 4.84 days (57–101 days), but in cows with RFM, the average DPL was 60 ± 4.38 days. For analysis, the dry period of cows was divided as follows: shortened DPL averaging 30 ± 5.20 days (4–46 days), 20% of cases; traditional or standard DPL averaging 63 ± 1.58 days (47–70 days), 60% of cases; and prolonged DPL averaging 79 ± 4.46 days (71–108 days), 20% of cases. The authors of various articles have defined the dry period of dairy cows differently. No set standard exists, but there are common trends: < 49 days is considered a shortened DP or shorter than the conventional DP; a period of 49–70 days is considered a traditional, standard, or conventional DP (Gulay et al., 2003; Kuhn et al., 2006; Pezeshki et al., 2008; Steeneveld et al., 2013; van Knegsel et al., 2014; Sawa et al., 2015; Hamidreza et al. 2017; O'Hara et al., 2017; O'Hara et al., 2019), and over 70 days is a prolonged DP (Sawa et al., 2015; Hamidreza et al. 2017; O'Hara et al., 2020).

In the present research, the DPL was different between herds ($P < 0.05$) (Table 5). The first herd had more cows with a prolonged DPL (6 out of 12). In contrast, the second herd had more cows with a shortened DPL (5 out of 18). Therefore, the effects of DPL on MY and SCS were statistically corrected for the herd influence.

Table 5. Evaluating dry period length by a herd

Herd	Shortened DPL	Traditional DPL	Prolonged DPL	Fisher`s exact test	Total
1	$n = 1$	$n = 5$	$n = 6$	$P = 0.018$	$n = 12$
2	$n = 5$	$n = 12$	$n = 1$		$n = 18$
Total	$n = 6$	$n = 17$	$n = 7$		$n = 30$

In the present research, the effect of shortened or prolonged DPL on MY_{30} and SCS_{30} was compared to the traditional DPL. When comparing shortened DPL with the traditional DPL, the cows with shortened DPL had significantly lower MY by 222.45 ± 96.05 kg ($P < 0.05$). In contrast, no differences were found between prolonged DPL and traditional DPL ($P > 0.05$). O'Hara et al. (2019) mentioned that a 30–day DP might not be long enough for the mammary tissues to adapt to the coming lactation, especially if the DPL is only a few days. Also, in their study, Sawa et al. (2015) indicated that excessively shortened DP in older cows resulted in a daily MY decrease of more than 10% and have a significant effect on udder health during the first 30 days of subsequent lactation. This was confirmed for MY in the present study, but there were not found significant differences in SCS_{30} between the shortened and traditional DPL ($P > 0.05$).

Evaluating the effect of shortened or prolonged DPL on MY_{100} and SCS_{100} was compared to the traditional DPL. Cows with the shortened DPL continued to show the same tendency as in the first 30 days PP. Respectively, MY_{100} was 385.69 ± 272.26 kg lower than in cows with traditional DPL ($P > 0.05$). In cows with prolonged DPL, no significant decrease was observed if compared to a traditional DPL. In their studies, O'Hara et al. (2017) pointed out that milk yield during the first 100 days PP was reduced for the shorter 30-day DP cows compared with 60-day DP cows, but there was no significant difference in total MY. This tendency was also confirmed in the present study that cows with the shortened DP period had a lower MY than those of the traditional DP. Even van Knegsel et al. (2014) emphasised in their research that MY in the first 100 days PP was less in cows with a short or no dry period ($P < 0.01$) compared to cows with a conventional DP. This was also confirmed by Steeneveld et al. (2013) study. However, all the studies of the previously mentioned authors were performed in clinically healthy cows. In contrast, this study evaluated milk production and milk SCC in cows with RFM. When evaluating present study SCS_{100}, no significant differences were observed between cows with different DPL ($P > 0.05$).

Further evaluating the effect of DPL on MY_{305} and SCC_{305} in the standard lactation, it was concluded that the shortened DPL negative impact on MY_{305} continued. Respectively, MY_{305} in shortened DP was 914.05 ± 642.56 kg lower than in cows with traditional DPL ($P > 0.05$). However, no significant decrease was observed in cows with prolonged DPL from cows with traditional DPL ($P > 0.05$). This is in line with O'Hara et al. (2020) investigations indicating that MY in the 305-days lactation was low if the DP was 30–39 days long, which corresponds to the present research with shortened DPL. However, our research differed concerning the effect of prolonged DPL. Unlike no difference in the present study, O'Hara et al. (2020) found the milk yield in the 80–89 day group was the lowest ($P < 0.001$). Also, Hamidreza et al. (2017), in their study, described that reducing DPL to less than 45 days had an adverse effect on MY, milk components, and health status. Whereas, Pezeshki et al. (2008) declared that a 28–days DP had no adverse effect on 305–days MY and on the status of multiparous dairy cow health compared with a standard DP (49–days).

When evaluating present study SCS_{305}, neither shortened DPL nor prolonged DPL showed a significant effect ($P > 0.05$). Van Knegsel et al. (2014) and Steeneveld et al. (2013) indicated that there are no differences in SCC between short and conventional DPL in subsequent lactation, which is also confirmed in the present study. However, Kuhn et al. (2006) indicated that prolonged DPL decreases SCC in the subsequent lactation, which has not been detected in the present study.

CONCLUSION

In the present study, the hypothesis on the effects of the RFM treatment strategies and DPL influence on MY and SCS in cows subsequent lactation was partially confirmed.

No statistically significant differences were observed between the various RFM treatment strategies regarding MY_{30}, MY_{100}, and during the standard lactation. Similarly, no significant differences were observed between the various RFM treatment strategies regarding SCS_{30} and SCS_{100}. There was a tendency ($P = 0.07$) for SCS_{305} to be higher in cows with RFM, where it was removed manually, and the IU antibiotics were applied (group 2). The highest economic losses, calculated from the decrease in MY, were also detected in group 2.

Statistically significantly lower MY was observed in cows with a shortened DPL ($P < 0.05$) during the first 30 days PP. The DPL did not affect the SCS.

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Characterization of different wood species as potential feedstocks for gasification

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Abstract. This paper provides an extended overview of the chemical characteristics of 19 different wood species originating from Estonia. The variation of chemical composition in wood and bark was investigated using a variety of analytical techniques including WD-XRF, ICP-MS, and elemental analysis. Principal component analysis (PCA) was used to observe clustering in the sample set. It revealed a clear data clustering in terms of the wood and bark samples. Wood characteristics exhibit quite narrow ranges, on the other hand the composition of wood bark samples is significantly different and more distributed. The correlations and associations among 27 chemical parameters, including 16 ash-forming elements, were studied. Several significant positive correlations between Cr-Ni-Fe, Ca-Sr, Al-Na-Si-Ti, K-Mg-P, Fe-Zn-Cr-Ni-Cu, Ash-Ca, N-S-P and O-volatile matter were found. Most of the metallic components are negatively correlated with volatile matter, C, H, O and heating value and are positively related to each other, or no significant correlation was identified. Results are compared to literature data and technical quality standards for biomass. Biomass feedstocks availability and composition for gasification process was discussed. Wood samples had higher volatiles content than in bark which is an indication that higher conversion rate and lower gasification temperature can be used. Spruce, pine and black alder barks have higher fixed carbon content than other common species that may increase biochar yield. Commonly available woods like Scots pine, Norway spruce, aspen, birch, black alder and grey alder may considered as suitable feedstocks for gasification because of their low N, S, Cl, and ash content together with high volatile matter, however, relatively high total heavy metals content were found from birch and grey alder barks compared to other hardwoods.

Key words: biomass, biomass ash, chemical composition.

INTRODUCTION

In order to reduce the share of oil shale in the Estonian energy sector, renewable fuels like woody biomass are increasingly becoming a prospective alternative to produce energy and reduce environmental impacts like greenhouse gas (GHG) emissions and the amount of ash in landfills (Konist et al., 2013). Moreover, renewable energy directive sets targets for EU Member States to raise the share of renewable sources to at least 32% by 2030 (Official Journal of the European Union, 2009; Amanatidis, 2019). There is a huge perspective for increasing natural biomass use and for co-gasification with solid fossil fuels in the energy sector (Vassilev et al., 2013). Via gasification, biomass can be converted to synthesis gas (syngas) which is a platform for heat and power production and an intermediate to produce chemicals. The co-gasification of many biomass/coal combinations were investigated by Koppejan & van Loo (2016), Thengane et al. (2019), Ma et al. (2019), Mallick et al. (2020). For Estonian, biomass co-combustion with oil shale would be a great alternative and offers near-term solutions to cut down CO_2 emissions.

Biomass is considered a highly reactive fuel (Koppejan & van Loo, 2016) and is easy to ignite at relatively low temperatures (García et al., 2012), however in industrial plants fuel feeding requires special measures (Caillat & Vakkilainen, 2013). From previous work of our research group (Maaten et al., 2019), biomass co-combustion with oil shale was found to be technologically promising. However, the characteristics of woody biomass and the possible effects of its composition on gasification were not included in the previous work.

Biomass composition is highly variable and depends on many factors, like the type of biomass, plant species and part of the plant; growth, transport and deposition conditions; geographic location; harvesting time and technique; pick-up of extraneous material (dust, dirt, soil) etc (Vassilev et al., 2010). A lot of different data about biomass composition are available, especially about major species in Nordic countries (Eriksson et al., 2004; Antero, 2006; Werkelin et al., 2010, 2011; Pesonen et al., 2014), but there is a lack of information involving Estonian biomass characteristics that may influence thermochemical biomass conversion processes like gasification. In order to estimate the Estonian renewable energy potential, it is necessary to investigate and map biomass characteristics that are related to regional biomass including all major species in Estonia. Furthermore, the present work includes also trace elements in local wood species which is important because Estonia is one of the major wood pellets exporters (Kaup, 2018).

A direct link exists between the chemical composition of biomass and the gas obtained by gasification. For example, the calorific value of syngas depends on the heating value and moisture content of the fuel (Kirsanovs & Žandeckis, 2015). Gil et al. (2019) statistically analysed biomass properties that influence gasification and found that carbon and hydrogen contents and the heating value of the biomass promote gas production. Also, important constituents that should be considered in gasifier selection are silica, chlorine, sulphur and alkali metals, which can cause slagging, fouling, hazardous emissions and high-temperature corrosion.

The gasification process is strongly influenced or controlled by the catalyst, which leads to high selectivity towards desired products. Catalysts can be expensive and rapidly deactivated when in direct contact with biomass, and therefore, are not in common use in industrial biorefineries. Using biomass with a suitable mineral composition that acts as a catalyst may improve the syngas composition. It is reported that alkali and alkaline earth metals are the best catalysts for promoting char gasification (Nzihou et al., 2013). K and Ca in feedstocks may exhibit a catalytic effect, enhancing the char gasification reactivity and leading to a higher carbon conversion rate. Early work on the importance of alkali catalysts was done by Elliott et al. (1984), who claimed the optimum concentration of catalyst to be 3×10^{-4}–1.5×10^{-3} mol of alkali per gram of biomass. Sueyasu et al. (2012) soaked feed biomass in a K solution before gasification to minimize tar emissions. Also, transition metals can participate as catalysts: Fe accelerates gasification and Ni prevents carbon deposition, which helps in conditioning biomass-derived syngas. Many studies have reported Ca to be a promising catalyst for increasing the gasification rate of the char (Nzihou et al., 2013; Perander et al., 2015; Link et al., 2018). On the other hand, high alkali contents are considered a serious problem in biomass gasification due to contamination of equipment and increase maintenance costs (Ren et al., 2019).

The aim of this study was to analyse different types of wood species harvested from Estonia and analyse their suitability as gasification feedstock based on their characteristics and chemical composition. The selection of wood species was based on the principle that all dominant species harvested in Estonia should be covered in this study. Dominant wood species in Estonia are Scots pine (36%), silver birch (25.6%), Norway spruce (19.7%), aspen (7.0%), grey alder (6.8%) and black alder (3.8%) (Raudsaar et al., 2018). Additional tree species like common laburnum, hawthorn, European crab apple, blackthorn, common lilac, European ash, Norway maple, common plum, rowan, white willow, bird cherry, wych elm and common juniper were also selected for the current study. These generally have a small volume (1.2%) of the total growing stock compared to the dominant species. However, the wider selection of wood species gives a more detailed overview of biofuel characteristics and their relationships.

It is known that physicochemical properties of bark and wood differ (Olanders & Steenari, 1995; Frandsen et al., 2007; Vassilev et al., 2010; Werkelin et al., 2010; Johansson et al., 2016; Koppejan & van Loo, 2016); therefore, a comparison between bark and wood was made. Most studies have focused only on common wood species or analysed whole tree parts that contains both wood and bark and limited information on the separate components. Wood species were characterized by proximate and ultimate analysis, like ash content, heating value, volatile matter, C, H, N, S, Cl content and concentration of microelements/trace components. The data were compared to literature sources where possible. The results are compared with the data of Technical Specification (ISO 17225), which defines the biomass requirements and typical values and ranges for coniferous and broad-leaf wood and bark (results are obtained from a combination of research from Sweden, Finland, Denmark, Netherland and Germany). This study enhances our understanding of biomass composition as feedstock for gasification and will be beneficial to developing energy efficient processes.

MATERIALS AND METHODS

In total, 19 different wood species were investigated. These include 16 broad-leaf species: common laburnum (*Laburnum Anagyroides),* hawthorn (*Crataegus),* European crab apple (*Malus sylvestris*), blackthorn (*Prunus spinosa*), common lilac *(Syringa vulgaris),* European ash *(Fraxinus excelsior),* Norway maple *(Acer platanoides),* common plum *(Prunus domestica),* rowan (*Sorbus aucuparia),* white willow *(Salix alba),* bird cherry *(Prunus padus),* wych elm *(Ulmus glabra),* grey alder *(Alnus incana),* silver birch *(Betula pendula),* aspen *(Populus tremula),* black alder *(Alnus glutinosa).* 3 coniferous wood species were also included: common juniper *(Juniperus communis),* Scots pine *(Pinus Sylvestris),* Norway spruce *(Picea abies).* Samples originated from northern and southern Estonia in order to represent a wider population of tree species.

Wood samples were provided as air-dried logs. Wood and bark were separated mechanically before further analyses. The wood and bark ratios were determined gravimetrically after drying. Samples were grinded with a cutting mill and homogenized according to ISO 14780 solid biofuel sample preparation standard.

Wood samples were dried at 105 °C to a constant weight (ISO 18134-3). Ash content was determined at 550 °C (ISO 18122). Volatile matter was determined by burning the sample in a muffle furnace at 900 °C, following the procedure described in ISO 18123. Fixed carbon is the mass remaining after volatilization, excluding ash and moisture component. It can be calculated as subtracting from 100% the sum of the volatile matter and ash in percentage (Telmo et al., 2010).

Gross calorific value at constant volume ($q_{V,gr}$) was measured with IKA C2000 and IKA C5000 bomb calorimeters (ISO 18125). Gross calorific value on a dry basis ($q_{V,gr,d}$) includes the condensation enthalpy of water. Net calorific value, also called the lower heating value ($q_{p,net,d}$), is obtained when the condensation enthalpy of water is not included.

A Vario MACRO CHNS Elementar Analyser System was used to measure the C, H and N contents of the dry matter (ISO 16948). Oxygen content was calculated from the C, H, N, S, Cl and ash. Cl and S contents were measured with a Dionex 1000 ion chromatograph (ISO 16994). A Rigaku WD-XRF and Thermo Scientific iCAP Qc ICP-MS were used to determine ash forming elements and trace components in the biomass (ISO/TS 16996, ISO 16968).

Analysis results are presented according to the requirements of ISO standards. Uncertainties for every method is determined by the Nordtest (Magnusson et al., 2017) or ISO GUM method (Joint Committee For Guides In Metrology, 2008) or the relative standard deviation is given. Uncertainty estimations according to (a) the Nordtest approach (Uc, k = 2): HV – 3.9%; Ash – 43.0%; S – 3.2%, Cl – 4.2%, Volatiles – 4.6%; C – 1.10%; H – 4.1%; N – 19.1%; Mg – 9.4%; Fe – 9,1%; Mn – 5.4%; Zn – 11.4%; Cr – 14.8%; Ni – 16.4%; Sr – 6.6%; Ti – 11.3%; Cu – 7.4%; Pb – 8.1%; K – 19.8%; Na – 75.5% (b) ISO GUM approach (Uc, k = 2): O – 3.0%, FC – 27.7% and (c) RSD (rel%): Ca – 1.1%; Al – 29.2%; P – 2.2%; Si – 14.8%.

PCA was carried out with the aim of highlighting possible clusters of wood types characterized by similar properties. PCA is a chemometric technique for analysing the structure of the observations (wood and bark samples) and the variables (proximate, ultimate and trace metal analyses). PCA finds lines, planes and hyperplanes in the K-dimensional space that approximate the data as well as possible (Jackson, 1991; Jollife & Cadima, 2016). PCA was conducted via XLSTAT Statistical Software for MS Excel.

Chemical correlations between variables were determined in order to understand some of the fundamental relationships and trends. Data from the analysed woody biomass were subjected to the Pearson's correlation test (Plata, 2006) to calculate correlation coefficient values between 26 characteristics. The calculated correlation coefficient values include the statistically significant as well as statistically insignificant R^2 relationships, at a 95% confidence level.

RESULTS AND DISCUSSION

Proximate, ultimate analysis and heating value results are presented in Table 1. Minor and trace elements are shown in Table 2. Overall, 26 different parameters were measured for every sample. For better understanding, results are grouped as common and all wood species. Scots pine, silver birch, Norway spruce, aspen, grey alder and black alder are largely the most available resource in Estonia (Raudsaar et al., 2018), and therefore, some special attention is given to them.

Table 1. Proximate and ultimate analysis and heating value of different wood species. Literature data is included and refer to the wood type in the row above. (A – ash; VM – volatile matter; FC – fixed carbon; C – carbon; H – hydrogen; N – nitrogen; S – sulfur; O – oxygen; Cl – chlorine; HHV – higher heating value; LHV – lower heating value, wt% db – weight percent of dry basis)

Feedstock **Wood**	Content of wood or bark, %	Proximate analysis (wt% db)			Ultimate analysis (wt% db)						Heating value (MJ kg^{-1})	
		A	VM	FC	C	H	N	S	O	Cl	HHV	LHV
1. Broad-leaf wood (BLW)												
Aspen	87.2	0.4	87.0	12.6	50.2	6.2	0.1	0.01	43.1	< 0.01	20.07	18.72
Pesonen et al. (2014)		0.8			47.8	5.9	-				20.04	18.76
Bird cherry	90.1	0.5	86.4	13.1	48.1	6.5	0.1	< 0.01	44.8	0.01	19.11	17.69
Black alder	86.9	0.4	86.1	13.5	50.6	6.2	0.2	0.03	41.8	< 0.01	19.79	18.45
Blackthorn	81.9	0.6	84.4	15.0	48.4	6.3	0.2	< 0.01	44.5	0.01	19.24	17.86
Common laburnum	90.1	0.5	81.2	18.3	47.8	6.6	0.3	0.07	44.6	0.02	19.59	18.15
Common lilac	89.4	0.5	85.0	14.5	48.7	6.5	0.2	< 0.01	44.2	0.01	19.55	18.14
Common plum	57.9	0.5	83.2	16.3	49.5	6.4	0.2	< 0.01	43.4	0.01	19.65	18.26
European ash	94.9	0.4	84.9	14.7	49.1	6.5	0.1	< 0.01	43.8	0.01	19.44	18.03
European crab apple	96.3	0.8	83.5	15.8	47.0	6.5	0.1	0.02	45.6	0.01	19.22	17.81
Grey alder	90.8	0.4	85.7	13.9	49.9	6.6	0.2	< 0.01	43.0	< 0.01	19.95	18.52
Pesonen et al. (2014)		0.33			48.5	6.1	0.2				20.44	19.11
Hawthorn	92.9	1.6	81.6	16.8	48.1	6.5	0.2	0.05	43.6	0.01	19.82	18.40
Norway maple	92.7	0.6	86.2	13.3	48.7	6.5	0.1	< 0.01	44.1	0.01	19.36	17.95
Rowan	92.4	0.8	85.0	14.2	48.5	6.5	0.1	< 0.01	44.1	< 0.01	19.07	17.65
Silver birch	87.3	0.3	86.9	12.8	49.3	6.6	0.1	< 0.01	43.7	0.01	19.94	18.10
Pesonen et al. (2014)		0.4			48.2	6.1	-				19.80	18.46
White willow	92.7	0.5	85.2	14.3	48.4	6.5	0.1	< 0.01	44.5	< 0.01	19.72	18.31
Wych elm	89.8	1.8	79.9	18.4	49.6	6.5	0.2	< 0.01	42.1	0.01	19.69	18.29
Common BLW												
Mean	88.0	0.4	86.4	13.2	50.0	6.4	0.2	0.02	43.1	< 0.01	19.94	18.45
Minimum	86.9	0.3	85.7	12.6	49.3	6.2	0.1	0.01	42.6	< 0.01	19.79	18.10
Maximum	90.8	0.4	87.0	13.9	50.6	6.6	0.2	0.03	43.7	0.01	20.07	18.72
All BLW												
Mean	87.8	0.7	84.5	14.8	48.9	6.5	0.2	0.01	43.9	0.01	19.58	18.15
Minimum	57.9	0.3	79.9	12.6	47.0	6.2	0.1	0.01	42.1	< 0.01	19.07	17.65
Maximum	96.3	1.8	87.0	18.4	50.6	6.6	0.3	0.03	45.6	0.01	20.07	18.72

Table 1 (continued)

Feedstock	Content of wood or bark, %	Proximate analysis (wt% db)			Ultimate analysis (wt% db)						Heating value (MJ kg^{-1})	
		A	VM	FC	C	H	N	S	O	Cl	HHV	LHV
2. Coniferous wood (CW)												
Common juniper	86.2	1.2	82.3	16.6	48.8	6.8	0.2	< 0.01	43.1	0.01	20.65	19.16
Norway spruce	92.2	0.3	85.5	14.2	50.3	6.6	0.1	< 0.01	42.7	0.01	19.79	18.35
Scots pine	89.3	0.3	85.2	14.5	50.1	6.6	0.2	< 0.01	42.8	0.01	20.04	18.61
Mason et al. (2015)		2.0	82.5	17.5	47.4	5.3	1.3		45.9		18.6	
Common CW												
Mean	90.7	0.3	85.4	14.3	50.2	6.6	0.1	0.01	42.7	0.01	19.92	18.48
All CW												
Mean	89.2	0.6	84.3	15.1	49.8	6.7	0.1	0.01	42.8	0.01	20.16	18.71
Minimum	86.2	0.3	82.3	14.2	48.8	6.6	0.1	0.01	42.7	0.01	19.79	18.35
Maximum	92.2	1.2	85.5	16.6	50.3	6.8	0.2	0.01	43.1	0.01	20.65	19.16
Bark												
Broad-leaf wood (BLW)												
Aspen	12.9	3.5	79.8	16.7	53.4	6.4	0.9	0.07	35.8	< 0.01	21.37	19.99
Bird cherry	9.9	4.9	72.8	22.4	48.6	5.9	1.0	0.06	39.7	0.01	19.36	18.07
Black alder	13.1	3.2	71.3	25.5	55.1	5.9	1.3	0.05	34.4	0.02	21,97	20,69
Blackthorn	18.2	6.8	76.3	16.9	52.6	6.7	0.9	0.06	33.0	0.03	22.77	21.32
Common laburnum	9.9	2.7	77.0	20.3	49.2	6.4	2.5	0.10	39.0	0.02	21.02	19.62
Common lilac	10.6	3.2	76.3	20.5	48.6	6.1	0.9	0.07	41.1	0.01	19.85	18.52
Common plum	42.1	5.9	67.5	26.6	48.6	5.5	1.0	0.05	38.9	0.01	19.58	18.37
European ash	5.1	9.3	74.8	15.9	45.0	5.5	0.6	0.06	39.6	0.01	17.86	16.66
Zajac et al. (2018)		9.14										
European crab apple	3.7	16.9	69.2	13.9	44.1	5.2	1.0	0.07	32.8	0.01	16.47	15.34
Grey alder	9.2	2.7	74.0	23.4	53.5	6.5	1.1	0.05	36.2	< 0.01	22.27	20.86
Hawthorn	7.1	13.7	68.8	17.5	47.9	5.7	0.9	0.11	31.6	0.03	19.46	18.22
Norway maple	7.3	10.4	74.0	15.6	43.9	5.5	0.8	0.03	39.4	0.01	17.14	15.94
Bryers, (1996)		4	76.6	19.4	52	6.2	0.4	0.11	41.3			
Rowan	7.6	4.1	77.9	18.0	49.4	6.5	0.6	0.04	39.4	0.01	20.63	19.22

Table 1 (continued)

Feedstock	Content of wood or bark, %	Proximate analysis (wt% db)			Ultimate analysis (wt% db)						Heating value (MJ kg^{-1})	
		A	VM	FC	C	H	N	S	O	Cl	HHV	LHV
Silver birch	12.7	1.4	80.1	18.5	56.3	7.0	0.4	0.02	34.8	0.02	24.18	22.66
Bryers, (1996)		2.1	78.5	19.4	57.0	6.7	0.5	0.10	35.7			
White willow	7.3	9.0	69.6	21.4	45.8	5.4	0.6	0.08	39.1	0.01	18.48	17.30
Wych elm	10.2	13.4	72.0	14.6	44.8	5.5	0.9	0.07	35.4	0.01	17.31	16.12
Bryers, (1996)		8.1	73.1	18.8	50.9	5.8	0.7	0.11	42.5			
Common BLW												
Mean	12.0	2.7	76.3	21.0	54.6	6.4	0.9	0.05	35.3	0.01	22.45	21.05
Minimum	9.2	1.8	71.3	16.7	53.4	5.9	0.4	0.02	34.4	< 0.01	21.37	19.99
Maximum	13.1	3.5	80.1	25.5	56.3	7.0	1.3	0.07	36.3	0.02	24.18	22.66
All BLW												
Mean	11.5	6.8	73.5	19.8	49.3	6.0	0.9	0.06	37.0	0.01	19.97	18.68
Minimum	3.7	1.4	67.5	13.9	43.9	5.2	0.4	0.02	31.7	< 0.01	16.47	15.34
Maximum	42.1	16.9	80.1	26.6	56.3	7.0	2.5	0.11	41.2	0.03	24.18	22.66
Coniferous wood (CW)												
Common juniper	13.8	7.2	73.0	19.8	49.2	6.0	0.6	0.07	37.0	0.03	19.66	18.35
Norway spruce	7.9	7.9	71.1	21.0	48.4	5.6	0.6	0.02	37.6	0.01	18.72	17.50
Frandsen et al. (2007)		4.9			49.9	5.8	0.5	0.04		0.02		
Antero (2006)		2.3	77	23	51.1	6	0.4	0.03			20.3	19.0
Brunner et al. (2013)		4.8			49.8	5.7	0.34	0.03	40.8	0.01		
Scots pine	10.7	3.1	70.1	26.9	52.8	6.1	1.2	0.05	36.9	0.01	21.40	20.08
Jerzak, (2020)		1.56	67.98		55.95	5.29	0.05	0.03	37.03	0.012		
Antero (2006)		2.3	74.3	25.7	53.4	5.8	0.4	0.03			21.3	20.0
Vassilev et al. (2010)		1.9	73.7	24.4	53.8	5.9	0.3	0.07	39.9	0.01		
Common CW												
Mean	9.3	5.5	70.6	24.0	50.6	5.8	0.9	0.04	37.2	0.01	20.06	18.79
All CW												
Mean	10.8	6.0	71.4	22.6	50.1	5.9	0.8	0.05	37.2	0.01	19.93	18.64
Minimum	7.9	3.1	70.1	19.8	48.4	5.6	0.6	0.03	36.9	0.01	18.72	17.50
Maximum	13.8	7.9	73.0	26.9	52.8	6.1	1.2	0.07	37.6	0.03	21.40	20.08

Table 2. Minor and trace elements (ppm, db)

Feedstock	Al	K	Na	Ca	Si	Mg	Fe	P	Mn	Zn	Cr	Ni	Sr	Ti	Cu	Pb
Wood																
Broad-leaf wood (BLW)																
Aspen	51	479	20	890	51	648	47	100	3	6	-	0.2	2.3	-	0.9	< 0.1
Werkelin et al. (2010)	6	1,370	15	998	63	286	5		49							
Bird cherry	22	547	17	1,482	68	280	46	98	9	5	1.5	0.2	2.3	2.7	0.4	0.1
Black alder	17	354	6	1,171	35	354	43	104	10	6	-	0.1	2.9	-	1.5	-
Blackthorn	23	779	29	1,920	55	415	40	96	1	2	1.1	0.1	7.6	0.5	0.4	-
Common laburnum	29	766	32	1,146	95	338	41	139	2	3	0.4	0.1	4.8	-	0.6	-
Common lilac	24	693	34	1,127	61	388	41	194	2	15	0.1	-	3.3	-	0.1	-
Common plum	23	699	31	1,060	66	297	43	143	1	3	0.2	0.1	3.4	1.9	1.8	-
European ash	17	597	114	891	46	360	40	81	0.2	2	0.1	0.4	3.0	-	1.5	-
European crab apple	29	869	41	2,551	58	365	42	124	1	5	0.2	0.1	10.1	2.4	0.8	-
Grey alder	19	496	12	1,091	35	268	41	137	8	8	0.4	0.1	2.2	-	0.9	0.3
Hawthorn	40	1,124	232	5,522	100	394	45	372	2	12	0.5	0.2	6.9	0.9	2.9	-
Norway maple	20	769	22	1,316	48	417	40	162	13	2	0.2	0.2	1.4	0.1	0.4	-
Rowan	17	1,093	40	1,692	38	513	41	79	114	7	1.3	1.0	3.1	1.1	0.4	0.4
Silver birch	14	473	12	1,036	21	421	39	51	44	14	0.3	0.3	2.5	2.1	0.2	0.5
Werkelin et al. (2010)	2	315	4	636	77	92	6		102							
White willow	28	1,192	31	975	51	333	43	288	2	17	0.1	0.1	2.2	2.4	1.0	-
Wych elm	21	4,847	47	3,371	175	391	43	172	1	2	0.2	0.0	10.4	0.3	2.0	-
Common BLW																
Mean	25	451	13	1,047	36	423	43	98	16	8	0.2	0.2	2.5	0.5	0.9	0.2
Minimum	14	354	6	890	21	268	39	51	3	6	0.0	0.1	2.2	0.0	0.2	0.0
Maximum	51	496	20	1,171	51	648	47	137	44	14	0.4	0.3	2.9	2.1	1.5	0.5
All BLW																
Mean	25	986	45	1,703	63	386	42	146	13	7	0.4	0.2	4.3	0.9	1.0	0.1
Minimum	14	354	6	890	21	268	39	51	0	2	0.0	0.0	1.4	0.0	0.1	0.0
Maximum	51	4,847	232	5,522	175	648	47	372	114	17	1.5	1.0	10.4	2.7	2.9	0.5

Table 2 (continued)

Feedstock	Al	K	Na	Ca	Si	Mg	Fe	P	Mn	Zn	Cr	Ni	Sr	Ti	Cu	Pb
Coniferous wood (CW)																
Common juniper	30	591	43	5,817	49	371	42	181	5	5	0.4	0.2	3.0	1.7	0.9	-
Norway spruce	14	246	13	1,118	26	233	38	17	11	5	0.3	0.2	8.9	1.2	0.3	1.4
Werkelin et al. (2010)	3	235	7	752	93	99	5		100							
Scots pine	16	530	16	718	28	241	41	103	6	6	0.3	0.1	1.4	-	1.0	0.1
Werkelin et al. (2010)	4	407	15	641	123	189	8		81							
Common CW																
Mean	15	388	15	918	27	237	40	60	9	5	0.3	0.1	5.2	0.6	0.7	0.7
All CW																
Mean	20	456	24	2,551	34	282	40	100	7	5	0.3	0.2	4.5	1.0	0.7	0.5
Minimum	14	246	13	718	26	233	38	17	5	5	0.3	0.1	1.4	0.0	0.3	0.0
Maximum	30	591	43	5,817	49	371	42	181	11	6	0.4	0.2	8.9	1.7	1.0	1.4
Bark																
Broad-leaf wood (BLW)																
Aspen	70	4,727	86	9,527	164	1,618	55	812	25	123	-	0.1	14.1	0.3	2.1	-
Bird cherry	316	1,787	142	22,762	616	645	391	589	215	108	254.7	128.5	27.8	14.5	36.3	3.5
Blackthorn	84	1,959	125	26,708	222	1,268	65	615	11	13	1.4	0.9	87.4	3.5	6.2	0.2
Black alder	85	122	66	13,191	255	470	60	195	75	27	1.0	0.5	26.8	1.6	9.5	1.2
Common laburnum	154	5,468	73	6,479	431	1,451	115	1381	11	52	3.7	0.8	22.1	6.0	3.9	0.4
Common lilac	209	3,972	197	10,259	659	1,154	167	757	21	119	1.5	37.6	24.0	9.2	3.4	0.6
Common plum	209	2,968	120	29,078	492	934	261	490	18	38	78.7	44.6	66.3	14.5	20.8	1.2
European ash	605	1,503	359	34,067	2,930	962	1377	300	103	960	407	236.7	64.1	37.5	41.6	2.7
European crab apple	393	4,039	297	63,910	1,379	1,434	125	985	28	135	9.9	1.7	189.6	31.6	6.7	1.8
Grey alder	393	1,414	63	8,715	247	476	494	404	62	109	191.7	92.3	14.3	7.1	26.7	4.5
Hawthorn	2,810	2,432	1,204	38,453	9,487	946	581	777	61	139	8.2	2.0	48.4	160	14.8	4.6
Norway maple	343	3,817	181	43,797	3,068	1,198	154	619	233	99	3.3	1.9	29.3	9.4	6.1	0.8
Rowan	117	3,131	123	15,863	345	1,246	120	505	833	139	6.9	2.7	21.7	2.8	9.6	0.8
Silver birch	102	606	38	4,489	163	703	184	195	201	157	59.9	31.0	10.5	2.4	5.2	0.4

Table 2 (continued)

Feedstock	Al	K	Na	Ca	Si	Mg	Fe	P	Mn	Zn	Cr	Ni	Sr	Ti	Cu	Pb
Werkelin et al. (2010)	19	1,710	14	7,860	114	323	24		534							
White willow	146	5,741	289	33,966	910	1,481	71	800	16	332	0.3	0.2	24.2	2.6	4.6	0.1
Wych elm	153	5,067	149	49,587	4,140	1,102	73	870	11	13	1.8	1.2	138.3	8.7	8.6	0.6
Common BLW																
Mean	163	1,717	63	8,981	207	817	198	402	91	104	84.2	31.0	16.4	2.9	10.8	1.5
Minimum	70	122	38	4,489	163	470	55	195	25	27	1.0	0.1	10.5	0.3	2.1	0.0
Maximum	393	4,727	86	13,191	255	1,618	494	812	201	157	191.7	92.3	26.8	7.1	26.7	4.5
All BLW																
Mean	356	2,800	207	25,421	1,416	995	250	598	116	152	70.7	37.9	54.1	17.7	12.6	1.4
Minimum	70	122	38	4,489	163	470	55	195	11	13	0.3	0.1	10.5	0.3	2.1	0.0
Maximum	2,810	5,741	1,204	63,910	9,487	1,618	1,377	1,381	833	960	407.2	236.7	189.6	160	41.6	4.6
Coniferous wood (CW)																
Common juniper	405	1,574	302	27,033	1,094	566	151	424	41	39	5.3	1.2	15.4	18.5	4.0	3.1
Norway spruce	79	786	86	32,175	193	795	114	333	193	231	98.5	45.3	188.9	2.8	14.3	0.7
Frandsen et al. (2007)	616	2,493	103	13,196	3,866	781	377	361	428							
Werkelin et al. (2011)	98	2,030	26	8,350	171	865	39	452	714							
Antero (2006)		3,003	89													
Brunner et al. (2013)		1,825	79	13,450	1,810	822		338		85						
Scots pine	87	2,095	29	12,947	102	459	186	310	48	50	138.2	91.4	15.7	3.5	14.4	0.2
Ragland et al. (1991)	2,149	1,333	280	5,282	5,278	1,137	609									
Werkelin et al. (2010)	908	3,180	22	6,350	60	874	52		343							
Common CW																
Mean	83	1,441	58	22,561	148	627	150	322	120	141	118.4	68.3	102.3	3.2	14.3	0.4
All CW																
Mean	190	1,485	139	24,052	463	607	150	356	94	107	80.7	45.9	73.3	8.3	10.9	1.3
Minimum	79	786	29	12,947	102	459	114	310	41	39	5.3	1.2	15.4	2.8	4.0	0.2
Maximum	405	2,095	302	32,175	1,094	795	186	424	193	231	138.2	91.4	188.9	18.5	14.4	3.1

It is noticeable that in most of the species bark is less than 10% of the overall stem wood (Table 1). However, there are some exceptions like common plum where the bark ratio reached over 42%. This result may be explained by the factors that affect bark thickness: wood species, the age and growing conditions (Sonmez et al., 2007; Koman & Feher 2015). It should be considered that wood chips are generally produced from whole trees; therefore, the prominent component is wood and bark makes only a small contribution to the average. However, bark is a main source of ash, different extractives and elements that may affect the biomass gasification process and need careful consideration.

Ash yield

Ash content is an important quality parameter of biomass. A high content of ash is undesirable in most conversion processes as it causes problems related to utilization and process design, reduces the heating value (Elbersen et al., 2017) and affects dust emission (Koppejan & van Loo, 2016). Biomass with an ash content over 10% is considered to have a high risk of slag formation (Ren et al., 2019). However, there is no evidence that the ash content in a gasification process directly influences gas composition, although ash can cause a variety of problems like slagging, which is influenced by the presence of alkaline metals in biomass.

Results show that the wood part of most of the species has a relatively low ash content ranging between 0.3 to 1.8%. Only hawthorn and wych elm have a higher ash content, 1.56% and 1.75%, respectively, compared to typical broad-leaf wood values (0.2–1.0%). The ash content of pine and spruce (0.2–0.3%) matches standardized values of 0.1–1.0% according to ISO 17225. However, common juniper has a higher ash content than other softwoods. Mason et al. (2015) report much higher ash values for pine and willow, 1.9% and 2.0%, respectively. On the other hand, the alder ash content is similar to that from Pesonen et al. (2014). It is stated that the ash content in untreated wood of different species is 0.1% to 0.6% and in bark 3% to 5% (Ragland et al., 1991), but it can reach up to 20% for some biomasses (Caillat & Vakkilainen, 2013). In gasification mostly a mixture of bark and wood is used; therefore, ash content is primary dependent on the bark content of the feedstock. Results show that broad-leaf trees have higher ash contents that coniferous wood, which is similar to earlier findings (Alakangas et al., 2016).

The ash content of bark (1.4–16.9%) was significantly higher than that of stem wood (0.3–1.8%). Silver birch, alder and common laburnum have the lowest ash contents in the bark: 1.4%, 2.7% and 2.7%, respectively. These values lie between the typical broad-leaf wood bark ash content values of 0.8–3.0% according to ISO 17225. The bark ash content of birch and European ash, 1.4 and 9.3%, respectively, are similar to the values of 2.1% (Bryers, 1996) and 9.14% (Zając et al., 2018) reported in previous studies. However, most of the analysed Estonian hardwood barks have higher ash values than typical hardwood. The wood species with the highest bark ash content are European apple tree 16.9%, hawthorn 13.7%, wych elm 13.4% and Norway maple 10.4%. High ash content may be due to mossy bark. Contaminants from soil can lead to severe problems like fouling and slagging (Caillat & Vakkilainen, 2013). The ash content of 3.1% for pine bark was within the typical range of values for softwood bark (< 1.0–3.0%) and comparable to the value reported by Antero (2006) (2.3%). Norway spruce and common juniper have higher ash contents of 7.9 and 7.2%, respectively.

A high ash content is normally undesirable since it complicates the operational process. Moreover, it is stated that higher ash content promotes the growth of CO and H_2 concentration in the syngas and the decrease of CH_4 content (Kirsanovs & Žandeckis, 2015). On the other hand, it is possible to use biomass with a suitable ash composition that may act as a natural catalyst for char gasification and tar removal. Therefore, ash yield alone is not a sufficient parameter for estimating the suitability of different woody samples. The chemical composition, especially alkali and alkali-earth metals concentrations, should additionally be considered (discussed in the following paragraphs).

Heating value

Heating value (HV) is an expression of the energy content released when burnt in air (McKendry, 2002). HV is not highly variable from one tree species to another (Table 1). The HHV of wood lays between 19.07 and 20.65 MJ kg^{-1}. These results are similar to earlier findings, where no significant differences in HVs of different wood species and barks were found (Pesonen et al., 2014). On contrary, HHV of bark is much more distributed and values range between 16.47 and 24.18 MJ kg^{-1}. Moreover, silver birch bark has the highest HV of 24.18 MJ kg^{-1} exceeding a typical hardwood bark HV of 20.0 MJ kg^{-1} (according to ISO 17225). This result may be explained by the fact that birch bark is rich in extractives and suberin (Pinto et al., 2009) that may contribute to higher HV. Also, it is reported that lignin content has strong positive correlation to HV (Demirbaş, 2001). However, Pesonen et al. (2014) states that the HV of silver birch wood HV is much lower, 18.46 MJ kg^{-1}, but aspen and alder have very similar results to current results. The lowest HV determined was for European crab apple bark, 16.47 MJ kg^{-1}, where a negative correlation is seen between ash content (16.91%) and HV. The HV of the pine bark analysed is similar to those reported by Antero (2006), McKendry (2002) and Phyllis2 Database. In contrast, spruce bark has a lower HV than reported by Antero (2006) and the Phyllis2 Database, but still lies between values that are typical for softwood barks. Overall, the mean HVs of hardwood and softwood are very similar, although softwood samples tend to have a slightly higher HV, as supported by previous findings (Ragland et al., 1991; Nurmi, 2000).

The HHV of the biomass along with C and H content were found to promote the concentrations of CO and combustible gas, the calorific value of the product gas and the gasification conversion (Gil et al., 2019). Therefore, bark samples that have much higher HHV than wood samples may appear to be more suitable feedstocks for gasification.

Volatile matter

Data for the volatile matter (VM) of the biomass samples indicates that there is no significant difference between broad-leaf and coniferous wood. The same trend is seen in bark samples. Wood samples have higher VMs compared to barks (79.9–86.6% versus 67.5–80.1%). According to the literature, VM in woody biomass lies between 70.20 and 93.99% (Miles et al., 1996; Koziński & Saade, 1998; McKendry, 2002; Cuiping et al., 2004; Demirbas, 2004; Eriksson et al., 2004; Grammelis et al., 2006; Saidur et al., 2011; García et al., 2012; Miccio et al., 2012; Rizvi et al., 2015; Lu et al., 2018). The VM of willow and pine wood is similar to that result reported by Mason et al. (2015). Silver birch bark had the highest VM among all barks, similar to results obtained by Bryers (1996). The VM content of Norway spruce coincides with the content reported by Antero (2006).

According to the literature, a higher volatile matter increases the biomass conversion rate in comparison to biomasses with higher fixed carbon content (Roldan, 2018). Therefore, the conversion rate of wood samples in thermochemical processes should be much higher than bark samples. Kurkela (1996) found that a lower volatiles content decreases the reactivity of pine bark compared to wood, and therefore, pine bark gasification needs an extra 100 °C increase in the temperature. These findings suggest that samples with higher VM matter, like wood samples, are preferred for gasification when a lower gasification temperature is required.

Fixed carbon

Fixed carbon (FC) is the carbon found in the material that remains after volatile materials are emitted. FC content is linked to the biochar yield which is reported to be higher for biomasses with high FC (Roldan, 2018). FC in wood and bark samples varies between 12.6–18.4% and 13.9–26.9%, respectively. It is possible to hypothesise that gasification of bark samples yields more biochar. Biochar may be a valuable by-product that can be used as a soil amendment or in carbon sequestration applications (Hansen et al., 2015). Therefore, from the analysed data set the most suitable samples for a high biochar yield would be spruce and black alder bark that have by far the highest FC, low ash content and belong to prevalent wood species in Estonia.

Carbon

The typical range of carbon (C) content in softwood is between 47–54% and in hardwood 48–52%, on a dry basis, according to ISO 17225. The present study shows that there is no significant difference in C content between hardwood and softwood wood, as in hardwood it varies between 47.0 to 49.9% and in softwoods from 48.8 to 50.3%. On the other hand, bark samples have a much higher variation between different species – 43.9 to 56.3%. Silver birch bark stands out as having the highest C content of 56.3%, which is similar to results reported in the literature (Bryers, 1996). Wych elm, white willow, European ash, European crab apple and Norway maple have C content below the typical hardwood bark range of 47 to 55% (ISO 17225). Norway spruce, however, has a lower C content than Scots pine, 48.4% and 52.8%, respectively. In the literature the same pattern is apparent between pine and spruce bark (Antero, 2006; Frandsen et al., 2007; Vassilev et al., 2010; Brunner et al., 2013; Jerzak, 2020).

Hydrogen

Hydrogen (H) is one of the parameters that has a higher influence on the heating value along with carbon, oxygen and sulfur (Telmo et al., 2010). According to the results, most of the species have higher H content in the wood than the bark with a few exceptions like silver birch, blackthorn, rowan and aspen. The H content of the analysed samples in broad-leaf wood is slightly higher than reported in the literature (Pesonen et al., 2014; Mason et al., 2015) lying between 6.3 and 6.6%. Hardwood wood, on the other hand, have a wide distribution in H content, varying between 5.2–7.0%. The H content of softwood bark is similar that reported in the literature (Antero, 2006; Vassilev et al., 2010; Brunner et al., 2013; Phyllis2 Database).

A higher H content is preferred in biomass as it results in a higher concentration of H_2 and a higher H_2/CO ratio in the gasification outlet gas according to hierarchical cluster and principal component analyses conducted by Gil et al., (2019).

Oxygen

Lignocellulosic fuel has a comparatively high oxygen (O) content that contributes to low heating values. Oxygen content is one of the most significant parameters along with gasification temperature, that determine the chemical efficiency of the gasification (Schuster et al., 2001). O content is very similar in all woods and ranges between 42.1 to 45.6%, but in bark samples it lies between 31.6 and 41.3%. O content in the literature is reported mostly on the basis of the whole wood and the content varies between 40.6 and 44.6% among different wood species (Miles et al., 1996; Koziński & Saade, 1998; Cuiping et al., 2004; Demirbas, 2004; Eriksson et al., 2004; Saidur et al., 2011; García et al., 2012; Miccio et al., 2012; Brunner et al., 2013; Lu et al., 2018; Phyllis2 Database). Kirsanovs & Žandeckis (2015) point out that lower O content in the feedstock necessitates a higher amount of air to be injected into the gasifier in order to achieve the required equivalent ratio. In addition, a higher C/O mass ratio in the fuel increases the heating value of the syngas. Therefore, common hardwood barks like grey alder, black alder, silver birch and aspen that have higher C/O mass ratios, ranging between 1.43 to 1.62, may be a more suitable feedstock when the highest HV of syngas is required.

Nitrogen

Results show that nitrogen (N) content in bark is remarkably higher than in wood, varied from 0.1 to 0.3% and 0.4 to 2.5%, respectively. Typical N content in hardwood and softwood lays between < 0.01 to 0.5% (ISO 17225). Common laburnum bark differs greatly from other species and the N content reaches 2.5%. A relatively high N content (1.2%) was also found in Scots pine bark. On the one hand, this is similar to the result for pine wood, which has a N content of 1.3%, as reported by Mason et al. (2015). However, Brunner et al. (2013), Frandsen et al. (2007), Antero (2006) report much lower N contents in softwood bark ranging between 0.05–0.5%. N is an important characteristic that should be considered in gasification and design of gas cleaning section. N is converted into N_2, NH_3 and HCN during gasification. The N_2 content in the syngas decreases the calorific value of the syngas produced. Ammonia can be eliminated from product gas by increasing the gasification temperature so it decomposes to nitrogen (Devi et al., 2003). Also, NH_3 can be separated from the gas flow by adding H_2SO_4 (Zisopoulos et al., 2018), where the product ammonium sulphate can be further used as fertilizer. Samples that have a higher N value should be avoided if possible, e.g. common laburnum, black alder and Scot’s pine bark.

Sulphur and chlorine

The advantage of untreated woody biomass is the low content of sulphur (S) and chlorine (Cl). The presence of Cl and S in the feedstock often leads to the formation of sulphates and facilitates fouling (Miles et al., 1996). The problem is that HCl formation and condensation can occur on the cooler parts of equipment, often on heat exchangers, which will lead to corrosion of the metal surface. In gasification, S is transformed to H_2S or COS, but as the concentrations are low, the issue is not very common in biomass gasification. It is seen that most of the sulphur originates from the bark as a majority of wood samples have an S content below 0.01%. Common laburnurm, hawthorn and European crab apple stand out as they have relatively high S contents both in wood and bark compared to other tree species, and in order to avoid S and Cl related problems, these samples can be excluded from the selection of possible feedstocks for gasification.

The S content of fuel increases when the bark content is higher. Cl content in barks is slightly higher compared to woods, ≤ 0.01% and 0.01–0.03%, respectively. Higher Cl contents can be found when woody biomass contains needles or leaves (Alakangas et al., 2016), however these parts of the tree were out of the scope of this study.

Minor and trace elements

According to Zevenhoven & Kilpinen (2001), the elements can be classified as follows: major (> 1.0%), minor (0.1–1.0%) and trace (< 0.1%) elements. The present results show that elemental classification is slightly different and changeable in the case of wood and bark. Major elements in both are C, H, N, O, but also Ca can be classified as a major component in bark where its concentration may exceed 6%. However, in stem wood Ca is a dominant minor element and all other determined elements: Al, Na, Si, Mg, Fe, P, Cu, Mn, Ni, Zn, Sr, Cr, Ti, Pb, belong to the trace elements range. K is found as either a minor or trace element depending on the wood type. In the bark samples, there is variation between different species and K, Mg, Si can be classified as minor elements. All in all, the major and minor elements in biomass, in decreasing order of abundance, are C > O > H > N > Ca > K > Mg > P > S > Cl > Si > Na > Fe > Al > Mn > Zn > Sr > Cr > Ni > Ti > Cu > Pb.

Many of the tested wood and bark samples contain high concentrations of Ca, K, Mg and Na that, according to different studies, improve the gasification process (Elliott et al., 1984; Sueyasu et al., 2012; Perander et al., 2015; Ren et al., 2019). These results support earlier findings that the majority of ash forming elements are found within the bark (Kleinhans et al., 2018). Also, Werkelin et al. (2011) investigated the chemical composition of different parts of Norway spruce and reported lower Al, K, Si and Mn in wood than in the present work and much higher values of Mn and K in bark. Scots pine and silver birch, on the other hand, show small amounts of Al, Na and Si. Generally, common wood species stand out as having relatively low contents of ash-forming elements compared to all analysed species.

Ca is the dominate ash forming element in biomass, especially in bark samples where concentrations were up to twelve times higher than those of the wood samples. Similar findings have been reported by Kleinhans et al. (2018). The Ca content of the wood species ranged within rather wide limits, 718–63,910 ppm, depending on the wood type. The highest Ca content of 63,910 and 49,587 ppm was found from the European crab apple and wych elm bark samples, respectively. The lowest Ca content was observed in Scots pine, aspen and European ash wood samples: 718, 890 and 891 ppm, respectively. A higher Ca content in the feedstock is advantageous. In some cases, limestone as a source of calcium is added because it acts as a sulphur capture technique and prevents agglomeration (Miles et al., 1996). Koppejan & van Loo (2016) indicate that ashes of agricultural crops with low concentrations of Ca and high concentrations of potassium (K) start to sinter and melt at significantly lower temperatures than woody biomass. In this study, the Ca content of most of the samples outweigh K content, but there are two exceptions: willow and wych elm wood – these samples have a much higher probability to cause lower ash melting behaviour.

K is the second most abundant alkali element in biomass. K is stated as one of the elements that in high concentrations together with the high silica and chlorine content, is known to create ash deposit problems (Kurkela, 1996). K is detected as KCl, K_2SO_4, K_2CO_3, K_3PO_4 in biomass ash (Mlonka-Mędrala et al., 2020). Results show that broad-leaf wood species tend to have higher K contents than softwood. On the other hand, K is reported as an effective catalyst in gasification and it enhances tar reforming (Sueyasu et al., 2012; Nzihou et al., 2013). Gil et al. (2019) also found that a high H_2 content and high H_2/CO ratio in the gas product are related to the higher H/O ratio and K_2O in biomass. Therefore, samples that have a higher K content may be favoured instead, when a low content of tar in the syngas is required, although sintering problem should be considered.

It has been found that the Si content in hardwood bark may reach up to 2% and in case of softwood up to 0.5% (ISO 17225). Interestingly, all analysed samples had lower Si content than typical values, from 0.002 to 0.41% (except hawthorn bark with 0.9% Si). Although, a similar pattern exists where bark has majority of Si content compared to the whole wood. These results seem to be consistent with other research which found that the silica content of clean bark is higher than that of wood (Ragland et al., 1991). The high silica content in bark or wood originates from soil components and sand.

Iron (Fe) content varied between 38 ppm and 1,377 ppm. It is stated that the typical Fe content in woods lies between 0–100 ppm and in bark it may reach up to 800 ppm (ISO 17225). European ash wood was enriched in Fe (1,377 ppm), but all other samples are comparable to typical values.

Al content in most of the woods and barks are in accordance to technical standard, that propose values of < 10 to 1,200 ppm (ISO 17225). Smallest amount of Al is found from common species (< 393 ppm). On the other hand, hardwood barks show higher concentrations than recommended values in standard, from 70 to 2,810 ppm. Na content in woods and barks are below 200 ppm and 1,200 ppm, respectively and are in accordance to typical values.

Woody biomass has higher amounts of heavy metals than annually harvested crops because wood has a longer rotation period for accumulation of heavy metals (Koppejan & van Loo, 2016). Barks of hawthorn, European ash, bird cherry, and rowan stand out as they have relatively high amount of Mn, Zn, Cr, Ni, Sr, Ti, Cu and Pb. Silver birch and grey alder have higher concentration of heavy metals than other common hardwoods. In addition, Norway spruce bark has twice as high of a heavy metals concentration as Scots pine bark.

Correlations between characteristics

The Pearson correlation coefficient was used to assess linear associations between biomass parameters in order to elucidate which feedstock features have an impact on each other (Table 3). The strength of the correlation is described using the guide that Evans (1996) suggests for the absolute value of r: 0.40–0.59 as ’moderate’; 0.60–0.79 as ‘strong’; 0.80–1.0 as ‘very strong’. Knowing correlations between variables gives the opportunity to predict biomass composition without a need to carry out a full set of analyses.

The present data show very strong positive correlations ($r \geq 0.8$) between the following characteristics: Cr-Ni-Fe, Ca-Sr, HV-C, Al-Na-Si-Ti, K-Mg-P, Fe-Zn-Cr-Ni-Cu, Ash-Ca, N-S-P and O-VM. Most of the ash forming elements like Si, Ca, K, P, Al, Mg, Fe, S, Sr, Na and Ti have moderate to very strong correlations with ash. Also, Mn is classified as an ash-forming element according to Werkelin et al., (2011). Cr and Ni together with Ca, Fe and Cu are non-volatile metals that are found to exit principally in equal concentrations in the bottom ash and the fly ash (Nzihou & Stanmore, 2013). However, this was not supported in the present study where no significant relationships between ash yield and Mn, Cr and Ni were determined.

Table 3. Pearson's correlation matrix between any two variables ($P \leq 0.05$)

	Ash	VM	FC	C	H	N	S	O	Cl	HHV	Al	K	Na	Ca	Si	Mg	Fe	P	Mn	Zn	Cr	Ni	Sr	Ti	Cu	Pb	
Ash	1.0	-0.8	0.2	-0.5	-0.8	0.5	0.7	-0.7	0.5	-0.5	0.6	0.6	0.7	1.0	0.7	0.7	0.4	0.7	0.1	0.4	0.2	0.2	0.8	0.6	0.4	0.5	+1
VM	-0.8	1.0	-0.7	0.1	0.8	-0.7	-0.8	0.8	-0.5	0.1	-0.5	-0.6	-0.5	-0.8	-0.5	-0.6	-0.4	-0.7	-0.2	-0.4	-0.4	-0.4	-0.6	-0.4	-0.6	-0.5	
FC	0.2	-0.7	1.0	0.3	-0.3	0.6	0.4	-0.5	0.3	0.3	0.1	0.2	0.1	0.3	0.0	0.2	0.2	0.3	0.2	0.2	0.3	0.3	0.3	0.1	0.5	0.3	
C	-0.5	0.1	0.3	1.0	0.5	0.1	-0.1	-0.2	0.1	0.9	-0.2	-0.3	-0.3	-0.5	-0.4	-0.2	-0.2	-0.3	0.0	-0.3	0.0	0.0	-0.4	-0.2	-0.1	0.0	
H	-0.8	0.8	-0.3	0.5	1.0	-0.4	-0.6	0.5	-0.1	0.6	-0.4	-0.5	-0.5	-0.8	-0.5	-0.5	-0.4	-0.5	0.0	-0.5	-0.3	-0.3	-0.7	-0.4	-0.5	-0.4	
N	0.5	-0.7	0.6	0.1	-0.4	1.0	0.8	-0.7	0.4	0.2	0.3	0.6	0.3	0.5	0.3	0.6	0.3	0.8	0.1	0.2	0.2	0.2	0.4	0.2	0.4	0.4	
S	0.7	-0.8	0.4	-0.1	-0.6	0.8	1.0	-0.8	0.5	-0.1	0.6	0.7	0.6	0.7	0.6	0.8	0.4	0.9	0.1	0.4	0.2	0.2	0.4	0.5	0.4	0.5	
O	-0.7	0.8	-0.5	-0.2	0.5	-0.7	-0.8	1.0	-0.6	-0.2	-0.5	-0.4	-0.5	-0.7	-0.5	-0.6	-0.3	-0.6	-0.2	-0.3	-0.2	-0.2	-0.6	-0.5	-0.4	-0.5	
Cl	0.5	-0.5	0.3	0.1	-0.1	0.4	0.5	-0.6	1.0	0.2	0.5	0.1	0.6	0.4	0.5	0.3	0.2	0.4	0.0	0.1	0.0	0.0	0.3	0.5	0.1	0.4	
HHV	-0.5	0.1	0.3	0.9	0.6	0.2	-0.1	-0.2	0.2	1.0	-0.1	-0.2	-0.2	-0.5	-0.3	-0.1	-0.1	-0.1	0.1	-0.2	0.0	0.0	-0.4	-0.2	-0.1	0.0	
Al	0.6	-0.5	0.1	-0.2	-0.4	0.3	0.6	-0.5	0.5	-0.1	1.0	0.2	1.0	0.4	0.9	0.2	0.5	0.3	0.1	0.3	0.2	0.2	0.2	1.0	0.4	0.7	
K	0.6	-0.6	0.2	-0.3	-0.5	0.6	0.7	-0.4	0.1	-0.2	0.2	1.0	0.3	0.5	0.3	0.8	0.1	0.8	0.1	0.2	0.0	0.0	0.3	0.1	0.1	0.1	
Na	0.7	-0.5	0.1	-0.3	-0.5	0.3	0.6	-0.5	0.6	-0.2	1.0	0.3	1.0	0.6	0.9	0.4	0.5	0.4	0.1	0.3	0.1	0.1	0.3	1.0	0.3	0.6	
Ca	1.0	-0.8	0.3	-0.5	-0.8	0.5	0.7	-0.7	0.4	-0.5	0.4	0.5	0.6	1.0	0.6	0.7	0.4	0.6	0.2	0.4	0.3	0.2	0.8	0.5	0.5	0.5	
Si	0.7	-0.5	0.0	-0.4	-0.5	0.3	0.6	-0.5	0.5	-0.3	0.9	0.3	0.9	0.6	1.0	0.3	0.5	0.4	0.1	0.3	0.1	0.1	0.3	0.9	0.3	0.6	
Mg	0.7	-0.6	0.2	-0.2	-0.5	0.6	0.8	-0.6	0.3	-0.1	0.2	0.8	0.4	0.7	0.3	1.0	0.2	0.9	0.3	0.4	0.1	0.1	0.5	0.2	0.2	0.1	
Fe	0.4	-0.4	0.2	-0.2	-0.4	0.3	0.4	-0.3	0.2	-0.1	0.5	0.1	0.5	0.4	0.5	0.2	1.0	0.2	0.1	0.9	0.9	0.9	0.2	0.5	0.8	0.7	
P	0.7	-0.7	0.3	-0.3	-0.5	0.8	0.9	-0.6	0.4	-0.1	0.3	0.8	0.4	0.6	0.4	0.9	0.2	1.0	0.1	0.2	0.0	0.0	0.5	0.3	0.2	0.3	
Mn	0.1	-0.2	0.2	0.0	0.0	0.1	0.1	-0.2	0.0	0.1	0.1	0.1	0.1	0.2	0.1	0.3	0.1	0.1	1.0	0.2	0.2	0.1	0.1	0.0	0.3	0.2	
Zn	0.4	-0.4	0.2	-0.3	-0.5	0.2	0.4	-0.3	0.1	-0.2	0.3	0.2	0.3	0.4	0.3	0.4	0.9	0.2	0.2	1.0	0.7	0.8	0.3	0.3	0.7	0.4	
Cr	0.2	-0.4	0.3	0.0	-0.3	0.2	0.2	-0.2	0.0	0.0	0.2	0.0	0.1	0.3	0.1	0.1	0.9	0.0	0.2	0.7	1.0	1.0	0.2	0.1	0.9	0.5	
Ni	0.2	-0.4	0.3	0.0	-0.3	0.2	0.2	-0.2	0.0	0.0	0.2	0.0	0.1	0.2	0.1	0.1	0.9	0.0	0.1	0.8	1.0	1.0	0.2	0.1	0.9	0.5	
Sr	0.8	-0.6	0.3	-0.4	-0.7	0.4	0.4	-0.6	0.3	-0.4	0.2	0.3	0.3	0.8	0.3	0.5	0.2	0.5	0.1	0.3	0.2	0.2	1.0	0.2	0.3	0.2	
Ti	0.6	-0.4	0.1	-0.2	-0.4	0.2	0.5	-0.5	0.5	-0.2	1.0	0.1	1.0	0.5	0.9	0.2	0.5	0.3	0.0	0.3	0.1	0.1	0.2	1.0	0.3	0.7	
Cu	0.4	-0.6	0.5	-0.1	-0.5	0.4	0.4	-0.4	0.1	-0.1	0.4	0.1	0.3	0.5	0.3	0.2	0.8	0.2	0.3	0.7	0.9	0.9	0.3	0.3	1.0	0.7	
Pb	0.5	-0.5	0.3	0.0	-0.4	0.4	0.5	-0.5	0.4	0.0	0.7	0.1	0.6	0.5	0.6	0.1	0.7	0.3	0.2	0.4	0.5	0.5	0.2	0.7	0.7	1.0	-1

Strong positive correlations (0.60 ≤ r ≤ 0.79) occurred also between Ash-Na-Si-Mg-P-Sr-Ti, VM-H, N-Mg, S-Ash-K-Na-Ca-Si-Mg, HV-H, K-P-Pb, Ca-Si-Mg-P. Compared to previous studies there were similar findings, for example strong positive correlation for Al-Ti-Si, N-S-P and K-Mg-P (Vassilev et al., 2010), S-N-Cl (Jenkins et al., 1998), Ash-Ca-Na-Si, K-P-Mg, Ca-S-P (Monti et al., 2008).

Very strong negative relationships occur between Ash-VM, Ash-H, VM-Ca and Ca-H. Strong negative correlations are mainly associated with VM, C, H, O and HV which are mostly negatively correlated with trace elements. VM has a negative correlation with most of the parameters, except with O, H, C and heating value. Same pattern is seen with O, where a positive correlation is only observed with volatiles and a moderate correlation with H. This finding was also reported by Vassilev et al. (2010) who stated there is a strong negative relationship between ash-O, O-N, O-S, O-P. In contrast to earlier findings, however, no evidence of negative correlations between trace elements (e.g. Si-Ca, Si-Mg, Si-K, Si-Mn, Ca-K, Ca-Al, K-Al, Ca-Al, Fe-Ca) was detected. In this study, several strong correlations between metallic elements were found, for example Al-Na-Si-Ti, Ca-Sr, Ca-Si, Ca-Mg, Si-Pb, Fe-Pb, Zn-Cr-Ni, Cu-Pb. According to Vassilev et al. (2012) the Si-Al-Fe-Na-Ti association is related mostly as detrital silicates and oxyhydroxides, excluding authigenic opal and Ca-Mg-Mn is commonly related to authigenic oxalates and carbonates.

VM correlates with H content in the data set, which is in agreement with the conclusion of Caillat & Vakkilainen (2013) that a higher VM content is due to a high hydrogen content and depends on the nature of the material and thermochemical process conditions. It has also been suggested that a high VM and fixed carbon content increase the heating value (Saidur et al., 2011). This does not appear to be the case and no correlation between VM and HV was detected in the current study.

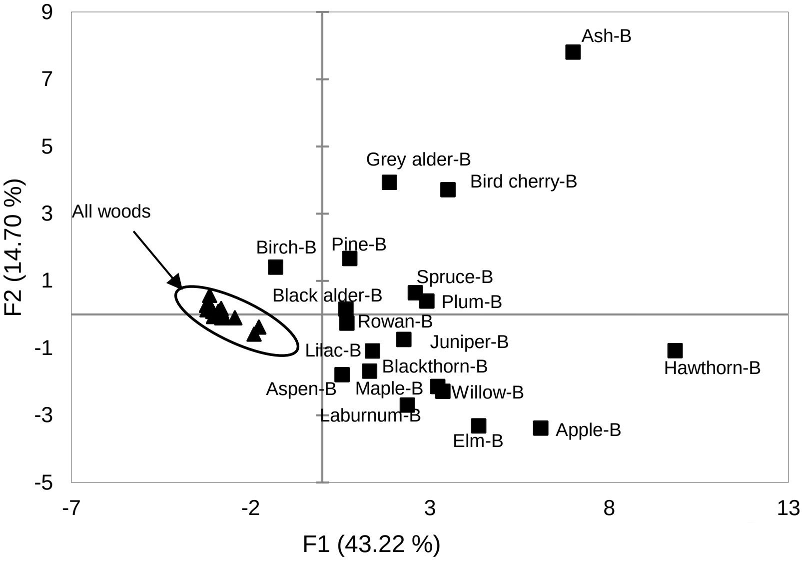

Figure 1. Graphical dispersion based on the PCA obtained from 26 variables (autoscaled): B – bark; W – wood.

A principal component analysis (PCA) was conducted on the variables studied in order to observe possible groupings and/or separation among wood species. PCA scatter plot (Fig. 1) revealed a clear data clustering in terms of the sample type using the scores of the first two principal components with a total variance of 57.9%. Generally, a total variance of 70% is considered as satisfactory in PC analysis. In this case, a lower total variance was accepted as satisfactory because descriptive purpose was desired, and it gave sufficient interpretation and scattering of the samples. Wood samples form a relative compact cluster that is characterized by high O, H, VM and HHV and low ash, while the bark samples are much more diverse and distributed over a wider range that is characterized by high ash and ash-forming element concentration. This suggests that different woods have similar compositions and there is no significant difference between species. It is possible, therefore, that the grouped biomass samples will show similar behaviours even if they are namely different. This means that if the process conditions are similar, the same behaviour can be expected among wood samples. However, the layout of bark samples reflects their variability and differences in composition. Interestingly, birch bark belongs to the wood cluster. This can be explained by the very high heating value (24.18 MJ kg^{-1}), volatiles content (80.1%) and carbon content (56.34%), makes it similar to wood samples. Results show that there are some significant differences and exceptions between wood species. For instance, hawthorn bark had remarkably high Al, Na, Ca and Si content compared to others.

The loading plot (Fig. 2) illustrates the connections of the variables to each other and gives an impression about correlations between features. The loading unravel the magnitude (large or small correlations) and in which way (positive or negative correlation) the variables contribute to the scores (Jackson, 1991; Jollife & Cadima, 2016). Sample distributions follow the loading values that wood samples spread in horizontal direction due to contents of volatiles, hydrogen, oxygen, Al, Ti, Na, Si, Ca, S and ash, and in the vertical direction due to mainly Cr, Ni, K content. It reveals that VM, H and O exhibit negative loadings in the negative side of the first principal component (F1). On the other hand, Al, Ti, Na, Ca, S and ash show significant positive loading contributions to the first principal component (F1).

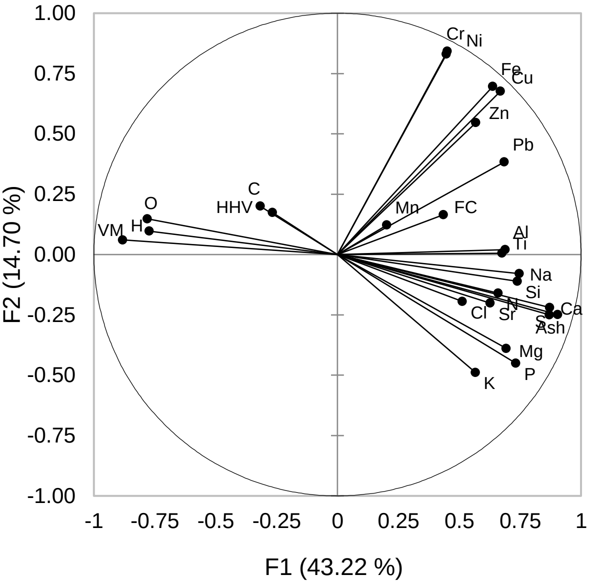

Figure 2. Correlations between variables according to PCA.

CONCLUSIONS

Based on results obtained experimentally and information available in the literature, this paper gives an overview of the characteristics of different Estonian wood species.

Statistical analysis supports the knowledge that woody biomass is heterogenous and its composition depends on its origin, growing environment and wood-specific mineral uptake.

Some conclusions based on complete proximate, ultimate and ash analyses of 19 different wood and bark samples can be made:

1) Wood samples have a relatively predictable composition where parameters vary in a narrow range. On the other hand, wood bark composition is more diverse and depends on the wood species.

2) Birch bark composition is more similar to wood samples than other bark samples, mainly due to its very high heating value, high content of C, H and volatiles and low ash content.

3) Main parameters that cause wood and bark samples clustering are volatiles, hydrogen, oxygen, ash, Al, Ti, Na, Si and Ca.

4) Very strong positive correlations were found, namely: Cr-Ni-Fe, Ca-Sr, HV-C, Al-Na-Si-Ti, K-Mg-P, Fe-Zn-Cr-Ni-Cu, Ash-Ca, N-S-P and O-VM.

5) Strong positive correlations occurred between Ash-Na-Si-Mg-P-Sr-Ti, VM-H, N-Mg, S-Ash-K-Na-Ca-Si-Mg, HV-H, K-P-Pb, Ca-Si-Mg-P.

6) Most of the ash forming elements have moderate to very strong correlation with ash, except Mn, Cr, Ni.

7) Very strong negative relationships occur between Ash-VM, Ash-H, VM-Ca and Ca-H.

8) Results show that not all wood species coincide with typical wood or bark. Most of the analysed hardwood barks have higher ash yields compared to a typical hardwood.

9) Gasification feedstock should be chosen considering the aim of gasification. To increase the yield of biochar, bark should be used because of the high content of fixed carbon. When a high reactivity and conversion rate is required, the wood part should be used because of its high volatile matter content.

10) Common wood species (Scots pine, Norway spruce, aspen, birch, grey alder and black alder) are perspective feedstock for gasification because of the low S, N, Cl, and ash content and high volatile matter.

11) Hardwood species have higher heavy metal contents (Mn, Zn, Cr, Ni, Sr, Ti, Cu, Pb) than softwoods. Of the common species, birch and grey alder have over three times higher total heavy metal content than other common hardwoods. In addition, spruce bark has more than two times higher total heavy metals content than pine bark.

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Matrix quality variability of oilseed radish (*Raphanus sativus* L. var. *oleiformis* Pers.) and features of its formation in technologically different construction of its agrophytocenosis

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Abstract. Overview of the formation of matrix variability of oilseed radish seeds at two levels: within a single pod and within the generative part of the plant, given the presence of vertical layering in the placement of fruit elements. The peculiarities of formation of variation component of morphological parameters of seeds from different zones of inflorescence were estimated, basing on their electrical scanning for oilseed radish agrophytoconosises of different technological construction and sowing rate against the background of four options of mineral nutrition (without fertilizer to the level 90 kg ha^{-1} with an equal interval of 30 kg ha^{-1} of the primary material). The results of the value of matrix variability in morphological and weight characteristics were grounded on the basis of the analysis of the structure of selected seed fractions and their intra-group variation. The main features of formation of morphometric variability of seeds from different zones of inflorescence depending on different technological construction were formulated.

Key words: heterospermy, matrix variability, morphological parameters of seeds, oilseed radish, seed fractions, separation variability.

INTRODUCTION

Modern approaches to seed production technologies provide for the transition from general concepts of qualitative categories of seeds in general to the study of complex patterns of its formation. This provides prediction of its yield, sowing and adaptive properties and allows obtaining seeds with a high level of genetic (matrix), ecological-adaphic and morphological uniformity. Seed uniformity in this aspect is considered as a factor in ensuring optimal and uniform germination rates, which forms a high-intensity agrophytocenosis of the culture with the same standing density, the desired level of coenotic stress and feeding conditions (Scarisbrick, 1982; Wulff, 1986; Bouttier & Morgan 1992; Aicher, 2011; Balodis & Gaile, 2016; Stankowski et al., 2019; Wilczewski et al., 2020).

Heterospermia of seeds is considered as its difference in morphological characteristics, biochemical composition and physiological state, the ability to germinate and certain provide productivity of plants in offspring (Venable, 1985; Geritz et al., 1999; Alonso-Blanco et al., 2009; Li et al., 2014, 2014a; Yang et al., 2017).

Scientific practice distinguishes three types of heterospermy, namely ecological, matrix and genetic (genotypic) (Kizilova, 1974; Makrushin, 1994; Zhu & Weir, 1994; Yizhyk, 2000, 2000a; Rahman & Vetty, 2011; Hua et al., 2012). Ecological quality variability is the result of interaction of seeds in the process of its formation with environmental factors and belongs to not hereditary form, but it is an important aspect of the formation of biological and morphological properties of seeds and levels of seed productivity in the format of implementation of the genotype-environment combination (Leishman, 1995; Rees, 1996; Zhu, 1996; Fox et al., 1997; Nikolayeva, 1999; Imbert, 2002; Yang & Midmore, 2005; Gunasekera, 2006; Silveira et al., 2012; Sadras et al., 2013; Zhang et al., 2013; Ivanova & Sarmosova, 2014; Gnan et al., 2014; Yang et al., 2017; Li et al., 2020).

Genotypic variability is the result of a combination of hereditary signs of parental forms. In these conditions, while preserving the general type of heredity (varietal characteristics), each seed has differences due to the insemination process. This type of variability is also caused by mutagenic factors (Tayo & Morgan, 1978; Clarke, 1979; Morgan, 1980; Kindruk, 1990; Smith & Scarisbrick, 1990; Batygina, 1999; Ali et al., 2002; Wolfe & Mazer, 2005; Kaushik et al., 2007; Kennedy et al., 2011; Würschum et al., 2012; Li et al., 2014; Fu et al., 2015; Li & Li, 2015a; Li et al., 2015b; Li et al., 2019).

Matrix variability is one of the most common options and is formed as a result of different placement of flowers within the inflorescence on the mother plant. As a result, conditions are created for different nutrition conditions of different inflorescence levels and different influence of the plant as a mother body. Even if genetic and environmental factors are identical, the location of the seeds leads to the heterospermy detection (Olsson, 1960; Cavers & Steel, 1984; Inanaga & Kumura, 1987; Roach & Wulff, 1987; Smith & Scarisbrick, 1990; Habekotté, 1996; Makrushin, 1994; Donohue & Schmitt, 1998; Mazer & Wolfe, 1998; Nikolayeva, 1999; Sundaresan, 2005; Sadeghietal., 2011; Bukharov & Baleev, 2012; Hua et al., 2012; Zhang et al., 2016: Zheng et al., 2017).

The primary reasons for the matrix variability are the differences associated with the placement of individual seeds in different parts of the inflorescence in the vertical and horizontal orientation, namely: different flowering time, insemination and seed formation, different input intensity of plastic substances in the process of seed formation due to the reutilization of their transformation from the vegetative part of the mother plant fruits and seeds, as well as different degree of protection of the seedor fruit from abiotic environmental factors (Tayo & Morgan, 1978; McGregor, 1981; Roach & Wulff, 1987; Chay & Thurling, 1989; Kindruk, 1990, Makrushin, 1994; Donohue & Schmitt, 1998; Lemontey et al., 2000; Zlobin, 2009; Faraji, 2010; Kennedy et al., 2011; Wang et al., 2011; Gomez & Miralles, 2011; Gnan et al., 2014; Khan et al., 2014; Xing et al., 2014; Yang et al., 2016, 2017). This changes the physical and mechanical, chemical, sowing and yield properties of seeds. For these reasons, the creation of a control system for matrix heterospermia is a more important prerequisite for obtaining high-quality seed (Berry & Spink, 2006; Faraji, 2012; Monty et al., 2016).

As biological and physiological aspects, the matrix quality variability of seeds is important in the analysis of adaptability of technology and optimality of formation of agrophytocenosis both from the position of plant density in the unit area and from the position of mineral nutrition (Tsytsiura, 2019). For the group of cruciferous crops, among which is the object of our study, the signs of heterocarpy and heterospermy were studied by separate indicators in a number of studies. Thus, the results of the study of peculiarities of flowering stages of the generative part of rapeseed (McGregor, 1981; Habekotté, 1996.) and mustard (Vovchenko & Fursova, 2012) were made public. The peculiarities of forming the spatial structure of winter and spring rapeseed are highlighted (Tayo & Morgan, 1978; Smith & Scarisbrick, 1990; Habekotté, 1996). Issues of modeling the architectonics of rapeseed inflorescence and estimation of peculiarities of fruits and seeds uniformity within the inflorescence in such modeling are considered (Wang et al., 2011; Li et al., 2016). The influence of fractional composition of seeds of a number of cruciferous crops on the consistent formation of its sowing and yielding qualities was grounded (Olsson, 1960; Nikolaeva et al., 1999, Vyshnivsky, 2014). The evaluation of the general stage of flowering period of individual cruciferous crops and their influence on the formation of conditioned seeds was carried out (Smith & Scarisbrick, 1990; Polowick & Sawhney, 1986).

It is also noted for many cruciferous crops that the degree of variability of seeds is determined by the level of mineral nutrition of plants (Gattelmacher et al., 1994; Ozturk, 2010; Béreš et al., 2019), as well as technological factors such as the nature of the combination of fertilizer (Vujaković et al., 2014), the relationship between macro and micronutrients in the fertilizer system (Jankowski et al., 2014). In the formation of morphological features of seed development within the inflorescence a significant influence on this indicator of the technological features of nitrogen nutrition. In terms of flowering duration, the nature of flower formation, the use of phosphorus fertilizers and trace elements, as well as their ratio (Gomez & Miralles, 2011; Grant et al., 2013; Ragab et al., 2015; Sieling et al., 2017; Béreš et al., 2019; Zou et al., 2020).

The area of plant nutrition in the agrocoenosis due to the regulation of the value stress in turn determines the morphological development of plants, reproductive effort and seed productivity. The increase in standing density per unit area is directly proportional to the decrease in the generative part of plants with a decrease in the reaction rate interval in terms of morphological and weight characteristics of fruits and seeds (Hocking & Stapper, 2001; Takashima et al., 2013). As a result, the morphometric and weight alignment of seeds for many cruciferous crops, including oilseed radish, will be determined by the optimal ratio of technological parameters of subsowing formation of agrocenosis at the appropriate value of background mineral nutrition (Rathke et al., 2005; Al-Doori & Hasan, 2010; Qian et al., 2012; Sieling et al., 2017; Porter et al., 2020). For each plant species, these parameters are individual and rather extent unique (Berry et al., 2010; Qian et al., 2012). On the other hand, this conditions are different for different soil-climatic zones, but tend to be similar (Hocking et al., 1997; Horst et al., 2003; Marjanovi´c-Jeromel et al., 2011). It is also noted that considering the aspects of the matrix variability of seeds, given its fractional composition during harvesting and separation, provides a reduction of yield losses up to 30–39% and significantly improves the homogeneity of seed material and provides optimization of growth processes for agrophytocenosises basing on its consistent reproductive use (Makrushin, 1994; Kyrpa, 2011).

On the other hand, it was noted (Hasanuzzaman, 2020) that despite the agro-technological value of a number of crops from the cruciferous family. These factors are studied in detail on winter and spring rape and little-studied on white and black mustard, spring colza, and the object of our study oilseed radish as well.

For this group of crops, which also includes oilseed radish (*Raphanus sativus* var. *oleifera* Pers.), in addition to the above types of seed variability within its matrix type, its individual subtypes are distinguished: gravimorphic (differences in seed weight, size, shape, pattern) and enantiomorphic (differences in symmetry and asymmetry of a seed or fruit) (Dorofeyev, 2004; Tsytsiura & Tsytsiura, 2015). A number of questions remain to be discussed concerning the estimation of the general level of variation of morphological parameters of seeds in cruciferous crops and their influence on the value of realization of individual seed productivity of plants. It is also important to consider the peculiarities of layering in the flowering of different orders of the inflorescence of cruciferous crops (Schiessl et al., 2015) and, as a consequence, the corresponding stage in the formation of fruits from different tiers in the vertical placement of the last from the base of the inflorescence to its apex. This feature is noted by a number of researchers on winter and spring rapeseed (McGregor, 1981; Scarisbrick et al., 1982; Habekotté, 1996; Wang et al., 2011; Vyshnivsky & Slisarchuk, 2014) but is poorly studied on oilseed radish plants. This leads to well-known technological problems in the time diversity of seed maturation and choice of pre-harvest preparation of plants, associated with the need for separate harvesting or the use of desiccation, the latter has certain limitations in the application for the oilseed radish agrophytocenosises (Tsytsiura, 2018a). Given the above facts, the assessment of the level of matrix variability of oilseed radish varieties will make it possible to determine the adaptation strategy of plants, and use this parameter as an indicator of the optimization of agrophytocenosis formation.

MATERIALS AND METHODS

The research on the formation of indicators of the matrix variability of oilseed radish varieties of plants with changes in technological parameters of the construction of its agrophytocenosises was carried out during 2013–2018 on the experimental field of theVinnytsia National Agrarian University (N 49°11′31″, E 28°22′16″) on dark gray forest soils (Luvic Greyic Phaeozem soils (Working Group WRB, 2015)) of the medium loam mechanical compositi on with fluctuating basic agrochemical indicators in the rotation section: humus content 2.16–2.52%, pH 5.8–6.7, lightly hydrolyzed nitrogen content 71–77 mg kg^{-1}, mobile phosphorus 187–251 mg kg^{-1}, exchangeable potassium 95–143 mg kg^{-1}. The basic technological scheme of technological construction of oilseed radish agrophytocenosises provided for the application of the interval of technological solutions adopted in the region in terms of row width and sowing rate in

Table 1. The range of acceptable common options for the formation of oilseed radish agrophytocenosis in the study area (Tsytsiura, 2019)

Planting method and seeding rates (million germinable seeds ha^{-1})		Fertilization (of the active substance), kg ha^{-1}
Row method (15 cm)	Wide-row method (30 cm)	Without fertilizers
1.0	0.5	$N_{30}P_{30}K_{30}$
2.0	1.0	$N_{60}P_{60}K_{60}$
3.0	1.5	$N_{90}P_{90}K_{90}$
4.0	2.0	

accordance with zonal recommendations of oilseed radish cultivation in the zone of theforest-steppe of moderately continental belt (Table 1). Term of sowing of all variants corresponded to the period of the end of the first-middle of the second ten-days period of April.

Three recognized varieties of oilseed radish, namely, ‘Zhuravka’, ‘Raiduha’ and ‘Lybid’, were used in the research. These varieties belong to the varieties of combined use for fodder purposes and seeds with wide adaptive potential for cultivation in different soil and climatic zones.

The hydrothermal regime of oilseed radish vegetation during the period of seed formation and filling differed (Table 2).

Table 2. Precipitation amount and average daily temperature in comparison with the long-time average annual regime for the research period

Months	Ten-days period	Years						Long-time average annual value (30-year averaging period)
		2013	2014	2015	2016	2017	2018	
		Average daily temperature, °C						
June	I	17.2	18.1	20.4	15.9	18.0	19.2	15.9
	II	19.9	16.3	19.2	18.7	18.1	20.7	16.7
	III	20.8	15.7	18.2	23.7	21.4	17.9	17.5
July	I	19.7	19.2	21.5	19.3	18.2	18.6	18.2
	II	18.6	19.7	19.0	20.6	19.2	19.1	18.8
	III	18.6	21.3	22.7	21.6	21.7	21.5	19.0
Average for the selected period, °C		19.5	18.5	19.7	20.8	19.8	19.1	17.7
		Precipitation amount, mm						
June	I	36.0	43.1	3.2	15.0	1.8	0.5	22.8
	II	71.0	0.0	28.8	38.9	11.9	91.3	24.7
	III	37.0	33.4	9.3	18.3	15.2	156.3	25.9
July	I	0.2	28.5	3.0	17.4	5.7	10.0	25.2
	II	11.1	39.0	11.9	44.0	18.6	44.0	23.8
	III	15.4	15.0	5.8	6.7	67.7	52.3	29.2
Amount forthe selected period, mm		134.7	216.8	56.2	125.3	119.1	302.1	151.6
		HTC**						
May		1.305	2.783	0.719	1.227	0.645	0.258	1.460
June		2.202	1.078	0.613	0.893	0.349	3.124	1.416
July		0.377	1.137	0.230	0.682	0.806	1.349	1.396
August		1.047	0.750	0.061	0.486	0.563	0.349	1.234
Average for the vegetation period		1.232	1.437	0.406	0.822	0.591	1.270	1.377

* – grey color marks the period from flowering (BBCH 63) to the yellow-brown pod phase (BBCH 86) of the oilseed radish for phenological conditions of the study area; ** $HTC = \frac{\sum R}{0.1 \cdot \sum t_{>10}}$, where the amount of precipitation (ΣR) in mm over a period with temperatures above 10 °C, the sum of effective temperatures ($\sum t_{>10}$) over the same period, decreased by a factor of 10 (Polyovy & Bozhko, 2007).

Variation of morphological parameters of seeds was estimated with a sample of 25 plants in non-contiguous repetitions (total sample of 50 plants). Total number of repetitions for each variant 4. Plant analysis provided for the evaluation of a group of 5 plants in 5 places by the length of the line stochastically by the width of the area with a displacement in the line horizontal to the phase of brown maturity of the pod BBCH 87 (Hocking, 1997; Meier, 2001; Skinner & Moore, 2007) when all the pods have reached a typical size.The formed seeds were selected separately from the three zones of the oilseed radish inflorescence (evenly with the same interval on the total height for each inflorescence according to the general recommendations for determining the vertical level and mosaic of agrophytocenosises (Rabotnov, 1992)). Peculiarities of oilseed radish fruit anatomy due to the evaluation of heterospermy level were carried out taking into account scientific developments in the field of carpology for cruciferous crops (Puri, 1941; Zohary, 1948; Levina, 1987; Lotova & Rudko, 1999; Dorofeyev, 2004; Ilyinska, 2013.

Seed mass characteristics were determined with the use of laboratory scales RADWAG PS 1000.R1with 0.001 g discretion.Analysis of fractional composition of seeds was carried out by sifting the selected average sample of seeds on testing sieve sets with a round cell according to Specifications 23.2.2068-94 typeIandwitha rectangular cell according to Specifications 5.897–11722–95, TC 23.2.2068-94 type II. The fractional composition was also determined by the method of processing a single-layer scanned image of seeds using the Digimizer image analysis software package (v 4.2). Weight of 1000 seeds was determined according to the state national standard (State standard of Ukraine 4138-2002, 2003).

Seed parameters were estimated based on such characteristics as projection surface area (PS, mm^2), surface perimeter (P, mm), seed length (L, mm), seed width (W, mm), roundness (R, mm), seed sorting index (SI) (ratio of seed length (L, mm) to its width (W, mm)).

Study of the morphological structure of fruits was conducted in accordance with the general recommendations of morphological and anatomical analysis of cruciferous fruits (Puri, 1941; Zohary, 1948; Levina, 1987; Lotova & Rudko, 1999; Dorofeyev, 2004; Ilyinska, 2013).

To determine these morphometric characteristics, used Didital Caliper (measurement accuracy of 0.01 mm), USB microscopy SigetaMCMOS 5100 5.1 MPUSB 2.0 (in combination with a digital microscope for ×10 and ×40 optical magnification formats) and OotdtyDM–1600, 2 MP with corresponding software and using ImageJ 1.52 software.

The matrix variability index (MVI) in comparison with the morphological parameters of seeds from the lower and upper inflorescence zone was determined in relation to the average coefficient of variation (CV,%). Measurements and observations were performed using field and laboratory photography with the camera Canon EOS 750DKit and lenses Canon EF 100 mmf/2.8 LUS Mand CanonMP-E 65 mmf/2.8 1-5 x Macro.

General methodology of researches, consideration of phenological phases in the system of signs of oilseed radish bearing phase and other related observations and records were conducted in accordance with the basic recommendations of researches on cruciferous crops (Sayko, 2011; Test Guidelines…, 2017) using the package of statistical software Statistica 10, Exel 2013, Past 324 and biometric methods of statistics

(Zar, 1984). The level of variability of morphological features and grouped indicators was conducted on the scale (Zaytsev, 1984): very low (CV < 7%); low (CV = 8–12%); medium (CV = 13–20%); elevated (CV = 21–30%); high (CV = 31–40%); very high (CV > 40%). Given the massiveness of the data and general similarity in the values of formation of features for the studied oilseed radish varieties. The data are presented by key indicators for ‘Zhuravka’ variety.

RESULTS AND DISCUSSION

According to our preliminary estimates (Tsytsiura, 2018b), there is a great variation in the seeds of oilseed radish varieties. The seed of this crop itself has a number of morphological forms, which, in average, make it possible to attribute it to the egg-shaped one with different index of length to width ratio. This is clearly demonstrated in Fig. 1.

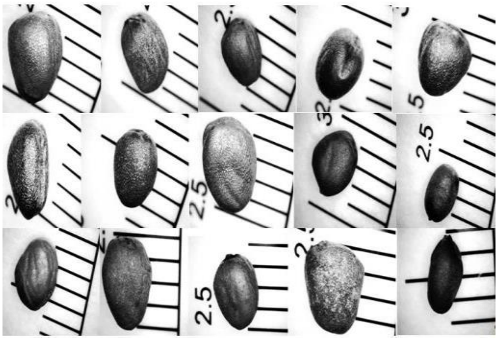

Figure 1. Quality variability of oilseed radish seeds of the ‘Zhuravka’ variety by typological linear sizes and shape, 2015.

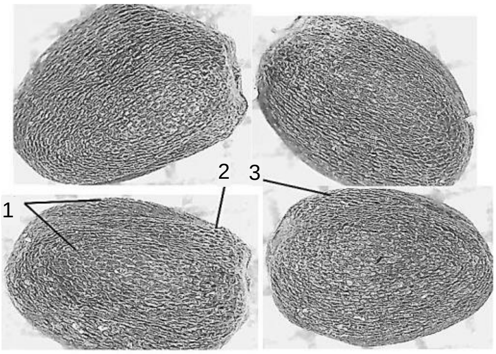

Figure 2. General appearance of oilseed radish seeds of the ‘Zhuravka’ variety from different positions, 2018.

According to the presented typological forms of seeds there are egg-shaped, elongated egg-shaped, flattened egg-shaped, oval, globe-shaped and various combined forms. Seed color is also a variable indicator (gravimorphic variability) from grey and brown to dark brown with a dark red shade. The color tends to change from light brown for newly harvested seeds to brownish red for seeds after long-term storage. Common external morphological features can be identified for any oilseed radish seed of any shape (Fig. 2). The seed is divided into two symmetrically cotyledonary lobes, separated by a shallow hollow of hypocotyl and coleorhiza elements. These elements are most noticeable on seeds of the middle zone of the pod. The formation of seeds of different sizes within the pod for oilseed radish is determined by several reasons. Some of them are associated with the peculiarities of the formation of different morphotypes of pods in the context of different zones of inflorescence, determined by a certain layering of formation of fruit elements the gradient of height (Tsytsiura & Tsytsiura, 2015; Tsytsiura, 2018, 2019). Depending on the tier (lower, middle and upper) (Fig. 3) the oilseed radish pods differ in length, diameter and their ratioas well as by the wall thickness of the pod itself. In particular, the pods of the upper zone in comparison with the pods of the lower zone have thicker walls, significantly shorter length of the pod itself and overall higher variability in its morphology. The staging of the phenological formation of pods of the upper zone also has signs of desynchronization in the format of accelerated formation and maturation. It causes to deformation of the fruit walls, and to the appearance of such morphotypes as twisted, spiral, crescent, etc.

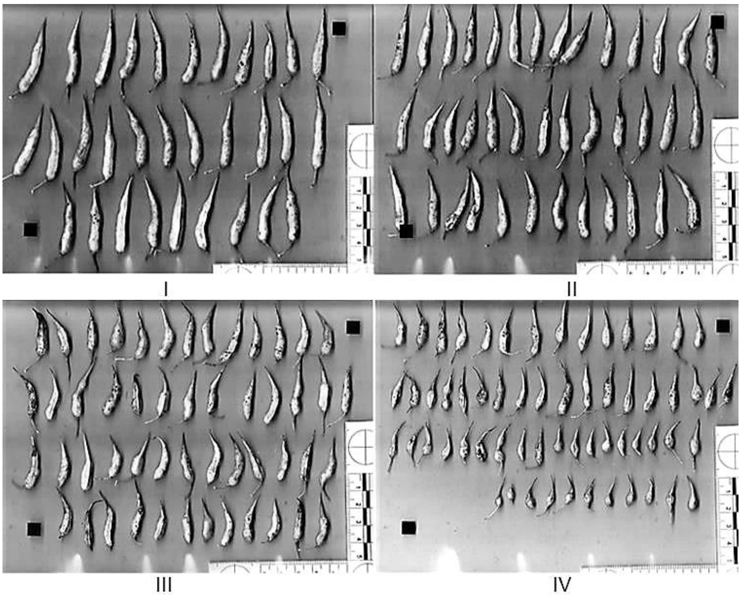

Figure 3. Morphotypes of oil seed radish pods of the 'Zhuravka' variety (I – pods of the lower zone; II – pods of the middlezone; III, IV – pods of the upper zone) for thetechnological variant of 2.0 mlnpcs. ha^{-1} of the germinable seeds with wide-row sowing against the back ground $N_{90}P_{90}K_{90}$ (for the phenological phase BBCH 87–88) (black square with dimensions 2×2 cm), 2018.

This nature of change in the morphology of the fruit provides not only a general decrease in the number of seeds in the pod in the direction from the base to the apex of the inflorescence at the oil radish. This is the scope of variation in the shape and size of the seed and its individual weight characteristics. Such features of pod formation for oilseed radish create prerequisites for reducing the favorability of the formation of the seed itself. This eventually leads to a change in the shape of the seed itself. Similar features were noted on a number of agricultural crops, including those of the cruciferous family (Levina, 1987; Pechan, 1988; Bouttier & Morgan, 1992; Mazer & Wolfe, 1998; Faraji, 2010, Hua et al., 2012; Gnan et al., 2014; Li et al., 2014, Li & Li, 2015a; Li et al., 2015b; Yang et al., 2016, Li et al., 2019, 2020).

The matrix variability of seeds in oilseeded radish pods within one inflorescence zone is also due to the peculiarities of its formation from the earliest stages after successful insemination. Conducted anatomical and microscopic studies at the stage of initiation of the formation of oilseed radish pods (BBCH 68-71), which indicate marked differences both in the size of the seed at the beginning of its formation and in the staging of its development. With these features, the format of the matrix variability by the height placement factor of a seed in a pod can be defined as differently directed. Over the period of many years of observations, I have found a gradual decrease in linear sizes of seeds from the base of the pod to its apex and a high probability of seeds formation with changed morphotype in the lowest parts of the pod. It should be noted that for all zones of oil radish inflorescence, which were determined during the evaluation of fruit elements by morphotype (Fig. 3), similar peculiarities in seed formation were noted. Such features are clearly shown in Figs. 4–5.

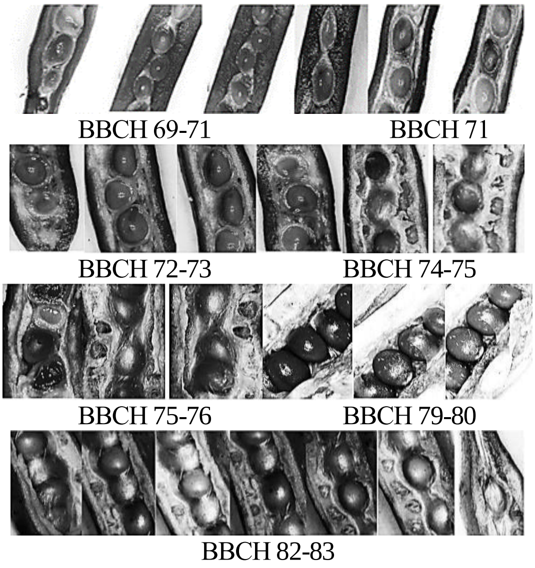

Figure 4. Nature of variability of oilseed radish seed size of the 'Zhuravka' variety in a pod at various microstages of its formation (in each line position of the figure from left to right: from the base of the pod to its apex), 2017.

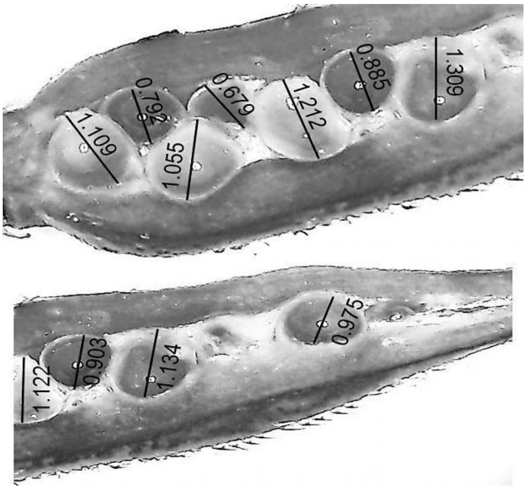

Figure 5. Nature of variability of oilseed radish seed size of the'Zhuravka' variety in a pod at various microstages of its formation position – seed size difference in the formation phase (BBCH 69-71, the dimension of values of mm), 2017.

As a result, for the period of waxy ripeness of seeds (brown pod phase BBCH 85-87) in the dynamic row of assessment of linear sizes of seeds, placed similarly to the pod from its base to the top, there is a gradual reduction of seeds in sizes and the appearance of atypical seeds in shape in the row of seeds by pod height. At the same time, the total number of seeds in the pod has an overall stable tendency to decrease. It was 1–3 seeds on average in comparison with the lower zone pods to the upper zone pods. This is clearly illustrated in Fig. 6. It should be noted that in contrast to rapeseed and mustard, for which the general nature of the variation of linear seed size is most significant in the upper and lower pod zones (Clarke, 1979; Smith & Scarisbrick, 1990; Bouttier & Morgan, 1992; Angadi et al., 2000; Garcia et al., 2005; Gunasekera et al., 2006; Vovchenko & Fursova, 2012; Zheng et al., 2017; Luo et al., 2018; Li et al., 2019), for oilseed radish such variability is more pronounced zonally and includes some seeds of the middle zone of the pod.

The data array obtained in the automated scanned images processing mode (Table 3) has confirmed the general conclusion about the presence of significant levels of matrix quality variability of seeds. In comparison with seeds from pods with different placement on the mother plant, respectively, for the lower and upper zones against the background of increased variation (according to the values CV (Zaytsev, 1984) projection surface area ofseeds (SA), medium and elevated levels of seed surface perimeter variation (P), medium variation level in length (L), width (W) and seed roundness (R) and, respectively, elevated variation level in seed sorting index (SI) – variation coefficient value was 0.7–3.6% higher for seeds from the upper zone pods.

It was also determined that the studied technological parameters influenced the value of variation of the specified morphological features of seeds. Thus, for all the stand densities under study, mineral fertilizer application contributed to the growth of the overall overall value of all the features without exception, with increasing dynamics, in line with the increasing rate of fertilizer application. This nature of influence is more pronounced in the formation of seeds from the pods of the upper zone with an increase to the unfertilized variant in the context of the studied technological variants of sowing rates from 1.3 to 2.4% in comparison to seeds from the pods of the lower zone in the range from 1.0 to 1.9%. The gradual increase in variation of morphological signs of seeds with a decrease in plant stand density and an increase in the area of their nutrition with a maximum expression at the application of fertilizers in the norm of 90 kg ha^{-1} of the primary material are determined. Thus, the difference in the average variation coefficient ($CV_{aver.}$, %) between the variant with the maximum stand density at the rate of 4.0 million of germinable seeds ha^{-1} with the fertilizer $N_{90}P_{90}K_{90}$ and the variant with the minimum stand density at the rate of 0.5 million of germinable seeds ha^{-1} was 7.2% for seeds from the pods of the lower zone and 10.1% for seeds from the pods of the upper zone.

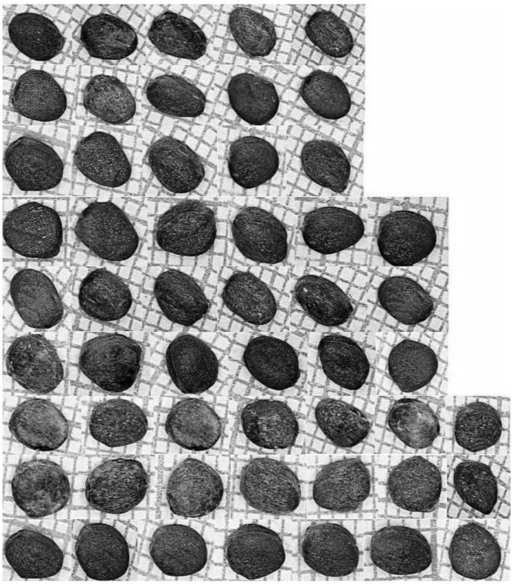

Figure 6. Qualityvariability of oilseed radish seeds of the‘Zhuravka’ variety in the order of their placement in the pods of various zones of the generative part (three upper positions - pods of the upper zone, three middle positions - pods of the middle zone, three lower positions - pods of the lower zone; placement of seeds on the rows of the figure from left to right in each row from the base of the pod to its apex), sampling selection of 2017.

As a result, according to the matrix variability index (MVI), the most significant gap in the morphology of seeds of pods of the lower and upper zones with the value of 0.86 was noted exactly for the variant of 0.5 million of germinables seeds ha^{-1} with the maximum fertilization with $N_{90}P_{90}K_{90}$. Similar features are noted in other cruciferous crops (Kitaeva, 1952; Tayo & Morgan, 1978; Morgan, 1980; McGregor, 1981; Voytenko, 1989; Lovett-Doust, 1989; Rao et al., 1992; Habekotté, 1996; Susko & Lovett-Doust, 2000; Faraji, 2010; Wang et al., 2011; Li et al., 2016, 2019, 2020).

Table 3. Variation coefficients (CV, %) of the main signs of oilseed radish seeds of the 'Zhuravka' variety according to the results of scanning of seeds for the brown pod phase (BBCH 85-86) in different stages of inflorescence at different variants of formation of agrophytocenosis on the average for 2013–2018, % (for *N* in the scanning group from 4,200 to 18,000)

Sowingrate and sowing method	Fertilizer	Morphological signs of seeds that were observed in the Digimizer image analysis software (v 4.2)														MVI
		PS, mm^2	P, mm	L, mm	W, mm	R, mm	SI	CV_{aver}, %	PS, mm	P, mm	L, mm	W, mm	R, mm	SI	CV_{aver}, %	
		Seeds from the pods of the upper generative zone							Seeds from the pods of the lower generative zone							
4.0 million, row	1	22.1	18.9	15.2	12.3	17.8	13.2	16.6	20.8	18.2	14.6	11.6	17.1	12.8	15.9	0.96
	2	22.7	19.1	15.8	12.6	18.2	13.9	17.1	21.2	18.4	15.1	11.8	17.3	13.2	16.2	0.95
	3	23.1	19.7	16.4	13.4	19.1	14.5	17.7	21.6	19.2	15.1	12.4	17.1	13.1	16.4	0.93
	4	25.2	20.4	17.2	14.3	20.6	15.4	18.9	22.2	19.5	15.3	12.6	17.4	13.5	16.8	0.89
3.0 million, row	1	23.5	20.4	16.2	13.7	18.4	14.9	17.9	22.6	19.1	15.1	12.3	17.9	13.4	16.7	0.94
	2	24.2	20.9	17.5	14.2	18.9	15.3	18.5	22.9	20.3	15.7	12.5	18.2	13.8	17.2	0.93
	3	25.1	21.4	18.2	14.9	19.5	15.8	19.2	23.2	20.6	15.9	12.8	18.3	13.9	17.5	0.91
	4	25.8	22.2	18.7	15.5	20.4	16.2	19.8	23.5	20.9	16.4	13.2	18.6	14.4	17.8	0.90
2.0 million, row	1	26.7	22.9	19.5	15.9	20.9	16.8	20.5	23.8	21.5	16.2	13.5	18.4	14.8	18.0	0.88
	2	27.4	23.4	19.8	16.7	21.3	17.2	21.0	24.2	21.8	16.7	14.2	18.8	15.1	18.5	0.88
	3	27.8	24.5	20.2	16.9	21.7	17.6	21.5	24.6	21.9	16.9	14.7	19.3	15.5	18.8	0.88
	4	28.3	25.2	20.7	17.3	22.4	17.9	22.0	24.9	22.3	17.2	14.9	19.7	15.8	19.1	0.87
1.0 million, row	1	27.9	23.5	20.8	16.7	21.7	18.2	21.5	25.2	23.7	17.2	15.1	20.6	16.7	19.8	0.92
	2	28.2	24.2	21.5	16.9	22.4	18.8	22.0	25.8	24.2	17.9	15.6	21.2	17.1	20.3	0.92
	3	28.9	24.7	22.3	17.2	22.8	19.1	22.5	26.2	24.7	18.5	16.3	21.7	17.4	20.8	0.92
	4	29.2	25.1	22.6	17.7	22.9	19.3	22.8	26.8	25.3	19.1	16.9	22.5	18.4	21.5	0.94
2.0 million, wide-row	1	23.5	19.2	15.7	12.9	18.4	13.8	17.3	21.5	18.6	14.9	12.2	17.5	13.1	16.3	0.94
	2	24.2	19.6	16.4	13.5	18.8	14.3	17.8	21.9	19.3	15.5	12.6	17.9	13.5	16.8	0.94
	3	24.8	20.1	16.8	14.2	19.2	14.9	18.3	22.2	19.5	15.8	12.7	18.3	13.9	17.1	0.93
	4	25.1	20.8	17.4	14.8	19.6	15.2	18.8	22.5	20.3	16.7	13.2	18.8	14.5	17.7	0.94
1.5 million, wide-row	1	25.2	21.8	17.8	14.9	19.1	15.6	19.1	23.4	19.5	15.5	13.4	18.2	13.7	17.3	0.91
	2	25.8	22.3	18.4	15.5	19.8	16.2	19.7	23.8	20.4	15.9	13.8	18.7	14.4	17.8	0.91
	3	26.1	22.9	19.2	15.8	20.6	16.9	20.3	24.2	20.6	16.3	14.2	19.1	14.8	18.2	0.90
	4	26.9	23.2	19.6	16.4	20.9	17.4	20.7	24.8	21.2	16.9	14.8	19.6	15.5	18.8	0.91
1.0 million, wide-row	1	28.1	23.5	20.6	16.4	21.7	17.5	21.3	26.1	24.5	17.8	14.8	19.5	15.2	19.7	0.92
	2	28.8	24.2	21.4	16.9	22.3	17.9	21.9	26.4	25.2	18.1	15.3	19.8	15.7	20.1	0.92
	3	29.5	24.8	22.5	17.4	22.9	18.5	22.6	26.8	25.7	18.4	15.5	20.1	15.9	20.4	0.90
	4	30.2	25.1	22.9	17.9	23.7	19.4	23.2	27.2	26.1	18.8	15.9	20.4	16.1	20.8	0.89
0.5 million, wide-row	1	30.7	29.2	22.8	18.7	24.5	21.3	24.5	28.2	27.3	19.3	16.4	21.5	16.9	21.6	0.88
	2	31.6	30.4	23.5	19.2	24.1	20.7	24.9	28.9	27.8	19.6	16.8	21.9	17.4	22.1	0.89
	3	32.3	31.4	24.1	19.8	24.7	21.1	25.6	29.2	28.1	20.4	17.2	22.4	17.9	22.5	0.88
	4	33.1	32.2	25.6	21.8	25.7	22.6	26.8	30.1	28.7	20.9	17.9	22.7	18.3	23.1	0.86
Tukey test upper/lower zone p_{adj}		0.00096**	0.04152*	0.06317*	0.03942**	0.04752*										

1 – Fertilizer-free; 2 – $N_{30}P_{30}K_{30}$; 3 – $N_{60}P_{60}K_{60}$; 4 – $N_{90}P_{90}K_{90}$. Tukey test: * – *P* < 0.05; * – *P* < 0.001.

In my opinion, the obtained values are determined by the peculiarities of relations between plants in populations of different densities. Thus, at maximum technological density, the level of variation of indicators is under much higher coenotic tension than in the variants of minimum technological density. Due to these regularities, the average value of a feature has a smaller scale of deviations, also in terms of morphometry of seeds and fruit elements. Mineral fertilizers should be considered in our system as stress regulators, which reduce the coenotic tension (Zlobin, 2009). For these reasons, the range of deviations from the average for the same density level of cenosis indicated by us also increases dynamically with a reduction of the coenotic tension at lower sowing rates. An important aspect in this context is to determine the role of hydrothermal conditions of oilseed radish vegetation on the general manifestation of the level of variation of morphological features of seeds, which was noted by a number of studies conducted on rapeseed (Rao et al., 1992; Morrison & Stewart, 2002; Gan et al., 2004; Lester et al., 2004; Luo et al., 2018). Comparison of factors of such influence (Fig. 7) allowed noting that decrease of hydrothermal coefficient (HTC) of oilseed radish vegetation period contributes to growth of general variability of seeds and expression of the effect of its matrix variability.

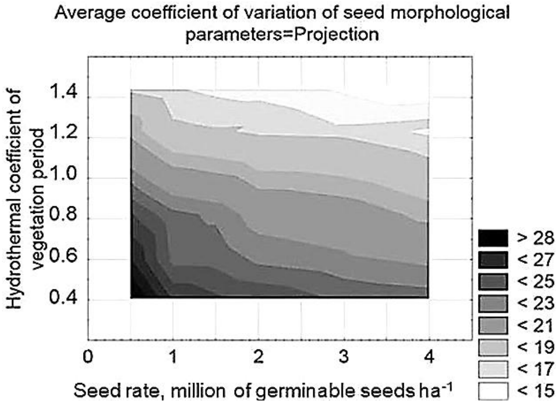

Figure 7. Dependence between the level of variation in the morphological parameters of oilseed radish seeds of the ‘Zhuravka’ variety, hydrothermal regime of the vegetation period and sowing rate of million germinable seeds ha^{-1} in conjunction with the width of the row spacing for the average for the fertilizer variants (consolidated data for the 2013–2018 period).

The maximum level of variation variability of morphological features of seeds at the level of CV = 27–30%, obtained at HTC in the range of 0.400–0.800 at sowing rate of 0.5–1.0 million germinable seeds ha^{-1}. Accordingly, the minimum level of variability of morphological features of seeds CV = 12–15% - when combined with the following parameters: HTC 1.3–1.4, 3.0–4.0 million germinable seeds ha^{-1}. Such results are quite consistent with our conclusions about the role of stress levels in the combination of density growth. An important component of the evidence of the above conclusions is the conducted fraction separation of oilseed radish seeds on the basis of the studied technological parameters of the construction of its agrophytocenosises. The results of seed separation on two types of sieves with round holes ((mm): 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5; 5.0; 6.0; 7.0) (Table 4) and with rectangular holes (1.0×20; 1.2×20; 1.5×20; 1.7×20; 2.0×20; 2.2×20; 2.5×20; 3.0×20) (Table 5) allow us to generalize the peculiarities of the matrix variability of oilseed radish seeds.

Table 4. Fraction composition of oilseed radish seeds by its separation on sieves with round holes for the ‘Zhuravka’ variety on the brown pod phase (BBCH 85-87) in different tiers of inflorescence with different variants of agrophytocenosis formation on the average for 2013–2018,% (A factor - year)

Sowingrate (C), sowing method (B)	Fertilizer (D)**	Fractions by seed separationon sieves with rectangular holes, mm (%)															
		0.5	0.5	0.5	0.5	0.5	0.5	0.5	$m_{1,000}$, g	0.5	1.0	1.5	2.0	2.5	3.0	3.5	$m_{1,000}$, g
		Seeds from the pods of the upper generative zone								Seeds from the pods of the lower generative zone							
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*
4.0 million, row	1	7.2	20.6	26.3	25.8	20.1	0.0	0.0	7.6	3.8	14.2	17.3	30.8	33.9	0.0	0.0	8.3
	2	6.6	19.1	27.2	27.6	19.5	0.0	0.0	7.7	3.2	12.1	17.5	31.6	35.6	0.0	0.0	8.5
	3	6.2	18.5	28.4	30.1	16.8	0.0	0.0	8.2	2.1	10.7	18.1	33.2	35.9	0.0	0.0	8.8
	4	5.4	17.7	29.2	31.3	16.4	0.0	0.0	8.4	1.8	8.5	18.7	34.4	36.6	0.0	0.0	8.9
3.0 million, row	1	6.4	19.4	28.4	26.7	19.1	0.0	0.0	8.0	2.6	13.4	18.2	31.6	34.2	0.0	0.0	8.6
	2	5.6	18.8	29.1	27.4	19.1	0.0	0.0	8.3	1.6	12.0	18.6	32.7	35.1	0.0	0.0	8,9
	3	4.9	18.2	29.5	29.2	18.2	0.0	0.0	8.7	0.9	10.6	18.9	33.1	36.5	0.0	0.0	9.3
	4	4.6	17.4	30.2	30.7	17.1	0.0	0.0	9.0	0.6	9.2	19.2	33.8	37.2	0.0	0.0	9.5
2.0 million, row	1	5.3	16.8	29.3	27.9	20.7	0.0	0.0	9.2	1.1	13.8	19.2	31.4	34.5	0.0	0.0	9.7
	2	4.5	15.9	29.6	28.1	21.9	0.0	0.0	9.4	1.0	11.9	19.5	32.9	34.7	0.0	0.0	10.0
	3	3.4	14.5	30.2	28.4	23.5	0.0	0.0	9.6	0.4	10.5	20.1	32.6	36.4	0.0	0.0	10.3
	4	2.7	13.8	30.8	29.2	23.5	0.0	0.0	9.8	0.4	8.3	20.7	32.4	38.2	0.0	0.0	10.6
1.0 million, row	1	3.7	15.9	30.6	28.2	21.6	0.0	0.0	9.7	0.0	11.7	20.4	32.4	35.5	0.0	0.0	10.0
	2	3.5	14.5	31.2	28.7	22.1	0.0	0.0	10.0	0.0	9.5	20.8	31.8	37.9	0.0	0.0	10.4
	3	2.7	13.7	31.6	29.3	22.7	0.0	0.0	10.1	0.0	7.8	21.3	31.2	39.7	0.0	0.0	10.6
	4	1.9	12.9	32.2	29.7	23.3	0.0	0.0	10.4	0.0	5.7	21.5	31.6	41.2	0.0	0.0	10.8
2.0 million, wide-row	1	6.1	18.4	26.9	27.3	21.3	0.0	0.0	8.2	2.1	13.1	18.5	33.4	32.9	0.0	0.0	8.7
	2	5.8	17.5	27.4	27.7	21.6	0.0	0.0	8.5	1.8	10.9	18.9	34.8	33.6	0.0	0.0	9.0
	3	4.8	16.8	28.5	28.2	21.7	0.0	0.0	8.8	0.8	9.2	19.4	36.5	34.1	0.0	0.0	9.2
	4	4.5	16.2	29.2	28.6	21.5	0.0	0.0	9.0	0.5	7.8	19.7	37.1	34.9	0.0	0.0	9.4
1.5 million, wide-row	1	5.2	17.3	27.4	28.5	21.6	0.0	0.0	9.0	1.2	12.3	19.2	30.5	36.8	0.0	0.0	9.4
	2	4.8	16.7	28.1	29.1	21.3	0.0	0.0	9.2	0.8	11	19.6	30.4	38.2	0.0	0.0	9.6
	3	4.4	16.4	28.9	29.4	20.9	0.0	0.0	9.3	0.6	9.5	19.9	29.8	40.2	0.0	0.0	10.0
	4	3.9	15.9	29.5	29.8	20.9	0.0	0.0	9.7	0.4	6.8	20.6	29.5	42.7	0.0	0.0	10.2
1.0 million, wide-row	1	4.5	15.2	29.2	23.3	27.8	0.0	0.0	9.5	0.0	11.5	20.9	25.7	41.9	0.0	0.0	10.2
	2	3.8	13.8	31.3	23.9	27.0	0.2	0.0	9.7	0.0	9.8	21.3	25.1	43.3	0.5	0.0	10.5
	3	3.5	12.6	32.6	24.2	26.8	0.3	0.0	9.9	0.0	7.2	21.6	24.3	46.1	0.8	0.0	11.0
	4	2.7	11.5	33.4	24.7	27.2	0.5	0.0	10.4	0.0	5.7	22.1	23.5	47.6	1.1	0.0	11.3
0.5 million, wide-row	1	2.3	13.8	31.5	17.7	34.4	0.3	0.0	10.4	0.0	9.3	22.5	19.5	48.3	0.4	0.0	11.2
	2	1.9	12.7	32.7	17.9	34.3	0.5	0.0	10.8	0.0	7.8	22.8	19.3	49.3	0.8	0.0	11.5
	3	1.7	13.2	33.4	18.4	32.6	0.7	0.0	11.0	0.0	5.5	23.4	19.2	50.7	0.9	0.3	11.7
	4	1.5	11.3	34.3	18.6	33.4	0.9	0.0	11.1	0.0	3.8	23.8	19.4	51.6	1.1	0.3	12.0
$LSD_{05}A^{*}$		0.08	0.31	0.18	0.42	0.38			0.14		0.18	0.31	0.29	0.60			0.15
$LSD_{05}B$		0.05	0.18	0.10	0.24	0.22			0.08		0.10	0.18	0.16	0.35			0.09
$LSD_{05}C$		0.07	0.25	0.15	0.34	0.31			0.12		0.14	0.25	0.23	0.49			0.13
$LSD_{05}D$		0.07	0.25	0.15	0.34	0.31			0.12		0.14	0.25	0.23	0.49			0.13
$LSD_{05}AB$		0.12	0.43	0.25	0.59	0.53			0.20		0.25	0.44	0.40	0.86			0.22
$LSD_{05}AC$		0.17	0.61	0.36	0.84	0.75			0.29		0.35	0.62	0.57	1.21			0.31
$LSD_{05}AD$		0.17	0.61	0.36	0.84	0.75			0.29		0.35	0.62	0.57	1.21			0.31
$LSD_{05}BC$		0.10	0.35	0.21	0.48	0.43			0.17		0.20	0.36	0.33	0.70			0.18

Table 4 (continued)

1	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*
LSD$_{05}$BD	0.10	0.35	0.21	0.48	0.43			0.17		0.20	0.36	0.33	0.70			0.18
LSD$_{05}$CD	0.14	0.50	0.29	0.68	0.61			0.23		0.29	0.51	0.47	0.99			0.25
LSD$_{05}$ABC	0.24	0.86	0.51	1.19	1.06			0.41		0.50	0.88	0.81	1.71			0.44
LSD$_{05}$ABD	0.24	0.86	0.51	1.19	1.06			0.41		0.50	0.88	0.81	1.71			0.44
LSD$_{05}$ACD	0.34	1.22	0.72	1.68	1.50			0.57		0.71	1.25	1.14	2.42			0.62
LSD$_{05}$BCD	0.20	0.70	0.41	0.97	0.87			0.33		0.41	0.72	0.66	1.40			0.36
LSD$_{05}$ABCD	0.48	1.73	1.01	2.37	2.13			0.81		1.00	1.77	1.62	3.42			0.87
Share of influence A																
B	10.30	36.87	64.61	40.34	26.69			56.4		17.12	67.90	59.06	43.74			56.8
C	6.38	10.54	1.19	12.55	23.26			5.00		10.25	6.50	6.35	14.39			5.54
D	64.18	40.75	0.99	17.75	37.11			31.7		21.50	20.90	28.37	23.38			30.8
AB	15.15	9.88	1.89	2.55	0.20			4.99		47.41	3.40	3.61	5.48			5.24
AC	0.10	0.23	9.52	0.26	0.28			0.07		0.18	0.09	0.20	0.16			0.08
AD	0.91	0.65	7.87	0.26	0.59			0.56		0.30	0.43	0.52	0.41			0.54
BC	0.18	0.12	0.34	0.12	0.03			0.19		0.59	0.17	0.09	0.18			0.19
BD	0.88	0.13	1.28	23.64	8.18			0.48		1.09	0.32	0.53	11.29			0.46
CD	0.46	0.05	0.22	0.33	0.52			0.02		0.33	0.02	0.01	0.03			0.01
ABC	0.63	0.40	0.24	0.93	0.82			0.17		0.34	0.05	0.35	0.35			0.13
ABD	0.02	0.05	9.24	0.51	0.10			0.10		0.05	0.08	0.12	0.12			0.07
ACD	0.01	0.00	0.36	0.01	0.01			0.00		0.01	0.00	0.07	0.00			0.00
BCD	0.01	0.01	1.01	0.04	0.02			0.02		0.01	0.03	0.18	0.02			0.02
	0.76	0.31	0.12	0.67	2.15			0.13		0.78	0.08	0.33	0.41			0.04
	0.02	0.02	1.11	0.02	0.04			0.02		0.02	0.03	0.21	0.02			0.02

* – in the system of value expression at deprivation of values,%; ** – 1 – Fertilizer-free; 2 – $N_{30}P_{30}K_{30}$; 3 – $N_{60}P_{60}K_{60}$; 4 – $N_{90}P_{90}K_{90}$.

According to the data presented in both tables morphological row of oilseed radish seeds of the ‘Zhuravka’ variety is placed in the range of 0.5–3.5 mm for the sieves with round holes and 1×20–3×20 for the sieves with rectangular holes. This confirmed the affiliation of seeds to the general group a rounded and flattened. It also allowed recommending requires the introduction of a system of combined sieves for technological cleaning and calibration of oilseed radish seeds in production conditions. In addition, the array of data obtained indicates that the fraction composition of the size of seeds from pods of different zones of the plant is significantly different according to certain difference indices *LSD*$_{05}$ for the main factors and their interaction. Thus, when separating seeds on round sieves (Table 4), given the significance of the difference between the studied variants, it should be noted a reliable increase in the percentage of fine fraction 0.5 mm in the seeds from the pods of the upper zone in the context of all the variants of standing density and fertilization. In fact, for variants 0.5–1.0 million germinable seeds ha^{-1} of this pod zone, this fraction was absent. As well as it is practically absent the fraction 3.0 and 3.5 mm for all technological variants. The distribution between the seed fractions in the range of 1.5–2.5 mm for seeds of the lower and upper tier of pods also differed significantly. Thus, on the average for the studied technological variants the seed fraction share of 2.0 mm and 2.5 mm for seeds from the pods of the lower zone was 29.83% and 39.40%, which is 11.8% and 69.0% higher than for seeds from the pods of the upper zone. At the same time, the actual nature of fractional distribution in all variants differed: the sum of fractions' share of 0.5–1.5 mm for seeds from pods of the lower zone averaged 30.57%, which is 19.33% lower than for seeds from pods of the upper zone. Seeds of 3.0 and 3.5 mm fractions in the research variants are stably noted for technological variants of 0.5 and 4.0 million germinable seeds ha^{-1} in the variant of $N_{60}P_{60}K_{60}$ and $N_{90}P_{90}K_{90}$ application. The role of mineral fertilizers in changing fractional composition of oilseed radish seeds also had its own peculiarities. Thus, for the variants of evaluation of fractional composition of seeds from the pods of the upper zone, the dynamic growth of fertilizer rates provided a decrease in the share of 0.5 and 1.0 mm seed fractions with adequate growth of other fractions. For seeds from the pods of the lower zone, the effect of mineral fertilizers was of two-level direction: a decrease in the share of the 0.5 mm fraction, 1.0 mm with the growth of other fractions. In case of wide-row sowing variants assuming the sowing rate of 1.5 million germinable seeds ha^{-1}, a decrease in the share of 2.0 mm seed fraction with an increase in the intensity of 2.5 mm share growth by 8.9–10.3% was noted. This indicates that on more dissolved sowings the character of formation of fractional composition of oilseed radish seeds has a more complex interfactional nature with the allocation of two or three main fractions. It should also be noted that the general regularity in the formation of fractional composition on the round sieves, despite the peculiarities in the fertilization section of specific technological variants, has its own characteristics for seeds from pods of both zones. Under these conditions, it is established that a stable growth of the share of large fractions of 2.0, 2.5 mm with a corresponding decrease of fractions of 0.5–1.0 mm. For certain technological variants, the impact of fertilizers on the proportion of the respective fractions had certain features, particularly for seeds from the pods of the upper zone for variants of 3.0–4.0 million germinable seeds ha^{-1} with normal row sowing and 1.5–2.0 million germinable seeds ha^{-1} with variants of wide-row sowing. It is proved that an increase in fertilizer rates ensured an increase in the share of seed fractions of 1.5 and 2.0 mm by 4.0–5.5 and 1.8–2.9%, respectively, while the share of seeds of 2.5 mm decreased by 2.0–3.7%.

The results of calculating the weight of 1,000 seeds are also important, as the matrix variability has weight expression and affects the level of individual seed productivity of plants (Makrushin, 1994). At a varietal idiotype of oilseed radish of the ‘Zhuravka’ variety with the index of mass of 1,000 seeds within 9–12 g, the studied technological approaches had essentially different efficiency of index formation including for seeds from pods of different zones. The average value of this indicator for the studied variants was 9.94 g for seeds from the pods of the lower zone and 9.33 g for seeds from the pods of the upper zone, which makes a difference of 6.5%. It should be noted that the difference in the value of the indicator differed within the technological options from 3.8% to 11.1%. Application of mineral fertilizers at a gradually increasing rate reduced the difference in weight of 1,000 seeds in comparison with seeds from pods of different zones by 1.3–3.7%. The maximum average difference in the value of the indicator for the two zones of pods was noted for the two extreme technological parameters in 4.0 and 0.5 million germinable seeds ha^{-1} - 8.8 and 8.2%, respectively. As a result, the weight of 1,000 seeds had a stable growth dynamics with an increase in the feeding area of one plant against the background of fertilizer increase to $N_{90}P_{90}K_{90}$ with a maximum value for both zones on the level of 11.1–12.0 g and a minimum value in the range of 7.6–8.3 for the non-fertilized background of 4.0 million germinable seeds ha^{-1}.

Table 5. Fraction composition of oilseed radish seeds by its separation on sieves with rectangular holes for the ‘Zhuravka’ variety on the brown pod phase (BBCH 85-87) in different tiers of inflorescence with different variants of agrophytocenosis formation on the average for 2013–2018, %

Sowingrate (C), sowing method (B)	Sowingrate (C), sowing method (B)	Fractions by seed separationon sieves with rectangular holes, mm (%)													
		1.0×20	1.2×20	1.7×20	2.0×20	2.2× 20	2.5×20	3.0×20	1.0×20	1.2× 20	1.7×20	2.0×20	2.2×20	2.5×20	3.0×20
		Seeds from the pods of the upper generative zone							Seeds from the pods of the lower generative zone						
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*8*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
4.0 million, row	1	25.7	21.4	7.2	26.3	5.1	14.3	0.0	16.9	16.8	5.4	36.1	6.2	18.6	0.0
	2	25.0	21.9	7.6	25.2	5.5	14.8	0.0	16.3	17.4	5.7	35.2	6.7	18.7	0.0
	3	21.5	22.9	7.9	24.7	7.4	15.6	0.0	14.8	17.8	5.9	34.7	7.2	19.6	0.0
	4	19.7	23.4	8.2	24.2	8.3	16.2	0.0	11.6	18.1	6.1	35.6	8.5	20.1	0.0
3.0 million, row	1	23.3	22.8	7.7	27.2	6.8	12.2	0.0	15.5	17.5	6.1	34.8	7.5	18.6	0.0
	2	22.6	23.1	8.1	26.8	7.2	12.2	0.0	14.7	18.2	6.7	33.7	7.9	18.8	0.0
	3	21.3	23.8	8.5	26.2	7.8	12.4	0.0	12.5	18.7	7.1	32.1	9.3	20.3	0.0
	4	20.2	24.2	8.9	25.8	8.2	12.7	0.0	11.4	19.1	8.2	31.1	8.8	21.4	0.0
2.0 million, row	1	22.9	23.4	7.4	28.7	7.5	10.1	0.0	14.2	18.8	6.9	31.9	8.5	19.7	0.0
	2	21.5	23.9	8.1	27.4	7.9	11.2	0.0	12.3	19.1	7.3	32.2	8.9	20.2	0.0
	3	20.1	24.6	8.5	26.5	8.3	12.0	0.0	10.2	19.8	8.2	31.5	9.4	20.9	0.0
	4	17.7	25.4	9.2	25.7	8.9	13.1	0.0	9.3	20.2	8.7	30.8	9.7	21.3	0.0
1.0 million, row	1	20.3	23.9	8.7	28.3	7.8	11.0	0.0	12.6	19.9	7.7	30.2	9.1	20.5	0.0
	2	19.2	24.2	9.2	27.8	8.1	11.5	0.0	11.4	20.4	8.8	28.6	9.4	21.2	0.2
	3	18.5	24.8	9.6	27.4	8.5	11.2	0.0	10.8	20.6	9.1	27.1	10.3	21.8	0.3
	4	15.8	25.9	9.8	26.8	9.9	11.8	0.0	9.1	20.9	9.6	26.7	10.7	22.5	0.5
2.0 million, wide-row	1	17.9	16.9	9.1	28.5	9.4	18.2	0.0	9.3	13.9	7.9	34.1	8.3	26.5	0.0
	2	16.8	17.2	9.5	27.8	9.8	18.9	0.0	8.3	14.1	8.2	33.5	9.1	26.8	0.0
	3	15.7	18.2	9.8	26.9	8.6	20.8	0.0	6.6	14.5	8.8	33.2	9.7	27.2	0.0
	4	14.2	18.8	10.1	26.2	9.5	21.2	0.0	4.9	14.7	9.1	33.5	10.2	27.6	0.0
1.5 million, wide-row	1	16.3	16.6	10.4	27.4	7.8	21.5	0.0	8.9	12.6	8.4	32.6	8.9	28.4	0.2
	2	15.9	16.9	10.7	27.2	7.4	21.9	0.0	8.7	12.8	8.7	31.3	9.4	28.8	0.3
	3	14.7	17.5	11.3	26.8	5.9	23.8	0.0	6.4	13.9	8.9	30.9	6.9	32.7	0.3
	4	13.5	18.2	11.6	26.4	5.1	25.2	0.0	4.3	13.3	9.3	30.7	7.2	34.8	0.4
1.0 million, wide-row	1	14.7	15.8	15.3	24.8	7.9	21.2	0.3	7.5	11.8	9.3	30.8	9.6	30.4	0.6
	2	13.2	15.3	15.9	23.9	8.5	22.7	0.5	7.2	12.4	9.6	28.6	10.5	30.9	0.8
	3	12.7	15.2	16.5	22.6	9.3	23.2	0.5	6.1	12.9	9.7	27.4	11.2	31.9	0.8
	4	11.9	14.6	16.9	22.4	9.8	23.7	0.7	4.7	13.3	9.9	27.3	11.8	32.2	0.8
0.5 million, wide-row	1	13.8	14.8	16.3	20.9	11.2	22.5	0.5	7.4	9.3	9.8	29.6	12.3	30.9	0.7
	2	12.5	14.6	16.9	20.2	11.9	23.2	0.7	5.7	9.5	10.6	28.4	13.5	31.4	0.9
	3	11.6	14.2	17.5	19.4	12.6	24.0	0.7	5.3	10.3	10.8	24.1	14.8	33.8	0.9
	4	10.8	13.9	18.2	18.9	12.9	24.5	0.8	3.4	10.6	11.2	24.3	15.2	34.3	1.0
LSD_{05} A*		0.30	0.32	0.19	0.40	0.14	0.29		0.18	0.26	0.15	0.48	0.35	0.40	
LSD_{05}B		0.17	0.18	0.11	0.23	0.08	0.17		0.11	0.15	0.08	0.28	0.20	0.23	
LSD_{05}C		0.25	0.26	0.15	0.32	0.12	0.24		0.15	0.21	0.12	0.39	0.28	0.33	
LSD_{05}D		0.25	0.26	0.15	0.32	0.12	0.24		0.15	0.21	0.12	0.39	0.28	0.33	
LSD_{05}AB		0.43	0.45	0.27	0.56	0.20	0.42		0.26	0.37	0.21	0.68	0.49	0.57	
LSD_{05}AC		0.60	0.63	0.37	0.80	0.29	0.59		0.37	0.52	0.29	0.97	0.69	0.81	
LSD_{05}AD		0.60	0.63	0.37	0.80	0.29	0.59		0.37	0.52	0.29	0.97	0.69	0.81	

Table 5 (continued)

1	*3*	*4*	*5*	*6*	*7*	*8*	*8*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
LSD_{05}BC	0.35	0.36	0.22	0.46	0.17	0.34		0.21	0.30	0.17	0.56	0.40	0.46	
LSD_{05}BD	0.35	0.36	0.22	0.46	0.17	0.34		0.21	0.30	0.17	0.56	0.40	0.46	
LSD_{05}CD	0.49	0.51	0.31	0.65	0.24	0.48		0.30	0.42	0.24	0.79	0.57	0.66	
LSD_{05}ABC	0.85	0.89	0.53	1.12	0.41	0.83		0.52	0.73	0.41	1.37	0.98	1.14	
LSD_{05}ABD	0.85	0.89	0.53	1.12	0.41	0.83		0.52	0.73	0.41	1.37	0.98	1.14	
LSD_{05}ACD	1.21	1.26	0.75	1.59	0.58	1.18		0.74	1.04	0.58	1.93	1.39	1.61	
LSD_{05}BCD	0.70	0.73	0.43	0.92	0.33	0.68		0.43	0.60	0.34	1.12	0.80	0.93	
LSD_{05}ABCD	1.71	1.78	1.06	2.25	0.82	1.66		1.04	1.47	0.83	2.73	1.96	2.28	
Share of influence														
A	20.4	22.3	12.3	55.01	22.8	13.8		8.74	21.41	30.21	57.43	20.33	23.03	
B	55.0	59.7	46.6	7.13	11.8	74.4		63.0	62.58	30.41	4.68	17.00	65.64	
C	12.7	3.51	24.1	8.39	32.4	0.31		8.26	0.60	27.53	30.28	35.53	4.68	
D	9.12	3.25	2.13	2.78	4.64	3.01		16.1	1.75	7.93	3.54	6.45	3.11	
AB	0.85	0.95	0.59	0.19	0.14	0.99		0.95	1.00	0.36	0.14	0.21	0.85	
AC	0.19	0.06	0.35	0.16	0.55	0.03		0.11	0.03	0.46	0.50	0.56	0.10	
AD	0.10	0.06	0.06	0.05	0.13	0.08		0.19	0.07	0.20	0.06	0.15	0.10	
BC	0.17	4.25	13.5	25.22	16.5	6.29		1.27	12.06	1.43	0.60	8.62	1.16	
BD	0.60	0.21	0.01	0.02	3.14	0.40		0.05	0.05	0.24	0.17	0.48	0.19	
CD	0.41	3.98	0.06	0.29	3.63	0.26		0.63	0.02	0.47	1.61	4.81	0.75	
ABC	0.02	0.13	0.16	0.61	0.24	0.07		0.01	0.28	0.10	0.09	0.12	0.04	
ABD	0.01	0.01	0.00	0.00	0.04	0.01		0.00	0.00	0.00	0.01	0.01	0.01	
ACD	0.01	0.07	0.01	0.02	0.08	0.01		0.01	0.01	0.02	0.05	0.10	0.01	
BCD	0.36	1.47	0.03	0.10	3.86	0.32		0.54	0.13	0.61	0.81	5.54	0.33	
ABCD	0.01	0.03	0.00	0.02	0.05	0.01		0.01	0.01	0.02	0.04	0.08	0.02	

* – in the system of value expression at deprivation of values,%; ** – 1 – Fertilizer-free; 2 – $N_{30}P_{30}K_{30}$; 3 – $N_{60}P_{60}K_{60}$; 4 – $N_{90}P_{90}K_{90}$.

Under these conditions have also determined that the share of influence of the studied factors had different efficiency in the formation of values of different fractions of seeds separated on the round sieves, and within certain zones of pods placement. The most determinant factors in the formation of indicators of fractional composition of seeds were determined as the conditions of the year (factor A) with the share of influence in the range 10.3–67.9, sowing rate (factor C) 0.99–64.18. For the share of row width (factor B) and fertilizer (factor D) 5.0–23.26 and 0.2–47.41, respectively.

The certain non-uniformity of influence of the studied factors and their interaction indicates the complex mechanism of formation of linear and weight characteristics of seeds within the limits of fruit elements at different levels of combination of factors of the research. However, the regulatory factors of fractional composition of seeds and the expression of its matrix quality variability can be placed in the following order of importance A>C>D>B>BC. Similar results were obtained with respect to the quality variability of seeds in fractional composition within different zones of oilseed radish fruit elements at its separation on the sieves with rectangular holes (Table 5). The presented nature of fraction distribution has a wider interval nature, which indicates in favor of technologically restricted variant of oilseed radish seed separation on round sieves. This is indicated particularly by the corresponding share of seed fractions with the size of 1.7×20 mm and 2.2×20 mm. The nature of the fractional composition was different for seeds from pods of different zones.

For the average variant on technological variants in seeds from pods of the lower zone of the generative part the share of fraction 1.0×20 mm is 45.1% lower than for the variant from pods of the upper zone. For fractions 1.2×20 and 1.7×20, respectively, it was 21.2% and 23.6% lower. However, the total increase in the share of fractions in the range from 2.0–2.5×20, was noted, respectively, by 21.7%, 13.3% and 45.5%. In addition, considering certain technological variants, the formation of fractional composition has the same features as in the case of separation on round sieves.These features include a consistent increase in the share of large seed fractions (2.2 and 2.5 mm) with an increase in a single plant's feeding area and an increase in background fertilizer. In addition, the nature of growth has two levels: one is observed in the range of normal line sowing. The other is observed in the range of wide-row sowing. Due to these reasons, the factor interaction of the research data system has a different nature of relationships among their values: B>A>C>D>BC. That is, the main components that determine the fractional distribution of seeds by separation on rectangular sieves is the width of row spacing and conditions of the year. It should also be noted that the sowing rate factor has two realizable components both as a single component and as its interaction with the width of row spacing.

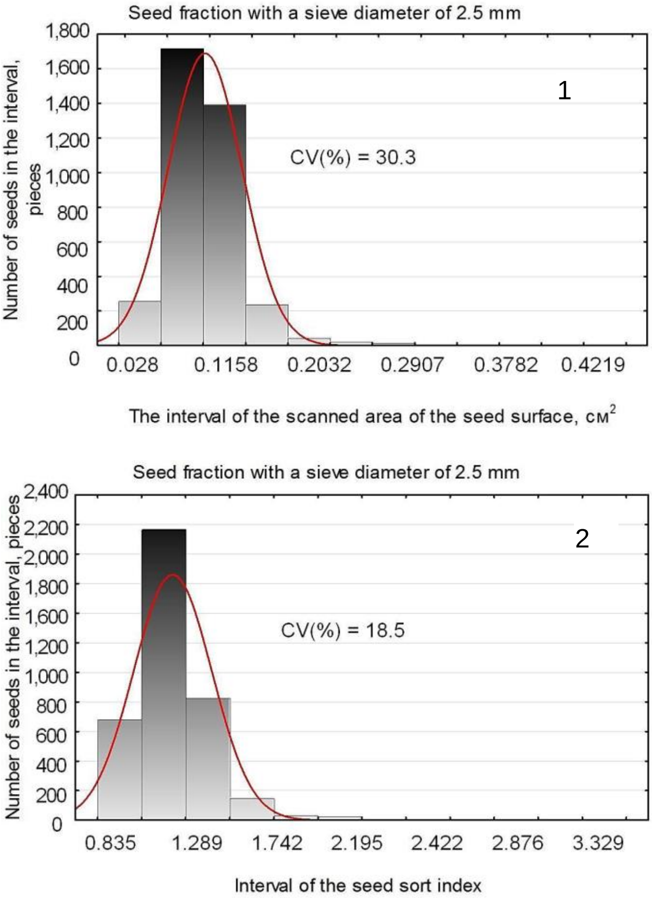

Figure 8. (a) Interval distribution of seeds of the ‘Zhuravka’ variety by indicators of the area of the scanned surface and sorting index of seeds for different fractions of seeds from the pods of the upper zone (position 1–2) of the generative part of plants with a technological variant of 2.0 million germinable seeds ha^{-1} with fertilizer N90P90K90, 2013.

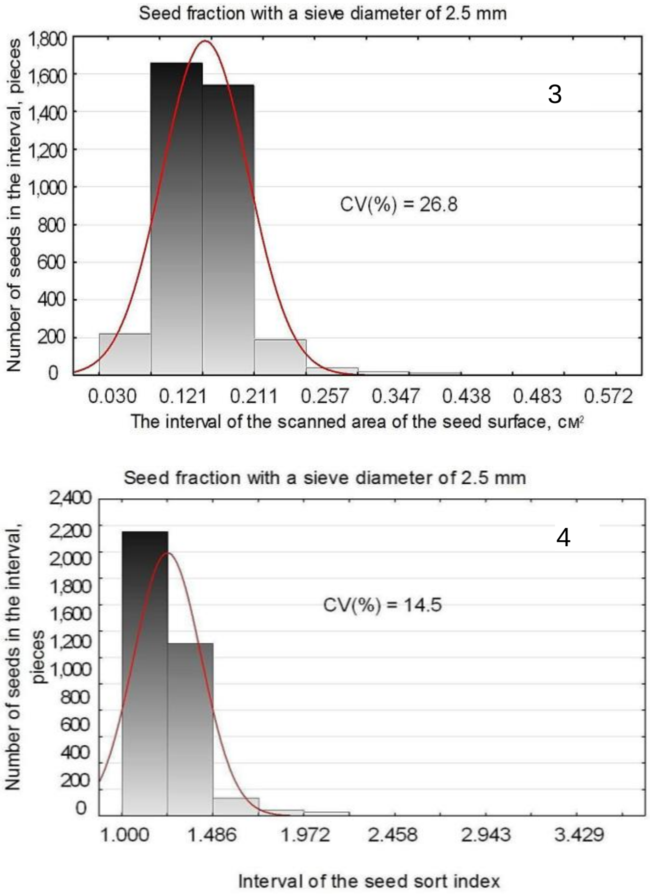

Figure 8. (b). The same from the pods of the lower zone (position 3–4), 2013.

On the other hand, it is important for practical seed production of cruciferous crops to understand the internal alignment of the received fractions of seeds (Würschum et al., 2012; Yang et al., 2016, 2017; Zheng et al., 2017). By applying the same electronic scanning approach to the separated seeds, we obtained its interval values of the morphological features of the seeds used to assess their variability (Table 3). So, for example, for the technological variant of 2.0 million germinable seeds ha^{-1} with fertilizer N90P90K90 (conditions of 2016), the nature of the interval row of seeds by the size of the area of the scanned surface (SA) andsorting index (SI) for two fractions of 2.5 mm (round sieves) (Fig. 8 (a, b)) and 2.5×20 (rectangular sieves) (Fig. 9) for different zone was different I also have found that the general variability of certain intervals for both given indicators on the value of the variation coefficient (CV) both for the fractions sieves is higher in the variant of seeds from the pods of the upper zone.

In particular, Figs 8, 9 gives an example of conditions for 2013 as approximate to the long-time average annual hydrothermal regime of vegetation of oilseed radish plants. Moreover, it should be noted that the difference in the variation coefficient between seeds from both pod zones was 3.5–9.7% higher for seeds from the upper zone. It was determined a higher value in the range of annual values for these deviations in the stress dry years (2015, 2017), and a lower value in the same range of values for conditions of years with moderate or optimal moistening (2013, 2014).

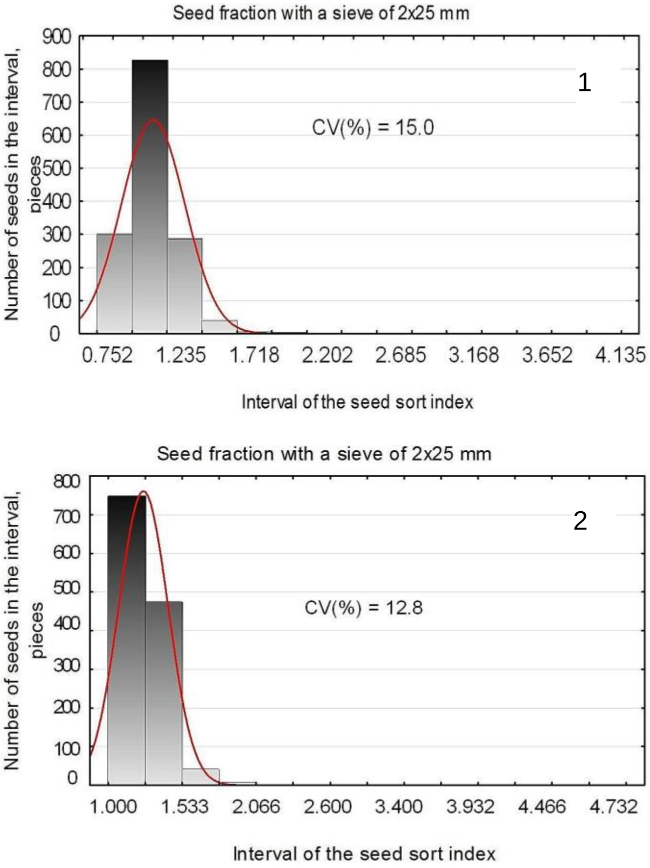

Figure 9. Interval distribution of seeds of the 'Zhuravka' variety by indicators of sorting index of seeds for seed fractions 2×25 mm from the pods of the upper zone (position 1) and from the pods of the lower zone (position 2) of the generative part of plants with a technological variant of 2.0 million germinable seeds ha^{-1} with fertilizer $N_{90}P_{90}K_{90}$, 2013.

It is also important that the general alignment of seeds from both zones of the generative part of plants is higher than the separation of seeds on rectangular sieves. For example, in Figs. 8 (a b) and 9, the coefficient of variation in the separation of seeds on round sieves was 2.2–5.4% higher, compared to a similar indicator for the separation of seeds on rectangular sieves.

CONCLUSION

Thus, on the basis of a comprehensive analysis of long-term data we have determined the presence of matrix quality variability of oilseed radish seeds both in morphological and weight characteristics according to the nature of placement of pods in the generative part of plants. The level of seed quality by matrix type in oilseed radish is determined by the nature of inflorescence formation and the corresponding morphotype of fruit elements, variable according to their height. The difference in the coefficient of variation of linear seed sizes was on average 6.7% higher for the upper zone of oilseed radish inflorescence compared to the lower zone. The maximum matrix variability of oilseed radish seeds was observed in the variant with a sowing rate of 0.5 million germinable seeds ha^{-1} against the background of the application of 90 kg ha^{-1} of mineral fertilizers. The integrated indicator of seed variability for this variant was the coefficient of 1.45 and 1.61 in comparison for the lower and upper inflorescence zones, respectively, in the system of the ratio of the two variants 0.5 and 4.0 million germinable seeds ha^{-1}. Mineral fertilizers contributed to the growth of seed variability in the range of 4.5–10.0% for the upper inflorescence zone depending on the cenosis design variant and 5.6–14.0% for the lower inflorescence zone. The heterogeneity of oilseed radish seed fractions in both round and elongated sieves was maximal at the minimum seeding rate on the background of maximum fertilizer. The summary result of the researchwas defined a decrease in the total linear individual size of oilseed radish seeds within the pod from the lower to the upper zone with a consistent reduction in the range of 3.8–8.3%, within the inflorescence in the range of 6.8–15.8%. An increase in the variation of seed morphoparameters in the comparison of the lower and upper zones of the inflorescence and pod by 7.2–10.2% with the maximum values of the growth of differences in the variants of the lowest seeding rate against the background of the maximum fertilizer $N_{90}P_{90}K_{90}$ was established.

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