

Response of spring and facultative triticale on microbial preparation (*Azospirillum brasilense* and *Bacillus polymyxa*) by different nitrogen nutrition

Bohdan MAZURENKO (✉), Nataliia NOVYTSKA, Liubov HONCHAR

Department of Plant Science, Agrobiological Faculty, National University of Life and Environmental Sciences, Kyiv, Ukraine

✉ Corresponding author: mazurenko.bohdan@nubip.edu.ua

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ABSTRACT

The use of phosphate-mobilizing and nitrogen-fixing bacteria can increase the productivity of spring triticale and its resistance to abiotic factors. Peculiarities of microbial preparations influence on yield formation was studied in 3 triticale cultivars. Seed treatment by *Azospirillum brasilense* and *Bacillus polymyxa* had a different effect on elements of triticale productivity. Grain yield was increased on 7.2% in average by *Azospirillum brasilense* seed treatment and to 8.6% by *Bacillus polymyxa*. Their combined application gave 11.5% raise to grain yield. Positive effect of microbial preparations on number of productive shoots, grain weight per spike and thousand kernel weight was observed for traditional fertilization system ($P_{36}K_{72} + N_{80}$) and system without nitrogen application ($P_{36}K_{72}$). Correlation between elements of productivity showed differences between cultivars. Indicators of productive shoots and grain weight per spike had main effect on grain yield in all cultivars. Raising all elements of triticale performance has led to decrease in protein content for treated variant by *Azospirillum brasilense* and *Bacillus polymyxa*. Combined use this microbials allowed to keep protein content high while yield was increasing.

Keywords: facultative, fertile system, phosphate-mobilizing, protein, spring, yield

INTRODUCTION

Productivity of spring triticale depends more on weather conditions than winter cereals one. Spring cultivars have far less moisture available, that why they need to use effectively. Different seed treatments can be used to increase viability and accelerate growth during the initial stages of development. Application of phosphate-mobilizing and free-living nitrogen-fixing bacteria for seed treatment can improve the nutritional regime of plants and increase their resistance to adverse conditions.

Microbial preparations are based on free-living nitrogen fixers *Azospirillum* spp. can capture free nitrogen from the air and synthesize several phytohormones (Perrig et al., 2007). *Bacillus polymyxa* (*Paenibacillus polymyxa*)

produces many polysaccharides that can dissolve hard-to-reach phosphates in the soil and improve plant nutrition (Yegorenkova et al., 2013).

Content of available phosphorus compounds in the soil after harvesting remains at the pre-sowing level due to the activity of phosphate-mobilizing bacteria (Arkhipova et al., 2019). Efficiency of using these bacteria together depends on the weather conditions and the sensitivity of the variety (Caceres et al., 1996). Different types of bacteria may compete for a nutrient environmental and spread of some species may inhibit the development of other ones (Shcherbakova et al., 2017; Yegorenkova et al., 2016). Establishing varietal sensitivity to certain types of bacteria and their interaction is an important way to improve plant productivity.

MATERIALS AND METHODS

Field experiments were carried out in 2017-2019 at the Right-bank Forest-Steppe area of Ukraine (49°46' N, 30°44' E). The soil of the experimental plot is typical low-humic chernozem. It is characterized by the following indicators: the humus content in the arable layer – 4.31-4.63%, the reaction of the soil is close to neutral (pH 7.2), the content of easily hydrated nitrogen – 15.2-16.7 mg, mobile phosphorus and exchangeable potassium – 10.9-14.2 mg and 12.7-13.2 mg per 100 g of soil, respectively. This type of soil is typical for significant part of the Forest-Steppe area of Ukraine.

Weather conditions

First year (2017) of research characterized by dry condition. Summary precipitations during spring triticale vegetation was 170.7 mm. Average daily temperature exceeded multi-annual parameter on 1-2 °C. Second year (2018) was more humid and warmer than previous – summary precipitations was 331.6 mm and daily temperature in few mounts exceeded multi-annual parameter more than 3 °C. Final year (2019) was hot and wet – summary precipitation was 379 mm, but average temperature in June was 22.6 °C (multi-annual – 18.0 °C).

Experimental procedure

The experiment had a randomized block design, with four replications. Total area of an individual plot was 32 m², harvest area – 25.2 m². Seeding rate was 450 seeds/m² with 15-cm row spacing. The previously crop was soybean. Soil tillage included ploughing after soybean harvesting (1st or 2nd decade of September) and presowing cultivation in 4 cm depth in spring. Sowing was carried out when available water capacity was 60%, but date depend on year condition (25.03.2017, 14.04.2018, 30.03.2019). Pesticides was applied for requirement.

Experimental design

Field experiment design included 3 factors and their combination (Table 1). 3 triticale cultivars were examined for spring sowing. There were Pidzimok kharkivskyi

(facultative, long-term vernalization), Vuiko (facultative, short-term vernalization) and Landar (spring) in the field experiment.

Fertilization system included 2 variants. First variant of fertilization system (without nitrogen) involved the application P₃₆K₇₂ before ploughing, without use of nitrogen fertilizers. Second variant is traditional fertilization system for triticale in climatic area. It was included the application P₃₆K₇₂ before ploughing and N₈₀ in spring. Nitrogen fertilizers were applied in 2 terms: 25 kg/ha N in pre-sowing applying and 55 kg/ha in tillering phase (BBCH 23-25).

Seed treatment included 4 variants of microbial preparations (Factor C). There were *Azospirillum brasilense* 18-2 (1 L/t seed, 1 mL contained 2×10⁹ cfu), *Bacillus polymyxa* KB (1 L/t seed, 1 mL contained 5×10⁹ cfu), compatible applying (1 L *Azospirillum brasilense* 18-2 + 1 L *Bacillus polymyxa* KB per 1 t seed) and control variant without microbial treatment (water). Seed was treated for 1 day before sowing.

Sampling

Grain yield was evaluated by harvesting all plants from plot. Productive shoots were evaluated before harvesting in 4 marked plots (0.25 m²) for each variant. Sample of 30 plants was selected from each plot to determine the thousand kernel weight, grain weight per spike. Grain yield was calculated to 14% moisture. Protein content was established by near infrared spectroscopy.

Statistical analysis

The results were expressed as the means (n = 4) and standard errors (SE). The data were statistically analyzed by Statistica 12.5 software. Significantly differences between variants were assessed using Fisher's LSD test for thousand kernel weight, productive shoots per m², grain weight per spike and grain yield at P<0.05 and P<0.01. Bonferroni test was used to determine the significance of the difference between variants depending on the protein content.

Table 1. Scheme of field experiment

Cultivars (Factor A)	Fertilization system (Factor B)	Microbial preparation (code) (Factor C)
A1. Landar	B1. Without nitrogen P ₃₆ K ₇₂ (fone)	C1. Without treatment (Wt)
A2. Vuiko		C2. <i>Azospirillum brasilense</i> (Az.)
A3. Pidzimok kharkivskyi	B2. Traditional P ₃₆ K ₇₂ (fone) + N ₂₅ (BBCH 00) + N ₅ (BBCH 23-25)	C3. <i>Bacillus polymyxa</i> (Bac.)
		C4. <i>Azospirillum brasilense</i> + <i>Bacillus polymyxa</i> (Az.+ Bac.)

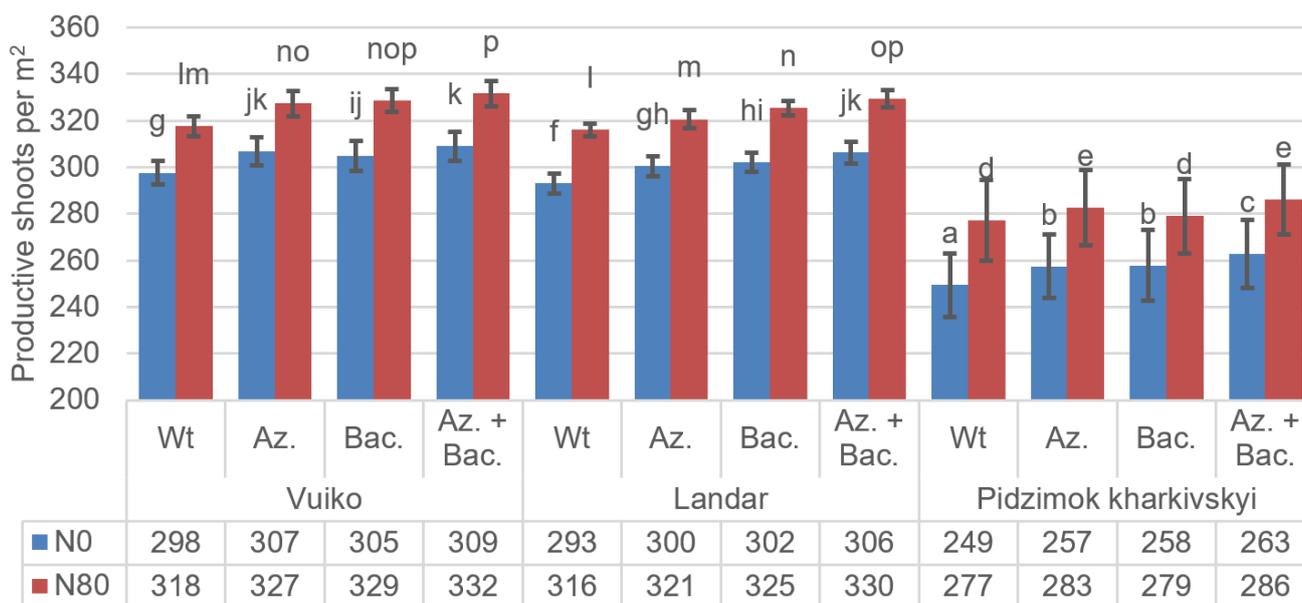
RESULTS AND DISCUSSION

Number of productive shoots per m²

Number of productive shoots per m² describes the response of the variety to environmental conditions. Weather conditions have the greatest impact on this indicator, especially during plant tillering. Abortion of part of the shoots is a natural process, which indicates the availability of plant resources. Increasing the availability of nutrients allows to increase the number of productive shoots. The influence of the studied factors on the number of productive shoots differently manifested in different varieties (Figure 1).

Vuiko cultivar had little sensitive to microbial preparation for both fertilization systems. They had no significant effect on the number of productive shoots, and main factor was fertilization system.

Seed treatments increased the number of productive shoots in Landar cultivar independent on fertilization system. It formed a relatively equal number of shoots under the same conditions of nutrition, regardless of weather conditions. Seed treatments by *Bacillus polymyxa* separately and in combination with *Azospirillum brasilense* gave a significant increase in the number of productive shoots compared to the control without treatment (+3.1% in Bac. and +4.6% in Az.+Bac.) for fertilization system without nitrogen. Number of productive shoots for traditional fertilization system(N₈₀) increased by 3.0% in Bac. and 4.3% Bac.+Az. The single use of *Azospirillum brasilense* had no significant effect, but slightly increased the number of productive shoots (+2.5% in N₀ and +1.5% in N₈₀).



Means followed by the same letter do not differ by the Tukey test at 5% probability

Figure 1. Number of productive shoots depends on cultivar, microbial preparations and fertilization system (average 2017-2019), items/m²

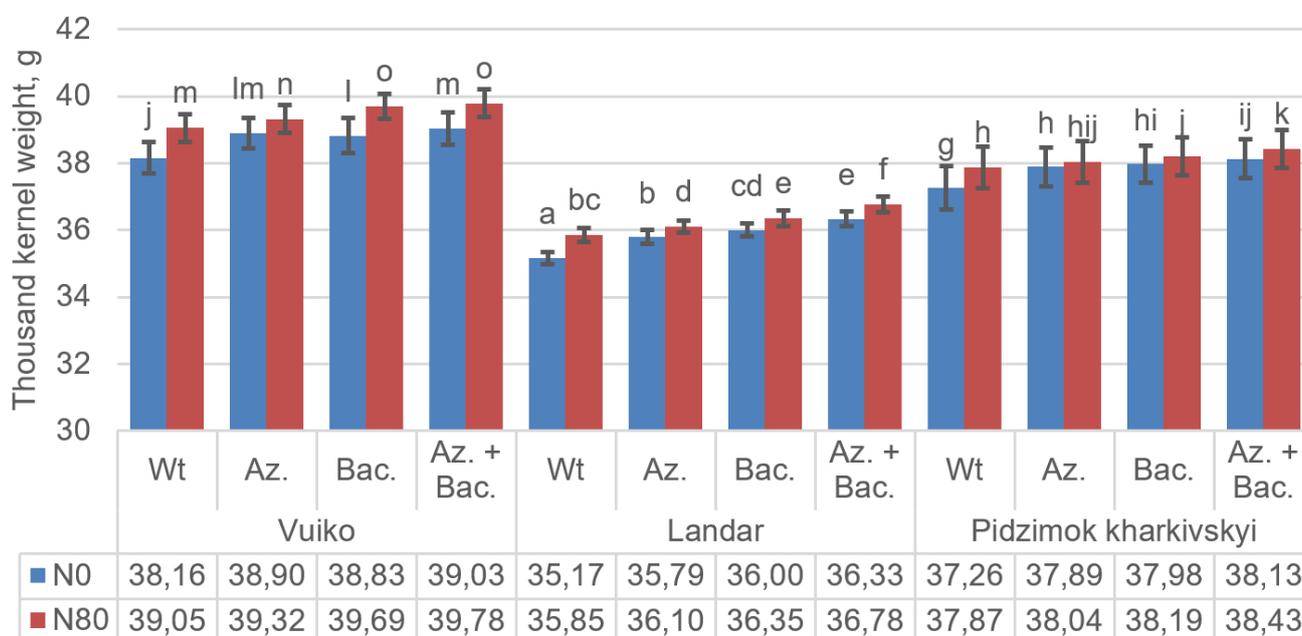
Microbial preparation treatments did not have significant difference in cultivar Pidzimok kharkivskyi, because the number of productive shoots was influenced by weather conditions. Temperature regime during the spring vegetation changed the processes of growth and development of this cultivar. A distinctive feature of Pidzimok kharkivskyi was the long period of vernalization. That was affected to the late beginning of stem elongation.

Weather conditions in the early spring in the Forest-Steppe area of Ukraine were typical in 2017, but they were characterized by sharp increases in average daily temperatures in 2018 and 2019 (Mazurenko et al., 2020). Beginning of stem elongation (BBCH 31) occurred during the flowering period of other cultivars, which led to the reduction of a large number of shoots in 2 last years of research. As a result, the number of shoots was significantly more dependent on weather conditions than on other factors. Application of microbial preparations increased the number of productive shoots much less than the increase in the rate of nitrogen in the fertilizer system. Zorita and Canigia (2009) noted that the number of productive shoots increased on 3.6% by inoculation

Azospirillum brasileense, but sensitive cultivar Landar in field experiment had a lesser response to it. Other cultivars had no significant sensitivity to the number of productive shoots. Long duration of vegetation in cereals leads to reduction of a large part of spikes and decreased in deviation this parameter (Moeller and Rebetzke, 2017). Shoot reduction exceeded the effect of seed treatment by microbial preparation.

Thousand kernel weight

Thousand kernel weight depends significantly on the variety and less on the growing conditions (Figure 2). Cultivar Vuiko had no significant reaction in increasing the thousand kernel weight to seed treatment with microbial preparations for both fertilization systems. Cultivar Landar had a significant difference between microbial preparation for both fertilization systems. Thousand kernel weight did not depend on microbial treatments and fertilization system in cultivar Pidzimok kharkivskyi, but biological characteristics and reaction to environmental conditions had a significant effect on its variation.



Means followed by the same letter do not differ by the Tukey test at 5% probability

Figure 2. Thousand kernel weight depends on cultivar, microbial preparations and fertilization system (average 2017-2019), g

Thousand kernel weight is an important marker of nutrition and the impact of weather conditions on their absorption. It had a lesser variation among all elements of plant productivity. Inoculation increases the thousand kernel weight significantly or insignificantly depending on weather conditions and cultivar sensitivity (Piccinin et al., 2013). Seed treatment with microbial preparations increased thousand kernel weight in all cultivars, but the significance of this increase was different. Statement "Wt < Az. < Bac. < Az.+Bac." was true in a lot of cultivars, indicating greatest effect of *Bacillus polymyxa* on grain filling process. According to Cakmakci et al. (2014) effect of seed treatment by *Bacillus polymyxa* can be manifested by optimization phosphorus nutrition, improving drought resistance or enzyme balance. Thousand kernel weight is almost unchanged due to the seed treatment by *Azospirillum brasilense*, because their influence was manifested at other stages (Zorita and Canigia, 2009)

Grain weight per spike

Grain weight per spike is one of the most variable indicators, which depends on thousand kernel weight and the number of grains in the spike. Seed treatments of microbial preparations have the greatest impact on

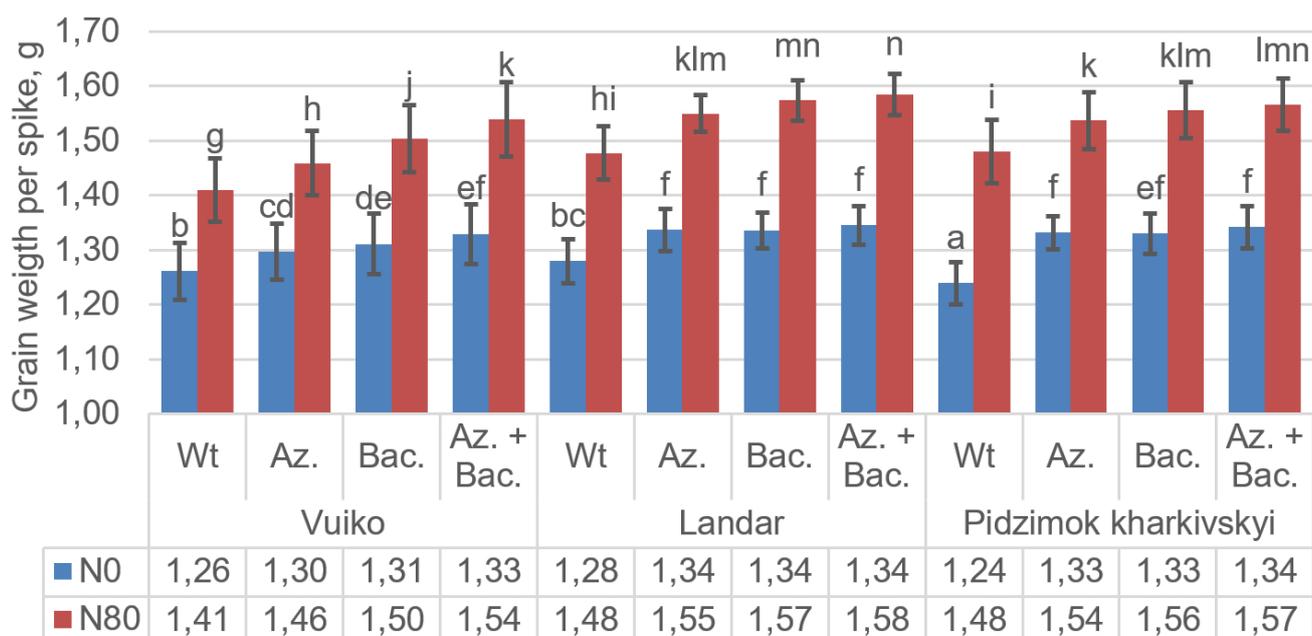
plant development on this period, so their effect on grain weight per spike is greater than on the thousand kernel weight (Figure 3).

All cultivars had similar reactions to the treatment of microbial preparations on grain weight per spike, but they were differed in absolute values. Seed treatment of microbial preparations significantly increased the grain weight per spike compared to variant without treatment for fertilization systems without nitrogen, but there was no difference between types of microbial preparations. Microbial preparation increased the grain weight per spike in traditional fertilization system (N_{80}) too, but their effect was not significant.

Arkhipova et al. (2019) indicated that seed treatment with phosphate-mobilizing *Bacillus* spp. contributes to increasing the grain weight from main shoots and tillers.

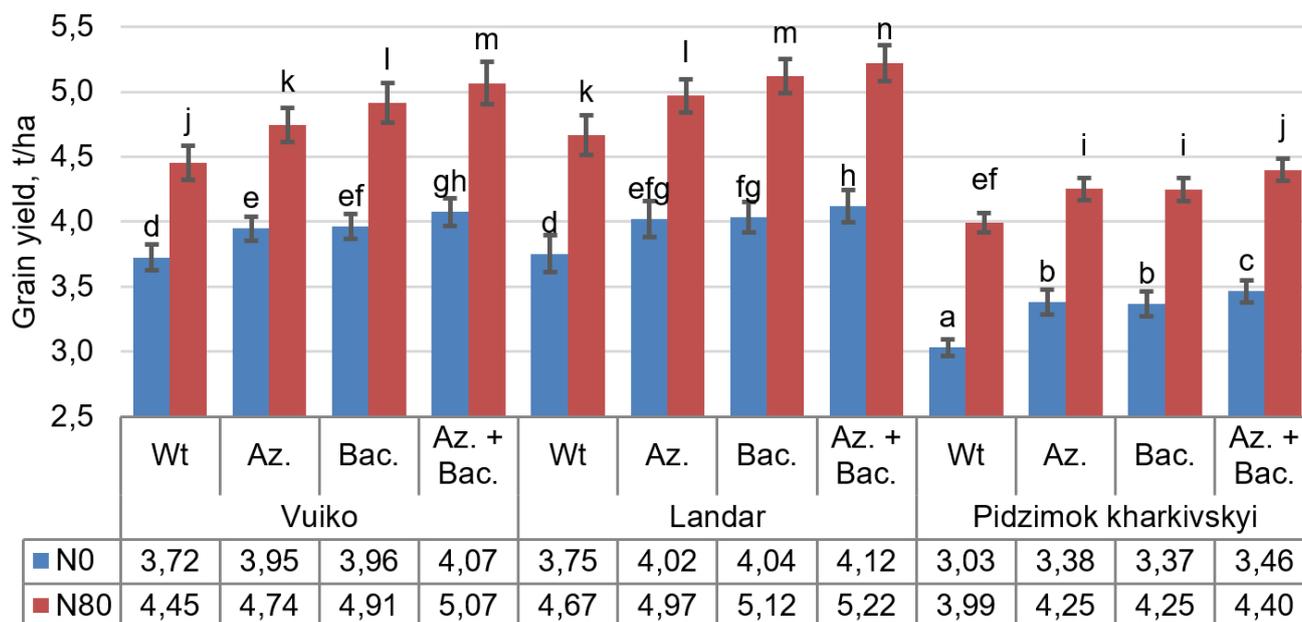
Grain yield

Seed treatment of microbial preparations allows to increase the yield compared to the variants without treatment (Figure 4), but its efficiency is much lesser than effect from nitrogen fertilization. Seed treatment with microbial preparations significantly increased grain yield of cultivars Landar and Vuiko compared to variant



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Figure 3. Grain weight per spike depends on cultivar, microbial preparations and fertilization system (average 2017-2019), g/spike



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Figure 4. Grain yield depends on cultivar, microbial preparations and fertilization system (average 2017-2019), t/ha

without treatments for both fertilization systems. Seed treatment by *Bacillus polymyxa* allowed for higher yields than *Azospirillum brasilense* treated variant for traditional fertilization system. Differences between Az. and Bac. variants were absent for fertilization systems without nitrogen. synergistic effect of the joint application was manifested in all variants of fertilization system and cultivars. Facultative triticale Pidzimok kharkivskiy formed significantly lower grain yield than other cultivars due to the peculiarities of its development.

Seed treatment by *Azospirillum brasilense* can significantly increase the yield of cereals, but their highest efficiency observes on condition without the application of nitrogen fertilizers (Piccinin et al., 2011; Braccini et al., 2012). Grain yield may increase up to 18.1% under favorable weather conditions compared to the untreated variant (Hungria et al., 2010). Seed treatments increases the yield of cereals, but at high nitrogen norms decreases efficiency of *Azospirillum* spp. (Santa et al., 2005; Zorita and Canigia, 2009). Seed treatment by *Azospirillum* spp. is most effective when applying nitrogen fertilizers (Namvar and Khandan, 2013). Seed treatment by nitrogen-fixing bacteria provides plant with a certain amount of nitrogen, but its effectiveness depends on cultivar, weather

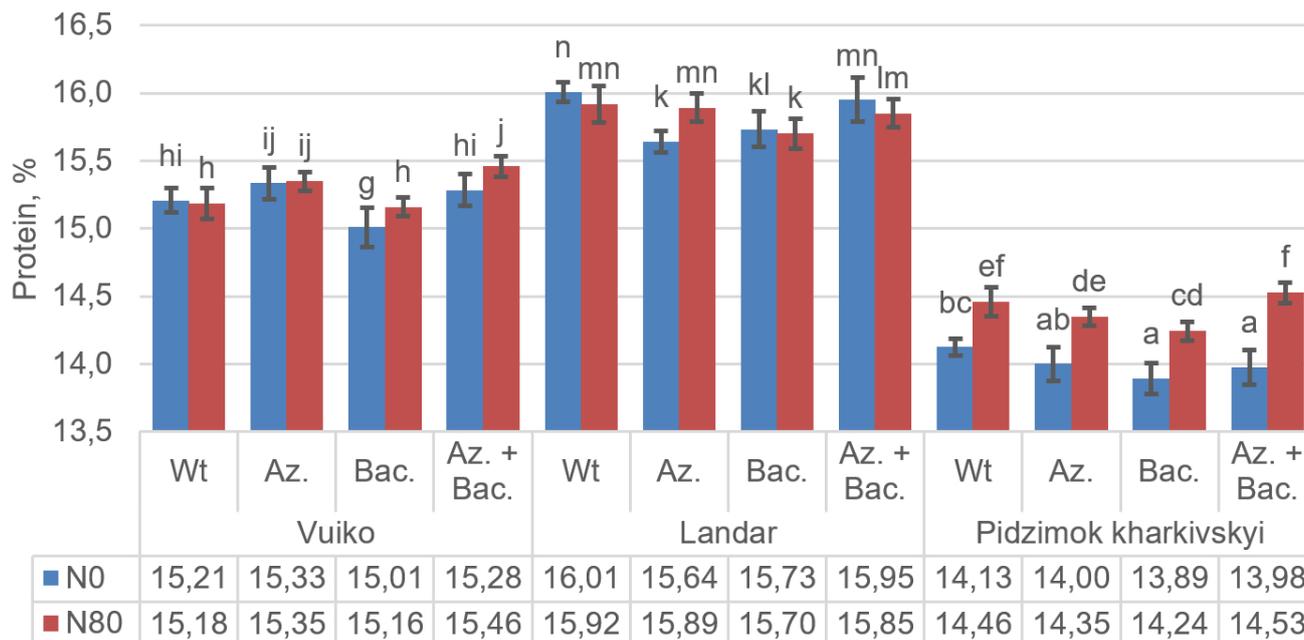
conditions and nitrogen rate (Munareto et al., 2017). Efficiency of treatment by phosphate-mobilizing bacteria depends on the characteristics of the soil, which is manifested in the change in the thousand kernel weight, grain weight per plant and grain yield (Akbar et al., 2019).

Protein content

Protein content depends on many factors during the growing season. Triticale cultivars had different reactions to the treatment with microbial preparations and the fertilizer system (Figure 5).

Seed treatment by *Bacillus polymyxa* significantly reduced the protein content in cultivar Vuiko for fertilization system without nitrogen. *Azospirillum* treatment had no significant difference the protein content compared to variant without treatment. Seed treatment by *Azospirillum brasilense* separately and in combination with *Bacillus polymyxa* increased the protein content for traditional fertilization system.

Cultivar Landar had no significant difference between protein content of the fertilizer system. Seed treatment by *Azospirillum brasilense* and *Bacillus polymyxa* separately led to a significant reduction in protein content, but their



Means followed by the same letter do not differ by the Tukey test at 5% probability

Figure 5. Protein content depends on cultivar, microbial preparations and fertilization system (average 2017-2019), %

combine application had no difference compare to variant without treatments for fertilization system without nitrogen. Treatment by *Bacillus polymyxa* decreased protein content in traditional fertilization system.

Combined use of *Azospirillum brasilense* and *Bacillus polymyxa* optimized protein accumulation in cultivar Pidzimok kharkivskiyi. Separable seed treatment had an effect decreasing protein content for both fertilization system. Protein content of the triticale grain is determined by the processes that take place at the end of the vegetation, so technology elements can only have an indirect effect. Seed treatment by *Azospirillum brasilense* increases summary mass of accumulated nitrogen in plant, but nitrogen reutilization to seed depends on a lot of another factors (Correia et al., 2020).

Analysis of variance

The studied factors had different effects on the elements of yield structure (table 2). The weather condition(year) was the main factor that caused the variation of shoots per m². Cultivar had a similar MS to year, interaction "cultivar - year" and factor "fertilization system" were less in 2 times. Microbial preparation had

lesser effect on variation of shoots per m². Almost all factor interactions had a significant effect on the variation of SPM², but the influence of the interaction "fertilization system-microbial preparation" did not affect the variation of this parameter.

All factors and their interactions had a significant effect on variation of thousand kernel weight, but influence of cultivar and interaction "cultivar-year" were highest. Fertilizing ationght per plant. Influence of other factors and their interactions was lesser All factors and their interactions had a significant effect on variation of thousand kernel weight, but influence of cultivar and interaction "cultivar-year" were highest. Fertilization system and weather conditions (year) had a highest effect on variation of grain weight per plant. Influence of other factors and their interactions was lesser in decades and hundred times.

Fertilization system had the greatest impact on triticale grain yield. Influence of variety factor, weather conditions and their interaction also were significant. Other interactions had a significant effect on yield variation, but their effect was much smaller. Factor "cultivar" significantly affected the protein content, and

Table 2. Analysis of variance for different elements of yield structure

Effect	df	MS				
		SPM ²	TKW	GWPS	Yield	Protein
Cultivar (C)	2	63778	228.3	0.0559	14.218	66.32
Fertilization system (FS)	1	37663	17.9	3.1193	62.475	1.85
Microbial preparation (MP)	3	1903	9.3	0.1188	2.675	0.69
Year (Y)	2	75575	54.4	3.3634	5.249	11.35
C x FS	2	48	1.1	0.0159	0.134	1.03
C x MP	6	45	0.2	0.0020	0.019	0.14
C x Y	4	34745	127.1	0.0551	7.500	0.60
FS x MP	3	8 ^{ns}	0.6	0.0045	0.093	0.07
FS x Y	2	118	1.3	0.0314	0.128	0.77
MP x Y	6	26	0.05	0.0048	0.045	0.16
Other		666	0.8	0.024	0.184	1.071
MSE		6 ^{ns}	0.01 ^{ns}	0.002 ^{ns}	0.003 ^{ns}	0.01 ^{ns}

Means without index are significant on $P < 0.05$, ^{ns} – no significant

the effect of weather conditions was several times lower. Other factors and their interactions were significant, but with significantly lower impacts.

Namvar and Khandan (2013) showed that interaction "nitrogen rate – biofertilizers" has a low effect on elements of structure, but it was significant in grain yield. Grain yield depends on N rate more than microbial preparation in few times (Diosnel et al., 2019). Interaction "year–fertilization system" has a significant effect on grain yield and grain weight per plant independent on other interactions (Rial-Lovera et al., 2016).

Correlations between structural elements of grain yield of triticale

Relationships between the elements of productivity were similar in different triticale cultivars (Table 3-5). Correlation coefficients in relations "grain yield – grain weight per spike" and "grain yield – productive shoots per m²" were close to functional relation ($r > 0.90$). Thousand kernel weight had positive correlation with grain yield

and grain weight per spike, but they had different power depend on cultivar and growing season conditions.

Correlation coefficients between almost all elements of productivity (except for protein) in all cultivars were similar because processes of growth and development were similar in 2017 (Table 3). Main differences between cultivar were observed between protein content and other elements of productivity. Cultivar Landar had no significant correlation between protein content and other. Cultivar Vuiko had a negative correlation that indicated a decrease in protein content with increasing quantitative elements. Cultivar Pidzimok kharkivskiy had positive correlation between all elements of productivity include protein content in 2017.

Weather conditions in 2018 were different with more precipitation than previous year. Relationships between elements of productivity in cultivar Landar were similar to 2017 (Table 4). Cultivar Vuiko differed in relationships between protein content and other elements. These relations became positive, that shows sensitivity of

protein accumulation to changes in growing conditions. Correlation coefficients in cultivar Pidzimok kharkivskiy were similar to 2017 except relation between protein content and thousand kernel weight.

Table 3. Correlation between structural elements of grain yield (2017)

Cultivar		SPM ²	TKW	GWPS	GY
Landar	TKW	0.72**	1		
	GWPS	0.93**	0.67**	1	
	GY	0.96**	0.69**	0.99**	1
	PC	0.10 ^{ns}	0.20 ^{ns}	0.04 ^{ns}	0.05 ^{ns}
Vuiko	TKW	0.76**	1		
	GWPS	0.90**	0.58**	1	
	GY	0.96**	0.65**	0.99**	1
	PC	-0.43*	-0.19 ^{ns}	-0.65**	-0.59**
Pidzimok kharkivskiy	TKW	0.51**	1		
	GWPS	0.84**	0.73**	1	
	GY	0.95**	0.67**	0.98**	1
	PC	0.66**	0.41*	0.50**	0.58**

* P<0.05; ** P<0.01; ^{ns} – no significant; SPM² – spike per m²; TKW – thousand kernel weight; GWPS – grain weight per spike; GY – grain yield; PC – protein content

Table 4. Correlation between structural elements of grain yield (2018)

Cultivar		SPM ²	TKW	GWPS	GY
Landar	TKW	0.76**	1		
	GWPS	0.87**	0.50**	1	
	GY	0.93**	0.59**	0.99**	1
	PC	0.17 ^{ns}	-0.19 ^{ns}	0.25 ^{ns}	0.24 ^{ns}
Vuiko	TKW	0.87**	1		
	GWPS	0.96**	0.93**	1	
	GY	0.98**	0.92**	0.99**	1
	PC	0.80**	0.75**	0.79**	0.80**
Pidzimok kharkivskiy	TKW	0.43*	1		
	GWPS	0.91**	0.63**	1	
	GY	0.97**	0.55**	0.98**	1
	PC	0.74**	-0.17 ^{ns}	0.46**	0.60**

* P<0.05; ** P<0.01; ^{ns} – no significant; SPM² – spike per m²; TKW – thousand kernel weight; GWPS – grain weight per spike; GY – grain yield; PC – protein content

Table 5. Correlation between structural elements of grain yield (2019)

Cultivar		SPM ²	TKW	GWPS	GY
Landar	TKW	0.73**	1		
	GWPS	0.98**	0.65**	1	
	GY	0.99**	0.68**	0.99**	1
	PC	-0.65**	-0.57**	-0.62**	-0.62**
Vuiko	TKW	0.93**	1		
	GWPS	0.92**	0.91**	1	
	GY	0.97**	0.94**	0.99**	1
	PC	0.19 ^{ns}	0.19 ^{ns}	0.25 ^{ns}	0.24 ^{ns}
Pidzimok kharkivskyi	TKW	0.91**	1		
	GWPS	0.81**	0.85**	1	
	GY	0.90**	0.90**	0.98**	1
	PC	0.62**	0.54**	0.81**	0.80**

* P<0.05; ** P<0.01; ^{ns} – no significant; SPM² – spike per m²; TKW – thousand kernel weight; GWPS – grain weight per spike; GY – grain yield; PC – protein content

Correlation coefficients between protein content and other elements of productivity in cultivar Landar were negative that shows the predominance of the formation of quantitative indicators in qualitative (protein content) in 2019 (Table 5). Relationships between protein content and other elements of productivity were insignificant in cultivar Vuiko, Cultivar Pidzimok kharkivskyi had same relations in 2019 compared to previous years.

Silva et al. (2020) manifested that protein content and grain yield have a weak negative correlation, but water availability has a large effect on protein content. Positive correlation coefficient in "protein-yield" may be a consequence of moisture deficiency and cultivar response on it. Correlation coefficient was negative in the most optimal year for moisture (2019) in cultivar Landar, but relation between protein content and grain yield was insignificant in other years. Cultivar Vuiko had a negative correlation under arid conditions, and it became positive or insignificant with improvement of moisture regime. Relation was positive in cultivar Pidzimok kharkivskyi, because protein content fluctuated in a smaller range

than in other cultivars. Increasing yields under the action of nitrogen fertilizers and seed treatment also increased the protein content. Protein accumulation and nitrogen outflow process depends on cultivar (Noorka et al., 2009).

CONCLUSIONS

Established that the effect on grain yield from the application microbial preparations was manifested in all cultivars but the mechanisms of this manifestation differed. Cultivars Vuiko and Landar formed a similar number of productive shoots per m² independent on weather condition. Cultivar Pidzimok kharkivskyi reduced some part of shoots during vegetation and the number of productive shoots per m² decreased and finally had not deviations between the variants. Microbial treatment had significantly less efficiency than application of 80 kg/ha N. Seed treatment of microbial preparations increased some elements of individual performance of triticale plants, sometimes without significant difference, but it had significant effect on grain yield. Effect of microbial preparation on grain yield was similar in cultivars Landar and Vuiko, although manifested in different ways. Seed

treatment by *Azospirillum brasilense* increased grain yield on 6.3-6.7% and *Bacillus polymyxa* increased it on 8.5-8.7%. Combined application this microbials increased grain yield on 11.8% in Vuiko and 10.9% in Landar. Seed treatment by *Azospirillum brasilense* increased grain yield on 8.7% and 8.5% for variants with *Bacillus polymyxa* in cultivar Pidzimok kharkivskiyi. Combined application two strains of microbial preparations increased grain yield on 12.0% in this cultivar.

Studied cultivars differed in their response to the weather conditions of the year, which was manifested in the change of correlation coefficients between some elements of productivity. Main difference between cultivars are the formation protein content in different weather condition and their relations with other elements of productivity.

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