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Article

Influence of Microbial Preparations on *Triticum aestivum* L. Grain Quality

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Abstract: Gluten, protein and amino acid composition play an important role in grain quality assessment. Areas of interest of our research include essential amino acids, which are not synthesized in the human body. It is a commonly known fact that large doses of mineral fertilizers increase grain crops' yield and quality. However, fertilization leads to undesirable effects—in particular, environmental pollution. This creates a need to replace mineral fertilizers, at least partially, with alternative methods. One such method is the use of microbial preparations in modern technologies for growing cereals. This research, therefore, aimed to study the effect of presowing seed inoculation with a microbial preparation (based on phosphate-mobilizing bacterium *Lelliottia nimipressuralis* CCM* 32-3) on *T. aestivum* grain quality, namely the content of gluten, protein and amino acids. The analysis of three-year field experiments showed that the highest values were obtained when using the microbial preparation against the background of mineral fertilizers at the rate of P_{30}. Presowing seed inoculation contributed to a significant increase in grain productivity (by 31.5% compared to control). The content of protein and gluten in the grain also increased up to 12.5 % and 28.0%, while in the control, these figures were 10.8% and 21.2%, respectively. Moreover, the total content of amino acids in wheat grain in the variant inoculation + fertilizers (P_{30}) was the highest compared to those without inoculation. The following excess was noted: by 52% compared to control (without fertilizers); and by 29%, 17% and 10% in variants with mineral fertilizers at the rate of P_{30}, P_{60}, and P_{90}, respectively. The obtained research results indicate that the combined application of mineral fertilizer Ammophos (at the rate of P_{30}) and microbial preparation based on the phosphate-mobilizing bacterium *L. nimipressuralis* CCM 32-3 for presowing seed inoculation is an effective technique that improves the yield and quality indicators of winter wheat grain under the conditions of southern regions with insufficient moisture supply.

Keywords: *Triticum aestivum* L.; gluten; protein; amino acids; microbial preparation; mineral fertilizers

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important crops in the world and one of the most important cereals in the human diet. According to the United Nations, by 2050, the world population will grow by nearly 30% [1], and thus, the importance of *T. aestivum* is expected to increase. Winter wheat helps to solve the food supply problems. Its grain has a high content of protein (14–17% or more) and carbohydrates (80%), and it is widely used in baking, pasta and confectionery industries.

The optimal seeding rate for obtaining appropriate plant density is one of the important factors that allows you to control wheat yield increment [2–5]. To improve the productivity and quality of cultivated crops, it is necessary to apply large doses of mineral fertilizers [6]. However, high doses of mineral fertilizers, especially under conditions of extensive agricultural production and the predominance of monoculture, lead to undesirable effects—in particular, environmental pollution [7]. This creates a need to replace mineral fertilizers, at least partially, with alternative methods based on natural processes of self-renewal. One such environmentally safe and resource-saving element of the functioning and sustainable development of agroecosystems is the introduction of microbial preparations into modern crop cultivation technologies [8–12].

Today, a sufficiently large number of biological preparations based on microorganisms are known. They improve the mineral nutrition of plants and stimulate their growth. All known microbial preparations are based on the naturally occurring phenomena of nitrogen fixation and phosphate mobilization, which are typical for epiphytic and soil microorganisms [13–18]. The most popular microbial preparations (based on nitrogen-fixing microorganisms) significantly increase the productivity of agricultural crops: grain yield improvement reaches, on average, 15–20%, while vegetable crops' yield increases by 20–30% [19–21].

Along with nitrogen, phosphorus is an equally important macronutrient. The widespread use of biopreparations based on microorganisms able to transform sparingly soluble phosphates into forms available to plants has not only an ecological but also economic priority. The role of biologization in modern crop cultivation technologies increases significantly in the context of more severe soil–climatic and weather conditions [22–27]. One of the most promising directions for improving the phosphorus nutrition of agricultural crops is biological phosphate mobilization. It is carried out by soil microorganisms (bacteria and micromycetes) that contribute to the transformation of sparingly soluble phosphorus compounds from soil and fertilizers into forms available to higher plants [11,14,15,26–30]. The presence of phosphate-dissolving microorganisms in the soil is the main pathway for the solubilization and release of inorganic phosphates into soluble forms that can be directly used by plants. One of the ways to introduce effective strains into the plant's rhizosphere microbiocenosis is presowing inoculation when suspension of microorganisms is applied to the surface of seeds. Experience in this field indicates that the use of bacterial preparations in crop cultivation technologies has a beneficial effect on the mineral nutrition of plants and increases the yield of high-quality products with rational consumption of mineral fertilizers, as well as improves both the ecological state of soils and their fertility [10,18,19].

Among the quality indicators of winter wheat grain, gluten content, as one of the most important economic and price features, is of particular importance. According to Torikov et al. [6], the largest winter wheat yield increase compared to control (without agrochemicals) was obtained against the background of mineral fertilizers ($N_{98}P_{64}K_{124}$) applied in autumn and two top dressings applied in spring (during the resumption of plant growth and at the beginning of the stem elongation phase) at the rate of N_{30}. This option of applying the calculated norms of mineral fertilizers ensured yield at the level of 5.6 t/ha and content of raw gluten in the grain over 28%. The contents of protein and amino acids are the most important characteristic of the biological value of grain. Proteins perform a vast array of functions within the cell: enzymatic, building, regulatory, etc. Amino acids are the structural units of protein molecules that take part in all processes occurring in the human and animal body. One of the most important amino acids is proline. It plays a key role both as a free amino acid and as a structural component of proteins. It is known that stress conditions promote its accumulation in plants [31,32].

Based on the foregoing, the development of environmentally friendly biological methods of plant cultivation under conditions of insufficient moisture using phosphate-mobilizing bacteria to improve plants' mineral nutrition, productivity, as well as obtained product quality is an urgent task. Thus, this research aimed to study the effect of presowing seed inoculation with a microbial preparation of Phosphostim (based on phosphate-mobilizing bacterium *L. nimipressuralis* CCM 32-3) on *T. aestivum* yield and grain quality, namely the content of gluten, protein and amino acids under the soil and climatic conditions of Crimea.

2. Materials and Methods

2.1. Experimental Setup

Field experiments were carried out in 2016–2019 at the experimental fields of Agro-Industrial College—structural unit of V.I. Vernadsky Crimean Federal University (Simferopolsky district). Soil—chernozem southern calcareous heavy loamy; agrochemical characteristics: humus content −2.5%, mobile phosphorus −2.6 mg/g of soil, exchangeable potassium −25.0 mg/g of soil, soil solution pH −7.0–7.2. Winter wheat (*Triticum aestivum* L.) was grown on four backgrounds: without mineral fertilizers (Variant I), application of mineral fertilizer Ammophos at the rate of P_{30} (Variant II), P_{60} (Variant III), P_{90} (Variant IV). The area of the experimental plot was 1500m^2, fourfold replication. The scheme of experiments on each background included the following options: control (seed treatment with water); presowing seed inoculation with a microbial preparation of Phosphostim: based on phosphate-mobilizing bacterium *L. nimipressuralis* CCM 32-3 deposited in "All-Russian Collection of Industrial Microorganisms" (ARCIM) number 12783. The microbial preparation was in the form of an aqueous suspension [33]. It contained cells of phosphate-mobilizing bacterium *L. nimipressuralis* ARCIM-12783; titer −1.0–2.0 × 1010 cells/mL at a dose of 2% of seed weight.

2.2. Determination of Protein and Gluten

Grain quality (protein and gluten mass fraction) was determined according to the method of near-infrared spectroscopy (NIRS). This method is based on the use of the dependences of the spectral characteristics of absorption, transmission or reflection of light in the infrared region of the spectrum on the content of grain constituents [34].

2.3. Amino Acid Analysis

Amino acid composition in winter wheat grain proteins was determined via the method of complete acid hydrolysis using 6N HCl; quantitative determination of all amino acids in the hydrolysate—on biochemical analyzer "Hitachi". The determination of free proline in the grain was carried out using acidic ninhydrin reagent according to the method by Bates et al. [35], while that of amino acids containing sulfur (methionine, cysteine, cystine) according to the methodology [36].

2.4. Statistical Analysis

Field experiments were carried out according to generally accepted methods [37]. In the process of evaluating the results obtained, we used the method of multivariate variance analysis of data, as well as multiple correlation coefficient, which were calculated using Statistica 7.0 software (Development by StatSoft (USA)).

3. Results

3.1. Grain Productivity

As a result of the conducted experiments, it has been determined that the application of mineral fertilizers contributed to an increase in the yield of winter wheat up to 3.78 (Variant II) and 4.08 (Variants III and IV) t/ha compared to the variant without mineral fertilizers (2.55 t/ha). Data on the winter wheat yield under conditions of field experiments are presented in Table 1.

Analysis of the obtained results (average for 3 years) showed that the use of a microbial preparation for presowing seed treatment is an effective method for increasing winter wheat grain productivity. Depending on the background of fertilizers, it increased by 0.21–1.19 t/ha or 8–31% compared with the variants without seed inoculation.

However, the highest yields of winter wheat were noted when mineral fertilizers were applied at the rate of P_{30}. Microbial preparation for seed inoculation significantly increased grain yield (by 1.19 t/ha or 31.5%) compared to the control values.

Table 1. Winter wheat yield, t/ha (field experiments, average for 3 years).

Variant	t/ha	Yield Increase	
		t/ha	%
Without Mineral Fertilizers			
Without inoculation	2.55	-	-
Phosphostim	2.76	0.21	8.2
LSD_{05}	0.27		
P_{30}			
Without inoculation	3.78	-	-
Phosphostim	4.97	1.19	31.5
LSD_{05}	0.53		
P_{60}			
Without inoculation	4.08	-	-
Phosphostim	4.95	0.87	21.3
LSD_{05}	0.43		
P_{90}			
Without inoculation	4.08	-	-
Phosphostim	4.83	0.75	18.4
LSD_{05}	0.48		

LSD_{05} least significant difference at $p \leq 0.05$.

3.2. Gluten and Protein

Gluten content in winter wheat grain is presented in Figure 1. Our studies point out that high doses of mineral fertilizers have a positive effect on the gluten content in wheat grain. When mineral fertilizers were applied at the rate of P_{60} and P_{90}, the gluten content increased up to 27.3% vs. 19.2% (plot without fertilization). The amount of gluten in the grain also increased in all variants with microbial preparation, both when it was combined with mineral fertilizers, and on the site without their application. The best efficiency of the biological product was revealed when it was combined with mineral fertilizers at the rate of P_{30}: gluten content in grain increased to 28.0% vs. 21.2% in the control (without inoculation).

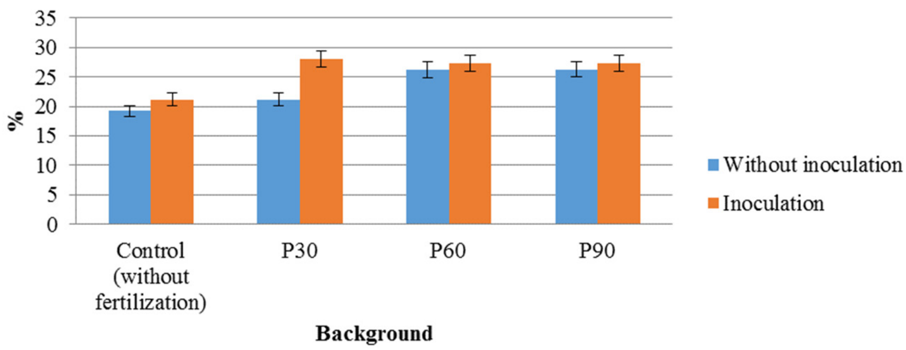

Figure 1. Gluten content in winter wheat grain, % (average content with standard errors).

The results of our experiments showed that protein content in winter wheat grain depends both on the dose of mineral fertilizers and on the use of microbiological preparation (Figure 2). The application of fertilizers at the rate of P_{30}, P_{60} and P_{90} contributed to an increase in the protein content in the grain to 12.4%, 12.5% and 12.8%; in the control variant (without fertilization), this indicator reached only 9.9%. The most favorable dose of mineral fertilizers, which provides a positive effect of presowing seed bacterization, is P_{30}. In this variant, protein content in grain increased up to 12.5% vs. 10.8% in the control one (without inoculation).

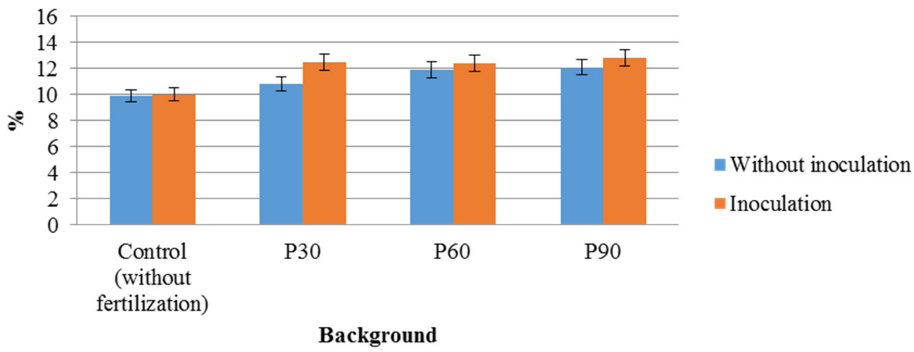

Figure 2. Protein content in winter wheat grain, % (average content with standard errors).

To conclude, the use of a microbial preparation based on the bacterium *L. nimipressuralis* ARCIM-12783 for presowing bacterization of winter wheat seeds under soil and climatic conditions of the Crimean Peninsula improves grain quality (protein and gluten content increases). The most favorable dose of mineral fertilizers, which provides a positive effect of presowing seed bacterization, is P_{30}. In this variant, protein content in grain increased up to 12.5%, gluten content—up to 28.0% vs. 10.8% and 21.2% in the control, respectively.

3.3. Amino Acids

3.3.1. Essential and Critical Amino Acids

It should be noted that the results of our experiments confirmed the positive effect of mineral fertilizers and microbial preparation of Phosphostim on the amino acid composition of plant protein in winter wheat grain (Table 2).

Table 2. Amino acid content in winter wheat grain, mg/100 mg of the initial substance.

Amino Acids	**Variant**				
	Control (Without Fertilizer)	P_{30} Without Inoculation	P_{30} Inoculation	P_{60}	P_{90}
	Essential amino acids, including:				
Valine	0.236	0.268	0.317	0.287	0.293
Isoleucine	0.173	0.202	0.227	0.208	0.227
Leucine	0.502	0.579	0.745	0.641	0.667
Lysine	0.183	0.209	0.250	0.215	0.221
Methionine	0.189	0.197	0.189	0.243	0.275
Threonine	0.177	0.256	0.242	0.209	0.215
Tryptophan	0.128	0.147	0.136	0.154	0.152
Phenylalanine	0.226	0.262	0.314	0.289	0.288
	Critical amino acids, including:				
Alanine	0.185	0.216	0.244	0.214	0.226
Arginine	0.256	0.324	0.327	0.300	0.307
Aspartic acid	0.340	0.369	0.469	0.380	0.412
Histidine	0.113	0.134	0.164	0.139	0.147
Glycine	0.292	0.339	0.421	0.366	0.377
Glutamic acid	2.760	3.324	4.815	3.893	4.171
Cystine	0.154	0.172	0.170	0.178	0.202
Serine	0.301	0.339	0.457	0.382	0.400
Tyrosine	0.134	0.149	0.168	0.160	0.174
The amount	6.349	7.486	9.655	8.258	8.754

Mineral fertilizer application contributed to an increase in the amount of each of the amino acids in wheat grain, both essential and non-essential, which indicates product quality improvement. The greatest increase in the content of amino acids in wheat grain occurred thanks to critical amino acids, namely glutamic acid. Its content in the grain of bacterized plants (background with mineral fertilizers at the rate of P_{30}) increased by

1.7 times and amounted to 4.815 mg/100 mg of the initial substance; in the control variant, this indicator reached only 2.760 mg/100 mg of the initial substance. It is important to point out that the content of amino acids in the grain was the highest in the experimental variant "microbial preparation Phosphostim + mineral fertilizers Ammophos at the rate of P_{30}". It not only exceeded the control variant (without fertilizer in the experimental variant "microbial preparation Phosphostim + mineral fertilizers Ammophos at the rate of P_{30}"); we also noted an increase in the total content of amino acids in wheat grain: their total amount reached 9.655 mg/100 mg of the initial substance and exceeded that of other variants (control, P_{30}, P_{60} and P_{90}) by 52%, 29%, 17% and 10%, respectively. In addition, presowing seed inoculation contributed to an increase in the total content of both essential and critical amino acids in the grain by 33% and 59% compared to control or up to 2.420 and 7.235 mg/100 mg of the initial substance (Table 3). This circumstance led to a change in the ratio of essential/critical amino acids in the direction of its reduction.

Table 3. Total content of amino acids and their ratio in winter wheat grain, mg/100 mg of the initial substance.

Amino acids	Variant				
	Control (Without Fertilizer)	P_{30} Without Inoculation	P_{30} Inoculation	P_{60}	P_{90}
Essential amino acids	1.814	2.120	2.420	2.246	2.338
Critical amino acids	4.535	5.366	7.235	6.102	6.416
Total	6.349	7.486	9.655	8.258	8.754
Ratio: essential amino acids/critical amino acids	0.400	0.395	0.334	0.373	0.364

3.3.2. Proline

As noted above, the amino acid proline plays an important role in the adaptation of plants to various stress factors, and in particular to drought, which is very relevant for the climatic conditions of Crimea. The results of our studies have shown that the application of mineral fertilizers helps to reduce the content of free proline in winter wheat grain compared to the control: from 4.27 mg/% to 3.33 mg/% (Figure 3). The use of the microbial preparation of Phosphostim for presowing inoculation of seeds reduced the accumulation of free proline in wheat grain both in the control variant and when combined with Ammophos—up to 3.91 mg/% and 3.53–3.64 mg/%, respectively.

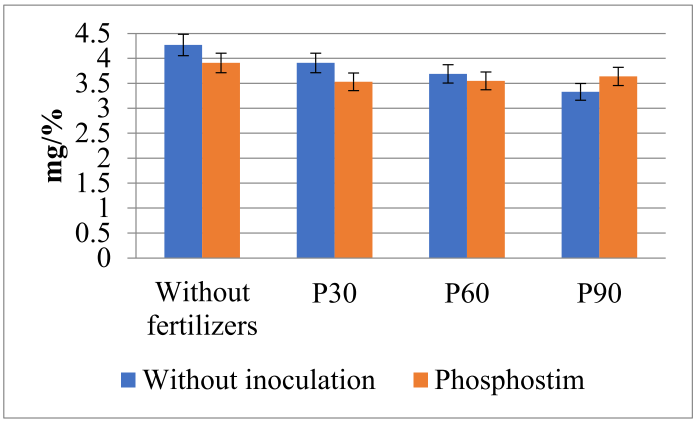

Figure 3. Free proline content in winter wheat grain, mg/% (average content with standard errors).

Statistical analysis of our data showed that an increase in the content of free proline in winter wheat grain due to stress exerted by soil and climatic conditions had a negative effect on *T. aestivum* yield and crop quality. Thus, with an increase in the level of free proline in winter wheat grain, there is a decrease in protein content (r = −0.69) and gluten (r = −0.70), as well as a decrease in the grain productivity of winter wheat (r = −0.72)—the inverse relationship of average tightness (Table 4). It was also revealed that an increase in the protein content in winter wheat grain contributed to an increase in the gluten content in wheat: the relationship is direct and close (r = 0.95). We have established a direct, close relationship between grain productivity and the quality of the products obtained; the correlation coefficient is 0.92.

Table 4. Influence of free proline on winter wheat grain quality indicators and productivity.

	Free Proline, mg/%	Protein, %	Gluten, %	Grain Productivity, t/ha
Free proline, mg/%	1.00	–	–	–
Protein, %	−0.69	1.00	–	–
Gluten, %	−0.70	0.95	1.00	–
Grain productivity, t/ha	−0.72	0.92	0.92	1.00

Thus, the combined use of the microbial preparation of Phosphostim (based on the phosphate-mobilizing bacterium *L. nimipressuralis* ARCIM-12783) for presowing seed inoculation and mineral fertilizers at the rate of P_{30} contributes to the accumulation of amino acids in wheat grain in an amount equivalent to the application of fertilizers at the rate of P_{90}.

4. Discussion

The use of fertilizing microbial preparations in crop cultivation technologies is one of the elements of modern agriculture. Our studies have shown a positive effect of the combined use of the bacterial preparation of Phosphostim and the mineral fertilizer Ammophos on the grain productivity of winter wheat. Thus, the results of three-year field studies showed the prospect of joint use of a biological preparation based on the phosphate-mobilizing bacterium *L. nimipressuralis* ARCIM-12783 and mineral fertilizers at the rate of P_{30} when growing winter wheat in the agroclimatic conditions of Crimea.

Gluten determines the baking properties of grain: its higher content improves bread quality. Grain cereal crops play a leading role as the main protein-containing raw material. On a global scale, about 70% of humanity's need for proteins is covered with grains. In Russia, winter wheat is the main food crop. Its sown area varies from 8 to 11 million hectares over the years, accounting for 20% to 24% of the gross grain harvest. However, it should be noted that the study of varieties of winter cereal crops (wheat, rye and triticale) growing on the territory of the forest-steppe of the southeast of Western Siberia showed that most of them do not meet the region's need for the accumulation of complete protein and, hence, biologically adequate nutrition [38]. Wheat grain quality indicators largely depend on the hereditary characteristics of the variety and, therefore, can serve as criteria in the selection of the most promising lines at the early stages of the breeding process. Therefore, in 2018–2020, under the agroclimatic conditions of the North Caucasus, studies were carried out to search for the source material among the variety of genotypes of soft winter wheat to select the most promising lines when creating new varieties for regions with a dry period of grain filling [39]. The most important criteria for the quality of grain of soft winter wheat were mass fraction of gluten and its quality, mass fraction of protein, as well as sedimentation rate. To conclude, the use of a microbial preparation based on the bacterium *L. nimipressuralis* ARCIM-12783 for presowing bacterization of winter wheat seeds under soil and climatic conditions of the Crimean Peninsula improves grain quality (protein and gluten content increases). The most favorable dose of mineral fertilizers, which provides a positive effect of presowing seed bacterization, is P_{30}. In this variant, protein content in grain increased up to 12.5%, gluten content—up to 28.0% vs. 10.8% and 21.2% in the control, respectively.

Amino acid composition is used as a biochemical criterion for the biological value of feed and food products (according to the total content of essential amino acids). The results of our research indicate that presowing seed inoculation contributed to an increase in the total content of both essential and critical amino acids in the grain by 33% and 59%, respectively, compared to control, or up to 2.420 and 7.235 mg/100 mg of the initial substance. Similar results were noted in the literature sources. For instance, use of the microbial preparation Kaliplant (containing potassium-mobilizing bacteria *Bacillus circulans BIM* V-376D) on sod-podzolic sandy loamy soil contributed to an increase in the protein content in the grain of winter rye and winter triticale by 0.4–0.5% and 0.7–1.3%, respectively. An improvement in the amino acid composition of the protein was also detected. The score of critical amino acids in winter rye increased by 5–8%, and essential amino acids by 7–11%, while in winter triticale, by 2–7 and 2–6%, respectively [40]. In the southeast of Western Siberia, our colleagues assessed the effect of diazotrophic presowing seed bacterization on the biological and nutritional value of barley grain. *H. vulgare* seeds were bacterized with the biopreparation Rizoagrin-B (peat form). As a result, protein content increased by 1.9% (up to 13.70%), and the number of amino acids by 23.3% (up to 10.79 g/100 g), as well as the content of all essential amino acids [41].

A number of studies provide data on an increased content of proline in plants under the influence of various stresses: soil salinity [42–46], heavy metal exposure [47,48] and drought [49–51], as well as high and low temperatures [52–54]. Our research showed that the use of the microbial preparation of Phosphostim for presowing seed inoculation reduced the accumulation of free proline in wheat grain both in the control variant (up to 3.91 mg/%) and in variants with Ammophos (3.53–3.64 mg/%). It can be assumed that the use of Phosphostim contributes to an increase in the adaptive potential of winter wheat plants to arid growing conditions.

5. Conclusions

A positive effect of the microbial preparation of Phosphostim (based on the phosphate-mobilizing bacterium *L. nimipressuralis* ARCIM-12783) on the yield and quality indicators of winter wheat grain was established. It contributed to the increment of such indicators as protein content, gluten content and amino acid content. The combined use of the microbial preparation of Phosphostim for presowing seed inoculation and mineral fertilizers at the rate of P_{30} contributes to the grain productivity (by 31.5% compared to control) and quality growing winter wheat in the agroclimatic conditions of Crimea. The content of protein and gluten in the grain also increased up to 12.5% and 28.0%, while in the control, these figures were 10.8% and 21.2%, respectively. Moreover, the total content of amino acids in wheat grain in the variant inoculation + fertilizers (P_{30}) was the highest compared to those without inoculation. The following excess was noted: by 52% compared to control (without fertilizers); and by 29%, 17% and 10% in variants with mineral fertilizers at the rate of P_{30}, P_{60}, and P_{90}, respectively. Furthermore, the rate of application of mineral fertilizers is significantly reduced, and consequently, material costs decline and anthropogenic load on the environment decreases. The obtained research results indicate that the combined application of mineral fertilizer Ammophos (at the rate of P_{30}) and microbial preparation based on the phosphate-mobilizing bacterium *L. nimipressuralis* CCM 32-3 for presowing seed inoculation is an effective technique that improves the yield and quality indicators of winter wheat grain under the conditions of southern regions with insufficient moisture supply.

The conducted research became the basis for obtaining a patent [55].

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