

The influence of agricultural biologization means on the content of essential amino acids in winter wheat grain

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Abstract. The effect of biological soil activation against the background of mineral and organic fertilizers (green manure) on the content of essential amino acids in winter wheat grain was studied. The technique is a pre-sowing application of a consortium of microorganisms (*Pseudomonas fluorescense*, strain AP-33, *Azotobacter vinelandii* IB-4) and microscopic fungi (*Trichoderma harzianum* and *Trichoderma viridae*) using a boom sprayer. It was found that the application of green manure and mineral fertilizers to non-activated soil is accompanied by a decrease in the amount of essential amino acids by 15.7 and 15.6%, respectively, in variants without mineral fertilizers and with ½ of their calculated dose. It has been established that the use of biological soil activation with the incorporation of green manure helps to increase the concentration of essential amino acids in variants with doses of mineral fertilizers 0 NPK and ½ NPK by 8.8 and 14.9%, respectively. An idea has been formed about the role of soil biological activity as a factor influencing not only the quality of agricultural products, but also a factor limiting the level of its productivity. Thus, it was found that the use of soil bioactivation helps to increase the yield of winter wheat by 11.7-17.7%, depending on the type of experiment.

1 Introduction

The soil is a self-regulating system that strives to ensure the sustainability of its functioning. At the same time, it is known that intensive anthropogenic impact on the soil significantly upsets the balance in the system, in which its microbiological component is especially affected. The basis of existing programs for the biologization of agriculture is the use of means and techniques that help increase the level of biological activity of the soil, which in turn affects the ability of soils to regenerate. Thus, fresh plant biomass entering the soil leads to an increase in the functional response of soil microflora [1], which

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apparently is one of the mechanisms of the soil-fertilizing effect of green fertilizers (green manures). The choice of cruciferous crops, in particular winter rapeseed, as a green fertilizer is due to a number of properties, such as cold resistance, intensity of accumulation of green mass, high concentration of protein in biomass and low costs of their use [2].

In addition to the activation of native soil microflora by organic substances of green fertilizers [2], the direct application of a consortium of agronomically valuable strains of microorganisms is also a recognized method of biologization of agriculture [3-5] and can improve the quality of products [6]. The quality of the soil, the improvement of which is the goal of using methods of its bioactivation, largely affects both the quantity and quality of crop products produced [7], determined in particular by the protein indicator. It is important to note that the protein nutritional value of feeds can be reduced if they contain insufficient essential amino acids, since their deficiency cannot be compensated for by other amino acids [8-10].

Table 1. Characteristics of essential amino acids [10].

No.	Amino acids	Consequences of shortage
1	Threonine	Stunted body growth
2	Valin	Impaired coordination of movements
3	Methionine	Muscle wasting, risk of liver cirrhosis, anemia, obesity
4	Isoleucine	Decreased formation of immunoglobulins
5	Leucine	Pathologies of the endocrine system, decreased immunity
6	Phenylalanine	Decreased mental activity, pancreatic disease
7	Histidine	Delayed hemoglobin synthesis, delayed cognitive development in children
8	Lysine	Bone degradation
9	Arginine	Decreased immunity, tissue destruction

Increasing the amino acid composition of agricultural products through the use of appropriate techniques and means in the production process (biofortification) meets the goals and objectives of improving the quality of food, which contributes to the prevention and control of non-communicable diseases of the population [11].

The purpose of the work was to assess the influence of agricultural biologization means (green manure and microbiological fertilizers) at different levels of mineral nutrition on the content of essential amino acids in winter wheat grain.

2 Materials and methods

The studies were carried out on ordinary chernozem in the long-term observation area (since 1979) of Geonetwork No. 82 (Opytnoe village, Kabardino-Balkarian Republic). The winter wheat variety Yuzhanka was used. Bioactivation agents are represented by green manure (winter rapeseed), plowed under the previous crop and a consortium of soil microorganisms (*Pseudomonas fluorescense*, strain AP-33, *Azotobacter vinelandii* IB-4) and microscopic fungi (*Trichoderma harzianum* and *Trichoderma viridae*). The concentration of living cells is $2-4 \times 10^9$. Application is carried out before sowing seeds at a dose of 2 liters/ha. The impact of the products was assessed on two backgrounds of mineral nutrition (Table 1) – without fertilizers (0NPK) and $\frac{1}{2}$ calculated dose (NPK 23:12.5:24.5).

The concentration of essential amino acids in grain was determined by high-performance liquid chromatography (GOST 32195-2013).

Table 2. Experience scheme.

Options	Background		Bioactivation
Control	No organic fertilizers	0NPK	-
Reference			+
Option 1		1/2NPK	-
Option 1a			+
Option 2	Green manure	0NPK	-
Option 2a			+
Option 3		1/2NPK	-
Option 3a			+

3 Results and Discussion

Analysis of the data indicates the influence of agrobiological (bioactivation) and agrochemical factors (mineral fertilizers) during the production of winter wheat on the content of essential amino acids in the grain (Table 3).

Table 3. Content of essential amino acids in grain, %.

Amino acids	Background - Without organic fertilizers				Background - Siderata			
	0NPK		½ NPK		0NPK		½ NPK	
	Contro l	Standard (Control+Bio)	1	1a	2	2a	3	3a
Threonine	0.291 ±0.014	0.295 ±0.036	0.236 ±0.013	0.301 ±0.023	0.255 ±0.001	0.280 ±0.038	0.207 ±0.003	0.240 ±0.001
Valin	0.432 ±0.030	0.434 ±0.025	0.355 ±0.023	0.439 ±0.037	0.398 ±0.021	0.473 ±0.033	0.300 ±0.001	0.357 ±0.010
Methionine	0.137 ±0.011	0.138 ±0.002	0.080 ±0.005	0.126 ±0.020	0.099 ±0.003	0.051 ±0.025	0.101 ±0.015	0.092 ±0.005
Isoleucine	0.337 ±0.015	0.332 ±0.023	0.268 ±0.012	0.346 ±0.037	0.311 ±0.020	0.371 ±0.038	0.214 ±0.006	0.256 ±0.001
Leucine	0.713 ±0.037	0.707 ±0.064	0.544 ±0.032	0.720 ±0.064	0.623 ±0.039	0.738 ±0.037	0.456 ±0.009	0.527 ±0.003
Phenylalanine	0.482 ±0.006	0.474 ±0.012	0.359 ±0.012	0.474 ±0.010	0.411 ±0.001	0.428 ±0.012	0.283 ±0.006	0.324 ±0.002
Histidine	0.288 ±0.033	0.265 ±0.027	0.226 ±0.021	0.282 ±0.021	0.250 ±0.028	0.253 ±0.024	0.204 ±0.013	0.222 ±0.023
Lysine	0.294 ±0.038	0.289 ±0.0245	0.245 ±0.029	0.307 ±0.031	0.268 ±0.026	0.284 ±0.032	0.223 ±0.006	0.258 ±0.013
Arginine	0.511 ±0.035	0.474 ±0.043	0.344 ±0.029	0.470 ±0.007	0.402 ±0.057	0.401 ±0.028	0.301 ±0.056	0.353 ±0.029
Total	3.484	3.407	2.647	3.466	3.016	3.280	2.289	2.630

It is known that the use of green fertilizers in agriculture is an important method of enriching the soil with fresh organic matter, and one of the most common green manure crops is winter rapeseed [12]. At the same time, as can be seen from the table, the influence of green manure on the grain quality of winter wheat was expressed in a decrease in the total amount of essential amino acids. The decrease is especially noticeable against the background of mineral fertilizers (Figure 1).

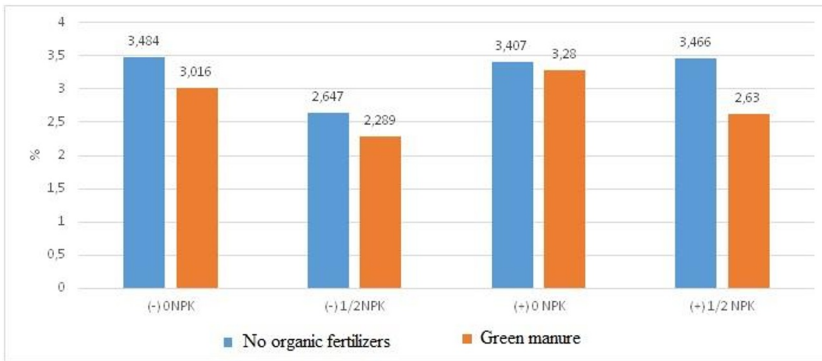


Fig. 1. The influence of green manure and mineral fertilizers on the amount of essential amino acids in grain.

A feature of green manure as a means of increasing the biological activity of soil is that it creates a high background of cellulose-decomposing activity [13], which possibly explains the decrease in the content of the studied amino acids, since the latter are organic compounds that are also subject to biochemical destruction in the environment. Thus, in the reference plot (without bioactivation) against the background of a zero dose of mineral fertilizers, the use of green manure was accompanied by a decrease in the content of the total essential amino acids by 15.7%. A similar trend is observed when using green manure together with mineral fertilizers. Thus, against the background of $\frac{1}{2}$ the calculated dose of mineral fertilizers, the decrease is 15.6%.

When considering the effect of mineral fertilizers as a separate factor, their more significant effect on the content of essential amino acids is noted, expressed in a decrease in their concentration, than from the effect of green manure. It is a generally established fact that mineral fertilizers are known for their properties that destroy soil organic matter [14], which can also cause a depletion of the amino acid composition of grain. Thus, the introduction of a calculated half-dose of mineral fertilizers in comparison with the control option leads to a decrease in the amount of amino acids by 24.0% - from 3.484 to 2.647%. A decrease in the amino acid content in option 3 to 2.289% indicates the manifestation of the cumulative effect of green manure and mineral fertilizers. Compared to the control variant, the decrease was 34.3%.

The discovered undesirable effect of green manures and mineral fertilizers associated with the deterioration of the amino acid status of grain cannot be the basis for the conclusion that their use is inappropriate for the purpose of obtaining high-quality agricultural products. Grain analysis data indicate the possibility of increasing the amount of essential amino acids by enriching the soil with agronomically valuable strains of microorganisms (Figure 2). An assessment of the effect of taking soil bioactivation within the considered options, both against the background of green manure and without their use, indicates a positive effect of the intake on the content of the total amount of amino acids.

The diagram shows an increase in the amount of essential amino acids against the background of green manure by 8.8% (0 NPK) and by 14.9% (0 NPK).

High values of the sum of essential amino acids in absolute terms are observed in the bioactivated area. Thus, in the variant without mineral fertilizers, the concentration of amino acids is 3.407%, which is only 2.2% lower than in the control. Against the background of mineral fertilizers, the intake of bioactivation leads to an increase in amino acids by 30.9%, which indicates the biological activity of the soil as a key factor determining the quality of the grain produced.

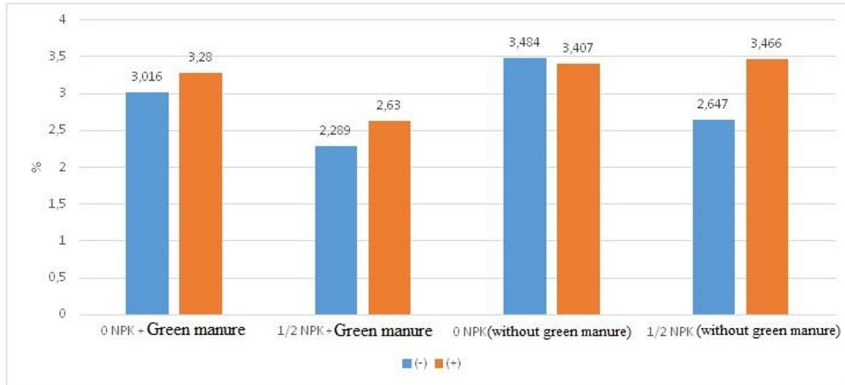


Fig. 2. The influence of soil bioactivation on the content of essential amino acids in grain.

In the structure of the total amount of essential amino acids, changes in the concentration of amino acids can be traced depending on the combination of mineral fertilizers and bioactivation agents (Table 4).

Against a background without organic fertilizers and a zero dose of mineral fertilizers, taking soil bioactivation does not affect the change in the concentration of amino acids such as threonine, valine, methionine, isoleucine, leucine and lysine. The spread is within 2%. A decrease in the concentration of histidine and arginine was revealed by 8.0 and 7.2%, respectively.

Table 4. Change in AMK concentration under the influence of soil bioactivation, %.

Amino acids	Background - Without organic fertilizers				Background - Siderata			
	0NPK		½ NPK		0NPK		½ NPK	
	Control	Standard (Control+Bio)	1	1a	2	2a	3	3a
Threonine		+1.3			27.5	9.8		15.9
Valin		0.5			23.7	18.8		19.0
Methionine		-			57.5	-48.5		-8.9
Isoleucine		-2.1			29.1	19.3		19.6
Leucine		-0.8			32.4	18.5		15.6
Phenylalanine		-1.7			32.0	4.1		14.5
Histidine		-8.0			24.8	1.2		8.8
Lysine		-1.7			25.3	6.0		15.7
Arginine		-7.2			36.3	-		17.3
r		-0.264						0.978

Bioactivation of soil fertilized with mineral fertilizers against a background without green manure demonstrates an increase in all amino acids in the range of 27.5-57.5%. Methionine shows the maximum increase.

Against the background of green manure, the effect of taking soil bioactivation in the variant with 0NPK is accompanied by an increase in the proportion of threonine, valine, isoleucine, leucine and lysine by 6.0-19.3% (Figure 3).

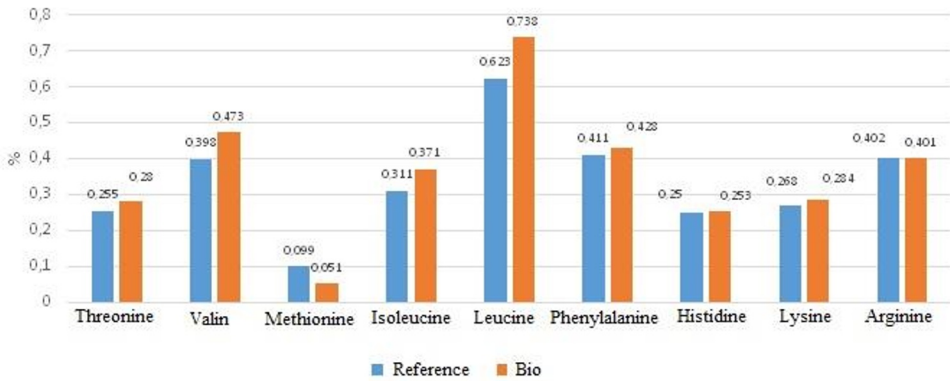


Fig. 3. Concentration of essential amino acids in grain. Background 0 NPK.

There were no noticeable changes (less than 5%) in the content of phenylalanine, histidine and arginine. In this variant, the concentration of methionine decreased significantly (by 48.5%).

The trend of changes in the content of amino acids against the background of green manure is repeated when using mineral fertilizers (Figure 4), which is confirmed by a high degree of correlation ($r = 0.978$).

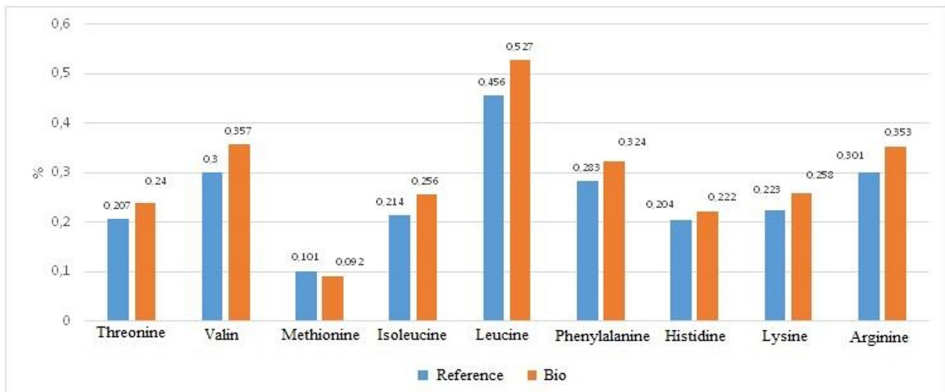


Fig. 4. Concentration of essential amino acids in grain. Background 1/2 NPK.

The diagrams (Figures 3 and 4) show duplication of the direction of change in methionine content, manifested in a decrease in concentration by 8.9%. The increase in the concentration of other amino acids is in the range of 8.8-19.6%.

One of the main purposes of soil fertilizers, which, along with mineral ones, include organic and microbiological fertilizers, is to increase the productivity of agricultural crops. At the same time, the main task of agriculture is to increase productivity without compromising its quality. The results of the study indicate the difficulty of achieving such an effect. The use of the studied fertilizers was accompanied by an increase in the yield of winter wheat (Table 5), but not in all cases it was possible to maintain the quality of the grain, assessed by amino acid composition.

Table 5. Winter wheat yield, c/ha.

Options	Productivity, c/ha				Increase, %
	Repetition		Average		
Control	23.71	23.37	23.01	23.36±0.87	17.7
Reference	27.05	26.7	28.76	27.50±2.74	
Option 1	27.7	29.2	28.97	28.62±2.01	16.4
Option 1a	33.26	33.58	33.09	33.31±0.62	
Option 2	25.89	24.46	25.42	25.26±1.81	11.7
Option 2a	28.11	28.35	28.2	28.22±0.30	
Option 3	29.67	30.49	30.37	30.18±1.10	15.2
Option 3a	35.2	33.95	35.13	34.76±1.74	

The table shows that the effect of taking biological activation of the soil within the evaluated options was accompanied by a noticeable increase in the yield of winter wheat - in the range of 11.7-17.7%. The effect of green manure had a lesser effect on the increase in yield, which was in the range of 4.4-8.1%. At the same time, the combined use of green manure and soil bioactivation (options 2a and 3a) made it possible to significantly increase the increase compared to the control. So, against the background of 0NPK, the increase increased by 20.8%, and against the background of ½NPK - by 48.8%.

4 Conclusion

The work revealed that the effect of biological soil activation agents on the amino acid composition depends on the nature of the agents themselves, as well as on their combination with other fertilizing substances. Thus, the use of green manure was accompanied by a decrease in the amount of essential amino acids in grain by 15.7 and 15.6%, respectively, in the options without the application of mineral fertilizers and with the application of their calculated half-dose.

The use of mineral fertilizers was also accompanied by a decrease in the concentration of amino acids, and if against the background without organic fertilizers it was 24.0%, then against the background of green manure it reached 34.3%.

The results of the study make it possible to evaluate the parameter of soil biological activity as a factor that determines not only the quality of agricultural products, but also limits the level of its productivity. It was found that soil bioactivation, promoting an increase in the concentration of amino acids in variants with green manure by 8.8 (0 NPK) and 14.9% (½NPK), led to an increase in agricultural yields by 11.7-17.7%.

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