Influence of biological preparations based on associative on
the yield of winter triticale in the conditions of the south-east
Central Chernozemic Area

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Abstract. The article presents the results of field studies to study the effectiveness of
biological preparations based on associative microorganisms in the crops of winter triticale on
ordinary chernozem in 2012-2015 years. The method of application of biological preparations
is pre-sowing inoculation of seeds. The experimental design included studies on a natural
background and when nitrogen fertilizers were applied at a dose of 30 kg / ha of the active
substance. According to the results of four years of research, the high efficiency of the use of
biological products for presowing treatment of seeds of winter triticale in the conditions of the
southeast of Central Chernozemic Area is shown. The productivity of winter triticale increased
on average by 1.5-5.5 c / ha. The maximum yield growth was detected using strain 18-5.
Mineral fertilizers in the form of nitrogen at a dose of N 30
in combination with presowing
inoculation of seeds did not contribute to a stable increase in the productivity of cultivated
crops. In the technologies of cultivation of winter triticale, it is possible to exclude presowing
introduction of nitrogen at a dose of N 30
and replace it with biological products based on
associative microorganisms.

1. Introduction
The search for alternative ways to optimize plant growth conditions and increase soil fertility based on
biological fixation of atmospheric nitrogen is one of the pressing issues in agricultural science. In
chernozem soils, despite their high natural fertility, during critical periods of plant growth and
development, a lack of mineral elements is noted [1, 2, 3]. One of the main macro-elements that
determine plant growth and development is nitrogen. The main factor in the regulation of soil fertility
until recently was the use of mineral fertilizers, but they cannot under all conditions meet the total
need of plants for nitrogen, phosphorus and potassium. A significant reduction in the use of mineral
and organic fertilizers in agriculture in the first place brings the problem of finding alternative sources
of plant nutrition. In this regard, the main role belongs to associative microorganisms that are able to
fix the molecular nitrogen of the atmosphere in the rhizosphere of cereal crops [4, 5, 6, 7, 8].
Atmospheric molecular nitrogen is a virtually inexhaustible source of soil enrichment to improve plant
nutrition. Unfortunately, until recently, the role of biological nitrogen as a factor in stabilizing soil
fertility, increasing yields and reducing the risk of ecological safety in crop cultivation has been underestimated [9, 10, 11, 12, 13].

In this regard, the search for ways to regulate the balance of soil nutrients through the use of biopreparations of associative microorganisms remains largely an issue that has not yet been studied. In the conditions of the Central Chernozemic Area, the influence of climatic features, the relationship between the provision of mineral nutrition elements and the activity of associative nitrogen fixators have not been revealed.

2. Materials and methods
The research was carried out in the Dokuchaev Research Institute of Agriculture in the Central Chernozemic Area in 2012 – 2015.

The soil of the test site is plain black (segregation) medium-weight medium-gum heavy-carbon on carbonate loess clays with humus content – 6.8%; pH_\text{water} = 7.2; Ng = 0.7 mmol eq/100 g soil. Exchange bases: Ca = 24-26 mmol eq/100 g; Mg = 4-5 mmol eq/100 g. The degree of saturation with bases is very high -96-98%. Gross content: nitrogen 0.321%, phosphorus 1.86%, potassium 0.193%.

Culture is a winter triticale. Variety - Doctrine 110. Predecessor - pair black.

The studies were carried out in two-factor experience. First order factor – fertilization levels: without fertilizers and N_30. Second order variants - microbiological strains of preparations: 1 – control (untreated seeds); 2 – mizorin (strain 7); 3 – azorisin (strain 8); 4 – strain 17 - 1; 5 – strain 18-5; 6 flavobacterin (strain 30); 7 – strain PG - 5; 8 – rhizoagrin (strain 204); 9 – strain 33-3; 10 – strain 2P - 7. Seeds were treated with active strains of bacteria on the day of sowing. Operating fluid flow rate – 1 l of solution per 1 t of seeds. Preparations are obtained from All-Russia Research Institute of Agricultural Microbiology.

Mineral fertilizers were introduced for pre-plant cultivation. The main soil treatment - ploughing to the depth of 20-22 cm. Pre-planting cultivation was carried out by KPS aggregate - 4. The sowing rate – 5.0 million germinating seeds per 1 ha. Depths of closure – 5 - 6 cm. Chemical agents for control of weed vegetation were not used.

The repetition of the experience is 6-fold. Sowing area is 7.0 m^2, accounting – 5.0 m^2. Location of variants - organized blocks with randomization inside the block.

Crop accounting - continuous dividing combine "Hege."

Agricultural machinery cultivating winter triticale is generally accepted for the Voronezh region.

The main integrating indicator of the level of effective soil fertility, growing conditions, as well as the efficiency of using different techniques of agricultural machinery, is productivity of cultivated crops.

3. Study of the influence of biological preparations based on associative microorganisms on the yield of winter triticale
The studies carried out in contrasting weather conditions have shown different efficacy of microbial preparations. Microbial preparations were the dominant element in the formation and increase of productivity of cultivated crops. Mineral fertilizers occupied a subordinate position. Their contribution to increasing yields is much lower than the actions of microbial drugs. Field studies showed that under the 2012 growing conditions, under an uncomfortable background, all the associated diazotrophic preparations used proved effective and increased the yield of the winter triticale relative to the control variant. The maximum crop increase was noted in the variant from strain 18-5 and was 6.1 c/ha relative to the control, which is 17.1% higher. A rather good increase was shown by strains 8 and PG-5 – 11.2 and 11.0%, respectively (4.0-3.9 c/ha). For the rest of the options, the yield increase was 2.5 to 9%.

The use of nitrogen fertilizers in pure form at a dose of 30 kg/ha active substance (a. s.) contributed to a smaller increase in productivity. The difference with the uncomfortable option was only 2.2 c/ha. Thus, the efficiency of the most effective strain 18-5 was 3.9 c/ha (table 1).
It should be noted that the use of mineral nitrogen fertilizers together with biopreparations of associative diazotrophies has not produced a reliable positive result this year. For all variants there was a tendency to decrease the yield of winter triticale relative to control (table 1). On average, the decrease in productivity on a fertilized background for all variants was a reliable value of 1.6 c/ha.

Table 1. Influence of biological preparations based on associative microorganisms on the yield of winter triticale in 2012 year

| Factor A  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Average, c/ha |
|-----------|---|---|---|---|---|---|---|---|---|--------------|
| without fertilizer | 35.6 | 36.5 | 39.6 | 38.8 | 41.7 | 37.7 | 39.5 | 38.4 | 38.1 | 38.4 |
| \(N_{30}\) | 37.8 | 36.6 | 36.9 | 37.4 | 36.8 | 36.9 | 36.5 | 35.7 | 36.6 | 36.8 |

1 - control; 2 - strain 7 misorin; 3 - strain 8 azorisin; 4 - strain 17-1; 5 – strain 18-5; 6 - strain 30 flavobacterin; 7 – strain PG - 5; 8 - strain 204 rhizoagrin; 9 - strain 33-3

The assessment of the main effects indicates that the additional application of nitrogen fertilizers did not have a positive effect on the increase in the yield of winter triticale. The difference between the fertilization backgrounds was 1.6 c/ha. Microbiological preparations mainly provided a reliable increase in yield (except for strains 7 (mizorin) and 204). The excess with respect to the control was 0.6- 2.6 c/ha.

In 2013, there was also a tendency to increase the yield of winter triticale against an unfertilized background when seeds were inoculated with associative diazotrophs in all cases. The maximum grain yield is noted on the variant with strain 17-1 and amounted to 35.2 c/ha, which is 21.4% higher than the control (table 2). In variants with strains 18-5 and 30 (flavobacterin), the yield increase was slightly less and amounted to 19.0 and 17.6%, respectively strain 8 (azorisin) and PG-5 increased the yield of winter triticale by 13.8 - 14.1%. For other strains, the increase was from 1.4 to 7.3%.

Table 2. Influence of biological preparations based on associative microorganisms on the yield of winter triticale in 2013 year

| Factor A  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Average, c/ha |
|-----------|---|---|---|---|---|---|---|---|---|--------------|
| without fertilizer | 29.0 | 30.9 | 33.0 | 35.2 | 34.5 | 34.1 | 33.1 | 30.5 | 29.4 | 31.1 | 32.1 |
| \(N_{30}\) | 33.7 | 29.5 | 35.5 | 27.9 | 28.8 | 29.8 | 27.9 | 29.5 | 29.1 | 30.1 | 30.2 |

1 – control; 2 - strain 7 misorin; 3 - strain 8 azorisin; 4 - strain 17-1; 5 – strain 18-5; 6 - strain 30 flavobacterin; 7 – strain PG – 5; 8 - strain 204 rhizoagrin; 9 – strain 2P-7; 10 - strain 33-3

We also found that the introduction of additional mineral nitrogen into the soil inhibited the activity of associative diazotrophs in most variants, with the exception of strain 8 (azorisin). There is a tendency to increase the yield of winter triticale by 1.8 kg / ha or 5.3%. The use of pure nitrogen fertilizers increased the productivity of winter triticale by 4.7 c / ha.

Analyzing the statistical data on the assessment of the main factor, we can say that associative diazotrophs provided a significant increase in the yield of winter triticale for most inoculants. The excess in relation to the control was 2.9 kg / ha. Differences between fertilizer backgrounds are insignificant and equal to 1.9 kg / ha. The introduction of additional mineral fertilizers did not reliably lead to an increase in the yield of winter triticale. Associated diazotrophs had the main effect on the yield of winter triticale. Their contribution amounted to 45.9%. The contribution from the application of mineral nitrogen fertilizers amounted to 18.2%.

In 2014, our studies noted the positive effect of inoculants both on an unfertilized background and when using mineral nitrogen.
Table 3. Influence of biological preparations based on associative microorganisms on the yield of winter triticale in 2014 year

| Factor A | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| without fertilizer | 24.9 | 27.2 | 28.7 | 29.1 | 28.7 | 30.8 | 29.4 | 29.4 | 28.9 | 29.4 |
| N<sub>30</sub> | 26.7 | 28.3 | 28.6 | 28.8 | 27.3 | 29.9 | 31.0 | 30.0 | 30.1 | 30.2 |

1 – control; 2 - strain 7 misorin; 3 - strain 8 azorisin; 4 - strain 17-1; 5 - strain 18-5; 6 – strain 30 flavobacterin; 7 – strain PG – 5; 8 - strain 204 rhizoagrin; 9 – strain 2P-7; 10 - strain 33-3

Nevertheless, relative excesses were higher against the natural background. The maximum increase in yield is observed when treating seeds with strain 30 (flavobacterin). Without the use of mineral fertilizers, productivity growth was 23.7%. Strains 204 (rhizoagrin), PG-5 and 33-3 gave an increase in the yield of winter triticale equal to 18.1%. For other strains against an unfertilized background, the increase was from 9.2 to 16.9%.

Under the conditions of 2014, a positive effect from microbial preparations was also noted against a fertilized background. The maximum increase against the background of N<sub>30</sub> of 4.3 c / ha was provided by the option with pre-sowing seed treatment with strain PG-5, which is 16.1% higher than the control. Inoculation of seeds with other strains of associative diazotrophs was less effective. The increase in productivity varied between 0.6–3.5 c / ha, from 2.2 to 13.1%.

The analysis of the main effects shows that the yield of winter triticale slightly changed under the influence of mineral nitrogen fertilizers. Differences between fertilizer backgrounds were insignificant and amounted to only 1.0 kg / ha. Microbiological preparations provided a significant increase in productivity. The excess in relation to control was 2.0 - 4.6 c / ha. As a result of statistical processing of experimental data, it was found that biological products had the main influence on the change in the productivity of winter triticale. Their contribution to the main resulting feature is 47.4%, mineral fertilizers account for 20.0%.

Table 4. Influence of biological preparations based on associative microorganisms on the yield of winter triticale in 2015 year

| Factor A | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| without fertilizer | 29.2 | 31.9 | 33.9 | 34.5 | 35.8 | 35.5 | 34.5 | 34.8 | 35.3 |
| N<sub>30</sub> | 33.7 | 35.7 | 34.4 | 34.8 | 36.9 | 38.0 | 38.3 | 35.4 | 38.2 |

1 – control; 2 – strain 7 misorin; 3 - strain 8 azorisin; 4 - strain 17-1; 5 - strain 18-5; 6 – strain 30 flavobacterin; 7 – strain PG – 5; 8 - strain 204 rhizoagrin; 9 – strain 2P-7

As in previous years, in 2015, associative diazotrophs had a positive effect on the yield of winter triticale both against an unfertilized background and against the background of N30. However, relative excesses were higher against the natural background of fertilizer. So, the maximum effect was achieved on the option with strain 18-5. The yield increase was 22.6% relative to the control (table 4). The control is 29.2 c / ha. In addition, a significant increase in yield was noted on variants with strain 30 (flavobacterin) and 2P-7 and is 6.3 c / ha and 6.1 c / ha, respectively, which is 21.6 and 21% higher than the control, respectively. The remaining strains also gave a significant increase from 9.2% to 19.2%.
When using mineral nitrogen fertilizers for pre-sowing cultivation at a dose of 30 kg a.a. per 1 hectare the maximum effect in increasing the yield of winter triticale showed strain PG-5. The increase was 4.6 c / ha, which is 13.6% higher than the control. A significant increase was noted by us in the version with pcs. 2P-7 and amounted to 13.3%. For other strains, the increase in winter triticale productivity relative to the control was 0.7 - 4.3 c / ha (table 4). The use of mineral nitrogen in comparison with the natural background increased the yield by 4.5 c / ha.

The assessment of the main effects indicates that mineral nitrogen fertilizers had a significant enough impact on the yield of winter triticale in 2015 conditions. Thus, the difference between fertilization backgrounds was 2.3 c/ha. All strains of associative diazotrophes have reliably increased the yield of winter triticale. And the increase was more significant than mineral nitrogen.

Statistical data processing shows that the contribution of associative diazotrophes to the change in the productivity of the winter triticale is 41.5%, while the contribution from the addition of additional mineral nitrogen fertilizers was only 15.9%.

As a result of our research in 2012-2015, it can be concluded that the most effective associative diazotrophies affected the yield of winter triticale against an uncomfortable background. The most active was strain 18-5 (table 5). The average yield for the years of studies on the variant with this strain was 35.2 c/ha, which is 18.5% higher than the control. On average, during the years of research against the background of N_{30}, strains of associative diazotrophes did not have a reliable positive effect on the grain harvest of winter triticale. However, in certain years (2015), under the influence of the current favorable conditions, mineral nitrogen fertilizers can positively affect the productivity of winter triticale.

**Table 5.** Influence of biological preparations based on associative microorganisms on the yield of winter triticale in 2012-2015 years

| Factor A          | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9* | 10** | Average, c/ha |
|-------------------|----|----|----|----|----|----|----|----|----|------|--------------|
| without fertilizer| 29.7 | 31.6 | 33.8 | 34.4 | 35.2 | 34.5 | 34.1 | 33.3 | 31.2 | 32.8 | 33.1         |
| N_{30}            | 33.0 | 32.5 | 33.8 | 32.2 | 32.4 | 33.7 | 33.4 | 32.6 | 32.5 | 32.3 | 32.8         |
| Average           | 31.4 | 32.1 | 33.8 | 33.3 | 33.8 | 33.8 | 34.1 | 33.8 | 33.0 | 31.9 | 32.6         |

1 – control; 2 - strain 7 misorin; 3 - strain 8 azorisin; 4 - strain 17-1; 5 - strain 18-5; 6 – strain 30 flavobacterin; 7 – strain PG – 5; 8 - strain 204 rhizoagrin; 9 – strain 2P-7; 10 - strain 33-3

*experimental data 2013-2015 years
**experimental data 2012-2014 years

According to the results of four years of research, the high efficiency of using microbial preparations for pre-sowing treatment of seeds in the conditions of the south-east of the Central Chernozemic Area has been established. On average, the productivity of winter triticale increased by 1.5-5.5c/ha. Maximum yield growth was observed using strain 18-5.

Mineral fertilizers in the form of nitrogen addition in the dose of N_{30} in combination with pre-sowing inoculation of seeds did not contribute to stable growth of productivity of cultivated cereals. In their effectiveness, they were close to microbial drugs. Therefore, the use of expensive nitrogen fertilizers and more environmentally friendly microbiological preparations can be avoided in the cultivation technologies of these crops.

**4. Conclusion**

According to the results of four years of research in the cultivation of grain crops, the high efficiency of the use of microbial preparations for pre-sowing seed treatment in the conditions of the southeast of Central Chernozemic Area was established. The productivity of winter triticale increased on average by 1.5-5.5 c / ha. The maximum yield growth was observed when using strain 18-5.
Mineral fertilizers in the form of nitrogen at a dose of N$_{30}$ in combination with presowing inoculation of seeds did not contribute to a stable increase in the productivity of cultivated crops. In their effectiveness, they were close to microbial drugs. In this regard, in the technologies for cultivating these crops, it is possible to abandon the use of expensive nitrogen fertilizers and more widely use environmentally friendly microbiological preparations.

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