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ECOLOGICALLY BASED DISEASE MANAGEMENT TECHNIQUES IN BARLEY CULTIVATION IN THE CENTRAL BLACK SOIL REGION

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ABSTRACT

At present, there are microbiological Bacillus-based products used to preserve valuable micro biota and improving the level of biological soil productivity as well as sustaining the local environment. The research focuses on discovering how some particular biological products and autochthonous microorganisms influence the yield capacity of barley grown in the Black-Earth region of Russia. The research objectives included a search for autochthonous strains of microorganisms that improve resistance to diseases, estimation of how biological products contribute to the quality of barley seeds, biological products effect on the spread and disease resistance and estimation of how biological products enhance the yield capacity of barley. The paper describes the results of identification of an autochthonous *Bacillus* strains. PCR diagnostic methods were used to confirm the strain specific origin of two sample cells extracted from soil (S1 and S2). The study involved the analysis of micro biota of leached chernozem, which revealed the autochthonous strain of *Bacillus* S1 having a germicidal effect. The S1 strain revealed *Bacillus subtilis* and *Bacillus cereus*, while S2 revealed only Bacillus subtilis, as detected by the method of molecular diagnostics based on using species-specific primers. Biological treatment of the seeds improved their sowing qualities, namely, germination readiness and germination capacity. In addition, it was found out that such treatment improves the resistance to disease affection and spread. *Bacillus* S1, in particular, reduces the disease affection by 16,5 % and the disease spread 3,5 as much. Finally, the experiment demonstrated that biological treatment can contribute to sustaining healthy environment for the plants and thus increase their yield capacity.

Key words: *identification of Bacillus, the polymerase chain reaction (PCR), sowing quality of seeds, barley disease control.*

INTRODUCTION

One of the most popular crops in Russia today is barley, which is actively involved in intensive agrarian technology. Barley covers about 8 bl ha of the territory of the country and about 180.000 ha in the Central region of Russian Federation.

Introduction of new highly productive cultivars, application of fertilisers and modern farming techniques undoubtedly account for barley high yield capacity. However, it is not enough to ensure the increase in the yield due to different diseases (Lukina et al., 2013).

The most common barley diseases today include *Alternaria* blight, Fusarium blight, Helminthosporium blight, and barley smut diseases. One of the methods of dealing with these barley diseases is the chemical one, but we should also take into account the side-effects that fungicides have on other elements of farming ecology. Chemicals produce both a direct, fungicidal and indirect effect causing changes in plant nutrient sources or environmental conditions required by a particular taxonomic group of microorganisms. In other words, they affect the entire microbial community in soil. Alternatively, we can use microorganisms, which can affect the development of pathogenic population of phylloplane and pathogenic elements in the soil. In the natural environment microorganisms live in communities based on complicated relationships of both symbiosis and antagonism. (Ponyatayev V. et al., 1999). One of the well-known species of microorganisms that has antagonistic characteristics is Bacillus. Many Bacillus-based products are used today in farming and help to reduce the effect of opportunistic pathogenic or pathogenic microorganisms. (Maraeva et al., 2015)

Thus, Bacillus subtilis, for example, is antagonistic to saccharomyces, salmonella, proteus, staphylococcus or streptococcus. Also, Bacillus influence synthesis of vitamins, amino acids as well as immune-active factors. These bacteria are involved in producing enzymes which eliminate the saprogenic, or putrefactive, products.

At present, there are quite effective microbiological Bacillus-based products used to preserve valuable micro biota and improve the level of biological soil productivity as well as sustaining the local environment.

The present study aimed at establishing how some particular biological products and autochthonous microorganisms influence the yield capacity of barley grown in the Black-Earth region of the Russian Federation.

The research objectives included: search of autochthonous **s**trains of microorganisms that improve resistance to diseases; estimation of how biological products contribute to the quality of barley seeds; estimation of how biological products affect the spread and resistance to diseases; estimation of how biological products contribute to the yield characteristics of barley.

MATERIAL AND METHODS

In 2014 – 2015, a number of micro plot tests were conducted on the territory of the Botanical garden named after B.A. Keller (the Voronezh State Agricultural University). Also, some tests were carried out on one of the local farms.

The micro plot tests included the following variants: 1. C; 2. C+B1+$N_{60}P_{60}K_{60}$; 3. C+B2+$N_{60}P_{60}K_{60}$; 4. C+B3+$N_{60}P_{60}K_{60}$; – where C is the control variant; B1 – biological product Baikal ЭМ-1; B2– biological product Phytosporin M; B3– biological product with an autochthonous **s**train of Bacillus.

The experimental design (germination was in rolls) was as follows: 1. Control - water; 2. Baikal ЭМ–1; 3. Phytosporin M; 4. autochthonous strain of Bacillus. The seeds were treated with the solution at 10 litres per ton at the seeding rate of 500 seeds/m^2.

The biological products used in the experiment were analysed in the context of the barley micro plot tests. The biological treatment was applied at different stages, namely, at the stage of seeding, tillering and booting.

A traditional PCR analysis involved DNA purification, for which we used 0,2 ml of bacterial culture (DNA-sorb developed by Gamaleya Research Institute of Epidemiology and Microbiology of RAMS, Russia). The quality of the DNA was tested by electrophoresis on agarose gel containing ethidium bromide (Gowdaman et al., 2014).

The PCR-analysis with specific primers for Bacillus spp. was conducted in the PCR thermocycler "Tercik" ("DNA-technology", Russia). The nucleotide sequences of the primers for *Bacillus sp.* were as follows: direct - 5'-TCACCAAGGCRACGATGCG-3', reverse - 5'-CGTATTCACCGCGGCATG-3', for *Bacillus subtilis*: direct (Bsub5F) - 5'-AAGTCGAGCGGACAGATGG-3 ', reverse (Bsub3R) - 5'-CCAGTTTCCAATGACCCTCCCC-3', for *Bacillus cereus*: direct (BCFomp1) - 5'-ATCGCCTCGTTGGATGACGA-3', reverse (BCRomp1) - 5'-CTGCATATCCTACCGCAGCTA-3' and *Bacillus thuringiensis*: direct (Un4d) - 5'- GCATATGATGTAGCGAAACAAGCC-3 ', reverse (Un4r) - 5'-GCGTGACATACCCATTTCCAGGTCC-3'. The amplification parameters were as follows: preliminary denaturation at 94°C for 3 minutes, followed by 40 cycles at 94°C – 30 sec, 65°C (B. subtilis), 54.5°C (B. cereus), 60°C (B. thuringiensis) – 30 sec., 72°C – 60 sec. and the final stage of elongation of the chain was at 72°C for 3 min (Guidi et al., 2010, Wattiau et al., 2001).

The microorganisms using organic nitrogen were grown by plate method on meat-and-peptone agar (MPA). The mineral nitrogen was assimilated on starch-and-ammonia agar (SAA) (Selyavkin et al., 2015).

RESULTS AND DISCUSSION

The autochthonous strain was detected in the soil samples while they were studied for ammonification. Figure 1 demonstrates the growth of the colony showing the zone of bactericidal activity in relation to other microorganisms.

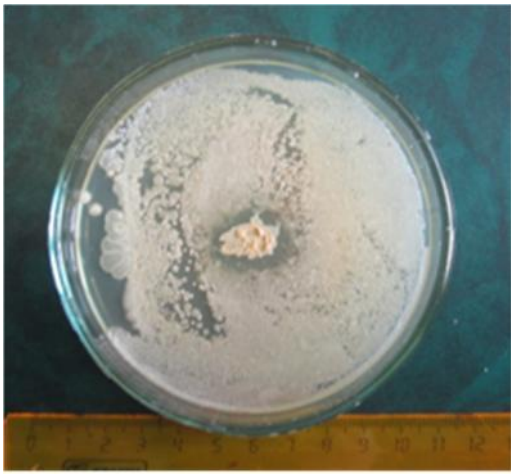

Figure 1. Mixed growth of Bacillus subtilis and Bacillus cereus colony

The strain obtained during the experiment was purified and pure culture was obtained. To define its Bacillus origin, we used the method of molecular genetic diagnostics based on using generic species-specific gen 16s pRNA.

The PCR analysis based on generic-specific primers for Bacillus sp. and the analytic agarose gel electrophoresis demonstrated the presence of the amplification product in all of the studied samples (Fig. 2). Besides, the DNA markers showed that the length of the amplicon is 1100 base pairs, which is characteristic of Bacillus and already established. (1)

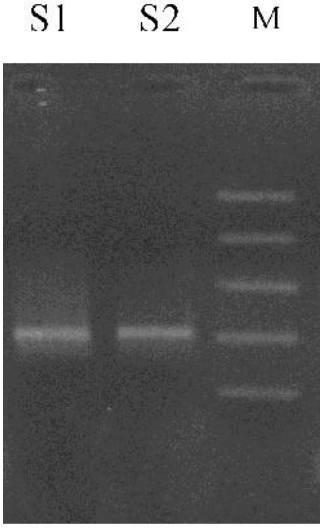

Figure 2. The results of the DNA amplification based on generic-specific primers for Bacillus sp. S1, S2, M – markers of the DNA length (base pairs): moving downwards – 5000, 3000, 2000, 1000, 500.

The results of the study demonstrate that samples S1 and S2 contain Bacillus DNA, which is proved by the presence of only one specific amplicon in both samples. In each of the samples the amplicon is 1140 base pairs long.
The amplification of the same samples with species-specific primers for Bacillus subtilis (2), Bacillus cereus (3) and Bacillus thuringiensis (4) varued among the samples. For example, samples S1 and S2 demonstrated amplification with species-specific primers for Bacillus subtilis, which proves the presence of genome DNA of this species in the sample. Also, it was found that the DNA of S1 contained the products of amplification with primers for Bacillus cereus, while S2 demonstrated the absence of PCR-products (Fig. 2). Finally, none of the samples gave amplification products with primers specific for Bacillus thuringiensis.

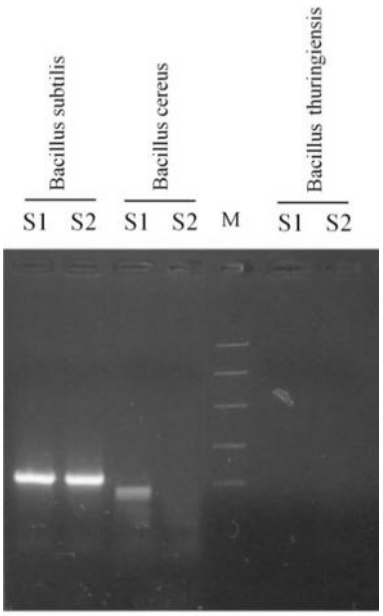

Figure 3. The results of the amplification of the DNA with species-specific primers for Bacillus subtilis, Bacillus cereus and Bacillus thuringiensis. S1, S2, M – markers of the DNA length (base pairs): moving downwards – 5000, 3000, 2000, 1000, 500.

The results obtained on the basis of using genus specific primers show the presence of DNA of Bacillus genus in S1 and S2. Species-specific analysis of S1 and S2 demonstrated the presence of Bacillus subtilis as well as Bacillus cereus. Presumably, S1 contains bacteria species, namely Bacillus subtilis and Bacillus cereus. However, neither of the studied samples revealed Bacillus thuringiensis, which was proved by using the respective species-specific primer. The obtained autochthonous strain (Bacillus subtilis, Bacillus cereus.), Bacillus S1, was used for treatment of the seeds and barley plants according to the methods described in the literature. (Lukin et al., 2015)

To define the laboratory germination, the seeds were germinated in rolls at 20 ^{0}C. Table 1 shows the results of the analysis of the rolls effect on laboratory germination and germination readiness of the barley seeds (the sort Vakula) after biological treatment.

Table 1. The effect of biological products on germination readiness and laboratory germination of barley seeds

Variants	germination readiness		laboratory germination	
	number	%	number	%
Control	46,0	92,0	46,6	93,3
Phytosporin M	47,0	93,5	47,0	94,0
Baikal EM-1	47,3	94,0	47,0	94,0
Autochthonous strain of Bacillus S1	47,0	94,4	47,0	94,0

As shown in the table, the germination readiness of the control seeds is lower than that of the biologically treated. The same is observed in regard to laboratory germination index.

While studying the germs of the seeds affected by diseases (in the rolls), spores of causal agents of helminthosporiose, fusariose, alternaria and barley smut were detected. The analysis of barley seeds resistance to diseases and disease affection was conducted in accordance with the well-established methods. Table 2 describes data on the influence that biological products have on the seeds capacity to resist diseases as well as spread of the disease.

Table 2. Index of barley seeds disease affection and capacity to resist disease

Variants	Affected seeds (%)	Disease spread (%)
Control	25,8	15,0
Phytosporin M	20,5	14,8
Baikal EM-1	22,5	14,2
Autochthonous strain of Bacillus S1	16,5	11,5

As shown in Table 2, the treatment of seeds with the autochthonous strain of Bacillus S1 helps to reduce the rate of disease affection and increases capacity to resist disease .

The control of the plants affected by barley smut was carried out visually on the basis of elimination of the affected ears (Fig.4). Table 3 demonstrates data on affection by barley smut.

Figure 4. The plant ear affected by barley smut

Table 3. Affection by barley smut

Variants	Number of ears affected by barley smut (%)
Control	3
Phytosporin M	2
Baikal EM-1	1
Autochthonous strain of Bacillus S1	1

As shown in the table, biological treatment contributes to healthy environment affecting phytopathogens. Also, the affection by barley smut reduced after application of biological products and the autochthonous strain of Bacillus S1 1,5-3 as much.

Table 4 demonstrates data on yield capacity and the mass of 1000 seeds, which are important elements of the yield formula.

Table 4. Yield capacity and the mass of 1000 seeds (dt/ha)

Variants	Yield capacity per year on average		Average per 2 years	the mass of 1000 seeds (gram)		Average per 2 years
	2014	2015		2014	2015	
Control	20.1	22.6	21.35	42.5	54.3	48.4
Phytosporin M	21.6	26.6	24.1	43.8	58.4	51.1
Baikal EM-1	24.9	28.4	26.65	44.2	58.6	51.4
Autochthonous strain of Bacillus S1	28.0	29.9	28.95	44.2	59.0	51,6
The least significant difference (0,05)	3.24					

All biologically treated variants demonstrated the increase in the barley yield, which was 3-7 dt/ha and thus provides confirmation of the data presented above.

CONCLUSIONS

1. The study of micro biota of leached chernozem revealed the autochthonous strain of Bacillus S1, which has a germicidal effect.
2. The S1 strain revealed Bacillus subtilis and Bacillus cereus, while S2 revealed only Bacillus subtilis, as detected by the method of molecular diagnostics based on using species-specific primers.
3. Biological treatment of the seeds improves the seed quality: germination readiness and germination capacity.
4. Biological treatment improves the capacity to resist disease and reduces spread of the disease. Bacillus S1, in particular, reduces the disease affection by 16,5 % and the disease spread by 3,5.
5. Biological treatment contributes to sustaining healthy environment for the plants and thus increases their yield capacity.

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