Chapter 9

Presowing with Bacteria Improved the Productivity and Resistance to Fungal Root Pathogen in Wheat and Barley

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Additional information is available at the end of the chapter

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Abstract

It is well known that reducing the extent of damage to grain crops by root rot causing agents is one of the most effective ways to increase the yield of agricultural grain crops and improve their quality. These diseases are especially harmful for hard wheat, barley, soft spring wheat, and winter rye. Yield losses due to these diseases may reach 19–20% or more for wheat and 25–30% or more for barley. In order to assess the effectiveness of the bacteria isolated from earthworm coprolites as biological control agents, we conducted a series of field tests in Western Siberia from 2011 to 2015. We compared growth and development indicators of spring wheat (*Triticum aestivum* L., Irgina variety) and barley (*Hordeum vulgare* L., Acha variety) where seeds were treated with *Bacillus cereus* and two strains of *Pseudomonas*. The results showed that the inoculation increased the grain yield by 0.2–1.0 t ha^{-1} for spring wheat and by 0.3–1.8 t ha^{-1} for barley. In addition, the prevalence of the disease in spring wheat plants was significantly reduced from 18.1–61.1% in the control plots to 6.4–50.2% in the inoculated plots. Similarly, the index of root rot development decreased from 18.2–23.0% in the control plots to 13.2–15.8% in the inoculated plots. To understand the mechanism that induces the spring wheat resistance to fungal root rots under the influence of rhizobacteria, we investigated the effect on the guaiacol-dependent peroxidase activity. There was an inverse relationship between the peroxidase activity in wheat tissues and damage of plants caused by root rot agents indicating that the response of peroxidase enzymes to plant inoculation is a meaningful indicator that can be used to assess the potential of a particular strain as a biological agent for protecting spring wheat.

Keywords: bacteria inoculation, barley, *Bipolaris sorokiniana*, *Hordeum vulgare*, peroxidase, root rot, *Triticum aestivum*, wheat

1. Introduction

It is well known that in soil and climatic conditions of the West Siberian region, reducing the extent of damage to grain crops by root rot causing agents is one of the most effective ways to increase the yield of agricultural grain crops and improve their quality. Unstable weather factors, high probability of spring frosts, high humidity, and heavy soil texture create the most favorable conditions for the rapid development of root rots caused by soil phytopathogenic fungi. A review of literature data [1] and results of annual phytosanitary inspections of grain plantings by responsible federal services indicates that the most destructive diseases of grain crops in the West Siberian region are root rots caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker and *Fusarium oxysporum* Schlecht. These diseases are especially harmful for hard wheat, barley, soft spring wheat, and winter rye. Yield losses due to the diseases may reach 19–20% or more for wheat and 25–30% or more for barley [2, 3].

The concept of "high farming culture" implies not only scientifically based and environment-friendly application of chemical fertilizers and pesticides, but also their partial replacement with biological preparations with a similar spectrum of action. Therefore, the development and use of biological preparations for plant protection is one of the key priorities of modern agrobiotechnology. Search for new strains of antagonistic bacteria that can be effectively used as biological control agents and research of antifungal mechanisms of the bacteria are both important tasks.

Long-term studies of microbiological aspects of vermiculture demonstrate that the percentage of *Pseudomonas* and *Bacillus* bacteria significantly increases in the microbial community of earthworm coprolites. These bacteria are known to be one of the most active producers of plant growth stimulants and, at the same time, antagonists of lower soil fungi. Also, the studies show an increased antifungal activity of the bacteria isolated from coprolites of *Eisenia fetida* worms compared to the strains isolated from the original organic substrate [4].

Many rhizosphere bacteria are well known to have fungistatic properties, since this feature provides bacteria with a significant trophic advantage when growing on substrates populated with a mixed (bacterial and fungal) flora. There are a number of mechanisms through which bacteria inhibit the growth of lower soil fungi, for example, competition for nutrients and production of siderophores, antibiotics, enzymes, and a number of other compounds [5–8].

A number of studies also indicate that growth-stimulating and antifungal activities of the bacteria *in vitro* usually positively correlate with the metabolic activity of bacteria in field experiments [5, 9, 10]. However, there is also abundant evidence that the metabolic activity of the bacteria revealed in the laboratory conditions cannot always be reproduced in practical application of the biological preparations based on them [11, 12]. In order to assess the effectiveness of the bacteria isolated from earthworm coprolites as biological control agents, we conducted a series of field tests from 2011 to 2015.

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2. Materials and methods

2.1. Materials

The main objects of our research were spring wheat (*Triticum aestivum* L., Irgina variety) and spring barley (*Hordeum vulgare* L., Acha variety). We compared growth and development indicators of spring wheat plants and barley whose seeds were treated with enrichment cultures of microbial strains with a titer of 10^6–10^7 cells per 1 ml. In this experiment, one *Bacillus cereus* strain and two *Pseudomonas* (*Ps*.sp.GS4 and *Ps*.sp.PhS1) strains were tested. Bacterial strains were isolated from coprolites of earthworms *Eisenia fetida*. All strains are producers of plant growth stimulants. In addition, one of the strains (PhS1) is capable of phosphate mobilization. Bacterial preparations were used at a dose of 100 ml/10 kg of grain. Seeding rates were 6.5 million seeds per hectare for wheat and 5.5 million seeds per hectare for barley. Bacteria were used both as a monoculture of each strain (separately) and as a mixed culture of all the three strains.

2.2. Field experiments

Field experiments were conducted on gray podzolic medium-loamy soil with the following physical and chemical properties: pH—5.0; humus content—4.87%; total absorbed bases—24.9 mg per 100 g of soil on a dry weight basis; and N—NH_4, N—NO_3, P_2O_5, and K_2O—2.66, 8.48, 236.5, and 99.2 mg/kg dry weight of soil on a dry weight basis, respectively. Each experiment was repeated three times in all years of the tests. The variants were arranged in a systematic way. The total area of the plot was 40 m^2. The area of the record plot was 32 m^2. In order to optimize the mineral background, mineral fertilizers were applied to the soil at a dose of $N_{45}P_{30}K_{30}$.

The effectiveness of bacterial application was assessed in terms of field germination, some morphometric parameters of plants (height and green mass of plants), content of photosynthetic pigments in leaves, the extent of damage caused by root rot causing agents, as well as grain yield, yield structure, and grain quality. Morphometric parameters of the plants were taken into account in the flowering stage, and the extent of disease damage in the tillering and early flowering stage. The total protein content in the grain was determined with an Infrared FT-10 IR spectrometer. In order to determine the content of photosynthetic pigments in the leaves in the flowering stage, we analyzed alcohol extracts from the leaves with a UV-1601 SHIMADZU spectrophotometer at wavelengths of 665, 649, and 440.5 nm, and then made calculations using the Vernon formula [13]. The statistical validity of the estimated effects was tested by the Student's test and the nonparametric Mann-Whitney test using Statistica 10.0 for Windows.

2.3. Weather and soil conditions

Throughout the field test period, there were significant fluctuations in temperature and precipitation. While the growing season of 2011 could be described as moderately warm and humid, the season of 2012 in the south of Western Siberia was characterized by extremely

unfavorable weather conditions: June was abnormally hot, with precipitation of 48% below normal. The average monthly air temperature was 5.3°C above normal. The topsoil under spring grain crops was insufficiently moistened (5–22 mm). High temperatures and lack of moisture caused the accelerated development of grain crops at different stages. The weather conditions in the growing season of 2013 were not sufficiently favorable for the growth and development of crops as well. The beginning and end of the growing season were characterized by low temperatures with frequent and heavy precipitation, while late July and early August were hot and dry. The early growing season both in 2013 and 2014 was characterized by unfavorable weather conditions for field work, growth, and development of crops: cold May and early June with frequent and heavy precipitation. The weather conditions of the entire growing period of 2015 were relatively favorable, with high total precipitation between April and September, above-normal precipitation in May, July, August, and September, and a short dry period in June.

2.4. Inoculation study in model experiments

The model experiments were based on a liquid 24-hour culture of the bacteria grown in 250 ml flasks with 100 ml of GRM broth at +28…+29°C to a titer of 1–9 × 10^9 cells/ml. The experiments were carried out in laboratory conditions. We studied the effect of seed inoculation on the peroxidase activity in the presence of phytopathogen using a model of the simplest terrestrial ecosystem consisting of four links: sand-host plant-bacterial strain-phytopathogenic fungus [12]. Seeds pretreated for 3 min with 70% ethanol were washed with sterile water and germinated in a wet chamber at +18... +20°C. After the appearance of a germ (1 mm), the seeds were inoculated with suspensions of the experimental strains for 20 min at the rate of 10^4 cells per seed. In the control variant, the seeds were soaked in distilled water. After treatment, the seeds were placed in plastic containers (1200 ml, 45 seeds per container) filled with coarse sterile river sand (800 ml), and evenly moistened with a sterile Knop's solution for hydroponic and sandy cultures: $Ca(NO_3)_2$—1.0 g/l, KH_2PO_4—0.25 g/l, $MgSO_4 \times 7H_2O$—0.125 g/l, KNO_3—0.25 g/l, $FeCl_3$—0.012 g/l.

In order to create infection background, we used agar strips with a 6-day mycelium of *Bipolaris sorokiniana* (Sacc.) Shoemaker, grown on potato-glucose agar at +22… +24°C. This micromycete is a common agent of root rots in grain crops. The *B. sorokiniana* culture was provided by employees of the Faculty of Agronomy of the Novosibirsk State Agrarian University. The agar strips with the fungus mycelium were placed in rows with the plant seeds and embedded in the sand.

2.5. Model experiment in growth chamber

The plants were grown in an environmental chamber (Growth Chamber GLK-300, Korea) under fluorescent lamps with an illumination intensity of 12 klux (200 μmol quanta/m^2 × sec, PAR) with a 16-hour photoperiod at +18… +20°C (daytime temperature), +14… +16°C (nighttime temperature), and 75% humidity. The total duration of the experiments was 12 days. At the end of the experiment, the germination capacity, plant length, peroxidase activity in the leaves and roots of the plants, as well as the prevalence of the disease and index of root rot development (disease index) were determined [14].

The guaiacol-dependent peroxidase activity was determined spectrophotometrically in the total protein extract (centrifugate) produced from wheat leaves and roots (concentration in the hydrogen peroxide reaction mixture—7.35 mM and guaiacol—0.672 mM). The enzyme activity was calculated by the Boyarkin's method [15], taking into account the molar extinction coefficient of tetraguaiacol and expressed in mM of guaiacol/(min × g of fresh weight).

3. Results and discussion

3.1. Effects on morphology of plants

The results of field experiments for different years show that the inoculation usually stimulates the growth of wheat and barley plants, which is expressed in the accelerated development at main plant development stages and a statistically significant increase in the basic morphometric parameters of plants, such as height, the number of productive stems, the number of leaves, as well as the dry green mass compared to the control variant (non-inoculated plants treated with water before planting).

In different years, the height of wheat and barley plants increased, respectively, by 8–14% and 6–40% under the influence of bacteria monocultures and by 6–16% and 5–20%, respectively, under the influence of complex inoculation. On average over all years, the flag leaf area of wheat increased by 5–6%, while the sub-flag leaf area of barley increased by 5–15%. On average over all years, the dry mass of wheat and barley plants increased, respectively, by 10–40% and 15–73%, for monoculture variants, and by 7–54% and 23–63% for the variants with complex inoculation. In general, the plants responded to the inoculation by increasing not so much their length as their green mass, which contributed to an increase in the stem thickness and, therefore, indicated an increased lodging resistance of the plants.

The analysis of the moisture content in the green mass of wheat and barley shows that the water content of the inoculated plants tends to be higher compared to the control plants, which suggests a positive effect of the bacteria on the development of the root system. Rhizobacteria are known to be capable of controlling parameters of the root system by producing a wide range of hormone-like metabolites. Therefore, they contribute to an increase in length of both main and lateral roots, thereby increasing the catchment area [16].

These physiological effects are especially important in the context of the recent climate changes that increase the risk of extreme weather events (sudden changes in air temperature and humidity, increased dry periods during vegetation, etc.). It was no coincidence that the positive effects from the inoculation of spring grain crops were most noticeable in 2012—the year marked by an extremely dry summer period. The moisture content in the inoculated wheat plants was significantly (8–10%) higher than that of the control plants. Notably, the differences with the control variant are most noticeable at the flowering and tillering stages where the need of spring wheat for soil moisture is maximal and the water consumption makes up 50–60% of the total water consumption for the entire growing season.

For barley, the differences with the control variant are also most noticeable at the tillering (booting) stage when this crop has the maximum need for soil moisture. The root system of the inoculated barley plants absorbed 18.5–28.4% more moisture compared to the control plants. Lack of soil moisture in this period is known to lead to an increased number of sterile spikelets in a barley ear, thereby significantly reducing its yield. The more noticeable response of barley to the inoculation, compared to spring wheat, may be attributed to a less developed root system of barley and, therefore, to a larger "compensatory effect" of inoculation.

3.2. Effects on physiology and productivity of plants

Stimulation of the root system with the bacteria increased the feeding area and, therefore, the total content of nitrogen and phosphorus in the green mass of both tested crops. Improvement of nitrogen and phosphorus nutrition of the plants under the influence of inoculation is noted by many researchers [17, 18]. In our studies, the complex inoculation of spring wheat increased the total nitrogen content by 10–12% and the total phosphorus content by 50–54% on average over all years. The inoculation of barley contributed to a significant increase in the total nitrogen (24–30%) and phosphorus (11–13%).

Photosynthesis processes, along with mineral nutrition processes, are known to be decisive factors of grain maturing. At the same time, optimization of mineral nutrition is known to have a positive effect on photosynthetic activity of plants [19]. It is probably for this reason that the inoculation has also contributed to a significant increase of photosynthetic pigments in wheat and barley leaves. The inoculation of wheat with the *Bacillus cereus* monoculture contributed to a significant average increase in chlorophyll *a* and *b* contents in the leaves: 19–20% and 21–23%, respectively. Application of the *Pseudomonas* sp. monoculture (PhS1 and GS4 strains) increased the chlorophyll *a* and *b* contents in the leaves by 22–27% and 31–33%, respectively. The greatest effect was demonstrated by the PhS1 strain, which was capable of phosphate mobilization. The use of the bacteria in combination with bacilli did not provide a significant additional effect: an average increase in the contents of chlorophyll *a* and *b* was 28–31% and 32–34% over the years.

The assessment of presowing seed treatment of spring wheat and barley conducted from 2011 to 2015 showed that, with rare exceptions, the inoculation in the climatic conditions of Western Siberia increased the grain yield of spring wheat by 8–37% compared to the control variant. It is equivalent to 0.2–1.0 tonnes per ha. The greatest increase was provided by the inoculation of wheat with a monoculture of *Pseudomonas* strains. In different years, the inoculation of barley increased the grain yield by 8–26%, which was equivalent to 0.3–1.8 tonnes per ha. The complex inoculation demonstrated the best results (**Table 1**).

In addition to the increased yield of the grain crops per hectare, the inoculation contributed to improving its quality. For example, in different years, the inoculation of spring wheat contributed to an increase in the protein content in the grain from 15.3–17.4% in the control variant to 16.5–17.7% in the experimental variants (**Table 1**).

The analysis of the yield structure shows that the bacteria contribute, first of all, to an increase in the productive tillering capacity and grain content. For example, on average over the years,

Variant	Research period, year							
	2011		2012	2013	2014		2015	
	Grain yield (ton/ha)	Protein content (%)	Grain yield (ton/ha)	Grain yield (ton/ha)	Grain yield (ton/ha)	Protein content (%)	Grain yield (ton/ha)	Protein content (%)
Wheat								
Control (water treatment)	2.7 ± 0.1	16.2 ± 1.2	0.9 ± 0.04	2.5 ± 0.1	3.4 ± 0.2	15.3 ± 1.4	2.3 ± 0.1	17.4 ± 1.1
Bacterization of *Bacillus cereus*	3.0 ± 0.1	16.5 ± 1.2	1.2 ± 0.1	—	—	—	—	—
Bacterization of *Ps*.sp. GS4	3.7 ± 0.2*	16.6 ± 1.3	1.0 ± 0.1	2.7 ± 0.3	—	—	—	—
Bacterization of *Ps*.sp. PhS1	—	—	1.2 ± 0.1	2.8 ± 0.3	3.6 ± 0.1	16.8 ± 0.9	3.3 ± 0.2*	17.5 ± 0.8
Complex bacterization (mixed culture)	3.1 ± 0.1*	16.9 ± 1.3	—	3.2 ± 0.2*	3.4 ± 0.03	17.0 ± 1.2	2.5 ± 0.2	17.7 ± 0.9
Barley								
Control (water treatment)	8.1 ± 0.4	8.2 ± 0.5	1.5 ± 0.2	3.9 ± 0.3	7.5 ± 0.2	11.8 ± 1.1	3.9 ± 0.2	12.5 ± 0.7
Bacterization of *Bacillus cereus*	9.5 ± 0.3*	9.3 ± 0.3*	2.4 ± 0.3	—	—	—	—	—
Bacterization of *Ps*.sp. GS4	9.7 ± 0.3*	9.1 ± 0.2	2.4 ± 0.2	4.2 ± 0.2	—	—	—	—
Bacterization of *Ps*.sp. PhS1	—	—	2.5 ± 0.1	4.3 ± 0.3	7.8 ± 0.3	11.5 ± 1.0	4.4 ± 0.3	12.6 ± 0.6
Complex bacterization (mixed culture)	9.9 ± 0.4*	9.4 ± 0.2*	—	4.3 ± 0.1	8.0 ± 0.3*	11.5 ± 1.1	4.9 ± 0.2*	12.7 ± 0.7

*The difference with the control by the Mann-Whitney test is reliable for $p < 0.5$.

Table 1. Effect of presowing treatment of spring wheat and barley seeds with bacterial strains on yield and grain quality.

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the productive tillering capacity of spring wheat increased from 1.04–1.37 in the control variant to 1.12–1.55 in the inoculated variants. The grain content of spring wheat increased from 23–26 grains per year in the control variant to 24.4–32.4 grains per year in the experimental variants. The inoculation with the monoculture of phosphate-mobilizing strain *Ps*.spPhS1 or the complex with these bacteria turned out to be the most effective solution. Weight of 1000 grains, another important indicator of the yield structure, also slightly increased under the influence of inoculation—from 27.9–34.0 g in the control variant to 29.1–35.4 g in the experimental variants.

3.3. Effects on the resistance of plants to pathogens

One of the most important factors that makes a significant contribution to grain crop yield is the development of diseases on plants, including root rots. Five-year field tests showed that the inoculation enhanced the resistance of spring wheat and barley plants to agents of root rots. Except for the dry year of 2012, the prevalence of the disease in spring wheat plants was significantly reduced—from 18.1–61.1% in the control variant to 6.4–50.2% in the inoculated variants. Severity of the disease was also significantly reduced in most cases. For example, in different years, the index of root rot development decreased from 18.2–23.0% in the control variants to 13.2–15.8% in the inoculated variants (**Table 2**).

The capability of rhizobacteria to increase the resistance of plants to phytopathogens is widely discussed in the scientific literature. However, mechanisms responsible for increasing this resistance remain understudied. Most researchers have come to the conclusion that nonpathogenic rhizobacteria can activate resistance mechanisms of plants in the same way as phytopathogens, in particular through the synthesis of PR proteins, including peroxidase [7, 20, 21].

To clarify the mechanism that induces the spring wheat resistance to the agent of *Helminthosporium* root rots under the influence of rhizobacteria, we studied the effect of inoculation of spring wheat seeds on the guaiacol-dependent peroxidase activity in the presence of artificial infection background. The main test objects were wheat plants (*Triticum aestivum* L., Iren variety, susceptible to root rots) and two *Pseudomonas* sp. (GS4 and PhS1) strains isolated from earthworm coprolites. These strains showed significant antifungal and growth-stimulating activities in laboratory conditions and confirmed this activity in field experiments [4].

It was no coincidence that microorganisms of the *Pseudomonas* genus were selected for inoculation. Researchers have paid particularly close attention to *Pseudomonas*. The capability of these bacteria to increase the resistance of a host plant to phytopathogens is demonstrated in studies by Choudhary et al. [22] Van Loon [23], and other researchers. This type of induced resistance is known as rhizobacteria-induced systemic resistance of plants [24].

Bacteria are known to have a positive effect only if they successfully colonize the rhizosphere [22, 25]. The effectiveness of wheat inoculation in our model experiment was assessed in terms of decreased development and prevalence of root rot agents in variants with and without infection background. The prevalence of the disease in the experimental variant without infection background and inoculation was at the level of 26%. In variants with artificial *B. sorokiniana*

Variant	Research period, year								
	2011	2012		2013		2014		2015	
	Prevalence of the disease (%)	Prevalence of the disease (%)	Index of disease development (%)	Prevalence of the disease (%)	Index of disease development (%)	Prevalence of the disease (%)	Index of disease development (%)	Prevalence of the disease (%)	Index of disease development (%)
Wheat									
Control (water treatment)	18.1 ± 3.4	53.1 ± 10.7	23.0 ± 3.0	51.7 ± 3.6	19.9 ± 3.8	56.4 ± 10.5	18.2 ± 3.4	61.1 ± 17.4	21.7 ± 7.1
Bacterization of *Bacillus cereus*	14.3 ± 1.2	43.3 ± 6.4	21.7 ± 3.9	—	—	—	—	—	—
Bacterization of *Ps*.sp. GS4	6.4 ± 0.8*	45.2 ± 7.1	14.7 ± 2.9*	44.4 ± 2.6*	15.8 ± 3.1*	—	—	—	—
Bacterization of *Ps*.sp. PhS1	—	50.2 ± 11.4	21.1 ± 2.1	36.7 ± 5.7*	13.2 ± 2.6*	39.4 ± 12.4	13.9 ± 1.7*	50.0 ± 17.8	15.8 ± 3.3
Complex bacterization (mixed culture)	10.0 ± 2.2*	—	—	38.3 ± 6.7*	11.2 ± 0.6*	47.2 ± 9.8	16.0 ± 2.5	26.6 ± 15.8*	9.2 ± 1.7*
Barley									
Control (water treatment)	13.9 ± 1.7	79.2 ± 17.3	45.5 ± 6.1	44.4 ± 2.9	14.7 ± 2.6	87.0 ± 11.3	41.2 ± 9.7	66.7 ± 16.8	28.3 ± 2.7
Bacterization of *Bacillus cereus*	5.8 ± 1.3*	71.5 ± 10.7	31.6 ± 2.7*	—	—	—	—	—	—
Bacterization of *Ps*.sp. GS4	11.7 ± 2.7	73.7 ± 11.0	33.7 ± 2.3*	24.4 ± 8.07*	8.3 ± 2.5*	—	—	—	—
Bacterization of *Ps*. sp. PhS1	—	61.9 ± 8.8	30.6 ± 1.8*	—	—	73.1 ± 11.6	34.3 ± 5.1*	46.6 ± 7.8*	14.2 ± 2.0*
Complex bacterization (mixed culture)	6.5 ± 1.9*	—	—	41.0 ± 7.8	13.3 ± 2.5	71 ± 6.7	30.1 ± 3.4*	57.7 ± 12.6	20.8 ± 3.7*

*The difference with the control by the Mann-Whitney test is reliable for $p < 0.5$.

Table 2. Influence of presowing treatment of spring wheat and barley seeds with bacterial strains on the infection of plants with root rot.

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infection background, there was a significant increase in the number of wheat seedlings with signs of the disease. This finding demonstrates the effectiveness of the infection background method. In case of inoculation, we observed 1.4- to 2.5-fold decrease in the prevalence of disease and disease index compared to the control variant with and without infection background, with the greatest decrease demonstrated by the inoculation with the *Pseudomonas* sp. PhS1 bacteria. The same strain provided the maximum (2- to 4-fold) decrease in the disease index compared to the control variant with and without infection load (**Figure 1**).

3.4. Effects on peroxidase activity in model experiment with an artificial infectious load

According to some authors, the peroxidase activity can be used as a marker of plant resistance to phytopathogens [26, 27]. Since roots (rather than stems and leaves) are the first to contact the phytopathogenic fungus when the plants are infected with root rots, a separate assessment of oxidative stress enzyme activity in roots and aerial parts of plants is a matter of scientific interest.

The results of the peroxidase activity analysis showed that, in general, the inoculation significantly increased the enzyme activity in wheat leaves, both with and without the *B. sorokiniana* phytopathogen in the system. The maximum increase in the peroxidase activity was induced by the *Pseudomonas* sp. PhS1 strain in the variants without infection load and by the *Pseudomonas* sp. GS4 strain with infection load. Similar effects were found by Manikandan et al. [28] after the treatment of tomato plants with a liquid *Ps. fluorescence* Pf1 culture, which significantly increased peroxidase and polyphenol oxidase activities, and inhibited the development of *Fusarium oxysporum* f. sp. *lycopersici* in plant roots. Garcia-Cristobal et al. [25] demonstrated

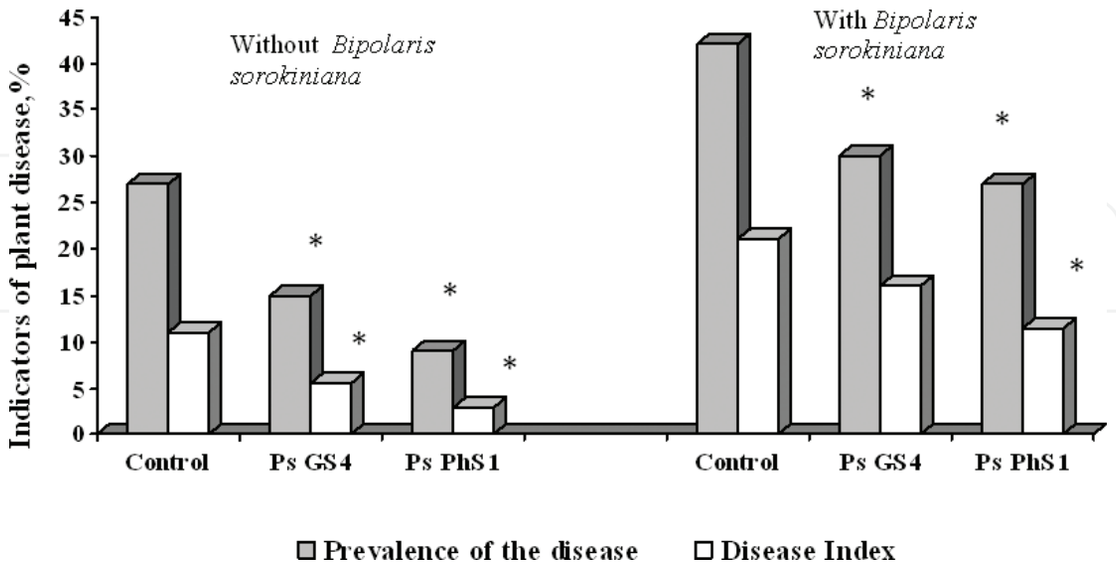

Figure 1. The prevalence of root rot and the disease index in a model experiment with the bacterization of wheat seeds with and without *Bipolaris sorokiniana.* Control—without bacterization; *Ps*.sp.GS4—bacterization of *Pseudomonas* sp. strain GS4; *Ps*.sp.PhS1—bacterization of *Pseudomonas* sp. strain PhS1, *—statistically significant difference with the corresponding control by the Mann-Whitney test ($p < 0.05$).

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an increase in germination and resistance of rice plants to *Xanthomonas campestris* after seed treatment with the *Bacillus* sp. L81 strain and, at the same time, increased peroxidase activity as early as the first 48 hours after the treatment.

In our experiment, wheat plants responded to the infection with *B. sorokiniana* by significantly (11%) increasing the peroxidase activity in their roots (**Figure 2**). The analysis of the enzyme response to plant inoculation revealed an interesting relationship: the response of the peroxidase activity to seed inoculation without a phytopathogen was insignificant (4% increase in the enzyme activity when using the *Pseudomonas* sp. PhS1 strain). The inoculation with *B. sorokiniana* increased the enzyme activity in plant roots by 14% when the seeds were treated with the *Pseudomonas* sp. GS4 strain and by more than 40% when the *Pseudomonas* sp. PhS1 was used (**Figure 2**).

A comparison of the peroxidase activity in leaves and roots of uninfected and infected plants showed that the free peroxidase activity in the plant roots was, respectively, 60 and 23% higher than that in the leaves. At the same time, the response of enzyme systems to the inoculation in the presence of the causing agent was found to be much higher in the roots than the response of free peroxidase in the leaves. For example, the inoculation of wheat seeds with *Pseudomonas* increased the peroxidase activity by 29–58% in the roots and only by 4–20% in the leaves of the infected plants (**Figure 2**). This pattern was not identified in the uninfected plants.

The correlation analysis of the data obtained in the variants without a causing agent *B. sorokiniana* revealed a direct relationship between the peroxidase activity in the roots and tissues of wheat leaves, as well as a feedback between the peroxidase activity in the leaves and roots of wheat, disease index, and prevalence of the disease, that is, the parameters reflecting the

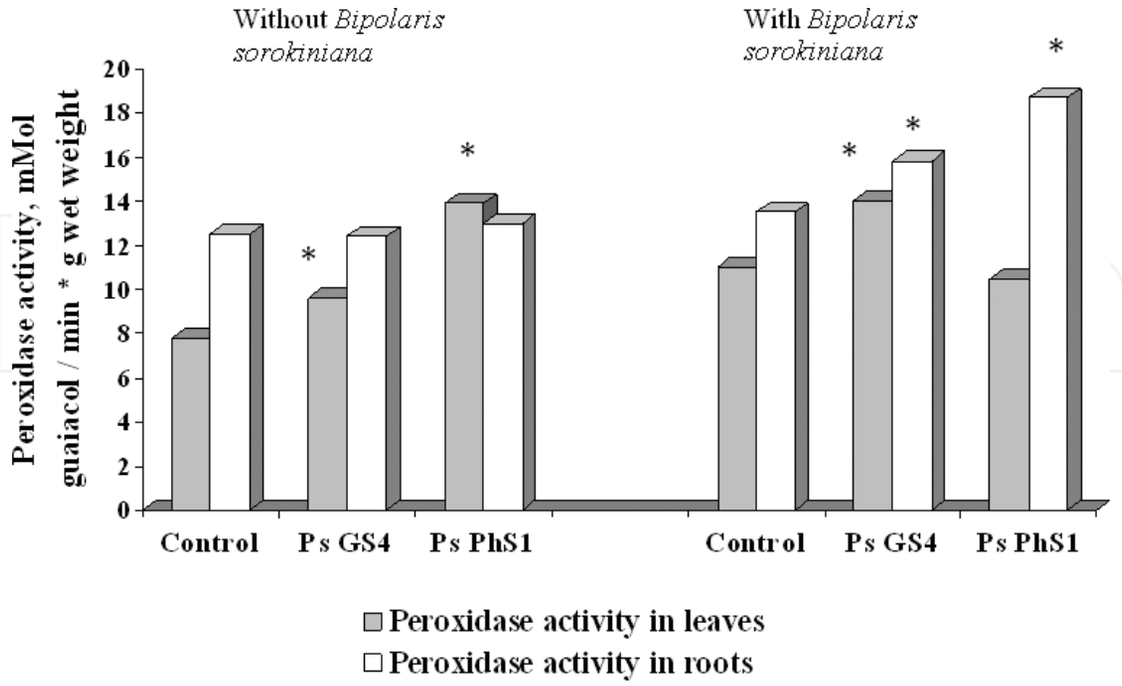

Figure 2. Peroxidase activity in wheat leaves and roots in a model experiment with seed bacterization with and without *Bipolaris sorokiniana*.

susceptibility of plants to root rots (r = −0.833 and −0.889, respectively). These findings suggest that, with increasing peroxidase activity in plant tissues, the resistance to root rot causing agents increases significantly. This confirms the available data on a relationship of peroxidase activity and content with the plant resistance to phytopathogens [25, 28–30].

The *B. sorokiniana* infection background significantly changed the relationship between the peroxidase activity in roots and tissues of wheat leaves. The coefficient of correlation between these parameters became negative and statistically insignificant (r = −0.330). This may be associated with a change in the plant's strategy in response to its contact with the phytopathogen: the peroxidase activity significantly increases in the roots and decreases in the leaf tissues. The lignification catalyzed by peroxidase is known to play an extremely important role in protecting plant tissues from phytopathogens. The resulting mechanical barrier limits the water exchange and supply of nutrients to the zone of penetration of pathogenic microorganisms [22, 31]. Since the root rot causing agents, including *B. sorokiniana*, penetrate a plant through its roots and root neck, similar rearrangement of the activity can play a decisive role in inducing the systemic resistance of plants to this group of diseases.

Thus, the experimental findings suggest that there is an inverse relationship between the peroxidase activity in wheat tissues and damage of plants caused by root rot agents, and that the response of peroxidase enzymes to plant inoculation is a meaningful indicator that can be used to assess the potential of a particular strain as a biological agent for protecting spring wheat.

4. Conclusion

The results of a series of field tests in the climatic conditions of Western Siberia showed that the inoculation of spring wheat seeds with three bacterial strains isolated from earthworm coprolites increased the grain yield of spring wheat by 0.2–1.0 t ha^{-1}. In different years, the inoculation of barley increased the grain yield by 0.3–1.8 t ha^{-1}. In addition, the inoculation contributed to improving grain quality where the inoculation of spring wheat contributed to an increase in the protein content in the grain from 15.3–17.4% in the control variant to 16.5–17.7% in the variants with bacterization. Besides, field experiments showed that the grain bacteria inoculation enhanced the resistance of spring wheat and barley plants to root rots. For example, the prevalence of the disease in spring wheat plants subjected to bacterization was reduced from 18.1–61.1 to 6.4–50.2%. Severity of the disease was also significantly reduced in most cases where the index of root rot development decreased from 18.2–23.0% in the control variants to 13.2–15.8% in the inoculated variants. The results of model experiment clarified a number of mechanisms for increasing the plants' resistance to root rots under the influence of rhizobacteria in the presence of artificial infection load (*Bipolaris sorokiniana* (Sacc.) Shoemaker). The experimental findings suggest that there is an inverse relationship between the peroxidase activity in wheat tissues and damage of plants caused by root rot agents, and that the response of peroxidase enzymes to plant inoculation is a meaningful indicator that can be used to assess the potential of a particular strain as a biological agent for protecting spring wheat.

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