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Application of Associative Rhizobacteria for Increasing the Soft Wheat Productivity and Reducing the Diseases Harmfulness

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ABSTRACT

Background: The search for environmentally friendly biological approaches to increase the productivity and resistance to phytopathogens of wheat is an urgent task for agriculture. For this purpose, the effect of plant growth-promoting rhizobacteria on yield and disease development of soft wheat was studied.

Methods: Two soft wheat varieties (Trizo and Sudarynya) were inoculated with *Bacillus subtilis* 124-11, *Pseudomonas fluorescens* SPB2137 and *Sphingomonas* sp. K1B under field conditions during 2017-2021 years. Wheat development was monitored at different stages using a set of indicators characterizing morphological traits and yield structure. Susceptibility of plants to root rot pathogens and leaf diseases (brown and yellow wheat rust, powdery mildew, septoria-pyrenophorous spotting) was analyzed using a number of phytopathological indicators.

Result: The maximal increase in yield by 1,14 g plant^{-1} and 0,87 g plant^{-1} was revealed after treatments with *B. subtilis* 124-11 and *Ps. fluorescens* SPB2137. The minimal ecological variation in wheat productivity elements was observed when *B. subtilis* 124-11 was used. The bacteria reduced plant damage caused by helminthosporiotic root rot, leaf-stem infections, yellow and brown rust and septoria-pyrenophorous spotting. The effects of bacteria on wheat growth and biocontrol of phytopathogens significantly varied depending on meteorological conditions and plant cultivar.

Key words: *Bacillus subtilis*, *Pseudomonas fluorescens*, Rhizobacteria, *Sphingomonas*, *Triticum aestivum* L., Wheat diseases, Wheat productivity, Yield structure.

INTRODUCTION

The level of cereal yield and yield quality depend on crop management system, genetic potential of cultivar and on biotic factors causing damage to plants. Such factors include fungal diseases of cereals which may cause a reduction in yield in average by 15-20% and in some cases even by 60% (Różewicz *et al*., 2021). To reduce the impact of chemical pesticides on the environment, there are relevant efforts to enhance the possibility of controlling plant diseases using environmentally friendly biocontrol agents or natural products that show pathogen control capacity (Scortichini 2022). One of the key elements of plant growth-promoting rhizobacteria (PGPR) with biocontrol properties is the synthesis of biologically active compounds: phytohormones, antibiotics, lytic enzymes, siderophores, *etc*. (Carmona-Hernandez *et al*., 2019; Sharma *et al*., 2009). A diverse group of antibiotics against phytopathogens is formed by bacteria *Bacillus subtilis*. The first antibiotics of *B. subtilis* culture fluid was subtilin (Housusright, 1948), which is a short peptide. Then lipopeptide antibiotics of several classes were isolated from various strains of *B. subtilis*: subsporins (Loeffler *et al*., 1986), bacillomycins (Peypoux *et al*., 1984), fengicins (Loeffler *et al*., 1986) and other compounds including siderophores were also identified (Hoffmeister *et al*., 2004).

In addition to antagonistic abilities regarding phytopathogenic fungi, genus *Pseudomonas* exhibit other protective properties, such as improving phosphorus nutrition for plants (Satyaprakash *et al*., 2017), synthesizing plant growth stimulators (Pham *et al*., 2017), producing siderophores (Trapet *et al*., 2016), as well as substances responsible for resistance induction against phytopathogens (Pieterse *et al*., 2014). *Pseudomonads*, like typical soil bacteria, are capable of synthesizing a whole complex of antibiotics. The most well-studied antibiotics are phenazine group (Briard *et al*., 2015), phloroglucins (Kidarsa *et al*., 2011),

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pyolyuteorin (Hu *et al*., 2005), pyrrolnitrin (Park *et al*., 2011). The mechanisms of protective action of these bacteria explained by the synthesis of various fungistatic metabolites (Strunnikova *et al*., 2007), phosphorus solubilization (Selvakumar *et al*., 2011) and activity of enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, an inhibitor of plant ethylene synthesis (Belimov *et al*., 2007).

Wheat production potential can be defined as the maximum yield obtained annually taking into account limitations of climate conditions and mitigation the negative impacts of pests and diseases under particular management (Ali *et al*., 2011). As the wheat production is under the pressure of extreme climate and the demand from enhancing world's population, the application of different crop strategies without harming the environment is one of the main goals for 21st century.

Our research purpose was to investigate the effects of PGPR (*B. subtilis* 124-11, *Pseudomonas fluorescens* SPB2137 and *Sphingomonas* sp. K1B) on the soft wheat productivity and diseases development. The scientific novelty of this work consists in a comprehensive assessment of rhizobacterial effects on the plant morphological characteristics, grain yield and resistance to the most dangerous diseases. The practical significance of the research lies in obtaining information related to developing of environmentally friendly technologies for cultivating wheat, ensuring an increase in its productivity and minimizing the costs of plant protection measures.

MATERIALS AND METHODS

Strains *B. subtilis* 124-11, *Sphingomonas* sp. K1B and *Ps. fluorescens* SPB2137 were obtained from the Russian Collection of Agricultural Microorganisms (ARRIAM, Saint-Petersburg). Seeds of wheat cultivars Trizo (k-64981) and Sudarynya (k-66407) were obtained from the N.I. Vavilov All-Russian Institute of Plant Genetic Resources» (VIR, Saint-Petersburg).

The researches were carried out on the base of Plant Protection and Quarantine Department, Saint-Petersburg State Agrarian University. Field small-plot experiments (the total area of the plot is 32 m^2) were performed at the VIR experimental station (Saint-Petersburg) and arranged on a randomized complete block designed with four replicates and plot area 1.0 m^2 as described previously (Kolesnikov *et al*., 2021). Wheat development was studied at the germ shoot (stage 3-leaves), earing-flowering and maturation using a set of indicators that characterize morphological characteristics and yield structure as previously described (Kolesnikov *et al*., 2021). Data on meteorological conditions and solar activity were provided by the Department of Agrometeorology of the VIR.

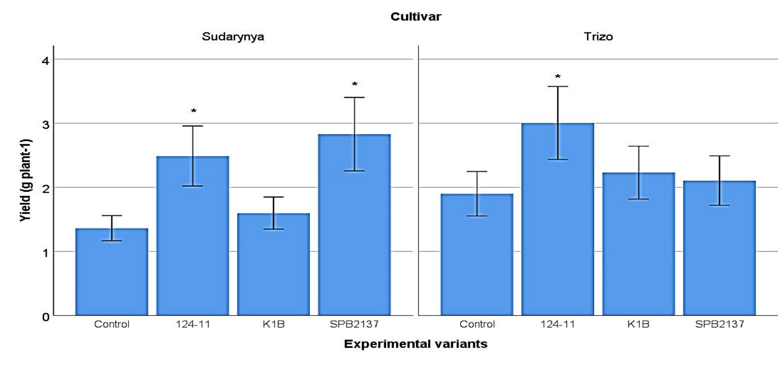
The development of root rot pathogens and leaf diseases (brown and yellow wheat rust, powdery mildew, septoria-pyrenophorous spotting) was analysed both using generally accepted phytopathological indicators (disease development, reaction type) and additionally by clarifying ones as previously described (Kolesnikov *et al*., 2021).

Statistical analysis was performed using the IBM SPSS Statistics software platform. Methods of parametric statistics (standard errors of mean, 95% confidence intervals and the Student's t-test) were used.

RESULTS AND DISCUSSION

Previously we showed that inoculation with the studied strains improved growth of wheat and protected the plants against a number of phytopatogens (Kolesnikov *et al*., 2021). Here we combined and summarized the results of field experiments applying these bacteria for 5 years (from 2017 to 2021) to estimate variation and stability of their beneficial effects on wheat plants.

Applying of bacteria on cultivars Sudarynya and Trizo on average for the period 2017-2021 caused an increase in yield ranging from 0.20 to 1.47 g $plant^{-1}$ (Fig 1). The maximal yield enhancement was observed when both cultivars were inoculated with *B. subtilis* 124-11. Significant effect of *Ps. fluorescens* SPB2137 was evident on Sudarynya. The effect of bacteria on yield of cultivars Sudarynya and Trizo depending on the year of the study is shown in (Fig 2). The maximal increase in yield was recorded in 2017 and 2019



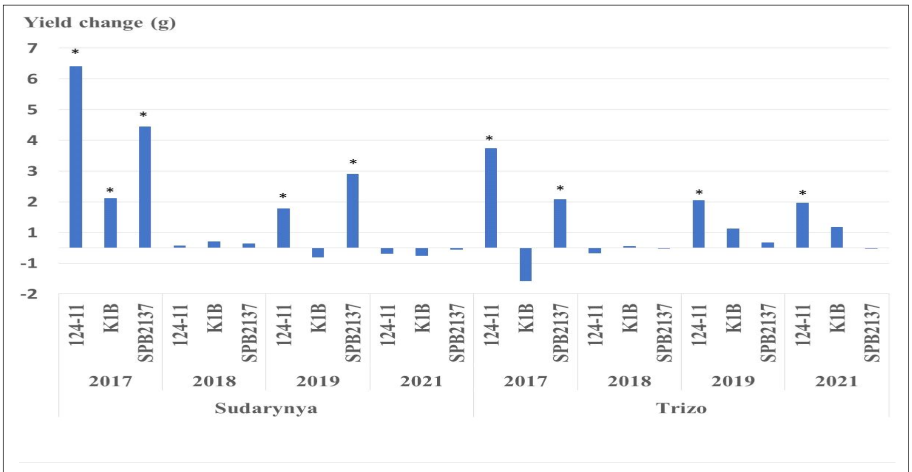
Vertical bars show standars errors. Asterisks show significant effect of rhozobacteria as compared with the control treatment according at P<0.05.

Fig 1: Mean values of the yield of cultivars Sudarynya and Trizo inoculated with rhizobacteria for period from 2017 to 2021.

when *B. subtilis* 124-11 and *Ps. fluorescens* SPB2137 were applied. Growing season 2018 and particularly season 2021 were characterized by elevated values of temperatures, but insufficient amounts of precipitation as compared to seasons 2017 and 2019 (data not shown). In addition, the number of sunspots for July 2021 was higher by 175% and 407% as compared to 2017 and 2019, respectively. As a result, minimum values of grain yield in the control treatment was registered in droughty 2021 season and only *B. subtilis* 124-11 increased the yield of cultivar Trizo (Fig 2). Application of *Sphingomonas* sp. K1B and *B. subtilis* 124-11 on cultivar Sudarynya increased the number of germinal and nodal roots by 16% and 41%, respectively (Fig 3).

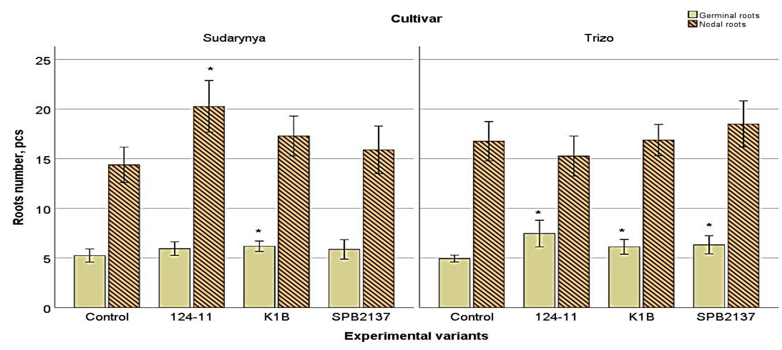
It is known that PGPR improve plant development and productivity due to the effects on the main physiological processes in plants through the production and destruction of phytohormones, production of siderophores, improving assimilation of nutrients from soil, production of antimicrobial metabolites and inducing of phytoimmunity responses (Di Benedetto *et al*., 2017; Fahad *et al*., 2015; Ghazy and El-Nahrawy, 2021; Syrova *et al*., 2022). The observed wheat growth promotion by *Ps. fluorescens* SPB2137 was probably due its ability producing auxins and possessing ACC deaminase activity, which was recently demonstrated on pea plants (Belimov *et al*., 2022). Strains *B. subtilis* 124-11 and *Sphinhomonas* sp. K1B were initially selected due to its ability for antagonism against phytopathogenic fungi and producing auxins, respectively (data not published). Further experiments are needed to establish the mechanisms of plant growth promotion by these strains.

In the control variant of the experiment (without treatment), the average long-term development of root rot was 34.8±2.8% (Sudarynya cultivar) and 42.1±4.3% (Trizo cultivar). The greatest efficiency against the development of root rot caused by the fungus *Bipolaris sorokiniana* was observed after inoculation with *Sphingomonas* sp. K1B and *Ps. fluorescens* SPB2137 (Fig 4). When the bacteria were applied for the period 2017-2021 a statistically significant



Asterisks show significant effect of rhozobacteria as compared with the control treatment according at P<0.05.

Fig 2: Yield change of wheat cultivars Sudarynya and Trizo by inoculation with rhizobacteria relative to uninoculated controls.

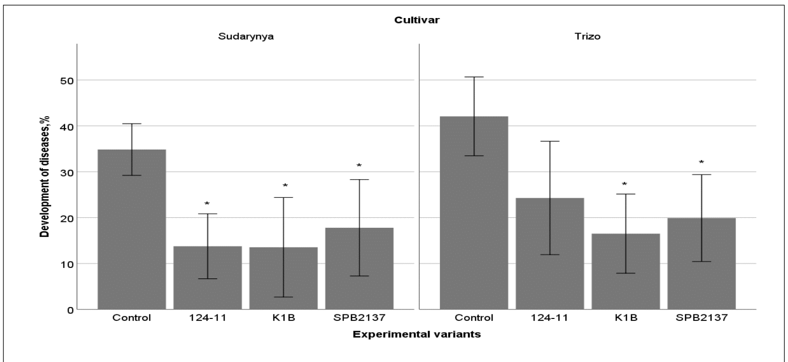


Vertical bars show standars errors. Asterisks show significant effect of rhozobacteria as compared with the control treatment according at P<0.05.

Fig 3: Roots number of wheat cultivars Sudarynya and Trizo inoculated with associative rhizobacteria in comparison with the uninoculated controls.

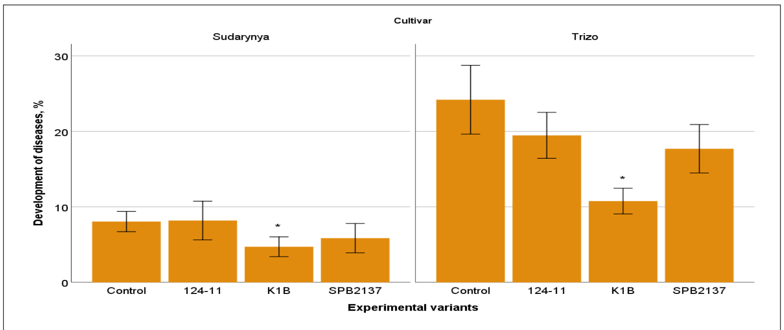
decrease in the development of this disease was registered on cultivar Sudarynya (by 61% and 49%) and on cultivar Trizo (by 61% and 53%), respectively. More intensive development of brown rust was registered in the control variant on the Trizo cultivar (2017-1.0±0.0%, 1.5±0.5 pustules per leaf; 2018-29.8±10.6%, 255.8±100.0 pustules per leaf; 2019-28.8±6.9%, 295.8±126.3 pustules per leaf; 2021-15.2±4.8%, 253.3±82.4 pustules per leaf) compared with the Sudarynya cultivar (2017-2018-6.2±4.7%, 80.0±59.7 pustules per leaf; 2019-6.9±1.9%, 48.0±20.0 pustules per leaf; 2021-12.3±3.2%, 208.5±76.8 pustules per leaf). On average, on these cultivars, the area of brown rust pustule on the flag leaves varied from 0.087 to 0.102 mm^2. A decrease in the intensity of brown list rust induced by the fungus *Puccinia triticina* Erikss. was obtained after inoculation with *Sphingomonas* sp. K1B of cultivar Sudarynya by 41% and cultivar Trizo by 56% (Fig 5). On average, on both cultivars inoculated with this strain showed decrease in the number of pustules by 85% and the values of the area of micromycete pustules by 25% (data not shown). The dynamics of the septoria-pyrenophorous spotting development in the control variant on cultivars Sudarynya and Trizo was as follows: 2017-22.3±9.5% and 18.0±6.5%, 2018 -24.3±5.6% and 14.8± 5.2%, respectively and in 2019-2021- there were no symptoms of the disease development. For the period 2017-2021. A7 significant decrease in septoria-pyrenophorosis spotting was achieved due to application of *Sphingomonas* sp. K1B and *Ps. fluorescens* SPB2137 on cultivars Sudarynya (by 54% and 80%) and Trizo (by 78% and 66%), respectively (Fig 6).

Strain *Ps. fluorescens* SPB2137 was previously characterized as a PGPR protecting barley plants against infection by *Fusarium culmorum* (Shaposhnikov *et al*., 2019). Species *Ps. fluorescens* and other species of this group of PGPR are also known as efficient biocontrol agents protecting plants against fungal and bacterial phytopathogens (Bhat *et al*., 2022; Biessy and Filion, 2021; Raio and Puopolo, 2021 and Zehra *et al*., 2021). In addition, some *Ps. fluorescens* strains also capable of producing salicylic acid leading to the expression of plant diseases resistance genes. (Syamala and Sivaji, 2017). Strain *B. subtilis* 124-11 had high antifungal activity *in vitro* against various phytopathogenic fungi (data



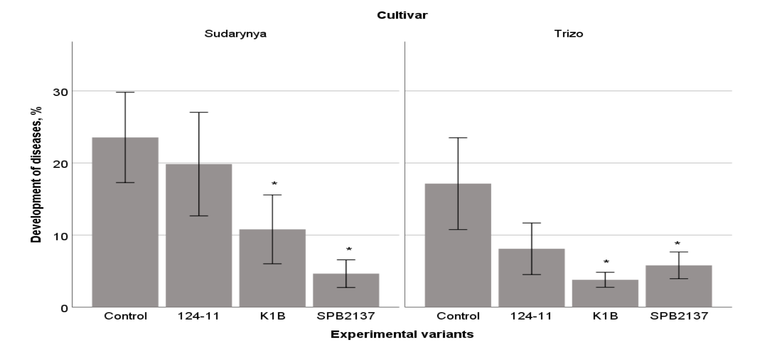
Vertical bars show standars errors. Asterisks show significant effect of rhozobacteria as compared with the control treatment according at P<0.05.

Fig 4: Development of root rot of wheat cultivars Sudarynya and Trizo inoculated with associative rhizobacteria in comparison with the uninoculated control.



Vertical bars show standars errors. Asterisks show significant effect of rhozobacteria as compared with the control treatment according at P<0.05.

Fig 5: Development of brown leaf rust of wheat cultivars Sudarynya and Trizo inoculated with associative rhizobacteria in comparison with the uninoculated control.



Vertical bars show standars errors. Asterisks show significant effect of rhozobacteria as compared with the control treatment according at P<0.05.

Fig 6: Development of septoria-pyrenophorous spotting of wheat cultivars Sudarynya and Trizo inoculated with associative rhizobacteria in comparison with the uninoculated control.

not published). The species *B. subtilis* is widely used in the biological control and induction of disease resistance in various plant species, including wheat. (Hashem 2019). Much less is known about biocontrol capability of bacteria belonging to the genus *Sphingomonas*. However, such bacteria were capable to inhibit the development of phytopathogenic micromycetes, including those causing fusarioses (*F. avenaceum, F. culmorum, F. tricinctum* and *F. graminearum*) on winter wheat (Wachowska *et al*., 2013).

CONCLUSION

The obtained results showed that application of the studied bacteria increase grain yield and promote growth of roots and vegetative parts of the inoculated wheat plants. These beneficial effects were consistent during the experimental period (from 2017 to 2021 years) with some variation depending on plant and bacteria genotypes, as well as on the environmental conditions. Biocontrol effects of the bacteria also varied depending on these factors and the species of phytopathogen also contributed to plant response to inoculation. It could be concluded that positive effects of these bacteria on plant growth and their biocontrol activity were partially reproducible in various natural conditions. The results can serve as a basis for recommending these strains for creation of biopreparations that increase yield and disease resistance of wheat.

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Declarations of interest

The authors declare no conflict of interest.

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