The impact of plant-associated rhizobacteria on germination capacity, growth and productivity of spring field mustard and emmer wheat

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Abstract. The paper reports the results of field experiments that analysed changes in germination capacity, growth indicators and productivity of two crops in response to seed inoculation: field mustard (Brassica campestris L.) of the Vostochnaya variety (k-274) and emmer wheat (Triticum dicoccum subsp. asiaticum Vavilov) of k-7516 variety. Morphometric parameters and productivity of dry mass were measured during the major stages of crop development. The seeds of field mustard were inoculated with the following bacterial products: Mizorin (Arthrobacter mysores, strain 7), Mobilin (Pseudomonas fluorescens, strain PG-5) and Flavobakterin (Flavobacterium sp., strain 30). The seeds of emmer wheat were inoculated with Pseudomonas (Pseudomonas fluorescens, strain PG-5) and Rizoagrin (Agrobacterium radiobacter, strain 204). In addition, we also tested binoculation (combined use of two bacterial products) for each crop. The study aimed to analyse changes in growth indicators and productivity of field mustard and emmer wheat in response to mono- and binoculation of seeds with plant-associated rhizobacteria. The experiments showed that the application of bacterial products and increasing doses of mineral nitrogen has a stimulating effect on plants. The study proved monoinoculation to be more effective than binoculation. This may be due to the competing effect of rhizobacterial strains from different bacterial products once the binoculated seeds are put in soil.

1. Introduction

Worldwide, modern agricultural practices are marked by intensive development often associated with chemical impact on the environment. For this reason, especially in view of the current trend towards environmentally-friendly agriculture, a special focus is given to biotechnologies [1, 2]. Among such biotechnologies are bacterial products based on plant-associated nitrogen-fixing rhizobacteria strains [3, 4]. It is known [5, 6], that apart from improving mineral nutrition, nitrogen-fixing bacteria have a
positive impact on root health. Therefore, the application of growth-promoting bacteria to the plant root zone increases plant productivity and resistance to such adverse external factors as soil drought [7]. This is especially relevant for the productivity of non-legumes, whose needs in nitrogen under favorable conditions have been shown to be provided for by almost 45% [8]. Products based on associative rhizobacteria are reported [9] to contribute to economic efficiency. They are also shown to have a positive impact on annual Brassicaceae species grown for the sake of green shoot mass used as manure and forage [10].

According to some reports, modern biotechnologies can partially [11] or even completely [12] replace chemical fertilisers. As noted in [13], responsiveness of plants depends not only on the crop species, but also on its variety. This highlights the necessity for experimental studies to confirm the effectiveness of an associative rhizobacterial strain.

At the same time, there is a dearth in research on the application techniques of bacterial products. Approaches to inoculation vary. Thus, we differentiate between seed, sprout, mixed, complex (using mycorrhizal fungi), monoinoculation (the use of one biological product) and biinoculation (combined use of two biologicals). It should be noted that the data on the combined use of different bacterial products are conflicting. Some studies [14] indicate a synergistic effect of biinoculation, while others [15] do not report similar results.

2. Materials and Methods
The study aimed to analyse changes in growth indicators and productivity of field mustard and emmer wheat in response to mono- and biinoculation of seeds with plant-associated rhizobacteria.

The study was conducted at the agrobiological research station of Herzen University using a common methodology of experimental research [16]. Field experiments were conducted on soddy podzolic sandy loams with an average content of mobile phosphorus (147 mg/kg), potassium (120 mg/kg) and humus (1.2%) [16]. The vials were stuffed with 5 kg of soddy podzolic sandy loam.

The study focused on field mustard (Brassica campestris L.) of the Vostochnaya variety (k-274) and emmer wheat (Triticum dicoccum subsp. asiaticum Vavilov) of k-7516 variety. These crops are minor for the North-West of Russia. However, they have high potential productivity of shoot mass in northern parts of non-black soil areas [17, 18]. The seeds of both species were inoculated with the following bacterial products: Mizorin (Arthrobacter myorens, strain 7), Flavobakterin (Flavobacterium sp., strain 30), Pseudomonas (Pseudomonas fluorescens, strain PG-5) and Rizoagrin (Agrobacterium radiobacter, strain 204). Bacterial products were provided by the Laboratory of Ecology of Symbiotic and Plant-Associated Rhizobacteria of the All-Russian Research Institute for Agricultural Microbiology. The seeds were mono- and biinoculated before sowing. Bacterial products were chosen in view of the earlier experiments with monoinoculation. Field mustard was inoculated with Mizorin and Flavobakterin. Emmer wheat was treated with Rizoagrin and Pseudomonas. The seeds from the control site (uninoculated) were treated with water.

Bacterial products were applied four times, each time following the indicated experimental design. We planted 20 seeds per vial. After the germination, the number of plants leveled off. The plants were watered daily. Morphometric parameters of growth and plant productivity were measured during the major stages of crop development. Statistical processing of data was carried out using the dispersion method [19].

3. Results and Discussion
The application of bacterial products in the experiments with field mustard proved to be effective already at the early stages of plant development. The obtained results showed that seed inoculation had a positive impact on germination capacity compared to the control plants (Table 1). Interestingly, both crops were more responsive to monoinoculation—it turned out to be more effective and statistically reliable than biinoculation. Field mustard showed an increase in germination capacity in response to Flavobakterin (84.3%) and Mizorin (80.0%) relative to their combined use (76.5%). Experiments with emmer wheat also returned an increase in germination capacity in response to
monoinoculation: 73.0% (Psevdomonas) and 71.0% (Rizoagrin) as compared to biinoculation (69.0%). The control plants performed more poorly showing significantly lower germination of 69.3% (field mustard) and 63.0% (emmer wheat).

**Table 1.** Changes in germination capacity and growth indicators of field mustard and emmer wheat in response to the inoculation of seeds with plant-associated rhizobacteria

| Type of impact | Germination capacity | Plant height |
|----------------|----------------------|--------------|
|                | %                    | D% cm %      |
| **Spring field mustard** |                     |              |
| Control plants | 69.3 -               | 31.5 100     |
| Mizorin        | 80.0 +16             | 42.8 136     |
| Flavobakterin  | 84.3 +22             | 43.5 138     |
| M + F<sup>a</sup> | 76.5 +10             | 33.5 106     |
| LSD<sub>05</sub> | 3.6 -                | 3.8 -        |
| **Emmer wheat** |                     |              |
| Control plants | 63.0 -               | 98.0 100     |
| Psevdomonas    | 73.0 +16             | 104.0 106    |
| Rizoagrin      | 71.0 +13             | 103.0 105    |
| P + R<sup>b</sup> | 69.0 +10             | 97.0 99      |
| LSD<sub>05</sub> | 3.9 -                | 2.0 -        |

<sup>a</sup> M + F—inoculation with Mizorin and Flavobakterin
<sup>b</sup> P + P—inoculation with Psevdomonas and Rizobakterin

Source: Compiled by the authors.

Plant height is an important growth indicator as it reveals changes in linear parameters of a plant. It is also an important structural element of shoot mass productivity. In addition, it is indicative of the intensity of physiological processes and hormonal state of plants.

Our study showed that experimental plants grew faster. Field mustard showed an increase in plant height in response to the inoculation of seeds with Flavobakterin and Mizorin—38% and 36% relative to the control plants, respectively. The application of Psevdomonas and Rizoagrin had a less pronounced yet reliable effect on emmer wheat height resulting in 6% and 5% increase against the control plants, respectively. However, the combined use of bacterial products (biinoculation) did not prove effective for either of the crops.

Leaf area is a key to effective assimilation and photosynthesis. Moreover, leaf area and a high number of leaves per plant contribute to dry shoot mass productivity. Among other elements of shoot mass, leaves have the highest value as forage.

Spring field mustard showed an increase in leaf area in response to monoinoculation with PGPR (Table 2). Compared to the control plants with the leaf area of 54.4 cm<sup>2</sup>/plant, monoinoculation of seeds with Mizorin (75.5 cm<sup>2</sup>/plant) and Flavobakterin (72.5 cm<sup>2</sup>/plant) was much more effective than their combined application (63.4 cm<sup>2</sup>/plant).

**Table 2.** Leaf area and dry shoot mass of spring field mustard and emmer wheat depending on the bacterial product

| Type of impact | Leaf area | Dry shoot mass |
|----------------|-----------|----------------|
|                | cm<sup>2</sup>/plant | % | g/vial | % |
| **Spring field mustard** |         |              |       |    |
| Control plants | 54.4 100 | 13.6 100     |
| Mizorin        | 75.5 139 | 19.9 147     |
| Flavobakterin  | 72.5 133 | 19.6 145     |
M + F^a 63.4 117 19.4 143
LSD_{05} 4.4 - 2.9 -

Emmer wheat

| Treatment         | Leaf Area (cm²/plant) |
|------------------|-----------------------|
| Control plants   | 602                   |
| Psevdomonas      | 618                   |
| Rizoagrin        | 608                   |
| + R^b            | 611                   |
| LSD_{05}         | 6.0                   |

^a M + F—inoculation with Mizorin and Flavobakterin
^b P + P—inoculation with Psevdomonas and Rizobakterin

Source: Compiled by the authors.

Relative to the control plants with the leaf area of 602 cm²/plant, significant increases in emmer wheat were noted after its monoinoculation. At the same time, pre-sowing inoculation with Psevdomonas (618 cm²/plant) was more effective than the use of Rizoagrin (608 cm²/plant). The combined use of bacterial products (biinoculation) did not have a significant effect on the leaf area of emmer wheat (611 cm²/plant).

Pre-sowing inoculation of spring field mustard resulted in higher productivity of shoot mass in all the experimental plants. The productivity of field mustard increased relative to the control plants (19.4 g/vial) after the application of Mizorin (19.9 g/vial), Flavobakterin (19.6 g/vial) and their combined use. Mono- and biinoculation in this experiment did not result in any significant difference.

Pre-sowing inoculation of emmer wheat with PGPR returned an increase in dry shoot mass: compared to the control plants with 14.4 g/vial, Rizoagrin and Psevdonomas exceeded the control indicator by 35% (19.4 g/vial) and 30% (18.7 g/vial), respectively. Biinoculation did not contribute to an increase in dry shoot mass of emmer wheat (14.0 g/vial).

4. Conclusion

The experimental study concluded that inoculation of field mustard (Brassica campestris L.) of the Vostochnaya variety (k-274) and emmer wheat (Triticum dicoccum subsp. asiaticum Vavilov) of k-7516 variety with plant-associated rhizobacteria strains stimulates plant growth and dry shoot mass productivity. In particular, the application of PGPR increased germination capacity of field mustard and emmer wheat: 22-16% and 16-13%, respectively. Plant height also showed an increase by 38-36% and 6-5% (field mustard and emmer wheat, respectively). In addition, an increase was noted in the leaf area of crops: up to 33-39% (field mustard) and up to 3% (emmer wheat). Productivity of shoot mass rose up to 47-45% (field mustard) and 35-30% (emmer wheat).

Spring field mustard benefited most from monoinoculation of seeds with Mizorin and Flavobakterin, while Psevdomonas and Rizoagrin performed best for emmer wheat. Relative to the non-inoculated control plants, the combined use of biological products did not stimulate growth of field mustard or emmer wheat. This lack of synergy may be due to the competing effect of rhizobacterial strains from different bacterial products once the biinoculated seeds are put in soil.

Anyway, bacterial products have to be chosen in view of the needs of a particular plant variety. It also means that further experiments with other varieties of spring field mustard are needed to identify the most effective rhizobacterial strains for soil and climatic conditions under study.

References
[1] Zanilov A Kh and Yakhtanigova Zh M 2016 Innovations in Agribusiness Industry: Challenges and Perspectives 1(9) 47-52
[2] Tikhonovich I A and Zavalin A A 2016 Fertility 5 28-32
[3] Ivanov A L 2016 2016 Bulletin Soil Institute of V.V. Dokuchaev 82 139-155
[4] Zavalin A A, Alferov A A and Chernova L S 2019 Agrochemistry 8 83-96
[5] Lebedev V N and Uraev G A 2015 Perm Agrarian Journal 3(11) 21-25
[6] Gupta G, Chaturvedi H, Snehi S K and Prakash A 2019 The role of plant growth promoting rhizobacteria (PGPR) for improvement of sustainable agriculture Plant Growth Promoting Microorganisms: Microbial Resources for Enhanced Agricultural Productivity (New York: Nova Science Publishers) 67-86

[7] Lebedev V N, Vorobeykov G A and Uraev G A 2021 Perm Agrarian Journal 3(35) 52-58

[8] Verma P P, Das S, Sharma P, Shelake R M and Kim J Y 2019 Microbial Interventions in Agriculture and Environment 1 281-311

[9] Uraev G A and Lebedev V N 2017 Assessment of ecological and economic risks impact on the environment of agricultural enterprises Environmental and geographical aspects of nature management, recreation and tourism sectors. Proceedings of the research and practice conf. marking the Year of Ecology in Russia 08-09 November 2017, Kurgan (Kurgan: Kurgan State University) 132-136

[10] Lebedev V N and Vorobeykov G A 2017 Transactions of the Karelian Research Centre of RAS 12 80-86

[11] Raj M, Rahul K, Lal K, Sirisha L., Chaudhary R and Patel S K 2020 International Journal of Chemical Studies 8(5) 105-110

[12] Uzma F., Chowdappa S. 2017 Agricultural Research Updates 18 187-251

[13] Ha-Tran D M, Nguyen T M, Hung S H, Huang C C and Huang E 2021 International Journal of Molecular Sciences 22(6) P1-38

[14] Basu A, Prasad P, Das S N, Kalam S, Sayyed R Z, Reddy M S and Enshasy H E 2021 Sustainability 13(3) 1-20

[15] Zavalin A A, Sokolov O A and Shmyreva N Ya 2019 Environmental Issues in Nitrogen Fixation (Saratov: ООО “Amirit”)

[16] Narusheva E A Research methods in agrochemistry: A learner’s guide (Saratov: FGBOU VPO “Saratovsky GAU”)

[17] Zhang T G, Lai J, Li P, Diao Z H, Wang J, Zheng S and Sun W C 2019 Chinese Journal of Ecology 38(1) 173-180

[18] Romanov B V, Pimonov K I and Voshedsky N N 2018 Bulletin of Don State Agrarian University 4-1(30) 28-33

[19] Lebedev V N and Uraev G A 2021 Osnovy obrabotki eksperimentalnih dannikh s ispolzovaniem tablichnogo protsessora Excel: uchebnoe posobie dlya studentov pedagogicheskikh spetsialnostey [Basics of data processing using Excel: A guideline for students on teacher training programmes] (Saint Petersburg: Herzen State Pedagogical University of Russia)