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CROP PRODUCTION

Responsiveness of *Triticum aestivum* L. Cultivars to Inoculation with Cells of Endophytic *Bacillus subtilis* Strains

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Abstract—The effect of two bacterial strains, *B. subtilis* 26D and 11VM, on three cultivars of wheat *Triticum aestivum* L., Omskaya 35, Kazakhstanskaya 10 (spring wheat), and Volzhskaya Kachestvennaya (winter wheat) was tested. The character of plants' response to endophytic inoculation depended on the strain of the microorganism, the concentration of cells in the preparation, and the wheat cultivar used in the experiment in Petri dishes. Both the strains showed a strong growth-stimulating effect when seeds were inoculated with bacterial suspensions at a concentration of 10^6 cells/mL. There was no growth-stimulating effect when seeds were inoculated with bacteria at a concentration of 10^9 cells/mL. Plants of the Omskaya 35 cultivar were most responsive to inoculation with endophytes. This cultivar was well responsive to the inoculation with bacterial cells at different concentrations. The Volzhskaya Kachestvennaya cultivar had the lowest growth stimulation. Plants of this cultivar responded well when grown in soil, unlike experiments in Petri dishes. The Kazakhstanskaya 10 cultivar was less responsive when growing plants in Petri dishes. There was no difference between the size of seedlings of inoculated and noninoculated plants of the Kazakhstanskaya 10 cultivar, and only stimulation of root growth was observed. It was concluded that there is pronounced responsiveness of wheat cultivars to the effect of endophytic strains of *B. subtilis* 26D bacteria, the basis of biofungicide (Fitosporin-M), and this should be considered when using this biofungicide for wheat cultivation.

Keywords: wheat, endophytic *B. subtilis* strains, growth, responsiveness of cultivars

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Endophytic *B. subtilis* strains are isolated from various plant species and can be used as PGPB (plant growth promoting bacteria) to stimulate growth, protect against pathogens, increase resistance of the macroorganism to adverse environmental factors, and for land reclamation [1–3]. Endophytic bacteria *B. subtilis* 26D, the basis of the Phytosporin-M biofungicide, are actively used by the BashIncom company as a part of various preparations for plant growing. Some properties of this strain, as well as those of *B. subtilis* 11VM deposited in the collection of the All-Russian Research Institute of Agricultural Microbiology (the ability to synthesize hormones, organic acids, antibiotics, etc.), were investigated. It was proven that these bacteria have growth-stimulating activity and are able to penetrate the roots and shoots of seedlings of various plants in a relatively short time (in the tissues of corn and pea seedlings, endophytic bacterial populations were found on the third day after inoculation, while that in pumpkin and beans was found on the fifth day) [4].

Bacteria *B. subtilis* strains 26D and 11VM belong to the same species, but phytohormone-like activity of the strains is different [4], and the response of crop cultivars to inoculation with their endophytes may vary. There is very little information on this subject in the literature. It is known, for example, that, out of seven studied bacterial strains of the *Bacillus* genus, S50 isolate stimulated stem growth in *Triticum durum* Desf. (cultivar Marzak (V1)), while isolate S48 promoted both lengthening of the root and increase in wet and dry mass of the plant. The greatest stimulation of the growth in Karim (V2) wheat plants was revealed upon inoculation with S35 isolate [5].

It is known that the nature of the response of plants under the action of any phytohormone as a signal molecule is determined by its concentration. In this case, the dose-response curve of the plant to the use of exogenous auxin may take the form of a two-vertex curve [6]. If the concentration of the hormone is not optimal, it enhances plant growth at lower concentrations [7, 8] and reduces it at high concentrations [8, 9]. Also, the same bacterial strain can stimulate and inhibit the growth of plants of certain species depending on the level of the hormone it synthesizes [7]. Therefore, the physiological response of plants may vary depending on the concentration of bacterial cells in the inoculum. The right choice of the PGPR strain

and the most "appropriate" host plants can improve the quality and productivity of various crops without the use of mineral fertilizers [5].

In this regard, we investigated the responsiveness of various wheat cultivars to seed treatment with cells of *B. subtilis* 26D and 11VM strains depending on the inoculum concentration, 10^5–10^9 cells in 1 mL of the preparation.

MATERIALS AND METHODS

The study was carried out using spring soft wheat *(Triticum aestivum* L.) of cultivars Omskaya 35 and Kazakhstanskaya 10 and winter wheat of the Volzhskaya Kachestvennaya cultivar. The Omskaya 35 cultivar is medium late, resistant to lodging (4.7–5.0 points), medium dry, and its growing season is 87–90 days. It is moderately susceptible to wheat leaf rust, susceptible to loose smut and highly susceptible to common bunt, stem rust, powdery mildew, and root rot. The Kazakhstanskaya 10 cultivar is early ripening, highly productive, resistant to lodging, shedding, and germination on root, and its growing season is 66–90 days. It is strongly affected by loose smut and moderately by leafy diseases. The Volzhskaya Kachestvennaya cultivar is midripening, drought tolerant, susceptible to wheat leaf rust, highly susceptible to snow mold and root rot, and its growing season is 304–348 days [10].

In the experiments, we employed two *B. subtilis* Cohn. Strains: 26D (collection of the All-Russia Research Institute of Agricultural Microbiology, VNIISKhM no. 128) and 11VM (VNIISKhM, no. 519) [11, 12]. The first strain was isolated from superficially sterilized tissues of cotton plants and the second strain was isolated from soft spring wheat [11]. In the experiments, calibrated seeds with a germination rate of at least 90% were used. Before germination, the seeds were washed in running tap water and then in freshly prepared distilled water [13]. The seeds were treated with bacteria in a laminar box. In the experiments, we used a 20-h bacterial culture grown in a shaker in meat-peptone broth at 37°C in 250-mL flasks. The cell suspension was adjusted to the required concentration with a solution of 0.01 M KCl by the optical density of the culture. Seeds were treated by shaking for 30 s in a round bottom flask at a ratio of 20 μL of the bacterial suspension/g of seeds. After treatment, the seeds were dried in an open flask for 1 h. The seeds of the control plants were treated with distilled water according to the same procedure. Before sowing, Petri dishes of various diameters along with filter paper were sterilized in an oven (GP-49-3 ZUPP, Vityaz, Belarus) for 45 min at a temperature of 120°C. Each Petri dish then received 30 seeds and freshly prepared distilled water in such a volume that the paper remained moist for 5 days during germination. Plants were grown in the dark at a 18–20°C.

When growing plants in the soil (leached chernozem), part of the soil was put into plastic vessels followed by seeds, which were covered with a 1-cm layer of the remaining soil. The soil was watered in such a way as to achieve 70% of the total field water capacity (TFWC). Plants were grown under illumination with fluorescent lamps (12 kL) and a 16-hour photoperiod for 30 days.

All experiments were carried out in three biological repetitions. Statistical processing of the results was carried out using standard programs of the Microsoft Excel package. In the tables, the data are presented as the arithmetic mean of the repetitions and the standard deviation. To identify statistically significant differences between plants inoculated and noninoculated with bacteria, Student's *t*-test was used. Differences between the control and experimental variants were evaluated as statistically significant at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Both the strains showed the strongest growth-stimulating effect when the seeds were inoculated with the bacterial suspension at a concentration of 10^6 cells/mL (Table 1). The treatment of seeds with endophytic cells at a concentration of 10^9 cells/mL was not effective for stimulating the growth of the Kazakhstanskaya 10 and Volzhskaya Kachestvennaya wheat cultivars.

Our results are consistent with literature data on the optimal concentrations of endophyte cells in the range of 10^5–10^8 cells/mL for inoculation of plants such as elephant grass (*Pennisetum purpureum* Schumach) and *Pennisetum* hybrids using bacteria of the species *Sphingomonas*, *Pantoea*, *Bacillus*, and *Enterobacter* (Li et al., 2016).

Most responsive to endophyte inoculation were plants of the Omskaya 35 cultivar. Thus, when treating seeds with bacteria at a concentration of 10^6 cells/mL, the shoot length increased by 28.6% when treated with cells of strain 26D and 40% when treated with cells of strain 11VM as compared to control plants. Growth stimulation of the above-ground part was higher than that of the roots. However, the ratio of root length to shoot length was more than one (1.0) (Table 2). The cultivar was responsive to inoculation over a wide range of cell concentrations in the bacterial suspension.

Sprouts of the Kazakhstanskaya 10 wheat cultivar responded to inoculation of seeds in a narrower concentration range: 10^5–10^8 cells/mL of strain 26D and 10^5–10^6 cells/mL of strain 11VM. The root growth stimulation in the plants of this cultivar when treating seeds at a concentration of 10^6 cells/mL was higher than that of the Omskaya 35 cultivar. When the concentration of cells of strain 11VM was more than 10^8 cells/mL, the growth of wheat seedlings of the Kazakhstanskaya 10 cultivar was inhibited. In Petri dishes, the least responsive to

Table 1. Growth of 5-day-old seedlings after seed treatment with endophytic cells (± % of this indicator in control plants)

Cultivar	Organ	Cell concentration in 1 mL				
		10^5	10^6	10^7	10^8	10^9
B. subtilis 26D						
Omskaya-35	Shoots	19.2*	28.6*	28.6*	25.0*	12.5*
	Roots	15.9*	22.9*	17.5*	17.1*	8.3*
Kazakhstanskaya 10	Shoots	−5.6	15.5*	12.7*	12.7*	0
	Roots	8.2	29.8*	27.9*	6.3	−3.4
Volzhskaya kachestvennaya	Shoots	1.4	11.4*	11.4*	−1.4	−12.9*
	Roots	0.7	8.5*	5.1	−0.7	−3.4
B. subtilis 11VM						
Omskaya-35	Shoots	28.6*	39.3*	28.6*	25.0*	0
	Roots	15.9*	17.1*	14.9*	13.0	7.3*
Kazakhstanskaya 10	Shoots	1.4	7.0*	−4.2	0	−15.5*
	Roots	23.1*	13.9*	11.5*	0	−3.8
Volzhskaya kachestvennaya	Shoots	14.3*	2.8	2.9	0	−28.6*
	Roots	5.1	10.5*	−1.4	0	−17.0*

* Differences between the indicators for plants inoculated and noninoculated with bacteria are significant at $P \le 0.05$.

Table 2. Ratio of the average root length to shoot length in 5-day-old wheat seedlings when treating seeds with endophyte cells

Cultivar	Control	Cell concentration in 1 mL				
		10^5	10^6	10^7	10^8	10^9
B. subtilis 26D						
Omskaya 35	1.12	1.10	1.07	1.06	1.05	1.08
Kazakhstanskaya 10	0.59	0.70	0.66	0.66	0.55	0.57
Volzhskaya kachestvennaya	0.84	0.84	0.82	0.80	0.85	0.93
B. subtilis 11VM						
Omskaya 35	1.12	1.02	0.95	1.00	1.01	1.20
Kazakhstanskaya 10	0.58	0.71	0.62	0.80	0.58	0.67
Volzhskaya kachestvennaya	0.84	0.77	0.90	0.81	0.84	0.98

inoculation of seeds with bacteria were winter wheat plants of the Volzhskaya Kachestvennaya cultivar; both the strains inhibited growth in seedlings when using preparations at a concentration of 10^9 cells/mL. When seeds were inoculated with cells of strain 11 VM, plant growth was inhibited several times stronger than when treated with cells of strain 26D.

It is known that damage or underdevelopment of a plant organ is compensated by increased growth of a similar organ or the emergence of new ones [14]. It is logical to assume that the root growth prevailing over the shoot growth in the seedling allows the plant to provide its water needs as the main necessary component for the further building of the organism; therefore, the ratio of the length or mass of the root/shoot towards the root prevalence can serve as one of the indicators of the plant's adaptation potential under the action of adverse factors.

The analysis of the root and shoot length ratio in wheat seedlings (Table 2) revealed that the root length to the shoot length ratio in all the cultivars increased in comparison with control plants when seeds were treated with cells of bacteria of strain 11VM at a concentration of 10^9 cells/mL and was greater than that in the strain 26D. This is consistent with the inhibition of the growth of wheat seedlings of the Kazakhstanskaya 10 and Volzhskaya Kachestvennaya cultivars when using a high concentration of bacterial cells (Table 1). Thus, these data confirm the opinion of the authors of [14] and may indicate a more "tough" effect of cells of

Table 3. Growth (cm) of 30-day-old plants in soil when treating wheat seeds with endophyte cells**

Cultivar	Control, no inoculation		*B. subtilis* 26D		*B. subtilis* 11VM	
	shoot	root	shoot	root	shoot	root
Omskaya 35	38.7 ± 0.6	46.2 ± 2.2	42.7 ± 0.8*	65.7 ± 3.9*	43.5 ± 0.5*	54.6 ± 2.9*
Volzhskaya kachestvennaya	35.4 ± 0.9	46.9 ± 0.9	38.8 ± 0.5	58.6 ± 2.6*	39.1 ± 0.8	55.4 ± 0.9*
Kazakhstanskaya 10	44.2 ± 2.5	43.3 ± 4.0	43.2 ± 2.2	51.2 ± 4.0*	44.0 ± 2.5	44.2 ± 3.8

* Differences between the indicators for plants inoculated and noninoculated with bacteria are significant at $P \leq 0.05$.
** The sum of lengths of main and lateral roots.

Table 4. Ratio of the average root length values to shoot length in 30-day-old wheat plants after inoculating seeds with *B. subtilis*

Cultivar	Control, no inoculation	*B. subtilis* 26D	*B. subtilis* 11VM
Omskaya 35	1.19	1.53	1.25
Volzhskaya kachestvennaya	1.32	1.5	1.42
Kazakhstanskaya 10	0.98	1.18	1.00

the 11VM strain, and also serve as a sign of activating the coordination reaction of physiological processes upon adverse environmental conditions, in this case, an increase in the density of bacterial cells in the inoculum.

When growing plants in soil under conditions of optimal load of bacterial cells on seeds (10^6 cells/mL), regular stimulation of seedling growth was observed (Table 3), similar to that obtained in experiments in Petri dishes. When seeds of the Omskaya 35 wheat cultivar were treated with bacterial strains 26D and 11VM, the length of shoots increased by 10–12% and that of roots by 41 and 18%, respectively, in comparison with control plants. In plants of the Volzhskaya Kachestvennaya cultivar, bacteria of strains 26D and 11VM stimulated shoot growth by 10% and root growth by 24.9 and 18%, respectively. The length of shoots of the Kazakhstanskaya 10 wheat cultivar did not significantly differ when treating seeds with bacteria from that indicator in control plants. Seed inoculation with cells of strain 26D stimulated root growth by 18% and with cells of strain 11VM by only 2%. Stimulation of root growth by inoculation with bacteria in all the cultivars was higher than that of shoots (Table 4).

In contrast to experiments with 5-day-old seedlings in Petri dishes, the Volzhskaya Kachestvennaya winter wheat cultivar, as well as the Omskaya 35 spring cultivar, responded to seed treatment by stimulating root growth, while there was almost no difference between the inoculated and control seedlings of the Kazakhstanskaya 10 cultivar in terms of the size of shoots and only the stimulation of root growth was observed. This effect may be associated with the known precocity of this cultivar. Over 30 days of growth, plants could approach developing maximum genetic organ sizes at a certain stage of ontogenesis, while late-ripening cultivars were still realizing their growth potential.

Thus, the studied wheat cultivars differ in the nature of their response to seed treatment with cells of the studied endophytic bacterial strains. The range of growth-stimulating concentrations of endophyte cells is narrower for plants of the early ripening cultivar Kazahstanskaya 10 in comparison with the midripening cultivar Omskaya 35. For an accurate assessment of the effective concentration of bacterial cells, it is better to use the experimental setup in a model close to the field one, growing plants in soil. In this case, the growth stimulation can be affected not only by the production of a phytohormone (growth regulator) by the bacterium or its induction of the synthesis of phytohormones by the plant [11] but also by the ability of the microorganism to mobilize nutrients in the soil.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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