New isolates of soybean nodule bacteria in Central Siberia

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Abstract. In a vegetation experiment, the effect of inoculation with new strains of symbiotic nitrogen fixers on the development of soybeans was assessed. The comparison was carried out with the industrial strain 634 b of the All-Russian Research Institute of Agricultural Microbiology and the autochthonous culture discovered in the Krasnoyarsk forest-steppe (Krasnoyarsk Territory). Two soybean samples were used: variety Zaryanitsa (characterized by high nodulation) and line G-23 with low nodulation capacity. Inoculation of five-day-old seedlings was carried out by immersing the roots in an aqueous solution obtained by washing the colonies from a semi-synthetic nutrient medium. The concentration of the microsymbiont is $1 \times 10^7$ cells/ml. The plants were planted in 5L pots filled with typical agrochernozem taken from soybean crops. The soil contained an average amount of mobile phosphorus, a high amount of potassium, and a low to medium amount of nitrogen. The count was carried out in the R3 phase of soybean development; for each variant, 16 plants in four vessels were analyzed. Significant differences in plant height, wet and dry weight of stem and root, maximum root length were established. In the G-23 line, the advantage was proven for strains 1–4 relative to the native culture (p < 0.003, 0.012, 0.006, 0.033), industrial drug (p < 0.002, 0.009, 0.004, 0.025), respectively. A significant difference in cultivar Zaryanitsa was obtained between strains 1, 2 and autochthonous culture (p < 0.036, 0.036), preparation 634 b and isolates 2, 3 (p < 0.012, 0.011). The maximum ranks were established for the Zaryanitsa variety for strains 1 (5.14), 2 (4.78), line G-23, strains 4 (5.4) and 3 (5.00). Consequently, new isolates of nodule bacteria are sources of increasing soybean productivity in northern agriculture.

1. Introduction

Nitrogen is the main element of mineral nutrition, which determines the value of the yield of agricultural crops, as well as the content of protein and oil. The main sources of nitrogen in crop production are mineral fertilizers, associative and symbiotic nitrogen fixation. In conditions of limited material resources, the need to reduce anthropogenic energy input to agricultural systems, the role of biologically fixed nitrogen increases. The close attention of researchers is focused on the factors of nitrogen nutrition of soybeans, as a crop that occupies first place in terms of cultivation area and distribution among legumes capable of symbiotic nitrogen fixation. The study of the effect of nitrogen-fixing nodule bacteria strains on the development of soybeans in Central Siberia is an urgent scientific task.

Numerous studies have established a high demand for soybeans in relation to nitrogen nutrition, due to the peculiarities of the chemical composition of the grain. According to the data presented in [1], it is known that 70–240 kg/ha nitrogen is biologically fixed in soybean crops. There is also a large variation in the amount of soil nitrogen consumed by soybeans. A significant number of factors influence the amount of biological and mineral nitrogen used by soybeans to form yield. These are the
level of soil fertility, the amount and uniformity of distribution of precipitation, doses, terms, rates and methods of applying mineral fertilizers, the presence of effective microsymbionts, and a number of others [2-3].

In modern practice of the soyivodship, the variety and its ability to effectively assimilate the nitrogen of fertilizers are the leading factors in obtaining high crop yields [4]. In a work summarizing research on the ratio of mineral and biological nitrogen in soybean nutrition [5], it is indicated that these processes are antagonistic. The maximum decrease in symbiotic nitrogen fixation was established in experiments in protected ground. The positive effect of inoculation on the intensity of physiological processes has been noted [6]. Seed treatment with effective local strains is a factor in increasing the productivity of soybeans and reducing the effect of abiotic and biotic stressors [7].

2. Materials and methods

2.1. Plant material

The experiment used two early maturing soybean genotypes, contrasting in their ability to form nodules when inoculated with an open autochthonous culture. Variety Zaryanitsa (originator of Omsk Agrarian Scientific Center and Krasnoyarsk State Agrarian University) - with a high nodulation rate, promising line G-23 (originator of Krasnoyarsk State Agrarian University) with a low nodule-forming ability. Soybean seeds were disinfected in a solution of ethanol (C₂H₅OH) and 15% hydrogen peroxide (H₂O₂) with a 1:1 component ratio for 1 minute. Washed with distilled water and germinated in filter paper rolls for 5 days. Normally germinated seeds with developed main and lateral roots were inoculated with strains of bradyrisobia by immersion in an aqueous solution for 2 hours with an inoculant concentration of 1 × 10⁷ cells/ml. After treatment with biological products, 6 seedlings were planted in 5-liter pots. A week after the emergence of seedlings, thinning was carried out, leaving 4 plants in a vessel. In this way for each variant, there were 4 vessels and 16 plants. After 45 days of vegetation, the formation of the symbiotic apparatus and the parameters of plant development (aboveground mass and root mass, plant height, maximum root length) were assessed. The dry weight was determined after drying for four hours at 60°C in a drying oven. The difference in dry matter mass in the control and experimental variants was used to judge the competitiveness of the strains [8]. At the time of the survey, line G-23 was in the stage of a flat bean at the first productive node; in the variety Zaryanitsa, the formation of an ovary was observed.

2.2. Data analysis

The obtained experimental data were analyzed using the STATISTICA program (version 13.3). The significance of the differences between the variants was determined by the method of analysis of variance, individual means by Duncan's rank test, the ranking of the variants was determined by the analysis of variance according to Friedman with a confidence level of P = 0.95. Correlation analysis was used to identify links between indicators. Microbial inoculant and soybean genotype were used as factors.

2.3. Soil characteristics

The soil for the experiment was selected on the experimental field of the educational and scientific center "Borsky" of the Krasnoyarsk State Agrarian University in a layer of 5-15 cm. According to the accepted classification, it belongs to typical agrochernozem, clayey-illuvial, cryogenic-mycelial on medium and heavy loams with high humus content (7.3%). The actual acidity (pH(H₂O) 6.7) is close to neutral, the content of mobile forms of phosphorus (P₂O₅) is average (190 mg/kg-1), potassium (K₂O) is high (230 mg/kg-1), nitrogen (NH₄+, NO₃-) from low to medium (17-18 mg/kg-1). To assess the competitiveness of the studied strains to the indigenous culture of the microsymbiont, we used soil from a plot containing a spontaneous nodule culture from a previous experiment with soybeans.

2.4. Bacterial strains

The industrial strain of bradyrizzobia 634 was obtained from the All-Russian Research Institute of Agricultural Microbiology. New isolates of nodule bacteria selected from the autochthonous culture (4 strains in total) were cultivated on a mineral-plant medium of the following composition, g/l: KH₂PO₄ – 0.5; K₂HPO₄ – 0.5; MgSO₄ – 0.1; CaSO₄ – 0.1; NaCl – 0.2; (NH₄)₆Mo₇O₂₄ - traces, mannitol – 20;
soy flour – 10; agar - 20 [8]. To obtain a microbiological fertilizer, after ten days of cultivation, the colonies were washed off with distilled water. To determine the number of bacteria, the washings were microscoped, the amount of microsymbiont in the inoculant, if necessary, was brought to the specified value (1 × 10^7 cells/ml). The comparison options were the treatment with the industrial strain 634 and the native crop isolated from the agrochernozem of the Krasnoyarsk forest-steppe in 2019.

3. Results
It is believed that with mixed inoculation, the accumulation of dry matter proceeds more intensively in variants with treatment with active strains. It was found that the open native nodule culture is characterized by a low symbiotic efficiency, without exerting a significant effect on the development parameters of soybean plants (leaf area, number of beans, plant height). The effectiveness of inoculation with a drug based on a spontaneous symbiotic culture and an industrial drug are presented in [9]. At the same time, autochthonous symbiotic microorganisms are able to suppress the activity of breeding strains, reducing the number of nodules forming, which leads to a decrease in the amount of biomass formed by the macrosymbiont [8].

According to the results of the analysis of the parameters of plant development, a significant difference in genotypes was established in plant height (p < 0.001), maximum root length (p < 0.001), number of formed nodules (p < 0.07), wet (p < 0.02) and dry weight root (p < 0.09), aboveground mass before (p < 0.09) and after drying (p < 0.06). The G-23 line in terms of the observed parameters, with the exception of the root length, surpasses the Zaryanitsa variety (table 1).

**Table 1.** Average values of the development parameters of soybean plants depending on the inoculation options.

| Option        | Plant height, cm | Maximum root length, cm | Weight, g | Number of nodules, pieces/root |
|---------------|------------------|-------------------------|-----------|-------------------------------|
|               |                  |                         | stalk raw, g | stalk dry, g | root raw, g | root dry, g |                   |
| grade Zaryanitsa |                  |                         |           |                 |             |             |                   |
| the control   | 19.3             | 32.6                    | 4.1       | 0.9             | 0.9         | 0.5         | 7.3             |
| strain 1      | 22.3             | 43.3                    | 8.9       | 2.0             | 3.5         | 1.3         | 16.3            |
| strain 2      | 21.3             | 43                      | 9.2       | 2.1             | 2.8         | 1.1         | 16.6            |
| strain 3      | 21               | 52                      | 7.7       | 1.6             | 2.7         | 1.02        | 12.6            |
| strain 4      | 21.3             | 53                      | 9.2       | 1.97            | 2.6         | 1.3         | 13.3            |
| strain 634 b  | 16.7             | 32.3                    | 2.2       | 0.5             | 0.7         | 0.3         | 5               |
| Line G-23     |                  |                         |           |                 |             |             |                   |
| the control   | 24               | 31                      | 3.1       | 0.5             | 0.9         | 0.2         | 5               |
| strain 1      | 25               | 36.3                    | 9.5       | 2.2             | 2.6         | 0.9         | 21.2            |
| strain 2      | 32.5             | 33.3                    | 7.9       | 1.8             | 2.7         | 1.1         | 18.7            |
| strain 3      | 29               | 37.3                    | 13.6      | 3.5             | 3.7         | 1.5         | 20              |
| strain 4      | 32.7             | 51.3                    | 13.6      | 3.1             | 4.4         | 1.8         | 16.6            |
| strain 634 b  | 16.7             | 27.3                    | 6.2       | 1.4             | 1.3         | 0.5         | 6.6             |

Line G-23 is distinguished by a higher maturity, being at the stage of a flat bean at the first productive node by the time of counting. Ovary formation was observed in the Zaryanitsa variety.

Plants of the studied samples upon inoculation with experimental strains formed a larger biomass in comparison with the control and the treatment with the industrial strain 634 b. The variant with the 634 b strain, which is characterized by minimal competitiveness under the prevailing conditions, had the minimum values for the studied parameters. Using the Duncan test for the G-23 line, significant differences were established between the new isolates with the control and with the 634 b strain. The differences between the industrial preparation and the primary culture are unreliable. In cultivar
Zaryanitsa, a significant difference was found for strains 1 and 2 with control and preparation 634 b. No significant differences were found between the experimental strains.

4. Discussion

By the method of Friedman's rank analysis of variance, a statistically significant difference \((p < 0.00005)\) of the options was established for the mean values of the features. The concordance coefficient \((W_k = 0.774)\) shows a significant closeness of the relationship between the investigated factors. The distribution of variants by average rank values is shown in Table 2.

**Table 2. Ranking of experimental soybean inoculation variants.**

| Inoculation option | Rank   | Sum of ranks | Average value | Standard deviation |
|--------------------|--------|--------------|---------------|--------------------|
| strain 4           | 5.357143 | 37.5         | 16.17143      | 18.24026           |
| strain 3           | 4.785714 | 33.5         | 14.80976      | 15.72034           |
| strain 1           | 4.285714 | 28           | 13.96796      | 14.22294           |
| strain 2           | 3.857143 | 27           | 13.89898      | 14.25841           |
| 634 b              | 1.571429 | 11           | 8.42531       | 10.99309           |
| the control        | 1.428571 | 10           | 9.32429       | 12.42789           |

When using the average values of variants by genotypes, a significant difference \((p < 0.00003)\) between the strains was noted. The degree of influence of drugs on the manifestation of signs in the studied samples is different. The maximum effect in the variety Zaryanitsa was obtained when treated with strains 1 and 2, lines G-23 3 and 4 (Table 3).

**Table 3. Comparison of ranks of inoculation variants in Siberian soybean samples.**

| Inoculation option | Zaryanitsa | G-23 |
|--------------------|------------|------|
| the control        | 2          | 1.285714 |
| strain 1           | 5.142857   | 3.857143 |
| strain 2           | 4.785714   | 3.714286 |
| strain 3           | 3.428571   | 5     |
| strain 4           | 4.642857   | 5.428571 |
| 634 b              | 1          | 1.714286 |
| Wk                 | 0.812      | 0.813 |

There is no statistically significant correlation between the ranks by genotypes, which confirms the maximum efficiency of inoculation of Zaryanitsa cultivar with strains 1 and 2, line G-23 with strains 3 and 4. Experimental preparations have a greater positive effect on soybean samples in comparison with industrial culture 634 b and autochthonous symbiotic culture, showing a high relative competitiveness to native nitrogen fixers. For the selection of effectively interacting macro- and microsymbionts, it is advisable to use the number of nodules and the raw mass of the root. The multiple regression equation describing this interaction is as follows: number of nodules = 3.652 + 4.115 × root wet weight. The coefficient of determination \(R^2 = 0.517\), the significance of the regression \(p < 0.00000\).

The efficiency of soybean nodulation is influenced by a number of factors, among which the temperature regime of the soil plays a significant role in the northern cultivation regions. In the study area, the soil temperature in the warmest month of July in the 0–20 cm layer was in the range of 16–18°C. On the example of beans \((Vicia faba)\) in \([10]\) and soybeans \([11]\), a decrease in the efficiency of symbiotic nitrogen fixation at low soil temperatures was shown. In this case, the screening of
microsymbionts tolerant to low soil temperatures can be an effective source of increasing the efficiency of nitrogen fixation.

The development parameters of the soybean root system can be used to assess the responsiveness of cultivars to intensification of cultivation. The Zaryanitsa variety is characterized by a resource-intensive ecological strategy, which presupposes a high responsiveness to the anthropogenic contribution of energy to the agrocnosis. Similar results were obtained when determining the plasticity and stability of the genotype in terms of yield, as shown in the study [12-13], indicates the development of plants for resource conservation, the variety is classified as an intensive one [12]. Less intensive development of the root system along the path. This strategy, in our opinion, is inherent in the G-23 line, which is characterized by the greatest early maturity in Central Siberia. This is confirmed by the absence of a relationship between the number of nodules and the maximum root length \( r = 0.279 \pm 0.325, p < 0.05 \) in the studied genotypes.

5. Conclusion

As a result of the experiment, a positive relationship was found between the number of formed nodules and the estimated development parameters of Siberian soybean samples (correlation coefficients 0.554–0.719, \( r \pm 0.325, p < 0.05 \)). New strains of symbiotic nitrogen fixers isolated from an open nodule culture can be used in the future as an important resource allowing to increase the proportion of symbiotically fixed nitrogen and increase productivity in the region.

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