

# Effectiveness of Soybean Inoculation with Indigenous Symbiotic Culture in the Yenisei Siberia

Sergey Khizhnyak  
Institute of Agroecological Technologies  
Krasnoyarsk State Agrarian University  
Krasnoyarsk, Russia  
skhizhnyak@yandex.ru

Andrei Churakov  
Institute of Agroecological Technologies  
Krasnoyarsk State Agrarian University  
Krasnoyarsk, Russia  
a-tjn@ya.ru

**Abstract**— Soybean inoculation aimed at increasing yield and optimizing nitrogen nutrition is a widely used method. However, it is not always possible to achieve a positive effect; this fact forces us to search for compatible strains and varieties, appropriate methods of using agents. Indigenous nodule bacteria (named Spontaneous by the authors) isolated in 2018 in the Krasnoyarsk Territory were tested in the field in 2019 in comparison with an industrial preparation based on strain 634 of the All-Russian Research Institute of Agricultural Microbiology. This study was carried out on the basis of Krasnoyarsk State Agrarian University in Krasnoyarsk forest-steppe (56° 26' 15" N, 92° 54' 11" E). Treatment with Spontaneous preparation significantly ( $F = 126.34$ ;  $p = 0.0013$ ) increases nodulation. The average number of nodules by varieties in the control variant (treatment with distilled water) was 10 pcs/root; when treated with strain 634 – 19 pcs; when treated with Spontaneous preparation – 86 pcs. The greatest effect of inoculation by the number of nodules was obtained in Zaryanitsa variety (44 pcs per plant according to the average value of experiment), with this parameter of Eos variety amounting to 33 pcs. The number of beans in Zaryanitsa variety increased by 1.4 times when treated with Spontaneous preparation and 2 times when treated with strain 634. Increase in EOS variety was obtained only during treatment with strain 634. Treatment with industrial preparation leads to a significant increase in the photosynthetic apparatus ( $F = 6.064$ ;  $p = 0, 0063$ ); for the Spontaneous strain, this effect is not statistically proven. Increase in the number of nodules per plant does not lead to the increase in leaves area, the number and weight of beans. Weight of one nodule is crucial for all these parameters. The resulting indigenous microorganisms are of interest as sources of increased nodulation and adaptability of soybeans to the harsh conditions of Siberia.

**Keywords**—nodulation, *Glycine max* (L.) Merr., *Bradyrhizobium*, symbiosis, nitrogen fixation.

## I. INTRODUCTION

Soybean being the main source of vegetable protein and oil in the world [1; 2] attracts increasing attention of Siberian producers. Only in the Krasnoyarsk Territory with its limited bioclimatic potential, the area under soybean over the past three years has increased 4 times now reaching 9 thousand hectares. Constraints for the development of soybean production in this region are unstable yield (200–1,100 kg/ha), and large amount of nitrogen required for crop formation. Soybean as a bean culture is able to satisfy up to 50% of the need for nitrogen due to symbiotic nitrogen

fixation. Absence of specific soybean microsymbionts in Siberian soils, the sensitivity of industrial microbiological preparations to the weather, soil conditions and applied pesticides – all this requires additional developments aimed at improving nitrogen nutrition of this culture. The problems identified allowed defining the goal of this study: evaluating the effectiveness of symbiotic nitrogen fixation of soybeans using industrial and isolated inoculants.

## II. LITERATURE REVIEW

Nitrogen as a biogenic element is an integral part of plant proteins, nucleic acids, enzymes and provides plant growth. On a global scale, biological productivity on our planet is determined mainly by the level of molecular nitrogen fixation. In agricultural ecosystems, mineral fertilizers are also the source of nitrogen, but their production is energy-intensive and their use – cost demanding. Biologization of agriculture provides for the creation of effective agricultural systems based on the interaction of symbiotic and associative microorganisms and cultivated plants. In this case, a special role should be given to the development of stable interactions of legumes and microsymbionts as it is the most effective system of nitrogen fixation. A close study of the genetics of symbiotic signs was started in the last decades of the XX century [3; 4; 5]. It was found that the formation of an effective bean-rhizobium symbiosis system is controlled by a complex of genes that determine virulence and nitrogen fixation activity [6].

The effect of horizontal gene transfer that takes place both within a species and between species, genera, or families is shown in [7]. This process can lead to the appearance of strains with different plant specificity and unequal nitrogen fixation efficiency [8].

For areas with short growing season, such as Eastern Siberia, activation of symbiotic nitrogen fixation is an urgent task; its solution will lead to an increase in soybean yield. The relationship between the duration of reproductive period and nitrogen fixation intensity may become an additional obstacle to it [2]. Increased proportion of symbiotic nitrogen in soybean crop can be achieved by screening indigenous strains of microsymbionts which appear in the course of prolonged cultivation of soybean in the Krasnoyarsk Territory.

## III. MATERIALS AND METHODS

Within this study, laboratory and field experiments were conducted in 2019 at the base of Krasnoyarsk State Agrarian

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University (Krasnoyarsk SAU), Krasnoyarsk Territory. Indigenous population of microsymbionts was isolated by the authors in soybean reseedings from breeding nurseries in 2018. Pure cultures were obtained using liquid medium with mineral base according to [9], yeast extract, mannitol according to [10] and Sabouraud agar growing media 50%, Czapek agar medium with 20 g/L of glucose and 2.5 g/L of yeast autolysate.

Field tests were performed at Borsky Academic and Research Center located in Krasnoyarsk forest-steppe (56°26'15"N, 92°54'11"E) in accordance with the recommendations [11]. Soybean varieties were sown in the plot where no inoculation of the culture during fallow row crop rotation performed; the predecessor was potatoes. The soil of the plot was leached agricultural chernozem, heavy loamy, the depth of arable horizon was 26 cm, with high humus content (7.3%). Reaction of medium is neutral (pH<sub>H2O</sub> 6.7), the content of mobile forms of phosphorus (P<sub>2</sub>O<sub>5</sub> 190 mg/kg) and nitrate nitrogen (N-NO<sub>3</sub> 18 mg/kg) was medium; of potassium – high (K<sub>2</sub>O 230 mg/kg); of ammonium nitrogen (N-NH<sub>4</sub> 17 mg/kg) – low. In a field study, the industrial strain *Bradyrhizobium japonicum* 634 (from the collection of the All-Russian Research Institute of Agricultural Microbiology) and the indigenous culture of symbiotic nitrogen fixers, called Spontaneous by the authors, were used to inoculate early varieties of soybeans (*Glycine max* L. Merrill): Zaryanitsa (Omsk Agrarian Scientific Center, Krasnoyarsk State Agrarian University) and Eos (Krasnoyarsk State Agrarian University). Location of variants was randomized (control part – treatment with distilled water, inoculation with industrial strain and indigenous culture) in triplicate; total plot area was 6.7 m<sup>2</sup>. Sowing was carried out on May 22 by the routine method using SSFK 7 selection seeder, soil temperature at the depth of seed placement was 9°C. Seed application rate was 80 seeds per m<sup>2</sup> adjusted for sowing suitability. Indigenous strains of microsymbiont isolated into a pure culture showed the highest growth rate in Sabouraud medium 50%. Cumulative passages from this medium into liquid one were used to inoculate seeds in the field. Inoculum dose was 1 × 10<sup>7</sup> cells/mL. The effectiveness of method under consideration was evaluated at R5 stage of soybean development (seeds with the diameter of 3 mm on one of the four upper tiers of plant). Nodulation, leaves area and the number of beans were evaluated in duplicate; a soil sample of 20 × 20 × 20 cm in size was taken on August 10 according to the method [12]; the number of analyzed plants in replication

was 4-5 pcs. Results were processed using StatSoft STATISTICA 6.0 software.

**IV. RESULTS**

The period from May to September 2019 was excessively warm. The maximum excess of normal temperature amounting to 3.5 °C and 4 °C was recorded in June and August, respectively. The warmest month was July; average daily temperature was 19.5 °C. Amount of precipitation in May–September exceeded the long-term normal range by 112% reaching a value of 275 mm. Moreover, the distribution of precipitation was extremely uneven. Lack of precipitation was observed in May and July, in August a slight excess of climatic norm was noted. In June, precipitation was 1.7 times higher than normal. However, 78% of precipitation (60 mm) during this month fell on June 24-25. Lack of precipitation delayed the emergence of seedlings for 1 day, and the beginning of flowering for 3 days compared with the timing of the start of these phases during previous three years of observation. Seed ripening occurred 5 days later. There was no difference in terms of soybean development between inoculation and control variants.

Development of soybean symbiotic apparatus depended on the inoculant used (Table 1). Isolated indigenous culture of Spontaneous microsymbiont was the most virulent (F = 126.34, p = 0.00127). Using of new isolate of nitrogen-fixing bacteria led to a four-fold increase in the number of nodules compared to control in Eos variety and 26-fold in Zaryanitsa. In the variant with treatment using industrial preparation, a six-fold increase in nodulation was obtained in Zaryanitsa variety, a 24% reduction was registered in Eos variety. According to the averaged weight of nodules per root, both inoculation variants exceeded the control; the largest increase was obtained with Spontaneous preparation based on indigenous culture. The nature of nodule arrangement on soybean roots also differs. Industrial strain 634 is characterized by the formation of nodules near the central root, while the nodules formed by indigenous strain were diffusely located on the main and lateral roots. The degree of nodule development in control part indicates not only the presence of the corresponding autochthones in soil, but also their low activity. Positive effect of inoculation on the development of photosynthetic apparatus was established. Maximal positive changes were found under inoculation with an industrial strain, however, the reaction of varieties to studied variants was different; this fact confirms the results of previous studies [13].

TABLE I. EFFECT OF INOCULATION ON NODULATION AND MORPHOMETRIC CHARACTERISTICS OF SOYBEAN VARIETIES (PER PLANT)

Variant	Number of leaves, pcs	Leaves area, cm <sup>2</sup>	Number of beans, pcs	Number of nodules, pcs	Weight of one nodule, g		Weight of nodules per root, g
					wet	dry	
<i>Eos</i>							
control	11	407	21	17	0.043	0.0099	0.731
indigenous	10	305	14	68	0.030	0.0091	2.040
634	14	710	34	13	0.062	0.0135	0.806
<i>Zaryanitsa</i>							
control	9	251	13	4	0.038	0.0115	0.152
indigenous	9	275	19	104	0.016	0.0054	1.664
634	14	587	26	24	0.052	0.0130	1.248

Response of plants to inoculation with Spontaneous was lower in comparison with strain 634 Taking into account the number of beans per plant, the effect of inoculation with an

indigenous preparation averaged 113% between varieties, and with industrial one – 203%.

Discussion. Significant competitive advantage of the industrial strain was obtained by the number of leaves ( $F = 3.343$ ;  $p = 0.048$ ) and leaves area ( $F = 6.064$ ;  $p = 0.0063$ ), weight ( $F = 8.474$ ;  $p = 0.0583$ ) and the number of beans ( $F = 6.5181$ ;  $p = 0.0313$ ) per plant (Fig. 1). A small number of nodules formed under treatment with strain 634 may indicate the inhibition of microsymbiont development by soybeans what is confirmed in [14]. Significant number of nodules is formed upon inoculation with preparation based on the indigenous culture of Spontaneous microsymbionts (Fig. 2). The significance level of effect was  $F = 126.34$ ;  $p = 0.0013$ . However, nodules formed by Spontaneous strain were relatively small in size. The observed increase in photosynthetic apparatus and the number of reproductive organs is not mathematically proven.

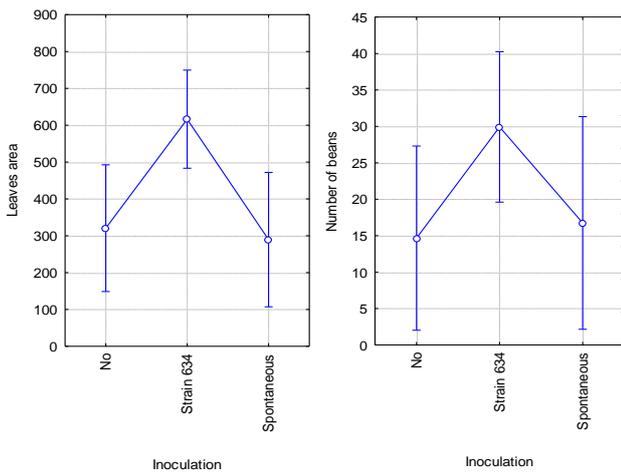


Fig. 1. The effect of inoculation on leaves area and the number of beans per plant. Vertical bars denote 0.95 confidence intervals

Positive effect of inoculation with industrial preparation and indigenous Spontaneous culture is shown in increased weight of leaves and stems. However, the statistical significance of effect is not confirmed ( $F = 0.452$ ;  $p = 0.673$ ;  $F = 0.275$ ;  $p = 0.777$ , respectively). Maximal nodulation obtained by inoculation of Zaryanitsa variety with indigenous culture is confirmed statistically; the level of significance of this effect was  $F = 11.493$ ;  $p = 0.043$  (Fig. 2). Zaryanitsa variety is characterized by a significantly greater susceptibility to inoculation with Spontaneous strain.

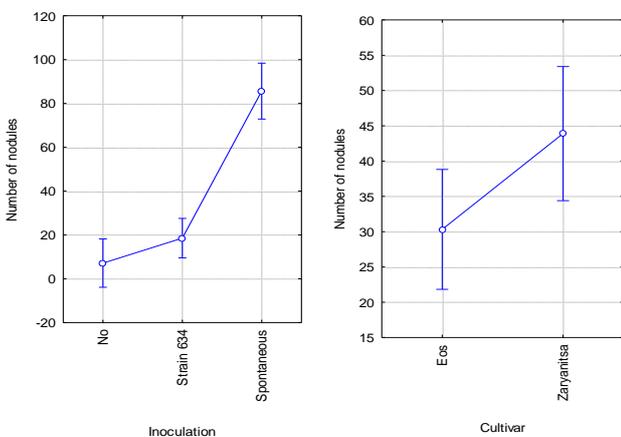


Fig. 2. Effect of inoculation on soybean nodulation. Vertical bars denote 0.95 confidence intervals

Calculation of correlation coefficients showed a high positive relationship between the size of the photosynthetic apparatus (the number of leaves, their area and weight) of the studied varieties and productivity elements (weight of stem, beans and the number of beans per plant). No reliable relationship was found between the number of nodules and mentioned parameters. However, a direct strong relationship was established between such parameters as wet and dry weight of one nodule, photosynthetic apparatus, and elements of crop structure. To assess the relationships between the studied variables, a factor analysis and varimax rotation of factors were performed (Table 2). The first group (Factor 1) includes the number, weight and area of leaves, weight of stems, beans, the number of beans, wet and dry weight of one nodule, area and weight of one leaf. The second “linkage group” (Factor 2) includes the number of nodules per plant, wet and dry weight, and moisture content of a nodule.

TABLE II. FACTOR ANALYSIS (VARIMAX) OF THE SET OF PARAMETERS PER PLANT,  $p > 0.700$

Parameter	Factor 1	Factor 2
Number of leaves	<b>0.912</b>	-0.095
Weight of leaves	<b>0.984</b>	-0.072
Weight of stems	<b>0.982</b>	-0.070
Weight of beans	<b>0.977</b>	0.066
Number of beans	<b>0.974</b>	0.049
Number of nodules	-0.276	<b>0.909</b>
Wet weight of nodules	0.148	<b>0.939</b>
Dry weight of nodules	-0.030	<b>0.979</b>
Wet weight of one nodule	<b>0.882</b>	-0.382
Dry weight of one nodule	<b>0.839</b>	0.066
Moisture of nodule	0.029	<b>-0.794</b>
Area of one leaf	<b>0.988</b>	-0.021
Weight of one leaf	<b>0.951</b>	-0.028
Total variance	9.085	3.479
Share of total variance	0.649	0.249

Variation of studied parameters is determined by two studied factors which include 89.8% of total variance.

The first factor (Factor 1) is positively associated with such parameters as the number, area and weight of leaves, area and weight of 1 leaf, weight of stems, number and weight of beans, and wet and dry weight of 1 nodule. The second factor (Factor 2) is positively associated with the total number and total weight (wet and dry) of nodules, and negatively – with the moisture content of nodule. The first factor can be interpreted as the size of nodules, the second – as the total number of nodules per 1 plant. Summarizing the results of factor analysis, it can be stated that the vegetative biomass and productivity are determined not by the total number of nodules, but by their size. At the same time, a simple increase in the total number of nodules does not significantly influence either biomass accumulation or yield.

Revealed patterns show the relationship of inoculation with industrial strain 634 with two groups of factors. Inoculation with Spontaneous preparation based on indigenous microsymbionts has a significantly lesser effect on the studied parameters.

## V. CONCLUSION

Inoculation is an important reserve for improving soybean nitrogen nutrition. In new areas of its cultivation, using industrial preparations of nitrogen-fixers does not always have a positive effect, thus leading to decreased productivity. Spontaneous strains isolated on the northern border of culture

area showed a high virulence for the varieties developed in the Krasnoyarsk Territory and significantly exceeded the industrial preparation by the number of nodules. Nodules that arise upon inoculation with Spontaneous strain contain less moisture and are significantly inferior to the nodules of industrial strain 634 by weight ( $F = 3.077$ ;  $p = 0.188$ ). According to one of the main parameters determining soybean productivity, i.e. the number of beans, a statistically significant effect of industrial strain 634 was found ( $F = 9.637$ ;  $p = 0.0134$ ). The development of soybean photosynthetic apparatus (quantity, area, weight of leaves) and productivity elements (number, weight of beans) is determined by the average weight of one nodule and does not depend on the total number of nodules formed on the plant. Found indigenous microorganisms are of interest in the sense of clarifying the nature of the interaction with macrosymbionts and developing effective biological products.

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