Variety-Specific Soybean Yield with Respect to Nitrogen-Fixing Symbiosis on the Northern Boundary of the Crop’s Geographical Range

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Abstract The effects of inoculating nitrogen-fixing symbionts on soybean yield were tested in the experimental field of the Krasnoyarsk State Agrarian University, 50 km north of Krasnoyarsk. Strain 634 was tested in 2017–2018 on the Zaryanitsa variety. Strain 634 and Spontaneous, discovered in Krasnoyarsk forest steppes, were tested in 2019 on the precocious Zaryanitsa and Eos varieties (Siberian ecotype). Treating Zaryanitsa soybean with Strain 634 induced a yield of 0.197 kg/m² compared to the control yield of 0.167 kg/m² (p = 0.037). The number of nodules peaked in Zaryanitsa inoculated with the biological Spontaneous: 104 nodules per root. Spontaneous reduced the height of Zaryanitsa plants (p = 0.0606) but increased the seed weight (p = 0.0282). Eos was inoculated with two strains; compared to the controls, it had lower productivity metrics such as the number of branches (p = 0.0771), beans (p = 0.0483), seeds (p = 0.0142), and seed weight (p = 0.0381). However, the preservation rates were 16% higher in case of Strain 634, 22% in case of Spontaneous. Factor analysis (varimax rotation) identified two factors: one comprised the indicators related to the development of photosynthesis organs (the number, weight, and area of leaves) and to nodulation (the number, weight, and moisture of nodules). The other one comprised the yield structure components and the number of plants per unit of area. Eos yield rose by 0.02 kg/m² and 0.048 kg/m² when inoculated with Strain 634 and Spontaneous, respectively.

1. Introduction
The achieved crop yields, especially in developed economies, are attributable to the extensive use of nitrogen fertilizers. Denitrification and leaching lead to a considerable loss of the applied nitrogen, making such application inefficient. These processes negatively affect the ecosystem, polluting groundwaters and the air [1]. Fertilizer placement in polymer shells reduces nonproductive loss, increases the availability of the fertilizers to plants thanks to slow release [2]. Symbiotic nitrogen fixation by symbionts observed in legumes and in some other crop families provides more available nitrogen. Soybean is the most common legume in the world, as it provides a source of complete proteins and oil. What else makes this crop valuable is that it makes tilling ecofriendlier thanks to fixing atmospheric nitrogen in symbiosis with Bradyrhizobia [3]. Of the substantial number of Bradyrhizobium species [4], B. elkanii, B. japonicum , and B. diazoefficiens are the most common isolates used for making commercial biologicals for soybean inoculation. Inoculation is deemed to improve soybean productivity by enhancing its nitrogen nutrition and adaptability. [5] Symbiotic machinery is important for farming a crop in a different soil and climate. Over the last decade, Russia
has been planting ever more soybean, including in European Russia and Western Siberia. Of all the
regions that have only recently begun to farm soil, Yenisei Siberia has the worst bioclimatic potential.
Krasnoyarsk Krai only began planting soybean on a regular basis as recently as in 2008; only in 2017
did soybean farmland exceed two thousand hectares. Yield increase could be an additional incentive to
expand such farming. According to [6], inoculating soybean plantations with a commercial microbial
symbiont did improve productivity in Western Siberia.

The goal of this study was to test the effects of autochthonous soybean bacteria cultures on the
yields of the precocious varieties in Krasnoyarsk forest steppes.

2. Research materials and methods
This research used two soybean varieties: Zaryanitsa (patent holder: Omsk Agrarian Research Center,
FSBEI HE Krasnoyarsk SAU), allowed for use in Eastern Siberia since 2018; and EOS (selected by
the Krasnoyarsk SAU Laboratory), currently under state-endorse
ded testing. Zaryanitsa is a precocious
variety; it takes 97-106 to ripen from sprouting to full maturity. The variety has determinant growth,
produces 35 to 75 cm high plants, yields 1.81 tons/ha on average and 3.7 t/ha at max. Absolute dry
matter content of protein and fat in seeds equals 34% and 19%, respectively. Eos is a variety that was
created by hybridization of Siberian and European precocious ecotypes. It matures in 105 days, the
average yield in competitive testing was 2.16 t/ha, a positive difference of 0.48 t/ha from the standard
SibNIIK 315 variety. The plants have determinant growth, are 52 to 101 cm high, and the first pods
are located at 16 cm. The protein content is 35%, and the oil content is 18.2%.

For inoculation, the research team used *Bradyrhizobium japonicum* 634, an effective strain
provided by the All-Russian Research Institute of Agricultural Microbiology (ARRIAM, Saint-
Petersburg, Pushkin 8), as well as an open-source aboriginal culture dubbed Spontaneous. The seeds
were moisturized with a water solution of these biologicals at 10 l/t with an inoculant concentration of
1 × 10⁷ cells/ml. The control seeds were treated with distilled water. Seeds were planted two hours
after treatment.

Research was carried out in 2017–2019 in the experimental field of Krasnoyarsk SAU, which is
located in Sukhobuzimsky Municipality in the northwest of Krasnoyarsk forest steppes. The
experiments were carried out using four-field crop rotation including cereals, fallow crops, and
intertilled crops. Soybeans were preceded by potatoes. The soil of this area consisted of typical clayey-
illuvial and cryogenic-micellar chernozem soils; in terms of particle size, it was medium / heavy loam.

As of the day of planting, soil was poor in mobile nitrogen, had moderate amounts of mobile
phosphorus, and rich to very rich in mobile potassium, featured neutral pH, had moderate to high
humus concentration (5.1% to 9.1%). Soil was prepared for planting by deep sweeping (20 to 22 cm)
in late September, harrowing in early spring, and cultivation to a depth of 5 to 6 cm before planting.
Soybeans were sown in rows at 15 cm with a sower while heating the soil at the planting depth to 8°C
at the end of the second third of May. Plot placement was randomized with a three-time repeat; for
accounting purposes, each plot was 5 m² in area. The sowing rate was 0.8 million of viable seeds per
hectare, adjusted for viability and purity. As the plants developed two trifoliate leaves, they were
treated with a Paradox herbicide (water-soluble concentrate, WRC) at 0.2 l/ha (this is an imazamox-
based herbicide solved at 120 g/l). Mature plants were harvested by a TERRION-SAMPO SR2010
combine. Yield was measured by weighing dried and cleaned seeds. The crop was reported fully
sprouted on June 2 (2017) and June 5 (2018 and 2019); observations and measurements followed the
guidelines in [7, 8].

Statistical processing was carried out in StatSoft STATISTICA 13 using analysis of variance,
correlation analysis, discriminant analysis, and factor analysis. The significance of differences
between individual means was tested by Duncan’s multiple range test (MRT).

Over the years of study, temperature in May through September averaged at 15.1°C to 15.6°C with
the climate norm of 12.9°C. June and August were the warmest months compared to the long-run
annual average. Precipitation varied significantly from year to year. 2018 was a year of drought, as it
only had 67% of the normal seasonal precipitation. 2017 and 2019 were excessively humid, having 115% and 111% of the long-run averages, respectively.

3. Results and discussion

Commercial nodule-associated bacteria that Zaryanitsa plants were inoculated with did induce active symbiosis, see Table 1. In 2018, root nodules were first found in the controls, a sign of the spontaneous emergence of a microbial symbiotic population.

Table 1. Zaryanitsa nodulation.

| Group       | Nodules per root, pcs. | Weight of root nodules, g | Single-nodule weight, g |
|-------------|------------------------|---------------------------|-------------------------|
|             |                        | fresh | dry   | fresh | dry   |
| 634         | 2.5                    | 0.259 | 0.068 | 0.1   | 0.03  |
| controls    | 2.5                    | 0.185 | 0.049 | 0.074 | 0.02  |
| 634         | 5.9                    | 0.42  | 0.105 | 0.071 | 0.02  |

The difference in nodule production by Strain 634-inoculated plants could be attributable to warmth and moist. May and June 2017 saw drought with only 45.3 mm of precipitation (60% of the norm) and a cumulative positive temperature of 950°C. The first and the last month of summer 2018 were more favorable with 76% of the normal precipitation and a cumulative positive temperature of 869°C.

Inverse correlation was found between the number and size of nodules. The coefficient of fresh nodule weight (single nodule) and the number of nodules per root had a coefficient of -0.579 (p<0.05, n-2 = 12, r ± 0.532).

Despite the number of nodules being small, they did have a positive effect on Zaryanitsa yields, see Figure 1.

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![Figure 1. Zaryanitsa yields from inoculated seeds (average for 2017–2018), kg/m², F = 5.75, p = 0.037.](image)

Inoculation had minimal effect on soybean yields in 2017 at 0.18 kg/m², cf. 0.17 kg/m² for controls. The uniform distribution of the little precipitation that 2018 had during the soybean growing season
helped produce substantial yield using the biological fertilizer (0.197 kg/m²): an additional 22%. On average, the two-year effects of Strain 634 on yields was positive and significant.

Native nodule-associated bacteria were isolated in a pure culture and selected by the colony growth rate in a mineral and vegetative medium for B. japonicum [8], then used to inoculate soybeans. Nodulation reporting during the bean filling stage helped identify the variety-specific processes in Siberian-selected soybean. Zaryanitsa was therefore found to be better at producing root nodules: 44 per plant. Eos was less responsive with 26% less nodules. On the other hand, it was more responsible to native bacteria inhabiting the soil. An average intact plant had 17 nodules, cf. 4 in Zaryanitsa plants, see Figure 2.

![Figure 2. Number and weight of root nodules, 2019.](image)

Treatment with the commercial Strain 634 produced few nodules (18 per plant on average). Nodulation peaked in plants inoculated with the selected Spontaneous culture. Where there were more nodules, they were lighter at a correlation coefficient of -0.817 (p<0.05, n=22, r ± 0.404). It is believed nodulation is more effective where soils have moderate amounts of nitrogen [9].

The tested varieties responded differently to inoculation, see Table 2. Eos was most productive in the control group, while Zaryanitsa’s productivity was boosted by Spontaneous. Difference between planting groups was due to the difference in the density of growth. Eos had 16% more preserved plants per square meter when treated with the commercial biological, 22% more in case of Spontaneous compared to the controls. Zaryanitsa showcased the same trend only when treated with Strain 634 (an increase of 9%). Spontaneous reduced the number of plants by 17%. Eos and Zaryanitsa controls had 66 and 68 plants per square meter, respectively.
Table 2. Yields and productivity metrics of soybean varieties, 2019.

| Group                  | Yield, kg/m² | Height, cm | Number, pcs/plant | Weight of seeds per plant, g |
|------------------------|--------------|------------|--------------------|-----------------------------|
|                        | plant        | first bean attachment point | branch | productive nodes | beans | seeds |
| EOS, control           | 0.293        | 70         | 15                 | 0.6 | 9.4 | 16 | 34 | 5.1 |
| Eos, 634               | 0.313        | 73         | 16.5               | 0.4 | 8.3 | 14 | 28 | 4.2 |
| Eos, Spontaneous       | 0.341        | 74         | 16.6               | 0.4 | 8.1 | 13 | 28 | 4.2 |
| Zaryanitsa, control    | 0.284        | 69         | 16.3               | 0.9 | 8.6 | 15 | 27 | 3.8 |
| Zaryanitsa, 634        | 0.283        | 72         | 18.7               | 1.4 | 10.3 | 18 | 35 | 5.1 |
| Zaryanitsa, Spontaneous| 0.282        | 77         | 14.3               | 1.2 | 10.9 | 20 | 41 | 6.0 |

Yield is an integral metric that defines the ultimate effectiveness of the bacterial cultures in use. Whilst the experimental microbiological did improve the productivity of Zaryanitsa plants, yield did not rise. Eos had better yields when treated with Strain 634 and Spontaneous.

Discriminant analysis showed significant effect of the biological on the plant height (F = 7.828, p = 0.00056), the number of seeds (F = 5.168, p = 0.00662), and the weight of seeds from a plant (F = 14.240, p = 0.000002).

It was found out that the fresh and dry weight of a single nodule were the decisive factor contributing to the weight of seeds from a single plant at r = -0.5305 and r = -0.7052, respectively (n=22, r = 0.404).

Paper [10] shows the significant effects of photosynthesis-related metrics (photosynthesis rate, leaf area, leaf area-to-weight ratio) on the productivity of a crop. In this research, the authors picked plants to determine the number, weight, and area of leaves so as to find out how nodulation would affect leaf development and productivity at Stage R5 (seeds of 3 mm in diameter on one of the plant’s four upper tiers). This stage is characterized by the maximized leaf growth and the completion of growth in the determinant varieties used in his experiment. The number, area, and weight of leaves were moderately positively linked to the plant height at determination coefficients of 40%, 36%, and 30%, respectively. The determination of these metrics against the number of plants to harvest was somewhat lower at 25%, 30%, and 31%. These metrics were not found to significantly affect the productivity or the yield structure components. The number of plants to harvest was in negative correlation with the number of productive nodes (r = 0.8031), beans (r = 0.7879), or seeds (r = 0.6904) as well as with the weight of seeds from a single plant (r = 0.7904). No significant correlation found between nodulation and leaf growth metrics. However, strong positive correlation was found between single-nodule weight and the number (r = 0.8594), area (r = 0.8762) and weight (r = 0.8460) of leaves. These correlation coefficients are significant at p < 0.05, n=22, r = 0.404.

Varimax rotation was applied to find out the correlation between the assimilating surface development metrics, nodulation, and productivity metrics. The first group comprised the metrics associated with the growth of vegetative organs and the development of symbiotic machinery. The second group comprised productivity-determined metrics of generative organs, which accounted for 28% of variance.
Table 3. Varimax factor analysis of the total of features per plant; highlighted loads are significant at p > 0.700.

| Metrics                              | Factor 1  | Factor 2  |
|--------------------------------------|-----------|-----------|
| variety                              | 0.232614  | 0.800715  |
| biological                           | -0.572636 | 0.415694  |
| plant height                         | 0.888930  | -0.233859 |
| first bean attachment height         | 0.347215  | 0.457830  |
| number of branches                   | 0.063185  | -0.900420 |
| productive nodes                     | -0.103051 | -0.948488 |
| number of beans                      | -0.100727 | -0.916934 |
| number of seeds                      | 0.008993  | -0.847117 |
| weight of seeds per plant            | -0.411316 | -0.527649 |
| number of plants to harvest per m²   | 0.455898  | 0.833210  |
| number of leaves                     | 0.836977  | 0.090804  |
| leaf area                            | 0.854101  | 0.180439  |
| weight of leaves                     | 0.825999  | 0.290625  |
| weight of stems                      | 0.853254  | 0.291988  |
| nodules per root                     | -0.845367 | 0.098470  |
| fresh weight of nodules per root     | -0.403371 | 0.564777  |
| dry weight of nodules per root       | -0.727251 | 0.349771  |
| fresh weight of a single nodule      | 0.986956  | 0.133496  |
| dry weight of a single nodule        | 0.921850  | 0.103964  |
| nodule moisture                      | 0.866187  | 0.106646  |
| single-leaf area                     | 0.842184  | 0.245331  |
| single-leaf weight                   | 0.815511  | 0.345158  |
| total variance                       | 9.889724  | 6.200517  |
| share of total variance              | 0.449533  | 0.281842  |

The number of fully formed nodules did not improve the assimilation metrics of soybeans (leaf number and weight, single-leaf weight and area). Optimal soy growth i.e. maximum seed productivity is observed where large nodules appear on the roots.

The variation of the tested features is attributable to two factors that account for 73.1% of the total variance.

4. Conclusions
In-field experiments carried out in 2017–2018 showcased significant positive effects of the commercial symbiotic nitrogen-fixing Strain 634 on the yields of the Zaryanitsa variety. In 2019, comparative testing of this biological against Spontaneous, a newly cultured microbial symbiont
derived from native bacteria, identified variety-specific response to inoculation. Strain 634- and Spontaneous-treated Zaryanitsa had more formed nodules than Eos (F = 8.220, p = 0.0606).

The weight of seeds from a single plant was significantly affected by the number of preserved plants per unit of area and the dry weight of a single nodule (correlation coefficients of -0.709 and -0.705, respectively).

Eos showcased the best preservation before harvest when treated with Spontaneous, while Zaryanitsa had the best values when treated with Strain 634. Eos yielded 0.048 kg/m$^2$ when treated with Spontaneous, 0.028 kg/m$^2$ more when treated with Strain 634 compared to the controls, meaning that Spontaneous inoculation produced the best yield. This experiment confirms the specificity of variety response to inoculation as well as the positive effects this technique has on yields. Given that Yenisei Siberia is a high-risk region for farming, soybean inoculation helps optimize the use of soils and climate for more cost-effective agriculture.

5. References

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