The effect of native strains of nodule bacteria on the development of symbiotic apparatus and on the productivity of new soybean cultivars

V A Tilba\textsuperscript{1*}, V L Makhonin\textsuperscript{1} and S V Zelentsov\textsuperscript{1}

\textsuperscript{1}V.S. Pustovoit All-Russian Research Institute of Oil Crops, 17 Filatova street, 350038, Krasnodar, Russian Federation

E-mail: vniimk@vniimk.ru

Abstract. In 2018 and 2019, we studied new strains of nodule bacteria of soybean (Bradyrhizobium japonicum) in comparison with the standard (Noktin A) in field experiments on the experimental field of V.S. Pustovoit All-Russian Research Institute of Oil Crops (VNIIMK), Krasnodar on a leached extra heavy loam and weakly humus chernozem. The new strains were the strain 5/1, isolated from the meadow-chernozem soil of the Amur region and the strain 7p, isolated from the leached chernozem of the Krasnodar region. The aim of the research was to evaluate their effectiveness on promising soybean cultivars Slavia, Bars, Irbis and Opus by their influence on the development of symbiotic apparatus, development of root system and above-ground vegetative mass of plants in the phase of soybean vegetation, flowering and seed-filling, as well as on the seed yield and its quality.

For 2 years, on average, all strains to varying degrees contributed to a more intensive development of a root system and an increase of above-ground mass of soybean plants at the beginning of the growth season. We observed the maximum increase in biomass by the seed-filling phase in the Bars cultivar with strains 5/1 and in the standard – by 45 and 48 %, respectively. However, the Slavia cultivar with bacterization variants in this phase had less dry above-ground plants mass than the control, by 30-51 %.

Overall, the bacterization provided an increase in soybean productivity but to varying degrees by year and with different combinations of symbiotic partners (soybean cultivar – rhizobia strain). We observed the highest increase in seed productivity in Slavia and Opus cultivars: on average over 2 years - by 0.17-0.27 t/ha (8.7-13.0 %) and by 0.06-0.35 t/ha (2.6-15.1 %), respectively. The cultivars Bars and Irbis were less responsive to bacterization. We noted a certain cultivar-strain relation of preliminary complementary pairs: the Slavia cultivar – strains 5/1 and 7p; the Bars cultivar – strain 5/1; the Irbis cultivar – strains 5/1 and the standard; the Opus cultivar – strains 7p and the standard.

1. Introduction

In all regions of industrial cultivation of soybean, which has become the main protein-oil crop, numerous researches are being conducted to determine the possibilities of increasing its productivity and economic efficiency. One of the key conditions is to optimize the supply of plants with necessary life factors, first of all, with available elements of mineral nutrition. The distinctive biological characteristics of soybean as a high-protein bean crop are the increased demand of nitrogen and, at the same time, the ability to provide itself with this element by absorbing it from the atmospheric air in symbiosis with specific soil microorganisms – nodule bacteria (rhizobia).
The molecular nitrogen that is found in the atmosphere is an almost inexhaustible source for replenishing soil reserves through biological fixation by various soil microorganisms. According to estimates of many scientists, the share of applied nitrogen fertilizers in agricultural production is only about 10% of the amount of nitrogen fixed by microorganisms [1]. Symbiotic nitrogen fixation is the most effective type of biological nitrogen fixation, during which rhizobia (microsymbionts) are introduced into the root tissue of a host plant (macrosymbiont), developing special formations – nodules [2]. It is ten times higher than non-symbiotic fixation in the amount of fixed nitrogen, which is used directly by macrosymbiont.

The application of preparations of nodule bacteria (bacterization) is usually recommended as an indispensable element in the agrotechnology of bean crops, including soybean. It is an inexpensive agricultural method, the cost of which is incomparably lower than the price of an equivalent amount of mineral nitrogen fertilizers that it is able to replace. According to economic estimation, bacterization is one of the most cost-effective methods in the agricultural complex of soybean cultivation, contributing to an increase in its yield, an increase in protein content of seeds and replenishment of soil nitrogen reserves [3, 4]. Unlike nitrogen of mineral fertilizers, biologically fixed nitrogen is safe in the environmental respect, since it is not subjected to depletion and weathering processes and does not lead to the accumulation in the products of compounds such as nitrates, nitrites, etc., harmful to humans and animals. In each case, the positive effect of bacterization is due to numerous factors: availability of accessible mineral nitrogen and soil moisture, weather conditions, cultivar and strain specific characters [5, 6], as well as the presence and activity in the soil of the local population (resident or spontaneous) of nodule bacteria. In the areas of long-term soybean cultivation, the nodule bacteria, characteristic for it, can be an integral part of soil-microbial coenosis in a free-living state [7, 8, 9, 10].

We selected various bacterial forms and new strains of rhizobia during conduction of field examinations; some of these forms and strains we studied in laboratory experiments. Based on the results of a laboratory assessment of their symbiotic qualities (growth characteristics at various sources of carbohydrates, competitiveness and ability to develop nodules on seedlings of different soybean cultivars), we selected 2 native strains (5/1 and 7p) that showed fairly stable virulence indicators. The aim of this research was to evaluate the effectiveness of these strains in field experiments on leached chernozem of the Krasnodar region by the effect on the development of symbiotic apparatus in promising soybean cultivars with increased biological potential, on the accumulation of vegetative biomass by plants, and also on the quantity of seed yield and its quality.

2. Materials and methods
In 2018 and 2019, we conducted the research on the experimental field of V.S. Pustovoit All-Russian Research Institute of Oil Crops on a leached extra heavy loam and weakly humus chernozem.

We studied the soybean cultivars of VNIIMK breeding: Slavia – early (ripening group 0.5), the growth period is 102-108 days, the protein content in seeds is 40-41%, the oil content in seeds is 23-24%; Ibis – early (ripening group 0.5), the growth period is 105-112 days, high-protein – the protein content in seeds is 43-45%, the oil content in seeds is 18-21%; Bars – medium early (ripening group 1), the growth period is 110-117 days, the protein content in seeds is 41-45%, the oil content in seeds is 18-22%; and the Opus cultivar of Canadian breeding – medium early (ripening group 1), the growth period is 105-114 days, high-protein – the protein content in seeds is 40-45%, the oil content in seeds is 22-23%.

We used native strains of slow-growing nodule bacteria of soybean \textit{(Bradyrhizobium japonicum)} in experiments: strain 5/1 was isolated from the meadow-chernozem soil of the Amur region, strain 7p was isolated from the leached chernozem of the Krasnodar region. We used Noctic A, an inoculant of Argentinean production, which is allowed for use in production, as a standard. Before sowing we treated seeds of soybean cultivars with an emulsion of nodule bacteria at the rate of up to 2 million cells per seed. In the control, soybean seeds were sown without bacterization.
The experiment was replicated 3 times, the area of one plot was 14 m². We selected plants with soil monoliths in 4-time replication on record plots on non-adjacent replications in 2 dates: during the flowering (June) and seed-filling (August) phases of soybean vegetation. We took into account the number and mass of nodules, the mass of roots and above-ground parts of plants according to the VNIIMK method [11]. We conducted the mathematical processing of results by the analysis of variance in the B.A. Dospekhov variant [12].

Winter barley was soybean predecessor in crop rotation. We did not apply mineral fertilizers on soybean. We carried out sowing with row spacing of 70 cm. We chose generally accepted soybean agricultural technology in accordance to existing recommendations [13]. We conducted harvest record from the plot area by the Wintersteiger breeding combine. We reduced yield data to 100 % purity and 14 % moisture.

The soil of the experimental plot is characterized by a high degree of structure (more than 55 % of agronomically valuable aggregates) with a steady-state density of 1.27-1.30 g/cm³ and high capacities of water absorption and retention. The arable layer has 3.49-3.61 % of humus, pHkcl is 5.6-5.9, nitrification ability is 15.9-18.8 mg of NO₃ per kg of soil, in 1 kg of dry soil the average reserves are: P₂O₅ - 26.1-27.6 mg; K₂O - 408-450 mg (the data is provided by the laboratory of agrochemistry of V.S. Pustovoit All-Russian Research Institute of Oil Crops).

The weather conditions of 2018 and 2019 characterized by sufficient moisture at the beginning of soybean vegetation (the precipitation amount in May was 86 and 68 mm, respectively, with a climatic norm of 57 mm); by dry, hot weather during the flowering-bean development (the total annual precipitation in June was only 11 and 17 mm, respectively, with average daily temperatures of 26.3 and 25.1 °C) and by wet weather in the seed-filling phase of July (the precipitation amount was 119 and 134, respectively, with a climatic norm of 60 mm). A distinctive characteristic of weather conditions of 2018 was a severe drought in the final stage of the soybean vegetation period, when during the whole of August not a single productive rain fell (only 6.8 mm for the whole month) against the background of high average daily temperatures (25.4 °C) and relatively low humidity (50 %), while in 2019 during this period 57 mm of precipitation fell, with a climatic norm of 48 mm.

3. Results
The data in Table 1 show the development of the nodules on plant roots in the control without bacterization, which indicates the presence of resident (spontaneous) rhizobia in soil. The weather conditions significantly influenced the process of development of the symbiotic apparatus in soybean. Thus, in 2018, the number of nodules at the beginning of vegetation was significantly lower in all variants, than in 2019; however, in the second half of the vegetation of 2018, the nodules developed more intensively. Cultivar differences appeared according to the reaction to bacterization: on average over 2 years, it contributed to the better development of the symbiotic apparatus in the seed-filling phase of high-protein cultivars Irbis and Opus; in case of the Bars cultivar it was expressed to a lesser extent, and the Slavia cultivar had neutral reaction.

The mathematical processing of the obtained results shows that most of the numerical values of the studied characteristics are within the experimental error and can be regarded as trends.

Table 1. The number and dry mass of nodules on the roots of soybean cultivars under bacterization of seeds with different strains of nodule bacteria (per 10 plants).

| Variant (strain) | The date of samples selection (phase of soybean vegetation) |  |
|-----------------|----------------------------------------------------------|--|
|                 | number of nodules, pcs. | mass of nodules, g | number of nodules, pcs. | mass of nodules, g |
|                 | years | average for 2 years | years | average for 2 years | years | average for 2 years | years | average for 2 years |
| Control         | 2018  | 172               | 2019  | 108               | 2018  | 0.29               | 2019  | 0.48              | 2018  | 391               | 2019  | 0.39              | 2018  | 330               | 2019  | 1.70              | 2018  | 1.01              |
Table 2 shows that, in general, studied cultivars responded positively to bacterization in terms of growth of the above-ground vegetative mass and roots. We observed the maximum growth in the Bars cultivar in variant with strains 5/1 and in standard – by 45 and 48 %, respectively. The exception is the Slavia cultivar, the dry above-ground mass of plants of which was by 30-51 % lesser than in control in the phase of seed-filling in variants with bacterization; the mass of roots was also less.

**Table 2.** The dry mass of above-ground part of plants and roots of soybean cultivars under bacterization of seeds with different strains of nodule bacteria (per 10 plants).

| Variant (strain) | The date of samples selection (phase of soybean vegetation) |  |
|------------------|-----------------------------------------------------------|---|
|                  | June (flowering)                                          | August (seed-filling) |
|                  | above-ground mass, g                                       | roots mass, g          | above-ground mass, g | roots mass, g |
|                  | years | average for 2 years | years | average for 2 years | years | average for 2 years | years | average for 2 years |
|                  | 2018  | 2019                | 2018  | 2019                | 2018  | 2019                | 2018  | 2019                |
| Slavia cultivar  |                                          |                                          |                                          |                                          |
| Control          | 24.9  | 105.7               | 65.3  | 11.8                 | 12.1  | 12.0                | 86.0  | 805.0               | 445.5 | 24.9               | 62.2  | 436                |
| Standard         | 30.0  | 116.9               | 73.5  | 12.1                 | 16.6  | 14.4                | 103.1 | 333.3               | 218.2 | 26.7               | 33.3  | 30.0               |
| Strain 5/1       | 26.2  | 117.1               | 71.7  | 11.9                 | 17.5  | 14.7                | 81.1  | 538.3               | 309.7 | 23.9               | 41.0  | 32.5               |
| Strain 7p        | 23.3  | 104.0               | 63.7  | 9.7                  | 16.6  | 13.2                | 67.3  | 389.2               | 228.3 | 23.3               | 39.8  | 31.6               |
| LSD<sub>0.05</sub> | 5.3   | 21.7                |       | 1.4                  | 4.6   |                    | 22.0  | 162.0               | 1.2   | 17.3               |
| Bars cultivar    |                                          |                                          |                                          |                                          |
| Control          | 27.2  | 78.6                | 52.9  | 10.3                 | 13.5  | 11.9                | 83.0  | 208.3               | 145.7 | 24.3               | 22.5  | 23.4               |
| Standard         | 29.9  | 113.9               | 71.9  | 14.3                 | 18.9  | 16.6                | 121.0 | 311.7               | 216.4 | 29.9               | 21.8  | 25.9               |
| Strain 5/1       | 26.9  | 101.7               | 64.3  | 12.7                 | 15.3  | 14.0                | 116.2 | 306.7               | 211.5 | 26.9               | 26.0  | 26.5               |
| Strain 7p        | 33.2  | 91.6                | 62.4  | 15.2                 | 14.8  | 15.0                | 121.1 | 205.0               | 163.1 | 32.1               | 19.8  | 26.0               |
| LSD<sub>0.05</sub> | 1.0   | 28.7                |       | 0.3                  | 3.9   |                    | 45.0  | 83.2                | 0.8   | 8.5                |
| Irbis cultivar   |                                          |                                          |                                          |                                          |
| Control          | 30.2  | 72.9                | 51.6  | 10.4                 | 11.6  | 11.0                | 64.3  | 251.7               | 158.0 | 28.9               | 18.5  | 23.7               |
| Standard         | 20.2  | 126.8               | 73.5  | 17.2                 | 18.6  | 17.9                | 149.1 | 240.0               | 194.6 | 21.2               | 18.2  | 19.7               |
| Strain 5/1       | 31.2  | 79.0                | 55.1  | 16.9                 | 12.3  | 14.6                | 95.0  | 245.0               | 170.0 | 31.3               | 17.1  | 24.2               |
| Strain 7p        | 34.9  | 54.2                | 44.6  | 14.9                 | 7.6   | 11.3                | 137.1 | 280.0               | 208.6 | 34.9               | 28.1  | 31.5               |
The data in table 3 shows that, in general, bacterization provided the increase in soybean productivity but to a different extent by the years and different combinations of symbiotic partners (soybean cultivar – rhizobia strain). The Slavia and Opus cultivars had the highest yield increase. The Bars and Irbis cultivars were less responsive to bacterization in terms of seed productivity. Depending on the year conditions, we noted different reaction of cultivars to bacterization by the protein and oil content in seeds, as well as by the productivity. For example, in 2018, the Slavia cultivar had more protein content in seeds, than in control, in variants of bacterization with strains of 5/1 and 7p, while in 2019 there were no differences in these indicators; on the contrary, in 2018, in case of the Bars cultivar there were no differences between the variants but in 2019 the variant with strain 7p had the lowest protein content.

Table 3. The effect of strains of nodule bacteria on the productivity of soybean cultivars and the protein and oil content in seeds.

| Variant (strain) | Seed yield, t/ha | Protein content in seeds, % | Oil content in seeds, % |
|-----------------|-----------------|----------------------------|------------------------|
|                 | 2018a 2019b     | average for 2 years        | 2018  2019 average for 2 years | 2018  2019 average for 2 years |
| Slavia cultivar |                 |                            |                        |                        |
| Control         | 2.00 1.94       | 2.07                       | 41.8 37.5 39.7         | 21.7 22.1 21.9         |
| Standard        | 2.36 2.26       | 2.31                       | 41.5 37.9 39.7         | 21.5 21.9 21.7         |
| Strain 5/1      | 2.51 2.17       | 2.34                       | 42.3 37.6 40.0         | 21.1 21.8 21.5         |
| Strain 7p       | 2.07 2.43       | 2.25                       | 42.3 37.5 39.9         | 21.0 22.0 21.5         |
| Bars cultivar   |                 |                            |                        |                        |
| Control         | 2.16 2.56       | 2.36                       | 42.3 38.0 40.2         | 21.1 21.8 21.5         |
| Standard        | 2.25 2.36       | 2.31                       | 42.4 38.4 40.4         | 20.7 21.7 21.2         |
| Strain 5/1      | 2.08 2.74       | 2.41                       | 42.3 38.1 40.2         | 21.2 21.9 21.6         |
| Strain 7p       | 2.04 2.48       | 2.26                       | 42.5 36.6 39.6         | 20.7 22.7 21.7         |
| Irbis cultivar  |                 |                            |                        |                        |
| Control         | 2.58 2.54       | 2.56                       | 44.2 41.5 42.9         | 20.2 20.0 20.1         |
| Standard        | 2.80 2.52       | 2.66                       | 44.1 42.1 43.1         | 20.6 19.2 19.9         |
| Strain 5/1      | 2.70 2.51       | 2.61                       | 43.8 40.8 42.3         | 20.8 20.2 20.5         |
| Strain 7p       | 2.51 2.68       | 2.60                       | 44.5 41.9 43.2         | 20.1 19.6 19.9         |
| Opus cultivar   |                 |                            |                        |                        |
| Control         | 2.18 2.40       | 2.29                       | 45.0 40.5 42.8         | 19.1 20.9 20.0         |
| Standard        | 2.65 2.62       | 2.64                       | 44.7 40.2 42.5         | 19.9 21.4 20.7         |
| Strain 5/1      | 2.29 2.41       | 2.35                       | 44.4 40.4 42.4         | 19.9 21.4 20.7         |
| Strain 7p       | 2.49 2.35       | 2.42                       | 45.3 40.6 43.0         | 18.9 21.3 20.1         |

LSD₀.₀5 by variants 0.31 t/ha.
LSD₀.₀₅ by variants 0.26 t/ha.

4. Summary
As a result of the field experiment of 2018 and 2019 on a leached chernozem of the Krasnodar region, when we assessed the effectiveness of native rhizobia strains 5/1 and 7p on soybean cultivars Slavia, Bars, Irbis, and Opus in comparison with the standard, we revealed rather complex and variable relations in the system soybean cultivar – rhizobia strain during the development of nodules and development of symbiotic relationships.
Given the high variability of the studied indicators under various weather conditions during the years of research (the regularly occurring lack of moisture), we revealed some varietal differences in the soybean vegetation and in the development of symbiotic apparatus. According to generalized data, at the beginning of the growth season, all strains to varying degrees contributed to a more intensive development of the soybean root system and an increase in the above-ground mass on all cultivars. The Bars cultivar with strains of 5/1 and standard had the highest increase – by 45 and 48 %, respectively. However, the dry above-ground mass of plants of the Slavia cultivar was by 30-51 % lesser than in control in the phase of seed-filling in variants with bacterization.

In general, bacterization provided the increase in soybean productivity but to a different extent by the years and different combinations of symbiotic partners (soybean cultivar – rhizobia strain). We observed the highest increases in seed yields in Slavia cultivar - on average over 2 years - by 0.17-0.27 t/ha (8.7-13 %), and in Opus cultivar - by 0.06-0.35 t/ha (2.6-15.1 %). The Bars and Irbis cultivars were less responsive to bacterization.

According to the average indicators of the yield calculated for each variant of bacterization, the studied native strains of 5/1 and 7p compared with each other and with standard did not differ much and provided an increase in the yield of soybean cultivars, on average over 2 years, by 0.08-0.13 t/ha (3.0-5.5 %). By the protein and oil content in seeds, as well as by the yield, we observed different reaction of cultivars to bacterization with different strains depending on weather conditions, nut on average over 2 years there was no significant effect of bacterization on the biochemical composition of seeds.

As a result of 2-year research, we can note a certain cultivar-strain relation according to the effect of studied rhizobia strain on the productivity of different soybean cultivars. We preliminary identified the following complementary pairs: the Slavia cultivar – strains 5/1 and 7p; the Bars cultivar – strain 5/1; the Irbis cultivar – strains 5/1 and the standard; the Opus cultivar – strains 7p and the standard.

5. References
[1] Dyakov A B 2019 Supraorganismal biologic systems and methods of their study (Krasnodar: Prosveshenie-Yug) 267
[2] Delaev U A, Kobozeva T P, Zuziev U G and Shishkhaev I Y 2018 Features of air nitrogen fixing by free-living microorganisms and club bacteria in symbiosis with pine cultures (The news of the Chechen State University) 4 (12) 68-71
[3] Baranov V F, Kochegura A V and Lucomets V M 2009 Soybean on Kuban/Scientific and production edition (Krasnodar) 320
[4] Zaitsev N I, Agafonov O M, Shabaldas O G and Vlasova O I 2017 The development of nodules depending on the presowing treatment of soybean seeds with bacterial preparations (Oil Crops. Scientific and technical bulletin of VNIIMK) 1 (169) 64-68
[5] Dega L A, Butovets E S and Lukyanchuk L M 2018 Complementary links of rhizobia and soybean varieties in Primorsky krai (Protection and quarantine of plants) 11 41-42
[6] Vasil'chikov A G and Akulov A S 2016 Increase in Soybean Productivity by Intensified Symbiotic Nitrogen Fixation (Zemledelie) 4 14-16
[7] Yakimenko M V and Begun S A 2016 Basic directions of researches of the Far Eastern natural populations of rhizobia (Vestnik of Far Eastern Branch of Russian Academy of Sciences) 2 (186) 45-49
[8] Tilba V A 2016 Virulence of legume bacteria on soybean and degree of symbiotic nitrogen accumulation in soils of Amur River region (Oil crops. Scientific and technical bulletin of VNIIMK) 4 (168) 61-66
[9] Roumiantsceva M L 2019 Root nodule bacteria: perspectives of monitoring symbiotic properties by applying genetic markers (Agricultural biology) 5 (54) 847-862
[10] Butovets E S, Lukyanchuk L M and Vasina E A 2019 Interaction of rhizobial bacteria with plants of soybean varieties developed in Primorsky Krai (Vestnik of Far Eastern Branch of Russian Academy of Sciences) 3 (205) 48-54
[11] 2010 *Methodology of agronomic field experiments with oil crops* Under the edit. of Lucomets V M (Krasnodar: VNIIMK) 327
[12] Dospekhov B A 1985 *Methodology of field experiment (with basics of statistical processing of research results)* (M.: Agropromizdat) 351
[13] Lucomets V M, Bochkaryov N I, Khatnyansky V I 2010 *Adaptive technologies of oil crops production in the Southern region* (Krasnodar) 160