ATLANTIS PRESS
Advances in Social Science, Education and Humanities Research, volume 393
The Fifth Technological Order: Prospects for the Development and Modernization of the Russian Agro-Industrial Sector (TFTS 2019)

Symbiotic Efficacy of Variety-Specific Strains of Nitrogen-Fixing Bacteria on Soybean Varieties of Omsk Selection

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***Abstract*—The experiments were performed in 2017-2018 in the fields of the Omsk Agrarian Scientific Center (Omsk, Russian Federation), with winter cereals as fore corp. The objective was to study and isolate strains of varietal-specific nodule bacteria that positively affect the development of soybean plants and nitrogen-fixing nodules. The experiment included soybean varieties of northern ecotype of the Omsk ASC selection: Zolotistaya and Cheremshanka. The seeds of cultivars were processed before sowing with microbiological fertilizers – four experimental varietal-specific isolates of nodule bacteria created in the Laboratory of Industrial Microbiology "PlantaPlus" (Tomsk, Russian Federation); control (K) experiment – clear water. Structural analysis (10 plants for each option) was carried out in late flowering (July) and in the phase of bean filling (August). Harvesting was in the 3rd decade of September with the Hege 125 combine. Biochemical parameters of seeds were determined in the Laboratory of pPlant Genetics, Physiology and Biochemistry at the Omsk ASC: nitrogen content – by automatic analyzer "KjeltekAuto 1030 Analyzer", nitrogen to protein conversion factor for soybean grain – 6,25; the content of crude fat is by the Soxhlet extractor. It was established that soybean varieties differ in the strength of response to the use of biological products, which are mainly effective. The Cheremshanka variety has the highest weight of plants and nitrogen-fixing nodules and seed yield in comparison with the Zolotistaya variety. The maximum experimental values (on average over 2 years) without loss of seed quality were obtained in the Cheremshanka cultivar variant + strain C.11/2017: the green mass of the plant at the end of flowering is 43,9 g (+13,6 g to K); at the bean filling phase – 90,1 g (+32,9 g to K); seed yield is 3,56 t/ha (+0,19 t/ha to K), weight of nitrogen-fixing nodules 1,27 g/plant (+1,00 g to K).**

Keywords—variety, biological product, nitrogen-fixing nodules, plant habit, seed yield, protein and fat content.

I. Introduction

Interest to profitable crops that bring more income per hectare than wheat has been growing in Russia in the past recent years. Such plants include soybean, which can be used in very wide ranges. The total soybean production in the Russian Federation reached 3,9 million tons in offset weight, which is 10% ahead of last year. In 2018, another record was set – soybean sowing on an area of 2,78 million ha (+141 thousand ha in comparison with year 2017) [1]. In the Omsk region, soybean sown areas increased from 6,7 thousand ha in 2016 to 10,7 thousand ha in 2018, but so far its yield is very low – from 0,82 to 0,92 t/ha.

The soybean breeding began in 1953 at the Siberian Scientific Institute of Agriculture (now the Omsk ASC), on the basis of samples and populations of the Amur Experimental Station obtained from V.P. Chernogolovin [2]. As a result of many years of research aimed at creating stress-resistant, early-ripening, highly productive and technologically advanced varieties that can effectively use the agroclimatic potential of the Siberian region, 8 soybean varieties are recommended for cultivation in the 10th region, including the Omsk Region: Omskaya 4, Altom, SibNIIISHKhoz 6, Dina, Eldorado, Zolotistaya, Sibiryachka, Cheremshanka [3].

Numerous studies have confirmed that increasing yields and quality of soybean depend on the realization of the potential of cultivated varieties under production conditions due to: optimal sowing dates, sowing standards and methods, fore crops, soil cultivation methods, proper use of organic and mineral fertilizers, micronutrient fertilizers, as well as techniques that contribute to the reproduction and increased activity of nodule bacteria [2].

Legumes, including soybean, have a unique ability to fix atmospheric nitrogen in symbiotic nodules as a result of interaction with rhizobia. The intensity of nitrogen fixation depends on a complex of natural, anthropogenic and biological factors: soil and climatic conditions, especially soil moisture; level of agricultural technology; genetic characteristics of leguminous crops and varieties; nodule bacteria strain [4]. Visible nodules on soybean roots begin to form from a phase of 2-3 true leaves, and the largest weight and total number of nodules, including active ones, are observed in the flowering phase – the beginning of bean formation [5].

But since the process of nodule formation requires energy, their quantity is limited by the plant [6]. Symbiotic efficiency or the ability to increase productivity (weight, seed quantity, protein accumulation) in leguminous host plants is the most important sign of nodule bacteria, characterizing their adaptive potential and practical significance [7]. The fixation of molecular nitrogen is controlled by the genotypes of both partners [8]. N.A. Provorov et al. suggest that in the system of nodule symbiosis, the regulatory effects of rhizobia on legumes significantly activate their symbiotrophic development (for example, improving the assimilation of N_2-fixation products by actively growing aerial organs) [9]. Scientists have proven that when entering into symbiosis, plant protection against bio- and abiotic stresses is enhanced [10].

The hypothesis: the use of variety-specific strains of nitrogen-fixing bacteria will increase the yield and quality of seeds of soybean varieties recommended for cultivation in the Omsk region.

The objective was to study and isolate strains of varietal-specific nodule bacteria that positively affect the development of soybean plants and nitrogen-fixing nodules.

II. Research Methodology

The experiments were conducted in 2017 and 2018 in the fields of the Omsk Agrarian Scientific Center (Omsk, Russian Federation) located in the forest-steppe zone. Winter cereals were the fore crop. The soil is weakly leached chernozem, of medium power, medium loamy, humus content of about 6%, pH_{kcl} of soil solution is 6,5. The content of $N\text{-}NO_3$ in the soil layer 0-40 cm before sowing is low, P_2O_5 (according to Chirikov) is high, K_2O is very high. The main tillage is subsurface fall-plow. In the spring there are two-track harrow and pre-sowing cultivation in two directions. Immediately before sowing, a starting dose of nitrogen fertilizer was introduced (ammonium nitrate – 100 kg/ha). Sowing is on May 17th with repetition of 4 times, with the “SSFK 7” seeder on plots with an area of 10 m^2, taking into account the weight of 1000 seeds, laboratory germination and the recommended sowing rate of 0.8 million germinating grains per hectare. Row spacing is 15 cm. “Pulsar” herbicide treatment – 1 l/ha (fluid consumption at 250 l/ha) in phase of 1-2 true leaves. Direct harvesting is at the end of September with the “Hege 125” combine in the phase of full ripeness. Accounting for harvested crops is made by weighing plots after picking the seeds from debris.

We studied soybean varieties of the Omsk ASC – Zolotistaya (included in the State Register of the Russian Federation of selection achievements in 2012) and Cheremshanka (2017). Microbiological fertilizers (4 experimental varietal-specific isolates of nodule bacteria) were provided by their originator – Laboratory of Industrial Microbiology “PlantPlus” (Tomsk, Russian Federation), control experiment (K) – with clear water. The seed treatment with biological products in the recommended doses was carried out in a room protected from direct sunlight. The time interval between seed treatment and sowing did not exceed 3 hours.

Biochemical parameters of seeds were determined in the laboratory of genetics, physiology and biochemistry of plants of the Federal State Budgetary Institution “Omsk ASC” in an absolutely dry sub-sample in triplicate. Grain grinding was carried out at the “Cyclotek 1092” mill. The nitrogen content in the grain was determined on the automatic “KjeltekAuto 1030 Analyzer”, the coefficient of conversion of nitrogen to protein for soybean grain is 6,25. The content of raw fat in the grain was determined by the Soxhlet extractor [11].

Structural analysis (10 plants from each variant) for 18 indicators was carried out at the end of flowering of agricultural crops (July) and in the phase of bean filling (end of August).

The forest-steppe zone is located at 55° north latitude in the central part of the Siberian Irtysh region and occupies a significant part of the Omsk region. The climate is sharply continental, characterized by harsh and lightly snowy winters, warm but short summers, short transitional seasons in spring and fall [12]. The frost-free period lasts for 116 days. The sum of average daily air temperatures is 1943 °C. The average date for the frost termination in the spring is at May 22nd, the average date for the onset of the first fall frosts is at September 17th.

The dynamics of hydrothermal support of the vegetation period (May – September) in 2018 significantly differed from 2017 (Fig. 1) [13]. The average daily air temperature over the period in 2017 was 15,8 °C (+0,7 °C to the long-term value), in 2018 it was 14,3 °C (-1,2 °C); the amount of precipitation is 173 mm (75% of the annual average) and 264,3 mm (112,4%), respectively. In September there were no frosts. Weather conditions during these years were generally quite favorable for soybean varieties of Siberian ecotype.

III. Results

It was established that soybean varieties of the Omsk selection – Zolotistaya and Cheremshanka, had a different degree of reaction to the use of bio-preparations for all analyzed indicators of plant habit and nodulation elements.

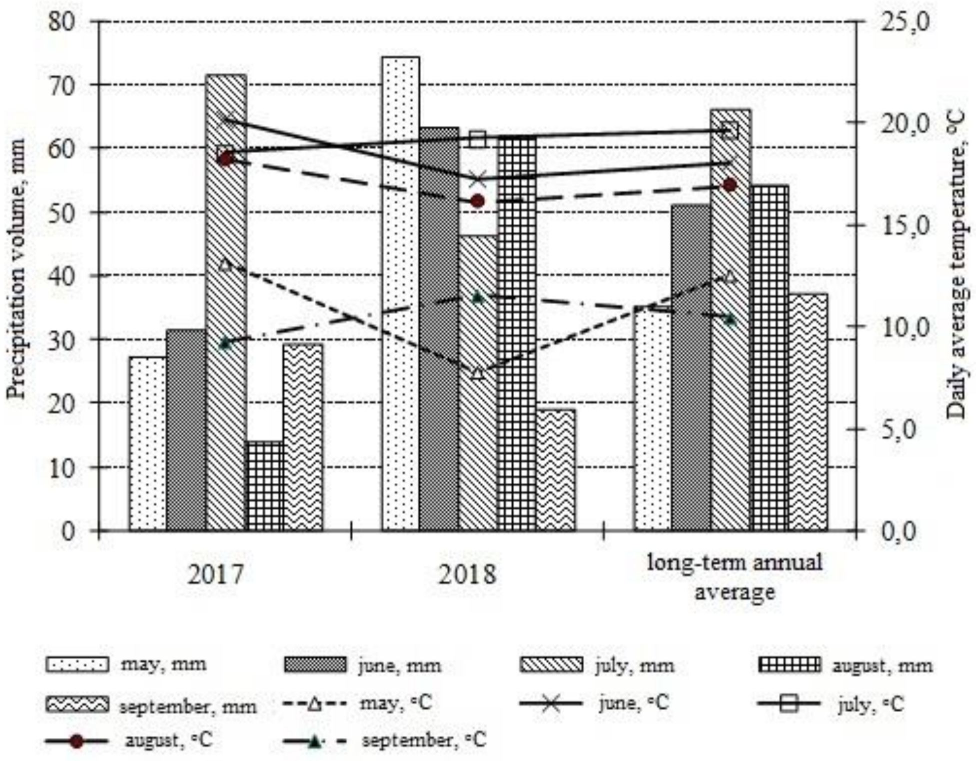

Fig. 1. Hydrothermal support of the growing season in 2017 and 2018, Omsk, Russian Federation [13].

The Cheremshanka variety is much stronger than Zolotistaya and is infected with native strains of nitrogen-fixing bacteria that live in the soil, but at the same time, it is more responsive to treatment with experimental strains (Fig. 2). In the Zolotistaya variety of the control experiment had nodules' weight that was stable in two phases of development – 0,46 g/plant, while this indicator in the Cheremshanka variety increased at the bean filling phase from 0,63 g/plant to (during flowering) up to 1,00 g/plant.

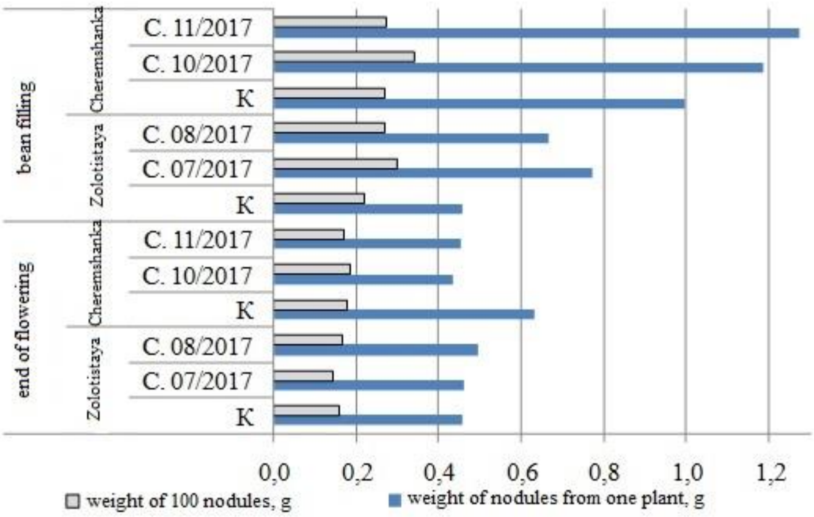

Fig. 2. Mass of nitrogen-fixing root nodules of soybean varieties, g/plant (average for 2017 and 2018)

During the period of bean filling, a significant increase in the control by weight of nitrogen-fixing nodules was found to be from 19% to 68%, variants of the Cheremshanka variety had indicators significantly higher than in the Zolotistaya variety. There were no significant differences in the size of the nodules (weight of 100 pieces).

By the time of massive bean filling, the plants doubled the vegetative weight in the variants of the Cheremshanka variety due to using both experimental variety-specific strains; and Zolotistaya + strain C.08/2017 (89% and 43% in the control experiment, respectively).

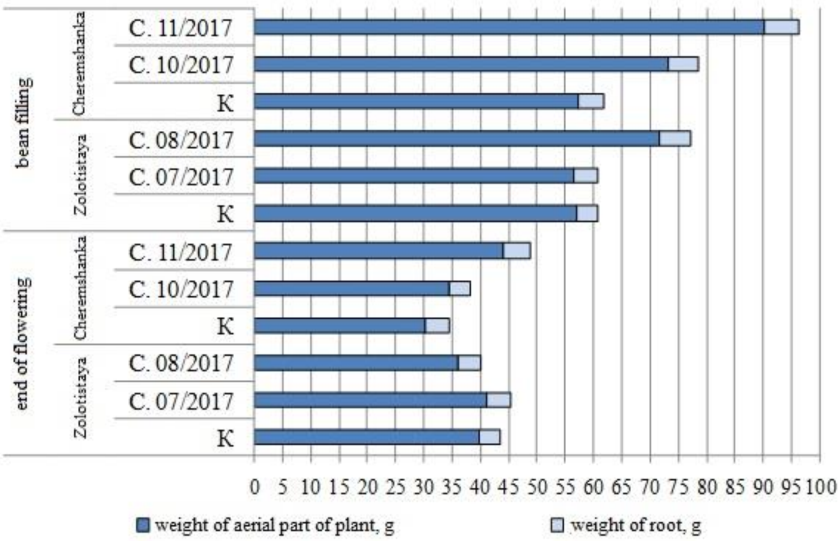

Fig. 3. Green mass of the plants' aerial parts and roots in soybean varieties, g/plant (average for 2017 and 2018)

In the phase of full ripeness, the experimental variants were distinguished by the highest plant density – 72,4-79,0 pcs/m^2 (Table I). In terms of plant height and the number of productive nodes and beans on the plant, reliable additions to the control experiment are only in the Zolotistaya variety. The use of biological products on the Cheremshanka variety contributed to an increase in seed size.

TABLE I. YIELD INDICATORS OF SOYA VARIETIES (AVERAGE FOR 2017 AND 2018)

Variety	**Plants for harvest, pcs/m^2**	**Plant height, cm**	**Quantity per plant, pcs**		**Mass of 1000 seeds, g**
			productive nodes	***beans***	
Zolotistaya variety					
control	63.6	91.4	8.8	14.5	154.8
C.07/2017	72.6	98.4	11.5	20.2	150.0
C.08/2017	79.0	97.2	14.4	27.9	149.4
Cheremshanka variety					
control	66.8	97.1	9.9	17.8	166.8
C.10/2017	72.4	97.7	10.5	19.4	176.1
C.11/2017	73.9	99.7	12.0	20.1	178.9
HCP_{05}	6.4	5.2	4.8	6.7	15.1

In the abovementioned two variants of the variety Cheremshanka and Zolotistaya + strain C.08/2017, the seed yield was significantly higher than in the control experiment (Fig. 4). The maximum value in the experimental study (3,73 t/ha) was obtained in 2017 from the Cheremshanka variety when treated with strain C.11/2017 (control experiment – 3,38 t/ha).

On average, over two years, the protein and fat content in soybean seeds in the experimental variants was at the control level – 40,8% and 16,0%, respectively. In 2017, the maximum protein percentage in seeds was obtained in the Cheremshanka variety in the experiment – 44,7% when treated with strain C.10/2017 (control – 40,7%) and in fat – with strain C.11/2017 – 18, 3% (control – 17,9%).

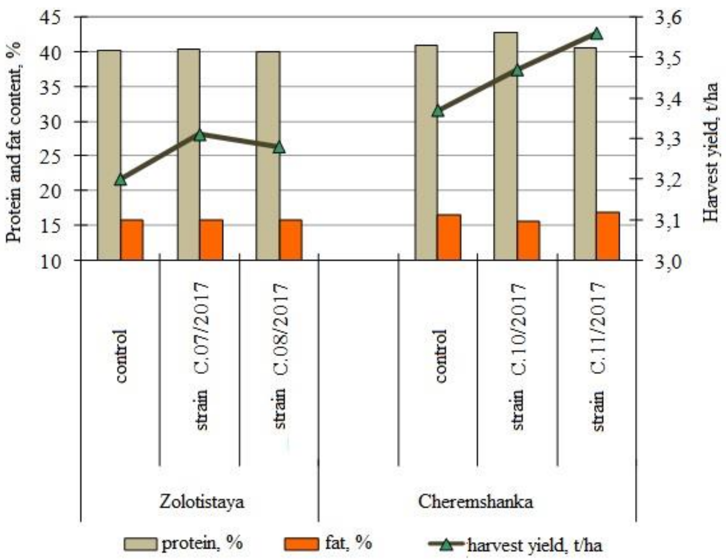

Fig. 4. Yield and quality of seeds of soybean varieties (average for 2017 and 2018)

IV. Conclusion

The soybean varieties included in the experiment differ in the strength of the response to the use of bio preparations, which are mainly effective.

The Cheremshanka variety has a better vegetative weight, seed yield and weight of nitrogen-fixing nodules compared to the Zolotistaya variety. The maximum experimental values (on average over 2 years) without loss of seed quality were obtained in the Cheremshanka cultivar in the variant with strain C.11/2017: the green mass of the plant at the end of flowering is 43,9 g (+13,6 g compared to the control experiment); in the bean filling phase – 90,1 g (+32,9 g to control); root weight – 4,8 g (+0,6 g), 6,1 g (+1,6 g), respectively; seed yield –g 3,56 t/ha (+0,19 t/ha to control), weight of nitrogen-fixing nodules is 1,27 g/plant(+1,00 g to control)

Given the weather conditions uniqueness in 2017 and 2018, comparatively favorable for soybeans, the study of the effect of experimental microbial preparations on the development of soybean varieties was continued on three register varieties – the Sibiryachka variety was added (included in the State Register of the Russian Federation of selection achievements in 2013).

The research results obtained in the conditions of risky farming in the forest-steppe of Western Siberia prove the effectiveness of variety-specific strains of nitrogen-fixing bacteria, the use of which will improve the yield and quality indicators of Omsk varieties of soy – an economically and environmentally significant agricultural crop of world level.

References

[1] Results of the year 2018. Oilseeds – sunflower, soybean, canola, mustard, flax, safflower, camelina. Moscow cold storage №14. https://www.moshol14.ru/press-centr/novosti-rynka/maslichnye/

[2] L.V. Omelyanyuk, Selection of peas and soybeans for the conditions of Western Siberia: Authors Abstract of Candidate of Science (PhD) Dissertation (Agricultural Science), Tyumen, 2015. (in russ.)

[3] L.V. Omelyanyuk, A.M. Asanov and A.Yu. Karmazina, "Novelties of breeding of leguminous crops at the FSBSI "Omsk ASC"," Federal Research Center "Krasnoyarsk Scientific Center of the Siberian Branch of the Russian Academy of Sciences", pp. 164-168, 2019 [Optimization of the selection process - a factor of stabilization and growth of crop production in Siberia. OSB – 2019, 2019]. (in russ.)

[4] R.D. Magomedov, N.G. Tsekhmeistruk, V.A. Shelyakin, S.S. Ryabukha and S.V. Didovich, "The influence of various strains Rhizobium japonicum (Kircher) Buchanan on soybean yield," Maslichnye kultury. Nauchno-tehnicheskij byulleten VNIIMK (Oil crops. Scientific and technical Bulletin of VNIIMK), No. 2(148-149), pp. 159-162, 2011. (in russ.)

[5] E.N. Ozyakova, Yield and features of the formation of the symbiotic apparatus of peas and soybeans in the southern forest-steppe of Western Siberia: Authors Abstract of Candidate of Science (PhD) Dissertation (Agricultural Science), Omsk, 2009. (in russ.)

[6] A.V. Tsyganova and V.E. Tsyganov, "Negative hormonal regulation of symbiotic nodule development. II. Salicylic, jasmonic and abscisic acids," Selskochozyajstvennaya biologiya (Agricultural biology), Vol. 53, No. 1, pp. 3-14, 2018 (in russ.) https://doi.org/10.15389/agrobiology.2018.1.3rus

[7] E.N. Mishustin and V.K. Shilnikova, Nodule bacteria and inoculation process. Moscow: Nauka, 1973.

[8] J.J. Terpolilli, G.A. Hood and P.S. Poole, "What determines the efficiency of N_2-fixing Rhizobium-legume symbioses?" Advances in Microbial Physiology, Vol. 60, pp. 325-389, 2012. https://doi.org/10.1016/B978-0-12-398264-3.00005-X

[9] N.A. Provorov, O.P. Onishchuk and O.N. Kurchak, "Impacts of inoculation with Sinorhizobium meliloti strains differing in salt tolerance on the productivity and habitus of alfalfa (Medicago sativa L.)," Selskochozyajstvennaya biologiya (Agricultural biology), Vol. 51, No. 3, pp. 343-350, 2016. (in russ.) https://doi.org/10.15389/agrobiology.2016.3.343rus

[10] M.J. Dilworth, E.K. James, J.I. Sprent and W.E. Newton, Nitrogen-fixing leguminous symbioses. Dordrecht: Springer, 2008. https://doi.org/10.1007/978-1-4020-3548-7

[11] B.P. Pleshkov, Workshop on Plant Biochemistry. Moscow: Kolos, 1976.

[12] A.R. Makarov and A.E. Snitsar, Moisture accumulation and yield of field crops in the arid conditions of Western Siberia. Omsk: Omsk State Agrarian University, 2000.

[13] The climate of Omsk. Wikipedia. https://ru.wikipedia.org/wiki/Климат_Омска