PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

A INTRODUÇÃO DA SOJA (GLYCINE MAX) COMO OBJETO ECONÔMICO E ECOLÓGICO NA REGIÃO NOROESTE DA RÚSSIA

SOYBEAN *(GLYCINE MAX)* AS AN ECONOMIC AND ECOLOGICAL OBJECT OF INTRODUCTION TO THE NORTHWESTERN REGION OF RUSSIA

СОЯ *(GLYCINE MAX)* КАК ХОЗЯЙСТВЕННО-ЭКОЛОГИЧЕСКИЙ ОБЪЕКТ ВВЕДЕНИЯ В СЕВЕРО-ЗАПАДНОМ РЕГИОНЕ РОССИИ

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RESUMO

Introdução: O avanço da soja para as regiões do norte está associado à necessidade de aumentar a produção de proteína vegetal para ração. **Objetivo:** melhorar o solo através da fixação biológica de nitrogênio feita durante o cultivo da soja. **Métodos:** O estudo foi realizado na região de Kostroma de 2007 a 2017. Os objetos do estudo foram: as variedades de soja cultivada Mageva, Svapa, Lancet, Light, Kasatka e microrganismos específicos utilizados para ativar o processo de fixação de nitrogênio. Vários métodos de montagem de experimentos de laboratório, campo e produção foram aplicados em conformidade com os requisitos de coleta e processamento dos dados obtidos. Experimentos de laboratório de inoculação artificial de sementes de soja com microflora fixadora de nitrogênio foram realizados de acordo com o método de G. S. Posypanov. A configuração, plotagem, condução de experimentos de campo e produção e processamento dos dados experimentais coletados foram realizados de acordo com os métodos geralmente aceitos na Federação Russa por B. A. Dospekhov e V. E. Eshchenko. **Resultados e Discussão:** Foi estabelecida a possibilidade de obtenção de material de sementes maduras de soja de até 1,8-2,0 t/ha, com rendimento de proteína bruta de até 800 kg/ha. A inoculação de sementes de soja com cepas de melhoramento de bactérias aumentou seu rendimento em 14-30%, enquanto 80-90% de nitrogênio biológico ecologicamente correto foi acumulado na massa das sementes, aumentando a qualidade forrageira e nutricional dos produtos. Uma cultura local (Russa) de bactérias fixadoras de nitrogênio (K-2) foi utilizada com sucesso. O efeito simbiótico leva à assimilação do nitrogênio atmosférico, que é gasto tanto para a formação das culturas quanto para a reposição das reservas do solo desse elemento químico mais importante. Esse nitrogênio é ecologicamente limpo e é considerado como "nitrogênio biológico" (BN). Calculou-se a oferta de nitrogênio biológico no sistema solo-planta (60-70 kg/ha). Esses valores não compensam totalmente a remoção de nitrogênio com a cultura da soja, mas permitem reduzir o uso de fertilizantes minerais mais caros e ambientalmente menos seguros. **Conclusões:** Os custos econômicos da produção de proteína alimentar equivalente diminuíram duas vezes em comparação com o cultivo de culturas tradicionais, como aveia e cevada. Pela primeira vez, foram estabelecidos resultados específicos sobre a viabilidade de introdução das variedades de soja estudadas na região de Kostroma da Federação Russa.

Palavras-chave: *Variedades de soja, rendimento, proteína, microrganismos fixadores de nitrogênio, nitrogênio biológico.*

ABSTRACT

Background: The advancement of soybeans to the northern regions is associated with the need to increase the production of plant feed protein. **Aim:** to improve the soil through biological nitrogen fixation done during the cultivation of soybeans. **Methods:** The study was conducted in the Kostroma region from 2007-2017. The objects of the study were: the crop soybean varieties Mageva, Svapa, Lancet, Light, Kasatka, and specific microorganisms used to activate the process of nitrogen fixation. Various methods of setting laboratory, field,

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and production experiments were applied in compliance with the requirements of collecting and processing the obtained data. Laboratory experiments on artificial inoculation of soybean seeds with nitrogen-fixing microflora were carried out according to the method of G. S. Posypanov. Setting, plotting, conducting field and production experiments, and processing of the collected experimental data was carried out according to the generally accepted methods in the Russian Federation by B. A. Dospekhov and V. E. Eshchenko. **Results and Discussion:** The possibility of obtaining soybean mature seed material up to 1.8-2.0 t/ha, with gross protein yield up to 800 kg/ha, was established. Inoculation of soybean seeds with breeding strains of bacteria increases their yield by 14-30%, while 80-90% of environmentally friendly biological nitrogen was accumulated in the seed mass, increasing the fodder and nutritional quality of products. A local culture (from Russia) of nitrogen-fixing bacteria (K-2) was successfully used. The symbiotic effect leads to the assimilation of atmospheric nitrogen, which is spent both for crop formation and for replenishment of soil reserves of this most important chemical element. Such nitrogen is ecologically clean and is considered as "biological nitrogen" (BN). The supply of biological nitrogen in the soil-plant system (60-70 kg/ha) was calculated. These values do not fully compensate for nitrogen removal with the soybean crop but allow us to reduce the use of more expensive and less environmentally safe mineral fertilizers. **Conclusions:** The economic costs of producing equivalent feed protein decreased twofold compared to growing traditional crops such as oats and barley. For the first time, specific results on the feasibility of introducing the studied soybean varieties into the Kostroma region of the Russian Federation have been established.

Keywords: *soybean varieties, yield, protein, nitrogen-fixing microorganisms, biological nitrogen*.

АННОТАЦИЯ

Актуальность: Продвижение сои в северные районы связано с необходимостью увеличения производства растительного кормового белка **Цель:** оздоровления почвы за счет биологической азотфиксации. **Методы:** Исследование проводилось в Костромской области в 2007-2017 гг. Объектами исследования являлись: сельскохозяйственная культура соя сортов Магева, Свапа, Ланцетная, Светлая, Касатка и специфические микроорганизмы, применяемые для активизации процесса азотфиксации. Применялись различные методы постановки лабораторных, полевых и производственных опытов с соблюдением требований сбора и обработки полученных данных. Лабораторные опыты по искусственной инокуляции семян сои азотфиксирующей микрофлорой проводились по методике Г.С. Посыпанова. Постановка, закладка, проведение полевых и производственных опытов, обработка собранных экспериментальных данных проводилась по общепринятым в РФ методикам Б.А. Доспехова и В.Е. Ещенко. **Результаты и обсуждение:** Установлены возможности получения зрелого семенного материала сои до 1,8-2,0 т/га с валовым выходом протеина до 800 ц/га. Инокуляция семян сои селекционными штаммами бактерий повышает их урожайность на 14-30%, при этом (80 – 90%) экологически чистого биологического азота аккумулируется именно в семенной массе, повышая кормовые и пищевые качества продукции. С успехом использовали местную культуру азотфиксирующих бактерий (К-2). Симбиотический эффект приводит к усвоению атмосферного азота, который расходуется и на формирование урожая, и на пополнение почвенных запасов этого важнейшего химического элемента. Такой азот является экологически чистым и расценивается как «биологический азот» (БА). Рассчитана обеспеченность биологическим азотом в системе почва-растение (60-70 кг/га). Эти величины не полностью компенсируют вынос азота с урожаем сои, но позволяют сократить применение дорогостоящих и экологически небезопасных минеральных удобрений. **Выводы:** Экономические затраты на производство эквивалентного кормового белка снижаются в два раза по сравнению с выращиванием традиционных культур, таких как овес и ячмень. Впервые были установлены конкретные результаты по целесообразности интродукции исследуемых сортов сои в Костромскую область Российской Федерации.

Ключевые слова: *сорта сои, урожайность, белок, азотфиксирующие микроорганизмы, биологический азот.*

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1. INTRODUCTION:

1.1. Setting the issue

Of all the legumes, soybeans are the most well-known crop due to its biological features and the economic factors connected – the ability to grow on different types of soils, the rich chemical composition of seeds (high content of valuable vegetable protein and fat), and the possibility of widespread use for food, feed, and technical purposes (Posypanov, 1990, 2007). From the environmental point of view, soybeans are the most active symbiont that, together with the specific microflora, can absorb nitrogen from the air. In this case, a large proportion of the crop is formed due to free and environmentally friendly biological nitrogen (BN). In regions of traditional soybean cultivation, biological nitrogen provides seed yields and enters the soil in significant quantities, enriching it and cultivating subsequent crop rotations. Prospects for obtaining more protein with significant savings in expensive nitrogen fertilizers and reducing the negative environmental load on the soil ensure the desire of scientists to promote soybean cultivation in the northern regions of the Russian Federation. In recent years, this opportunity emerged thanks to the achievements of breeders who created a whole group of soybean varieties of the northern ecotype; plants of these varieties have intensive metabolic processes and time to go through all stages of maturation with limited thermal and light resources. Such varieties were: Mageva, Svetlaya, Svapa, Lancet, Kasatka, and others.

1.2. Literature review

Soybean has a wide distribution range; heat requirements (according to the sum of active temperatures) range from 1700-1800 °C to 3500-4000 °C. The first group includes early-ripening varieties with a growing season of 75-90 days; the second includes late-ripening varieties growing 135-150 days (Posypanov, 2007).

Soybeans are quite unpretentious and can be grown on various soils, allowing crop cultivation on all continents. Such widespread cultivation is due to its natural ecological plasticity and the high responsiveness of its genotypes to various breeding techniques, up to genetic transformation methods (Baranov and Baranova, 2011). In addition, an important feature of soybeans is that the crop, being a member of the legume family, has a high potential for symbiotic nitrogen fixation, which increases its ecological significance.

The active advancement of soybeans from traditional places of origin to the more northern regions of the globe (including the Russian Federation) was due to specific values. According to Tomkina E. A., Vodorezova N. N., and Gorodneva N. V. (2008), at the beginning of the 2000s, 19 different varieties of soybeans of domestic and foreign selection from Sweden, France, Poland, and Germany were tested in the experimental field of Yaroslav the Wise Novgorod State University.

The advancement of soybeans to the north in the Russian Federation is gradual. The sown area occupied by soybeans in 2006 in Yaroslavl Oblast was only 20 ha; according to Grinev N. F. (2012), by 2010, the area under soybeans increased sharply, and the share of this crop in the structure of sown areas was also increasing. According to the Russian Statistical Yearbook (FSSS, 2010), in 2009-2010, 60 ha of soybeans were sown annually in Vologda Oblast, and experimental and industrial crops appeared in Novgorod and Pskov Oblasts. Since 2007, introducing soybeans in crop production in Kostroma Oblast has been studied (Bortsova, 2013).

Growing this crop in the northwest involves adapting to stress factors such as the return of spring frosts, excessive humidity, high acidity and low buffering of sod-podzolic soils, a fairly short growing season, and low levels of effective temperatures. These conditions are extreme for the plants and bacteria entering symbiotic relationships (Dozorov and Ermoshkin, 2007).

Breeders working on new varieties are searching for a solution to these problems; researchers-agronomists study the possibilities of cultivating soybeans under different technological regimes, and microbiologists engage in selecting symbiotic microorganisms resistant to the conditions of the non-chernozem zone of Russia. This allows obtaining a wide range of strains of nodule bacteria with diverse physiological activity and different levels of adaptive and symbiotic properties (Kozhemyako, 2011; RAS, 2011). Notably, not only representatives of typical nodule bacteria of the genus *Rhizobium* and *Bradyrhizobium* can enter a relationship with the root system. Other types of associative and symbiotic microorganisms can absorb atmospheric nitrogen both in the root zone of plants and inside the root tissues. In this regard, an interesting study was conducted by a Vietnamese graduate student Phung Thi Mi under the supervision of Professor V.T. Emtsev – in 2015, Phung Thi Mi discovered and proved that bacteria of the genus *Agrobacterium*, living in the root zone of various vegetable plants, have a pronounced and effective form of nitrogen fixation (Phung, 2015). Species of these microorganisms are also present on soybean roots; there is reason to believe that such relationships are formed during the lifespan of the crop and contribute to the enrichment of agricultural systems with biological nitrogen.

Using biological nitrogen in agriculture reduces energy consumption, saves material resources, and reduces environmental pollution by transforming nitrogen fertilizers. In addition, the cultivation of legumes in general, and soybeans in particular, helps optimize the microbiological situation in the soil and improve a number of its physicochemical properties, resulting in significantly increased soil fertility and increased crop yields in crop rotation (Posypanov *et al.,* 1990).

The nodule and associative bacteria activity require a fairly high energy cost provided by a certain part of carbohydrates synthesized in the groundmass of the plant. The active work of the symbiotic microflora thus becomes a supplier of nitrogen-containing compounds, which are assimilated by all parts of the plant and provide high protein content in the seed mass. The process is dynamic and constant, making it very difficult to separate biological and soil nitrogen flows. The study and accounting of the arrival of biological nitrogen in the process of symbiotic nitrogen fixation were given much attention in the works by scientists of K.A. Timiryazev Moscow Agricultural Academy – E.N. Mishustin, V.T. Yemtsev, V.K. Shilnikova, and G.S Posypanov (Mishustin and Shilnikova, 1968; Mishustin, 1985). This question remains relevant; I.A. Chiampitti and F. Salvaggiotti (2018) analyzed data on the productivity and symbiotic activity of soybeans in 733 studies conducted in different countries and in different growing conditions. The analysis showed that the average share of biological nitrogen (BN) in the total nitrogen balance of soybeans ranges from 50 to 80%. However, 100% provision of the needs of the crop with biological nitrogen, even in favorable conditions, is observed only at average yields at the level of 3 t/ha; the higher the seed yield, the higher the additional uptake of soil nitrogen by the crop, and the greater its need for mineral nitrogen fertilizers (Chiampitti and Salvaggiotti, 2018).

The range of values of yield and arrival of biological nitrogen and, consequently, economic and ecological significance of soybean introduction into new areas is not sufficiently studied; this determined the aim of the present study – to assess the feasibility of introducing soybeans in crop production in Kostroma Oblast as a typical region of northwest Russia.

This particular study focused on five issues:

1. Determining the adaptive capacity and productivity of several new soybean varieties (name the varieties here) in Kostroma Oblast;

2. Studying the reaction of soybeans to artificial inoculation of seeds with strains of nitrogen-fixing bacteria and their comparative evaluation;

3. Establishing the value of the active symbiotic potential of soybeans and studying the dynamics of its formation;

4. Finding out the size of the arrival of biological nitrogen in the system plant-soil and, if any, its share in the total balance of soil nitrogen;

5. Production tests of inoculation efficiency of soybean seeds by the most active strains of nodule bacteria and a comparative analysis of the costs of soybean cultivation and the main fodder crops of the region.

2. MATERIALS AND METHODS

2.1. Objects of research

The research was conducted from 2007 to 2017 in Kostroma Oblast, part of the northwestern region of Russia.

The study area was located at the level of 57 degrees north latitude and characterized by a temperate continental climate and soils of sod-podzolic type with medium loamy particle size distribution and medium degree of cultivation:

- Power of the arable horizon 22-23 cm;
- Humus content in the arable horizon 1.82%;
- Available phosphorus content 250-255 mg/kg of soil;
- Mobile potassium content 123-164 mg/kg of soil;
- Acidity (pH) – 5.0-5.6;
- Degree of base saturation 75-78%;
- Absorbed bases 10-12 mg/eq. per 100 g of soil.

Two research object groups were identified: early-ripening soybean varieties of the northern ecotype: Mageva, Svapa, Svetlaya, Kasatka, created at Ryazan Research Institute of Agriculture and the Lancet variety of Belgorod selection. Elite seeds of the above varieties were provided to the authors of the present research on a contractual basis by the above organizations.

Second, the objects of study were specific microorganisms used to activate symbiotic nitrogen fixation. Most of them in the form of experimental batches of soy Nitragin, which is a bacterial preparation containing active races of nodule bacteria (Bacterium radicicicola), which live on the roots of leguminous plants and assimilate nitrogen from the air; it is used to inoculate soybean seeds with microorganisms. It is produced by the enterprise LLC "STC BIO" in Belgorod region of the Russian Federation. It was provided by the Laboratory of Ecology of Symbiotic and Associative Rhizobacteria of the All-Russian Research Institute of Agricultural Microbiology of the Russian Academy of Sciences. The study tested four species of Nitragin based on different strains of bacteria of the genus *Bradyrhizobium*; these were designated under conditional numbers: 626, 634, 640, and 645.

In addition, one of the crops that belonged to the group of local soil microorganisms isolated from the 2012 soybean field was studied; the culture was named K-2 (Figures 1, and 2). Isolation and subsequent cultivation of microorganisms were carried out, taking into account the recommendations of the Research Institute (Khotyanovich, 1991), and own methodological modifications were also used. The microorganisms were grown on a soybean medium (prepared from soy milk, 90 g of soy flour dissolved in 1 liter of water and boiled for 10 minutes) with the addition of the bacterial dye gentian violet (10 mg per 1 liter of medium). Gentian violet has strong bacteriostatic and bactericidal properties, and foreign microorganisms do not grow on such a medium – the culture from the first crops was pure. A MOTIC-300 microscope equipped with a large display, a camera, and multimedia software “Motic Images Plus 2.0 ML” for determining the dimensional characteristics of the studied objects were used during microscopic work. Based on the study of morphological, cultural, and some physiological properties, this culture is preliminarily assigned to the genus *Agrobacterium* of the community of associative microorganisms (Figures 1 and 2). The samples were not kept.

2.2. Methods of field and production experiments

2.2.1. Field experiments

Adaptive and production features of soybeans during its introduction from 2007 to 2010 were studied. First, the crop was cultivated according to the common technology (Posypanov, 2007) with plowing, pre-sowing soil cultivation, and applying mineral fertilizers at the dose of two quintals of NPK/ha (16-16-16). Then, each variety was sown on experimental plots (1 ha); the sowing rate was 800 thousand germinated seeds/ha (120 kg/ha).

The results of the artificial inoculation of the soybean seeds with nitrogen-fixing microflora were studied from 2013 to 2014 according to a two-factor scheme: Factor A (Svetlaya and Kasatka) and Factor B (inoculation of soybean seeds before sowing with active strains of *Rhizobium*: 626, 634, 640, 645, and K-2).

Seeds were treated with nitragin as follows: preparations with appropriate strains of microorganisms: 626, 634, 640, 645, K-2 were applied to seeds on the day of sowing. The preparations were dissolved in water until a homogeneous suspension was formed and then mixed it with seeds. The preparations were dissolved in water until a homogeneous suspension was formed and mixed with seeds. The treated seeds were immediately sown by hand. An increased hectare rate of Nitragin was applied to each plot portion of seeds – 600 mg of the drug with the initial microbial concentration of 10 billion cells per gram, diluted in 20 ml of sterile tap water.

To treat seeds with K-2 culture, grown five-day colonies were washed from the surface of Petri dishes to the concentration of 10 billion cells/mL. Then, the suspension was adjusted to the appropriate microbial concentration of Nitragin and used in the same volume by dilution.

The seeds were thoroughly mixed and sown by hand; the experiment had the following scheme:

Factor A (Svetlaya and Kasatka);

Factor B (according to the drugs used): Option 1 – Control; Option 2 – Nitragin 626; Option 3 – Nitragin 634; Option 4 – Nitragin 640; Option 5 – Nitragin 645; Option 6 –K-2.

The experiment was repeated four times, and the placement of plots was randomized (Figure 3). The plots were located in 2 tiers; the area of each plot was 10 m^2, length – 2 m, width – 5 m. The registered area was 4 m^2 (Eshchenko *et al.*, 2009; Dospekhov, 2011).

2.2.2. Production experiments

Production tests of soybean cultivation

and the effectiveness of symbiotic microflora were conducted from 2015 to 2017 (Tables 10, 11). Crops were created in an agricultural enterprise of Kostroma Oblast - LLC Mechta. The studied soybean variety was Lancet; the sown area was 13 ha. To observe the variability in the common territory, several study sites were laid out for 6 variants of the two-factor type of experience. Factor A – soil pH (5.9 and 6.2), Factor B – Nitragin variants 640 and 645 against the control (without seed treatment) background. Nitragin pH was not determined in the experiment.

2.3. Methods of observations and accounting

When conducting all the field experiments, a unified scheme of observations and accounting was followed according to the methodological recommendations of V. E. Eshchenko *et al*. (2009) and B. A. Dospekhov (2011). In addition to visual observations of the crop conditions and plant growth, test bundles of plants with a root system were selected from an area of 0.25 m^2 for each replicate of any experiment. The number of plants in the sheaf was determined in the laboratory, and 10 random specimens were isolated for complete morphometric analysis on 13 indicators, including the number and weight of nodules, of which the number of active ones (with leghemoglobin) was also counted. Morphometric analysis was performed three times during the growing season of the culture – at the stage of the third leaf during the flowering period, at the beginning of bean formation, and the stage of bean ripening. Thus, according to the two-factor experiment with 6 variants in 4-fold repetition during the growing period, 1440 plants were analyzed.

To determine the yield, the seeds were collected and weighted from each accounting bundle of each repetition, followed by recalculation of indicators per unit area of sowing with corrections for standard seed moisture – 14-15% (see Figure 4).

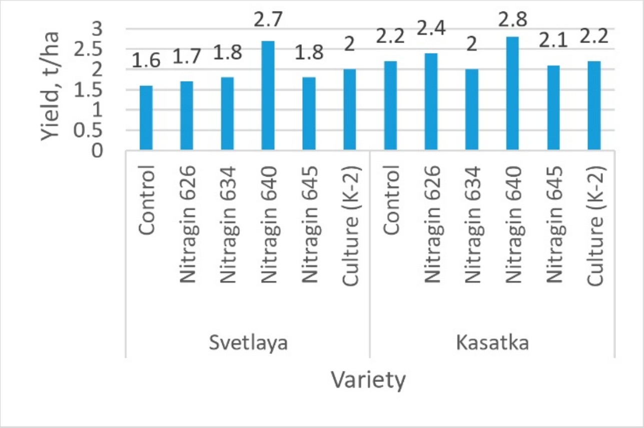

Figure 4. *Graphical representation of the yields. Source: Authors*

2.4. Methods of calculations in determining the arrival of biological nitrogen in the soybean agrocenosis

To assess the intensity of the symbiotic apparatus of culture according to the method by G. S. Posypanov (1991), the index of Active Symbiotic Potential (ASP) was calculated by Equation 1.

$$t * (M_1 + M_2)/2 = ASP \qquad \text{(Eq. 1)}$$

Where t is the time between two analyzes, days, and $(M_1 + M_2)/2$ is the average mass of active nodules, kg/ha.

The product of the mass of active nodules (AN) for the duration of their work is expressed in ASP units kgAN/day.

To determine the effectiveness of the symbiotic potential, the Specific Synthetic Potential (SSP) was calculated according to the method by G.S. Posypanov (1991), equal to the amount of nitrogen absorbed by the nodules per unit of ASP.

$$SSP = \text{the amount of nitrogen absorbed by the nodules / unit of ASP} \qquad \text{(Eq. 2)}$$

The amount of nitrogen fixed from the air for a certain period is calculated by the Equation 3:

$$N = 10 * y * t \qquad \text{(Eq. 3)}$$
$$y = (y_1 + y_2)/2$$

where t is the determination period (days),
y is an average specific nitrogen-fixing activity, g/m^2

10 is the conversion factor from g/m^2 to kg/ha.

SSP (Specific Synthetic Potential) is determined per unit area (g/m^2 to 1 day) or, in our case, per mass of nodules unit (g/kg of active raw nodules in 1 day).

In experiments with artificial inoculation of seeds, the arrival of biological nitrogen in the soil-plant system was determined by the product of the value of the action potential by the value of the specific potential and was referred to as the gross arrival of biological nitrogen. Furthermore, the proportion of biological nitrogen in the seed yield was calculated from the difference in nitrogen content in the seed mass of inoculated and control variants plants, as in Equation 4.

$$ASP * SSP = BN \quad \text{(Eq. 4)}$$

where *ASP* is active symbiotic potential, kg/day, *SSP* is specific synthetic potential, g/kg per day, *BN* is the income of biological nitrogen, kg/ha.

2.5. Statistical data processing

Mathematical analysis of experimental data was performed by variance according to the recommendations of B. A. Dospekhov (2011). The analysis was mainly aimed at identifying the significance of differences between the studied indicators and was expressed by the value of the smallest mean difference (SMD) due to the statistical variance of the experimental results. The digital material was processed using the Statistica Excel software package.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. The results of the study of productivity indicators in soybean cultivation in Kostroma Oblast

Several soybean varieties of the northern ecotype (Mageva, Svetlaya, Svapa, Lancet) were tested at the first stages. The first experiments demonstrated the prospects of a new culture in the region. Seed yields obtained at the thermal resources of the growing season at the level of 1900^0 - 2000^0C were 2.0-2.5 t/ha (Table 1).

The test results showed that the highest seed yield was obtained with the Svapa variety; therefore, it is more adaptable to different weather conditions of the study period. Furthermore, all the crops successfully passed all the stages of plant development before seed ripening; Table 2 shows an example of the process.

3.1.2. The results of studying the effectiveness of pre-sowing inoculation of seeds

According to the observations and calculations conducted in 2013-2014 for crops with pre-sowing seed treatment with experimental varieties of Nitragin and local culture K-2, soybean responds positively to this in the northwestern territories (Table 3).

Seed yields of the Svetlaya variety were, on average, two years higher than those of the Kasatka variety – 1.9-2.5 t/ha, respectively. The positive reaction to seed inoculation by variety was similar, averaging 17.6% for Kasatka and 18.4% for Svetlaya.

The established differences are determined by the work of the photosynthetic and symbiotic apparatus of the studied plants.

The dynamics of leaf surface growth, reflecting the photosynthetic potential of the crop, had certain differences both in varieties and in the applied bacterial cultures (Table 4).

In inoculated plants, changes in leaf mass and area suggest that the outflow of assimilates into the root system increases at the beginning of growth. By the end of the growing season, greater consumption goes to terrestrial organs, especially noticeable on Kasatka plants.

3.1.3. The results of studying the symbiotic apparatus formation of soybeans in Kostroma Oblast

The dynamics of nodule formation on the roots of young plants differed greatly depending on the growing conditions. In particular, 2014 was a much more favorable year in humidification and the distribution of thermal resources. Thus, in the period of sowing until the appearance of shoots and formation of the first trifoliate leaf, 2014 had twice as much precipitation as 2013 (Table 5).

In this regard, the roots had many nodules at the first plant life stages in 2014. However, their number, size, and weight increased significantly during the growth and development of plants (Figs. 4 - 7).

Figure 4. *Nodules on the roots of young soybean plants in 2014 (Svetlaya)*

Figure 5. *Nodules on the roots of young soybean plants in 2014 (Kasatka)*

Figure 6. *Nodules on soybean roots at the stage of seed ripening (Svetlaya)*

Figure 7. *Nodules on soybean roots at the stage of seed ripening (Kasatka)*

Based on the calculation of nodule mass at different stages of plant development, the values of the ASP were determined. Notably, these values for both Kasatka and Svetlaya in 2014 were significantly higher than in 2013 (Table 6).

The presence of nodules and an increase in the yield of soybean seed mass during its cultivation in the northwestern region allowed comparing the amount of biological nitrogen input into the plant-soil system with the consumption of this element from soil reserves. As a result of observing the change in the nitrogen content in the plant mass of inoculated and control plants, the value of specific symbiotic potential (SSP) was determined. For the conditions in the present research, it averaged 6 g of pure nitrogen per ASP unit. The actual data on the nitrogen content in the seed and in the vegetative mass were determined in the State Agrochemical Service 'Kostromskaya' chemical laboratory. Vegetative residues from different experiment variants had similar indicators for the nitrogen content in the absolute dry mass and averaged 2.0%. The characteristics of the seed mass varied somewhat (Table 7).

The data show that the seeds obtained in 2013 had lower protein content and higher fat content. From the studied variants of Nitragin, according to the provision of seeds with fats in 2013, strain 645 stood out in the Kasatka variety. In the more favorable conditions of 2014, the protein content increased while the fat percentage decreased. The priority indicators were again observed when strain 645 was used on both varieties. High protein content indicates a greater amount of nitrogen removed from the soil.

3.1.4. *The results of assessing the size of biological nitrogen entering the ecosystem*

Based on the data obtained, the authors of the present research calculated the ratio of biological and soil nitrogen in the soil-plant system. For clarity, these definitions are demonstrated with Kasatka and Svetlaya grew in 2014. At the same time, indicators of nitrogen removed in the yield at the control took into account that it was formed mainly with soil nitrogen and a certain share of biological nitrogen due to a small number of random symbionts. Thus, the removal of soil nitrogen with the yield in control was determined by the difference between total removal and the share of biological nitrogen equal to 10 kg/ha. The volumes of biological nitrogen in the total removal on plots with inoculation were calculated from the difference between the experimental plots and the control parameters, assuming that the increase in yield and, consequently, in the removal was due to symbionts.

The total nitrogen balance was determined by the difference between the total removal and the total input of biological nitrogen, calculated by the ASP value (Tables 8 and 9).

The amount of biological nitrogen, determined by the ASP value, was considered as 'gross input'.

The calculations show that the gross input of biological nitrogen into the soybean agrocenosis in the conditions of Kostroma Oblast for both varieties ranged from 50 to 90 kg/ha. Of the tested Nitragin strains of microorganisms, 640 and 645 demonstrated the highest effectiveness. Using local culture K-2 for inoculation provided the input of biological nitrogen in 62 kg for the Kasatka variety and 53 kg/ha for the Svetlaya variety.

3.1.5. Production test results and economic calculations

In addition to small-plot experiments to study the effectiveness of artificial inoculation of soybean seeds in 2015, 2016, and 2017, experimental and production crops were created at Mechta, Kostroma Oblast. Strains of nodule bacteria 640 and 645 and soybean varieties Lancet and Svetlaya were used; the sowing area was 13 ha in 2015, 46 ha in 2016, and 50 ha in 2017. The soils were sod-podzolic with high content of phosphorus and potassium (>250 mg/kg soil, according to GOST R 54650-2011) and a pH of 5.9 and 6.2 (according to GOST R 26483-85). The values of the seed yield in these experiments reached from 1.6 t/ha in the control plots to 4.6 t/ha in the experimental variants. The greatest inoculation efficiency was observed when Nitragin was used with bacterial strain 645 (Table 10). At the same time, strain 645 significantly increased the analyzed parameters at both acidity levels. Notably, the activity of strain 640 is expressed in a significant increase in both the mass and the number of nodules; in this variant, the nodules were much larger. The proportion of large nodules in variant 640 was the highest of all – 68% and 61% at pH 5.9 and 6.2, respectively (24% and 28% increase to the control). The large nodules inside were pink, indicating the presence of leghemoglobin. At the same time, ASP reached more than 7000 units, and the estimated input of biological nitrogen averaged 43 kg/ha.

On-farm crops in Mechta LLC, the seed yield in granary weight was 1.5 tons in 2015, 2.2 tons in 2016, and 2 tons/ha in 2017 (Table 11), which allowed Kostroma Oblast to enter the "Rating of subjects of the Russian Federation for the production and yield of soybeans" (Demianova-Roy and Lugovkina, 2016).

The results of observations and calculations reveal that in the conditions of the northwest region, a certain part of the obtained soybean yield is due to biological nitrogen from the air; its amount does not fully compensate for the removal of nitrogen from the soil, yet undoubtedly reduces the needs of the culture in mineral nitrogen. Considering the high protein content in seed products, this circumstance allows recognition of certain economic advantages of soybean cultivation over traditional forage crops in the cultivation zone (Table 12).

As mentioned above, the introduction of soybeans into atypical cultivation areas in Kostroma Oblast was carried out for the first time. It began with defining general indicators of the soybean adaptive capacity to local conditions and its production characteristics. The results showed that almost all the studied varieties of the northern ecotype, with a sufficiently short growing season in this region, managed to complete the physiological development stage and reach the seed maturation stage. Furthermore, shoots, branching, and formation of the leaf apparatus were simultaneous in different plants, unaffected by any diseases and pests. Notably, with sufficiently high yield rates, the seeds obtained from local crops were characterized by high sowing qualities. In particular, the weight of 1000 seeds, depending on the variety, was 110-114 g, and the laboratory germination rate was 90-95% (Demianova-Roy and Bortsova, 2013).

The main, albeit expected, disadvantage in the early years of cultivation was the absence of root nodules. As a result, the entire crop was formed at the expense of soil nitrogen reserves. This was natural for crops on atypical soil plots devoid of the appropriate microflora. Thus, in 2012-2013 studies aimed to activate nitrogen fixation by artificial inoculation of soybean seeds (before sowing) with variants of Nitragin and with K-2 identified by the authors.

A comparative assessment of the effectiveness of various strains of microorganisms revealed that the Kasatka variety had the minimum yield with strain 634. The remaining microorganisms used for seed treatment provided an advantage over the control at 14 to 30%; the most noticeable increases in yield in this variety were with strains 645 and 640. In the Svetlaya variety, the greatest inoculation efficiency was manifested with strains 640 (Table 3). Moreover, in both varieties, inoculating seeds with K-2 led to an increase in the yield by 31% (Kasatka) and 26% (Svetlaya). These values show efficiency at the level of the most active breeding strains.

The vital activity of nodule and associative bacteria requires rather high energy costs. The active work of the symbiotic microflora simultaneously becomes a supplier of nitrogen-containing compounds for all parts of the plant. The work of the photosynthetic apparatus ensures plant productivity; the photosynthetic and the symbiotic apparatus in leguminous plants are closely related. Setting the parameters for the region in question was a key work point. For this, it was necessary to follow the soybean root system colonization with the strains of microorganisms studied by the authors and the dynamics of the ASP indicator.

Notably, the intensity of nodules was different in both soybean varieties and differed in the activity of variants of microorganisms and in the conditions of the growing periods. Thus, under the conditions of 2014, the number and weight of nodules and the ASP value for both varieties were significantly higher than in 2013 (Table 6). Assessment of the dependence of this important indicator on the three factors determined by the authors in the variance analysis showed that the influence of the variety is 17.9%, the effect of drugs is 18.2%, and the external influence is 34.0%.

Evaluating biological nitrogen fixation effectiveness by calculating the ASP indicator is not a sufficiently accurate method; this delicate process can be detailed with sophisticated laboratory equipment. Unfortunately, in the available conditions, the authors of the present research were deprived of such opportunity, yet the method developed by G.S. Posypanov (1991) is still employed in relevant studies, in particular, by A.V. Anisimov in his work on the varieties of peas – the ASP ranged from 10 to 14 thousand units and rose to the maximum of 16.5 thousand when seeds were inoculated with specific forms of Nitragin (Anisimov, 2008).

Having data on the ASP of crops and the nitrogen content in the seed mass allows for assessing the key issue of the present research – the positive effect of soybean crops on the soil and plants and the economic feasibility of introducing the crop into the northwest region.

According to ASP, if the value of the gross input of biological nitrogen is estimated, it reaches 50-90 kg/ha; however, it is only 30-40% of the total removal. Therefore, between 30 and 80% of this biological nitrogen is accumulated in the yield. The highest values were for strains 640, 645, and K-2. Thus, in the Kasatka variety (version with Nitragin 645), most of the gross input of biological nitrogen (90%) went to seeds. In the Svetlaya variety, the maximum input of biological nitrogen in the seed yield was observed when using strain 640 (80%). In the variant with K-2, the gross input of biological nitrogen was identically distributed on both varieties – more than 80% of its total amount was spent on the seed mass (Tables 3.7, 3.8).

Comparing the values of biological nitrogen with the size of its total removal with the yield shows that (under the conditions of the said region) the bulk of the yield obtained is still formed due to soil nitrogen. The nitrogen balance of the soil remains negative in all the variants, and in the Svetlaya variety, it is more pronounced. The gross input of biological nitrogen, determined by the ASP, is about 30% of the total nitrogen removal from the crop. The conclusions of the authors coincide with Chiampitti and Salvaggiotti (2018) data – even in more favorable growing conditions, the full provision of the crop of soybean seeds with biological nitrogen is quite rare. In other words, the hope that the cultivation of soybeans on sod-podzolic soils will lead to their enrichment with nitrogen is not justified. However, it reduces the consumption of mineral nitrogen fertilizers and the area under crops to obtain more feed protein.

3.2. Discussion

The work carried out in Kostroma Oblast for almost ten years (both in experimental and in production mode) indicates that the soybean varieties of the northern ecotype created by breeders are new genetic material, which allows using soybeans as a valuable legume in areas where it was not previously grown. Furthermore, its application can improve the fodder base of livestock farming in the northwest region by obtaining more protein and fat.

Observations of the ability of soybeans to enter into a symbiotic relationship with nitrogen-fixing bacteria revealed the prospects of this characteristic feature. Its systematic cultivation will ensure the accumulation of natural symbiotic and associative microflora in sod-podzolic soils. In the first stages, a specific soy Nitragin created by the employees of the All-Russian Research Institute of Agricultural Microbiology gives favorable results, some of which can also be obtained by local microorganisms of associative nature, isolated from the soil of soybean crops and accessible to the simplest methods of creating enrichment crops. However, the arrival of biological nitrogen in the soil under the conditions in the present research is only about 30% of the total removal of this element with the crop and, therefore, does not lead to nitrogen filling of the soil. However, even with a large removal of nitrogen from one hectare, soybeans can significantly reduce the cost of fodder production by reducing the cost of cultivating the area and of expensive mineral fertilizers. This demonstrates the ecological significance of cultivating this crop.

The greatest difficulty in introducing soybeans is the acquisition of seeds due to their high cost. However, the experiments show that the conditions of the northwest region of the Russian Federation allow obtaining conditioned soybean seeds and designing a system of its seed production. This is facilitated by the engineering group of scientists of the Academy, who created an effective device for drying soybean seeds in the post-harvest mode (Volkhonov *et al.*, 2019).

The prospects of the present research are supported by the general trend of expanding both foreign and domestic research work on this culture. In this regard, the breeding work associated with obtaining early maturing varieties for the northern regions is crucial (Shushkevich and Kust, 2013; Gerasimova, 2021). In addition, deep work is constantly being carried out to create specific bacterial preparations for activating the symbiotic nitrogen fixation of soybeans grown on various types of soils (Kokorina and Kozhemyakov, 2010; Kozhemyakov *et al.,* 2015; Matveeva and Otten, 2019).

Interesting works were presented by scientists from the University of Michigan studying the interaction of soybeans with natural microbial communities in the soil in traditional and organic farming (Longley *et al.,* 2020), and from Viçosa Federal University, in Brazil that have investigated, identified, quantified, and determined the antioxidant activity of flavonoids of soybean (Nagem *et al.*, 1993). All this indicates that the work on introducing soybeans in the northern regions of Russia is widespread and versatile and tends to continue.

4. CONCLUSION

The following specific conclusions can be drawn:

1. When using soybean varieties of the northern ecotype, the cultivation of soybeans in the northwest region allows obtaining the yield of mature and viable seeds on average at the level of 1.8-2.0 t/ha. The total yield of crude protein is 800-1000 kg/ha.

2. Inoculating soybean seeds (before sowing) with active forms of soy Nitragin and strains of local associative microorganisms significantly accelerates the formation of legume-Rhizobium complexes. Among the studied options, Nitragin strains 640, 645, and K-2 are most effective, increasing the seed yield by 14-30%.

3. Intense formation of the legume-Rhizobium complex of soybeans under the studied conditions depends on the type of culture, on the activity of the used strains of nodule bacteria, and, to a greater extent, on the conditions of heat and moisture supply of crops. The ASP on soybean crops in 2014 for both varieties was 7-10 times higher than in 2013 (9 to 16 thousand units on the Svetlaya variety and 10-14 thousand units on the Kasatka variety).

4. The amount of biological nitrogen input into soybean agrocenoses in the northwest region is on average 30% of the total nitrogen removal from soil ecosystems. Moreover, when using the most active inoculants, most (80-90%) of environmentally friendly biological nitrogen is accumulated in the seed mass; this increases the feed and nutritional quality of soy products.

5. In Kostroma Oblast, economic costs are twice as high for oats and barley when producing an equivalent amount of fodder protein as for soybeans. However, this is not to say all the local crops be replaced with soybeans – partial introduction of soybeans into the crop-growing industry of the northwest regions is advisable.

5. DECLARATIONS

5.1. Study Limitations

The study is limited to the samples used in the case.

5.2. Acknowledgements

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5.3. Funding source

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5.4. Competing Interests

The authors declare that the study was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

5.5. Open Access

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FIGURES

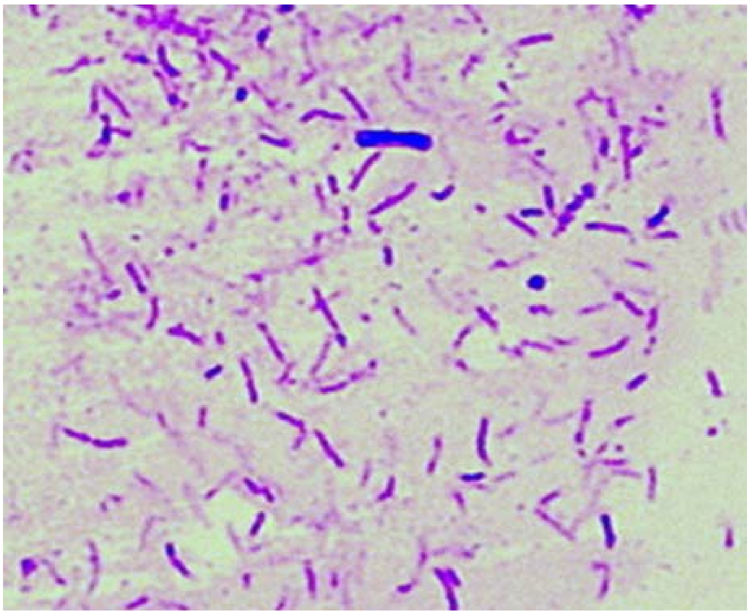

Figure 1. *Morphology of nitrogen-fixing bacteria of strain 645 (MOTIK-300 microscope, 2500-magnification)*

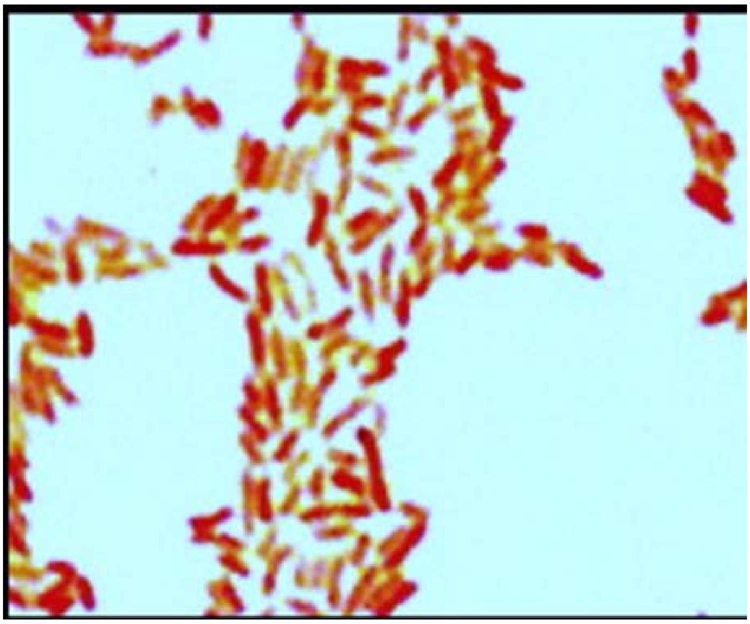

Figure 2. *Morphology of nitrogen-fixing bacteria of culture K-2 (MOTIK-300 microscope, 2500-magnification)*

Figure 3. *Soybean crops in 2013 at the beginning of branching*

TABLES

Table 1. *Seed yield (t / ha) of different soybean varieties in 2007-2010 (Bortsova, 2013)*

Variety	Yield, t/ha				Average for 4 years	Deviation from st.
	2007	2008	2009	2010		
Svetlaya (st)	–	2.1	2.1	1.1	1.8	–
Mageva	2.5	–	1.8	1.3	1.9	+0.1
Lancet	2.2	2.0	2.2	1.4	2.0	+0.2
Svapa	3.0	2.1	2.0	1.5	2.2	+0.4

Table 2. *Phases of soybean development on the example of the Lancet variety in 2010*

Stages of development (beginning of the phase)	Indicators					
	Date	∑ active temperatures, °C	Interphase period, days	Plant height, cm	Number of leaves, pcs/plant	Number of lateral shoots, pcs/plant
Sowing	23.05	38.5		-	-	-
Shoots	02.06	155.8	10	6.0	-	-
Branching	16.06	231.8	14	12.0	3.2	2.0
Beginning of flowering	22.07	529.0	36	23.0	5.2	5.2
Formation of beans	22.08	1438.0	30	50.0	9.0	6.5
Ripening	13.09	1864.0	22	47.0	8.0	7.4
Fully ripe	26.09	1950.0	13	47.4	0.8	-

Table 3. *Seed yield (t/ha) of soybean varieties Kasatka and Svetlaya in the experimental field (2013-2014)*

Seed inoculation options	Kasatka				Svetlaya			
	2013	2014	Average for 2 years	% to the control	2013	2014	Average for 2 years	% to the control
Control (without seed treatment)	1.5	2.0	1.75	100	1.7	2.1	1.9	100
626	1.6	2.4	2.00	114.3	2.0	2.6	2.3	121.0
634	1.9	1.8	1.75	100.0	1.9	2.4	2.2	115.8
640	2.0	2.4	2.20	125.7	2.1	3.0	2.5	131.6
645	2.0	2.7	2.35	134.3	1.8	2.6	2.2	115.8
K-2	2.2	2.4	2.30	131.4	2.3	2.6	2.4	126.3
HCP_{05}	0.15	0.23	0.19		0.22	0.29	0.2	

Table 4. *Dynamics of change in the relative area of leaves (m^2/m^2) of soybean varieties Svetlaya and Kasatka in 2014*

Seed inoculation options	Date					
	30.06.2014		17.07.2014		16.08.2014	
	Variety					
	Svetlaya	Kasatka	Svetlaya	Kasatka	Svetlaya	Kasatka
Control (without seed treatment)	1.07	2.08	3.61	4.55	3.58	4.41
626	1.39	1.81	4.14	4.70	3.36	3.46
634	1.88	1.43	4.48	3.47	3.71	2.93
640	1.86	2.10	3.18	3.98	7.18	3.17
645	1.87	1.77	4.52	4.57	4.43	3.55
K-2	1.47	1.28	6.54	4.12	1.60	3.33

Table 5. *Brief description of weather conditions in 2013-2014*

Growing months	Average daily air temperature, °C		∑ effective temperatures per month, °C		Precipitation, mm		HTC indicator	
	Years							
	2013	2014	2013	2014	2013	2014	2013	2014
May	14.5	14.2	449	440	48.6	59.7	1.08	1.35
June	18.5	15.0	555	450	40.2	113.4	0.72	2.52
July	18.7	18.6	579	477	44.0	59.3	0.76	1.02
August	17.3	18.1	550	562	44.1	92.3	0.8	1.64
September	13.5	11.1	405	333	78.0	52.8	1.9	1.58
Average for the season	16.5	15.5	507.6	452.4	51.0	75.5	1.05	1.62
∑ for the growing season	-	-	2538	2262	254.9	377.5	-	-

Table 6. *Raw weight of active root nodules (kg/ha) and ASP (units) of soybean varieties Kasatka and Svetlaya in the experiments of 2013-2014, the average for the growing season*

Seed inoculation options	Kasatka				Svetlaya			
	2013		2014		2013		2014	
	mass	ASP	mass	ASP	mass	ASP	mass	ASP
Control (without seed treatment)	6.0	365	56.3	3380	0.75	45	35.5	2130
626	38.3	2299	207.4	12447	16	960	160.05	9603
634	31.9	1917	157.2	9432	9.0	540	219.6	13175
640	32.5	1950	204.6	12276	39	2340	268.3	16100
645	32.5	1950	243.9	14634	28.2	1695	262.4	15745
К-2	2.5	150	173.2	10394	14.25	855	138.2	8292

Table 7. *Qualitative indicators of soybean seeds in 2013-2014experiments (% of absolutely dry matter)*

Variety	Options	Fat		Protein		Nitrogen	
		2013	2014	2013	2014	2013	2014
Svetlaya	Control (without seed treatment)	21.14	18.83	30.00	39.65	4.80	6.34
	634	20.54	19.36	32.25	41.25	5.16	6.60
	645	18.27	17.08	39.38	44.19	6.03	6.60
	К-2	20.65	17.28	30.46	40.10	4.87	6.41
Kasatka	Control (without seed treatment)	21.50	18.62	28.69	37.29	4.59	5.96
	634	21.36	18.67	29.75	38.83	4.76	6.21
	645	30.90	17.41	28.31	40.21	4.53	6.41
	К-2	23.28	18.80	27.13	38.12	4.34	6.10

Table 8. *The ratio of soil and biological nitrogen in the plant-soil system in the Kasatka variety in 2014*

Indicators	Seed inoculation options					
	Control (without seed treatment)	626	634	640	645	К-2
Seed yield, t/ha	1.7	2.13	1.79	2.43	2.66	2.4
Total nitrogen removal with the yield, kg/ha	91.2	127.8	107.4	145.8	170.5	146.4
Biological nitrogen (BN) in the yield, kg/ha	10.1	36.6	16.2	54.6	79.3	55.2
Share of BN in the yield, % of the total removal	9.9	28.6	15.1	37.4	46.5	37.7
Average ASP for the period, (units)	3380	12447	9432	12276	14634	10394
BN input according to SSP, kg/ha	20.3	74.6	56.6	73.6	87.8	62.4
Share of BN in the yield, % from the gross income	49.8	49.1	28.6	74.2	90.3	88.5
Nitrogen balance, kg/ha	- 81.10	-53.2	- 50.8	-72.2	-82.7	-84.0

Table 9. *The ratio of soil and biological nitrogen in the plant-soil system in the Svetlaya variety in 2014*

Analyzed	Seed inoculation options				
	626	634	640	645	K-2
Seed yield, t/ha	2.6	2.4	3.0	2.6	2.6
Total nitrogen removal with the yield, kg/ha	164.8	158.4	198.0	171.6	166.4
Biological nitrogen (BA) in the yield, kg/ha	44.7	38.3	78.0	51.5	46.3
Share of BN in the yield, % of the total removal	27.3	24.2	39.4	30.0	27.8
Average ASP for the period, (units)	9603	13175	16100	15745	8880
BN input according to SSP, kg/ha	57.6	79.1	96.6	94.5	53.3
Share of BN in the yield, % from the gross income	77.6	48.4	80.7	54.5	86.9
Nitrogen balance, kg/ha	- 107.2	- 79.3	- 101.4	- 77.1	- 113.1

Table 10. *Number and weight of nodules per plant and the active symbiotic potential of the Lancet variety soybean crops (kg * days/ha), on average for 2015-2017.*

Variety		Number of nodules per plant, pcs/plant	Weight of nodules per plant, g	SSP	ASP
pH 5,9	Control (without seed treatment)	1.3	0.91	73.6	50.6
	Strain 640	6.6	1.85	5520.0	4912.5
	Strain 645	4.6	1.34	4468.8	4303.2
pH 6,2	Control (without seed treatment)	1.7	1.04	117.3	98.9
	Strain 640	6.7	1.87	7535.0	7120.0
	Strain 645	6.5	1.71	6589.2	6939.4

Table 11. *Elements of structure and yield of soybean crops of the Lancet variety, on average for 2015-2017, t/ha*

Variety		Elements of the yield structure of soybean plants			
		Number of beans, pcs/plant	Number of seeds in a pod, pcs.	Seed weight, g/plant	Biological yield, t/ha
pH 5,9	Control (without seed treatment)	21.3	2.5	10.7	1.5
	Strain 640	25.1	2.9	24.7	2.7
	Strain 645	39.3	2.8	22.8	4.6
pH 6,2	Control (without seed treatment)	20.2	2.4	9.8	2.9
	Strain 640	30.7	2.7	27.9	4.2
	Strain 645	38.6	3.0	23.5	4.3

***Table** 12. Comparative assessment of the cost of producing 1000 kg of protein in the cultivation of soybeans and traditional forage crops in the northwest region of the Russian Federation*

Analyzed indicators	Crops		
	Soybeans (*Glycine max*)	Oats (*Avena sativa*)	Barley (*Hordeum distichon*)
Grain yield, t/ha	2.0	2.0	2.0
Average protein content in grain,%	40.0	11.0	10.0
Protein yield, kg/ha	800	220	200
Removal of nitrogen with grain mass, kg/ha	120	40	40
Biological nitrogen input, kg/ha	50	–	–
Missing amount of nitrogen, kg/ha	70	40	40
Area required for the production of 1 ton of protein, ha	1.25	3.5	4.0
Total mineral nitrogen required, kg/t of protein	87.5	140	160
Total mineral fertilizers (in the form of Nitrophoska) required	547	875	1000
Expenses for mineral complex fertilizers (in the form of Nitrophoska), RUB	10000	17500	20000
Seed costs for the entire area, RUB	3750	5250	6000
Cost of crop cultivating to obtain 1 ton of protein, RUB	8444	47250	54000
Total costs for the production of 1 ton of protein, RUB	22194	53000	54000