UDK 631.461.5:633.34

Evaluation of the effect of symbiotic nitrogen fixation on the yield of soybean and nitrogen state of soil

A.V. Revtie-Uvarova

NSC «Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky», *Kharkiv, Ukraine*

ARTICLE INFO

Received 29.03.2019
Received in revised form 02.05.2019
Accepted 19.08.2019
Available online 01.09.2019

Keywords:

chernozem typical;
nitrogen;
inoculation;
mineral nitrogen reserves;
soybean plants development;
symbiotic nitrogen fixation.

ABSTRACT

Under the conditions of the low-field experiment on chernozems typical low humus, it was estimated the agronomic efficiency of symbiotic nitrogen fixation for growing soybean by comparing indicators of soil condition and morphometric characteristics of soybean plants, the seeds of which were treated with a preparation based on nodule bacteria *Bradyrhizobium japonicum* with untreated seeds (non-inculcated). It was established that symbiotic nitrogen fixation contributes to the formation of a more powerful root system with active nodules, an increase in the growth in intensity of growth of the vegetative mass by 42 %, and an improvement in the supply of nitrogen to soybean plants, as assessed by chlorophyll content in leaves. The yield of soybean grain increases by 29 %, which, like vegetative mass and roots, contains more nitrogen and is characterized by a tendency to a decrease in the content of phosphorus and potassium. It was determined that against the background of a decrease in the ratio of secondary products to soybean grain (1.5 : 1), nitrogen removal with vegetative mass (2.2 times) and grain (by 32 %) are increased. It was established that part of the biologically fixed nitrogen in the formation of the biological yield of soybean obtained on chernozem typical under the weather conditions of 2018 is 43 %, and the available soil nitrogen is 57 %. To diagnose the availability of soil with nitrogen in the aftereffect of symbiotic nitrogen fixation, it is proposed to determine the «net» nitrogen-fixation by the actual reserves of mineral nitrogen under inoculated soybean (minus its reserves under non-inoculated) and by the potential amount of nitrogen that additionally comes with the total vegetative mass (by the difference between the nitrogen content of vegetative mass inoculated and non-inoculated soybean). In the weather conditions of 2018 in the typical chernozem «net» nitrogen fixation under soybean was 69 kg N/ha, of which 40 kg/ha are the reserves of mineral nitrogen in the soil and 29 kg/ha are nitrogen coming from the vegetative mass.

*E-mail: *alina_rev@meta.ua*

Citing: Revtie-Uvarova A.V. 2019. Evaluation of the effect of symbiotic nitrogen fixation on the yield of soybean and nitrogen state of soil. Agrochemistry and Soil Science. Collected papers. No. 88. Kharkiv: NSC ISSAR, P. 79-88. DOI: https://doi.org/10.31073/acss88-11.

1. Introduction

A unique feature of leguminous crops, which ensures the functioning of one of the links of the nitrogen cycle and is of particular practical interest, is the ability to enter into symbiotic relationships with nodule bacteria capable of fixing atmospheric nitrogen. By value for nature, nitrogen fixation, which is characterized by a complex biochemical mechanism, is equated to another global process – photosynthesis. [1]. It should also be noted that legumes are characterized by exceptional nutritional benefits and favorable agrotechnical properties. Celebrating the role of these cultures in securing world food security at the 68th session of the UN General Assembly, 2016 was proclaimed the International Year of leguminous crops; FAO has been instructed to coordinate and facilitate special events [2].

For the first time, direct evidence of the existence of microorganisms that capture nitrogen, obtained in the late XIX century by a German researcher H. Helgerigel. He proved that legumes can, in addition to mineral nitrogen, have the ability to absorb nitrogen from the air due to the presence of tubers on the root system, which is a unique ecological niche for rhizobia supplying nitrogen fixation products to the host plant, which, in turn, provides bacteria with carbohydrates and other compounds [3]. Microorganisms, who can assimilate molecular nitrogen, are called diazotophs. Biochemical mechanism of fixings is to restore of N_2 to NH_3 with the participation the enzyme of nitrogenase, which is located in the inner cell membranes [1].

Essential developments are the study of biological, biochemical and physiological mechanisms of symbiotic nitrogen fixation and the influence of various anthropogenic and natural factors on the functioning of the legume-rhizobial complex. The development of biopreparations for inoculation and selection of new strains of bacteria was carried out at Zabolotny Institute of Microbiology and Virology of the NASU (Patyka V.P.) [4], in the department of symbiotic nitrogen fixation at the Institute of Plant Physiology and Genetics National Academy of Sciences of Ukraine (Morgun V.V., Kots S.Ya.) [5] and at the Institute of Agricultural Microbiology and Agricultural Production NAAS (Volkogon V.V) [6]. A lot of scientists' attention is devoted to finding a universal method for determining the true extent of nitrogen fixation, disclosing the mechanism of molecular nitrogen reduction, determining the principles of action and regulation possibilities of enzyme systems associated with this process, in order to improve conditions and improve the efficiency of biological fixation of molecular nitrogen [7, 8].

In the agronomical sense, the question remains of a practical plan regarding the direct effect of symbiotic nitrogen fixation on the growth and development of the host plant and on the actual amounts of nitrogen that remain in the soil after growing legumes. The complexity of the experimental solution of this issue is due to the significant influence on the flow of symbiotic fixation of atmospheric nitrogen as anthropogenically controlled factors, that is, elements of the cultivation technology (tillage, the use of macro- and micronutrients, bacterial preparations and biostimulants, plant protection system), and a purely natural effect. This causes significant differences and variations in the results obtained in research.

Among other leguminous crops, it is believed that soybeans (*Glicine hispida L.*) assimilate significantly more atmospheric nitrogen, second only to perennial legumes, such as clover or alfalfa [9]. Its dominance is traced in the structure of the acreage of legumes. In 2017 by soybeans 81 % were occupied, which is 4.4 times more than the area from which other leguminous crops were harvested (454.9 thousand hectares). In the period from 1990 to 2017 (Fig. 1), the area of soybean crops increased from 87.8 to 1981.9 thousand hectares, and the improvement of cultivation technologies, taking into account the weather conditions of the year, ensured an increase in yield over this time from 9.7 to 23 centners per hectare [10].

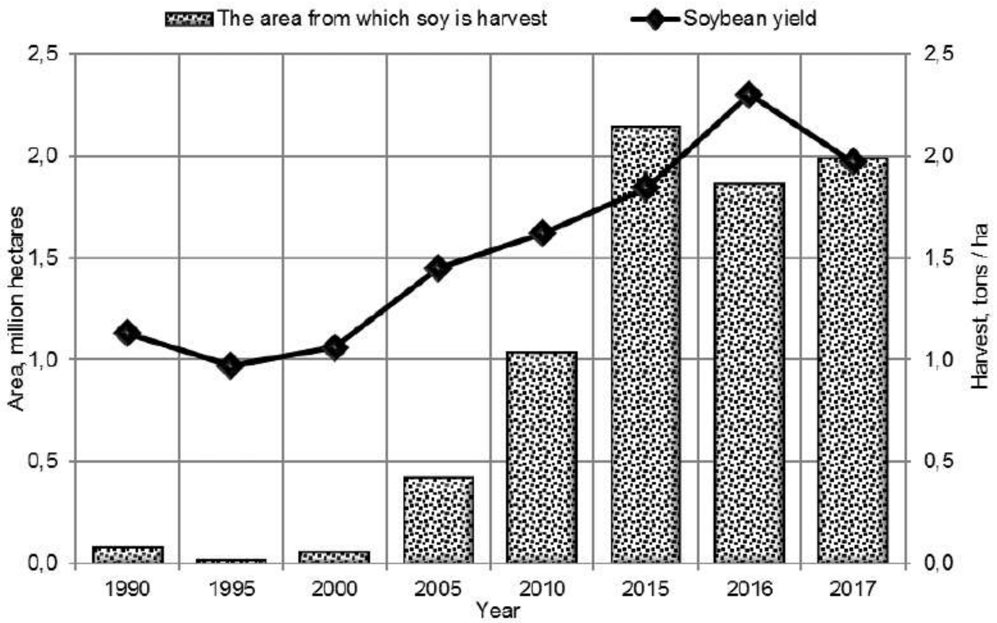


Fig. 1. *Dynamics of the areas from which the soybeans are harvested and its yield in Ukraine [10]*

According to the results of studies conducted in Ukraine and abroad, it was found that the amount of nitrogen that can be fixed under soybean in symbiosis with nodule bacteria ranges from 90 to 240 kg / ha [11]. According to other data, for the south of Ukraine, under optimal conditions of growth and development, soybean plants are able to fix 70–280 kg / ha of nitrogen, from which from 20 to 35 % enters the soil with crop residues [12]. So, the soybean of the northern ecotype with a yield of 3.1 t / ha fixes more than 200 kg of nitrogen of air per 1 ha [13].

In the conditions of Polissia Ukraine, on medium-secured nutritional elements light-gray soils the use of elements of intensive soybean cultivation technology makes it possible to enrich the soil with biological nitrogen, which corresponds to the equivalent of ammonium nitrate – 151.5 kg / ha [14]. It is known from analytical generalization of information performed by the team headed by V. Patyka [4] that the dimensions of symbiotic nitrogen fixation under soy are equivalent to a nitrogen fertilizer rate of 25–35 kg / ha and are 50–90 kg of nitrogen / ha / year, which 10-20 kg / ha remains in the soil.

In studies of African colleagues with fourteen soybean varieties, was found of variations in accumulated symbiotic fixed nitrogen from 51 to 148 kg / ha [15 When growing soybeans of the domestic variety Podolskaya-1 in favorable conditions for active symbiosis of bacteria and plants, about 104 kg / ha of nitrogen are recorded, in the best moisture content of the year –

110-133 kg / ha [16]. For the Gorlitz variety, depending on the weather conditions of the growing year, the nitrogen fixation volumes vary from 63.6 to 110.6 kg / ha, for the Vinnichanka variety – 78.3–133.1 kg / ha, reaching an average of 83.7 and 99,4 kg / ha, respectively [17].

The significant discrepancies in the volumes of symbiotically fixed nitrogen under the same crop are due to differences in research (soil and climatic conditions of the territory, varietal characteristics and various agrotechnological methods of cultivation, methods for determining the volumes of fixed nitrogen), so the most representative information can be obtained in a local context for specific conditions.

The aim of our study was to evaluate the agronomic efficiency of symbiotic nitrogen fixation stimulated by selective strains of bacteria when soybean is grown in the soil and climatic conditions of the Left Bank Forest-Steppe on chernozem typical by measuring some biometric parameters of plants during the growing season, actual and potential amount of nitrogen entering the soil due to symbiotic nitrogen fixation.

2. Materials and methods

Experimental work was conducted in the low-field experiment established in 2018 (according to DSTU 7080) on the territory of ISSAR State Enterprise «Experimental Farm «Grakivske», which is located near the village of Korotych in Kharkiv region, in the Left-Bank Forest-Steppe of Ukraine. The soil cover is represented by chernozem typical low humus heavy loam on loess-like loam. Soil in the arable layer, contains total humus – 4.3 %; total nitrogen – 0.22 %; total phosphorus – 0.12 %; total potassium – 2.2 %; exchangeable Calcium – 258 mmol / kg of soil; exchangeable Magnesium – 46 mmol / kg of soil. The total exchangeable cations are 308 mmol / kg of soil, the content of granulometric fractions with size <0.01 mm is 51.1 %, $pH_{(KCl)}$ 5.4.

To perform the tasks, among the spectrum of existing methods for determining the amount of fixed nitrogen was chosen the method of comparing the yield and state parameters of soybean plants, the seeds of which were treated with a preparation based on nodule bacteria with untreated seeds (non-inculcated) [18, 19]. For its implementation by the scheme of low-field experiment, two variant are provided: 1. Soybean (control) is not-inoculated; 2. Inoculated soybean. The experience is laid in quadruplicate; the total number of plots is 8. The rate of the elementary plot is 6 m^2 (2 m x 3 m). Protective side strips – 0.5 m, protective end strips – 1.0 m. In the experiment grown early maturing variety soybean Avatar, entered in the register of sorts of Ukraine in 2017.

Soybean seed treatment with an inoculum, which contains specific virulent strains of the nodule bacteria *Bradyrhizobium japonicum* (dose – 300 ml of the drug per 100 kg of seeds), was carried out on the day of sowing.

Elements of the technology of soybean cultivation were common for the region of research. According to crop rotation barley was the predecessor of soybean. Since 1970, soybean has not been grown on the field. From the family of legumes sowed vetch as part of mixture vetch (*Vicia sativa L.*) and oat (*Avena sativa L.*) last time in 2013. Plant protection from segetal vegetation was performed mechanically (manually).

During the period of vegetation of soybeans, three surveys of plant biometrics were carried out by phase: branching (leaf development) (20.06.2018), flowering (04.07.2018) and development of beans and seeds (07.31.2018).

Under field conditions, plant express diagnostics was performed with using the portable device «SPAD-502 Plus» («N-tester») designed to determine the need of plants for nitrogen by the color of the leaves. The measurements carried out in three replications on each variant of the experiment, where 30 plants were measured to obtain one mean value [20]. Also the intensity of growth of the vegetative mass and the mass of the root system of soybean plants was determined by directly weighing them, the number of nodules was considered (representative sample of 5 plants).

Analytical studies included the determination of mineral nitrogen in soil samples according to DSTU 4729 [21] and the determination of total nitrogen, phosphorus and potassium in plants materials of soybean, differentiated by vegetative (leafy mass and roots) and generative (seed) organs in accordance with MVV 31-497058-019-2005 [22].

Harvesting was carried out manually, by solid cutting of plants from each elementary site with their subsequent threshing and recalculation of the obtained grain weight into yield (tons per hectare) [23]. The yield index was calculated as the fraction of the grain weight in the total vegetative mass of the plant (%) at the time of harvest [19].

3. Results and discussion

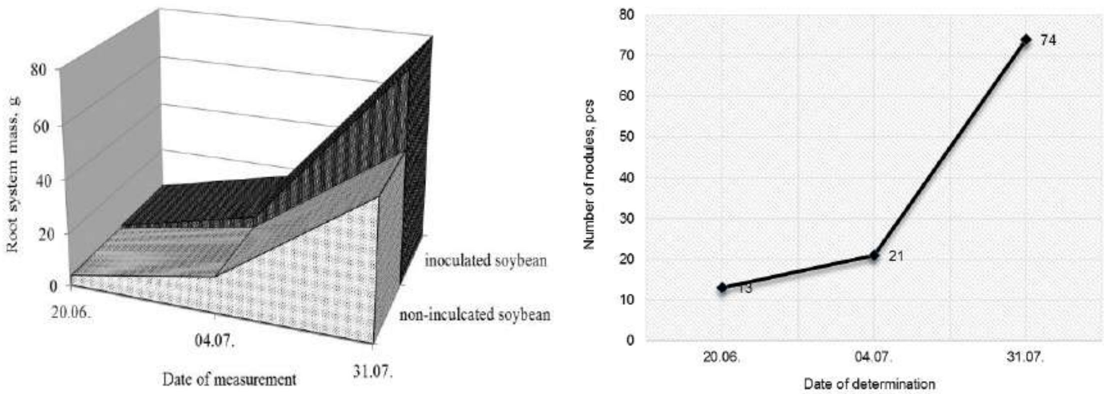
3.1 Evaluation of the effect of symbiotic nitrogen fixation on plant development

The intensity of symbiotic nitrogen fixation depends on a number of factors, among which acidity, temperature and soil aeration, moisture and nutrient supply of plants, specific strains of microorganisms and varietal characteristics of plants play a significant role [24, 25]. pH values of 6.5–7.5 are considered optimal for most species of nodule bacteria, soil moisture in the range of 40–80 % of field capacity (with a lack of moisture, the intensity of photosynthesis in plants decreases, and when waterlogging – nodules do not have enough oxygen) and an optimum temperature is about 20 °C. Under these conditions, the biological fixation nitrogen of soybean may reach 390 kg / ha [26].

In soils of agrocenoses, where soybean has not been grown for a long time, among the native soil microflora, virulent strains of rhizobia are usually absent, therefore the symbiotic nitrogen fixation process does not actually occur in the first year of seeding. An artificial factor in the activation of plant symbiosis with nodule bacteria is seed inoculation with selective strains of nodule bacteria of a specific genotype [26–29]. Guided by this fact and the principle of the only difference in conducting the experiment, we can distinguish the effect of symbiotic nitrogen fixation on the studied parameters. The plots were compared with the cultivation of non-inoculated soybean, where the symbiotic apparatus are not actually formed and functioning, with the plots with inoculated soybean where recognition occurs, infection of the root system *Bradyrhizobium japonicum*, nodulation (formation of root nodules) and, accordingly, symbiotic fixation of nitrogen out in the air [1, 30, 31]

Soybean plants have a well-developed taproot system, which consists of a relatively short primary taproot, from which a large number of secondary, tertiary and further orders long roots extend in the upper part [25]. According to research results, in weather conditions 2018, inoculated soybean plants are characterized by a more powerful root system. The average weight of the roots of five plants of inoculated soybean (Fig. 2.A) increased from 4.3 g at the first determination to 80.0 g at the last, while the weight roots of the control soybean (not-inoculated) from 3.7 to 51, 3 g, respectively.

It is believed that the process of nitrogen fixation begins from the phase trifoliate leaf development to mass flowering, is intense during the phase formation and the beginning of development of beans and weakens towards the end of the plant ontogenesis. Usually, the active work of nodules lasts about 40–45 days [24, 25]. During the 41 days that passed from the first to the third metering, an increase in the number of bubbles (Fig. 2) was found on the roots of one inoculated soybean plant (Fig. 2.B), on average, 61 pieces (from 13 to 74), while on the roots of some control plants only single nodules.



A. Dynamics soybean root mass

B. Dynamics of the number of nodules on the roots of inoculated soybeans

Fig. 2. *Dynamics of the development of the root system of soybean plants*

It should be noted that when calculating the nodules were in the active phase, as evidenced their red color, determined by visual diagnosis, which provides for their dissection. It is believed that the more saturated the color, the more intense the fixation of atmospheric nitrogen proceeds. The red color of the nodules is due to the formation of leghemoglobin, a red iron-containing protein that is a product of the symbiosis of two organisms and consists of heme synthesized by nodule bacteria (bacteroids) and globin formed by cells of a higher plant. With the cessation of nitrogen fixation, leghemoglobin is destroyed, and green pigments, choleglobin, are formed, which is a sign of the cessation or absence of nitrogen fixation [32].

At the time of harvesting, which corresponds to the phase of senescence, the root system of inoculated plants according to the content of macronutrients was characterized by a tendency to an increase in the nitrogen content and a decrease in the phosphorus and potassium content. Nodules contained an average of 3.05 % nitrogen, 0.84 % phosphorus, and 0.34 % potassium (Table 1).

Table 1
The content of nitrogen, phosphorus and potassium in the underground part of the vegetative organs of soybean plants

Variant	Part of the plant	Contents, %		
		nitrogen	phosphorus	potassium
Non-inoculated soybean	roots	0,65	0,41	0,53
Inoculated soybean	roots nodule	0,87 3,05	0,23 0,84	0,24 0,34
LSD $_{0,95}$		0,37	0,11	0,24

For evaluate the influence of symbiotic nitrogen fixation on growth and development of plants, an indicator of intensity of growth of vegetative mass is used as indicator, which can be different even with the same of soil and weather conditions. From inoculation of soybean seeds before sowing, it can be traced (Fig. 3), a natural gradual increase in the intensity of vegetative mass growth of plants during their vegetation compared to control plants. From the phase of branching to the phase of development of beans and seeds, the vegetative mass of inoculated soybeans increased in 13 times, while control plants – 11 times. In general, at the time of the last measurement, due to symbiotic nitrogen fixation, the vegetative mass of soybean increased by 42 %.

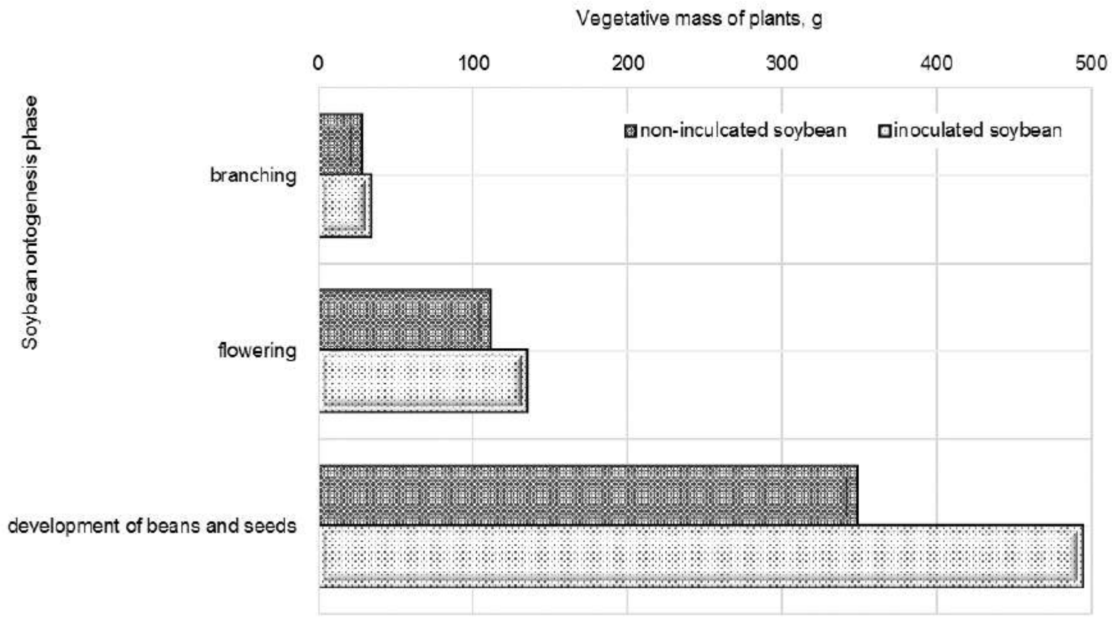


Fig. 3. *Intensity of growth of vegetative mass*

According to the results of plant express diagnostics, it was determined that the content in non-inoculated soybean leaves from the flowering phase to the phase of development of beans and seeds reduced the chlorophyll content from 40.4 to 35.0 units, while the leaves of inoculated soybean characterized by N-tester values of 42.6 and 41.3, respectively (Fig. 4). Better development of plants is confirmed by intense green coloration and greater uniformity in height in visual observations.

Fig. 4. *Chlorophyll content in terms of N-tester indicator*

In this way, symbiotic nitrogen fixation improves the nitrogen nutrition of inoculated plants, as evidenced by the indicator of the intensity of growth of the vegetative mass and the content of chlorophyll in the leaves. Nitrogen, fixed by nodule bacteria from the air, enters the tissues of the host plant in the form of ammonium and is included in its biomass [24]. In the phase of senescence (determined by the dying off of the stems and the complete drying of the beans), the nitrogen content in the vegetative mass of non-inoculated soybean plants was 0.71 % compared to more than twice as much in inoculated plants as 1.59 %. In the grain of inoculated soybean was 1.27 % more nitrogen – 4.01 and 5.28 %, respectively.

An integrated indicator of the efficiency of growing crops, the result of biological and biophysical processes occurring in plant organisms, depending on the genetic nature of the plant itself and the environmental conditions, is yield. Due to the inoculation of soybean seeds before sowing, the yield increase was 5 c / ha (29 %) compared with the control. The effectiveness of the treatment of soybean seeds before sowing *Bradyrhizobium japonicum* is confirmed by studies in the Americas, where in most of the experimental plots – 187 in the USA and 152 in Argentina, substantial increases were obtained in soybean yield [33].

In our studies, the yield index, which is calculated as the proportion of seed mass of the total vegetative mass of plants (primary and secondary production) at the time of harvest [19], increased by 9.6 % compared with the control. In relation of secondary products to soybean grain, an inverse relationship is observed: 1.5 : 1 at inoculation and 2.2 : 1 under control (Table 2), which indicates a more rational consumption of nutrient against the background of additional supply of nitrogen, which does not contribute to the formation excessive vegetative mass.

Table 2
Soybean productivity

Variant	Secondary production, t / ha	Grain harvest, t / ha	Yield index, %	Relation of secondary products to soybean grain
Non-inoculated soybean	3,9	1,7	30,4	2,3
Inoculated soybean	3,3	2,2	40,0	1,5
LSD $_{0,95}$	0,74	0,37	-	-

Studies by V.I. Netis [34] found that inoculation of seeds before sowing stimulates growth processes in soybean plants and contributes to the accumulation of a greater above-ground mass, as a result of which the removal of nutrients increases from the soil. Optimization of symbiotic nitrogen-fixing activity when growing early maturing variety soybean on typical chernozems contributed to an increase in nitrogen removal to form 1 ton of both vegetative mass (2.2 times) and grain (by 32 %) compared to non-inoculated soybeans (Fig. 5).

There is a clear tendency to reduce the removal of phosphorus and potassium in vegetative (leafy mass and roots), and generative (seed) organs, at additional provision of plants with biologically fixed nitrogen.

To produce 1 ton of biological yield, the removal of nutrients at inoculation was 68.7 kg of nitrogen, 16.6 kg of phosphorus, 40.6 kg of potassium, while in non-inoculated soybeans, respectively, 47.2 kg, 21.7 kg and 45.2 kg. Herewith the share of removal of nutrients by soybean grain (per ton of biological yield) at seed inoculation is 77 % for nitrogen compared to 85 % for non-inoculated soybean, for phosphorus – 76 and 76 %, and potassium – 57 and 55 %, respectively.

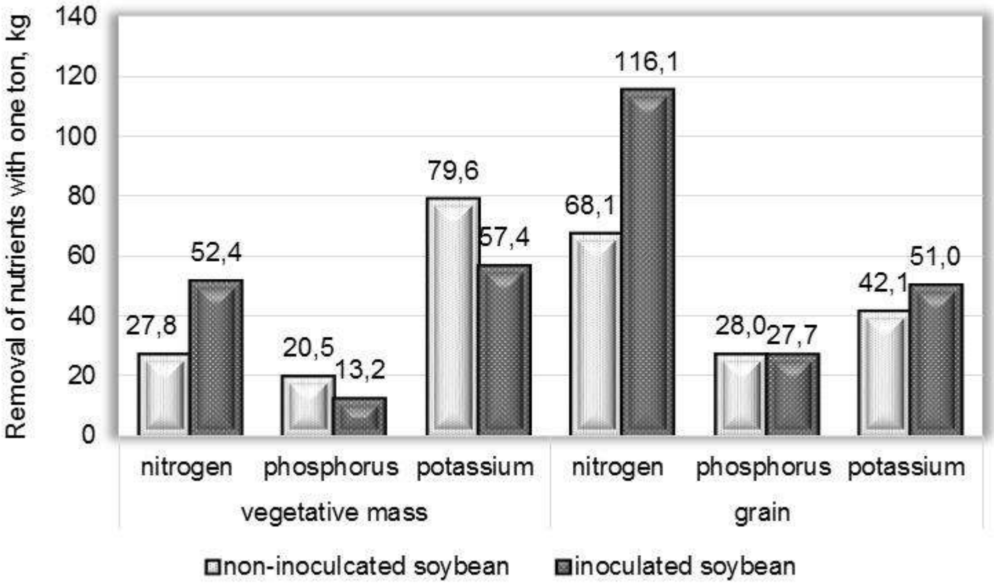


Fig. 5. *Removal of nutrients with one ton of vegetative mass and soybean grain*

It is believed that due to the symbiosis of soybeans with nodule bacteria, up to 70 % of its nitrogen requirement is satisfied [35, 36]. Studies by F. Salvagiotti and colleagues [37] found that leguminous plants satisfy 36–69 % of the total nitrogen requirement due to symbiotic bonds with *Bradyrhizobium japonicum*. In our case, 43 % of the removal of nitrogen from the biological yield of soybeans per hectare of area was satisfied by biological nitrogen, 57 % is available soil nitrogen.

So, due to symbiotic nitrogen fixation, the yield of soybean grain increases by 29 %, in which an increase in the nitrogen content and a decrease in phosphorus and potassium are recorded. The vegetative mass of inoculated soybean plants accumulates more organic bound nitrogen, which is a potential source for replenishing the soil's nitrogen stock.

3.2. The effect of symbiotic nitrogen fixation on the nitrogen state of chernozem typical

In the soils of agrocenoses, the regulation of the nitrogen regime is carried out primarily through the use of mineral fertilizers. When growing legumes, it becomes possible to additionally accumulate atmospheric nitrogen in the soil by symbiotic fixation. That is why, in agronomic practice, legumes are considered to be good predecessors for other crops, since some nitrogen remains after them, which makes it possible to adjust the doses of nitrogen fertilizers in the direction of reduction. However, the question remains about the actual amount of biologically fixed nitrogen, which remains in the soil after the cultivation of leguminous crops.

It is believed that nitrogen fertilizer inhibits nitrogen-fixing ability, and the stronger, the higher the dose of nitrogen. With sufficient provision of soil nitrogen, soybean plants, first of all, consume nitrogen of mineral compounds, as the least energy-intensive in the physiological sense, then – biologically fixed nitrogen, which is a minor source of nutrition. Therefore, symbiotic nitrogen fixation either does not occur or is inhibited [24]. In scientific circles, about the fertilizer soybean points of view are diametrically divergent: from the inexpediency of introducing nitrogen fertilizers to applying them in high doses, regardless of the effect on symbiotic nitrogen fixation [38].

In accordance with the objectives of our research, in order to exclude the effect of technical nitrogen from fertilizers on the course of symbiotic nitrogen fixation, on the content and reserves of mineral nitrogen, the borders of the low-field experiment were located in an area where mineral fertilizers have not been applied since 1970. This allows you to quantify the amount of mineral nitrogen accumulated by biological fixation of nitrogen.

In the first decade of October, after harvesting, the content of mineral nitrogen in the 0-20 cm layer of chernozem typical under inoculated soybean was on average 18.4 mg / kg of soil higher than under non-inoculated soybean, that is, an increase from very low of amount to the average [39]. Mineral nitrogen reserves increased, respectively, from 5.8 kg / ha under non-inoculated soybeans to 46.1 kg / ha of soil when inoculated with seeds before sowing. The difference between these values determined the value of the actual accumulation of mineral nitrogen due to symbiotic nitrogen fixation after the cultivation of legumes, which is 40.3 kg / ha (Table 3).

Table 3
The content and reserves of mineral nitrogen in the soil remaining after soybean cultivation

Variant	Content of mineral nitrogen, mg / kg of soil			Degree of provision of plants by nitrogen according to DSTU 4362	Reserves of mineral nitrogen in the 0-20 cm layer of soil, kg / ha
	nitrate	ammonium	total		
Non-inoculated soybean	2,2	0,4	2,6	very low	5,8
Inoculated soybean	9,2	11,8	21,0	average	46,1
LSD $_{0,95}$	5,49	9,62	7,13	-	15,69

The potential source of additional nitrogen input due to symbiotic nitrogen fixation is plant residues of legumes that enrich the soil with fresh organic mass, which undergoes intensive humification and mineralization. So, with residues of annual leguminous plants in the soil 15.8–32.6 kg / ha of nitrogen, 5.8–22.4 kg / ha of phosphorus and 8.6–27.5 kg / ha of potassium can accumulate [40]. Therefore, in assessing the actual volumes of nitrogen fixation, in our opinion, it is objective to include nitrogen entering the soil along with the vegetative mass when it is plowed.

In our experiment, the incident vegetative mass of inoculated plants at harvest time was 3.3 t / ha. The difference in total nitrogen content in the vegetative mass of inoculated and non-inoculated plants is 0.88 %, that is, with each ton of vegetative mass, 8.8 kg of N are fed into the soil due to nitrogen fixation. According to 3.3 t / ha of vegetative mass, 29 kg / ha of nitrogen is added to the soil in the organic matter, after which mineralization it replenishes the mineral nitrogen base of the soil.

So, the «net» nitrogen fixation, which includes the actual and potential income, when growing soybeans is 69 kg/ha.

4. Conclusions

Due to the symbiotic nitrogen fixation, which is activated by seed inoculation before sowing, soybean plants are formed, characterized by high studied parameters compared to non-inoculated, in particular:

– a more powerful root system with nodules develops, due to which plants are additionally provided with biological nitrogen, which is reflected in an increase in intensity of growth of the vegetative mass by 42 % and the level of plant availability of nitrogen, assessed by the content of chlorophyll in the leaves;

– the yield of soybean grain increases by 29 %, which, like vegetative mass and roots, contains more nitrogen and is characterized by a tendency to a decrease in the content of phosphorus and potassium;

– against the background of a decrease in the ratio of secondary products to soybean grain (1.5 : 1), nitrogen removal increases for the formation of 1 ton of both vegetative mass (2.2 times) and grain (by 32 %).

Part of the biologically fixed nitrogen in the formation of the biological yield of soybean obtained on chernozem typical under the weather conditions of 2018 is 43 %, and the available soil nitrogen is 57 %.

To assess the nitrogen-fixing ability of soybean in the aftereffect, it is proposed to determine the “net” nitrogen-fixation by the actual reserves of mineral nitrogen under inoculated soybean (minus its reserves under non-inoculated) and by the potential amount of nitrogen that additionally comes with the total vegetative mass (by the difference between the nitrogen content of vegetative mass inoculated and non-inoculated soybean).

At the time of harvesting inoculated soybean, the actual reserves of mineral nitrogen in the 0-20 cm soil layer were 40 kg/ha, which ensured an increase in the Degree of provision of plants by nitrogen from very low to medium. The potential amount of nitrogen entering the soil with a vegetative mass and becomes available after its mineralization is 29 kg/ha. Accordingly, the "net" nitrogen-fixation under soybeans on chernozem typical under weather conditions in 2018 is 69 kg N/ha.

References

1. *Umarov M.M., Kurakov A.V., Stepanov A.L.* 2007. Microbiological transformation of nitrogen in the soil. Moscow GEOS. 138 p. (Rus.).

2. *Altobelli F., Amanullah, Benedetti A., Calles T., Caon L., Charrondiere R., Giri S.P., Grande F., Muthuraman R.P., Pisante M., Pramar B., Vargas R., Verma D., Vishwakarma A.K., Wiese L., Xipsiti M.* 2016. Soils and pulses: Symbiosis for life. Rome: FAO, 104 p. URL: http://www.fao.org/3/a-i6437e.pdf.

3. *Volobueva O.G*. 2011. Symbiotic nitrogen fixation as a factor of ecological safety and soil fertility. *Vestnyk RUDN*. No 1. P. 53060. (Rus.).

4. *Patyka V.P., Gnatjuk T.T., Buleca N.M., Kyrylenko L.V.* 2015. Biological nitrogen in the system of agriculture. *Zemlerobstvo*. V. 2. P. 12020. (Ukr.).

5. *Morgun V.V., Kots S.Ja*. 2018. Biological nitrogen on present agriculture. *Plant Varieties Studying and Protection*. Vol. 14, No 3. P. 2850294. DOI: 10.21498/2518-1017.14.3.2018.145293. (Ukr.).

6. *Volkogon V.V., Sal'nyk V.P.* 2005. The importance of growth regulators in the formation of active nitrogen fixing symbiosis and associations. *Fiziologija i biohimija kul'turnyh rastenij*. No 3. P. 1870197. (Ukr.).

7. *Kots S.Ja.* 2011. The current state of research on the biological fixation of nitrogen. *Fiziologija i biohimija kul't. rastenij*. Vol. 43, No 3. P. 212-225. (Ukr.). URI: http:// dspace.nbuv.gov.ua/handle/123456789/66368.

8. *Pervachuk M.V., Vradij O.I.* 2016. Intensification of the process of fixation of atmospheric nitrogen under the influence of microbial preparations on crops of perennial legumes. *Sil's'ke gospodarstvo ta lisivnyctvo*. No 4. P. 2200230. (Ukr.). URL: http://socrates.vsau.org/repository/getfile.php/15746.pdf.

9. *Babych A.O., Babych-Poberezhna A.A.* 2011. Selection, production, trade and use of soybeans in the world. Kyiv: Agrarna nauka. 548 p. (Ukr.).

10. *Plant Growing* of Ukraine : Statistical Collection 2017. 2018. Kyiv. 222 p. URL: http://www.ukrstat.gov.ua/. (Ukr.).

11. *Provorov N.A., Simarov B.V*. 1992. Genetic polymorphism of legumes on the ability to symbiosis with nodule bacteria. *Genetika*. Vol. 28, No 6. P. 5-14. (Rus.).

12. *Gamajunova V.V., Nazarchuk A.A*. 2013. Formation of soybean productivity depending on variety, mineral nutrition and seed treatment with biopreparations in southern Ukraine. *Agropromyslove vyrobnyctvo Polissja*. V. 6. P. 70-73. (Ukr.).

13. *Posypanov G.S., Kobozeva T.P., Tazin I.I., Beljaev E.V., Delaev U. A.* 2006. Modern methods for determining the amount of fixed nitrogen in the field. *Izvestija TSHA*. V. 2. P. 129-134. (Rus.).

14. *Didora V.G., Stupnic'ka O., Didora L.D*. 2015. The effectiveness of symbiotic activity of soybean crops in the conditions of the Polissya of Ukraine. *Bulletin of Agricultural Science*. No 8. P. 56–60. (Ukr.). URL: http://agrovisnyk.com/archive_ua_2015_08_11.html

15. *Zoundji C.C., Houngnandan P., Kouelo F.A., F.E. Boko, J.J. Adu Gyamfi.* January 2016. Symbiotic nitrogen fixation and nitrogen budget of Brazilian soybean [Glycine max (L.) Merril] varieties introduced in Benin using ^{15}N isotopic dilution method. *African Journal of Agricultural Research*. P. 7-15. URL : https://www.researchgate.net/publication/290449458_Symbiotic_nitrogen_fixation_and_nitrogen_budget_of_Brazilian_soybean_Glycine_max_L_Merril_varieties_introduced_in_Benin_using_15N_isotopic_dilution_method.

16. *Petrychenko V.F., Sereda L.M.* 2000. Features of formation of soybean productivity depending on hydrothermal resources and influence of agrotechnical measures. *Zb. naukovyh prac' Vinnyc'kogo derzhavnogo agrarnogo universytetu*. V. 8. T. 1. P. 53–57. (Ukr.).

17. *Zabolotnyi G.M., Cygansyij V.I.,* Cygans'ka O.I. 2015. Symbiotic productivity of soybeans depending on the level of fertilization in the right-bank forest-steppe. *Zbirnyk naukovyh prac' NNC «Instytut zemlerobstva NAAN».* Vyp. 4. P. 66–71. (Ukr.). URL: http://nbuv.gov.ua/UJRN/znpzeml_2015_4_11.

18. *Trepachev E.P.* 1981. About methods of study of nitrogen-fixing capacity of legumes. *Agrohimija*. No 12. P. 129-141. (Rus.).

19. *Posypanov G.S*. 1991. Determination of the quantity of fixed nitrogen of air in the field. In the book : Methods of studying the biological fixation of nitrogen in air. Moscow: Agropromizdat. P. 27-30. (Rus.).

20. *Dotsenko O.V., Solokha M.O., Nikonenko V., Vinnichenko L.M*. 2018. Recommendations for operational evaluation and correction of nitrogen supply of basic cereal crops under production conditions with the use of terrestrial and remote methods of plant diagnostics. Kharkiv. 24 p. (Ukr.).

21. *Soil quality.* Determination of nitrate and ammonium nitrogen in modification of NSC ISSAR named after O.N. Sokolovsky: DSTU 4729:2007. 2007. [Existing with 2008-01-01]. Kyiv: Derzhcpozhyvstandart Ukraine. 14 p. (Ukr.).

22. *Plants.* Determination of common forms of nitrogen, phosphorus, potassium in one stress of vegetative material : MVV 31–497058–019–2005. 2005. In: Methods of determining the composition and properties of soils. Book 2. Kharkiv. Drukarnja No 13. P. 189–208. (Ukr.).

23. *Judin F.A.* 1971. Methodology of agrochemical research. Moscow: Kolos. 271 p. (Rus.).

24. *Hvorova L., Topazh A.G., Abramova A.V., Neupokoeva K.G.* 2015. Approaches to the description of symbiotic nitrogen fixation. Part 1. Analysis and selection of a list of factors with an assessment of their priority. *Izvestija Altajskogo gosudarstvennogo universiteta*. No 1/1 (85). P. 187–191. (Rus.). DOI: 10.14258/izvasu(2015)1.1-34.

25. *Bahmat O.M*. 2012. Simulation of adaptive technology of soybean cultivation [monograph]. Kam'janets–Podil's'kyj. 436 p. (Ukr.).

26. *Prokopchuk S.V.* 2017. Efficiency of application of nitrogen fertilizers and bacterial preparations for soy. In: Symbiotic nitrogen fixation and crop. Uman: «Sochins'kyj M. M.». P. 217-259. (Ukr.).

27. *Zimmer S., Messmer M., Haase Th. Piepho H.-P., Mindermann A., Schulz H., Habekuß A., Ordon F., Wilbois K.-P., Heß J.* 2016. Effects of soybean variety and Bradyrhizobium strains on yield, protein content and biological nitrogen fixation under cool growing conditions in Germany. *Europ. J. Agronomy*. № 72. P. 38-46. DOI: 10.1016/j.eja.2015.09.008.

28. *Coskan A., Dogan K.* 2011. Symbiotic Nitrogen Fixation in Soybean. *Soybean Physiology and Biochemistry*. P. 167-182. DOI: 10.5772/20073.

29. *Tamiru S., Lalit M. P., Tsige A.* 2012. Effects of Inoculation by Bradyrhizobium japonicum Strains on Nodulation, Nitrogen Fixation, and Yield of Soybean (Glycine max L. Merill) Varieties on Nitisols of Bako, Western Ethiopia. ISRN *Agronomy*. Vol. 2012, Article ID 261475, 8 pages. DOI: 10.5402/2012/261475.

30. *Long S.R.* 1996. Rhizobium symbiosis: Nod factors in perspective. *Plant Cell*. № 8. P. 1885-1898. DOI : 10.1105/tpc.8.10.1885.

31. *Mus F., Crook M.B., Garcia K., Garcia Costas A., Geddes B.A., Kouri E.D., Paramasivan P., Ryu M.H., Oldroyd G. E. D., Poole P. S., Udvardi M. K., Voigt C. A., Ane J. M., Peters J. W.* 2016. Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. *Appl Environ Microbiol*. № 82. P. 3698–3710. DOI: 10.1128/AEM.01055-16.

32. *Serova A.T., Cyganov V.*E. 2014. Aging of the symbiotic nodule in legume plants: molecular genetic aspects. *Sel'skohozjajstvennaja biologija*. No 5 P. 3-15. (Rus.). DOI: 10.15389/agrobiology.2014.5.3rus .

33. *Leggett M., Diaz-Zorita M.,Koivunen M.,Bowman R.,Pesek R., Stevenson C.,Leister T.* 2017. Soybean Response to Inoculation with Bradyrhizobium japonicum in the United States and Argentina. *Agronomy Journal*. Vol. 109. No 3. P. 1031–1038. DOI: 10.2134/agronj2016.04.0214.

34. *Netis V.I.* 2018. Formation of elements of soybean productivity for different growing practices. Tavrijs'kyj naukovyj visnyk. Vyp. 99. P. 100-107. (Ukr.).

35. *Babych A.*O. 1998. Soybeans for health and life on planet Earth. Kyiv: Agrarna nauka. 272 c. (Ukr.).

36. *Podobedov A.V. Tarushkin V.I., Sajko V.F.* 1998. World soybean production. *Agrarna nauka*. No 6. C. 12-15. (Rus.).

37. *Salvagiotti F., Cassman K.G., Specht J.E., Waltersa D.T., Weissb A., Dobermanna A.* 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. Field Crops Research. No 108 (1). P. 1-13. DOI: 10.1016/j.fcr.2008.03.001.

38. *Ogurcov Je.M., Myhjejev V.G., Bjelins'kyj Ju.V., Klymenko I.V.* 2016. Adaptive Soybean Growing Technology in the Eastern Forest-Steppe of Ukraine: [monograph]. Ed. by Bobro M.A. Kharkiv: Machulin. 272 p. (Ukr.).

39. *Soil quality.* Fertility indexes of soils: DSTU 4362:2004. 2006. [Existing with 2006-01-01]. Kyiv: Derzhcpozhyvstandart Ukrai'ny. 22 p. (Ukr.).

40. *Azarov B.F., Akulov P.G., Azarov V.B., Solovichenko V.D.* 2008. Contribution of the symbiotic nitrogen of legumes in the fertility of the soils of the central black earth. *Dostizhenija nauki i tehniki APK*. No 9. P. 9-11. (Rus.).

УДК 631.461.5:633.34

Оцінка впливу симбіотичної азотфіксації на продуктивність рослин сої та азотний стан ґрунту

А.В. Ревтьє-Уварова

ННЦ «Інститут ґрунтознавства та агрохімії імені О.Н. Соколовського», *Харків, Україна*
E-mail: *alina_rev @meta.ua*

В умовах польового дрібноділянкового досліду на чорноземі типовому малогумусному оцінено агрономічну ефективність симбіотичної азотфіксації за вирощування сої шляхом порівняння індикаторів стану ґрунту та морфометричних характеристик рослин, насіння яких перед сівбою було оброблене інокулянтом на основі вірулентних штамів бульбочкових бактерій *Bradyrhizobium japonicum*, з варіантами з необробленим насінням.
Встановлено, що симбіотична азотфіксація сприяє формуванню більш потужної кореневої системи з активними бульбочками, збільшенню на 42 % інтенсивності наростання вегетативної маси та поліпшенню забезпеченості рослин сої азотом, оціненого за вмістом хлорофілу в листях. Підвищується на 29 % урожайність зерна, яке містить, як і листостеблова маса та корені, більше азоту та характеризується тенденцією до зниження вмісту фосфору та калію. Визначено, що на фоні зменшення відношення побічної продукції до зерна сої (1,5 : 1) збільшується винос азоту на формування як листостеблової маси (в 2,2 раза), так і зерна (на 32 %). Встановлено, що частка біологічно фіксованого азоту, винесеного з біологічним урожаєм сої з гектару площі, становить 43 %, азот ґрунту становить 57 %. Для діагностики забезпеченості ґрунту азотом у післядії симбіотичної азотфіксації пропонується визначати «нетто» азотфіксації за фактичними запасами мінерального азоту у ґрунті під інокульованою соєю (за мінусом його запасів під неінокульованою) та за потенційною кількістю азоту, який надходить додатково з загальною листостебловою масою (за різницею між вмістом азоту в листостебловій масі інокульованої та неінокульованої сої). В погодних умовах 2018 року на чорноземі типовому «нетто» азотфіксації під соєю становило 69 кг N/га, з яких 40 кг/га – запаси мінерального азоту в ґрунті та 29 кг/га – азот, що надходить із листостебловою масою.

Ключові слова: *азот; запаси мінерального азоту; інокуляція; розвиток рослин сої; симбіотична азотфіксація; чорнозем типовий.*

Форма цитування: Revtie-Uvarova A.V. Evaluation of the effect of symbiotic nitrogen fixation on the yield of soybean and nitrogen state of soil. *Agrochemistry and Soil Science*. Collected papers. 2019. No. 88. Kharkiv: NSC ISSAR, P. 79-88. DOI: https://doi.org/10.31073/acss88-11.