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# The effect of soil tillage on symbiotic activity of soybean crops

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## Abstract

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This study is devoted to consideration of the practical aspect of atmospheric nitrogen biological fixation by soybean plants. This fixation is considered in terms of physiological-biochemical processes taking place at binding the inert nitrogen molecule in nitrogenous compounds accessible to plants. Searching the ways of intensifying the process of nitrogen fixation to obtain maximal yields of high-quality protein products appears to be an important practical task. It has been demonstrated that the role of soil tillage practices connected with the possibilities of soybean symbiotic nitrogen fixation, being among many factors capable of affecting this process, has not been sufficiently studied. To study effect of soil tillage and seed inoculation on soybean productivity field trials during the period from 2016 to 2018 were conducted. The following main soil treatment techniques were used: deep tillage (20–22 cm), shallow tillage of two types: up to 14–16 cm and to 12–14 cm depth. Various types of cultivators forming different degree of soil dispersity by its crumbling, fluffing, partial mixing, and also flattening the field surface were used for soil shallow tillage. It was established that inoculated soybean plants are able to uptake the maximal quantity of fixed nitrogen181.2 kg/ha under shallow tillage (12–14 cm), flattened and moderately compacted soil surface. Obtaining the highest soybean yield of 2.42 t/ha as compared with other soil tillage practices as shallow tillage (14–16 cm) and deep tillage under which the yields were 2.24 and 2.01 t/ha, respectively, was the result of such farm practices.

*Keywords:* soybean; pre-sowing tillage; symbiotic activity; yield

## Introduction

The interest to the problem of microbiological atmospheric nitrogen fixation by plants is stipulated not only by the importance of this process in nitrogen balance of the Earth biosphere, but also by its prospect as a source of biological nitrogen to ensure the growing needs of agriculture. The complete harmlessness of nitrogen fixating microorganisms for the environment and comparatively small energy consumption are the main arguments in favor of this process. Rational using the process of molecular nitrogen fixation in the technologies of legume crops cultivation will enable to obtain high yields of high-quality protein products together with simultaneous decreasing the level of using synthetic nitrogen.

Soybean (*Glycine max* (L.) Merr.) is one of legume crops which can effectively supply itself with nitrogen owing to the process of fixing atmospheric nitrogen? This process, called nitrogen fixation, is performed by symbiotic bacteria in soybean plants. These bacteria are settled in their roots and can regenerate molecular nitrogen $N_2$ into ammonia $NH_3$ with the help of nitrogenase enzyme. Ammonia is used for biosynthesis of amino acids and other nitrogen containing substances in bacteria cells. After bacteria destruction, organic nitrogen is mineralized and becomes available for plants. In turn, symbiotic bacteria receive carbohydrates and mineral salts from plants. Due to the biological nitrogen fixation soybean plants satisfy their requirements in nitrogen by 70% – 75% from the air by nodule bacteria which develop on their root system creating symbiosis (de Luca & Hungría, 2014). It is necessary to have not less than 25–50 nodules on one plant for obtaining optimal soybean yield. It has been established that the yield of soybean by 36%–82% depends on the efficiency of the nitrogen fixation by plant root system (Schipanski et al., 2010). It is known that a symbiotic nitrogen fixation is functionally connected with photosynthesis. 10%–20% of general photosynthesis production, necessary for plant growth, is used to ensure symbiotic nitrogen fixation. Nitrogen fixation intensity is regulated by the plant itself, which, unlike autotrophic nutrition with mineral nitrogen, excludes nitrates' accumulation in farm products.

In order to form a nitrogen-fixing system and ensure of soybean plants with air molecular nitrogen the seed inoculation by bio-preparations containing rhizobia bacteria was conducted before sowing. The effectiveness of this method depends on the quality of bacterial preparation and technology of application. In some cases, symbiotic microorganisms ensure effective microelement entry the plant root system even under the latter's' deficit (Khaitov, 2018). Rhizobia bacteria assist in effective plant growth by improving nutrient uptake, stimulating hormone production. Alternatively this assistance may occur by decreasing pathogens' inhibiting effect as a result of strengthening plant resistance against diseases or other stress factors (Zarei et al., 2012; Ahemad & Kibret, 2014).

Different kinds of *Bradyrhizobium* and *Sinorhizobium*, including microorganism strains, which are adapted to particular soil and climatic conditions, can be effective inoculants. Due to inoculation, the number of nodules formed on the plants root system may exceed by 28–178 pieces the number of nodules on plants without inoculation. Grain yield is also considerably increased by 34%–109% due to seed inoculation (Thilakarethna & Raizada, 2017).

It has been established that soybean plant symbiosis with *Bradirhizobium* can fix about 300 kg of nitrogen per hectare. This leads to increasing a soybean yield at the expense of seed number in the bean and their weight (Zarei et al., 2012). Using *Bacillus cereus*, UW85 strain also stimulates soybean plant development, but the difference in dry weight, concentration and nitrogen quantity becomes noticeable only in 90 days. The yield of inoculated plants increases by 9%, and nitrogen content in seeds exceeds the control by 14% (Bullied et al., 2002). Seed inoculation with *Rhizobium* sp. bacteria favors increasing plant height, the number and weight of nodules, roots and aboveground part. Considerably higher nitrogenase activity is observed in inoculated plants, which increases nitrogen fixation and assimilation activity. As a result, plants form considerably higher biological and economic yields (Alam et al., 2015). The similar results were also obtained by Hungria et al. (2015a) without applying mineral nitrogen fertilizers.

Adding microelement component to the complex of bacterial fertilizers contributes to increasing the quantity of fixed nitrogen by 34%–49% (Dozorov et al., 2015). In this regard, now the search for varieties with the best response to the use of biological fertilizers is carried out especially actively (Tesfaye et al., 2018).

Inoculation is important in process of cultivating soya in dry conditions. Soil dehydration results in slowing down the rate of nitrogen fixation through decreasing the activity of nitrogenase in nodules. Several authors noticed that there are genetic variations as to nitrogen fixation by soya (Sall & Sinclair, 1991; Streeter, 2003). Plural soybean varieties differently react on drought. It is reflected in the size and number of nodules on root system plants and their physiological properties (King & Purcell, 2001).

At soya cultivation on nitrogen poor soils plants may suffer from nitrogen deficiency, so the inoculation with nitrogen fixing bacteria strains can be a key factor in yield formation (Chibeba et al*.,* 2015).

Though seed inoculation is the most widely spread and effective method of increasing nitrogen fixation (Bashan et al., 2014; Chibeba et al., 2015), foliar application of bacterial preparations can be also effective (Puente, 2019). But, as it is mentioned in the paper (Mahmood et al., 2016), applying inoculants in the soil is more efficient, as in this case the risk of useful microorganisms' contact with their antagonists considerably decreases.

However, there are several factors, which can negatively affect the functioning of symbiotic system in soybean plants. The processes of wind erosion result in decreasing the effectiveness of inoculant activity (Mason et al., 2015). Apart from unfavorable weather conditions, mineral nitrogen fertilizers and pesticides may also have the same effect. The Phosphorus deficiency in the soil effects negative on nodule weight, nodule number, and total plant nitrogen in soybean (Jabborova et al., 2018).

It is known that applying mineral fertilizers under soya causes negative impact because it leads to the fact that the process of nitrogen fixing nodule formation slows down. It has been established that nitrogen in mineral fertilizers inhibits nitrogen fixation process by plants. It should be noted that the recommendations on the rates of applying mineral fertilizers at grain legume crops' cultivation are rather contradictory. Even the initial quantities of nitrogen can negatively affect nodulation. However, the reaction of various varieties on inoculation and simultaneous application of mineral fertilizers can be different (Hardarson et al., 1984). Thus, biological nitrogen fixation enables to minimize the application of nitrogen mineral fertilizers and stabilize the level of yield, which is economically important and should be considered as environmentally friendly resource (Peoples et al., 1995; Masson-Boivin et al., 2009).

Inoculants can correct the effect of pesticides, which are used in significant quantities in soybean cultivation. As known, the pesticides negatively affect the symbiosis of rhizobia and plants (Hungria et al., 2015b).

Methods of pre-sowing soil preparation also considerably influence the effectiveness of inoculants' activity and these methods have not been sufficiently studied. It has been established that under shallow soil tillage the yield of soybean seeds is 2.64 t/ha as compared with 2.13 t/ha under deep plowing. In addition, at shallow soil cultivation, the quality of the seeds of culture was significantly better in terms of fat content, which is equal to 20.34% compared to 19.94% under deep tillage. The protein content is practically equivalent in both methods of soil cultivation (Chetan et al., 2016).

There are different views on relationship between the soil tillage methods and seed inoculation. Some authors confirm the absence of any relationship between these two factors (Marburger et al., 2015). Others researchers, however, support the opinion about positive impact of these factors on the intensity of nitrogen fixation (Okoth et al., 2014; Alburquerque et al., 2015). Considerable positive effect of zero soil tillage (No-Till) on nitrogen fixation process (Smith et al., 2016), root system formation (Li et al., 2017), agro-physical indices and soil water availability was established (Tan et al., 2015; Anikwe et al., 2016).

Thus, at present there is no common theory, which would explain all the aspects of the nitrogen fixation process by plants. Therefore, the further investigations related to the regulation of biological nitrogen fixation, in particular, the role of tillage practices, optimal parameters of water content and soil aeration, and also nitrogen fixing by bacteria strains appear to be of significant importance (Schulze, 2004).

Thus, the current study aim is to determine the effect of main and pre-sowing soil tillage at seed treatment with active strain of nitrogen fixing bacteria on the dynamics of nodule formation on root system of soybean plants, their weight, and also the crop yield.

## Materials and Methods

Field experiment was carried out during 2016–2018 at Poltava State Agricultural Experimental Station named after M. I. Vavilov (Ukraine) and soybean variety *Bilosnizhka* was used as test crop. The presented soybean variety is the grain feed crop and belongs to the early-ripening groups. Soil characteristics of experimental sites are given in Table 1.

The experiment was conducted as a split plot arranged in randomized complete block design with three replications. The sub-plot size of experimental field was 160 $m^2$. Soybean seeding rate is 550 thousand viable seeds per hectare. The soybean seeds were not treated with fungicidal preparations before sowing. The soybean seeds before sowing were inoc-

**Table 1. Agrochemical soil properties of experimental site**

| Soil characteristics | 0–22 cm depth |
|---|---|
| Soil type | Black soil with low-humus content |
| Textural class | Heavy loamy |
| pH | 6.3 |
| Absorption capacity (mg-eq. per 100g of soil) | 33.0–35.0 |
| Bulk density | 1.18 |
| Hydrolytic acidity (mg-eq. per 100 g of soil) | 1.6–1.9 |
| Humus content (%) | 4.9–5.2 |
| Easily hydrogenated nitrogen content (mg per 100 g of soil) | 5.44–8.10 |
| Mobile phosphorus content (mg per 100 g of soil) | 10–15 |
| Potassium content (mg per 100 g of soil) | 16–20 |
| Average annual precipitation (mm) | 569; 30% rainfall is received in winter (Dec. to March) |
| Average air temperature | 7.6°C |

ulated with *Bradiorhizobium japonicum 634b* nodule bacteria strain at the rate of 0.2 kg of the bio-preparation per 150 kg of seeds. To suppress the growth of weeds, the treatments of main plots was conducted with herbicide, the active substances of which were *Imazethapyr* 450 g/kg and *Chlorimuron-ethyl* 150 g/kg at the rate of 100 g/ha. The herbicide was used single time in the phase of early weed development (up to 2–3 leaves). The any another agricultural measures were not involved.

Growth phases of soybean consist of 3 phase namely: budding, blooming, and bean formation phases. The number and weight of nitrogen fixing nodules on plant root system were determined in budding, blooming, and bean formation phases.

The following main soil treatment techniques were used: deep tillage (20–22 cm) and shallow tillage of two types: up to 14–16 cm and up to 12–14 cm depth.

After the main soil treatment the pre-sowing tillage was carried out for breaking clods, loosening soil compressed as a result of atmospheric precipitations and own weight as well as compacting excessively loosened soil.

Various cultivator types were used for pre-sowing tillage. Such cultivators are capable of creating different degrees of soil dispersity by crumbling, loosening and partial mixing of the soil. They also ensure the complete of weeds destruction and smoothing the field surface.

Steam cultivator (StC-4.0) creates soil loosening up to 4–5 cm deep, complete cutting of weed sprouts, and forms comb-like soil surface. On soil surface a comb is formed for melt water retention. Comb index may be equal to 2.1; 2.4; 2.9 cm. The degree of soil dispersity is from 5 to10 cm after tilling with this cultivator.

Universal cultivator (UC-5.4) creates a finely dispersed soil surface in order to provide a favorable water-air and thermal regimes. The diameter of the soil parts is from 0.5 to 1.0 cm after tilling with this cultivator.

Soil cultivator (SC-4) provides leveling and compacting of the soil surface. This technique contributes to raise moisture from the lower soil layers to the top layer, and the destruction of the soil crust to reduce moisture loss due to evaporation. The creation of hard soil surface ensures the seeds sowing at the necessary depth and its closer contact with the soil.

The desirable type of cultivator should be chosen taking into consideration the specific characteristics of the soil surface.

## Results and Discussion

The formation of high soybean yield is achieved by interrelated complex of agro-technical practices, which are united in the whole cultivation technology. The methods of main and pre-sowing soil tillage and seed inoculation turned out to be one of the decisive factors among the elements of soybean cultivation technology studied by us.

The results of investigating the effect of main and pre-sowing soil tillage systems on the number and weight of nitrogen fixing nodules on the soybean root system during the vegetation are presented in Table 2. The analysis of the dependence of the efficiency nodulation process on the root system of plants during the vegetation on the technique of main and pre-sowing soil tillage showed that they affected the indices of the crop symbiotic system functioning differently.

So, increasing the number of nitrogen fixing nodules on the crop root system without inoculation on average by 2.5-fold, depending on the development phase of plants, was observed at deep tillage. The number of nitrogen fixing nodules on the root system of non-inoculated plants during the vegetation period varied on the average from 34 pieces in budding phase to 88 pieces in bean formation phase under using of any cultivators. The similar tendency was observed for inoculated plants, but the number of nodules was calculated higher by 20.6% and 10.2%, in the phases of budding and bean formation, respectively.

When soil cultivator SC-4 was used for pre-sowing tillage, the number of nodules significantly increased depending on the development phase from 37 pieces in the budding phase to 94 pieces in the bean formation phase for non-inoculated plants and from 47 pieces to 101 pieces for inoculated plants, respectively. However, the increase of the nodule number depending on the vegetation phase was by 5.5% less in inoculated plants compared with the non-inoculated ones. This may be due to the fact that pre-sowing seed inoculation contributed to more active nodulation on soybean plant root system even at early vegetation stages. Nodulation on the plants from non-inoculated seeds occurred more intensively from the middle of the crop vegetation period when the proliferation of bacteria is sufficiently for population of the plant root system by them.

The inoculation effectiveness under pre-sowing tillage by StC-4.0 cultivator was follows: 18.2% in budding phase, 17.1% in blooming phase, and 12.8% in bean formation phase. Using UC-5.4 cultivator and SC-4 cultivator resulted in similar inoculation efficiency indices during the vegetation period: UC-5.4 – from 19.3% to 8.9% and SC-4 – from 27% to 7.4%. The highest index of inoculation effectiveness, equal to 27% in budding phase, was obtained while soil tilling was performed by SC-4 cultivator.

The lowest number of nitrogen fixing nodules on the root system of non-inoculated plants during vegetation was formed under shallow tillage (14–16 cm) compared with the deep tillage (20–22 cm) and shallow tillage (12–14 cm). Its quantity varied from 27 pieces in budding phase to 75 pieces in bean formation phase under all the techniques of pre-sowing soil tillage. The increasing of nodule number as a result of inoculation was 30% during budding phase and 13.3% during bean formation phase.

The inoculation effectiveness in budding phase depends on the system of pre-sowing soil tillage: it was about 29.6% under using StC-4.0 and about 18.5% under using UC-5.4. The highest index of inoculation effectiveness in budding phase 41.1% was obtained while soil surface treatment with SC-4 cultivator. But during the process of plant development the inoculation effectiveness gradually decreased and reached 11.2% in bean formation phase.

The maximum nodule number was formed as a result of using shallow tillage (12–14 cm) irrespective of the variants of pre-sowing soil tillage by any type of cultivators. Thus, the number of nodules on non-inoculated plants varied from 35 pieces in budding phase to 88 pieces in bean formation phase. The seeds inoculation leads to an increasing in the number of nodules by 20% in the budding phase and by 17% in the bean formation phase.

It should be noted several main reasons for the change in the number and weight of nitrogen fixing nodules on the root system of soybean. Such reasons include: different soil moisture content, the intensity of the soil-environment gas exchange process, soil temperature. Mainly, they form the different conditions for the symbiotic activity of soybean root system.

In our opinion, however, the number of nodules should not be considered as a sufficient parameter of the symbiosis activity, as the nodules may have different weight and be active or insufficiently active or even not active at all. It was founded that the number of nodules on the root system of plants cultivated in deep tilled (20–22 cm), shallow tilled (14–16 cm) soils and any variant of pre-sowing treatment does not differ significantly (Table 2).

In our trials the minimal number of nitrogen fixing nodules with minimal weight during vegetation was formed by soybean plants under shallow tillage (14–16 cm) and pre-sowing soil surface preparation by UC-5.4 cultivator. Under conditions of using such technique for main and pre-sowing soil tillage, the nodule weight on the plant root system in budding phase was 0.55 g/plant (27 pieces) and

**Table 2. Effect of tillage practices and seed inoculation on total number of nodules (piece/plant) and weight of raw nodules (g/plant) of soybean plants**

| Tillage practices | | Total number of nodules, piece/plant | | | Weight of raw nodules, g/plant | | |
|---|---|---|---|---|---|---|---|
| | | Bud formation | Bloom | Bean formation | Bud formation | Bloom | Bean formation |
| Deep tillage (20–22 cm) | | | | | | | |
| StC-4.0 | *a** | 33 | 70 | 86 | 1.55 | 3.14 | 4.23 |
| | *b* | 39 | 82 | 97 | 1.67 | 3.50 | 4.36 |
| UC-5.4 | *a** | 31 | 67 | 83 | 1.48 | 2.87 | 3.52 |
| | *b* | 37 | 73 | 93 | 1.68 | 3.39 | 3.89 |
| SC-4 | *a** | 37 | 73 | 94 | 1.58 | 3.26 | 4.23 |
| | *b* | 47 | 80 | 101 | 2.02 | 3.76 | 4.42 |
| Shallow tillage (14–16 cm) | | | | | | | |
| StC-4.0 | *a** | 27 | 57 | 72 | 0.54 | 1.58 | 3.08 |
| | *b* | 35 | 66 | 81 | 0.92 | 2.22 | 3.26 |
| UC-5.4 | *a** | 27 | 56 | 74 | 0.55 | 1.67 | 3.15 |
| | *b* | 32 | 66 | 84 | 1.11 | 2.21 | 3.40 |
| SC-4 | *a** | 29 | 62 | 80 | 0.96 | 2.04 | 3.19 |
| | *b* | 41 | 76 | 89 | 1.39 | 2.54 | 3.68 |
| Shallow tillage (12–14 cm) | | | | | | | |
| StC-4.0 | *a** | 34 | 75 | 88 | 1.75 | 3.01 | 3.71 |
| | *b* | 43 | 84 | 101 | 2.07 | 3.41 | 3.94 |
| UC-5.4 | *a** | 30 | 71 | 82 | 1.75 | 3.03 | 3.53 |
| | *b* | 44 | 85 | 97 | 2.10 | 3.19 | 3.73 |
| SC-4 | *a** | 40 | 79 | 95 | 1.96 | 3.24 | 4.10 |
| | *b* | 51 | 90 | 110 | 2.27 | 3.91 | 4.47 |
| V% | | 18.9 | 13.1 | 11.2 | 34.1 | 23.9 | 12.1 |

*Note: a** – non-inoculated seeds, *b* – seeds inoculated

1.11 g/plant (32 pieces) for non-inoculated and inoculated plants, respectively. The number of nodules and their weight during bean formation phase increased to 3.15 g/plant (74 pieces) in non-inoculated plants and to 3.40 g/plant (84 pieces) in inoculated plants. However, these are the lowest weight results compared with the obtained while using deep tillage (20–22 cm), shallow tillage (12–14 cm), and other studied techniques of pre-sowing soil tillage.

The maximum mass of nitrogen-fixing nodules from one plant during the growing season was provided by the shallow tillage (12–14 cm) under using of soil cultivator SC-4 for the leveling and compacting of the soil surface. So, the weight of nodules on plants' root system in budding phase was 1.96 g/plant (40 pieces) on non-inoculated plants and 2.27 g/plant (51pieces) on inoculated plants. During bean formation phase this index increased for both non-inoculated and inoculated plants to 4.10 and 4.47 g/plant, respectively.

The quantity of fixed atmospheric nitrogen is a useful measure of efficiency functioning of plant symbiotic system. Through using this index, it is possible to determine the effectiveness of used agro-technical methods, including both main and pre-sowing soil tillage. The indices of fixed nitrogen by soybean plants obtained as a result of our studies are presented in Table 3.

The results showed that during the research's years the quantity of fixed nitrogen depending on main and pre-sowing soil tillage and seed inoculation on average varied from 74.7 to 181.2 kg/ha. Besides, only under creating favorable conditions for active symbiosis of bacteria and soybean plants, the maximal quantity of nitrogen can be obtained.

Under shallow tillage (14–16 cm) the quantity of fixed atmospheric nitrogen varied from 92.9 to 115.1 kg/ha irrespective of pre-sowing soil preparation technique. Seed inoculation leads to increasing the fixed atmospheric nitrogen quantity up to 113.9–148.6 kg/ha which made 59.4%–65.6% of the total nitrogen content in biomass.

The lowest indices of fixed nitrogen per unit of soil area were obtained under deep tillage (20–22 cm) and all variants of pre-sowing soil tillage. With this method of land preparation, the quantity of nitrogen, fixed by inoculated plants, turned out to be 20% less in comparison with shallow tillage (14–16 cm), and 48% less in comparison with shallow tillage (12–14 cm).

The maximum quantity of fixed nitrogen was obtained for all methods of main tillage and pre-sowing treatment by soil cultivator SC-4 (Table 3). Thus, under deep tillage (20–22 cm), the nitrogen quantity, which accumulates by soybean non-inoculated plants, is 51.5% from the total nitrogen content in biomass. As for the nitrogen quantity accumulated under shallow tillage (14–16 cm) and shallow tillage (12–14 cm) it was 59.7% and 65.2%, from the total nitrogen content in biomass, respectively. Seed inoculation contributed to an increase the quantity of atmospheric nitrogen absorbed by soybean plants, which increased to 61.0% in areas treated with deep tillage (20–22 cm), up to 65.6% in areas treated with shallow tillage (14–16 cm), and up to 70.0% in areas treated with shallow tillage (12–14 cm).

**Table 3. Effect of tillage practices on nitrogen accumulation from atmosphere by soybean plants**

| Tillage practices | | Total nitrogen content in biomass, kg/ha | Amount fixed nitrogen from the atmosphere | |
|---|---|---|---|---|
| | | | kg/ha | % |
| Deep tillage (20–22 cm) | | | | |
| StC-4 | *a** | 152.5 | 74.7 | 49.0 |
| | *b* | 171.6 | 93.8 | 54.7 |
| UC-5.4 | *a** | 158.6 | 80.8 | 50.9 |
| | *b* | 187.0 | 109.2 | 58.4 |
| SC-4 | *a** | 160.5 | 82.7 | 51.5 |
| | *b* | 199.3 | 121.5 | 61.0 |
| Shallow tillage (14–16 cm) | | | | |
| StC-4 | *a** | 170.7 | 92.9 | 54.4 |
| | *b* | 191.7 | 113.9 | 59.4 |
| UC-5.4 | *a** | 183.8 | 106.0 | 57.7 |
| | *b* | 205.5 | 127.7 | 62.1 |
| SC-4 | *a** | 192.9 | 115.1 | 59.7 |
| | *b* | 226.5 | 148.6 | 65.6 |
| Shallow tillage (12–14 cm) | | | | |
| StC-4 | *a** | 194.4 | 116.6 | 60.0 |
| | *b* | 213.3 | 135.5 | 63.5 |
| UC-5.4 | *a** | 207.3 | 129.5 | 62.5 |
| | *b* | 240.7 | 162.9 | 67.7 |
| SC-4 | *a** | 223.9 | 146.1 | 65.2 |
| | *b* | 259.0 | 181.2 | 70.0 |
| *V*% | | | 24.4 | 9.8 |

*Note: a** – non-inoculated seeds, *b* – seeds inoculated

Thus, shallow tillage (12–14 cm) and the creation of a moderately compacted soil surface using the SC-4 cultivator leaded to the conditions for the most efficient functioning of the plant's symbiotic system. This method of soil treatment contributes to maximum soil moisture content, favorable thermal conditions, intensive aeration, and the soil biological activity. This conclusion was confirmed by the highest quantity of fixed atmospheric nitrogen. Inoculated soybean plants can absorb up to 70% or 181.2 kg/ha of atmospheric nitrogen under such technique of soil preparation.

According to results of correlation analysis (Figure1), the total nitrogen content in biomass depends on the number of nodules per plant which are formed during inter-phase period from budding to bean formation ($r$ = 0.59 … 0.68). The

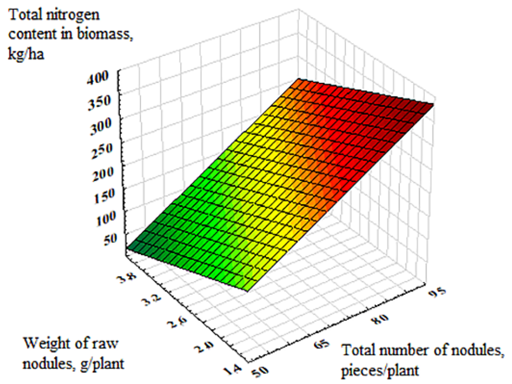


**Fig. 1. The effect of the weight of raw nodules and total number of nodules at soybean root system on total nitrogen content in biomass**

weight of raw nodules considerably affected this index only in the budding phase ($r$ = 0.52). Multiple regression analysis shows that the total nitrogen content in the biomass is directly proportional to the number of nodules per plant and inversely proportional to their raw weight (Figure 1).

That correlation is determined using the following equation:

$$Y = -38.22 + 5.1618x_1 - 48.11x_2,$$

where $Y$ – total nitrogen content in biomass, kg/ha; $x_1$ – total number of nodules, piece/plant; $x_2$ – weight of raw nodules, g/plant.

Crop yield is the criteria of estimating the effectiveness of the processes of photosynthesis, biological nitrogen fixation, and plant productivity formation. Yield is the final index, the main criteria of evaluating all the agro-technical measures and others factors of influence. Only under all these factors and optimal conditions of plants' vital activity, it is possible to obtain the significantly higher crop yield. Symbiotic nitrogen fixation positively affected the crop yields (Table 4).

The highest grain yield increase, resulting from using this agronomic practice, made 13.0% (0.23 t/ha) under deep tillage (20–22 cm) and pre-sowing tillage with SC-4 cultivator, while under shallow tillage (12–14 cm) the yield increase was only 7% (0.16 t/ha). The lowest level of 1.62 t/ha was obtained from non-inoculated seeds under deep tillage (20–22 cm) with pre-sowing soil loosening with StC-4.0 cultivator. Pre-sowing seed inoculation ensured grain increase by 12.3%–13.0% under other techniques of pre-sowing soil preparation.

The highest average annual soybean yield was obtained from plants under using active nitrogen fixing strain and pre-sowing tillage with SC-4 soil cultivator. The maximal yield 2.42 t/ha was received from inoculated seeds under shallow tillage (12–14 cm), whereas under shallow tillage (14–16 cm) and deep tillage (20–22 cm) the yields were only 2.24 and 2.01 t/ha, respectively.

**Table 4. Soybean yield depending on main and pre-sowing soil tillage systems, t/ha**

| Tillage practices | | Years | | | |
|---|---|---|---|---|---|
| | | 2016 | 2017 | 2018 | Average for 2016–2018 |
| Deep tillage (20–22 cm) | | | | | |
| StC-4 | *a** | 1.95 | 1.32 | 1.58 | 1.62 |
| | *b* | 2.22 | 1.49 | 1.74 | 1.82 |
| UC-5.4 | *a** | 2.07 | 1.40 | 1.61 | 1.69 |
| | *b* | 2.30 | 1.53 | 1.91 | 1.91 |
| SC-4 | *a** | 2.16 | 1.48 | 1.70 | 1.78 |
| | *b* | 2.39 | 1.7 | 1.98 | 2.01 |
| Shallow tillage (14–16 cm) | | | | | |
| StC-4 | a* | 2.25 | 1.48 | 1.79 | 1.84 |
| | *b* | 2.51 | 1.64 | 1.94 | 2.03 |
| UC-5.4 | *a** | 2.38 | 1.52 | 1.86 | 1.92 |
| | *b* | 2.54 | 1.72 | 1.98 | 2.08 |
| SC-4 | *a** | 2.47 | 1.49 | 2.02 | 1.99 |
| | *b* | 2.66 | 1.76 | 2.31 | 2.24 |
| Shallow tillage (12–14 cm) | | | | | |
| StC-4 | *a** | 2.39 | 1.70 | 1.95 | 2.01 |
| | *b* | 2.55 | 1.78 | 2.11 | 2.15 |
| UC-5.4 | *a** | 2.50 | 1.79 | 2.05 | 2.11 |
| | *b* | 2.74 | 1.91 | 2.31 | 2.32 |
| SC-4 | *a** | 2.76 | 1.80 | 2.23 | 2.26 |
| | *b* | 2.92 | 1.96 | 2.38 | 2.42 |
| S | | 2.4 | 1.6 | 2.0 | 2.0 |
| *V*, % | | 10.3 | 11.0 | 12.0 | 10.8 |
| X ± *Sx* | | 2.4 ± 0.06 | 1.6 ± 0.04 | 2.0 ± 0.06 | 2.0 ± 0.05 |

*Note: a** – non-inoculated seeds, *b* – seeds inoculated

Figure 2 demonstrates the effect of different factors on the soybean yield formation. It is seen, that factor of weather conditions, which made 59.1%, considerably influenced soybean productivity formation. In the same time, the main soil tillage and pre-sowing tillage influenced soybean productivity formation only by 15.2% and 4.2%, respectively. The seed inoculation process influenced yield formation by 5.6%.

As seen in Figure 2, the components of yield crop structure considerably depend on the weather conditions. The rainfall data during growing season of soybean is presented in Figure 3. Average monthly temperature distribution during growing season of soybean is shown in Figure 4.

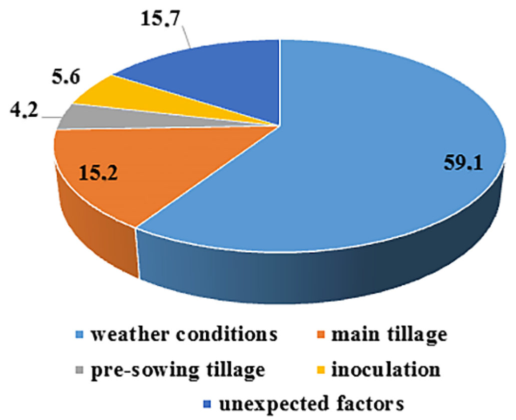


**Fig. 2. The participation of different factors in soybean yield formation, %**

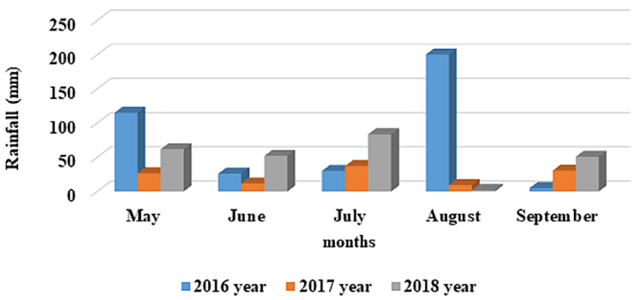


**Fig. 3. Rainfall during soybean vegetation period in 2016–2018**

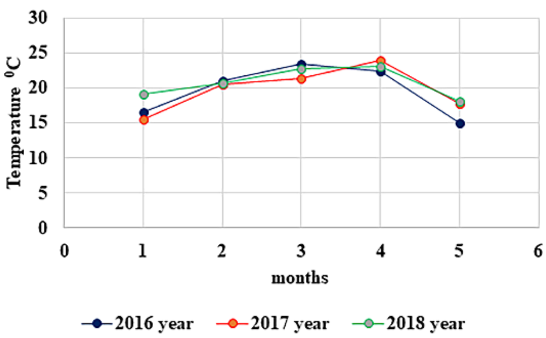


**Fig. 4. Average monthly air temperature during soybean vegetation period in 2016–2018**

As follows from presented data, the weather conditions during the vegetation in 2016 were the most favorable. The precipitation level reached 376.3 mm at the average monthly temperature 19.7°C. These factors influenced soybean yield, which was the highest that year as compared with all the years during the investigation period (Table 4). Under seed inoculation and deep tillage (20–22 cm), the yield was within 2.22–2.39 t/ha; under shallow tillage (14–16 cm) the yield reached 2.51–2.66 t/ha, and under shallow tillage (12–14 cm) to 2.55–2.92 t/ha, which was stipulated by using different techniques of pre-sowing soil preparation.

In 2017, the formation of the crop high productivity was limited by high air temperature (20.6°C, 21.4°C and 24.0°C, for June, July and August, respectively) and the deficit of precipitation in June, July and August (11.8, 37.2 and 9.3 mm, respectively). The negative effect of weather conditions in 2017 was manifested in inhibiting vegetation and reproductive development. It was reflected in decreasing plant height and formation fewer beans and grains in them. Accordingly, the yield decreased a little and made 1.49–1.67 t/ha under deep tillage (20–22 cm), 1.64–1.76 t/ha under shallow tillage (14–16 cm), and 1.78–1.96 t/ha under shallow tillage (12–14 cm).

Practically complete absence of precipitation (2.2 mm) in August 2018 resulted in untimely and accelerated soya ripening and, accordingly forming fine seeds. However, the harvest 2018 turned out to be higher than in 2017 due to a shorter period of unfavorable weather conditions and effective nitrogen fixation, favored by different pre-sowing soil tillage techniques. The highest soybean yield 2.38 t/ha was harvested in 2018, exceeding the similar index of 2017 by 21.4%. Such result was achieved under shallow tillage (12–14 cm) and pre-sowing soil surface flattening with SC-4 cultivator.

Mathematical processing shows that there is objective regularity between the yield and total nitrogen content in biomass, which is described by linear relationship between these two parameters for the given conditions (Figure 5).

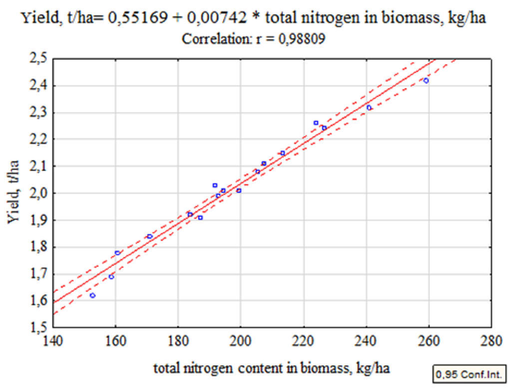


**Fig. 5. Plot for effect of nitrogen content in biomass on soybean yield, t/ha**

## Conclusions

The present investigation demonstrated that above all the pre-sowing soil preparation mainly influence on the formation of soybean plants' nitrogen fixation system. It was also found, that shallow tillage (12–14 cm) and moderate soil surface compaction as a result of pre-sowing preparation ensure up to 181.2 kg/ha of nitrogen uptake by inoculated plants. Our findings showed that the highest soybean yield of 2.42 t/ha was obtained from inoculated seeds under shallow tillage (12–14 cm), as a main method of soil tillage with further flattening and compacting soil surface with corresponding soil tilling implements.

## References

**Ahemad, M. & Kibret, M.** (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *Journal of King Saud University – Science, 26*(1), 1–20.

**Alam, F., Bhuiyan, M. A. H., Alam, S. S., Waghmode, T. R., Kim, P. J. & Lee, Y. B.** (2015). Effect of *Rhizobium* sp. BARIRGm901 inoculation on nodulation, nitrogen fixation and yield of soybean (*Glycine max*) genotypes in gray terrace soil. *Bioscience, Biotechnology, and Biochemistry, 79*(10), 1660–1668.

**Alburquerque, M. A., Dieckow, J., Sordi, A., Piva, J. T., Bayer, C., Molin, R., Pergher, M. & Ribeiro-Junior, P. J.** (2015). Carbon and nitrogen in a Ferralsol under zero tillage rotations based on cover, cash or hay crops. *Soil Use and Management, 31*(1), 1–9.

**Anikwe, M. A. N., Eze, J. C., Chima, M. C. & Ikenganyia, E. E.** (2016). Soil physicochemical quality in contrasting tillage systems and its effect on nodulation and nodulation effectivity of groundnut, Bambara groundnut and soybean in a degraded Ultisol in Agbani, Enugu Southeastern Nigeria. *Rhizosphere, 1,* 14–16.

**Bashan, Y., de-Bashan, L. E., Prabhu, S. R. & Hernandez, J. P.** (2014). Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). *Plant and Soil, 378*(1–2), 1–33.

**Bullied, W. J., Buss, T. J. & Kevin, J. V.** (2002). Bacillus cereus UW85 inoculation effects on growth, nodulation, and N accumulation in grain legumes: field studies. *Canadian Journal of Plant Science, 82*(2), 291–298.

**Chetan, C., Rusu, T., Chetan, F. & Simon, A.** (2016). Influence of soil tillage systems and weed control treatments on root nodules, production and qualitative indicators of soybean. *Procedia Technology, 22,* 457–464.

**Chibeba, A. M., de Fátima Guimarães, M., Brito, O. M., Nogueira, M. A., Araujo, R. S. & Hungria, M.** (2015). Co-inoculation of soybean with *Bradyrhizobium* and *Azospirillum* promotes early nodulation. *American Journal of Plant Sciences, 6,* 1641–1649.

**de Luca, M. J. & Hungría, M.** (2014). Plant densities and modulation of symbiotic nitrogen fixation in soybean. *Scientia Agricola (Piracicaba, Braz.), 71*(3), 181–187.

**Dozorov, A. V., Naumov, A. Y. & Yermoshkin, Y. V.** (2015). Organic nitrogen in soy nutrition. *Indian Journal of Science and Technology, 8*(27), 1–9.

**Jabborova, D. P., Enakiev, Y. I., Davranov, K. D. & Begmatov, S. A.** (2018). Effect of co-inoculation with *Bradyrhizobium japonicum* and *Pseudomonas putida* on root morph-architecture traits, nodulation and growth of soybean in response to phosphorus supply under hydroponic conditions. *Bulgarian Journal of Agricultural Science, 24* (6), 1004–1011.

**Hardarson, G., Zapeta, F. & Danso, S. K. A.** (1984). Effect of plant genotype and nitrogen fertilizer on symbiotic nitrogen fixation by soybean cultivars. *Plant and Soil, 82*(3), 397–405.

**Hungria, M., Nogueira, M. A. & Araujo, R. S.** (2015a). Soybean seed co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: a new biotechnological tool to improve yield and sustainability. *American Journal of Plant Sciences, 6*, 811–817.

**Hungria, M., Nogueira, M. A. & Araujo, R. S.** (2015b). Alternative methods of soybean inoculation to overcome adverse conditions at sowing. *African Journal of Agricultural Research, 10*(23), 2329–2338.

**Khaitov, B.** (2018). Effects of *Rhizobium* inoculation and magnesium application on growth and nodulation of soybean (*Glycine max* L.). *Journal of Plant Nutrition, 41*(16), 2057–2068.

**King, C. A. & Purcell, L. C.** (2001). Soybean nodule size and relationship to nitrogen fixation response to water deficit. *Crop Science, 41*(4), 1099–1107.

**Li, H., Mollier, A., Ziadia, N., Shi, Y., Parent, L-E. & Morel, C.** (2017). Soybean root traits after 24 years of different soil tillage and mineral phosphorus fertilization management. *Soil and Tillage Research, 165*, 258–267.

**Mahmood, A., Turgay, O. C., Farooq, M. & Hayet, R.** (2016). Seed biopriming with plant growth promoting rhizobacteria: a review. *FEMS Microbiology Ecology, 92*(8), 1–14.

**Marburger, D. A., Mourtzinis, S., Gaska, J. M. & Conley, S. P.** (2015). Do crop rotation and tillage influence soybean seed-applied inoculant decisions? *Agronomy Journal, 108*(1), 402–406.

**Mason, S., Galusha, T. & Kmail, Z.** (2015). Soybean yield and nodulation response to crophistory and inoculation. *Agronomy Journal, 108*(1), 309–312.

**Masson-Boivin, C., Giraud, C.E., Perret, X. & Betut, J.** (2009). Establishing nitrogen-fixing symbiosis with legumes: how many Rhizobium recipes? *Trends in Microbiology, 17*, 458–466.

**Okoth, J. O., Mungai, N. W.,Ouma, J. P. & Baijukya, F. P.** (2014). Effect of tillage on biological nitrogen fixation and yield of soybean (*'Glycine max* L. Merril') varieties. *Australian Journal of Crop Science, 8*(8), 1140–1146.

**Peoples, M. B., Herridge, D. F. & Ladha, J. K.** (1995). Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? *Plant and Soil, 174*(1–2), 3–28.

**Puente, M. L., Zawoznik, M., de Sabando, M. L., Perez, G., Gualpa, J. L., Carletti, S. M. & Cassán, F. D.** (2019). Improvement of soybean grain nutritional quality under foliar inoculation with *Azospirillum brasilense* strain Az39. *Symbiosis, 77*(1), 41–47.

**Sall, K. & Sinclair, T. R.** (1991). Soybean genotypic differences in sensitivity of symbiotic nitrogen fixation to soil dehydration. *Plant and Soil, 133*(1), 31–37.

**Schipanski, M. E., Drinkweter, L. E. & Russelle, M. P.** (2010). Understanding the variability in soybean nitrogen fixation across agro ecosystems. *Plant and Soil, 329*(1–2), 379–397.

**Schulze, J.** (2004). How are nitrogen fixation rates regulated in legumes? *Journal of Plant Nutrition and Soil Science, 167* (2), 125–137.

**Smith, C. R., Blair, P. L., Boyd, C., Cody, B., Hazel, A., Hedrick, A., Kathuria, H., Khurana, H., Kramer, B., Muterspaw, K., Peck, C., Sells, E., Skinner, J., Tegeler, C. & Wolfe, Z.** (2016). Microbial community responses to soil tillage and crop rotation in a corn/soybean agroecosystem. *Ecology and Evolution, 6* (22), 8075–8084.

**Streeter, J. G.** (2003). Effects of drought on nitrogen fixation in soybean root nodules. *Plant, Cell and Environment, 26* (8), 1199–1204.

**Tan, C., Cao, X., Yuan, S., Wang, W., Feng, Y. & Qiao, B.** (2015). Effects of long-term conservation tillage on soil nutrients in sloping fields in regions characterized by water and wind erosion. *Scientific Reports, 5*, 17592.

**Tesfaye, T., Tarekegn, Y. & Walelign, W.** (2018). Biological and inorganic fertilizer applications improved growth, nodulation and yield of soybean (*Glycine max* L.) varieties. *International Journal of Current Research, 10* (05), 68855–68862.

**Thilakarethna, M. S. & Raizada, M. N.** (2017). A meta-analysis of the effectiveness of diverse rhizobia inoculants on soybean traits under field conditions. *Soil Biology and Biochemistry, 105*, 177–196.

**Zarei, I., Sohrabi, Y., Heidari, G. R., Jalilian, A. & Mohammadi, K.** (2012). Effects of biofertilizers on grain yield and protein content of two soybean (*Glycine max* L.) cultivars. *African Journal of Biotechnology, 27,* 7028–7037.

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