doi:10.14720/aas.2022.118.2.1867 Original research article / izvirni znanstveni članek

Symbiotic and physiological indicators of soybean inoculated of *Bradyrhizobium japonicum* single-strain in 7 days before sowing

Nadiya VOROBEY [1], Kateryna KUKOL [1], Petro PUKHTAIEVYCH [1, 2], Tetyana KOTS [1,3]

Received September 09, 2020; accepted June 13, 2022.
Delo je prispelo 9. septembra 2020, sprejeto 13. junija 2022

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Abstract: Results of investigation of soybean of the Almaz variety in inoculation with preparations based on nodule bacteria *Bradyrhizobium japonicum* (Kirchner, 1896), Jordan, 1982 B78, B157, D37, D87 are presented. Different periods of the soybean seeds inoculation were used - on the sowing day (control) and in 7 days before sowing (experimental variants). The differences between control and experimental plants in the formation and functioning of the symbiotic apparatus and its functional activity, depending on the period between from seed inoculation to sowing were analysed. It was determined that the number of root nodules in the control plants was higher. The mass of nodules at the stage of 3 true leaves exceeded the control by 1.5–2.0 times in plants inoculated in 7 days before sowing, and the intensity of nitrogen fixation by 1.7–6.6 times. At the budding-beginning of flowering stage, the mass and intensity of N_2 fixation by the nodules of control plants increased. As a result, the difference between the nitrogen fixing activity of control and experimental plants decreased significantly. Stimulating effect on aboveground mass of *Bradyrhizobium japonicum* strains with increased nitrogen fixing activity was noted. Optimal conditions for the formation and functioning of bean-rhizobial symbiosis were provided at the use of both of these terms of soybean inoculation. This reveals the possibility of effective application of early inoculation of soybean seeds with preparations based on nodule bacteria *Bradyrhizobium japonicum* active strains.

Key words: rhizobia; *Bradyrhizobium japonicum*; bacterial preparations; pre-sowing inoculation; nitrogen fixing activity; soybean

Simbiontski in fiziološki indikatorji soje, inokulirane sedem dni pred setvijo s sevom bakterije *Bradyrhizobium japonicum*

Izvleček: Predstavljeni so rezultati raziskave inokulacije soje, sorte Almaz, s simbiontsko bakterijo *Bradyrhizobium japonicum* (Kirchner, 1896), Jordan; sevi 1982 B78, B157, D37, D87). Inokulacija semen soje je bila izvedena na dan setve (kontrola) in sedem dni pred setvijo (različna obravnavanja v poskusu). Ugotovljene so bile razlike med kontrolo in različnimi obravnavanji v tvorbi in delovanju simbiontskega aparata glede na čas inokulacije. Ugotovljeno je bilo, da je bilo število koreninskih nodulov pri kontrolnih rastlinah večje. Masa nodulov je na razvojni stopnji soje tretjega pravega lista presegala kontrolo pri rastlinah inokuliranih sedem dni pred setvijo za 1,5–2,0 krat , vezava zračnega dušika pa za 1,7–6,6 krat. Na razvojni stopnji začetka cvetenja sta se masa nodulov in jakost vezave N_2 pri kontroli povečali, s čemer se je značilno zmanjšala razlika med kontrolo in obravnavanji. Opazen je bil tudi stimulacijski učinek inokulacije s sevi *Bradyrhizobium japonicum* na nadzemno biomaso soje zaradi povečane vezave dušika. Optimalne razmere za tvorbo in delovanje te rizobijske simbioze s sojo so se pojavile pri obeh načinih inokulacije. To nakazuje možnost učinkovite uporabe zgodnje inokulacije semen soje s pripravki aktivnih sevov bakterije *Bradyrhizobium japonicum.*

Ključne besede: rizobiji; *Bradyrhizobium japonicum*; bakterijski pripravki; predsetvena inokulacija; aktivnost vezave zračnega dušika; soja

1 Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine
2 Corresponding author, e-mail: azotfixation@gmail.com
3 Institute of the Ukrainian Language, National Academy of Sciences of Ukraine, Kyiv, Ukraine

1 INTRODUCTION

The nodule bacteria *Bradyrhizobium japonicum* (Kirchner, 1896), Jordan, 1982 are the basis of biological microbial preparations for inoculation of soybean, that are characterized by multifunctional effect on plants. Their application increases resistance of plant to abiotic and biotic factors, the number and mass of root nodules, the intensity of symbiotic nitrogen fixation, the chlorophyll content in leaves, improves crop productivity and grain quality. This reduces the use of expensive nitrogen fertilizers, and, as a consequence, reduces the negative impact on the environment *(*Patyka & Petrychenko, *2004;* Morgun & Kots*, 2008; Kots, 2011; Kots et al., 2016;* Kramarev & Artemenko, 2016*).*

Increasing the sown areas of soybean testifies its important role in the agricultural complex (Berbenets, 2019). Usually pre-sowing seeds inoculation is carried out on the sowing day or in the day before, which provides a period of 24 hours from preparation application on the seeds to getting inoculated seeds into the soil. It is difficult for growers with large sown areas to treat and sow seeds in the soil in one day (because it takes a long time, related consumables, equipment preparation, and human resources, etc.). Pesticides with which are treated seeds to kill fungal and bacterial infections can also have a negative effect on nodulating bacteria *Bradyrhizobium* (soybean inoculants). All this complicates the pre-sowing treatment of seeds with biologicals in industrial conditions. Therefore, in recent years, the soybean cultivation technology has begun to use seeds with pre-treatment by plant protection products and preparations of nodule bacteria *Bradyrhizobium japonicum*, which are compatible with fungicides and insecticides. Thus, today there is a problem of providing highly effective inoculants for early inoculation of pulses seeds, in particular soybean. They got the name pre-inoculants.

In the segment of soybean inoculants on the Ukrainian market there is a wide range of trade names of preparations of domestic and foreign production (Kokorina & Kozhemyakov, 2010; Kots & Mamenko, 2015). According to the main criteria (type of microorganisms, their titer), the domestic inoculants do not differ from the foreign ones. However, the presence of protectors (preservative, adhesive) in some foreign preparations, increases the treatment manufacturability, simplifies the process of soybean sowing (Slobodyanyuk, 2017).

Pre-inoculants are used as a special element of soybean growing technology, which is in demand today. Pre-inoculant has advantages over conventional inoculants, even with small seed volumes. It contains an additional component that is able to form a protective film and protect bacteria on the surface of seeds from harmful environmental factors. It promotes additional seeds nutrition, provides better germination, increases germination energy and allows pre-treatment of seeds with inoculant.

Currently known pre-inoculants, compatible with the original soybean seed treatment and with the possibility of early application of the preparation before sowing seed in soil in 24 hours – RhizoFlo 5 (Saatbau Linz, Austria), in 1–2 days – Rhizobophyte (Ukraine), in 7 days – Biobacter (Lallemand, Uruguay), in 5–15 days – Rizoform + Static (Schelkovo Agrohim, Russia), in 21 days – Bioboost Plus (Liquid, Canada), in 45 days – HiCoat Super Extender (Agrocenter BASF, Germany) and in 90 days – HiCoat Super (Becker Underwood, USA), Agribacter, Agribacter + Rise (Lallemand, Uruguay) and other (Agritema, 2017).

Improving the nodule bacteria biologicals for soybean in the direction of increase the time between seeds inoculation and their sowing will significantly increase their effectiveness and attractiveness as a tool for obtaining biological nitrogen (Laktionov et al., 2018), because biological nitrogen fixation is an unalterable way to provide plants with nitrogen and does not violate the natural environment ecology (*Kots et al., 2016)*.

Domestic preparations are also known on the Ukrainian market, including Rizoline + Rizosave inoculants with recommendation of early treatment of seeds (7–10 days before sowing). When using a tank mix with Rizoline 2 l t^{-1} + Rizosave 2 l t^{-1} and a fungicide Fever 0.4 l t^{-1}, there were obtained soybean yields higher than control on 2.8–3.3 c ha^{-1} (Slobodyanyuk, 2017).

Microbial preparations with natural film-forming compounds are known, whereby nodule bacteria on seeds are protected from negative external influences and remain viable for a long time. For example film-former for seed inlay, created on the basis of waste plant and animal origin, opens the possibility of seed inoculation in 25–30 days before sowing, maintaining the nodulating activity of rhizobia and increased yields depending on the variety by 12 % (Grishechkin & Golovina, 2014).

Many Ukrainian farmers recognized the advantages of modern inoculation processing technologies. The additional harvests gave them the opportunity to recoup costs, and to make significant profits. The most effective will be the preparation that provides the highest concentration of live bacteria on the seeds at the time of their entry into the soil. Bacterial titers of preparations for soybean inoculation, which are widely available on the domestic market, range from 1×10^9 to 5×10^9 at the time of production and 2×10^8 – (2–4) $\times 10^9$ at the end of the shelf life of the preparation. High-quality two-component preparation for soybean HiCoat®Super of the American company Becker Underwood has the highest of the *Bradyrhizobium* genus bacteria concentration (1×10^{10} cells per 1 g of preparation), and the longest among all known inoculants bacterial life on seeds. This allows seeds inoculation in 90 days before sowing and initiates nodules formation already in the initial stages of plant development.

In the development modern preparations of nodule bacteria for legumes and pulses at the first stage it is important to study the period of viable bacteria on seeds to sowing, virulence and nitrogen fixing activity (NFA) due to pre-inoculation of seeds. Regarding the research of this problem, there are few publications, and the results obtained are debatable. Martyniuk S. et al. (2002) observed a sharp drop in the number of bacteria *Rhizobium lupini* (Schroeter, 1886) Eckhardt et al., 1931 363a and 367a on inoculated lupine seeds Oligarch variety (creator – Leningrad Scientific Research Institute of Agriculture 'Belogorka', Russia), within 24–48 hours after inoculation. However, after added to inoculant of polymeric protector polyvinylpyrrolidone (PVP) at a concentration of 5 % even after 168 hours, sufficient bacteria quantity remained to form an effective symbiosis, possibly due to the protective properties of this polymer and its action as an "adhesive". That is, the use of PVP is potentially able to increase the allowable time between seeds inoculation and sowing up to six days.

The scientists point to several factors that affect the effectiveness of early seeds treatment by inoculants. So, the most important of them are the ability of bacteria to survive on seeds, seeds storage conditions, as well as the influence of other products (compounds) used in inoculation (Anghinoni et al., 2017). There are data in the literature on the treatment of soybean seeds with bulk peat preparations based on active strains of *Bradyrhizobium japonicum* SEMIA 5079 and SEMIA 5080 in 5 days before sowing and on the sowing day. The authors found that both methods of bacterization at the absence of the fungicides use provided the formation of nodules number at the level of control plants (Zilli et al., 2010). This indicated the ability of rhizobia to survive on the seeds of *Glycine max* L. (Merrill) for five days. Regarding the fixation of molecular nitrogen, these researchers did not find significant differences between the variants depending on the duration from inoculation of seeds to sowing.

Bacterial preparations based on active strains of nodule bacteria can be effective for pre-sowing treatment of soybean seeds without the use of extenders. Thus, the aim of our investigation was to study the effectiveness formations and functioning of the symbiotic apparatus, growth and development of soybean plants depending on the duration of the period from seeds inoculation with nodule bacteria *Bradyrhizobium japonicum* to sowing without excipients in microbial preparations.

2 MATERIALS AND METHODS

The experiments were performed with the soybean (*Glycine max* L. (Merrill) seeds of Almaz variety (originator – Poltava State Agrarian Academy, Ukraine), included in the Register of plant varieties of Ukraine since 2007 and recommended for cultivation in the forest-steppe of Ukraine (early ripening, high plasticity to climatic conditions).

Inoculation of seeds was carried out with a liquid-phase preparation made on the basis different in symbiotic activity of nodule bacteria B78, B157, D37, D87 strains (obtained as result of intergeneric conjugation between *Escherichia coli* S17-1 (pSUP5011::Tn5*mob*) and strains 646, 634b from the collection of N_2-fixing microorganisms of the Institute of Plant Physiology and Genetics NAS of Ukraine. Restoration of physiological activity of *Bradyrhizobium japonicum* nodule bacteria after storage in the museum collection was carried out by standard microbiological methods (Netrusov et al., 2005).

The nodule bacteria were grown in biological tubes on a nutrient medium yeast mannitol agar (YMA) (Netrusov, 2005) for 7 days at +28 °C for preparing a liquid-phase preparation. Thereafter, the biomass of bacteria were washed off from agar, and transfered in glass flasks with liquid YM environment (10 ml of suspension per 350 ml of YM) and cultured during 7 days at a temperature of 28 °C and constant aeration. The bacterial titer of the preparation, which were used for inoculation of soybean seeds was $4.1–4.5 \cdot 10^9$ CFU (colony forming units) per g of the preparation.

Soybean seeds were externally sterilized for 15 min with 70 % ethanol, washed with running water, inoculated for 1 h by prepared liquid microbial preparations and sowed in the substrate. Variants with inoculation of soybean seeds in 7 days before the day of sowing (experimental variants) and with inoculation of seeds in 1 hour before sowing (control) were included in the experimental scheme.

Soybeans were grown on a sandy substrate (10 kg washed river sand, 8 plants in each pot on) with the introduction of Hellriegel nutrient mixture with 0.25 of nitrogen norm (1 norm was 708 mg $Ca(NO_3)_2 \cdot 4H_2O$ per kg of sand) (Grodzinsky & Grodzinsky, 1964). River sand is the sand extracted from riverbeds, which is characterized by a high degree of purification and the absence of foreign inclusions. The pots with plants were placed on a specially equipped site of the Institute of Plant Physiology and Genetics NAS of Ukraine under conditions at natural light, temperature and artificial controlled irrigation (Figure 1). Sowing seeds – 18.05.2018, the first seedling – 23.05.2018. Repeatability of the experimental variants was 5 times.

Selection of plants for the analysis was carried out in the stages: 3 true leaves (on the 28th day after germination), budding-beginning of flowering (on the 35 days after germination), full flowering soybeans (on the 48 days after germination).

The nodulating ability of *Bradyrhizobium japonicum* was determined by counting the number and mass of root nodules in 10 plants of each variant of the experiment. Biometric indices – the mass of the aboveground part of plants and roots – in 15 plants of each variant of the experiment. N_2-fixation activity was determined by acetylene method in terms of acetylene regeneration activity by root nodules of soybean (Hardy et. al., 1968) and expressed in μmol of ethylene, produced by nodules of 1 plant for 1 hour. The roots with nodules were placed in hermetically sealed glass vials with a capacity of 75 cm^3, 10 % of acetylene of the total volume was injected through the rubber membrane. Incubation period with acetylene - 1 hour. A gas mixture containing ethylene, formed as a result of acetylene reduction by nitrogenase, was analyzed on Agilent Technologics 6850 Network GC System (USA) gas chromatograph with flame ionization detector. Separation of gases was performed on a column Supelco Porapak N at thermostat temperature +55 °C and detector +150 °C. The gas carrier was nitrogen (50 ml per 1 min). Sampling capacity for analysis was 1 cm^3. Pure ethylene was used as the standard. The amount of ethylene formed from acetylene for 1 h under the action of nitrogenase incubated sample (the nitrogen fixing activity) was represented in molar units of ethylene formed per 1 plant for 1 hour – μmol C_2H_4 (plant h)$^{-1}$. Experiment with determine of N_2-fixation activity was repeated five times.

The statistical processing of the obtained data was conducted using ANOVA and the Tukey HSD Test with the average values. The results were presented in the form of mean values and standard error (m ± SE). The difference between the data was considered significant, if $p \leq 0.05$.

3 RESULTS AND DISCUSSION

The ability to penetrate in the legume root through

Figure 1: The pots with plants on a specially equipped site of the Institute of Plant Physiology and Genetics NAS of Ukraine

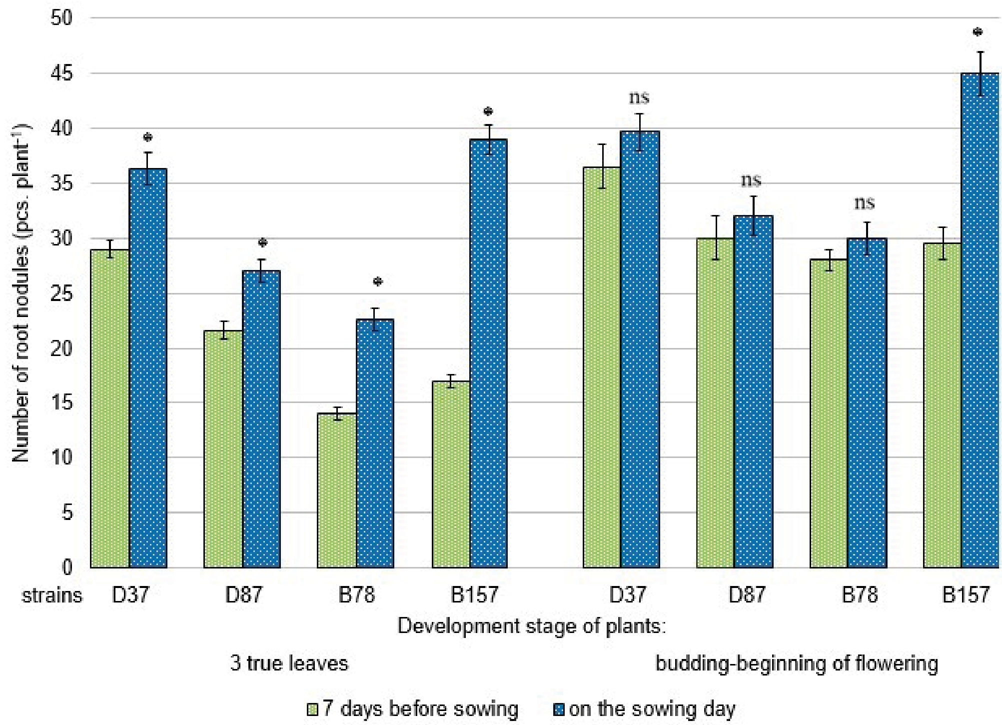

Figure 2: Root nodules number (pcs. plant^{-1}) in soybean Almaz variety depending on the period duration from seed treatment by strains of *Bradyrhizobium japonicum* (Kirchner, 1896), Jordan, 1982 D37, D87, B78, B157 to sowing (n = 10).
m ± SE. An asterisk (*) indicates statistically significant difference between treatments (paired columns) at $p \leq 0.05$; ns – no significant difference

root hairs, and in the process of complex morphophysiological changes to cause the formation of nodules indicates the virulence of nodule bacteria. Root nodules are the complex constructed organs of plants, the main structures of which are bacteria-infected tissue, where nitrogen is fixed, and the meristem.

We established that plants depending on the duration of the period from seeds inoculation to sowing and the inoculants on basis of *Bradyrhizobium japonicum* differed for number of root nodules formed. In the control plants (inoculated on the sowing day) formed more root nodules compared to the plants of the experimental variants (inoculation in 7 days before sowing). In particular, at stage of the 3 true leaves formation on soybean roots 22.6–39.0 nodules were counted. At the same time, in plants whose seeds were inoculation in 7 days before sowing, nodule number ranged from 14.0 to 29.0 per root (Figure 2). More intensive nodules formation was fixed in the budding-beginning of flowering period of soybean: 30.0–45.0 pieces per 1 root in variants with seeds inoculation in 1 hour before sowing and 28.0–36.5 pieces per 1 root in variants with seeds inoculation in 7 days before sowing.

Depending on the different time intervals between seed inoculation and sowing used in this study, no significant differences in the nodules location on the roots were observed. Symbiotic organs (nodules) formed mainly on the main root of soybean and branches of the first order at a depth of 1–17 cm and had a light pink color, indicating on the synthesis of leghemoglobin and nitrogen-fixing ability.

It should be noted the inoculating strains showed different virulence. When roots were infected by strain B78 the number of formed nodules was the lowest among the studied variants for both terms of preparations use. Probably due to the functional features of these rhizobia (reduced ability to survive on the seeds surface, less mobility and speed of penetration into the root meristem, etc.). Inoculation of soybeans with the preparation of nodule bacteria strain B157 was provided the largest number of root nodules in plants inoculated on the sowing day. In 7 days before sowing the largest nodules number was formed on the soybeans roots inoculated with nodule bacteria strain D37 (Figure 2).

It is known that after invasion of nodule bacteria in the roots of plants is realized by the formation of bacteroids and the growth of meristem due to which the mass of the nodules increases (Spaink et al., 2002). At the stage of 3 true leaves, in plants which seeds were inoculated in 7 days before sowing mass of nodules was 1.2–2.0 times higher than that of the variants at the sowing day. At the budding-beginning of flowering stage, the difference in this index between the variants has been decreasing due to a more intensive increase in the mass of root nodules in variants with seed inoculation on the sowing day (Table 1).

Thus, in the variants with a prolonged period of 7 days from seeds inoculation to sowing, the nodule bacteria strains retained their functional activity, which

Table 1: Root nodules mass (mg plant^{-1}) in soybean Almaz variety depending on the period duration from seed treatment to sowing (n = 10)

	Development stage of plants:					
	3 true leaves			budding-beginning of flowering		
Inoculant strain	7 days before sowing	on the sowing day		7 days before sowing	on the sowing day	
D37	0.152 ± 0.006b	0.122 ± 0.010c	*	0.235 ± 0.012a	0.214 ± 0.013b	ns
D87	0.203 ± 0.005c	0.101 ± 0.006b	*	0.311 ± 0.016b	0.241 ± 0.020c	*
B78	0.097 ± 0.003a	0.063 ± 0.002a	*	0.220 ± 0.011a	0.143 ± 0.010a	*
B157	0.198 ± 0.016c	0.113 ± 0.008bc	*	0.305 ± 0.012b	0.292 ± 0.016d	ns

m ± SE, * – significant difference at $p \leq 0.05$; ns – no significant difference; interaction: a – not significant. For each strain and each variable, different letters a, b, c, d indicate significant differences

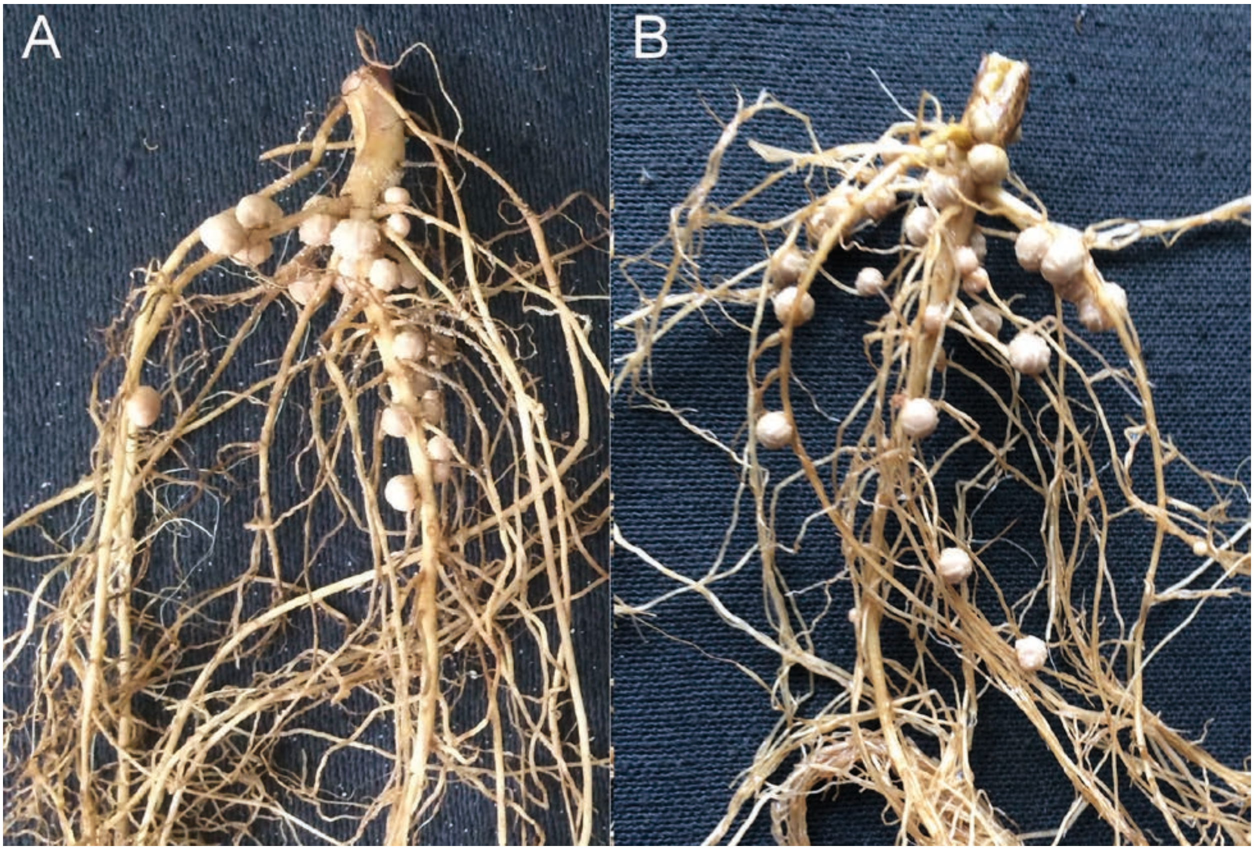

Figure 3: Nodules on soybean roots at seeds inoculation by the active strain *Bradyrhizobium japonicum* (Kirchner, 1896), Jordan, 1982 D87 at the budding-beginning of flowering stage, A – inoculation at the sowing day, B – inoculation in 7 days before sowing

was realized in the initial stages by active formation of root nodules (Figure 3).

Brazilian researchers, studying the effectiveness of early seeds inoculation of *Glycine max* L. (Merrill) in 5 and 10 days before sowing, using of chemical plant protection products, have established, that seeds treated with pesticides based on fludioxonil and thiamethoxane can be treated with bacterial preparations and stored for 10 days before sowing without negative impact on grain yield (Anghinoni et al., 2017). The same authors noted the influence of the duration from inoculation of soybean seeds to sowing in the soil on certain factors related to the nodulation. The issue of joint use of fungicides and inoculation requires the detailed study to ensure the effective formation and functioning of legume-rhizobial symbiosis and protection against phytopathogens of various etiologies. In model pot experiments in the Institute of Plant Physiology and Genetics NAS of Ukraine the effect of pesticides on the formation and functioning of the symbiotic apparatus of soybean plants was studied. A negative effect of a number of fungicides on the photosynthetic rate and nitrogen-fixing activity of soybean plants was studied. The strength of this effect depended on the preparation and the term of its use before sowing (Pavlyshche et al., 2017).

The time interval of 7 days between seeds inoculation and sowing is permissible in the case of preparations use based on mentioned active strains of nodule bacteria *Bradyrhizobium japonicum* with a high level of exopolysaccharides production. The latter can serve as natural substitutes for synthetic adhesive extenders, which are used in modern pre-inoculants. It is known that bacterial exopolysaccharides, forming a biofilm around rhizobial cells, provide their adsorption on the seed surface and protective function (Melnykova, 2019), thereby contributing to their preservation on seeds for a certain period of time and the restoration of physiological and symbiotic characteristics.

It is also actual studying the practical application of a wide spectrum water-soluble synthetic polymers as adhesive and film-formers as a part of biologicals for improvement of bacteria adhesion on a seed surface (by type of multicomponent formulations in the production of modern chemical treaters). Russian scientists have tested for this purpose low and high molecular mass sodium alginate (FMC polymer), hydroxypropyl methylcellulose (HPMC) (Colorcon, "Colorcon, Inc.", USA), polyethylene glycol (PEG), carbomer, polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP). Polymers are also capable prolonging the expiration date of microbial preparations, increase their compatibility with chemical plant protectors, resistance to ultraviolet radiation, temperature differences, drying, increase the survival of rhizobia on the seeds surface, which allows of pre-treatment. In the study of survival of 634b strain on the 'Belgorodska 7' (creator – Federal State Budgetary Educational Institution of Higher Education "V. Gorin Belgorod State Agricultural University", Russia) soybean variety seeds under the influence of polymers of different origin and composition it was shown that polyvinylpyrrolidone 10 % solution is the most effective of these compounds. Its use ensures the preservation of 10 times more number of viable rhizobia on the seeds in 10 days after inoculation compared to control (Laktionov et al., 2019).

The most important criterion for evaluating the effectiveness of symbiotic systems *Glycine max* – *Bradyrhizobium japonicum* is their molecular nitrogen fixation rate, which is based on the functioning of nodule bacteria enzyme nitrogenase and interrelated metabolic processes of symbiosis partners.

At the stage of 3 true leaves the nitrogen fixation of soybean root nodules was 1.7, 6.6, 4.5 and 1.8 times more intensive in plants which seeds were inoculated by D87, B78 and B157 strains in 7 days before sowing, compared with control plants (inoculation in 1 hour before sowing with similar preparations). The high level NFA of symbiotic systems when treating with rhizobia 7 days before sowing seems to be the result of a sufficient number of microbial cells for become infected after this period. Researchers have found that the viability of nodule bacteria on seeds without the use of pesticides depends on the plant species and the biological qualities of microorganisms (Gemell et al., 2005), as well as the duration and storage conditions of inoculated seeds (Deaker et al., 2012).

Pre-inoculation of soybean seeds (in 7 days before sowing) by preparation based on D87 strain provided the highest level of N_2 assimilation due to the formation of the largest nodules mass on the roots of plants (Figure 4).

At the budding-beginning of flowering stage the nitrogen fixation activity of soybean root nodules has increased in variants with seeds inoculation on the sowing day. As a result, the difference between the NFA indicators of control (inoculation at the sowing day) and experimental plants (inoculation in 7 days before sowing) has decreased and gradually to equalize. The identified features of the formation and functioning of the symbiotic apparatus of soybeans, formed due to the use of different terms between inoculation and sowing, indicate a significant role of adaptive properties of the microsymbiont during prolonged stay on the seeds before sowing and in the process of the formation of bean-rhizobial symbiosis. Therefore, the produce of microbial preparations for pre-inoculation of seeds requires proper selection of strains of *Bradyrhizobium japonicum* taking into account their adaptive and physiological characteristics.

Thus, as a result of the application of mentioned time periods from seed inoculation to sowing, a symbiotic apparatus was formed on soybean roots, the nitrogen fixation rate of which changed during the growing season, that affected the plant supply by biological nitrogen.

Intense assimilation of N_2 by root nodules provokes in plants a growing demand for photoassimilates and causes their redistribution (Kirizii et al., 2007). The regulatory role of nitrogen fixation in plant metabolism can stimulate or slow the growth of aboveground mass and rhizogenesis. At the stage of 3 true leaves, the aboveground and the root mass of inoculated plants (on the sowing day) outweighed the corresponding indices of plants bacterized in 7 days before sowing. During the soybean growing season, the difference in root mass between plants of control and experimental variants decreased and was not significant at the budding-beginning of flowering stage (Table 2). The root system of plants in all variants was well developed, with a large number of lateral roots, which provided an increase in the soybean nutrition area surface.

Plant mass is one of the indices that characterizes the conditions of growth and development in different stages of the growing season. An actively functioning symbiotic apparatus is a more powerful sink of assimilates compared to vegetative growth. Therefore the photosynthetic apparatus is not always fully able to provide the needs of all growth meristems in assimilates when the balance between growth, photosynthesis and nitrogen fixation is disturbed.

In the early period of functioning of the bean-rhizobial symbiotic system of soybean (3 true leaves stage) in control and experimental plants there was a positive relationship between the intensity of nitrogen-fixing activity and aboveground mass growth (Figure 4; Table 3).

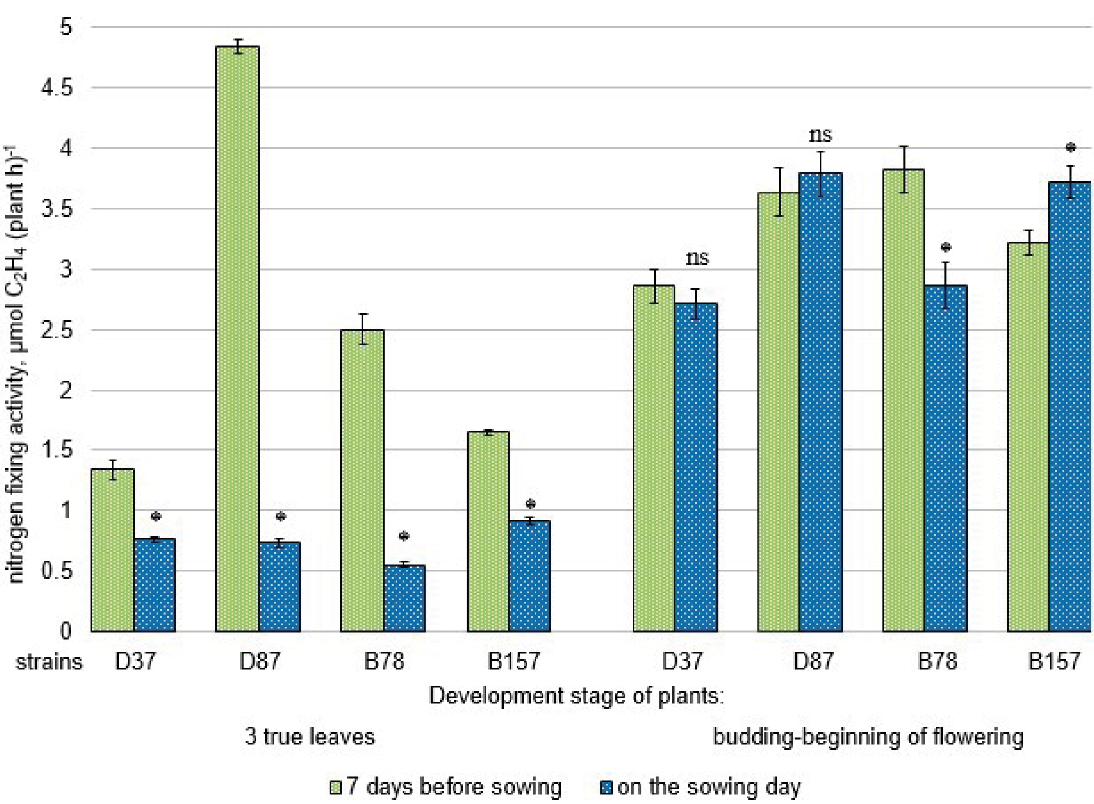

Figure 4: The nitrogen fixing activity, μmol C_2H_4 (plant h)$^{-1}$, of root nodules of the Almaz soybean variety plants depending on the period duration from seed treatment by *Bradyrhizobium japonicum* (Kirchner, 1896), Jordan, 1982 D37, D87, B78, B157 strains to sowing (n = 5).
m ± SE. An asterisk (*) indicates statistically significant difference between treatments (paired columns) at $p \leq 0.05$; ns – no significant difference

Table 2: The root mass (g plant^{-1}) of the Almaz soybean variety, under inoculation with nodule bacteria *Bradyrhizobium japonicum* (Kirchner, 1896), Jordan, 1982 (n = 15)

	Development stage of plants:					
	3 true leaves			budding-beginning of flowering		
Inoculant strain	7 days before sowing	on the sowing day		7 days before sowing	on the sowing day	
D37	2.09 ± 0.08a	2.30 ± 0.09a	*	3.34 ± 0.16a	3.08 ± 0.17a	ns
D87	2.04 ± 0.08a	2.28 ± 0.07a	*	3.33 ± 0.17a	3.45 ± 0.11a	ns
B78	2.35 ± 0.09b	2.50 ± 0.07ab	ns	3.19 ± 0.15a	2.95 ± 0.11a	ns
B157	2.08 ± 0.08a	2.31 ± 0.08a	*	3.14 ± 0.12a	2.86 ± 0.12a	ns

m ± SE, * – significant difference at $p \leq 0.05$; ns – no significant difference; interaction: a – not significant. For each strain and each variable, different letters a, b indicate significant differences

Table 3: The aboveground mass (g plant^{-1}) of the Almaz soybean variety, under inoculation with nodule bacteria *Bradyrhizobium japonicum* (Kirchner, 1896), Jordan, 1982 (n = 15))

	Development stage of plants:								
	3 true leaves			budding-beginning of flowering			full flowering		
Inoculant strain	7 days before sowing	on the sowing day		7 days before sowing	on the sowing day		7 days before sowing	on the sowing day	
D37	2.57a ± 0.12	2.50a ± 0.11	ns	3.86a ± 0.23	4.23a ± 0.13	ns	6.75a ± 0.28	6.82a ± 0.32	ns
D87	2.84a ± 0.18	2.73ab ± 0.11	ns	4.06a ± 0.28	4.60a ± 0.32	ns	7.62a ± 0.38	7.21a ± 0.30	ns
B78	2.87a ± 0.13	2.64ab ± 0.13	ns	3.77a ± 0.12	4.08a ± 0.30	ns	6.97a ± 0.32	7.18a ± 0.31	ns
B157	2.75a ± 0.13	2.93b ± 0.14	ns	3.70a ± 0.24	4.24a ± 0.32	ns	7.58a ± 0.23	7.23a ± 0.27	ns

m ± SE, * – significant difference at $p \leq 0.05$; ns – no significant difference; interaction: a – not significant. For each strain and each variable, different letters a, b indicate significant differences

Then, before the budding-beginning of flowering stage in control plants, the growth in aboveground mass accelerated and outpaced the growth of plants bacterized in 7 days before sowing with strains D37, D87, B78 and B157 by 9.6, 13.3, 8, 2 and 14.5 % respectively. There was an intensive increase in the vegetative mass of plants in all variants of the experiment from the budding stage to the full flowering stage. During the full flowering stage, which is related to the redistribution of assimilates and the formation of generative organs, the indicators of aboveground mass of control and experimental plants, taking into account the error of the experiment, also did not differ significantly. Thus, when applying seed bacterization on the sowing day, this indicator was in the range of 6.82–7.23 g plant^{-1} and with seed inoculation in 7 days before sowing – 6.75–7.58 g plant^{-1}. The dynamics of the aboveground mass formation of control and experimental plants was similar during the growing season. Optimal conditions for the formation and functioning of legume-rhizobial symbiosis for plants were provided using both of period of soybean inoculation.

In the vegetation experiment soybean were grown on a river sandy substrate with the introduction of Hellriegel nutrient mixture with 0.25 of nitrogen norm. It is probable that mineral nitrogen was used in the earlier stages of plant growth and development, and during the soybean flowering period the nutrition was mainly due to biologically N_2 (due to the functioning of the symbiotic apparatus of plants). Therefore, the stimulation of vegetative growth was more active in plants inoculated with preparations of nodule bacteria D87, B78 and B157 strains with increased nitrogen fixation intensity.

4 CONCLUSIONS

Thus, our studies have shown that inoculation of soybean seeds with microbial preparations based on

Bradyrhizobium japonicum on the sowing day caused the formation of more nodules on the plant roots. However, the mass of the formed nodules and the intensity of nitrogen fixation significantly dominated in plants inoculated in 7 days before sowing the seeds in the stage of the 3 true leaves only. The increase in the intensity of nitrogen fixation in control plants in the budding-beginning stage of flowering caused to the equalization of the difference of nitrogen fixation activity between the variants with different terms between inoculation and sowing. This allows the effective use of bacterial preparations based on active strains D37, B78, D87, B157 for pre-sowing treatment of soybean seeds (in 7 days before sowing) without the use of extenders. In the future, it is advisable to study the ability to nodulate and assimilate N_2 active strains of nodule bacteria (and preparations based on them without the use of extender) under conditions of longer delay of seeds sowing from their inoculation. The results obtained are important for elucidation of the possibility of introduction of nodule bacteria active strains obtained by biotechnological methods as a bacterial basis of preparations for pre-inoculation of soybean.

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