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BIODIVERSITY AND PHYSIOLOGICAL PROPERTIES OF BACTERIA FORMING SYSTEMS WITH GLYCINE MAX (L.) MERRIL

Biological nitrogen fixation provides a significant share of nitrogen nutrition of plants, including soybean. The efficiency of soybean-rhizobia systems depends on physiological properties and relationships between the introduced microsymbiont and indigenous microorganisms. Besides, the key of ecological balance in agrophytocenoses and their increased stress resistance and productivity lies in preservation of biodiversity.

*Evaluation of diazotrophic associations' composition of soybean rhizosphere by analyzing the diversity of *nifH* gene, that is a molecular marker of nitrogen fixation, has revealed representatives of phyla Firmicutes and Proteobacteria. Microorganisms belonging to the *Clostridium*, *Paenibacillus* and *Spirochaeta* genera were found to be predominant among them. It was shown that *Bradyrhizobium japonicum* was able to synthesize phytohormones with stimulating action, such as auxins, cytokinins and phytohormones-inhibitors (abscisic acid and ethylene). Cultivation in the presence of flavonoids genistein and naringenin has led to the narrowing of the range and reduction of the amount of synthesized phytohormones. The inhibiting effect of flavonoids on the synthesis of phytohormones by soybean rhizobia can be explained by the change in their metabolism toward the launch of effective nodulation mechanisms. The application of complex inoculation contributed to better development of agronomically useful microorganisms in rhizosphere, preservation of their diversity and increase of soil biological activity.*

K e y w o r d s: *soybean, rhizospheric microorganisms, biodiversity, *nifH* gene, phytohormones of *Bradyrhizobium japonicum*, flavonoids.*

Nitrogen-fixing microorganisms are important components of various ecosystems, which is due to their crucial role in the biogeochemical cycles of nitrogen, formation of soil fertility and preservation of nutrient resources within an ecosystem. The biological nitrogen fixation and photosynthesis provide the productivity of biosphere. In addition, diazotrophs supply biologically active compounds to the phytopartner, protect its root system from the aggression of pathogenic microorganisms etc. [1–3]. This contributes to improving conditions for plants development and increasing their immune status and physiological activity. As a result, the yield increases and its quality improve. However, our knowledge about the phylogenetic diversity of diazotrophs and their ecological importance is still incomplete.

Despite the constant interest of researchers in evaluation of nitrogen fixing soil activity, the composition of nitrogen fixing microbial communities remains insufficiently studied, and the existing data are based mainly on the use of traditional cultivation methods. While the majority of soil microorganisms are impossible to cultivate in a lab (no more than 1 % of all bacteria can be cultivated), in the last decades molecular methods of studying their diversity have been used. However, the data on metagenomic analysis of microbial

communities are limited. Therefore, the study of diazotrophs biodiversity by using molecular biological methods is promising.

Symbiosis is a complex multistage process with an exchange of signals between its partners. At the same time, extracellular metabolites of symbiotic partners are the basis of their signal and trophic relations and, therefore, are the cause of formation of legume-rhizobium systems and phytosphere microbiocenosis [2, 4].

One of the properties of rhizospheric symbiotic diazotrophic microorganisms is the ability to synthesize a wide range of biologically active substances, in particular, the compounds with phytohormonal properties. They act as signaling molecules that regulate metabolic reactions in the formation and functioning of symbiosis. It is known that microbial phytohormones regulate many important processes in symbiosis, such as stimulation of rhizogenesis, increase of roots surface area, enhancement of exometabolites exchange efficiency in the plant-soil-microorganisms systems, stimulation of plant cells proliferation (nodulation), control of the number of nodules and their mass, increase in the nitrogenase activity of bacteroides, enhancement of plant resistance to environmental stress factors [4–7]. Research of the ability of nodule bacteria of various efficiency to synthesize phytohormones is perspective in the view of plant hormonal balance optimization through the introduction of active strains.

It is known, that during the formation of symbiosis phenolic compounds of flavonoid nature are permanently secreted with exudates of legumes into the root zone. Only a few of them are specific molecular signal inducers of rhizobial nodulation genes (*nod*, *noe* and *nol*) [8–10] and catabolic rhizobia genes transcription [11]. The most active inducer of *nod*-genes for alfalfa rhizobia is the flavonoid luteolin, for pea rhizobia it is naringenin and for soybean rhizobia – daidzein and genistein [12]. The influence of plant flavonoids on physiological activity of symbiotic diazotrophs is insufficiently explored. In particular, in literature there are no data on the influence of flavonoids on synthesis of phytohormonal compounds by soybean rhizobia.

Therefore, the aim of our research in recent years was to study the biodiversity of diazotrophic microbial communities of soybean rhizosphere and to determine the ability to synthesize phytohormonal compounds by *Bradyrhizobium japonicum* strains with different efficiency, as well as under the action of flavonoids.

Significant progress in the study of diversity of soil microorganisms was achieved due to the development and improvement of methods for analysis of soil total microbial DNA. They include integrated evaluations based on the study of kinetics of DNA reassociation [13], hybridization *in situ* with fluorescent probes (FISH) [14–16], analysis of nucleotide sequences of the taxonomic marker 16S rRNA [17] or special functional genes-markers [18, 19]. Such approaches provide unique understanding of diversity and structure of microbial communities.

Presently, the analysis of evolutionarily conservative *nifH* gene is widely used for detection of nitrogen fixing microorganisms in natural microbial communities. A number of works have been published based on the analysis of *nifH* gene, which is a convenient phylogenetic gene-marker for diazotrophs studying in natural ecosystems. PCR analysis of the *nifH* gene fragments have been

successfully used for evaluation of nitrogen fixing communities composition in various soils [20–27]. However, the data on the composition of rhizospheric microbiocenoses of diazotrophs [28] and on the impact of the bioagents introduction, which are the components of microbial preparations, on diversity of the root zone microbiota are insufficient.

By analyzing the functional *nifH* gene [29], we studied the diversity of nitrogen fixing microorganisms in rhizospheric soil of soybean under pre-sowing inoculation of seeds with rhizobia monoculture of *B. japonicum* UCM B-6018 and its complex with phosphate mobilizing bacteria *Bacillus megaterium* UCM B-5724, that is included in the composition of the binary inoculant Ecovital (certificate of state registration ANo 05106) [30].

The performed phylogenetic analysis showed that in rhizosphere of both inoculated and control plants the majority of received nucleotide sequences were similar to those of *Firmicutes* members of the domain *Bacteria*. In addition, some sequences were identified as such belonging to the phylum *Proteobacteria*. Other authors have also identified them in the composition of rhizosphere microbial communities, using both microbiological and molecular approaches [20–24, 31].

According to the obtained results, the major part of *nifH* gene sequences formed a compact cluster with obligate and facultative anaerobic bacteria (*Clostridium*, *Paenibacillus* genera of the phylum *Firmicutes*). Diazotrophs that formed a cluster together with the representatives of α -*Proteobacteria* were also identified and found to be similar to the members of *Leptospirillum*, *Derxia*, *Azohydromonas* genera. The Phyla similar to *Clostridium pasteurianum* also formed a widespread band. The representatives similar to *Spirohaeta stenostrepta* were included into the cluster of anaerobic nitrogen fixators of the genus *Clostridium*. Sequences with high degrees of similarity (100 %) to the sequences of *Paenibacillus massiliensis* were also detected, which allowed to identify this phylotype as a representative of this genus.

When comparing our results with the data known from literature, it should be noted that in the majority of studies on rhizosphere nitrogen-fixing microbial communities the representatives of *Proteobacteria* dominated [31]. Researchers found representatives of anaerobic diazotrophs in the rhizospheric soil despite the fact that plants roots transport oxygen [32]. This can be explained by the presence of anaerobic microzones in the root zone. Facultative anaerobic representatives of *Paenibacillus* and *Natronobacillus* genera of nitrogen-fixing communities dominated in the rhizosphere of potatoes [33].

We determined that anaerobic organisms similar to clostridium, sulphate reducers and methanogens were dominant nitrogen-fixing bacteria in gray forest soil of soybean rhizosphere [29]. The dominance of anaerobes in this rhizospheric soil can be related to the specific composition of soil organic substance, soil mechanical composition, or features of microbial communities in those soils.

The performed molecular analysis showed that in rhizospheric nitrogen-fixing communities of soybean both known and unidentified nitrogen-fixing bacteria belonging to obligate anaerobes were present. These representatives exhibited the greatest similarity (92–95 %) to the sequences of natural clones which taxonomic identity was unclear. The obtained results do not contradict those available in literature. When analyzing natural communities, not more

than 30–40 % of clones of functional genes exhibit sufficiently high similarity to the known microorganisms, which can be cultivated [32].

At the same time, in order to determine the structure and metabolic potential of rhizosphere microbiocenosis, the composition of basic ecological and trophic groups was analyzed using microbiological methods. A positive effect of inoculation on the amount of ammonifying, amylolytic, phosphate mobilizing, oligoazotrophic and prototrophic microorganisms was shown [34]. The highest quantity of phosphate mobilizing and prototrophic microorganisms was revealed in the case of inoculation with the complex biopreparation Ecovital. Plants of this variant had better morphometric characteristics, photosynthetic apparatus and productivity. The application of this complex composition of rhizobia and phosphate mobilizing microorganisms for inoculation improved the phosphoric nutrition of plants and the efficiency of symbiotic systems.

Biosynthetic activity of rhizobia, along with their biodiversity, is important to form effective symbiosis. Synthesis of phytohormonal substances is considered as an important factor in the interaction of PGPR-bacteria (plant growth promoting rhizobacteria) with a plant, influencing the growth and development of the latter [35]. Particularly, it has been determined that the synthesis of physiologically active forms of auxins is inherent in most soil microorganisms that have associative relations with plants. Auxins act as signaling molecules in the microbial-plant interactions [36, 37]. On the other hand, it has been shown that even low level of indole-3-acetic acid (IAA) synthesis by associative bacteria was able to stimulate systemic plant resistance by speeding up the passage of sensitive to infection phases of plant development [38].

There are no data in literature on comparative research of phytohormones synthesis by soybean rhizobia with different symbiotic efficiency. We studied the content of auxins, cytokinins, abscisic acid and ethylene in the biomass and the cultural liquid supernatant of rhizobia. For this purpose we investigated 6 strains of soybean nodule bacteria with different activity of nitrogen fixation in the conditions of symbiosis, such as: the ineffective *Bradyrhizobium japonicum* 604k strain, forming nodules on soybean roots and lacking any nitrogen fixing activity, the *B. japonicum* 21110 strain with low efficiency. Also we investigated highly effective soybean rhizobia *B. japonicum* UCM B-6018, UCM B-6023, UCM B-6035 and UCM B-6036, which were used for obtaining microbial inoculants to improve soybean-rhizobial symbiosis.

Accumulation of phytohormones in the cells of rhizobia strains with different symbiotic efficiency was low and ranged between 0.004–2.0 µg/g of absolutely dry biomass (ADB). We can suggest that these phytohormones do not play a significant regulatory and physiological role in bacterial cells [39].

Simultaneously with the determination of phytohormones accumulation in bacterial biomass the ability of microorganisms to produce these compounds as exometabolites was studied. It was found that auxins synthesis by rhizobia is a strain feature not associated with their symbiotic activity [40]. For example, the ineffective *B. japonicum* 604k strain, which forms a large number of nodules with almost no nitrogenase activity in symbiosis, is able to synthesize high amounts of auxins (1067.0 µg/g ADB). The strain synthesizes such indole compounds as indole-3-carboxylic acid, indole-3-carbinol and indole-3-acetic hydrazide, but it does not synthesize the physiologically active for

plants auxin – IAA. The total level of auxins synthesis by the ineffective strain was almost equal to the corresponding amount produced by highly effective *B. japonicum* strains [40]. Another active soybean rhizobia strain – *B. japonicum* UCM B-6035 – synthesized only the IAA out of the mentioned indole compounds, though in large amounts that exceeded 770 µg/g ADB.

It is known that auxins synthesis by rhizobia at the stage of plant infection is associated with pathogenesis. Nodule bacteria are described in literature as “weak” pathogens; because the initial stage of symbiosis establishment represents an infectious process [36, 41]. *B. japonicum* strains with high virulence and nodulation activity were used in the work. Therefore, the significant synthesis of auxins was quite natural. When the nodulation apparatus is formed, rhizobial auxins are involved in a series of metabolic processes, including cell division, differentiation and formation of a vascular bundle [7, 42, 43]. These phases are necessary for the initial stages of root development and formation of nodules. Later other regulatory mechanisms of efficient symbiosis creation are triggered, among which there are other hormones-stimulants such as cytokinins and gibberellins playing an important role [36, 44].

The range of cytokinins produced by soybean rhizobia with various symbiotic activities differed by qualitative and quantitative composition. Thus, the highly effective symbiont *B. japonicum* UCM B-6018 produced the highest amount of cytokinins (~ 1555 µg/g ADB), among which *trans*-zeatin-riboside prevailed. The strain was also able to synthesize a significant amount of other cytokinins, in particular zeatin and isopentenyl adenosine. It should be noted that the amount of *trans*-zeatin-riboside synthesized by highly effective strains of soybean nodule bacteria was 1.7–7.9 times higher than the amount synthesized by ineffective and low active strains.

Trans-zeatin-riboside is a transport form of cytokinins with very low physiological activity. When forming symbiotic relationships, the synthesis of this form of cytokinins by bacteria allows a plant to obtain a ready-for-transport form that with an ascending current enters the aboveground plant parts. In a plant, *trans*-zeatin-riboside is transformed by hydrolysis to the active form – zeatin, which is involved in the regulation of cell metabolism, alters the pool of physiologically active cytokinins in plant tissues and strengthens certain links of metabolism.

The total amount of cytokinins, synthesized by effective strains of soybean rhizobia, was 4.4–9.4 times higher as compared to the low effective strain *B. japonicum* 21110, and it was 3.9–11.0 times higher as compared to the ineffective strain *B. japonicum* 604k. This gives a reason to suggest a direct positive correlation between the ability of soybean rhizobia strains to synthesize cytokinins and their symbiotic effectiveness. Based on this index it is possible to give a preliminary evaluation of activity of the strains and select them for further experiments on the efficiency of symbiosis in vegetation and field conditions [45, 46]. It is known that cytokinins play the key role in nodules formation and affect the further cell proliferation, elongation, differentiation or aging of nodules, depending on the stage of symbiosis development [5, 47–49].

In the formation and functioning of symbiotic systems, not only phytohormonal compounds of stimulatory action are involved, but also inhibiting phytohormones, such as abscisic acid (ABA) and ethylene. Their synthesis by symbiotic soybean bacteria is studied insufficiently and physiological significance for rhizobia is still debatable.

As the results of our research showed, the synthesis of ABA by symbiotic soybean bacteria is a strain-specific feature and is not related to their nitrogenase activity in symbiosis, but it directly correlates with high virulence of the strains and their ability to form a large number of nodules on the plant roots. Both the high effective soybean microsymbionts and the ineffective strain *B. japonicum* 604k are able to synthesize a significant amount of ABA (56.5–72.0 µg/g ADB).

According to literature, in different strains of rhizobia ABA can both increase and decrease activity of the nitrogenase complex, and, therefore, reduce the efficiency of symbiotic nitrogen fixation. At the same time, the ability of rhizobia to produce ABA is an additional factor that increases the potential activity of these strains in plant infections. In several cases, it was shown that ABA can reduce high concentrations of growth stimulating substances [50].

The role of ABA in legume-rhizobial symbiosis is not clearly determined. Some authors note the inhibitory action of the hormone on the formation of nodules [51]. ABA can interact with cytokinins during the division of cortical cells, inhibiting this process. It is also known that after inoculation with rhizobia the formation of nodules is slowed down under the influence of plant ABA and, on the contrary, the number of nodules increases abruptly when the concentration of hormone is reduced under the action of a specific inhibitor (9-cis-epoxycarotenoid dioxygenase). Observation of the root hair morphology revealed that ABA blocked the stage between the root hair swelling and its curling. It is assumed that in this way ABA controls the number of nodules on a plant's roots [51].

We obtained data on small amounts of gaseous phytohormone-inhibitor ethylene, which is produced by soybean symbiotic bacteria *B. japonicum* (from 0.046 to 3.46 nmol/g·h ADB) [52]. No direct correlation between the level of ethylene synthesis and nitrogenase activity of the strains in symbiosis was observed. But it was noticed that in highly effective strains *B. japonicum* UCM B-6023, UCM B-6018 and UCM B-6035 the amount of synthesized ethylene was 2.3–75.2 times lower as compared to the one in the strain *B. japonicum* 21110 with low efficiency.

Ethylene plays a dual role in formation of nitrogen-fixing nodules: it can inhibit their formation or under certain concentrations, it can stimulate a rhizobial infection [53, 54]. It is known that inhibition of ethylene synthesis increases the number of nodules formed on the roots of legumes [10]. The question of possible involvement of ethylene in inhibiting legume-rhizobia symbiosis under high doses of mineral nitrogen is being discussed. It has been shown that nitrate nitrogen promotes an activation of ethylene synthesis, high concentrations of which inhibit the formation of nodules in alfalfa [55]. Besides, ethylene is a negative regulator of *nod*-factor signaling [56]. Increasing the level of endogenous ethylene in cells of the host plant under high level of NO₃ can cause further limitation of rhizobial infection and nodulation [55].

A significant role in the functioning of the symbiosis also belongs to plant phenolic compounds – flavonoids, which are secreted into rhizosphere [4, 8, 9]. Flavonoid synthesis is mainly inherent in plants of *Leguminosae* family and *Papilionoideae* subfamily.

In plant-microbial relationships, the main function of flavonoids is their interaction with the products of rhizobial *nod* D-gene, and further activation

of transcription of the other *nod*-genes. Flavonoids activate the *nod*-genes that control the mechanisms of rhizobial infection of the legumes roots and the formation of nodulation apparatus. Flavonoids also stimulate chemotaxis and growth of bacteria [9, 57].

Previously, it was shown that flavonoids stimulate biomass accumulation and exopolymers production [58] while also activating nitrogen metabolism enzymes in soybean rhizobia with different symbiotic activity. Genistein and naringenin increased the activity of glutamate dehydrogenase enzyme in the highly effective microsymbiont *B. japonicum* UCM B-6035. Glutamine synthetase activity also increased significantly (by 60 times) in the active strain under the influence of genistein, but it was inhibited in the strain *B. japonicum* 21110 with low efficiency [59, 60].

The research of auxin and cytokinin synthesis by *B. japonicum* was performed under cultivation conditions in the presence of an isoflavonoid genistein, which was a signal molecule of soybean-rhizobia symbiosis, and of euflavonoid naringenin, which was a signal molecule for bean-rhizobia symbiosis.

It was determined that the synthesis of extracellular auxins by soybean rhizobia with different symbiotic activity in the presence of genistein and naringenin significantly decreased, and auxins composition changed. In particular, indole-3-carboxylic acid, indole-3-carbinol and indole-3-acetic hydrazide were not detected. Instead of that, indole-3-butyric acid was found. The amount of IAA synthesized by the highly efficient strain *B. japonicum* UCM B-6035 decreased 77–154 times under the action of these flavonoids. The amount of the synthesized hormone-inhibitor ABA also decreased 70-fold.

We observed a significant decrease in the synthesis of extracellular cytokinins by *B. japonicum* under the influence of genistein and naringenin flavonoids. In such conditions, the most physiologically active hormone zeatin was not found in soybean rhizobia and the amount of *trans*-zeatin-riboside in the supernatant of *B. japonicum* reduced 5–61 times. The total amount of cytokinins produced by soybean rhizobia was 6–80 times lower under the influence of genistein, and it was almost completely suppressed at the action of non-specific flavonoid naringenin. For example, the cytokinin synthesis by the strain *B. japonicum* UCM B-6018 decreased from ~ 1555 µg/g ADB without the addition of flavonoids to 19 µg/g ADB under the action of genistein and to 3 µg/g ADB under the action of naringenin.

The inhibiting effect of the studied flavonoids on phytohormones synthesis by soybean rhizobia may be explained by the change on the microsymbionts' metabolism that is directed not at the synthesis of secondary metabolites, but at the start of effective nodulation.

There are data in literature on flavonoids of different nature and of different concentrations being able to act as both stimulants and inhibitors of microsymbionts physiological activity [4]. The results of our work showed that the presence of genistein and naringenin flavonoids in the nutrient medium for cultivation of rhizobia in concentrations of 0.01 and 10 nmol resulted in a reduction of the range and amount of auxins and cytokinins synthesized by *B. japonicum*. Exactly in such quantities, plants secrete flavonoids into rhizospheric soil at the early stages of symbiotic systems formation and stimulate bacterial growth.

Therefore, the study of flavonoid compounds influence on the physiological activities of nitrogen fixing nodule bacteria, in particular on the synthesis

of phytohormonal compounds, is relevant and necessary for understanding the formation processes and finding the ways for increasing activity and effectiveness of symbiotrophic systems. The study is important and can be used to develop new bioformulations with phytostimulating activity for crop production. The use of microbial preparations with a balanced content of phytohormones can improve the efficiency of symbiosis and plant resistance to phytopathogens.

Thus, the obtained data of metagenomic analysis add to information about diazotrophs diversity in rhizosphere of cultivated plants and expand the knowledge about structure of rhizospheric microbial communities. The performed research indicates that the application of bioformulations based on highly effective strains of nitrogen fixing and phosphate mobilizing microorganisms is promising. The application of complex inoculation contributes to better development of agronomically useful microorganisms of the main ecological-trophic groups in rhizosphere, preservation of their diversity and increase of the soil biological activity. This is an indicator of ecological balance for natural cenoses and a key to increasing their stress resistance and productivity.

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БІОРИЗНОМАНІТНІСТЬ І ФІЗІОЛОГІЧНІ ВЛАСТИВОСТІ БАКТЕРІЙ, ЩО ФОРМУЮТЬ СИСТЕМИ З *GLYCINE MAX* (L.) MERRILL

Біологічна азотфіксація забезпечує значну частку азотного живлення рослин, зокрема, сої. Ефективність соєво-ризобіальних систем залежить від фізіологічних властивостей і взаємовідносин мікросимбіонта-інтродуцента з аборигенними мікроорганізмами. При цьому запорукою екологічної рівноваги в агрофітоценозах та їх підвищеної стресостійкості і продуктивності є збереження біорізноманітності.

Оцінка складу діазотрофних угруповань ризосфери сої шляхом аналізу різноманітності гену *nifH*, молекулярного маркера азотфіксації, виявила представників відділів *Firmicutes* та *Proteobacteria*. Домінуючими серед них були мікроорганізми, що відносяться до *Clostridium*, *Paenibacillus*, *Spirochaeta*. Показана здатність *Bradyrhizobium japonicum* синтезувати фітогормони стимулювальної дії: ауксини і цитокініни та фітогормони-інгібітори: абсцизову кислоту і етилен. Культивування у присутності флавоноїдів геністеїну і нарінгеніну призводило до звуження спектру та зменшення кількості синтезованих фітогормонів. Пригнічуючий вплив флавоноїдів на синтез фітогормонів у ризобій сої може пояснюватися зміною їх метаболізму у напрямку запуску механізмів ефективної нодуляції. Застосування комплексної інокуляції сприяло кращому розвитку в ризосфері агрономічно корисних мікроорганізмів, збереженню їх різноманітності та підвищенню біологічної активності ґрунту.

Ключові слова: соя, ризосферні мікроорганізми, біорізноманітність, *nifH* ген, фітогормони *Bradyrhizobium japonicum*, флавоноїди.

**БИОРАЗНООБРАЗИЕ И ФИЗИОЛОГИЧЕСКИЕ СВОЙСТВА БАКТЕРИЙ,
ФОРМИРУЮЩИХ СИСТЕМЫ С *GLYCINE MAX* (L.) MERRIL**

Биологическая азотфиксация обеспечивает значительную долю азотного питания растений, в частности, сои. Эффективность соево-ризобияльных систем зависит от физиологических свойств и взаимоотношений микросимбионта-интродукента с аборигенными микроорганизмами. При этом залогом экологического равновесия в агрофитоценозах и их повышенной стрессоустойчивости и продуктивности является сохранение биоразнообразия.

Оценка состава диазотрофных групп ризосферы сои путем анализа разнообразия гена *nifH*, молекулярного маркера азотфиксации, обнаружила представителей отделов *Firmicutes* и *Proteobacteria*. Доминирующими среди них были микроорганизмы, относящиеся к *Clostridium*, *Paenibacillus*, *Spirochaeta*. Показана способность *Bradyrhizobium japonicum* синтезировать фитогормоны стимулирующего действия: ауксины и цитокинины, и фитогормоны-ингибиторы: абсцизовую кислоту и этилен. Культивирование в присутствии флавоноидов генистеина и нарингенина приводило к сужению спектра и уменьшению количества синтезированных фитогормонов. Угнетающее влияние флавоноидов на синтез фитогормонов ризобиями сои может объясняться изменением их метаболизма в направлении запуска механизмов эффективной нодуляции. Применение комплексной инокуляции способствовало лучшему развитию в ризосфере агрономически полезных микроорганизмов, сохранению их разнообразия и повышению биологической активности почвы.

Ключевые слова: соя, ризосферные микроорганизмы, биоразнообразие, *nifH* ген, фитогормоны *Bradyrhizobium japonicum*, флавоноиды.

1. Umarov MM, Kurakov AV, Stepanov AL. Microbial transformation of nitrogen in soil. Moscow: GEOS. – 2007. 138 p. Russian.
2. Tytova LV, Leonova NO, Antipchuk AF. Nitrogen fixing microorganisms in microbial-plant systems. Bioregulyatsiya microbno-rastitel'nykh system (Bioregulation of Microbial-Plant Systems). Kiev: Nichlava. – 2010: 99–195. Russian.
3. Peoples MB, Herridge DF, Ladha JK. Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production. Plant Soil. 1995; 174: 3–28.
4. Spaink HP, Kondorosi A, Hooykaas PJJ, editors. The Rhizobiaceae. Molecular biology of model plant-associated bacteria. Springer Science & Business Media: Kluwer Academic Publ.; 1998.
5. Ariel F, Brault-Hernandez M, Laffont C, Huault E, Brault M, Plet J, et al. Two direct targets of cytokinin signaling regulate symbiotic nodulation in *Medicago truncatula*. Plant Cell. 2012; 24(9): 3838–52.
6. Davies PJ, editor. Plant hormones: biosynthesis, signal transduction, action! Dordrecht. Boston. London: Kluwer Acad. Publishers; 2004.
7. Spaepen S, Vanderleyden J, Remans R. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev. 2007; 31: 425–48.
8. Zhukov VA, Shtark OY, Borisov AY, Tikhonovich IA. Breeding to improve symbiotic ef-

- fectiveness of *Legumes*. In: Andersen SB, ed. Plant breeding from laboratories to fields. Rijeka, Croatia; 2013; 167–207.
9. Gonzalez JE, Marketon MM. Quorum sensing in nitrogen-fixing rhizobia. *Microbiol Mol Biol Rev*. 2003; 67(4): 574–92.
  10. Ferguson BJ, Mathesius U. Signaling interactions during nodule development. *J Plant Growth Reg*. 2003; 22(1): 47–72.
  11. Rao JR, Cooper JE. Soybean nodulating rhizobia modify *nod* gene inducers daidzein and genistein to yield aromatic products that can influence gene inducing activity. *Mol. Plant-Microbe Interact*. 1995; 8(6): 855–62.
  12. Pueppke SG, Bolaños-Vásquez MC, Werner D, Bec-Ferté M-P, Promé J-C, Krishnan HB. Release of flavonoids by the soybean cultivars McCall and Peking and their perception as signals by the nitrogen-fixing symbiont *Sinorhizobium fredii*. *Plant Physiol*. 1998; 117(2): 599–06.
  13. Ovreas L, Torsvik V. Microbial diversity and community structure in two different agricultural soil communities. *Microb Ecol*. 1998; 36: 303–15.
  14. Ludwig W, Bauer SH, Bauer M, Held I, Kirchhof G, Schulze R, et al. Detection and *in situ* identification of representatives of a widely distributed new bacterial phylum. *FEMS Microbiol Lett*. 1997; 153: 181–90.
  15. Weber S, Stubner S, Conrad R. Bacterial Populations Colonizing and Degrading Rice Straw in Anoxic Paddy Soil. *Appl And Environmental Microbiology*. 2001; 67(3): 1318–27.
  16. Watt M, Hugenholtz P, White R, Vinall K. Numbers and locations of native bacteria on field-grown wheat roots quantified by fluorescence *in situ* hybridization (FISH). *Environmental Microbiology*. 2006; 8(5): 871–84.
  17. Smit E, Leeftang GP, Wernars K. Detection of shifts in microbial community structure and diversity in soil caused by copper contamination using amplified ribosomal DNA restriction analysis. *FEMS Microbiol Ecol*. 1997; 23: 249–61.
  18. Zhan J, Sun Q. Diversity of free-living nitrogen-fixing microorganisms in wastelands of copper mine tailings during the process of natural ecological restoration. *J of Environmental Sciences*. 2011; 23(3): 476–87.
  19. Henckel T, Jäckel U, Schnell S, Conrad R. Molecular analyses of novel methanotrophic communities in forest soil that oxidize atmospheric methane. *Appl Environ Microbiol*. 2000; 66: 1801–08.
  20. Poly F, Ranjard L, Nazaret S, Gourbière F, Monrozier LJ. Comparison of *nifH* gene pools in soils and soil microenvironments with contrasting properties. *Appl Environ Microbiol*. 2001; 67: 2255–62.
  21. Rösch C, Mergel A, Bothe H. Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. *Appl Environ Microbiol*. 2002; 68: 3818–29.
  22. Diallo MD, Willems A, Vloemans A, Cousin S, Vandekerckhove TT, de Lajudie P, et al. Polymerase chain reaction denaturing gradient gel electrophoresis analysis of the N_2 -fixing bacterial diversity in soil under *Acacia tortilis* ssp. *raddiana* and *Balanites aegyptiaca* in the dryland part of Senegal. *Environ Microbiol*. 2004; 6(4): 400–15.
  23. Zhang Y, Li D, Wang H, Xiao Q, Liu X. Molecular diversity of nitrogen-fixing bacteria from the Tibetan Plateau, China. *FEMS Microb Ecol*. 2006; 260: 134–42.
  24. Wakelin SA, Colloff MJ, Harvey PR, Marschner P, Gregg AL, Rogers S. The effects of stubble retention and nitrogen application on soil microbial community structure

- and functional gene abundance under irrigated maize. FEMS Microb Ecol. 2007; 59: 661–70.
25. Sorokin ID, Kravchenko IK, Doroshenko EV, Boulygina ES, Zadorina EV, Tourova TP, Sorokin DYU. Haloalkaliphilic diazotrophs in soda solonchak soils. FEMS Microb Ecol. 2008; 65: 425–33.
  26. Zadorina EV, Slobodova NV, Boulygina ES, Kolganova TV, Kuznetsov BB, Kravchenko IK. Analysis of the diversity of diazotrophic bacteria in peat soil by cloning of the *nifH* gene. Microbiology (Microbiologiya). 2009; 78(2): 218–26.
  27. Widmer F, Shaffer BT, Porteous LA, Seidler RJ. Analysis of *nifH* gene pool complexity in soil and litter at a Douglas Fir Forest Site in the Oregon Cascade Mountain Range. Appl Environ Microbiol., 1999; 65: 374–80.
  28. Mehta MP, Butterfield DA, Baross JA. Phylogenetic diversity of nitrogenase (*nifH*) genes in deep-sea and hydrothermal vent environments of the Juan de Fuca Ridge. Appl Environ Microbiol. 2003; 69: 960–70.
  29. Kizilova AK, Titova LV, Kravchenko IK, Iutinskaya GA. Evaluation of the diversity of nitrogen-fixing bacteria in soybean rhizosphere by *nifH* gene analysis. Microbiology. 2012; 81(5): 621–25.
  30. Tytova LV, Leonova NO, Brovko IS, Iutynska GA. [Complex microbial formulation Ecovital for leguminous crops seeds inoculation]. Patent UA 101388 C2 (Ukraine), IPS (2013.01) C 05 F 11/100, C 12 P 39/00. Pub. 25.03.2013, Bull. N 6. Ukrainian
  31. Sarita S, Priefer UB, Prell J, Sharma PK. Diversity of *nifH* gene amplified from rhizosphere soil DNA. Current Science. 2008; 94(1): 109–15.
  32. Lovell CR, Piceno YR, Quattro JM, Bagwell CE. Molecular analysis of diazotroph diversity in the rhizosphere of the smooth cordgrass, *Spartina alterniflora*. Appl Environ Microbiol. 2000; 66(9): 3814–22.
  33. Boulygina ES, Zadorina EV, Kuznetsov BB. [Investigation of structure of diazotrophic communities of peat soil]. Agrarian science. 2008; 4: 13–5. Russian
  34. Tytova LV, Brovko IS, Kizilova AK, Kravchenko IK, Iutynska GO. Effect of Complex Microbial Inoculants on the Number and Diversity of Rhizospheric Microorganisms and the Yield of Soybean. International Journal of Microbiological Research. 2013; 4(3): 267–74.
  35. Maheshwari DK. Bacteria in Agrobiolgy: Plant Probiotics. Springer-Verlag Berlin Heidelberg; 2012.
  36. Ferguson BJ, Mathesius U. Phytohormone regulation of legume-rhizobia interactions. J Chem Ecol. 2014; 40(7): 770–90.
  37. Khalid A, Arshad M, Zahir ZA. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J Appl Microbiol. 2004; 96: 473–80.
  38. Berg G. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol. 2009; 84: 11–8.
  39. Dragovoz IV, Leonova NO, Biliavska LO, Yavorska VK, Iutynska GO. [Phytohormone production by some free-living and symbiotic soil microorganisms]. Reports of the NASU. 2010; 12: 154–59. Ukrainian
  40. Leonova NO. [Auxins and cytokinines synthesis by *Bradyrhizobium japonicum* under flavonoids influence]. Mikrobiol Z. 2015; 77(5): 95–103. Ukrainian

41. Silva T, Davies PJ. Elongation rates and endogenous indoleacetic acid levels in roots of pea mutants differing in internode length. *Physiol Plantarum*. 2007; 129: 804–12.
42. Fukaki H, Tasaka M. Hormone interactions during lateral root formation. *Plant Mol Biol*. 2009; 69(4): 437–49.
43. Youning W, Kexue L, Liang Ch, Yanmin Z, Haipei L, Yiping T, et al. microRNA167-directed regulation of the auxin response factors, GmARF8a and GmARF8b, is required for soybean (*Glycine max* L.) nodulation and lateral root development. *Plant Physiol*. 2015; 168(3): 984–99.
44. Dodd IC, Zinovkina NY, Safronova VI, Belimov AA. Rhizobacterial mediation of plant hormone status. *Ann Appl Biol*. 2010; 157: 361–79.
45. Dragovoz IV, Leonova NO, Iutynska GO. [Phytohormones synthesis by *Bradyrhizobium japonicum* strains with different symbiotic effectiveness]. *Mikrobiol Z*. 2011; 73(4): 29–35. Russian
46. Patent UA 95878 C2 MIIK A01N 63/02 C05F 11/08. Method for determining the biological activity of nodule bacteria *Bradyrhizobium* genus / Dragovoz IV, Leonova NO, Iutynska GO, Yavorska VK. Publ. 12.09.2011. Bull N 17.
47. Frugier F, Kosuta S, Murray JD, Crespi M, Szczyglowski K. Cytokinin: secret agent of symbiosis. *Trends in Plant Sci*. 2008; 13(3): 115–20.
48. Held M, Hou H, Miri M, Huynh C, Ross L, Hossain MS, et al. *Lotus japonicus* cytokinin receptors work partially redundantly to mediate nodule formation. *Plant Cell*. 2014; 26(2): 678–94.
49. Op den Camp RHM, De Mita S, Lillo A, Cao Q, Limpens E, Bisseling T, et al. A phylogenetic strategy based on a legume-specific whole genome duplication yields symbiotic cytokinin type-A response regulators. *Plant Physiol*. 2011; 157(4): 2013–22.
50. Mauch-Mani B, Mauch F. The role of abscisic acid in plant–pathogen interactions. *Cur Opin Plant Biol*. 2005; 8: 409–14.
51. Suzuki A, Akune M, Kogiso M, Imagama Y, Osuki K, Uchiumi T, et al. Control of nodule number by the phytohormone abscisic acid in the roots of two leguminous species. *Plant Cell Physiol*. 2004; 45(7): 914–22.
52. Leonova NO. Absciscic acid and ethylene production by biotechnological strains of *Bradyrhizobium japonicum*. *Biotechnologia Acta*. 2015; 8(5): 64–70.
53. Oldroyd GED, Engstrom EM, Long SR. Ethylene inhibits the *Nod* factor signal transduction pathway of *Medicago truncatula*. *Plant Cell*. 2001; 13(8): 1835–49.
54. Wang KLC, Li H, Ecker JR. Ethylene biosynthesis and signaling networks. *Plant Cell*. 2002; 14: 31–151.
55. Caba JM, Regalade L, Ligerio F. Nitrate-induced ethylene biosynthesis and the control of nodulation in alfalfa. *Plant Cell Environ*. 1998; 21(1): 87–93.
56. Geurts R, Bisseling T. *Rhizobium* Nod factor perception and signaling. *Plant Cell*. 2002; 14(suppl.): 239–49.
57. Sugiyama A, Shitan N, Yazaki K. Involvement of a soybean ATP-binding cassette-type transporter in the secretion of genistein, a signal flavonoid in legume-*Rhizobium* symbiosis. *Plant Physiol*. 2007; 144(4): 2000–08.
58. Leonova NO, Tytova LV, Tantsyurenko EV, Antipchuk AF. [The physiological activity of soybean rhizobia (*Bradyrhizobium japonicum*) for the flavonoids actions]. *Sci. Bul. Chernovtsy Univ.: Collection of scientific papers. Biology*. 2005; 252: 132–39. Ukrainian

59. Leonova NO, Titova LV, Tantsyurenko EV, Antipchuk AF. [Glutamate dehydrogenase activity of *Bradyrhizobium japonicum* in the presence of phyto regulators]. Mikrobiol Z. 2006; 68(4): 21–7. Ukrainian
60. Iutynska GO, Tytova LV, Leonova NO, Brovko IS. [Activity of ammonium assimilation basic enzymes at *Bradyrhizobium japonicum* under influence of plant flavonoid induc-tors]. Mikrobiol Z. 2010; 72(6): 23–9. Ukrainian

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