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INTERACTION BETWEEN LUPIN PLANTS AND STRAINS OF THE *BRADYRHIZOBIUM* GENUS

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Objective. *Study the peculiarities of the interaction between three species of lupin and nodule bacteria of the genus Bradyrhizobium, isolated from various leguminous plants: lupin, seradella, soybean and cowpea.* ***Methods.*** *Microbiological (isolation of rhizobia from nodules and their cultivation), serological (defining serological affiliation of strains), vegetation experiment (study of host specificity of bradyrhizobia, study of the formation and functioning of the symbiosis between lupin and nodule bacteria), gas chromatographic (determination of the activity of symbiotic nitrogen fixation), mathematical and statistical.* ***Results.*** *Under the conditions of vegetation experiments on sod-podzolic soil and sterile vermiculite, the peculiarities of the interaction between different species of lupin and specific and non-specific strains of nodule bacteria of the genus Bradyrhizobium were studied. It was established that rhizobia isolated from lupin nodules (B. lupini 367a, 30l, LD4 and Bradyrhizobium sp. LD8), seradella (Bradyrhizobium sp. CP1) and soybean (B. lupini 631) are able to form an active symbiosis with white and yellow lupin plants. Microsymbionts of soybean (B. japonicum KB11) and cowpea (B. diazoefficiens B22) did not infect these two species of lupin. In the experiment with narrow-leaved lupin, all strains of B. lupini (367a, 30l, LD4, 631) and LD8 Bradyrhizobium sp. ensured a reliable increase in the above-ground mass of plants by 14.3–22.6 % compared to the control. The maximum values of this indicator were obtained in variants inoculated with B. lupini LD4 and B. lupine 631 strains (1.03 and 1.02 g/plant versus 0.84 g/plant in the control). With the use of non-specific lupin strains B. japonicum KB11 and B. diazoefficiens B22, a significant increase in the nitrogenase activity of nodules was registered (1.5 and 1.9 times compared to the control), however, the increase in the above-ground mass of plants was insignificant — 7.1 % and 10.7 %, respectively.* ***Conclusion.*** *It has been established that lupin-specific bradyrhizobia of various origins (B. lupini and Bradyrhizobium sp.) have a positive effect on the formation of the symbiotic apparatus, the growth and development of white, yellow and narrow-leaved lupin plants. Rhizobia of soybean and cowpea (B. japonicum KB11 and B. diazoefficiens B22) non-specific for lupin, contribute to the activation of the interaction between narrow-leaved lupin and representatives of the soil population of B. lupini and act as PGPR microorganisms. B. lupini LD4 and B. lupini 631 strains are the best in terms of most symbiotic indicators and efficiency of symbiosis with narrow-leaved lupin.*

Key words: *nodule bacteria, Bradyrhizobium lupini, B. japonicum, B. diazoefficiens, lupin, symbiotic interactions.*

Introduction. Lupin (*Lupinus* L.) it is a genus of the legume family *Fabaceae* (*Leguminose*), which includes more than 200 species of annual and perennial plants (grasses, subshrubs, shrubs) [1; 2]. Due to its ability to symbiosis with nodule bacteria and fixation of molecular nitrogen, lupin is an important agricultural crop with significant biological and economic potential, which is widely used as a source of protein in human and animal nutrition and is a good green manure [3; 4]. In world agricultural practice, only a few annual species of lupin have become the most widespread: *L. albus* (white lupin), *L. luteus* (yellow lupin), *L. angustifolius* (narrow-leaved or blue lupin) and *L. mutabilis* (pearl lupin) [3]. Perennial species are mainly used as ornamental plants. In Ukraine, high-yielding varieties of white, yellow and narrow-leaved lupin are grown to solve the problem of fodder protein [5]. There are 7 species of lupin in the wild.

Analysis of recent studies and publications. Considering that in Ukraine, lupin has been grown as a sidereal and fodder crop since the beginning of the 20th century [5; 6], stable local populations of nodule bacteria capable of forming symbiosis with this plant have been formed in the soil. Lupin microsymbionts of only one species — *B. lupini* [7–9] are described in domestic literature. Previously, we isolated several strains from peanut nodules that, in addition to the host plant, infect white and yellow lupin, as well as a strain that additionally forms nodules on soybean roots [9]. These strains are assigned to the genus *Bradyrhizobium*, but their species needs clarification. In general, the diversity of lupin rhizobia in the soils of Ukraine remains poorly studied.

The analysis of literary sources devoted to the study of nodule bacteria of lupin in different countries shows that this plant is characterized by wide specificity and is able to form a symbiosis with many species of nitrogen-fixing bacteria [10]. The great diversity of lupin species reflects the great diversity of their microsymbionts. Currently, both slow-growing and fast-growing nodule bacteria capable of nodulating lupin have been described. It has been established that slow-growing representatives of the genus *Bradyrhizobium* predominate in the nodules of various lupin species: *B. lupini* [11–13], *B. japonicum* [11; 13; 14], *B. diazoefficiens* [13], *B. elkanii* [15], *B. canariense* [16], *B. valentinum* [17], *B. cytisi* [13] and *B. hipponense* [18]. It was also found that the roots of European lupin species are usually infected by *B. canariense* and *B. japonicum* [19], and American ones by *B. japonicum* and *B. elkanii* [17].

Despite that the predominant microsymbionts of lupin are bradyrhizobia, fast-growing nodule bacteria were also found in their nodule populations. For example, in Morocco and Poland, rhizobia belonging to the genera *Allorhizobium*, *Sinorhizobium* and *Rhizobium* were isolated from nodules on the roots of lupin plants [20; 21]. In the Tunisian soils, white lupin was infected by bacteria of the genera *Rhizobium* and *Neorhizobium* [22]. Representatives of genera are also described among fast-growing microorganisms, namely *Mesorhizobium* (*M. loti*) [23], *Agrobacterium* [22], *Phyllobacterium* (*P. trifolii*) [11; 24], *Ochrobactrum* (*O. lupini*) [25], *Microvirga* (*M. tunisiensis*, *M. lupini*) [11; 26; 27] and *Devosia* (*Devosia* sp.) [28].

It should be noted that the above microorganisms are able to infect not only lupin, but also other species of legumes, forming different groups of cross-inoculation. For example, nodule bacteria *B. japonicum*, *B. diazoefficiens*, *B. elkanii*, in addition to lupin, form nitrogen-fixing nodules on the roots of soybean, cowpea and mung bean plants [10; 29–31]. Among the bacteria of the species *B. lupini* (formerly *Bradyrhizobium* sp. (*Lupinus*)), microsymbionts of soybean [32], seradella [33] and peanut [9] have also been described. Representatives of other genera of nodule bacteria can also migrate between legumes and form different ranges of host plants. Under such conditions, the specificity of bacteria in relation to leguminous plants will be different, and the efficiency of symbiotic systems may differ significantly.

In the domestic literature, no information on the formation of an effective symbiosis between lupin and various species of specific and non-specific nodule bacteria is available. Considering the above, the objective of our work was to study the specifics of the interaction between three species of lupin and nodule bacteria of the genus *Bradyrhizobium* isolated from various leguminous plants: lupin, seradella, soybean and cowpea.

Materials and methods. The objects of research were strains of lupin nodule bacteria (*B. lupini* 367a (standard), *B. lupini* 301, *B. lupini* LD4, *B. lupini* 631, *Bradyrhizobium* sp. LD8); strains isolated from nodules of seradella (*Bradyrhizobium* sp. CP1), cowpea (*B. diazoefficiens* B22) and soybean (*B. japonicum* 46, *B. japonicum* KB11); plants of narrow-leaved lupin (*Lupinus angustifolius* L.), white lupin (*Lupinus albus* L.), yellow lupin (*Lupinus luteus* L.), truncated lupin (*Lupinus truncatus* Hook. & Arn.), fleshy lupin (*Lupinus subcarnosus* Hook.) and seradella (*Ornithopus sativus* Brot.). Lupin and seradella seeds were provided by the Department of Scientific Support for Agroindustrial Manufacture of the Institute of Agricultural Microbiology and Agroindustrial Manufacture of the National Academy of Agrarian Sciences (IAMAM NAAS), Polissia Institute of Agriculture of the National Academy of

Agrarian Sciences and Ternopil National Pedagogical University named after Volodymyr Hnatiuk. Bradyrhizobia strains are stored in the collection of the Laboratory of Plant-Microbial Interactions and in the Collection of Beneficial Soil Microorganisms of the IAMAM NAAS.

Isolation of nodule bacteria from lupin and seradella nodules and study of their morphological and cultural properties was carried out according to methodical recommendations [34].

The serological affiliation of the strains isolated from different leguminous plants was determined in the agglutination reaction according to the Gruber-Widal method [35]. Bradyrhizobia were grown on solid legume medium at 28 °C. In the logarithmic phase of growth, the bacterial mass was washed off the agar slopes, precipitated by centrifugation and washed twice with normal saline solution. 5 mL of saline solution and 5 mL of 2.5 % glutaraldehyde solution (to remove non-specific flagellar H-antigens) were added to the cell sediment and left in a refrigerator for a day. A day later, the bacterial cells (antigen) were washed three times from glutaraldehyde, the sediment was resuspended with saline solution and the antigen titre was brought up to $2 \cdot 10^9$ cells/mL. Specific immune anti-nodule bacteria obtained with lupin (367a, 631), soybean (46, M8, KB11, 634b, OR, HR, NR) and cowpea (B1 and B2) strains were used.

The ability of bradyrhizobia strains to enter into symbiosis with white lupin (Lybid variety) and yellow lupin (Chernihivets variety) was studied in a vegetation experiment. Plants were grown in 2 litre vessels on a nitrogen-free substrate (sterile vermiculite), which was moistened with a 0.2 % solution of KH_2PO_4. Before sowing, surface sterilized seeds were treated with a suspension of nodule bacteria (titre $2 \cdot 10^9$ cells/mL). The inoculation load was 200–300 thousand cells per 1 seed. The repetition of the experiment was fourfold. Humidity was maintained at 60 % maximum water-holding capacity.

The interaction between bradyrhizobia and narrow-leaved lupin was studied under growing conditions on sod-podzolic soil (soil samples were taken in the fields of the IAMAM NAAS). Nodule bacteria were cultivated for 72 hours in 750 mL flasks on a rocker (220 rpm) at 26–28 °C in a liquid bean medium [34]. Lupin seeds of the Lokomotyv narrow-leaved variety were treated with the studied strains. The inoculation load was 200–300 thousand cells per 1 seed. In the control variant, the seeds were moistened with tap water. The repetition of the experiment was fivefold. Humidity was maintained at 60 % maximum water-holding capacity [36].

The activity and efficiency of legume-rhizobial symbiosis was evaluated in the flowering phase according to the following parameters: plant height, weight of dry matter of the aerial part of plants, number and weight of nodules, activity of symbiotic nitrogen fixation.

Vegetation experiment on the stimulating effect of nodule bacteria of lupin and soybean on plants of the white lupin of Lybid variety was carried out on a nitrogen-free substrate (vermiculite) moistened with a 0.2 % solution of KH_2PO_4. Before sowing, the seeds were treated with strains of *B. lupini* 367a, *B. japonicum* 46 and *B. japonicum* KB11. The inoculation load was 200–300 thousand cells per 1 seed. In the control variant, the seeds were moistened with tap water. The repetition of the experiment was fivefold. Humidity was maintained at 60 % maximum water-holding capacity.

The nitrogenase activity of nodules was determined by the acetylene-ethylene method [37] on a Chrom-4 gas chromatograph with a flame ionization detector (column with β-β′-oxydipropionitrile).

Processing of experimental data was carried out using methods of mathematical statistics [38], Microsoft Office Excel 2016 and Statistica 8.0.

Results and discussion. To study the interaction between nodule bacteria and narrow-leaved lupin plants, we have selected strains of various species, representatives of which, according to literature data, are or may be potential microsymbionts of lupin. In the work, 8 strains of bradyrhizobia were studied, isolated from nodules of lupin, seradella, soybean and cowpea (Table 1). Both new and collection strains were used (*B. lupini* 367a, 30l, 631, *B. japonicum* KB11).

It should be noted that the four studied strains of lupin nodule bacteria are microsymbionts of different host plants. For example, strains *B. lupini* 367a and *B. lupini* 30l were isolated from nodules of narrow-leaved lupin and yellow lupin, respectively. It was established that despite their different origin, both strains belong to the same serogroup 367a (Table 1).

Table 1. ***Characteristics of nodule bacteria of the genus Bradyrhizobium isolated from nodules of various leguminous plans***

Species of microorganisms	Host plant	Serogroup	Formation of active nodules on roots:	
			Lupinus albus	*Lupinus luteus*
B. lupini 367a	*Lupinus angustifolius*	367a	+	+
B. lupini 301	*Lupinus luteus*	367a	+	+
B. lupini LD4	*Lupinus truncatus*	×	+	+
Bradyrhizobium sp. LD8	*Lupinus subcarnosus*	×	+	+
Bradyrhizobium sp. CP1	*Ornithopus sativus*	367a	+	+
B. lupini 631	*Glycine max*	631	+	+
B. japonicum KB11	*Glycine max*	KB11	–	–
B. diazoefficiens B22	*Vigna unguiculata*	B2	–	–

Notes: × — unspecified serogroup; + — formation of nitrogen-fixing nodules; – — no nodules.

We have isolated two new strains, LD4 and LD8, from truncated and fleshy lupin nodules, respectively. They form colonies 2–3 mm in diameter, which appear on solid bean medium at Day 5 to 6 of cultivation. According to the morphology, at Day 7 of growth, bacterial cells are mobile, slightly bent gram-negative rods, which do not form spores. Cultures acidify the milk with litmus, but do not form a zone of serum on the surface. No growth on MPA was registered. According to the morphological and cultural properties, the strains are classified as *Bradyrhizobium*. In previous studies, the LD4 strain was identified as *B. lupini* based on the results of sequencing of the ITS region [39]. Strains of *B. lupini* LD4 and *Bradyrhizobium* sp. LD8 did not react with any of the 11 antisera used, their serological affiliation remains uncertain.

According to our research, strain CP1, isolated from nodules of seradella, also had characteristic signs of bradyrhizobia. In the agglutination reaction, it reacted positively with antiserum 367a, obtained against the standard strain *B. lupini* 367a. Additional research is needed to determine its species affiliation.

It should be noted that *B. lupini* 367a, *B. lupini* 301 and *Bradyrhizobium* sp. CP1 showed a weak positive reaction with KB11 antiserum, which suggests the presence of antigenic determinants shared with the soybean rhizobia strain *B. japonicum* KB11.

We have also used the well-known strain of nodule bacteria *Bradyrhizobium* sp. 631 isolated from soybean nodules. It is interesting because it can form nodules both on soybean and lupin roots [32]. According to the results of sequencing of the intergenic ITS region, strain 631 was assigned to the species *B. lupini* [39]. According to antigenic properties, this strain belongs to serogroup 631.

Since it is known from the literature that nodule bacteria *B. japonicum* and *B. diazoefficiens* are able to nodulate lupin [11; 13; 14], we chose two strains that belong to these species for the study. They were isolated from soybean nodules (*B. japonicum* KB11) and cowpea (*B. diazoefficiens* B22) and belong to serogroups KB11 and B2, respectively.

Under the conditions of the vegetation experiment on vermiculite, it was established that all bradyrhizobia strains isolated from lupin nodules (*B. lupini* 367a, 301, LD4 and *Bradyrhizobium* sp. LD8) and seradella (*Bradyrhizobium* sp. CP1), as well as *B. lupini* 631, formed active red nodules on the roots of white and yellow lupin (Table 1). Microsymbionts of soybean (*B. japonicum* KB11) and cowpea (*B. diazoefficiens* B22) did not infect these two species of lupin.

The next stage of our work was to investigate the peculiarities of the interaction between microsymbionts of various leguminous crops and narrow-leaved lupin. The data obtained in the vegetation experiment are shown in the Table 2.

It was established that a significant number of nodules were formed on the roots of non-inoculated plants of narrow-leaved lupin, namely

Table 2. ***Influence of inoculation with Bradyrhizobium strains on symbiotic indicators of narrow-leaved lupin of Lokomotyv variety*** *(vegetation experiment, sod-podzolic soil)*

Variants of the experiment	Host plant	Number of nodules		Weight of nodules	
		units/plant	% versus control	g/plant	% versus control
Without inoculation (control)	–	54.73 ± 1.36	–	0.23 ± 0.01	–
Inoculation with *B. lupini* 367a	lupin	57.00 ± 1.41	4.1	0.29 ± 0.01*	26.1
Inoculation with *B. lupini* 301		59.73 ± 1.26*	9.1	0.28 ± 0.01*	21.7
Inoculation with *B. lupini* LD4		57.60 ± 1.09	5.2	0.31 ± 0.02*	34.8
Inoculation with *Bradyrhizobium* sp. LD8		57.27 ± 1.14	4.6	0.29 ± 0.01*	26.1
Inoculation with *Bradyrhizobium* sp. CP1	seradella	61.00 ± 1.10*	11.5	0.28 ± 0.01*	21.7
Inoculation with *B. lupini* 631	soybean	59.60 ± 1.26*	8.9	0.31 ± 0.01*	34.8
Inoculation with *B. japonicum* KB11	soybean	56.87 ± 1.53	3.8	0.29 ± 0.01*	26.1
Inoculation with *B. diazoefficiens* B22	cowpea	57.20 ± 0.63	4.5	0.31 ± 0.01*	34.8
HIP_{05}		3.52		0.04	

Note. * — significant increase versus control.

55 units/plant. This fact indicates the presence of a population of nodule bacteria capable of nodulating lupin in the soil.

Against the background of spontaneous infection of the roots of narrow-leaved lupin, all studied strains, regardless of their origin, contributed to the formation of a greater number of nodules compared to the control. A significant increase in this indicator by 9.1–11.5 % was registered after inoculation with *B. lupini* 301 and *B. lupini* 631, as well as with the strain of serogroup 367a isolated from the nodules of seradella — *Bradyrhizobium* sp. CP1. The fact that microsymbionts of lupin and seradella can cross-infect these leguminous plants is mentioned in the works of a number of researchers [16; 33].

Bradyrhizobia strains isolated from lupin, seradella, cowpea and soybean contributed to a significant increase in the weight of narrow-leaved lupin nodules by 21.7–34.8 % versus the control (Table 2). It is important to note that this indicator increased not only with the use of specific bacteria of *B. lupini* species, but also strains of other species: *B. japonicum* KB11 (by 26.1 %) and *B. diazoefficiens* B22 (by 34.8 %).

In addition to the positive effect on the number and weight of nodules, the inoculation of lupin seeds contributed to a significant intensification of the process of symbiotic nitrogen fixation (Fig. 1). A significant increase in nitrogen-fixing activity versus the control was registered when using strains of *Bradyrhizobium* sp. LD8 (by 58.2 %) and *B. lupini* 631 (by 85.8 %), capable of infecting lupins, as well as strains of soybean nodule bacteria *B. japonicum* KB11 (by 48.6 %) and *B. diazoefficiens* B22 (by 87.6 %). Other strains — typical nodule bacteria of lupin: *B. lupini* 367a, *B. lupini* 301, *B. lupini* LD4 increased this indicator only by 11.3–23.8 %.

The studied bradyrhizobia contributed to the improvement of the growth and development of lupin plants. Thus, the greatest reliable increase in plant height was registered when treating seeds with strains isolated from lupin nodules (*B. lupini* 367a, *B. lupini* 301,

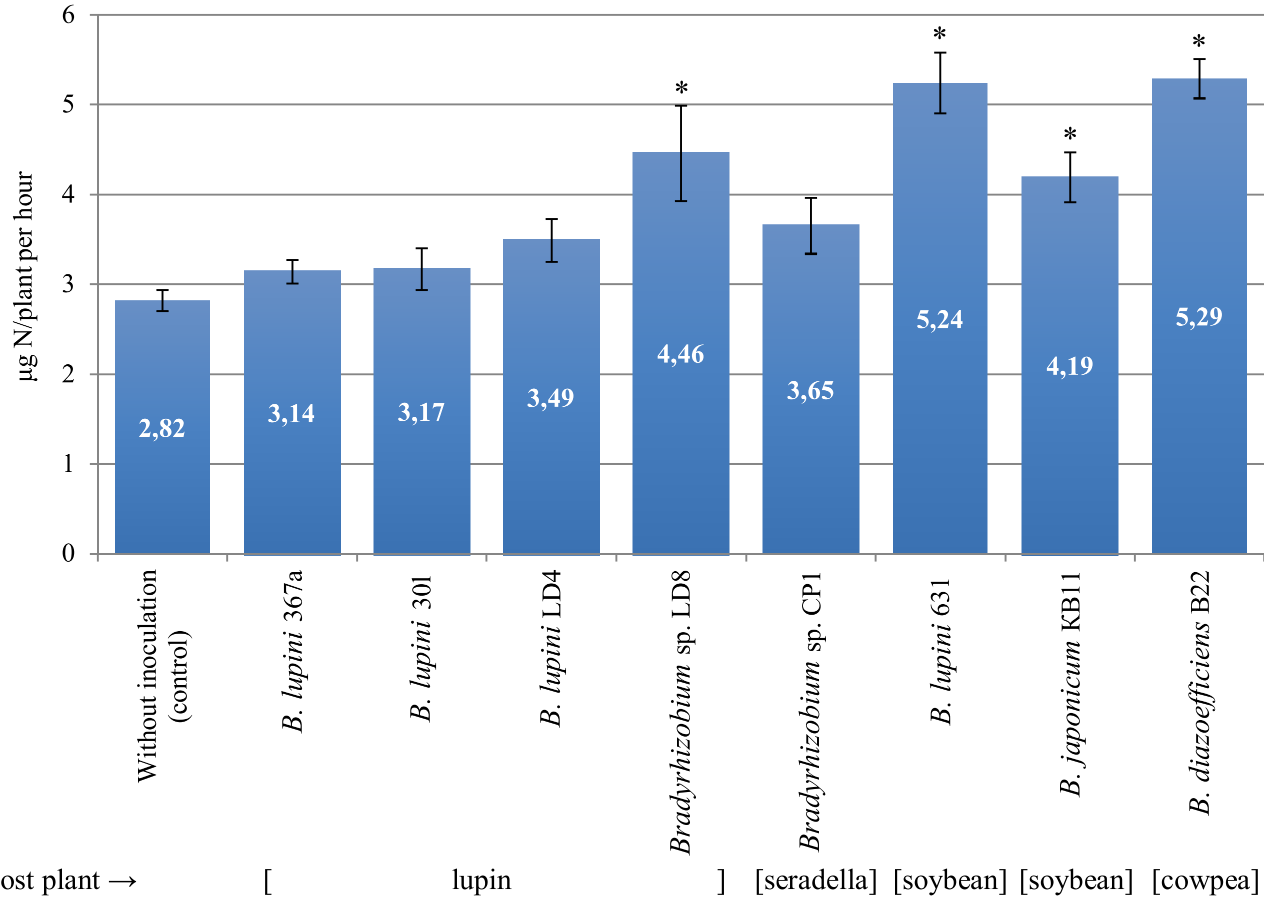

Figure 1. Nitrogenase activity of lupin nodules of the Lokomotyv narrow-leaved variety after inoculation with bradyrhizobia strains of different species (vegetation experiment, sod-podzolic soil). HIP$_{05}$ *= 0.84. * — significant increase versus control.*

B. lupini LD4, *Bradyrhizobium* sp. LD8) — 10.4–13.7 % and soybean nodules (*B. lupini* 631) — 12.9 % (Table 3). A slight increase in this indicator (by 5.8–6.8 %) was reported after inoculation with another strain from soybean nodules, but of a different species (*B. japonicum* KB11), as well as with microsymbionts of seradella (*Bradyrhizobium* sp. CP1) and cowpea (*B. diazoefficiens* B22).

The use of bradyrhizobia strains isolated from various leguminous plants had a positive effect on the content of dry matter in the above-ground mass of narrow-leaved lupin. Against the background of a large local population of lupin nodule bacteria, all specific strains of the species *B. lupini* (367a, 30l, LD4, 631) and *Bradyrhizobium* sp. LD8 provided a reliable increase in the above-ground mass of plants versus the control by 14.3–22.6 %. The maximum values of this indicator were obtained in variants inoculated with *B. lupini* LD4 and *B. lupini* 631 (1.03 and 1.02 g/plant vs. 0.84 g/plant in the control), which proved to be better for most symbiotic traits. Despite the high nitrogenase activity of lupin nodules with the strains *B. japonicum* KB11 and *B. diazoefficiens* B22, the increase in the above-ground mass of plants versus the control was lower and amounted to 7.1 % and 10.7 %, respectively.

We have also studied the influence of non-specific nodule bacteria of the species *B. japonicum* on white lupin plants. Lupin seeds were inoculated with two highly active strains of soybean rhizobia — *B. japonicum* KB11 and *B. japonicum* 46. The use of a sterile substrate (vermiculite) in the vegetation experiment made it possible to neutralize the effect of representatives of local populations of lupin microsymbionts present in the soil on plants.

The obtained data proved that the studied strains of soybean rhizobia are not capable of infecting white lupin (Table 4). Nitrogen-fixing nodules on the roots (22 units/plant) were formed only when seeds were inoculated with a specific strain of nodule bacteria — *B. lupini* 367a, while the above-ground mass of lupin plants increased by 60.0 % versus the control (without inoculation). In variants with soybean

Table 3. ***Influence of inoculation with Bradyrhizobium strains on growth and development of lupin plants of narrow-leaved Lokomotyv variety*** *(vegetation experiment, sod-podzolic soil)*

Variants of the experiment	Host plant	Plant height		Content of dry matter in the above-ground mass of plants	
		cm	% versus control	g/plant	% versus control
Without inoculation (control)	–	30.97 ± 1.17	–	0.84 ± 0.05	–
Inoculation with *B. lupini* 367a	lupin	35.15 ± 0.87*	13.5	0.96 ± 0.03*	14.3
Inoculation with *B. lupini* 301		34.71 ± 1.14*	12.1	0.99 ± 0.02*	17.9
Inoculation with *B. lupini* LD4		35.20 ± 0.70*	13.7	1.03 ± 0.22*	22.6
Inoculation with *Bradyrhizobium* sp. LD8		34.18 ± 0.71*	10.4	0.97 ± 0.02*	15.5
Inoculation with *Bradyrhizobium* sp. CP1	seradella	32.97 ± 1.11	6.5	0.91 ± 0.04	8.3
Inoculation with *B. lupini* 631	soybean	34.99 ± 0.78*	12.7	1.02 ± 0.05*	21.4
Inoculation with *B. japonicum* KB11	soybean	33.09 ± 0.56	6.8	0.90 ± 0.02	7.1
Inoculation with *B. diazoefficiens* B22	cowpea	32.76 ± 0.40	5.8	0.93 ± 0.03	10.7
HIP_{05}		2.50		0.10	

Note. * — significant increases versus control.

Table 4. ***Influence of soybean and lupin nodule bacteria on symbiotic indicators of white lupin*** *(vegetation experiment, vermiculite)*

Variants of the experiment	Number of nodules, units/plant	Weight of nodules, g/plant	Nitrogen fixation activity, μ N_2/plant per hour	Content of dry matter in the above-ground mass of plants, g/plant	Gain versus control, %
Without inoculation (control)	0	0	0	0.70 ± 0.02	–
Inoculation with *B. lupini* 367a	21.83 ± 0.64	0.13 ± 0.01	6.39 ± 0.47	1.12 ± 0.01	60.0
Inoculation with *B. japonicum* KB11	0	0	0	0.81 ± 0.02	15.7
Inoculation with *B. japonicum* 46	0	0	0	0.98 ± 0.01	40.0

rhizobia strains *B. japonicum* KB11 and 46, despite the absence of nodules, a significant increase of this indicator by 15.7 % and 40.0 %, respectively, was also registered.

The analysis of data from two vegetation experiments shows that strains of nodule bacteria species *B. japonicum* and *B. diazoefficiens*, not specific for lupin, are able to positively influence both symbiotic indicators and the growth and development of lupin plants. We believe this may be related to the production of biologically active substances by bradyrhizobia, in particular, phytohormones and exopolysaccharides (EPS) [40]. When interacting with lupin, the role of non-specific nodule bacteria can be similar to the role played by growth-promoting bacteria (PGPR) in the activation of symbiotic relationships under simultaneous inoculation with rhizobia [41–43]. There is evidence that symbiotic nitrogen fixers are also PGPR microorganisms and can be used as free-living rhizobacteria capable of forming associative relationships with non-leguminous plants [44; 45]. In his work Peňa-Cabrales J. et al. demonstrated that bacteria of the *Bradyrhizobium* genus develop well in the germinating zone of soybean, bean, clover, cowpea, oat, wheat and corn, stimulating root development in a similar way to free-living rhizobacteria [46]. Other researchers found a connection between the ability of nodule bacteria *B. japonicum* to stimulate the growth of non-leguminous plants and their production of indolyl-3-acetic acid (IAC) [47]. It is generally known that rhizobia, as PGP rhizobacteria, are able to produce a wide range of phytohormones: auxins, cytokinins, gibberellins, abscisic acid [48; 49]. Currently, the participation of all groups of phytohormones in the initiation, development and functioning of nitrogen-fixing nodules has been established.

Strains of *B. japonicum* КВ11 and 46 that we have used are also active producers of phytohormones of auxin and cytokinin nature, which was registered in previous studies [40]. In addition, it was shown that not only soybean nodule bacteria, but also the products of their metabolism are able to change the ratio of certain strains of rhizobia in nodules and positively influence the formation and functioning of symbiotic soybean systems. It should be also noted that *B. japonicum* КВ11 produces a significant amount of exopolysaccharides [40], which can exhibit hormone-like effects. It is known from the literature that EPS of rhizobia are able to change the virulence of other homologous strains, enhance the fixation of molecular nitrogen and increase the efficiency of symbiotic systems [50; 51]. Thus, possessing a complex of features characteristic of PGP microorganisms, the studied nodule bacteria *B. japonicum* and *B. diazoefficiens*, which are not specific for lupin, could directly or indirectly contribute to the activation of the symbiosis between this plant and representatives of the local population of *B. lupini*. Further study of the interaction between bradyrhizobia of various species and lupin will allow a better understanding of the principles of formation of effective symbiotic systems of this culture.

Conclusion. It was established that nodule bacteria isolated from lupin (*B. lupini* 367a, 30l, LD4 and *Bradyrhizobium* sp. LD8), seradella (*Bradyrhizobium* sp. CP1) and soybean (*B. lupini* 631) nodules are able to form an active symbiosis with white and yellow lupin. Microsymbionts of soybean (*B. japonicum* КВ11) and cowpea (*B. diazoefficiens* В22) did not infect these two species of lupin.

In the experiment with narrow-leaved lupin, all strains of *B. lupini* (367a, 30l, LD4, 631) and *Bradyrhizobium* sp. LD8 provided a reliable increase versus the control (without inoculation) of the above-ground mass of plants by 14.3–22.6 %. The maximum values of this indicator were obtained in variants inoculated with *B. lupini* LD4 and *B. lupini* 631 (1.03 and 1.02 g/plant vs. 0.84 g/plant in the control), which proved to be better for most symbiotic traits.

With the use of non-specific strains of *B. japonicum* КВ11 and B. *diazoefficiens* В22, despite the large mass of nodules and their high nitrogenase activity, the increase in the above-ground mass of narrow-leaved lupin plants was not significant — 7.1 % and 10.7 %, respectively.

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ВЗАЄМОДІЯ РОСЛИН ЛЮПИНУ ЗІ ШТАМАМИ БУЛЬБОЧКОВИХ БАКТЕРІЙ РОДУ *BRADYRHIZOBIUM*

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Мета. *Вивчити особливості взаємодії трьох видів люпину з бульбочковими бактеріями роду Bradyrhizobium, виділеними з різних бобових рослин: люпину, серадели, сої та вигни.* ***Методи.*** *Мікробіологічні (виділення ризобій із бульбочок та їх культивування), серологічні (визначення серологічної належності штамів), вегетаційного досліду (дослідження хазяйської специфічності брадіризобій, вивчення формування та функціонування симбіозу люпину з бульбочковими бактеріями), газохроматографічний (визначення активності симбіотичної азотфіксації), математично-статистичні.* ***Результати.*** *За умов вегетаційних дослідів на дерново-підзолистому ґрунті та стерильному вермикуліті досліджено особливості взаємодії різних видів люпину зі специфічними та неспецифічними штамами бульбочкових бактерій роду Bradyrhizobium. Встановлено, що ризобії, виділені із бульбочок люпину (B. lupini 367а, 30л, ЛД4 і Bradyrhizobium sp. ЛД8), серадели (Bradyrhizobium sp. СР1) та сої (B. lupini 631), здатні формувати активний симбіоз із рослинами люпину білого та жовтого. Мікросимбіонти сої (B. japonicum КВ11) та вигни (B. diazoefficiens В22) не інфікували ці два види люпину. У досліді з люпином вузьколистим усі штами B. lupini (367а, 30л, ЛД4, 631) і штам Bradyrhizobium sp. ЛД8 забезпечили достовірне збільшення до контролю надземної маси рослин на 14,3–22,6 %. Максимальні значення цього показника отримані у варіантах з інокуляцією штамами B. lupini ЛД4 і B. lupini 631 (1,03 і 1,02 г/рослину проти 0,84 г/рослину у контролі). За використання неспецифічних для люпину штамів B. japonicum КВ11 і B. diazoefficiens В22 спостерігали суттєве підвищення нітрогеназної активності бульбочок (в 1,5 і 1,9 раза проти контролю), проте приріст надземної маси рослин був незначним — 7,1 % і 10,7 % відповідно.* ***Висновки.*** *Встановлено, що специфічні для люпину брадіризобії різного походження (B. lupini та Bradyrhizobium sp.) позитивно впливають на формування симбіотичного апарату, ріст і розвиток рослин люпину білого, жовтого і вузьколистого. Неспецифічні для люпину ризобії сої та вигни (B. japonicum КВ11 і B. diazoefficiens В22) сприяють активізації взаємодії люпину вузьколистого з представниками ґрунтової популяції B. lupini та діють як PGPR-мікроорганізми. Кращими за більшістю симбіотичних показників та ефективністю симбіозу з люпином вузьколистим є штами B. lupini ЛД4 і B. lupini 631.*

Ключові слова: *бульбочкові бактерії, Bradyrhizobium lupini, B. japonicum, B. diazoefficiens, люпин, симбіотична взаємодія.*

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