

LECTINS AND THEIR ROLE IN FORMATION AND FUNCTIONING OF LEGUME-RHIZOBIAL SYMBIOSIS

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The paper covers the role of lectins of legumes in the establishing of nitrogen fixing symbiosis with nodule bacteria. The positive influence of these proteins in initiation of rhizobia binding to the roots of legume plants was proved. It was established that level of hemagglutination activity of lectins of soybean and lupine nodules directly relate on the activity of nitrogen fixation. Treatment of soybean seeds with the rhizobial suspension combined with homologous lectin promoted nitrogen fixation in nodules and has considerably increased crop yield thus indicating perceptiveness of use of legumes lectins in compositions of bacterial fertilizers for legume plants.

Key words: legume lectins, legume-rhizobial symbiosis.

It is well known that formation of symbiotic relations of legumes and nodule bacteria is the complex multi-step process. Most scientists consider lectins (carbon-binding proteins of legumes) to play special role in interaction of symbioses partners [1, 4, 7, 10]. Lectins are the group of non-immune proteins capable of selective and reversible binding of carbohydrates preventing their chemical transformation. They may interact both with mono- and oligosaccharides, as well as with carbohydrates residues entering the composition of glycoproteins and glycosides [8, 11]. Such interaction of cell surface components of different organisms can provide mutual recognition of certain species at the formation of various microbial-plant associations [3]. Occurrence of such tight contacts between these organisms allows use of their metabolites on their own needs. Nitrogen fixing legume-rhizobial symbiosis formed in the assistance of legume plants lectins and polysaccharides of nodule bacteria might be taken as an example of such interaction [2, 6]. The importance of studying of lectins role in the functionality of legume-rhizobial symbioses corresponds to the fact that the results obtained might clarify their functions and, furthermore, define means for optimization and improvement of symbiotic interrelations.

That, in turn, will certainly have the significant practical accent.

Hence, the main aim of our investigation was studying the role of lectins in the processes of formation and functioning of legume-rhizobial systems, as well as clarification of their application possibility in order to invoke symbioses and increase legume plants yield.

Materials and methods. In our experiments we have used soybean (*Glycine max* L. (Merr.)) and lupine (*Lupinus luteus* L.) plants. Seeds were sterilized during 15 minutes in ethanol, afterwards washed in flowing water and inoculated with the rhizobia strains of various efficiency prior to their sowing. The *Bradyrhizobium japonicum* strains 634b, 631 (active) and 604k (inactive) were used for inoculation of soybean seeds. The lupine seeds were inoculated with strains *Bradyrhizobium* sp. (Lupinus) 359a (active) and 400 (inactive).

Plants were grown in 11 kg Vagner pots. Washed river sand enriched with Hellriegel nutrient solution (0.25 of nitrogen norm) was used as the substrate. Study of the microbial preparations enriched with lectins was conducted in field experiments.

In order to determine indices of rhizobial cells adsorption the sterile seeds were grown on the poor agar, afterwards root cuts (1 cm) were incubated in the bacterial suspension (10^7 cells/ml) during 1 hour. Then seeds were washed out with sterile water and homogenised. The number of adsorbed cells was determined in homogenate.

Isolation of phytohemagglutinin from root nodules and other plant organs was performed by the ethanol elution method [11] with our modifications [9]. Hemagglutination reaction was made in immunological planes with U-shaped wells. Lectin titre was determined by wells with visible agglutination reactions and expressed in agglutination units (AU). Nitrogen fixation activity of root nodules was measured by acetylene reduction method [5]. Effect of exogenous lectin on nitrogen fixation activity and plants productivity was studied. The inoculum was prepared by 20 hours incubation of rhizobial suspensions with various lectin concentrations under the temperature $+28$ °C. Correlation of incubated components volumes was 1:1. Final bacterial concentration was no less than 10^7 cell per 1 ml of suspension.

Results and discussion. Binding of nodule bacteria to the roots of host-plant is considered to be one of the important stages preceding the bacterial infection of legumes. This is following by activation of enzymes synthesis, damaging cell walls of root fibrils resulting in its deformation and rhizobia penetration to the root cells of host-plant.

Experiments studying the ability of most specific to lectin sugars (haptens) to decrease the binding level of nodule bacteria to the roots of host-plant have proved the lectins participation in rhizobia adsorption to the roots of legume plants. As is seen from the table 1, incubation of lupine roots in the solution of lupine lectin's hapten, D-galactose, has resulted in the decrease of the number of adsorbed on their surface cells of *Bradyrhizobium* sp. (*Lupinus*).

Table 1. The influence of lectin hapten on the adsorption of *Bradyrhizobium* sp. (*Lupinus*) strain 359a on lupine roots

Variant	Number of cells per 1 cm of root, $\times 10^3$			
	time of incubation – 1 h	% to control	time of incubation – 2 h	% to control
Control (preincubation of roots in water)	62,8 \pm 2,3	100	82,8 \pm 2,3	–
Preincubation of roots in 0,1 mol D-galactose	25,9 \pm 1,5	41	13,8 \pm 1,3	16

Moreover, the number of the attached bacterial cells has depended on the duration of incubation. Thus, in control variant the number of adsorbed bacteria was 63×10^3 cells after the 1 hour incubation of roots in rhizobia suspension, while their preliminary treatment with D-galactose has decreased it to 26×10^3 cells. After 2 hours of rhizobia incubation in the solution of this lectin's hapten the number of bacterial cells adsorbed on the root surface was reduced to the 14×10^3 cells (table 1). Since galactose is a carbohydrate specific to the lupine's lectin, it blocks active centre of given protein and prevents its adsorption with the polysaccharides of bacterial cells followed by the decrease of their number on the root surface. Effect of lectin haptens on the adsorption level has confirmed their active role on the initial stages of symbiosis formation.

We have also studied the changes in lectin activity in different plant organs dependently to plants age. It was established that nodules have the most considerable activity of lectins.

Analysis of nitrogen fixation and lectin activity of protein extracts isolated from the lupine nodules has revealed the dependence of hemagglutination activity of nodules lectins on the activity of rhizobia strain used for plants inoculation (fig. 1). As is seen from the figure the

high level of nitrogen fixation activity of strains corresponds to the high lectin activity of protein isolated from the nodules. At the low level of nitrogen fixation (inoculation with inactive strains 604k *B. japonicum* – fig. 1A and 400 *Bradyrhizobium* sp. (*Lupinus*) – fig. 1B) the insignificant lectin activity of root nodules was observed. Direct relationship between nitrogen fixation and lectin activity testifies the direct participation of lectins in the processes of nitrogen fixation. In addition, as was shown for the first time, the activity of inoculum strain also influences on the level of lectin activity of protein extracts isolated from the other organs of host-plants. The results obtained have testified participation of lectins not only in fixation of atmospheric nitrogen in nodules but in various other physiological processes in plant.

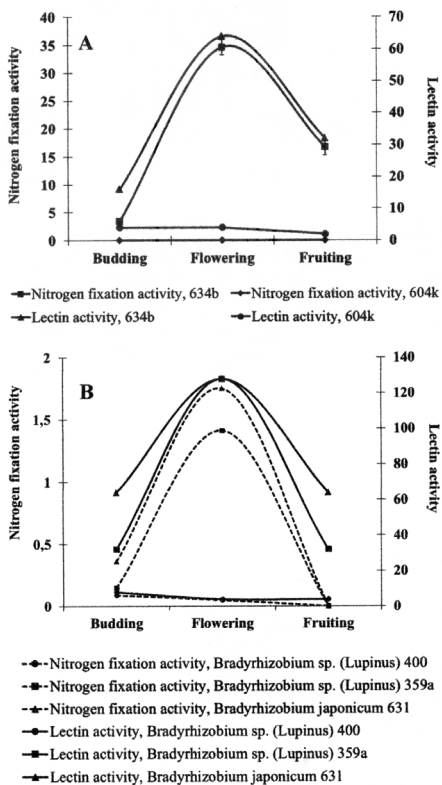


Fig. 1. Nitrogen fixation ($\mu\text{mol C}_2\text{H}_4\text{:plant}^{-1}\cdot\text{h}^{-1}$) and lectin activity (AU/50 μl) of soybean (A) and lupine (B) nodules inoculated by *Bradyrhizobium* strains with various activity

Taking into the account the high activity of lectin in different parts of lupine and soybean plants and basing on the literature data regarding their biological role in plants growth and development processes we have made a conclusion on the appropriateness of exogenous application of lectins at presowing inoculation of legume plants.

It was revealed that combined treatment of soybean plant seeds with suspension of *B. japonicum* 634b (specific) and 631 (non-specific, promotes nodule formation on both soybean and lupine) strains and lectins of soybean and lupine, homologous to these strains consequently activate nitrogen fixation activity of formed nodules.

Though, the soybean lectin promotes nitrogen fixation activity of both studied strains while lectin isolated from the lupine plants had increased the activity of 631 *B. japonicum* strain (used for inoculation of soybean and lupine plants) only (fig. 2). Enhancement of such activity might be related to the effect of homologous lectin of host-plant on the metabolism of bacterial cell, as well as on the synthesis of enzymes participating in the process of nitrogen fixation, and nitrogenase in first place.

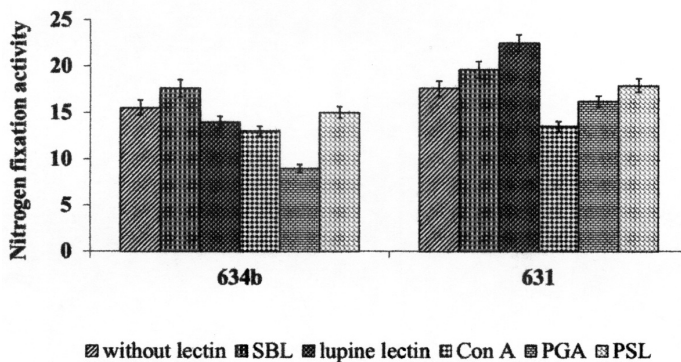


Fig. 2. The influence of specific and nonspecific soybean nodule bacteria lectins on soybean nitrogen fixation activity ($\mu\text{mol C}_2\text{H}_4:\text{plant}^{-1}\cdot\text{h}^{-1}$)

Preincubation of soybean rhizobia with the lectins not specific to the legume plants, namely lectins of bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.) and jack bean (*Canavalia ensiformis* L.) has showed the suppressive effect of these agglutinins on the nitrogen fixation activity of nodules. In particular, general nitrogen fixation activity of root nodules was decreased up to 40% depending on the lectin type and inoculum strain. At the same time heterologous lectins almost in all

variants has a positive effect on the mass of root nodules, in other words – on the «plant part» of the symbiotic system.

It can be assumed that presence of exogenous lectin in inoculum effects not only microsymbiont but also the host-plant. Thus, we can consider that lectins might be used as the biological active substance positively influencing on symbiosis productivity and plants yield.

Investigation of nitrogen fixation activity in nodules in plants inoculated with rhizobia suspensions with different lectin concentrations (2, 20, 200 mcg/ml) has revealed the 20 µg of lectin per 1 litre of bacterial suspension to be the most efficient dose. At budding and flowering periods this index in soybean plants was almost 4 times higher comparing to control value. Fruiting stage had revealed a little bit lower difference but still it has twice exceeded the control variant.

Table 2. The influence of different concentrations of specific lectin on nitrogen fixation activity ($\mu\text{mol C}_2\text{H}_4 \text{ plant}^{-1} \text{ h}^{-1}$) and yield (centner per ha) of soybean inoculated by strain *B. japonicum* 634b (field experiments)

Lectin concentration, µg/ml bacterial suspension	Nitrogen fixation activity (budding)	Yield	Yield increase to control	
			centner/ha	%
Control (without lectin)	5,13±0,24	27,9±1,6	–	–
50	4,37±0,29	32,3±2,2	4,4	15,8
100	4,93±0,15	32,7±1,7	4,8	17,2
200	9,85±0,38	33,3±1,9	5,4	19,4
300	7,78±0,28	34,5±2,1	6,6	23,6
LSD ₀₅		3,9		

In order to examine whether lectins preserve their effect (on nitrogen fixation, in particular) in natural conditions we conducted a number of field experiments. At this, analysis of nitrogen fixation activity of soybean plants has shown, that the application of bacterial suspensions containing 200 and 300 µg/ml of lectin was the most effective at budding stage (table 2). The level of nitrogen fixation in these variants was 91 and 51 % higher comparing to control. The same dependencies were observed during the other ontogenesis stages.

Influence of various factors on productivity of plants is measured by their yield. In our experiments use of exogenous lectins in different concentrations has ensured the considerable yield increase (table 2). At

this the tendency to its increase at the rise of lectin concentration in the inoculation suspension was noticed. Moreover, the most economical concentration was 200 µg of lectin per 1 ml of bacterial suspension.

Hence, lectins, as multifunctional compounds are considered to be the signal molecules used to control formation and functioning of legume-rhizobial system. The nature of their influence on nitrogen fixation and plants productivity allows their examination as the effectors of plant growth and development. In addition, the data obtained indicates the perspectives of the application of bacterial preparations including homologous lectin and might be used in the improvement of agricultural crops production technologies.

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ЛЕКТИНИ ТА ЇХ РОЛЬ У ФОРМУВАННІ І ФУНКЦІОНУВАННІ БОБОВО-РИЗОБІАЛЬНИХ СИМБІОЗІВ

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У статті розглядається питання ролі лектинів бобових у становленні азотфіксувальних симбіотичних систем цих рослин із бульбочковими бактеріями. Наводяться докази позитивного впливу досліджуваних білків на прикріплення ризобій до коренів бобових рослин. Було встановлено, що рівень гемаглютинуючої активності лектинів сої та бульбочок люпину прямо залежить від активності азотфіксації. Обробка насіння сої ризобіальною суспензією разом із гомологічним лектином сприяла підвищенню азотфіксації в бульбочках і значно підвищувала продуктивність рослин, що підтверджує перспективність застосування лектинів бобових у композиції з бактеріальними добривами для обробки насіння бобових рослин.

Ключові слова: лектини бобових, бобово-ризобіальний симбіоз.

ЛЕКТИНЫ И ИХ РОЛЬ В ФОРМИРОВАНИИ И ФУНКЦИОНИРОВАНИИ БОБОВО-РИЗОБИАЛЬНЫХ СИМБИОЗОВ

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В статье рассматриваются вопросы, касающиеся роли лектинов бобовых в формировании азотфиксирующих симбиотических систем этих растений с клубеньковыми бактериями. Приводятся доказательства позитивного влияния исследуемых белков на прикрепление ризобий к корням бобовых растений. Было установлено, что уровень геммагглютинирующей активности лектинов сои и клубеньков люпина напрямую зависит от активности азотфиксации. Обработка семян сои ризобиальной суспензией совместно с гомологичным лектином способствовала усилению азотфиксации в клубеньках и значительно повышала продуктивность растений, что подтверждает перспективность использования лектинов бобовых в композициях с бактериальными удобрениями для обработки семян бобовых растений.

Ключевые слова: лектины бобовых, бобово-ризобиальный симбиоз.