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❀ **The signal molecules produced by legume plants and soil bacteria rhizobia and involved in early steps of symbiosis regulation were identified through the evaluation of molecular mechanisms of plant-rhizobia communication. The molecular dialog between plants and rhizobia is initiated by plant flavanoids inducing the synthesis and secretion of lipochitooligosaccharide molecules Nod factors by rhizobial bacteria. Nod factors are N-acetylglucosamine oligomers, modified by fatty acid and certain chemical groups. Nod factors trigger a set of plant reactions resulting in a formation of root nodules — nitrogen fixing symbiotic organs. Fine chemical structure of signal molecules determines host specificity of the symbiosis. Nod factors are active in low concentrations and possess mitogenic and morphogenic activity, therefore they are recognized as the new class of growth regulators. In this paper the modern data about study of Nod factor perception mechanisms and signal transduction pathway in legume plants are presented and considered with perspective for future application of these knowledge for practical increasing of symbiosis efficiency from plant side.**

❀**Key words:** *Rhizobium* — legume symbiosis, signal molecules, plant flavanoids, Nod factors, LysM receptor-like kinases, signal transduction pathway, gene expression, infection structures, nodule organogenesis

ROLE OF SIGNAL EXCHANGE IN CONTROL OF *RHIZOBIUM* — LEGUME SYMBIOSIS SPECIFICITY

INTRODUCTION.

During last years many efforts were directed at elucidation of the molecular mechanisms by means of which the different extracellular signals trigger a biological response in target cells in many-celled animals. Signal binding with specific receptors activates transduction pathways that finally results in change of physiological conditions in the cell. Therefore, receptors transfer the signal into the cells, where it is amplified in a specific manner. Deciphering of signaling processes for representatives of different groups of eukaryotic organisms gives a key for understanding common biological processes: cell growth, homeostasis, apoptosis, cancer, pathogenesis, development and differentiation, so this question is intensively studied in many laboratories in the world.

Due to their permanent communication with soil microflora, plants have developed sensitive cellular mechanisms to respond to potentially beneficial or pathogenic organisms. One of the most important mechanisms is the chemoperception of signals emanating from the interacting organisms followed by the transduction of signals to induce appropriate response. Plants are able to recognize different molecules produced by microorganisms such as glycopeptides, oligosaccharides, chitooligosaccharides. Analysis of genomes of *Arabidopsis thaliana* and *Medicago truncatula* allowed to identify more than 500 specific receptors that significantly exceeds amount of receptors in animal (Yahyaoui et al., 2004). The nature of these signals and mechanisms of their binding by perception systems of plants are important aspects of plant-microbe interactions (Boller, 1995).

Soil bacteria, collectively referred to as rhizobia, are able to establish symbiosis with leguminous plants, resulting in a formation of a new organ, nitrogen-fixing nodule, on the plant root, where bacteria differentiate into bacteroids and realize their capacity to fix molecular nitrogen. Selectivity of mutual recognition of nitrogen-fixing bacteria and plants during endosymbiosis development is determined by secretion and perception of signaling molecules of the partners. Lipochitooligosaccharide signals emanating from rhizobia, Nod factors (NFs), trigger a complex of specific responses in root hairs, pericycle and root cortex of the plant, thereby providing the basis for subsequent bacteria entry, infection distribution and nodule morphogenesis. Nod factors are lipochitooligosaccharides (LCOs) — oligomers of N-acetylglucosamine (4-6 GlcNAc units) carrying fatty acid chain at non-reducing sugar and various modifications on chitin backbone. Additional substitutions may present on backbone of Nod factors produced by different rhizobial species (Perret et al. 2000). These substitutions as well as fatty acid structure determine the biological activity of Nod factors, while specific genes controlling nodulation (*Nod* genes) define types and location of these substitutions. For example, Nod factors produced by *Sinorhizobium meliloti*, microsymbiont of most *Medicago* species, are O-sulphated at the reducing sugar, O-acetylated at the non-reducing sugar and contain specific fatty acid C16:2. The presence of acetate at non-reducing sugar and fatty acid C18:4 is characteristic for Nod factors produced by *Rhizobium leguminosarum bv. viceae* infecting pea and vetch (Perret et al. 2000).

Specificity of Nod factor interaction with host plant and very low concentrations at which the biological activity of Nod factors is appeared suggest existence of specific receptors for Nod factors in legume plants. However, despite on progress in this area, molecular mechanisms of Nod factor perception on root surface of legume plants and signal transduction are still far from understanding.

Nod factor perception on root surface of legume plants and genes involved in control of this process

The process of nodulation is the result of tightly regulated biochemical and molecular interactions between the symbionts (Schultze and Kondorosi, 1998). Plants release flavonoids into the rhizosphere, which in turn stimulate the production of Nod factors by rhizobia (Denarie et al., 1996; Long, 1996). Successful development of nitrogen-fixing nodules is determined by activation of so-called early symbiotic reactions developing during several minutes or hours in response to Nod factor stimulation. Nod factor stimulation of signal transduction cascade elicits the early plant responses in root hair cells such as ion flux (Ehrhardt et al., 1992, 1996; Harris et al., 2003), calcium oscillations (Felle et al., 1999; Engstrom et al., 2002; Charron et al., 2004), cytoskeletal changes (Van Brussel et al., 1992; de Ruijter et al., 1999), root hair deformation (Lerouge et al., 1990), activation of a few initial rounds of mitotic divisions in root cortex (Spaink et al., 1991) and expression of Nod factor-dependent genes (ENOD genes or early nodulins) (Horvath et al., 1993; Albrecht et al., 1998). In model legume *Medicago truncatula*, the early nodulins *ENOD11* and *RIP1* have been valuable markers of early Nod factor activated signal transduction pathway (Cook et al., 1995; Journet et al., 2001).

Development of early symbiotic reactions precedes rhizobial entry into root hair cells and followed by subsequent infection progression. These later processes are developed in a few days after interaction between plants and rhizobia. In most species infection starts from formation of specific root hair curl, which entraps a bacterial microcolony (Caetano-Anolles and Gresshoff, 1991). At the next steps bacteria induce local degradation of cell wall and invagination of root hair plasma membrane that leads to formation of tubular structure, called by infection thread (IT). In parallel with initiation of IT growth, cortical cell division is resumed in root cortex resulting in nodule primordia and meristem formation (Timmers et al., 1999; Tsyganov et al., 2002). These processes are tightly interconnected although in some species like alfalfa (*Medicago sativa*) and soybean bacteria-free pseudonodule formation might occur, indicating an ability to provoke nodule organogenesis independently from bacterial infection (Truchet et al., 1989; Stokkermans and Peters, 1994).

Experiments with bacterial mutants in the *Nod* genes showed that the infection process (process of IT formation) in *M. truncatula* and pea is strictly dependent on structural peculiarities of Nod factors, while the induction of early physiological changes in cells (change in intracellular concentration of calcium, transcriptional activation of specific genes and reactivation of initial rounds of cortical cell division) are not strictly dependent (Ardourel et al. 1994, Oldroyd et al. 2001, Wais et al., 2000). In accordance with these data the hypothesis about existence of two types of receptors for Nod factors differing in specificity was suggested. It was predicted that strictly specific with respect to Nod factor structure receptor regulates infection of legume plant roots and it was called "entry receptor", while lower specific with respect to Nod factor structure "signaling receptor" regulates development of early symbiotic reactions preceding infection (Ardourel et al. 1994). This suggestion is being confirmed during subsequent study of Nod factor perception in legume plants.

During the last years study of mechanisms of Nod factor perception and signaling reached a new level due to the availability of the genome sequences of model legumes *M. truncatula* and *L. japonicus* and the development of new methodical approaches. Genetic analysis of non-nodulating plant mutants and subsequent map-based cloning of mutant genes in *M. truncatula* (Medicago) and *L. japonicus* (Lotus) allowed to identify genes which could be involved in control of Nod factor recognition and signal transduction to target genes. In legume plants, NF recognition seems to be mediated by receptor-like kinases (RLKs) possessing LysM extracellular domains (Radutoiu et al., 2003). LysM motifs were previously found in bacteriophage's enzyme lysine, mureine hydrolase that cleaves peptidoglycane mureine presenting in bacterial cell wall and constituting GlcNAc units as well as Nod factor (Bateman and Bycroft, 2000). Indeed a few receptor-like kinases with LysM extracellular domains were identified in *M. truncatula* and *L. japonicus*, is thought to be essential for Nod factor perception (Radutoiu et al. 2003, Madsen et al. 2003, Limpens et al. 2003, Arrighi et al. 2006, Mulder et al. 2006). In Lotus two genes *LjNFR1* and *LjNFR5* encoding putative receptors for Nod factors were identified (Radutoiu et al. 2003; Madsen et al. 2003). Mutations in either of genes blocked development of any responses to Nod factor application. Protein NFR1 is receptor-like kinase containing extracellular, transmembrane domains and a typical serine/threonine kinase domain, while NFR5, in contrast, contains atypical kinase domain without an important part — activation loop (Radutoiu et al. 2003). In addition, experiments with labeled ATP have shown that only the NFR1 protein is capable to be autophosphorylated, but not the NFR5 (Arrighi et al. 2006). Organization of kinase domains and analysis of mutant in the genes encoding appropriate proteins suggest that the NFR1 and NFR5 could be components of heterodimeric receptor complex, which may comprise the "signaling receptor" for Nod factor.

Two genes *MtNFP* and *MtLYK3* encoding receptors with LysM motifs in extracellular domains were also identified in Medicago (Limpens et al., 2003, Arrighi et al., 2006, Mulder et al., 2006) and in pea — *PsSym10* and *PsSym37* (Madsen et al., 2003; Zhukov et al., 2007). The genes *MtNFP* and *PsSym10* are orthologous to the *LjNFR5* and mutations in these genes led to almost complete blocking of plant responses to Nod factor stimulation (Ben Amor et al., 2003; Walker et al., 2000). Based on this, it was suggested that the NFP and SYM10 proteins

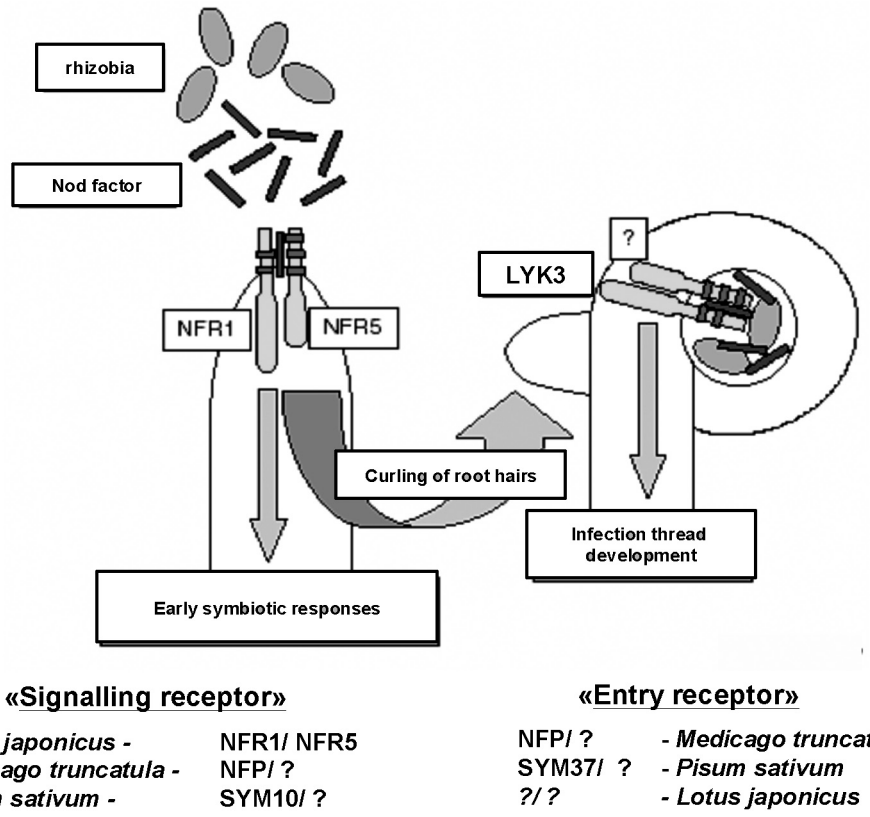

Fig. 1. **The model of plant recognition of bacterial signals Nod factors via LysM receptor-like kinases**

containing like the NFR5 non-functional kinase domains, may be one of the components in the "signaling receptor" for Nod factor.

Analysis of microsynteny and primary sequences of genes revealed that the *MtLYK3* and *PsSym37* are orthologous to the *LjNFR1* (Smit et al., 2007; Zhukov et al., 2007). However, in spite of the fact that structural resemblance between these genes in legume plants was shown, the operation mechanisms of the predicted receptor-like proteins LYK3 and SYM37 differ from those for Lotus. In pea mutation in the gene *PsSym37* has blocked only infection process in a specific manner, but not development of early symbiotic reactions (Tsyganov et al., 2002; Zhukov et al., 2007). Function of the *MtLYK3* was initially studied by RNA interference method (Limpens et al., 2003) followed by cloning and analysis of primary sequence of Medicago mutant gene *HCL (HAIR CURLING)* which encodes the LysM receptor-like kinase LYK3 (Smit et al., 2007). As well as in case of *sym37* pea mutant, suppression of *LYK3* expression by RNA interference or mutation in this gene induced specific blocking in symbiosis development at the stage of bacterial entry (Limpens et al., 2003; Smit et al., 2007). Besides a weak allele that controls infection thread formation in Nod factor structure-dependent manner was revealed (Smit et al., 2007). These data support the suggestion that the LYK3 in Medicago and the SYM37 in pea, may represent "entry receptor", but they are not components of "signaling receptor", because they regulate infection process in Nod factor structure-dependent manner. Accumulated data allowed suggesting the model of sequential activation of two complex receptors during Nod factor binding in legume plants (Geurts et al., 2005). In accordance with this model at the early stages of symbiosis development binding of Nod factors with first heterodimeric receptor induces the early symbiotic reactions including root hair deformations, calcium oscillations and reactivation of the cortical cells that leads to first rounds of mitotic divisions. In Lotus this heterodimeric receptor may be comprised by NFR5/ NFR1 proteins, while only particular components NFP/- and SYM10/- were characterized in Medicago and in pea, correspondently. At the later steps of symbiosis after curling of root hairs another receptor complex may be activated that triggers signaling cascade leading to infection process development (fig. 1). Proteins LYK3 and SYM37 are the most probable candidates on the role of "entry receptor" in Medicago and pea, while such candidates were not discovered in Lotus. Some researchers consider that "entry receptor" could be also represented by heterodimeric complex (Geurts et al., 2005; Arrighi et al.,. 2006). In this connection search of new mutants blocked in IT development is being continued in Lotus, Medicago and pea (Yano et al., 2006; Lombardo et al., 2006; Miwa et al., 2006; Borisov et al., 2007).

Previously it was suggested that the gene *Sym2* may be also specifically involved in control of infection process due to study of plants carrying different alleles of this gene (Geurts et al., 1997). Pea lines carrying the $Sym2^A$ allele (pea cultivars like Afghanistan) were characterized by infection abortion during inoculation with incompatible rhizobial strains. At the same time early symbiotic reactions preceding infection development were not disturbed in such lines. In contrast, normal symbiosis development resulting in effective nodule formation was found in pea lines carrying the $Sym2^C$ allele. Taking into consideration the significant resemblance between phenotypes of pea lines carrying the $Sym2^A$ allele and mutant lines in the gene the *PsSym37* it could not be excluded that the *Sym2* gene encodes other component of "entry receptor" in pea. However, until now the pea *Sym2* gene was not cloned and this suggestion should be verified in future.

Characterization of early signal transduction pathway components in legume plants

Basic data about components of signal transduction pathway activated by Nod factors were obtained using molecular-genetic approach. Analysis of non-nodulating Nod-mutants of legume plants showed that they were impaired at the different early stages of symbiosis development (Borisov et al., 2000; Tsyganov et al., 2002). Map-based cloning of these genes allowed finding the first components of signal pathway. It seems that Nod factor binding activates a whole complex of proteins involved in signal transduction, five from those may be components of a common pathway leading not solely to legume-rhizobial symbiosis development, but also to establishment of symbiosis between plants and arbuscular fungi (arbuscular mycorhiza) (Gianinazzi-Pearson, 1996; Catoira et al., 2000; Oldroyd and Long, 2003).

One of the first components of this pathway is a protein showing the properties of ligand- regulated cation channel. In *M. truncatula* this protein is encoded by the *DMI1 (DOES NOT MAKE INFECTION 1)* gene and its orthologs *Sym8* and *POLLUX* were found in pea and *L. japonicus*, correspondingly (Catoira et al., 2000; Ane et al., 2004; Imaizumi-Anraku et al., 2005). The presence of regions involved in binding with other proteins (proline rich) points out this cation channel as a constituent part of multicomponent complex with receptor transmitting signal from Nod factor to subsequent components of signal transduction pathway.

Next stage of signal cascade is operated by protein from family of receptor-like kinases with leucine-rich repeats in extracellular domain LRR-RLK (leucine-rich repeats receptor-like kinase) (Endre et al., 2002; Stracke et al., 2002). In *M. truncatula* this protein is encoded by the *DMI2 (DOES NOT MAKE INFECTION 2)* gene and its orthologs *Sym19* and *SYMRK (SYMBIOSIS RECEPTOR-LIKE KINASE)* were found in pea and *L. japonicus*, correspondingly (Schneider et al., 1999; Stracke et al., 2002). Plant receptor-like kinases with leucine-rich repeats in extracellular domain have evolutionary and functional resemblance with receptors of Toll family in *Drosophila* and Toll-like receptors of other animals. It is known that Toll-like receptors participate in recognition of pathogenic organisms and in development of immunity. Recently it was revealed that plant leucine-rich repeats receptor-like kinases participate in recognition of proteins regulating plant development and defence reaction activation.

The proteins composing the nuclear pore may be other components of signal transduction pathway in legume plants. By analogy with animal cells the nuclear pore comprised by a few proteins. However, until now only two such proteins the NUP85 and NUP133 were revealed in *L. japonicus* (Kistner et al., 2005; Kanamori et al., 2006), but no orthologous genes were found either in *M. truncatula* or in *P. sativum* (fig. 1). It means that other nucleopore proteins will be revealed nearest time. Mutations in the genes *Nup85* and *Nup133* blocked calcium oscillations which are activated during Nod factor dependent cascade, that demonstrates the importance of these proteins in generation of calcium signal.

Recently next key element of signal pathway activated by Nod factors the CCaMK, calcium/ calmodulin – dependent protein kinase was revealed (Levy et al., 2004; Mitra et al., 2004; Gleason et al., 2006). In *M. truncatula* this protein is encoded by the *DMI3 (DOES NOT MAKE INFECTION 3)* gene and its orthologs *Sym9* and *Sym15* were found in pea and Lotus (Levy et al., 2004; Mitra et al., 2004; Tirichine et al., 2006). Protein CCaMK is activated in response to change in intracellular calcium concentration in plant cells and transmits the signal to other proteins. It is known that the CCaMK acts at the checkpoint were pathways leading to legume-rhizobial symbiosis and arbuscular mycorhiza development are separated (Catoira et al., 2000; Parniske, 2004; Kistner et al., 2005).

After stage controlled by the CCaMK only specific for Nod factor activated signalling components are involved in subsequent signal transduction from Nod factor, but they are not related to arbuscular mycorhiza development. Probably, they participate in transcription activation of genes in nucleus of plant cells, which are targets of Nod factor activated signal transduction pathway. Recently two genes *NSP1 (NODULATION SIGNALING PATHWAY 1)* and *NSP2 (NODULATION SIGNALING PATHWAY 2)* encoding transcription factors of GRAS family were cloned and characterized in Medicago and Lotus (Kalo et al., 2005; Smit et al., 2005; Heckmann et al., 2006; Murakami et al., 2006). They are also key elements involved in legume-rhizobial symbiosis development. Pea *PsSym7* gene is orthologous to the *NSP1* (Kalo et al., 2005). Full-scale analysis of transcriptional changes in gene expression in plants during symbiosis development showed that activity of the NSP1 and NSP2 is absolutely required for expression induction of majority of symbiosis-specific genes (Mitra et al., 2004; Smit et al., 2005).

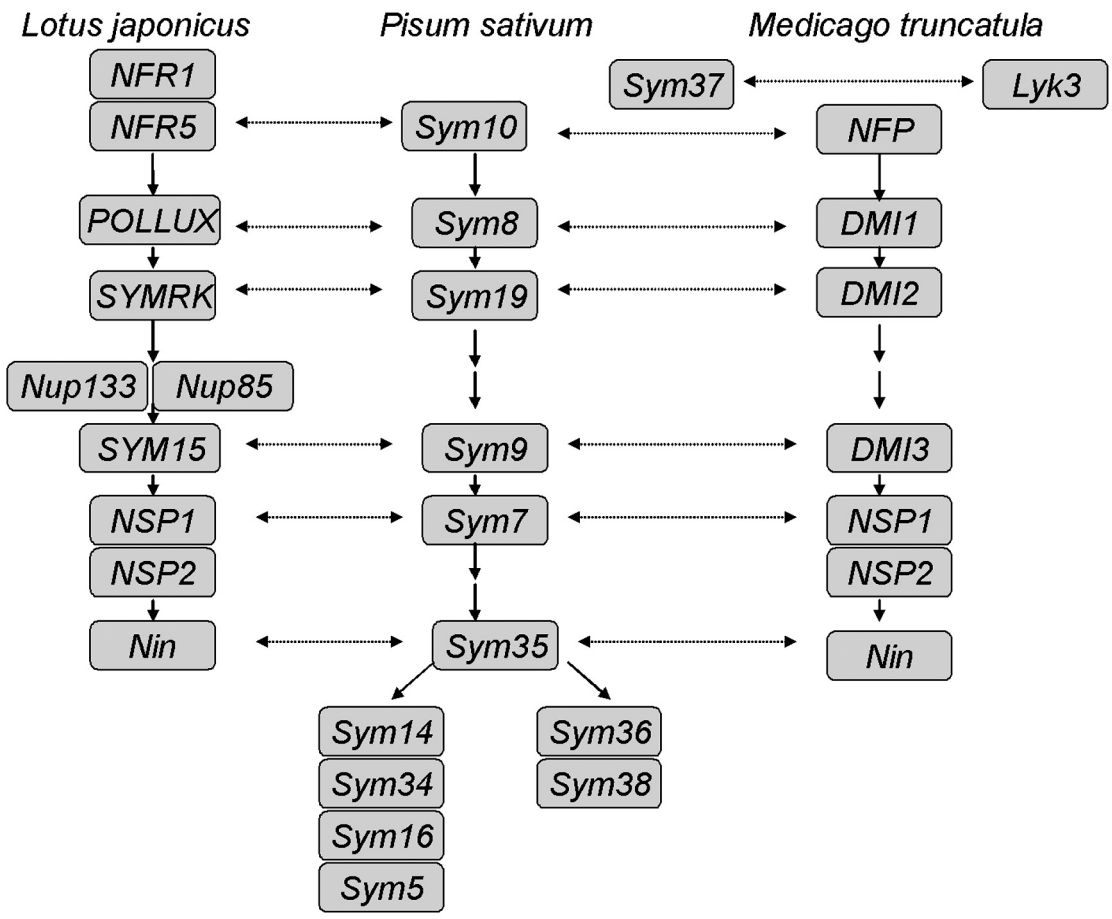

Fig. 2. **Genetic dissection of the Rhizobium – legume symbiosis**

Genes involved in control of later steps of symbiosis related to development of infection structures and nodule organogenesis

If the components controlling the early steps of symbiosis were partially characterized, it is noticeably less known about genes related to control of infection structure development and nodule organogenesis. Substantially it depends on what restricted number of Lotus and Medicago mutants blocked in symbiosis development at these stages are available. In contrast to Lotus and Medicago extensive genetic screening of symbiotic mutants was performed in *P. sativum* L. that resulted in identification of new loci required for infection and nodule organogenesis (Borisov et al., 2000; Tsyganov et al., 2002; Borisov et al., 2007). Among of them a few loci are orthologous to genes previously characterized in Lotus and Medicago, while the other ones were exclusively described in pea (fig. 2). Such material will be used for cloning and analysis of primary sequence of mutant genes.

Among of these the *NIN (NODULE INCEPTION)* gene should be remarked (Schauser et al., 1999; Borisov et al., 2003; Smit et al., 2005; Marsh et al., 2007). NIN protein, comprising several domains (I – VI), shows similarity to transcription factors. At present, no function can be suggested for domains I through III. Domain IV contains the hydrophobic stretches suggested to be either membrane-spanning regions or hydrophobic pockets (Schauser et al., 1999). Domain V is the most conserved region and makes up the previously identified RWP-RK region, suggested to serve in dimerization and DNA binding in this family of putative transcriptional regulators (Schauser et al., 1999). Detailed analysis of mutant plants has shown that the NIN is not required for early steps of symbiosis development, but may be involved in control of nodule organogenesis (Tirichine et al., 2006; Marsh et al., 2007).

CONCLUSION

The significant influence on the efficiency of symbiosis could be reached through applying stored knowledge about molecular mechanisms of rhizobia-plant signaling now. Modern programs aimed to improve the efficiency of symbiosis have assumed approaches exploring both microsymbiont and legume host. Summarizing published data about selected rhizobial strains with high efficiency and genetically modified stains harboring additional genes responsible for Nod factor synthesis it could be concluded that substantial progress in control of symbiotic performance from the side of

microsymbiont has been revealed. In contrast approaches to explore the control of symbiosis specificity and efficiency in plant partner are weakly designed. Recent progress in plant functional genomics is challenged the developing of new strategies to operate upon the system of symbiosis specificity. Identified plant genes responsible for plant-microbe signaling, perception of signals and nodulation could assume as a basis for evaluation of fundamental biochemical and genetic mechanisms potentially important for increasing symbiosis efficiency.

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Literature

1. *Ane J.-M., Kiss G. B., Riely B. K.* et al., 2004. *Medicago trancatula DMI1* required for bacterial and fungal symbioses in legumes // Science. Vol. 303, P. 1364–1367.
2. *Albrecht C., Geurts R., Lapeyrie F., Bisseling T.*, 1998. Endomycorrhizae and rhizobial Nod factors both require SYM8 to induce the expression of the early nodulin genes *PsENOD5* and *PsENOD12A* // The Plant Journal. Vol. 15, N 5, P. 605–614.
3. *Ardourel M., Demont N., Debelle F. D.* et al., 1994. *Rhizobium meliloti* lipooligosaccharide nodulation factors: different structural requirements for bacterial entry into target root hair cells and induction of plant symbiotic developmental responses // Plant Cell. Vol. 6, P. 1357–1374.
4. *Arrighi J.-F., barre A., ben Amor B.* et al., 2006. The *Medicago trancatula* lysine motif-receptor-like kinase gene family includes NFP and new nodule-expressed genes // Plant Physiology. Vol. 142, P. 265–279.
5. *Ben Amor B., Shaw S. L., Oldroyd G. E. D.* et al., 2003. The NFP locus of *Medicago trancatula* controls an early step of Nod factor signal transduction upstream of a rapid calcium flux and root hair deformation // Plant J. Vol. 34, P. 1–12.
6. *Bateman A, Bycroft M.*, 2000. The structure of a LysM domain from *E. coli* membrane bound lytic murein transglycosylase D (MltD) // J. Mol Biol. Vol. 299, P. 1113–1119.
7. *Borisov A. Y., Barmicheva E. M., Jacobi L. M.* et al., 2000. Pea (*Pisum sativum* L.) mendelian genes controlling development of nitrogen-fixing nodules and arbuscular mycorrhiza // Czech J. Genet Plant Breed. Vol. 36, P. 106–110.
8. *Borisov A. Y., Danilova T. N., Koroleva T. A.* et al., 2007. Regulatory genes of garden pea (*Pisum sativum* L.) controlling the development of nitrogen-fixing nodules and arbuscular mycorrhiza: a review of basic and applied aspects // Applied Biochemistry and Microbiology. Vol. 43, N 3, P. 237–243.
9. *Boller T.*, 1995. Chemoperception of microbial signals in plant cells // Annual Review of Plant Physiology and Plant Molecular Biology. Vol. 46, P. 189–214.
10. *Caetano-Anolles G., Gresshoff P. M.,* 1991. Plant genetic control of nodulation // Annu. Rev. Microbiol. Vol. 45. P. 345–382.
11. *Catoira R., Galera C., de Billy F.* et al., 2000. Four genes of *Medicago truncatula* controlling components of a Nod factor transduction pathway // Plant Cell. Vol. 12, P. 1647–1665.
12. *Charron D., Pingret J. L., Chabaud M.*, et al., 2004. Pharmacological evidence that multiple phospholipid signaling pathways link *Rhizobium* nodulation factor perception in *Medicago trancatula* root hairs to intracellular responses, including Ca^{2+} spiking and specific ENOD gene expression // Plant Physiol. Vol. 136, P. 3582–3593.
13. *Cook D., Dreyer D., Bonnet D.* et al., 1995. Transient induction of a peroxidase gene in *Medicago trancatula* precedes infection by *Rhizobium meliloti* // Plant Cell. Vol. 7, P. 43–55.
14. *Denarie J., Debelle F., Prome J. C.*, 1996. *Rhizobium* lipo-chitooligosaccharide nodulation factors: signaling molecules mediating recognition and morphogenesis // Annu. Rev. Biochem. Vol. 65, P. 503–535.
15. *De Ruijter N. C. A., Bisseling T., Emons A. M. C.,* 1999. *Rhizobium* Nod factors induce an increase in sub-apical fine bundles of actin filaments in *Vicia sativa* root hairs within minutes // Mol. Plant Microbe Interact. Vol. 12, P. 829–832.
16. *Ehrhardt D. W., Atkinson E. M., Long S. R.*, 1992. Depolarization of alfalfa root hair membrane potential by *Rhizobium meliloti* Nod factors // Science. Vol. 256, P. 998–1000.
17. *Ehrhardt D. W., Wais R., Long S. R.*, 1996. Calcium spiking in plant root hairs responding to *Rhizobium* nodulation signals // Cell. Vol, 85. P. 673–681.
18. *Endre G., Kereszt A., Kevei Z.* et al., 2002. A receptor kinase gene regulating symbiotic nodule development // Nature. Vol. 417, P. 962–966.
19. *Engstrom E. M., Ehrhardt D. W., Mitra R. M., Long S. R.*, 2002. Pharmacological analysis of Nod factor-induced calcium spiking in *Medicago truncatula*: evidence for the requirement of type IIA calcium pumps and phosphoinositide signaling // Plant Physiol. Vol. 128, P. 1390–1401.
20. *Felle H. H., Kondorosi E., Kondorosi A., Schultze M.* 1999. Nod factors modulate the concentration of cyto-

solic free calcium differently in growing and non-growing root hairs of *Medicago sativa L.* // Planta. Vol. 209, P. 207–212.
21. *Geurts R., Heidstra R., Hadri A-E.* et al., 1997. *Sym2* of *Pisum sativum* is involved in Nod factor perception mechanism that controls the infection process in the epidermis // Plant Physiology. Vol. 115, P. 351–359.
22. *Geurts R., Fedorova E., Bisseling T.,* 2005. Nod factor signaling genes and their functioning in early stages of *Rhizobium* infection // Curr. Opin. Plant Biol. Vol. 8, P. 346–352.
23. *Gleason C., Chaudhuri S., Yang T. B.*, 2006. Nodulation independent of rhizobia induced by a calcium activated kinase lacking autoinhibition // Nature. Vol. 441, P. 1149–1152.
24. *Gianinazzi-Pearson V.*, 1996. Plant cell responses to arbuscular mycorrhizal fungi: getting to the roots of the symbiosis // Plant Cell. Vol. 8, P. 1871–1883.
25. *Harris J. M., Wais R., Long S. R.*, 2003. *Rhizobium*-induced calcium spiking in *Lotus japonicus* // Mol. Plant Microbe Interact. Vol. 16, P. 335–341.
26. *Heckmann A. B., Lombardo F., Miwa H.* et al., 2006. *Lotus japonicus* nodulation requires two GRAS domain regulators, one of which is functionally conserved in a non-legume // Plant Physiology. Vol. 142, P. 1739–1750.
27. *Horvath B., Heidstra R., Lados M.*, et al., 1993. Lipooligosaccharides of *Rhizobium* induce infection related early nodulin gene expression in pea root hairs // Plant J. Vol. 4, P. 727–733.
28. *Imaizumi-Anraku H., Takeda N., Charpentier M.* et al., 2005. Plastid proteins crucial for symbiotic fungal and bacterial entry into plant roots // Nature. Vol. 433, P. 527–531.
29. *Journet E.-P., El-Gachtouli N., Vernoud V.* et al., 2001. *Medicago trancatula* ENOD11: A novel RPRP-encoding early nodulin gene expressed during mycorrhization in arbuscule-containing cells // Mol. Plant Microbe Interact. Vol. 14, N 6, P. 737–748.
30. *Kalo P., Gleason C., Edwards A.* et al., 2005. Nodulation signaling in legumes requires NSP2, a member of the GRAS family of transcriptional regulators // Science. Vol. 308, P. 1786–1789.
31. *Kanamori N., Madsen L. H., Radutoiu S.* et al., 2006. A nucleoporin is required for induction of Ca^{2+} spiking in legume nodule development and essential for rhizobial and fungal symbiosis // Proc. Natl. Acad. Sci. USA. Vol. 103, N 2, P. 359–364.
32. *Kistner C., Winzer T., Pitzschke A.* et al., 2005. Seven *Lotus japonicus* genes required for transcriptional reprogramming of the root during fungal and bacterial symbiosis // Plant Cell. Vol. 17, P. 2217–2229.
33. *Lerouge P., Roche P., Faucher C.* et al., 1990. Symbiotic host-specificity of *Rhizobium meliloti* is determined by a sulphated and acylated glucosamine oligosaccharide signal // Nature. Vol. 19, P. 781–784.
34. *Levy J., Bres C., Geurts R.* et al., 2004. A putative Ca^{2+} and calmodulin-dependent protein kinase required for bacterial and fungal symbioses // Science. Vol. 303, P. 1361–1363.
35. *Limpens E., Franken C., Smit P.* et al., 2003. LysM domain receptor kinases regulating rhizobial Nod factor-induced infection // Science. Vol. 302, P. 630–633.
36. *Long S. R.*, 1996. *Rhizobium* symbiosis: Nod factors in perspective // Plant Cell. Vol. 8, P. 1885–1898.
37. *Lombardo F., Heckmann A. B., Miwa H.* et al., 2006. Identification of symbiotically defective mutants of *Lotus japonicus* affected in infection thread growth // Mol. Plant Microbe Interact. Vol. 19, N 12, P. 1444–1450.
38. *Madsen E. B., Madsen L. H., Radutoiu S.* et al., 2003. A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals // Nature. Vol. 425, P. 637–640.
39. *Marsh J. F., Rakocevic A., Mitra R. M.* et al., 2007. *Medicago truncatula* NIN is essential for rhizobial-independent nodule organogenesis induced by autoactive calcium/calmodulin-dependent protein kinase // Plant Physiology. Vol. 144, P. 324–335.
40. *Mitra R. M., Shaw S. L., Long S. R.,* 2004. Six nonnodulating plant mutants defective for Nod factor-induced transcriptional changes associated with the legume-rhizobia symbiosis // Proc. Natl. Acad. Sci. USA. Vol. 101, P. 4701–4705.
41. *Miwa H., Sun J., Oldroyd G. E. D., Downie J. A.,* 2006. Analysis of Nod-factor-induced calcium signaling in root hairs of symbiotically defective mutants of *Lotus japonicus* // Mol. Plant Microbe Interact. Vol. 19, N 8, P. 914–923.
42. *Mulder L., Lefebvre B., Cullimore J., Imberty A.*, 2006. LysM domains of *Medicago truncatula* NFP protein involved in Nod factor perception. Glycosylation state, molecular modeling and docking of chitooligosaccharides and Nod factors // Glycobiology. Vol. 16, N 9, P. 801–809.
43. *Oldroyd G. E., Engstrom E. M., Long S. R.*, 2001. Ethylene inhibits the Nod factor signal transduction pathway of of *Medicago trancatula* // Plant Cell. Vol. 13, P. 1835–1849.
44. *Oldroyd G. E., Long S. R.,* 2003. Identification and characterization of *Nodulation-Signaling Pathway 2*, a gene of *Medicago truncatula* involved in Nod factor signaling // Plant Physiol. Vol. 131, P. 1027–1032.
45. *Parniske M.,* 2004. Molecular genetics of the arbuscular mycorrhizal symbiosis // Curr. Opin. Plant Biol. Vol. 7, P. 414–421.
46. *Perret X., Staehelin C., Broughton W. J.*, 2000. Molecular basis of symbiotic promiscuity // Microbiol Mol Biol Rev. Vol. 64, N 1, P. 180–201.

47. *Radutoiu S., Madsen L. H., Madsen E. B.,* 2003. Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases // Nature. Vol. 425, P. 569–570.
48. *Schauser L., Wieloch W., Stougaard J.*, 1999. A plant regulator controlling development of symbiotic root nodules // Nature. Vol. 402. P. 191–195.
49. *Schneider A., Walker S. A., Poyser S.* et al., 1999. Genetic mapping and functional analysis of a nodulation-defective mutant *(sym19)* of pea (*Pisum sativum* L.) // Mol. Gen. Genet. Vol. 262, P. 1–11.
50. *Schultze M., Kondorosi A.*, 1998. Regulation of symbiotic root nodule development // Annu Rev. Genet. Vol. 32, P. 33–57.
51. *Smit P., Raedts J., Portyanko V.* et al., 2005. NSP1 of the GRAS protein family is essential for rhizobial Nod factor–induced transcription // Science. Vol. 308, P. 1789–1790.
52. Smit P., Limpens E., Geurts R. et al., 2007. Medicago LYK3 an Entry Receptor in Rhizobial Nod Factor Signaling // Plant Physiology. Vol. 145, N 1, P. 183–191.
53. *Spaink H. P., Sheeley D. M., van Brussel A. A. N.* et al., 1991. A novel highly unsaturated fatty acid moiety of lipooligosaccharide signals determines host specificity of *Rhizobium* // Nature. Vol. 354, P. 125–130.
54. *Stokkermans T. J. W., Peters N. K.*, 1994. *Bradyrhizobium elkanii* lipo-oligosaccharide signals induce complete nodule structures on *Glycine soja* Siebold et. Zucc. // Planta. Vol. 193, P. 413–420.
55. *Stracke S., Kistner C., Yoshida S.* et al., 2002. A plant receptor-like kinase required for both bacterial and fungal symbiosis // Nature. Vol. 417. P. 959–961.
56. *Tirichine L., Imaizumi-Anraku H., Yoshida S.* et al.,, 2006. Deregulation of a Ca^{2+} /calmodulin-dependent kinase leads to spontaneous nodule development // Nature. Vol. 441, P. 1153–1156.
57. *Tsyganov V. E., Voroshilova V. A., Priefer U. B.* et al., 2002. Genetic dissection of the initiation of the infection process and nodule tissue development in the *Rhizobium*-pea *(Pisum sativum* L.) symbiosis // Annals of Botany. Vol. 89, P. 357–366.
58. *Truchet G., Roche P., Lerouge P.* et al., 1991. Sulphated lipo-oligosaccharide signals of *Rhizobium meliloti* elicit root nodule organogenesis in alfalfa // Nature. Vol. 351. P. 670–673.
59. *Van Brussel A. A. N., Bakhuizen R., Van Spronsen P. C.* et al., 1992. Induction of preinfection thread structures in the leguminous host plant by mitogenic lipooligosaccharides of *Rhizobium* // Science. Vol. 257, P. 70–72.
60. *Wais R. J., Galera C., Oldroyd G.* et al., 2000. Genetic analysis of calcium spiking responses in nodulation mutants of *Medicago trancatula* // Proc. Natl. Acad. Sci. USA. Vol. 97. P. 13407–13412.
61. *Walker S. A., Viprey V., Downie J. A.* 2000. Dissection of nodulation signaling using pea mutants defective for calcium spiking induced by Nod factors and chitin oligomers // Proc. Natl. Acad. Sci. USA. Vol. 97, P. 13413–14418.
62. *Yahyaoui F. E., Kuster H., Ben Amor B.* et al., 2004. Expression profiling in *Medicago trancatula* identifies more than 750 genes differently expressed during nodulation, including many potential regulators of the symbiotic program // Plant Physiology. Vol. 136, P. 3159–3176.
63. *Yano K., Tansengco M. L., Hio T.* et al., 2006. New nodulation mutants responsible for infection thread development in *Lotus japonicus* // Mol. Plant Microbe Interact. Vol. 19, N 7, P. 801–810.
64. *Zhukov V. A., Borisov A. Y., Kuznetsova E. V.*, et al., 2007. Genome synteny of pea and model legumes: from mutations through genetic mapping to the genes // 15th International Congress on Nitrogen Fixation & 12th International Conference of the African Association for Biological Nitrogen Fixation, Book of abstracts, Cape Town, South Africa. P. 62.

Роль обмена сигналами в контроле специфичности бобово-ризобиального симбиоза

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❀ **РЕЗЮМЕ: Результатом изучения молекулярных механизмов взаимодействия бобовых растений и почвенных бактерий ризобий при развитии внутриклеточного симбиоза стала идентификация сигнальных молекул, выделяемых партнерами по симбиозу. Молекулярный диалог инициируется флаваноидами растений, которые в свою очередь стимулируют синтез и выделение ризобиями липохитоолигосахаридных сигналов Nod факторов. Эти соединения представляют собой олигомеры N-ацетилглюкозамина, модифицированные жирной кислотой и определенными химическими группами. Nod факторы запускают ряд растительных реакций, которые ведут к формированию корневых клубеньков — симбиотических органов азотфиксации. Тонкая химическая структура этих молекул определяет хозяйскую специфичность симбиоза. Nod факторы активны при низких концентрациях и обладают митогенной и морфогенной активностью, что предполагает, что они являются новым классом регуляторов. В статье представлены современные данные об изучении механизмов рецепции Nod факторов и «сигналинга» у бобовых растений и рассматриваются перспективы дальнейшего использования этих знаний для практического увеличения эффективности симбиоза со стороны растений.**

❀**КЛЮЧЕВЫЕ СЛОВА:** симбиоз, *Rhizobium* — бобовые растения, молекулярные сигналы, флаваноиды растений, Nod факторы, LysM рецептор-подобные киназы, сигнальный каскад, экспрессия генов, инфекционные структуры, органогенез клубеньков.