EVOLUTIONARY and FUNCTIONAL STUDIES on NITROGEN FIXING SYMBIOTIC GENES of the MODEL LEGUME: *Medicago truncatula*

Ph.D. thesis summary

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2017
SZEGED

Introduction

Soil nutrient availability is a major limiting factor in plant growth and productivity at many areas under agricultural cultivation. The most frequent limiting elements are phosphorus (P) and nitrogen (N). An evolutionary answer to this demand is the symbiotic association of plants with soil microbes that provide valuable macronutrients (mostly P and N) to the host for a share of the sugar compounds of photosynthesis, as an exchange. An ancient type of coexistence is the arbuscular mycorrhiza symbiosis formed with fungi that provide mainly phosphates to the plant. Its first appearance is dated approximately 460 million years ago (mya), concomitant with the plants starting to colonize the land, and it is present in the majority of land plant species since then. Therefore it is hypothesized that the capability of forming mycorrhiza symbiosis was present in the common ancestor of all land species, hence the recent species that do not form mycorrhiza, most probably lost this capability during their evolution. Additionally, much more recently another symbiotic association showed up between plants and nitrogen fixing soil bacteria. During the evolution of land plants this nitrogen fixing root nodule symbiosis appeared multiple times (first approx. 65 mya), but always within a group of closely related species of the Fabales, Fagales, Cucurbitales és Rosales dicot clades. The ability to form nitrogen fixing symbiosis is most abundant among legume (Leguminosae or Fabaceae, Fabales) species. During the symbiosis a new organ, the root nodule is formed, where the symbiotic bacteria reside and fix atmospheric nitrogen, which is than converted to organic molecules that can be used by the plant.

By now many of the plant genes and gene products involved in nitrogen fixing symbiosis were described. These were identified mainly in two chosen nitrogen fixing model legumes: *Medicago truncatula* (*Mt*) and *Lotus japonicus* (*Lj*). Most of the known genes are so called early symbiotic genes functioning in the initial processes while forming the symbiotic association. LysM type receptor kinases (MtLYK3, MtNFP, LjNFR1, LjNFR5) and a LRR type receptor kiase (*Medicago sativa* NORK/MtDMI2/LjSYMRK) are needed in the perception of the bacterial signal molecules produced by the microsymbiont, and triggering the downstream signaling processes that lead to periodical perinuclear calcium (Ca) level oscillations in and around the nuclei of root hair cells. To form and maintain this so called Ca spiking nuclear pore complex elements (LjNUP133, LjNUP85) and putative cation channel proteins (MtDMI1/LjCASTOR and LjPOLLUX) are needed. This provides a signal to activate downstream processes. A Ca-calmodulin dependent protein kinase (MtDMI3/LjCCaMK), together with its interacting partner (MtIPD3/LjCYCLOPS), is decoding the Ca signature, and

forwards it towards e.g. transcription factors (MtNSP1/LjNSP1, MtNSP2/LjNSP2, MtERN, MtNIN/LjNIN). The *LjNAP1/MtRIT* and *LjPIR1* genes are involved in the actin cytoskeleton rearrangements during the forming and progression of the tubular shaped plant specific structure, the so called infection thread (IT), that promotes the infection process of the microsymbiont. The *MtLIN/LjCERBERUS* genes are coding for an E3 ubiquitin ligase that is indispensable for IT progression from the root hairs towards the underlying cell layers of the plant root as well as needed for the subsequent development of the nodule primordia. This symbiotic signaling network also crosstalks with the plant cytokinin signaling system through a symbiosis specific cytokinin receptor (MtCRE1/LjLHK1). From the very first steps during nitrogen fixing symbiosis the plant strictly and dynamically regulates nodule numbers. In this process subsequent LRR type receptor kinases are involved (LjHAR1/MtSUNN and LjKLV).

Molecular genetics studies on nitrogen fixing symbiosis led also to the discovery of many genes involved in mycorrhiza symbiosis. Analyzing the mycorrhiza phenotype of legume mutants that lost the ability to enter in nitrogen fixing symbiosis revealed that some of the mutants were unable to form mycorrhiza symbiosis either. The genes mutated in these plants are therefore indispensable for both symbioses. These are the genes of the so called common symbiotic pathway: *MsNORK/MtDMI2/LjSYMRK*, *MtDMI1/LjCASTOR* and *LjPOLLUX*, *LjNUP133*, *LjNUP85*, *MtDMI3/LjCCaMK* and *MtIPD3/LjCYCLOPS*. Therefore it is hypothesized that the phylogenetically much younger nitrogen fixing symbiotic system was developed on the molecular grounds of the more ancient mycorrhiza symbiosis, recruiting elements from the already existing signaling pathways.

By now multiple E3 ubiquitin ligases are known to play important roles during different stages of nitrogen fixing symbiosis. One of these is *LIN*, identified in the model legume *Medicago truncatula*, and its *Lotus japonicus* ortholog *CERBERUS*. Their protein products carry an E2 interacting U-box domain, and an Armadillo and WD40 repeats that are possibly involved in protein-protein interactions. Additionally, they carry a well conserved domain on their N-terminus that is specific for them and their orthologs, the so called LIN domain. The formation process of the nitrogen fixing symbiosis stalls early on both the *lin* and *cerberus* mutant roots. Nodule primordia are emerging, but early, supposedly due to the early abortion of symbiotic infection, their development stucks in the primordium phase. The gain-of-function mutant of the Ca-calmodulin dependent protein kinase MtDMI3/LjCCaMK is known to induce spontaneous nodulation on transformed wild type roots even in the lack of symbiotic bacteria. When this autoactive copy of the Ca-calmodulin dependent protein kinase was introduced into *lin* and

cerberus mutant plants spontaneous nodules could be detected also on these transformed mutant roots. This suggests that while LIN/CERBERUS is indispensable for symbiotic infection, it is not necessary for nodule development itself. The *lin* and *cerberus* mutant plants are able to form mycorrhiza symbiosis, however the *L. japonicus cerberus* mutant roots show reduced levels of mycorrhyza colonization, which may suggest *CERBERUS* a role in mycorrhiza symbiosis also.

Our study can be divided in two main units that are supplementing each other. Using public sequence databases we collected homologous sequences to known symbiotic genes from multiple plant species. By bioinformatics tools we analyzed the changes the different gene products went through while becoming part of the nitrogen fixing symbiotic system. In the other unit we performed evolutionary and functional analysis on the E3 ubiquitin ligase LIN, to reveal the changes that made the gene product indispensable for symbiosis.

Goals

Many of the plant genes indispensable for the forming of nitrogen fixing symbiosis are known now. Some of them were identified in our laboratory and still their analysis is the main task of our research group. Besides the identification and functional characterization of new symbiotic genes, due to the advancing plant genome sequencing projects, our research also covers the evolutionary analysis of these genes and gene products by now. We would like to know what makes these plants so special to be able to form nitrogen fixing symbiosis, while others are not. It is still enigmatic how these plants acquired this ability during the evolution of land plant species.

The evolutionary and functional analysis of plant symbiotic genes can be divided in two main topics in this work. These are the followings:

I. Due to the widespread genome sequencing projects more and more full plant genome sequences are available in public biological databases. Scanning for homologs of known nitrogen fixing symbiotic genes in these databases return hits not only from nodulating species but also from species incapable of nitrogen fixing nodule formation. Comparing the protein products of these genes we would like to know how the gene products involved in nitrogen fixing symbiosis specialized during their evolution. How conserved they are, and also how general the presence of symbiotic gene homologs in non-nodulating plant genomes. Our goals in this study were:

1. To collect the putative orthologs of nitrogen fixing symbiotic genes from fully sequenced plant genomes.

2. Assessing the level of conservation of the collected sequences, and describing their evolution. Pinpointing the genes whose changes were essential to be able to fulfill their function in nitrogen fixing symbiosis.

II. One of the genes identified in our laboratory that is indispensable for nitrogen fixing symbiosis is *LIN*, coding for an E3 ubiquitin ligase. Searching for *LIN* homologs in sequence databases led to the identification of its paralog, which we assigned as *LIN2*. No experimental data was available about the paralog before, so our goal was:

1. To reveal the evolutionary history for *LIN* and *LIN2*.

2. The functional characterization of *M. truncatula LIN2*.

Methods

1. Bioinformatics methods

- Data mining in public sequence databases, assembling the fragment sequences, determining consensus
- Prediction of coding sequences (FGENESH+)
- Assessment of sequence quality (Vector NTI, AlignX), manual repair
- Multiple sequence alignments, phylogenetic tree construction (MEGA, ClustallW)
- Synteny analysis (SyMAP)

2. Laboratory methods

- Plant genomic DNA and RNA extraction, cDNA synthesis
- Polymerase chain reactions (PCR)
- Molecular cloning: plasmid constructions for promoter analysis, protein localization and mutant complementation experiments
- *Agrobacterium rhizogenes* mediated *M. truncatula* „hairy root" root transformations
- *Agrobacterium tumefaciens* mediated *Nicotiana benthamiana* transient leaf transformations
- Fluorescence confocal laser scanning- and light microscopy
- Histochemical stainings (X-Gal, GUS)
- Quantitatve real-time PCR (qRT-PCR)

Results and discussion

Due to the research done on this field in the last decade many genes indispensable for nitrogen fixing symbiosis are known by now. For our evolutionary analysis we chose 16 *M. truncatula* genes coding for proteins fulfilling diverse functions during nitrogen fixing symbiosis. Most of them are specific for nitrogen fixing symbiosis, but some elements are part of the common symbiotic pathway. Additionally we chose 12 sequences with no proven symbiotic function, to use them as controls.

- The selected symbiotic and control *M. truncatula* sequences were used as queries while searching for their homologs in the available genome sequences of ten plant species from different groups of the angiosperm phylogenetic tree. Two other legume species, that are able to form nitrogen fixing root nodule symbiosis, were picked, and other non-nodulating dicot and also monocot species were selected for further analysis. All the selected species were capable of mycorrhiza symbiosis with the one exception of *A. thaliana* that is incapable of either the mycorrhiza or the nitrogen fixing symbiosis. After the BLAST searches with the *M. truncatula* amino acid sequences a single homologous sequence from each plant genome - that showed the highest similarity to the respective reference sequence - was chosen to work with. These homologous sequences were considered as putative orthologs. Reciprocal BLAST searches were used to identify and filter out the false ortholog hits. Our results suggest that the *A. thaliana* genome is missing the *LYK3*, *NFP*, *DMI3* and *IPD3* orthologs. Moreover the tested monocot genomes are also missing the *LYK3* orthologous sequence, while the putative ortholog is generally present in dicots. We suppose that the *LYK3* orthologous copy only appeared after the evolutionary split of monocots and dicots during plant evolution. The general model plant *A. thaliana* revealed the highest number of missing orthologous sequences. It is unable to form even mycorrhiza symbiosis, most probably lost this ability during its evolution, and supposedly, in connection with this, also lost the vast majority of its symbiosis related genes. Those orthologous sequences that are still present in the *A. thaliana* genome may have an important role in other, non-symbiotic function.
- First we compared the collected most similar sequences in their length on the amino acid level to see whether some of them went through larger changes in their length during their evolution that could possibly affect their domain composition. Considerable length differences could only be detected for orthologous sequences for some symbiotic proteins.

More than 20% difference in length compared to the respective reference *M. truncatula* sequence were shown for three *A. thaliana* paralogs, and the monocot DMI2 orthologous sequences. We know from the literature that DMI2 orthologs developed by extracellular domain acquisition during plant evolution. Among these, the ones with longer sequences were able to complement both the defective mycorrhiza and nitrogen fixing symbiosis phenotype of the respective *L. japonicus* mutants, while the shorter monocot orthologous sequence could only restore mycorrhiza symbiosis in trans-complementation experiments. Moreover, the two monocot NSP2 homologs and the *Z. mays* ERN1 sequence showed 10-15% difference in length compared to the respective reference sequence, but no extra domains in these proteins could be detected by InterPro database analysis. Also it is known from the literature that the extended length *O. sativa* NSP2 ortholog with extended length was able to fully complement the *L. japonicus nsp2* mutants.

- The selected putative orthologs were compared to the respective *M. truncatula* reference sequence at amino acid level one by one, in pairwise alignments. The percent proportion of the identical amino acids among the alignment positions resulted in the ID values, while similar amino acid substitutions in similarity (*SIM*) values that were all organized in a table. The more stringent ID values were used for subsequent analysis of the variations in the gene products from the chosen plant species through the phylogenetic tree. We arranged the ID values of the given putative orthologous proteins according to the species showing an increasing phylogenetic distance from the reference *M. truncatula*, then the modifications of the gene products were analyzed by following the changes in their ID values through the species tested. There were two positions where greater changes were experienced. The first one was between the values of nodulating and non-nodulating dicots along the row of tested species, while the second one was between dicots and monocots. Large drops in the changes of the ID values could be due to generally occurring fast modifications of a given gene product during plant evolution, which can be mirrored by the differences of the ID values between the dicots and the monocots. Alternatively, in some cases specifically acting evolutionary forces could cause particular differences in certain gene products for a particular function. In our example this latter is the ability to form nitrogen fixing symbiosis, which reflected in the ID value changes between the nodulating and non-nodulating dicot species. Based on the deviations in ID values detected in our data between the different groups of species the proteins fell into three categories. The **A** (symbiotic) and **A'** (control) categories consist of gene products for which the ID value

changes are both moderate between the nodulating and non-nodulating dicot species, and between the dicots and monocots. The amino acid composition of these gene products changed slowly during the evolution of angiosperm species, so their sequences are well conserved. The sequences of these proteins did not show great changes in the nodulating species compared to other dicots, i.e. they did not need extensive sequence modifications to be able to fulfill their function in nitrogen fixing symbiosis. This suggests that for symbiotic proteins in category **A**, the orthologous counterparts taken from non-nodulating species can possess a high probability for being capable of fulfilling the symbiotic functions. The results of trans-complementation experiments available in the literature seem to be confirming this hypothesis. The sequences that show considerable fall in the ID values between nodulating and non-nodulating species, but in the same time barely changed between dicots and monocots, belong to categories **B** and **B'**, respectively. These sequences are most probably members of slow evolving proteins showing extensive amino acid changes in the nodulating species. Therefore it is highly possible that the changes accumulated in their sequences during their evolution (at least in part) were needed for them to be able to achieve their function in nitrogen fixing symbiosis. If that was true, it is rather unlikely that their orthologs from non-nodulating species could accomplish the symbiotic functions of their legume counterparts. In accordance with this, several papers in the literature reported trans-complementation studies using orthologous sequences of symbiotic proteins of category **B** from non-nodulating genomes that could only partly, or could not complement at all the respective symbiotic mutant legume plants. Gene products of categories **C** and **C'** show remarkable fall in ID values at both borders of the above mentioned groups of species, suggesting fast evolution for these sequences during plant evolution. Despite of these variations if at the same time these proteins retained their original function, they demonstrate extensive compositional flexibility. Due to this flexibility they could easily change, and even gain new functional abilities through their changes. Such a new application of a function might be what the orthologous copies of the nodulating species perform during symbiosis. Considering this, there are two possibilities with the members of category **C**: 1) either these gene products went through sequence specialization during evolution to be able to fulfill functions in nitrogen fixing symbiosis, similarly to category **B** sequences, or 2) the changes in their sequences are simply due to their fast evolution and did not result in symbiosis-specific changes in their functional capabilities. Complementation studies available in the literature done with category **C**

sequences are without exception confirming so far the latter possibility. All of their tested orthologs from non-nodulating species showed to be fully successful in rescuing the respective legume symbiotic mutants. Each of the evolutionary categories specified in this study can be found among the symbiotic (**A**, **B**, **C**) and also the control (**A'**, **B'**, **C'**) sequences. However, while the majority of the control sequences belonged to the slow evolving, well-conserved category (**A'**), the symbiotic gene products mostly fell into categories **B** or **C**. This suggests that most of the sequences that are essential in nitrogen fixing symbiosis had to acquire specific sequence changes to be able to accomplish their symbiotic function, or alternatively, fast evolving sequences were preferentially recruited.

We performed a more detailed phylogenetic study and functional analysis on the members of the *LIN* gene family. The *M. truncatula LIN* gene codes for a protein indispensable for nitrogen fixing symbiosis. Searching for *LIN* homologs in sequence databases led to the identification of its paralogous counterpart in *M. truncatula* that was designated as *LIN2*. The LIN and LIN2 proteins are very similar in domain composition: both consist of a U-box and an Armadillo domain together with WD40 repeats at their C-terminus. Both are putative E3 ubiquitin ligases.

- The available LIN and LIN2 sequences were collected from representatives of the land plant species with sequenced genomes and a detailed analysis was performed with them. In the dicot species tested generally one *LIN* and one *LIN2* ortholog was identified. Beyond the sequence similarities the prepared Neighbor-Joining phylogenetic tree consisting of the available LIN homologs and synteny data from comparison of the chromosomes carrying the sequences confirmed the ortholog/paralog relationship between the particular gene products. The tested monocots carried only one *LIN* homolog counterpart, which proved to be orthologous with the *LIN2* genes according to the phylogram and the synteny analysis as well. *LIN* and *LIN2* orthologs can also be found in the most basal clade of angiosperms, in the *Aquilegia caerulea* (Ranunculales) genome, as well as among a group that diverged much earlier than the monocot-dicot split, the lycophytes, in the *Selaginella moellendorffii* genome. This implied that *LIN* and *LIN2* had to be present in the common ancestor of higher plants (Tracheophyta). Also, it suggests that either some monocot lineages or already the common ancestor of all monocot species lost the *LIN* orthologous copy. However, more information from fully sequenced genomes are needed from distinct groups of the monocot phylogenetic tree to resolve this.

We used orthologous copies of the *LIN* gene from non-nodulating species to analyze the functional aspects of the evolutionary changes in the symbiotic *LIN* gene.

- We cloned the *LIN* ortholog from the non-nodulating dicot *Vitis vinifera*, and the fern *Adiantum capillus-veneris*. Both showed highly conserved domain composition compared to the *M. truncatula* protein. Providing appropriate expression both orthologous copies were able to rescue the nitrogen fixing symbiosis-defective *lin* mutant plants in trans-complementation experiments. This indicated that the sequence variations found in LIN orthologs did not affect the function of the symbiotic LIN copies that is needed for nitrogen fixing nodule formation. In addition, this function appeared early during plant evolution, way before the first appearance of nitrogen fixing symbiosis, and so it was recruited for this process.

No experimental data was available about the *LIN2* gene (and gene product) earlier, so we performed its analysis.

- The activity of a 2156 bp DNA fragment from the 5' UTR promoter region of the *M. truncatula LIN2* gene was tested in fusion with a reporter gene using hairy root transformation. This promoter showed high activity in lateral root primordia starting from the very early cell divisions until the fully developed state. Similarly, the promoter showed to be active through the whole process of nodule development. At later steps this activity became restricted to the meristematic regions both for the developed lateral root and the nodule as well. Based on these results *LIN2* potentially could have a role during cell division or cell cycle control. Moreover the *LIN2* promoter was active during symbiotic infection. It was active in the cells surrounding the infection thread, but always preceding its path until the bacteria reached the nodule primordium. This may suggest a role for *LIN2* upon microsymbiont infection (also). The observed *LIN2* promoter activity is highly similar to the promoter activities of the genes *M. truncatula LIN* and its *L. japonicus* ortholog *CERBERUS* described upon nodulation. Quantitative real-time PCR analysis on *LIN2* expression showed extremely low transcript levels that were induced upon symbiotic bacterial inoculation, but even the induced transcript levels remained way below the *LIN* mRNA levels in the same samples. This suggests strict regulation on *LIN2* gene expression.
- In a heterolog system, we succesfully expressed the fluorescent tagged *M. truncatula* LIN2 protein in *N. benthamiana* leaves. We detected the gene product in the cytoplasm, the ER and the nucleus. The molecular mass of the *M. truncatula* LIN2 protein is 149 kDa. Molecules of this size only pass through the nuclear pore complexes by active transport

mechanisms, but we could not detect any nuclear localization signal on its amino acid sequence. It is possible that similarly to β-catenin LIN2 passes the nuclear pores through its Armadillo domain, even simultaneously binding to other molecules and carrying them as cargo.

- Screening the *M. truncatula* insertional mutant database we found several lines carrying a Tnt1 retrotransposon insertion in the *LIN2* sequence. We chose two of them for detailed analysis. The *lin2-1* and *lin2-2* mutant plants did not show any kind of general developmental anomalies or defects during nitrogen fixing symbiosis. This suggests that the *LIN2* gene itself is not indispensable for the establishment of functional nitrogen fixing symbiosis. Considering that the expression pattern and also the domain composition of LIN and LIN2 proteins are very similar, it is possible that LIN may be capable of fulfilling the function of LIN2, so conceals the phenotypic consequences of disrupting the LIN2 gene function in *lin2* mutant plants. This, at least in part, may be possible on the other way around too. In *lin* mutant plants where only *LIN* is defective, *LIN2* is present in an unaffected form in the *M. truncatula* genome, *LIN2* is not able to rescue the *lin* mutation. However it is possible that *LIN* and *LIN2* accomplish some functions redundantly. These functions are not lost completely in the *lin* mutants either. For their analysis *lin lin2* double mutants are needed, and we already started the plant crosses to create these mutant lines. Such a redundant function for the paralog gene pair may be during mycorrhiza symbiosis. Recently it was shown that the *LIN* ortolog mutant *L. japonicus cerberus* plants exhibit reduced levels of mycorrhyza colonization. It is posible that the mycorrhiza symbiosis phenotype of the *cerberus* mutant is due to the reduction of gene dosage. By disrupting the *LIN2* ortholog gene function too, the phenotype may become more severe, the plants would loose completely their ability to form mycorrhiza symbiosis.

Our research can help to understand how the genes involved in nitrogen fixing symbiosis developed during the course of plant evolution. The process by which a group of plant species earned the ability to form a symbiosis that enabled them to use the atmospheric elemental dinitrogen pool to satisfy their nitrogen nutrient needs crucial for their development, and this way deliberating themselves from the limited organic nitrogen stock in the soil.

List of publications

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PUBLICATIONS

Publications the PhD defense was based on:

Bozsoki Z, Cheng J, Feng F, Gysel K, Vinther M, Andersen KR, Oldroyd G, Blaise M, Radutoiu S, Stougaard J (2017) **Receptor-mediated chitin perception in legume roots is functionally separable from Nod factor perception.** Proc Natl Acad Sci 201706795
(IF: 9.661 - in 2016)

O'Rourke JA, Yang SS, Miller SS, Bucciarelli B, Liu J, Rydeen A, **Bozsoki Z**, Uhde-Stone C, Tu ZJ, Allan D, et al (2013) **An RNA-Seq Transcriptome Analysis of Pi Deficient White Lupin Reveals Novel Insights Into Phosphorus Acclimation in Plants.** Plant Physiol **161**: 705–724
(IF: 6.535)

Other publication:

Pénzes Zs, Melika G, **Bozsóki Z**, Bihari P, Mikó I, Tavakoli M, Pujade-Villar J, Fehér B, Fülöp D, Szabó K, et al (2009) **Systematic re-appraisal of the gall-usurping wasp genus Synophrus Hartig, 1843 (Hymenoptera: Cynipidae: Synergini).** Syst Entomol **34**: 688–711
(IF: 2.467)

Total **IF: 18.663**

Other book chapter:

Melika G, Pénzes Zs, Mikó I, Bihari P, Ács Z, Somogyi K, **Bozsóki Z**, Szabó K, Bechtold M, Fári K, Fehér B, Fülöp D, Csóka Gy, Stone GN (2007). **Oak gall wasps of the Carpathian Basin.** In: Forró L (editor) Forming of the fauna of the Carpathian Basin: Forming of the fauna of the Carpathian Basin and its zoological values. 399 p. Budapest: Hungarian Natural History Museum, 2007. pp. 165-174.
(ISBN:978-963-7093-99-9)

ACADEMIC MATERIALS RELATED TO THE SUBJECT OF THE THESIS

Conference talks:

Bozsóki Z, Kiss E, Oláh B, Endre G- **Homologs of *Medicago truncatula* symbiotic proteins and the evolution of nitrogen fixing root nodule symbiosis**
First Legume Society Conference, May 2013, Novi Sad, Serbia

Bozsóki Z, Kiss E, Endre G- **Relatives of symbiotic genes among plants**
Hungarian Society for Plant Biology - Lecture Series of Young Plant Scientists, January 2013, Szeged, Hungary

Bozsóki Z, Kiss E, Oláh B, Endre G- **Functional and structural studies on homologs of *Medicago truncatula* symbiotic genes**
"Genetics Workshops in Hungary" VIII. Mini Conference, September 2009, Szeged, Hungary

Poster presentations:

Homologs of *Medicago truncatula* symbiotic proteins in plants
Hungarian Molecular Life Sciences conference, April 2013, Siófok, Hungary

How could the *LIN* gene and its function evolve for symbiosis?
10th European Nitrogen Fixation Conference, September 2012, Munich, Germany

Evolutionary studies on symbiotic genes
IX. Hungarian Genetics Congress / XVI. Cell and Developmental Biology Conference, March 2011, Siófok, Hungary

Homologs of *Medicago truncatula* symbiotic genes in taxonomically distant species from nodulating to non-symbiotic plants
9th European Nitrogen Fixation Conference, September 2010, Geneva, Switzerland

Homologs of *Medicago truncatula* symbiotic genes in taxonomically distant species from nodulating to non-symbiotic plants
European Plant Science Organisation 5th EPSO Conference "Plants for Life", August 2010, Olos, Finland

Studies on the homologous counterparts of *Medicago truncatula* symbiotic genes in nodulating and non-nodulating plant species
8th International Symposium on "New development in green gene technology", September 2009, Szeged, Hungary

Structural analyses on Medicago truncatula symbiotic gene homologs in nodulating and non-nodulating plant species
Model Legume Congress, June 2009, Asilomar, CA, USA

Functional and structural studies on homologs of *Medicago truncatula* symbiotic genes from nodulating and non nodulating plant species
VIII. Hungarian Genetics Congress, April 2009, Nyíregyháza, Hungary

OTHER ACADEMIC MATERIALS
Conference talk:

Bozsóki Z, Cheng J, Blaise M, Stougaard J, Radutoiu S- **Symbiosis or defense: The molecular mechanism involving LysM receptors of the model legume *Lotus japonicus***
12th European Nitrogen Fixation Conference, August 2016, Budapest, Hungary (lightning talk)

Poster presentation:

A *Lotus japonicus* LysM kinase is a chitin receptor
2nd International Molecular Mycorrhiza Meeting, September 2015, Cambridge, UK