



Legume Root Symbioses: Natural History and Prospects for Improvement

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Summary: Legumes develop different mutually beneficial microbial-root symbioses such as arbuscular mycorrhiza (AM), rhizobium-legume symbiosis (RLS) and epiphytic or endophytic associations with plant growth-promoting bacteria (PGPB) which are distinguished in level of integration of the partners. Evidences of the role of AM as ancestral form of symbiosis which might be a source of the legume pre-adaptation to form some RLS are demonstrated. The RLS is supposed to evolve for a few times in ancient legumes in parallel ways based on the universal organization and regulatory mechanisms of the plant genetic material. Associations of plant roots with PGPB probably are the vestige of the early stages of evolution in morphologically differentiated RLS. Also, it is quite possible that “first” rhizobia have originated from bacterial endosymbionts of AM fungi; then AM fungi might operate as effective vectors for introducing bacteria into the plants. Thus, the legume root symbioses may be considered as a single “evolutionary plant-microbial continuum”. The acquired knowledge about evolution of plant-microbe symbioses would contribute to the creation of new commercial varieties of plants with the use of both bio-engineered methods and traditional plant breeding. An original conception of legume breeding to improve their symbiotic effectiveness is proposed.

Key words: legumes, rhizobia, Rhizobium-Legume symbiosis (RLS), nitrogen-fixing nodule, arbuscular mycorrhiza (AM), pre-adaptations, symbiotic specificity, mutualism, reciprocal altruism, symbiotic effectiveness, plant breeding

Introduction

Legumes (*Leguminosae*, syn. *Fabaceae*) are not only a key component of modern technologies for crop production, but also are unique models for different spheres of biology. They develop two highly integrated mutually beneficial endosymbioses, namely nitrogen-fixing root nodules with rhizobia bacteria (Rhizobium-Legume Symbiosis, RLS) and Arbuscular Mycorrhiza (AM) with Glomeromycotan fungi. Both symbioses are characterized by formation of special complex symbiotic compartments.

An ability to form nitrogen-fixing nodules with rhizobia is restricted to legumes; *Parasponia*

(*Ulmaceae*) is a single exception (Allen & Allen 1981). The majority of bacteria which can form RLS belong to the α -proteobacteria (e.g., *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, *Sinorhizobium*) (Sprent, 2001). Recently some β -proteobacteria, close to *Burkholderia*, *Cupriavidus*, *Pseudomonas* and *Ralstonia*, and even some γ -proteobacteria, able to form nitrogen-fixing nodules with legumes, have been discovered (Sprent 2007, Balachandar et al. 2007). The RLS is the most morphologically distinct symbiosis. The special symbiotic structure, the root nodule, provides conditions for the bacteria to fix atmospheric nitrogen. Bacteria inside the nodule cells are enclosed in a plant-derived membrane and form specialized symbiotic compartments – symbiosomes. Another feature of RLS is a high specificity (Brewin 2004, Dilworth et al. 2008).

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The AM is the most widespread terrestrial symbiosis. About 80-90% of land plants are able to form AM, including angiosperms, gymnosperms and some lower plants (Smith & Read 2008). All the fungal genera, which are able to form AM (*Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora*, *Paraglomus* and *Archaeospora*) belong to a single phylum *Glomeromycota* (Schüßler et al. 2001). A special intracellular compartment of AM is arbuscule, which is highly branched fungal hypha surrounded by membrane of plant origin similar to symbiosome of RLS. By contrast to RLS symbiosome, arbuscule remains bounded with inner and outer fungal mycelium (Genre & Bonfante 2005). Specificity of AM symbiosis is relatively low (Smith & Read 2008).

Legumes also interact with a variety of plant growth-promoting bacteria (PGPB) which are representatives of genera *Agrobacterium*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Erwinia*, *Flavobacterium*, *Paenibacillus*, *Pseudomonas* or *Streptomyces*. Despite the fact that associations of plants with these bacteria are known to be not only epiphytic (rhizospheric), but also endophytic ones, specialized symbiotic structures are not formed. Specificity of these interactions is not high (Bloemberg et al. 2001, Schulz et al. 2006, Bakker et al. 2007).

The RLS, AM and associations with PGPB are beneficial for the host plant and its environment supplying plant with nutrients (predominately nitrogen and phosphorus), protecting it from pathogens and stresses and improving soil biodiversity and fertility. In return, the microsymbiont receives the products of photosynthesis and an ecological niche (Bloemberg & Lugtenberg 2001, Schulz et al. 2006, Bakker et al. 2007, Dilworth et al. 2008, Provorov et al. 2008, Smith & Read 2008, Provorov et al. 2010). Exploitation of these systems in agriculture allows reducing the cost of agrochemicals and decreasing chemical burden on the environment that complies with the principles of sustainable agriculture. Unfortunately, the potential of the legume symbiotic systems is not widely used in crop production. Moreover, many modern legume varieties, selection of which was carried out against a background of high doses of agrochemicals, have lost the ability to interact effectively with microorganisms (Provorov & Tikhonovich 2003). Thus, one of the most important problems of modern crop production is improving the efficiency of legume symbiotic system.

In the last decade, thanks to modern advances in biology, substantial progress in understanding the mechanisms of beneficial plant-microbe

interactions has been made. It is established that genetic-metabolic integration of plants with different beneficial microbes involves similar mechanisms, which seem to have a common evolutionary origin. The new knowledge may be a fundamental basis for development of scientific approaches for the use of the legume root symbioses in sustainable agriculture. The author's own innovative conception of legume breeding to improve their symbiotic effectiveness is proposed.

The AM Evolution History

The AM fungi are probably the most ancient beneficial symbionts of land plants

The first palaeobotanical data suppose that AM symbiosis originated more than 400 millions years ago, in Silurian or Devonian, when the first land plants also appeared (Remy et al. 1994). With the use of recent methods of molecular phylogeny based on 18S rDNA nucleotide sequences polymorphism analysis, the AM fungi were shown might originate even before the land plants, in Ordovician, 500 million years ago (Redecker 2000), or even earlier, between 1,400 and 1,200 million years ago (Schüßler 2002).

The AM fungi are a monophyletic group (Schüßler et al. 2001), indicating they have originated from a common ancestor. Along with obligatory biotrophic AM fungi, the phylum *Glomeromycota* includes the genus *Geosiphon*, represented by a single species of *G. pyriformis*. This species is free-living and able to form symbioses with the photosynthetic and also nitrogen-fixing cyanobacteria *Nostoc*. The symbiosis could reflect an early evolutionary stage of an AM-like symbiosis, when lower plants had not yet colonised land and cyanobacteria would have been prominent under the prevailing environmental conditions (Schüßler 2002). Recent finding of genes of cyanobacterial origin in nuclear genomes of plants, both uni- or multicellular (Deusch et al. 2008), confirms the hypothesis of symbiotic beginning of plastids in the plant cell (Margulis 1998). Hence, metabolic interactions developed in ancient *Geosiphon-Nostoc*-like associations could be inherited by eukaryotic plant cell through cyanobacterial symbiont. This could form the basis of the relationship of the AM fungi and first plants. Therefore, it is possible that the *Geosiphon* symbiosis in evolutionary time have preceded AM, as supposed by Schüßler (2002) and Parniske (2008), but not those of marine ancestors of terrestrial plants with unicellular or possibly parasitic fungi, as suggested earlier

(Schüßler et al. 2001). Thus, ancient AM fungi and plants (algae) already formed symbioses in the aquatic environment and colonized the land together from the beginning (Schüßler 2002, Parniske 2008).

In this context it is very interesting that recent molecular analyses date back the origin of AM fungi (Heckman et al. 2001). At the same time, reappraisal of the origin of the first land plants puts the timing also much earlier, at around 900 million years ago. However, there is still some uncertainty because of the absence of fossils to calibrate the molecular data (Heckman et al. 2001, Smith & Read 2008).

The AM fungi are the constant satellites of land plants during their evolution

Importance of AM for the most ancient plants is evident from the fact that they lacked conventional root system but had rhizomes and rhizoids to anchor to substrates and not to uptake nutrients effectively. Thus, AM was considered to play a decisive role in plants achieving a terrestrial existence (Remy et al. 1994, Redecker 2000, Brundrett 2002). Obviously true roots co-evolved with AM-fungal partners and the anatomy of mycotrophic plants tends to confirm this (Brundrett 2002). Since then, the genetic system controlling the development of AM, apparently, largely overlapped with the genetic system of plants controlling root development and mineral nutrition.

Since fossil land plants obviously were obligate biotrophs, maintaining symbiotic fungi in roots was a more ancient function in comparison to the independent assimilation of nutrients from soil (Provorov & Vorobyov 2010). One of the novel strategies, acquired in the course of evolution, is the rejection of symbiotrophy and changing to autotrophy. An extreme case of this strategy is a complete rejection of symbiotrophy in, for instance, *Brassicaceae* (formerly *Cruciferae*). In many plant families, species have been recorded as occurring in both mycorrhizal and non-mycorrhizal states and members of some plant families typically form mycorrhizas of types other than AM or, indeed, more than one type of mycorrhiza. The reasons for failure of a potentially mycorrhizal species to become colonized are many, in particular environmental conditions (Smith & Read 2008). Still, most modern plants are mixotrophs capable of both autonomous and symbiotroph nutrition, forming different types of symbioses such as AM, ectomycorrhizas and nitrogen-fixing nodules (Smith & Read 2008, Provorov & Vorobyov

2010). The opposite strategy, that is, a complete denial of photosynthesis and the formation of myco-heterotrophic associations exists in some orchids, dicotyledons, ferns, bryophytes and liverworts (Bruns & Bidartondo 2002, Smith & Read 2008).

On the other side, ancient AM fungi were probably not obligatory biotrophs, gradually losing their capacity for autonomic existence. The suggestion is consistent with that the free-living *G. pyriformis* is the closest relative of AM fungi (Schüßler 2002). A recent study demonstrates that some contemporary AM fungi are capable of completing their life cycles in the absence of plant roots. Also, some substances, possibly enzymes or signal molecules, necessary to form fungal spores, can be provided by bacteria *Paenibacillus validus* (Hildebrandt et al. 2006). This discovery should shed light on the bottom of obligatory nature of AM fungi.

Since legumes originated long after AM, about 60 million years ago (Lavin et al. 2005), it may be assumed that all of them have the potential to produce this type of symbiosis. *Lupinus* is the only known genus where this ability had apparently been lost (Sprent 2007, Sprent & James 2007, Dilworth et al. 2008).

The RLS Origins

Parallel evolution of different RLS

In contrast to AM fungi, many other known plant microsymbionts including nodule bacteria are polyphyletic in their origins (Moncalvo et al. 2000, Sprent 2001, Sprent 2007, Balachandar et al. 2007). Consequently, plant-microbe symbioses with participation of those microorganisms evolved in parallel ways and most probably later than AM. Parallel evolution and convergence play an important role in the evolution, as plants possess universal gene systems for the control of basic functions required in symbiosis, including the maintenance of interior homeostasis, novel tissue and cellular structures (symbiotic compartments) and the utilization of nutrients provided by the partner organisms (Gualtieri & Bisseling 2000). Based on the universal organization and regulatory mechanisms of the plant genetic material (Cronk et al. 2002), the gene systems could have been rearranged in similar manners during co-evolution with different microbes including rhizobia.

In turn, different groups of plant-interacting microorganisms gained similar mechanisms to adapt to the existence in direct contact with various plant tissues using the older developmental

programs, for example, those being used for the development of AM (Provorov & Vorobyov 2010).

According to the phylogeny of flowering plants developed on the basis of plastid gene *rbcL* structure, differing from earlier systems based on plant morphology, all the plants able to form nitrogen-fixing nodules with rhizobia (RLS) as well as with actinobacteria *Frankia* sp. (actinorrhiza – symbiosis, close to RLS evolutionarily and functionally) belong to a monophyletic Eurosid I (former Rosid I) clade (Sprent 2007). A common ancestor of Eurosid I, obviously, could not form nodules with rhizobia but had some pre-adaptations to form nitrogen-fixing symbioses which developed in a parallel manner in several plant families evolving later. Within the four orders of the clade where these root-nodule symbioses occur, its distribution is scattered (Markmann & Parniske 2009). It could suggest that they either evolved several times independently or that the ability to form nodules arose from their common ancestor, later lost in some representatives of clade.

The two independent developmental programs in RLS formation

Nodule morphogenesis analysis allows subdividing the legume pre-adaptations to form RLS into two groups (Provorov et al. 2010). The first concerns the capacity for being colonized by AM fungi and of controlling the mycobiont once inside host tissues. The second type of the pre-adaptations includes capacities which determine the formation of special cellular, tissue and organ structures characteristic of nitrogen-fixing nodules (symbiotic organs and compartments).

With respect to the first group of the pre-adaptations, it has been revealed in some legume genera, particularly *Lotus*, *Medicago*, and *Pisum*, that similar molecular and cellular mechanisms are involved in infection process during both AM and RLS. Similarities are revealed starting with the stage of early recognition. For example, rhizobia and AM fungi produce low-weight lipochito-oligosaccharide signal molecules (Nod and Myc factors, respectively) of resemble chemical structures (D'Haese & Holsters 2002, Maillet et al. 2011). Both Nod and Myc factors induce expression of symbiotically specific plant gene *MiENOD11* (Kosuta et al. 2005) and elevation of Ca^{2+} concentration (Navazio et al. 2007) in root cells as well as root branching (Maillet et al., 2011).

Most of rhizobia start root colonization via infection threads extended through root hairs

(Sprent 2001). It was demonstrated that similar tubular apoplastic compartments are developed by plant to promote penetration both rhizobia and AM fungi, which are the result of formation of cytoskeletal pre-infection threads (Timmers et al. 2007, Fournier et al. 2008) or pre-penetration apparatus (Genre et al. 2005, Genre et al. 2008, Genre et al. 2009), consequently, and cytoplasmic membrane invagination (Fournier et al. 2008, Genre et al. 2009). Moreover, rhizobia and AM fungi are separated from cytoplasm with plant-derived membrane at the both stage: colonization and symbiotic compartment formation (Brewin 2004, Parniske 2008, Smith & Read 2008, Genre et al. 2009).

There are common regulatory plant genes (*Sym* genes) controlling development of AM and nitrogen-fixing nodules (Borisov et al. 2007, Parniske 2008, Provorov et al. 2010). Numerous plant housekeeping genes, nodulins and mycorrhizins, are co-induced during the both symbioses (Kistner et al. 2005, Küster et al. 2007). These are termed “symbiosins” (Küster et al. 2007). Sequencing of a number of common *Sym*-genes revealed that they encode key components common to both symbioses signaling cascade (Common Symbiotic Pathway, CSP) (Banba et al. 2008, Parniske 2008, Provorov et al. 2010, Shtark et al. 2010b). All the known CSP genes except one, encoding Symbiosis Receptor-Like Kinase (SYMRLK), are structurally conserved across dicot and monocot angiosperms (Markmann & Parniske 2009). In contrast, SYMRK has at least three distinct structural versions. All of these support AM symbiosis with fungi, while only the version with longest receptor domain is sufficient for functional root-nodule symbioses in both RLS and actinorrhiza (Markmann et al. 2008).

These commonalities suggest that AM might be a source of pre-adaptations for many other types of interactions responsible for the host regulatory reactions (Sprent & James 2007, Parniske 2008, Markmann & Parniske 2009, Provorov et al. 2010). These in turn may be controlled by plant genes recruited from AM programmes into those for nodule development.

Regarding the second type of these pre-adaptations, it is possible to propose that the ancestor of Eurosid I could form outgrowths (nodule-like structures) which could obey features of depository and systems of metabolic exchange with over-ground plant organs, which in consequence were used for integration of mechanisms of nitrogen fixation with energy and nitrogen-carbon balance of plant. Spontaneous formation of pseudo-nodules has been revealed

in a few present-day wild-growing legumes (Bonnier 1961) and some alfalfa and clover lines (Truchet et al. 1989). Several genes were revealed in the lines controlling the pseudo-nodules formation either under sterile conditions or in the presence of avirulent, Nod factor defective rhizobial mutants (Caetano-Anolles et al. 1992). Occurrence of nodule formation, induced by some rhizobia non-secreting host-specific Nod factors, may be considered an evidence of independence of this group of pre-adaptation on the first colonization (Deakin & Broughton 2009).

Thus, most probably, not all RLS developed on the base of the both groups of pre-adaptations. For example, successful root colonization of different plants by AM fungi is impossible without pre-penetration apparatus formation (Genre et al. 2008). Nevertheless, some legumes including *Lupinus* do not use the colonization programme *via* infection threads, but colonize root in intercellular manner. Moreover, *Lupinus* does not form AM at all (Sprent 2001, Sprent & James 2007, Dilworth et al. 2008). At the same time, several initial legumes develop infection threads but cannot form nodules (Sprent 2001). Obviously, the joint system of nitrogen-fixing nodules development, based on coordinated infection and nodule organogenesis programs, is an evolutionary achievement of most advanced legumes. Another achievement of advanced RLS is symbiosome, which is highly differentiated and autonomic structure in contrast to the other beneficial plant-microbial symbioses (Brewin 2004, Limpens et al. 2005), and is literally a tiny factory of nitrogen fixation for the host.

Evolution of nodule organogenesis and metabolism

It is possible to identify at least two major types of root nodules formed in legumes by rhizobia, namely those with indeterminate and with determinate nodule meristem type (Sprent 2007, Sprent & James 2007, Dilworth et al. 2008). The first nodule type is formed due to initiation of cortical divisions in the inner cortex, whereas the second one is initiated in the outer cortex. The indeterminate nodules are usually elongated, because of retaining of the nodule meristems activity during a long time. This activity results in a spatial gradient of cellular differentiation observed in the nodules and several anatomic (histological) zones can be distinguished readily. In contrast, the determinate nodules are usually spherical. At the latest stages they grow due to the extension of the cells completing their mitotic

cycle and predominantly have the time, not the spatial, gradient of cellular differentiation.

In spite of a simplified tissue structure, the determinate nodules possess an advanced biochemical strategy for assimilation of fixed nitrogen (Ohyama et al. 2008). In the central nodule zone, the rhizobia-infected plant cells do not excrete the primary assimilation products such as glutamine and asparagines, but use them as donors of N atoms for synthesis of uric acid. This acid is transported into the adjacent non-infected cells where the ureids such as allantoin and allantoic acid are synthesized and used as the transport form of N into shoots. This “ureide” assimilatory system has two great advantages over the “amide” system of indeterminate nodules, in which glutamine and asparagine are transported into the shoots: (a) N:C ratio in ureids is higher than in the amides and amino acids (1:1 as compared to 1:2 or 1:3); (b) synthesis of ureids provide the rapid removal of fixed N from the rhizobia infected plant cells avoiding the repression of the activity of nitrogenase, a key nitrogen fixation enzyme. The latter advantage allows the symbiotic system to provide highly effective symbiotrophic N nutrition without a deep differentiation of rhizobia into non-viable bacteroids. The relatively low specific nitrogenase activity of low differentiated bacteroids (typical for soybean or pole bean) is essential for plant N supply.

Evolution of symbiotic specificity

The conception of symbiotic specificity supposes that each host possesses a certain set of microsymbionts whereas each microsymbiont possesses a certain set of hosts. Usually, symbiotic specificity is associated with reciprocal perception of the partners as well as cross-regulation of their genes as a result of congruence of their signal and receptor molecules (Ovtsyna & Stachelin 2005).

The change from AM to the morphologically more pronounced symbiosis, RLS, was associated with increased levels of specificity of the interactions that resulted in a narrowing range of partners capable of forming symbiosis. Among RLSs, the most evolutionarily advanced symbioses formed by the galeoid legumes also exhibit the strongest specificity (Sprent 2001). The high specificity of RLS is due to the synthesis of a specific (Nod factor), successful perception of which leads to CSP starting in the plant roots and, consequently, the formation of nodule (Parniske 2008, Provorov et al. 2010, Shtark et al. 2010b). The decoration of Nod factor core

with different substitutes is often unique to a specific rhizobial strain (D'Haese & Holsters 2002, Ovtysna & Staehelin 2005).

In contrast, the specificity of AM is relatively low, since in the formation of this symbiosis rather universal and conserved regulatory systems of plant are involved (Markmann & Parniske 2009). Moreover, AM fungi release a signal molecule, Myc factor, structurally very close to the Nod factor, but much less complex (Maillett et al. 2011) requiring a less specific plant receptor.

How did the nodule bacteria evolve?

The nodule bacteria are polyphyletic group (Sprent 2001, Balachandar et al. 2007) and vary enormously in the overall genome organization, location of symbiotic genes as well as in their molecular organization and regulation (Spaank et al. 1998, MacLean et al. 2007).

The most striking example of adaptation of various taxonomically unrelated species of nodule bacteria to the common hosting program originated from AM is the acquisition of the ability to synthesize Nod-factor. This molecule is not typical for bacteria chitin-like compound. A question rises, where the bacteria had such property? It is assumed that the "first" rhizobia used a strategy of "molecular mimicry". Since different Gram-negative bacteria have homologues of many proteins involved in Nod factor synthesis, the unique system of its synthesis in rhizobia has obviously developed as a result of ancestral bacterial genes localization and regulation changing (Provorov 1998). Subsequently the ability to synthesize Nod-factor spread among soil bacteria through horizontal gene transfer (Terefework et al. 2000, Suominen et al. 2001).

Some nodule bacteria, not using the classic model of root hair invasion through root hairs and colonizing root in intercellular manner, also do not release Nod factors. These rhizobia probably share common strategies with pathogenic bacteria using protein secretion systems derived from flagella (type III secretion systems, T3SSs) or conjugation apparatuses (type IV secretion systems, T4SSs). Many different bacterial pathogens are known to inject special proteins called effectors into plants. The T3SSs and T4SSs conduct effectors from the bacterial cytoplasm to the plant's one. Genomics studies of plant-interacting bacteria have revealed similar secretion systems in some rhizobia as well. Most rhizobial effectors identified so far have homologues in pathogenic bacteria, although some, such as nodulation outer proteins NopL

and NopP, seem to be specific to few rhizobia (Deakin & Broughton 2009).

Conventionally, the rhizobia origins were considered mainly to have come from relative plant pathogenic bacteria or PGPB. For instance, some rhizobia are genetically related to a tumor-generating plant pathogen *Agrobacterium* (Sprent 2001). This is why rhizobia or agrobacteria recombinants can be produced in laboratory or be found in natural environment, which manifest phenotypes of each other in relation to their host plant (Provorov & Vorobyov 2010). At the same time slow-growing rhizobia might originate from *Azospirillum*-like strains, since there is a visible taxonomic relatedness between *Azospirillum* and *Bradyrhizobium* genera, and in the genomes of some *Azospirillum* strains the close homologues to some rhizobial *nod* and *fix* genes were found (Provorov & Vorobyov 2010). This suggestion is consistent with numerous data demonstrating that rhizobia can effectively operate as the beneficial rhizospheric or as endophytic symbionts in "non-host" (legume and non-legume) plants (Sessitsch et al. 2002, Hossain and Mårtensson 2008). Although the mechanisms of beneficial effects on plants may be non-typical for rhizobia (involving not nitrogen fixation but rather synthesis of hormones, defense of plants from pathogens, solubilization of soil phosphates), these endo- and epiphytic associations of rhizobia with plants may be considered the vestige of the early stages of evolution in morphologically differentiated nodular symbioses.

According to alternative hypothesis concerning the rhizobia origins, the potential precursors of the "first" rhizobia are bacterial satellites of AM fungi (Provorov et al. 2010, Shtark et al. 2010b), among which are the intracellular or surface-associated bacteria, including nitrogen-fixing ones (Barea et al. 2005, Artursson et al. 2006). So, it is the AM fungi that could serve as an effective means of delivering the bacteria into the plant tissue. It is consistent with presumption of Hirsch et al. (2001) that the "first" rhizobia have possibly got the genes necessary for synthesis of chitin-like signals directly from fungi. This hypothesis is also supported by the fact that the taxonomic relatedness of bacteria forming RLS with a relatively ancient subfamily of legumes *Mimosoideae* and some bacterial endosymbiont of AM fungi has been revealed, where both are *Burkholderia*-like ones (Minerdi et al. 2002, Balachandar et al. 2007). It is important to note that colonization of roots by mycorrhizal fungi is normally not associated with induction of bacteria-induced diseases (Smith & Read 2008).

Therefore, a sufficient selectivity probably occurs with respect to the bacterial satellites of fungi which are allowed by plants to come inside the roots. This selectivity may represent an important factor for maintenance of the multipartite symbiotic communities; otherwise they would have been eliminated by natural selection. In order to understand the role of these bacteria in the evolution of RLS, further comparative molecular genetic studies of various species of rhizobia and bacterial satellites of AM fungi is required.

Mutualistic And Parasitic Plant-Microbe Interactions: Commonalities And Principal Differences

As described before, a lot of similar processes occur in plant cell during both AM and nitrogen-fixing nodules development, which are regulated by the common symbiotic genes. Some of these features are similar to those involved in interactions of plants with biotrophic pathogens. In turn, beneficial bacteria (rhizobia, PGPB) and deleterious ones use common mechanisms for a dialogue with the plant and the invasion of its tissues. For example, bacterial protein secreting systems (see before) or universal mechanisms of bacterial attachment to eukaryotic cell (Sidorenko 2001; Jones et al. 2007; Downie 2010).

The Nod factor receptors are closely related to two receptors identified in the non-nodulating plants *Arabidopsis thaliana* and rice (*Oryza sativa* L.), which are required for the defense-related perception of chitin oligomers, fungal cell wall fragments (Wan et al. 2008).

Cytoplasmic aggregation (CA), a cytoskeleton (microtubules and microfilaments) driven accumulation of organelles including the plant nucleus, is also observed at the site of penetration of both compatible beneficial and pathogen fungi, as well as rhizobia. This may represent the cell's first defence strategy and anticipate hypersensitive and systemic responses. However, only during compatible interactions with rhizobia or AM fungi, CA leads to a pre-infection thread or a pre-penetration apparatus formation (Takemoto & Hardham 2004, Genre et al. 2009). It was shown in the model legume *Medicago truncatula* Gaertn. that CA is controlled by *MtDMI3* gene (Genre et al. 2009), which is component of CSP (see before in the text) and encodes Ca^{2+} /calmodulin dependent kinase (Lévy et al. 2004). In contrast, there are no genes homologous to *MtDMI3* in the genome of both non-nodulating and mycorrhizal plant

A. thaliana what suggest that the gene may be lost during the evolution (Zhu et al. 2005). At the same time, CA in interaction of *A. thaliana* with biotrophic pathogens is not accompanied by focusing of microtubules, but microfilaments only, at the infection site. No difference was evident between the responses of epidermal cells in the non-host, incompatible and compatible interactions (Takemoto et al. 2003). So, the CA involves probably different cell mechanisms. It is interesting that *MtDMI3* is also involved in the plant interaction with PGPB regulating intercellular bacterial colonization and expression of some plant housekeeping genes known earlier as mycorrhizins (Sanchez et al. 2005). It is the objective of future research to determine the role of the Ca^{2+} /calmodulin dependent kinase in the evolution of plant response to invasion by different microbes including pathogens.

Biotrophic pathogens and AM fungi form resembling attachment structures, appressoria -for AM is increasingly using the term "hyphopodia" (Genre & Bonfante 2007) - and intracellular structures, haustoria and arbuscules, essential for the absorption of nutrients from the plant (Parniske 2000, Parniske 2008), as well as like rhizobial symbiosomes, are surrounded by similar plant-derived membranes. The hallmark of the mutualistic symbioses is the presence of ATPase activity of the periarbuscular and peribacteroid membranes, generally lacking from the symbiotic membranes of pathogens (Parniske 2000).

In the nodules and mycorrhizal roots, an activation of the process similar to plant defence responses to pathogens is observed (Jones et al. 2007, Pozo et al. 2010). Similar reactions are observed in the interaction of plants with PGPB (Preston 2004, Vallad & Goodman 2004). It should be noted that these plant responses during mutualistic interactions are less pronounced than in the pathogenesis, and are probably under the control of symbiotic regulatory genes. Nod factor and specific surface polysaccharides of rhizobia (Jones et al. 2007), components of the cell wall of AM fungi (Garcia-Garrido & Ocampo 2002) and/or Myc factor, as well as a number of molecules on the surface or those secreted by PGPB (Preston 2004), can serve as elicitors of defence responses when interacting with beneficial soil microbes. The activation of plant defence responses when inoculating with beneficial microbes is supposed to be a reason for increasing systemic resistance of plants to pathogens (Preston 2004, Vallad & Goodman 2004, Pozo et al. 2010).

It is known, not all of those associations that are described as mycorrhizas (AM or others) have been shown to be mutualistic by experimental analysis of nutritional interactions or determination of fitness. Moreover, even in a single type of mycorrhiza, there may be a continuum of outcomes ranging from the mutualistic to the parasitic. Indeed, the relationship may change over the life of a single plant-fungus partnership. A major difference between the mycorrhizal symbiosis and those symbioses caused by parasites which lead to disease is that the mycorrhizal condition is the normal state for most plants under most ecological conditions (Smith & Read 2008).

Ecological Aspects of the Plant-Microbe Symbioses Evolution

From a perspective of the entire plant-microbe system, the mutually beneficial symbioses are represented as the products of interspecies (reciprocal) altruism which resemble to some extent antagonistic (pathogenic) interactions at the molecular and cytological levels. For the plant hosts, gene networks for regulating beneficial microbes and defending against pathogens, direct filiations between mutualism and antagonism are evident to exist. For the microbial side, this is unlikely, since beneficial and pathogenic microbes often demonstrate independent origins (Smith & Read 2008, Provorov & Vorobyov 2009, Provorov & Vorobyov 2010).

The evolutionary divergence of beneficial and deleterious micro-symbionts is suggested to result from specific selective pressures (different forms of group selection) maintaining genes for beneficial traits in microbial populations (Provorov & Vorobyov 2009).

Using the legume-rhizobia symbiosis as a model, it was proven that this selection might have two driving forces. The first one comprises the positive feedbacks operating between partners at the level of symbiosis metabolism (carbon and nitrogen compounds) and of the partners' co-evolution, that is, synergistic selective pressures supporting mutualism in plants and in microbes. The second one comprises the clonal structures of microbial populations in plants resulting in selection in favour of symbiotically effective strains (Provorov & Vorobyov 2009). However, using the mathematical model of multi-strain bacterial competition for inoculation of symbiotic (rhizospheric PGPB, nodule bacteria) habitats, it was demonstrated that an overall efficiency of symbiotic system is maximal when

the non-host-specific mutualists are present in rhizobia population. So, the construction of the economically valuable plant-microbe systems should provide the optimization of host-specific versus non-host-specific mutualists' composition in legume inoculants, combined with the clonal penetration of these mutualists into the nodules (Provorov & Vorobyov 2009).

Prospects

It is generally accepted that AM is an ancestral mutualistic plant-root symbiosis (Parniske 2008, Markmann&Parniske2009, Provorov&Vorobyov 2010) or, literally is "the mother of plant root endosymbioses" (Parniske 2008). Comparative data obtained recently by various up-to-date methods increasingly supports this hypothesis. Therefore, most probably, it is mutualism that was an initial form of plant-microbe symbiotic interactions (Provorov & Vorobyov 2010), and not antagonism, as sometimes postulated (Dyakov et al. 2007). However, recent data on the molecular and cellular processes occurring in plants and microsymbionts for various plant-microbe symbioses and their genetic control suggest that mutualistic and pathogenic systems might evolve in parallel way in different groups of plants and microorganisms. So, the question, what is mutualism primary strategy for the root-microbe symbiosis, remains open.

The RLS, in particular, might evolve in Eurosid I plant clade few times, as a result of parallel evolution and convergence based on the universal organization and regulatory mechanisms of the plant genetic material. Since almost all legumes form AM, we can assume that the majority of RLS came on the basis of pre-adaptation, formed in plants as a result of their co-evolution with AM fungi. An ability to form specialized tissue structures, supplying microsymbionts with energy and providing conditions for effective nitrogen fixation, is the most important evolutionary advantage of nitrogen-fixing symbioses compared to the AM. Importantly, a high specificity of legume-rhizobial interaction, represented by the discrete cross-inoculation groups, is typical for galeoid legumes, suggesting a coordinated increase of ecological efficiency and symbiotic specificity in the partners' co-evolution (Provorov & Vorobyov, 2009).

Rhizobia evolved from different groups of soil bacteria, which in our opinion, are also the ancestors of some contemporary pathogenic bacteria. There is a possibility that individual rhizobia symbiotic fungi originated from AM,

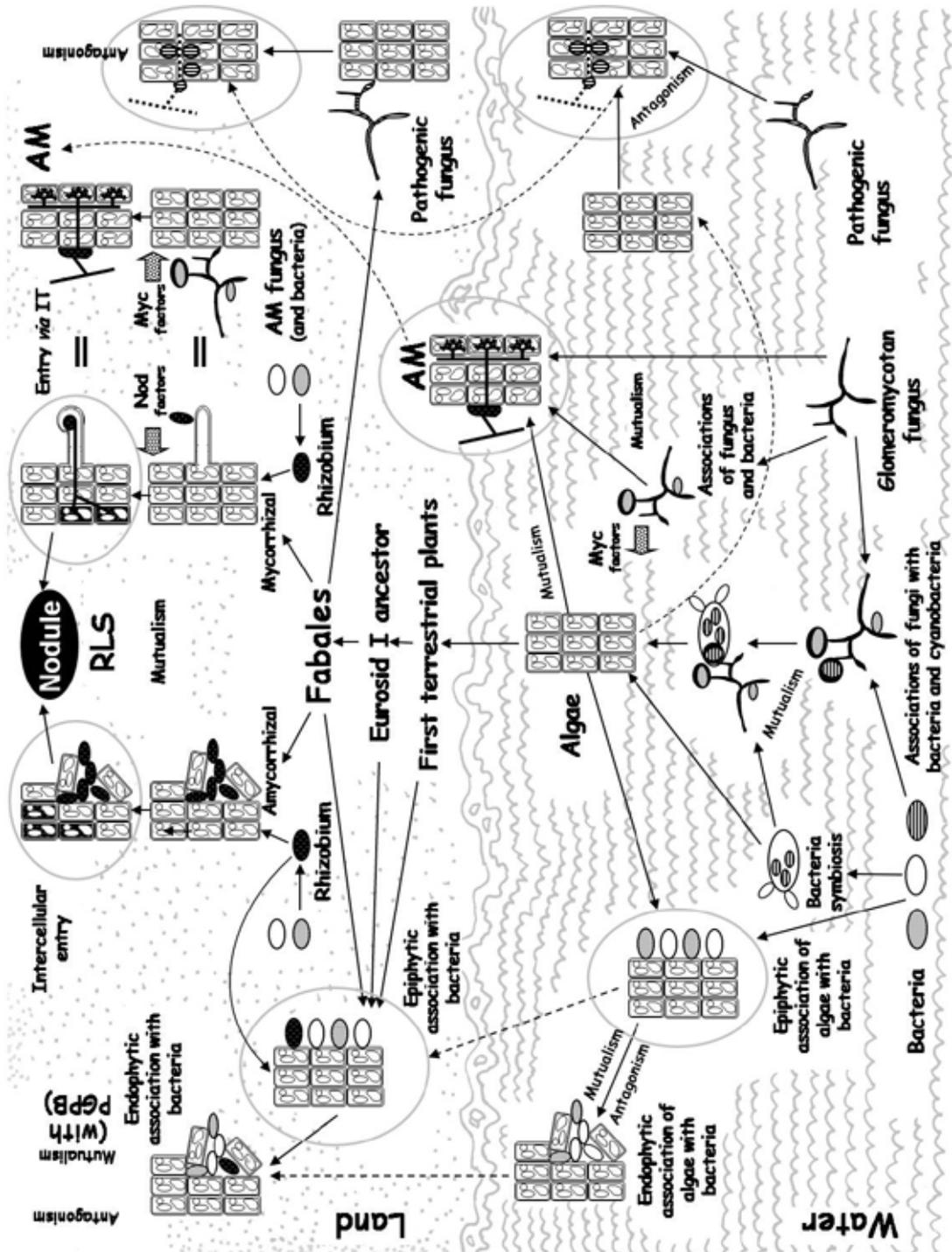


Figure 1. Hypothetical scheme of the evolution of legume root symbioses. Facts of symbioses occurrence in evolution are enclosed in ovals. The continuous arrows point to the novel microbial or plant-microbial interactions; broken arrows point to the different ways or stages of evolution of the interactions. Small ovals with horizontal shading - cyanobacteria, those with dark shading - rhizobia, those with light shading - fungal symbiotic bacteria and their probable derivatives, and those without shading - the other bacteria. AM - arbuscular mycorrhiza; RLS - rhizobium-legume symbiosis; PGPB - plant growth-promoting bacteria; IT - infection thread. Equals sign signifies commonalities between infection programmes during RLS and AM development.

but it can also be assumed that these rhizobia and related symbionts of AM fungi have evolved from a common ancestor. In accordance with the above-stated, a following scheme of the evolution of legume root symbioses can be constructed (Fig. 1).

Thus, it is assumed that the evolution of different mutualistic plant-microbe systems occurs through the conversion of AM to RLS and other symbioses. However, this transformation was not accompanied by the displacement of the original fungal symbiont. The existence of such a historical continuity allows us to consider these symbioses as a single “evolutionary plant-microbial continuum” (Provorov & Vorobyov 2010). In this case, multi-component symbiotic system (plant + fungi + bacteria, including rhizobia) can be regarded as ancestral with respect to the two-component one (e.g., plant + fungi, or plant + rhizobia).

Because RLS are still evolving, is it possible to construct new nodulating plants from non-nodulating ones or improve the efficiency of existing RLS with the use of bio-engineered methods (Sprent 2001). Basal but efficient forms of RLS (as well as actinorhiza) promise to involve a minimal set of host and bacterial genetic adaptations required for engineering artificial host systems. Although many factors can restrict nitrogen fixation within plant roots, some bacterial strains such as *Azorhizobium caulinodans* and symbiotic *Burkholderia*, having the capacity to subsist and fix nitrogen under both symbiotic and nonsymbiotic conditions, could provide a basis for artificially generating beneficial associations on a nonspecific or partially specific basis (Markmann & Parniske 2009).

A promising approach to improve efficiency of symbiotrophic N nutrition in legumes may be to combine the high nitrogenase activity of irreversibly differentiated bacteroids formed in nodules of galeoid legumes with the advanced N-assimilatory system of determined nodules. The plant genes responsible for bacteroid differentiation were identified by mutation analysis in several galeoid legumes, together with pea (*Pisum sativum* L.), alfalfa (*Medicago sativa* L.) and *M. truncatula* (Hadri et al. 1998, Starker et al. 2006, Borisov et al. 2007). A *M. truncatula* gene, *dnf1-1*, was transferred from *M. truncatula* to *Lotus japonicus* – a legume with determinate nodules lacking deep bacteroid differentiation. The resulted recombinants demonstrated the signs of much more deep bacteroid differentiation than the wild-type *L. japonicus* genotype (van de Velde

et al. 2010) demonstrating a compatibility of programs for determinate nodule development and irreversible bacteroid differentiation.

Since the use of genetically modified organisms in agriculture is limited by many difficulties, traditional plant breeding is the most simple and cheap way of increasing the efficiency of the legume symbiotic systems. Plant breeding to improve the efficiency of their interaction with beneficial microbes will “return” legumes to the natural symbiotrophic type of nutrition and improve efficacy of the use of mutualistic plant-microbe symbioses in the practice of sustainable plant production.

Existence of synergism of different microbes in the rhizosphere (Barea et al. 2005) within the context of common evolutionary “roots” of the various plant-microbe systems allows us to speak about the prospects of biotechnologies of creating of multi-component symbioses to increase agricultural productivity and quality of legumes as well as non-legumes. This is consistent with the results of mathematic modelling of mutualism evolution under the mixed nodule infections, which support necessity to use inoculants, composed of both host-specific and non-host-specific mutualists (Provorov & Vorobyov 2010).

Results of pot and field experiments with mixed inoculation of pea (Borisov et al. 2002, Borisov et al. 2004, Shtark et al. 2006) and soybean (*Glycine max* Merr.) (Labutova et al. 2004) demonstrated the possibility of using of potential of multi-component legume-microbe symbioses to increase productivity and quality of plant and to reduce the doses of mineral fertilizers. Innovation technology for the production and application of a new multifunctional biopreparation “BisolbiMix” (Chebotar et al. 2008), containing a complex of the most effective isolates of endosymbiotic microbes (AM fungi and rhizobia) and PGPB has developed. The efficacy of “BisolbiMix” was demonstrated in field trials with legumes, e.g. pea (Shtark et al. 2006), beans (Shtark et al. 2010a) or non-legumes (Zavalin & Kozhemyakov 2010). The use of microbial formulations containing rhizobia for non-legumes seems to be sensible because it is known that nodule bacteria which do not form nodules on a nonhost legume as well as non-legume roots can operate as PGPB (Sessitsch et al. 2002, Hossain and Mårtensson 2008). Thus, the selection of rhizobia with both PGPR activity and efficient symbiotic nitrogen fixation should be advantageous in crop rotations or intercropping systems using legumes and non-legumes.

A high level of genetic variability was demonstrated in the analyses of the symbiotic effectiveness under double inoculation with AM fungi and nodule bacteria of 99 pea genotypes from the collection N.I. Vavilov All-Russia Research Institute of Plant Industry, Saint-Petersburg, Russian Federation, with different geographical origins (Borisov et al. 2002). This suggests the possibility of legume breeding to increase their symbiotic effectiveness. Pea genotypes with the highest symbiotic efficiency were involved in breeding programs. As a result, in the whole history of legume breeding, the first pea cultivar Triumph, having increased potential of interaction with beneficial soil microbes, was intentionally developed (Borisov et al. 2008). Among the modern pea varieties, some genotypes were identified that have both high symbiotic efficiency and required plant architecture that can be directly involved into breeding programs (Shtark et al. 2006).

The innovation concept which offers fundamentally new approaches to plant production has been proposed. Firstly, it is necessary to consider plant genetic systems controlling interactions with different beneficial soil microbes in unison. Secondly, plants used as a component of this complex plant-microbe system controlling its effectiveness should be bred to improve the effectiveness of interactions with AM fungi, rhizobia and PGPB. Increases of plant biomass production due to plant-microbe symbiosis should be used as the main parameter for an evaluation of plant effectiveness in interactions with beneficial soil microbes. The plant production should be done with inoculation composed of multi-component microbial inocula consisting of foregoing beneficial microbes (Shtark et al. 2010b). In future, the sequence of plant genes that determine the stability and effectiveness of symbiosis may be used as DNA markers to facilitate breeding of legume varieties with high symbiotic efficacy (Zhukov et al. 2010).

Finally, taking into consideration the importance of legumes to global agriculture, greater emphasis should be placed on plant-microbial systems in the development of low-input agro-biotechnologies enabling wider cultivation of leguminous crops.

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