



Editorial

Rhizobial Symbiosis in Crop Legumes: Molecular and Cellular Aspects

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The production of high-value, environmentally friendly and healthy food has been the major global focus of sustainable agriculture in recent years [1,2]. Further progress in this area requires the development, testing and introduction of new agricultural technologies that would minimise environmental risks, help maintain or even improve the fertility of soils and support the creation of new types of agricultural products. To fulfil these conditions, new approaches are needed, including better use of genetic resources such as plants and microorganisms. The use of microorganisms will dramatically increase the diversity of genetic resources and contribute to increasing the resilience of agricultural systems [3].

Numerous studies show that during evolution, plants have used certain features of microorganisms to enhance their own adaptive capacities. For example, the plant genome incorporated some genetic factors that help create new ecological niches for microorganisms, wherein the genes providing the expression of adaptation remained in the genomes of microorganisms. Recent studies have shown that the symbiotic signalling pathways in plant species that form intracellular symbioses (including arbuscular mycorrhiza, ericoid and orchid mycorrhizae in angiosperms; ericoid-like mycorrhiza in bryophytes; legume–rhizobial and actinorhizal symbioses) is conserved [4]. The fact that symbiotic signalling has been conserved over 450 million years of evolution indicates the great importance of these associations for the successful spreading of land plants.

One of the best demonstrations of how the adaptive capacity of plants is expanded through co-evolution with microorganisms is the formation of symbiotic nitrogen-fixing nodules on the roots of legumes. Numerous genes in legumes are involved in the formation of the nodule. More than 40 regulatory symbiotic genes have been identified in the garden pea [5], while the process of nitrogen fixation is controlled by bacterial genes [6]. Therefore, the use of plant–microbe systems which are based on a nitrogen-fixing symbiosis between legumes and rhizobia is of significant interest for developing new approaches in sustainable agriculture [3,7–9]. The widespread use of legumes in sustainable agriculture will increase biological nitrogen fixation, reduce energy costs, improve the physical properties of the soil and increase soil microbial biodiversity [10,11]. In addition, legumes are important food and feed crops and are staples in some regions of the world [12].

The nitrogen-fixing nodule is a unique ecological niche for rhizobia in which micro-aerobic conditions enable the functioning of the main enzyme of nitrogen fixation, nitrogenase, which is highly sensitive to oxygen [13]. In the symbiotic nodule, specialised infected plant cells, which are increased in size due to endoreduplication, provide shelter to thousands of bacteria [14]. Bacteria are isolated from the cytoplasm of the plant cell through a membrane of plant origin, which has inclusions of bacterial proteins: the so-called symbiosome membrane. Within the symbiosome membrane, bacteria differentiate into a specialised form, bacteroids capable of nitrogen fixation, and together with the surrounding symbiosome membrane form a symbiosome [14]. The infected cells of the symbiotic nodule can be seen as a unique system in legumes that have appeared during



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their evolution to enable the adaptation of the plants to a lack of nitrogen in the soil and providing them with symbiotrophic nutrition. Therefore, it could be concluded that that the plant cell infected by rhizobia providing accommodation to numerous symbiosomes is the central component of nitrogen fixation. At the same time, it should be noted that the mechanisms that ensure the ability of plant cells to be filled by numerous symbiosomes, which are temporary cellular organelles of microbial origin, remain poorly understood.

In the current Special Issue, “Rhizobial Symbiosis in Crop Legumes: Molecular and Cellular Aspects”, we gathered seven articles with recent insights from studies into the development of legume–rhizobial symbiosis. These studies looked at both macro- and microsymbionts, with specific focus on how this knowledge could be applied in agriculture.

Recently, it was discovered that relict legumes could be considered as a source for new symbiotic genes. In current study, Safronova et al. [15] tested the effects of the co-inoculation of alfalfa (*Medicago varia* Martyn), common vetch (*Vicia sativa* L.) and red clover (*Trifolium pratense* L.) with commercial strains of *Sinorhizobium meliloti* and *Rhizobium leguminosarum* and with the strains of *Mesorhizobium japonicum*, *Bradyrhizobium* sp. or *M. kowhainii* isolated from the relict legumes *Oxytropis popoviana* Peschkova and *Astragalus chorinensis* Bunge. In some combinations, an increase in nodule number, plant weight and nitrogenase activity was reported. The authors attribute the positive effects on nodule development to the effect of rhizobial synergy, which occurs due to the presence of strains isolated from relict legumes with additional symbiotic genes, i.e., genes for the biosynthesis of phytohormones and genes encoding elements of the secretion systems. This study opens up new perspectives on the development of microbial biopreparations for the inoculation of legumes.

The studies presented by Zhukov et al. [16] revealed that in pea, the responses to double inoculation with rhizobial and arbuscular mycorrhizal fungi are increased in genotypes carrying the recessive allele *le* (*Le* encodes gibberellin 3-beta-dioxygenase). The increased responses are manifested by an increase in individual seed weight. This observation points out that the use of double inoculation could be a promising approach adopted in the cultivation of most modern pea varieties that carry the allele *le*. It could provide an important contribution for the development of sustainable agricultural technologies.

Given that legumes are a very large family, the number of species for which nodule development has been studied in detail is still limited. Therefore, studies of the development of symbiotic nodules in new legume species are of great interest. *Glycyrrhiza uralensis* Fisch. ex DC. is a well-known legume species, which is actively used in the pharmaceutical and food industries. Tsyganova et al. [17] conducted a detailed analysis of the development of nodules in this species (including tubulin cytoskeleton organization). In contrast to nodules of the other studied legumes, the walls of the infection threads are different from the cell wall in density and fibrillarity. A striking feature is the formation of indeterminate nodules in which infected cells are filled with multibacteroid symbiosomes, which is typical for nodules of a determinate type.

Medicago lupulina L. (black medic or hop clover) is another interesting legume species which is able to grow in adverse conditions. Roumiantseva et al. [18] performed a comprehensive analysis of the genome of its symbiont, *Ensifer* (*Sinorhizobium*) *meliloti*, strain L6-AK89. It revealed 53 *nod/noe/nol/nif/fdx/fix* genes and 32 genes involved in stress tolerance.

In recent years, the description of the transcriptional activity not only in the whole nodule but also in its individual zones has attracted increasing interest. Kusakin et al. [19] presented an analysis of differential gene expression in different zones of a symbiotic pea nodule isolated using laser microdissection. The maximum amount of differentially expressed genes was associated with the nitrogen fixation zone, with new genes involved in the nodule development being revealed.

Legume–rhizobium symbiosis is sensitive to various stresses, including salinity, which is one of the most widespread stress factors for plants. In this issue, two papers are dedicated to the study of genetic factors that may be involved in resistance to saline stress in rhizobia. Belfquih et al. [20] conducted a study of the *Ensifer aridi* strain isolated from the Moroccan Merzouga desert and demonstrating increased tolerance to drought and

saline stresses. The authors did not confirm the expected involvement of the alternative sigma factor RpoE2 in adaptation to saline stress. Therefore, revealing the alternative mechanisms of salt tolerance of this species of rhizobia requires further research. Muntyan and Roumiantseva [21] performed molecular phylogenetic analysis of salt-tolerance-related genes in 26 *Sinorhizobium meliloti* strains and revealed that megaplasmid pSymA carries not only genes of nitrogen fixation but also genes involved in salt tolerance. It means that pSymA is required for the formation of a stress-related gene pool in addition to genes regulating the nitrogen fixation function.

In conclusion, it should be noted that a detailed understanding of the molecular, genetic and cellular mechanisms of legume–rhizobial symbiosis is required for engineering new associations of non-legume plants with bacteria, which will acquire the ability to fix nitrogen [22,23].

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