



Editorial: Maximizing Nitrogen Fixation in Legumes as a Tool for Sustainable Agriculture Intensification

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Editorial on the Research Topic

Maximizing Nitrogen Fixation in Legumes as a Tool for Sustainable Agriculture Intensification

Today's agricultural systems are challenged by providing sufficient food for a growing world population while improving soil and water quality, maintaining farmer profitability (Stephens et al., 2018), and contributing to climate change mitigation (Niles et al., 2018). Nitrogen is an essential nutrient for plant growth and development, and intensification of agricultural systems typically encourages greater use of nitrogen to increase yields. Unfortunately, nitrogen is also directly linked to negative environmental impact *via* direct (N₂O) and indirect (NH₃) greenhouse gas emissions, ozone depletion (NO), and water pollution (NO₃⁻). Thus, nitrogen must play a central role in the sustainable transformation that global food production faces (Ying et al., 2017).

Most, if not all, agricultural production systems are limited by nitrogen availability, hence the widespread -and increasing- use of fertilizers (Rütting et al., 2018). Fertilizers are a source of nitrogen that is costly, of only moderate efficiency and with a relatively large CO₂ footprint, due to the energy intensity of the synthesis of chemical fertilizers by the Haber-Bosch process. Globally, the second largest source of agroecosystem nitrogen input is atmospheric N₂ fixation driven by a symbiotic relationship between legumes and soil bacteria collectively known as rhizobia. In this process, known as biological nitrogen fixation (BNF), rhizobia use plant assimilates to reduce N₂ to a plant-usable form through their nitrogenase enzyme. Compared to chemical fertilizers, BNF is cheaper and may result in a lower carbon footprint. Unsurprisingly, improving the effectiveness of BNF has been the focus of much research, and has risen in importance due to its critical role in sustainable agricultural systems. Improvement of the BNF process will improve our capacity to design sustainable diversified agroecosystems through the inclusion of legumes. This special issue addresses different approaches directed toward the exploitation of biological nitrogen fixation as a tool to develop more sustainable agricultural systems.

Rhizobia as soil inhabitants are important players in the legume rhizosphere microbiome, as supported by the minireview of Checcucci and Marchetti in this Research Topic. The signals involved in the rhizobium-legume interactions are shaped by plant exudates and rhizosphere microbiome composition. From the plant partner symbiotic perspective, Downie and Kondorosi discuss why different types of legume nodules, including both terminally differentiated rhizobia or

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not, depend on plant-encoded defense-like small peptides which include Nodule-specific Cysteine-Rich (NCR) peptides. Their minireview explores why NCR genes are absent from many legumes, and regulate bacteroid development and rhizobial strain discrimination in others.

Improving the biological nitrogen fixation (BNF) process has reinforced the attention to the rhizobia inoculation of legumes. Mendoza-Suárez et al. review the implications of rhizobial competitiveness for the establishment of a successful symbiosis. They describe ongoing approaches for inoculant development based on native strains to ensure optimal performance of both symbiotic partners.

While characterizing rhizobia partners for grain legumes, Missbah El Idrissi et al. report that the selection of lupine microsymbionts utilized as inoculants in the south Mediterranean should depend on soil type and pH. Some *Bradyrhizobium* spp. strains isolated from *Lupinus luteus* and *L. cosentinii* may constitute new genospecies while *nodA* and *nodC* nodulation genes correspond to members of the *genistearum* symbiovar.

Pigeon pea (*Cajanus cajan*) is a legume well-adapted to dry land areas and India is its major producer. Jorin et al. performed the first genomic study of pigeon pea endosymbionts in India. The major species nodulating pigeon pea was *Bradyrhizobium yuanmingense*, whereas nodulation-related features showed similarity to *B. cajani* and to *B. zhanjiangense*. They also recommend candidates for inoculant formulation in different agro-climatic regions.

Previous evidence supports the presence of indigenous soybean-nodulating bradyrhizobia in central Europe soils under cold-growing conditions, with Halwani et al. advancing our understanding of these strains by showing they remained viable in soil and were still symbiotically competent for up to 4 years after soybean cultivation. However, findings that their symbiotic performance was not sufficient in all cases makes inoculation with commercial products still necessary.

The symbiotic process is highly dependent on the dynamic exchange of signals and molecular nutrients between partners. The mini-review of Rey et al. highlights the importance of exopolysaccharide production and presence and possible function of type VI secretion systems, two key determinants in successful symbiotic interaction between rhizobia and pulse crops. Both exopolysaccharide composition and quantity play important roles in plant signaling and in bacterial protection to stressful conditions but type VI secretion systems are only recently studied in rhizobia.

Among other factors affecting N_2 fixation, Habinshuti et al. confirmed inhibition by N fertilization of common bean despite tissue N derived from BNF being high for bean plants receiving a double-dose of N fertilizer. In contrast, Bargaz et al. review how P bio-solubilization bacteria may enhance N_2 fixation in grain legumes. Rhizobial inoculation promotes growth of *Spartocytisus supranubius*, a keystone native legume species in a high mountain ecosystem on an oceanic island (Pulido-Suárez et al.). This article shows the importance of inoculation also for conservation purposes.

Nitrogenase activity results in the evolution of hydrogen but some rhizobia induce an uptake hydrogenase to recycle this hydrogen, improving the efficiency of the process. Sotelo et al. report the generation and symbiotic behavior of hydrogenase-positive *Rhizobium leguminosarum* and *Mesorhizobium loti* strains effective in vetch and birdfoot trefoil. The inoculation of these forage crops with the engineered strains leads to increases in the levels of nitrogen incorporated and indicates that hydrogen recycling has the potential to improve symbiotic nitrogen fixation in forage plants.

On the side of plant improvement, Puzozaa et al. report that black seedcoat pigmentation in Bambara groundnut, the most important food legume in Africa, is a biomarker for increased nodulation and N_2 fixation that can be used in breeding programs.

Plants MADS-domain/AGL proteins constitute a large transcription factor family that control root development among others, but their role in legumes was almost unexplored. Ayra et al. study the involvement of AGL from common bean as regulator of different stages of the rhizobia-legume symbiosis. They used composite plants with transgenic roots/nodules overexpressing or silencing AGL gene expression. The silencing plants were affected in the generation and growth of transgenic roots, decreased rhizobial infection with lower expression level of early symbiotic genes, and increased number of small ineffective nodules. This alteration in the autoregulation of nodulation symbiotic process is proposed to be related with the interplay with NIN, the master symbiotic regulator.

Non-specific lipid transfer proteins (LTPs) constitute a large protein family in plants while their role in mutualistic interactions is still unknown. Fonseca-García et al. find that genes of different classes of LTPs were expressed in roots inoculated with rhizobia and nodules of legumes. Specifically, common bean LTP genes are differentially expressed during the early and late stages of nodulation, and they might be regulated by ROS production.

Bottero et al. reported the high-efficiency of alfalfa mutagenesis by using public and regenerative alfalfa clone C23 and the CRISP/Cas9 system. This advance in the efficiency of CRISP/Cas9 genome editing suggests that this approach can be used to reduce the cost of production of edited cultivars for this important forage legume.

Finally, the integration of prokaryotic nitrogen fixation (*nif*) genes into the plastid genome for expression of functional nitrogenase components could render plants capable of assimilating atmospheric N_2 . However, N_2 fixation is a complex trait involving among others Nif proteins that are very sensitive to O_2 exposure. Aznar-Moreno et al. show that NifH protein expressed in transplastomic tobacco plants is functional when isolated from leaves collected at the end of the dark period. Non-unwanted negative effects from expression of functional NifH and NifM in plastids were observed, allowing to continue with attempts to engineer nitrogenase in crops.

DEDICATION

This Topic is dedicated to the memory of Tomás Ruiz-Argüeso in recognition for a lifetime of research on the improvement of symbiotic biological nitrogen fixation in legumes.

OBITUARY

Tomas Ruiz Argüeso (Villamol 1943–Madrid 2020) was Full Professor of Microbiology and, until his death, Emeritus Professor at the Universidad Politécnica de Madrid. After completing his Doctoral Thesis, focused on the study of the microbiology of honey, he became interested in the symbiotic nitrogen fixation process, which he would pursue throughout his long research career. He initially worked on the isolation and characterization of endosymbionts from soybean, a crop that was being introduced in Spain at that time and contributed to the development of the first rhizobial inoculants in the country. He later made postdoctoral stays at the laboratories of Harold Evans' lab at Oregon State University and at Donald Helinski's lab in La Jolla (UCSD). He devoted a long and fruitful research work to the study of the hydrogen recycling system of rhizobia, a subject on which he published over 50 research papers that covered from basic, mechanistic aspects of this metalloenzyme to its role in improving nitrogen fixation by legumes. In recent years he became interested in the characterization of new symbiotic systems, thus expanding our understanding on the high diversity of soil bacteria capable of establishing diazotrophic symbiosis.

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He published more than 80 articles in international journals, led over 30 research projects, presented almost 300 contributions at national and international conferences, and directed 16 Doctoral Theses. In parallel, he had a long teaching career in Microbiology, and a very notable task of service in different national and European research organizations and entities. And beyond his many achievements as a scientist and professor, Tomás always kept open the door of scientific curiosity and drawing pleasure from doing science, along with a friendly and close character, always ready to listen and help others. Those of us who have been fortunate and privileged to share these years with such an extraordinary researcher, teacher and friend will miss him. We are left with his many teachings and his memory. May he rest in peace.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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