HOW TO MANAGE LEGUME-RHIZOBLIA N2-FIXING SYMBIOSES AS ECOLOGICAL ENGINEERS?

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ABSTRACT Legumes have the capacity to fix large amounts of atmospheric N₂ into the biosphere through their capacity to establish a symbiosis with soil rhizobia. However, this legume contribution to the N biogeochemical cycle varies with the physico-chemical and biological conditions of the nodulated-root rhizosphere. In order to assess the abiotic and biotic constraints that might limit this symbiosis in an agro-ecosystem, a nodular diagnosis was performed in field-sites chosen with farmers of legume areas of production of the Mediterranean basin, with common bean as a model grain-legume, and a major source of plant proteins for world human nutrition. With this methodology, a large partial and temporal variation in the legume nodulation was found in cereal-cropping systems. In various reference areas of bean production, the nodular diagnosis showed that low P availability of soils is a major limiting factor of the rhizobial symbiosis. The relation with engineering the legume symbiosis was further addressed by participatory assessment of bean recombinant inbred lines contrasting for their efficiency in use of phosphorous for symbiotic nitrogen fixation. In situ RT-PCR of candidate gene on nodule sections has been developed in order to relate the field measurements with functional genomics of the symbiosis. It is concluded that various tools and indicators are available for developing the engineering of the rhizobial symbiosis for its beneficial contribution to the biogeochemical cycle of N, and also C and P in various Mediterranean and temperate climates.

Keywords: legume; N₂ fixation; rhizobia; symbiosis; biogéochimical cycle; nitrogen; phosphorus

INTRODUCTION

Farmers in most developed countries are recommended to minimize N fertilization for environmental sake, and farmers in less-developed agricultural areas of the world cannot afford it. Thus, symbiotic nitrogen fixation (SNF) by legumes that already contributes a major input into agricultural and natural ecosystems, may provide an ecologically acceptable substitute or complement to N fertilization.
However, P deficiency is a major limiting factor of the legume production where its N nutrition largely depends upon the rhizobial symbiosis. A possible explanation is that legumes, and common-bean especially, have higher P requirements than non-symbiotic plants, though it is possible to improve common-bean SNF potential and expression under P deficiency (Vadez et al., 1999).

In this study, the observation for nodulation and growth in field of common-bean recombinant inbred lines (RILs) most contrasting in P use efficiency (PUE) for SNF, from a screen in glasshouse hydro-aeroponic culture among the cross of parental lines DOR364 and BAT477, with multi-location participatory testing is presented as a tool for engineering the ecological services of the symbiotic legume. The relations with soil P and nodule phosphatase are also considered.

**MATERIALS AND METHODS**

Four RILs with most contrast in EUP for SNF were sown in Lauragais (France, 43-13°N, 02-19°E, 130 mas) with 6 farmers of the SPHC (Syndicat des Producteurs de Haricot à Cassoulet) during 4 successive years for multi-location field comparison with the local cultivar Linex. Each site consisted in 3 m rows per RIL within an homogenous area of the field ensuring similar canopy as for the local bean cultivar, and constituted one block of a randomized design, and/or was replicated as 3 block per farmer's field.

Sampling was performed at flowering stage during july, by excavating 20 cm in depth and around the root-system of 10 plants per block. Nodules and shoot were weighed after 48 h at 70 °C. The available P of the soil was measured by the Olsen method according to NF ISO 11263. Standard deviations were determined, and the analyzes of variance and regressions were performed. Nodule in situ reverse transcription and PCR amplification of *Phaseolus vulgaris* acid phosphatase (PvAC) was performed on nodule sections with modifications from Van Aarle et al. (2009).

**RESULTS AND DISCUSSION**

The RILs mean shoot-growth per field varied between 19 and 9 g SDW pl⁻¹. However the RIL104 show a lower growth potential than RIL115 whatever the field. An interesting low growth and leaf yellowing was observed with RIL147 (7 g SDW pl⁻¹) compared to RIL115 (12 g SDW pl⁻¹) in some fields (Fig. 1).

The nodulation varied considerably among fields, from 2 to 54 mg NDW pl⁻¹, corresponding to more than 50 nodules per plant. Also, nodulation varied much between years. In some filed, significant correlations were found between nodulation and growth, with higher ratio of growth/nodulation for RIL115 than 147.
In order to assess the RIL effects on soil P bio-availability, the Olsen P in soil extracted with nodulated-roots varied from a mean of 15 to more than 30 mg P Kg\(^{-1}\). It was significantly higher with RIL 115 than 147 in two fields. Also a large spatial variation within each field was found: e.g from 6 to 21 mg P Kg\(^{-1}\) soil versus from 24 to 65 mg P Kg\(^{-1}\) soil in two contrasting fields.

Since nodule phytase activity was found to increase under P deficiency (Apaújo et al., 2008), primers of phytase were designed from *Glycine max* and *Medicago truncatula* sequence, for phytase localization on nodule section. The green signal in figure 2 showed that phytases was widely expressed in nodules, and essentially localized in nodule cortex, both inner and middle.

**CONCLUSION**

Comparison of RILs contrasting in EUP for SNF in Lauragais fields confirm that screening hydro-aeroponic culture maximizing the rhizobial symbiosis under highly controlled P supply in glasshouse, is an innovative approach to select for the use of P by nodulated legumes. It complements to most other approaches that are focused on the acquisition of P. The correlation between plant growth and nodulation makes it possible to calculate the efficiency in use of P for N\(_2\) fixation. With plant N content, the fraction of N fixed from atmosphere can be extrapolated.

Thus, such RILs can be used to (i) assess the adaptation of the grain-legume in low N&P soils of agro-ecosystems, (ii) identify soils where P offer is deficient for the legume N\(_2\)-dependent growth, (iii) search for mechanisms and genes from the improvement of efficiency in use of P for N\(_2\) fixation, (iv) improve local or commercial varieties of adaptation to low P availability. Their participatory assessment with producers is pursued to assess the legume contribution to N and P bio-geochemical cycles in cereal cropping systems, and to generate technology to stabilize the legume yield for sustainability in agriculture.

**REFERENCES**

