'Nitrogen fixation in mycorrhizal symbioses of Ceratonia siliqua'

'Has Ceratonia siliqua formed a joint venture with mycorrhizal and nitrogen fixing symbioses? Potential for juvenile tree growth and orchard establishment'

ABSTRACT

'Among the legumes, three subfamilies are recognized: Papilionoideae, Mimosoideae and Caesalpinioideae. If the first two have overwhelmingly nodulated species, among Caesalpinioideae nodulation concern only 5% of genera. Among the different clades of Caesalpinioideae, the closest to Mimosoideae include the genus Ceratonia. The carob tree, Ceratonia siliqua, is cultivated in the Mediterranean area mainly for its fruits. It is not nodulated but mycorrhizal (arbuscular mycorrhizae). The objective of our work is to study the diversity of bacteria associated with the roots of this species in relation to mycorrhization.
Our approach comprised three steps:
(1) In different field sites, we collected leaves of carob, adjacent non-leguminous species and legumes to analyze the behavior of the carob tree with respect to biological nitrogen fixation by analysis of natural isotopic abundance of 15N. The analyses were carried out by mass spectrometry in UMR BPMP. The results showed a strong geographic variability with sometimes contradictory trends. A second campaign of sampling and analyses showed a particular behavior of the carob tree, different from that of non-legume and nodulated legumes. However, the variability in the analyses performed require to further increase the number of samples studied by multiplying the crop sites and considering well-distributed stands all around the Mediterranean sea, including the area of origin of the carob tree (especially Lebanon). It is in this context that the Mycamed project (ANR Jeune Chercheur and Apège INEE were submitted, and that a PHC Cèdre (Lebanon) project will be prepared.
(2) Roots and soil in carob were also collected to assess the presence of arbuscular mycorrhizal symbioses and to analyze the microbial communities associated with natural carob. Structure and diversity of bacterial communities and arbuscular mycorrhizal were revealed by PCR-DGGE of 16S and 28S rDNA. Carob showed a relatively high diversity of mycorrhizal compared to associated crops (barley, corn) and compared to the argan tree. Cloning / sequencing analyses reveal the presence of several species of the families Glomeraceae and Claroideoglomeraceae. Bacterial communities were also analyzed from the roots of carob (more than twenty field collected plants) to identify bands found in all or virtually all carob analyzed. These bands are currently under sequencing to identify the bacterial taxa constently associated to carob root system.
Microscopic observations revealed the presence of arbuscular mycorrhizal structures in roots and also root cells invaded by bacteria.
(3) From surface sterilized root fragments, bacterial isolation trials were performed. The isolates were selected on their ability to induce a phenotype of monoxenic nodulation or swelling of root portions in two legume with broad nodulation spectrum: Macroptilium atropurpureum and Mimosa pudica. Bacterial strains inducing one or the other of these phenotypes were characterized by their 16S rDNA sequencing. Bacterial taxa included the genera Rhizobium and Pseudomonas.'

Year : 200'9'
Project number : '0901-001'
Type of funding : 'AAP'
Project type : 'AAP'
Research units in the network : 'BPMP'
Start date : 2009-08-01
End date : 2012-12-31
Flagship project : no

Project leader : 'Yves Prin '
Project leader's institution : 'CIRAD'
Project leader's RU : 'LSTM'

Budget allocated : '30000' €
Total budget allocated (including co-financing) : '30000' €
Funding : 'RTRA'

GOAL

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ACTION

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RESULTS

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PERSPECTIVES

'Ceramyc has opened a number of very promising avenues of research on the possibility of an nitrogen acquisition by nitrogenase activity to be confirmed by a wider sampling to complete the data on natural isotopic abundance of 15N. These results have to be connected with the presence of endophytic bacteria of the genus Rhizobia. Tagging these bacteria with fluorescent markers in the presence (or not) of arbuscular mycorrhizal fungi should allow to follow the root interactions between the fungus and the tagged bacterial strains leading to bacterial endophytism. Ceramyc allowed the formation of two students from two masters, and helped to support the work of a thesis student of the University of Marrakesh. These studies have led to several posters and oral communications in national and international conferences, and should lead to several publications in peered-reviewed journals.'