

# '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  $^{15}\text{N}$ . 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 constantly 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*.'

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## CONTEXT

Over 10,000 years ago, our ancestors began domesticating wild plants to create crops capable of producing a more regular, predictable source of food. Taking advantage of existing plant biodiversity, they selected useful plant characteristics, such as disease and drought resistance, as well as grain or fruit traits. Crop biodiversity is vital to our societies. But the effects that humans and nature exert on crops can have unintended consequences for their biodiversity. And without sufficient biodiversity, crops are unable to adapt or be adapted by farmers and breeders to environmental or climate changes, hence putting the world's food supply at risk. It is therefore important to conserve crop biodiversity today and ensure it is sustainable by examining it at every level — from genes to populations — to understand how domesticated plants have adapted to human and environmental pressures over time.

ARCAD aims to be an innovative open multi-function (biological, technological, scientific, educational) resource center for agrobiodiversity in Montpellier, France, which is home to many of the world's leading research teams specializing in plant biotechnology, evolutionary biology, crop genomics and genetic resources in Mediterranean and African regions... ARCAD has three main complementary and interdependent components: research and training, genetic resource collections and technological platforms.

Supported by Agropolis Fondation, the Région Languedoc-Roussillon (France), and the European Regional , it is being jointly developed by CIRAD, INRA, IRD and Montpellier SupAgro, which are four French leading agricultural science establishments Development Fund. ARCAD is working in close partnership with Universities, Biological resource centers, research organization from various country and international centers.

{{A research and formation program on plant domestication and adaptation.}}

This component focuses on the harmonization, consolidation and animation of regional scientific community to develop ambitious research work on plant genetics and genomics. ARCAD's primary scientific work focuses on the relationship between crop biodiversity, crop domestication and adaptation to agricultural environments. By studying the history and patterns of crop domestication and adaptation, it shows how genes, genomes and populations of cultivated plants have been shaped by centuries of farming as well as environmental and societal changes. ARCAD's research examine urgent questions, such as those pertaining to crop adaptation to climate change and management of crop biodiversity by farmers in various farming systems in the Southern hemisphere. Its scientific endeavors will be conducted in collaboration with other international research teams. The knowledge and methods developed will be deployed across specific training modules, international training courses and participation to the existing courses provided by different universities in Montpellier.

{{An open and innovative platform for conservation and analysis of Mediterranean and tropical vegetal resources.}}

This component focuses on the acquisition, the development and the connection of technological platforms and biological resources, to increase the efficiency and attractiveness of research work for conservation and analysis of genetic resources. It is based on the reinforcement or creation of new platforms equipped with up to date technologies for seed conservation, seed phenotyping, cryopreservation, DNA extraction and banking, genotyping/sequencing as well as systems and web portal to access information on collections. A new building will host these platforms by the end of 2017.

{{ARCAD resources will serve agricultural research as well as local and national companies interested by the exploration and utilization of biodiversity. ARCAD is expected not only to contribute to reinforcing

local expertise but also to facilitating knowledge sharing on agrobiodiversity. } }

## GOAL

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## ACTION

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## RESULTS

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## PERSPECTIVES

'Ceramyx 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  $^{15}\text{N}$ . 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. Ceramyx 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 peer-reviewed journals.'