



## Breeding, agronomic and biotechnological approaches for reintegration and re-valorisation of legumes in Mediterranean agriculture



- ❖ Legumes are crops of an extraordinary importance for the agriculture and the environment. However, their cultivation is decreasing in most of the Mediterranean countries.
- ❖ Our main objective is to stabilize the yield and production of major food legume cultivars adapted to different pedoclimatic conditions encountered in the Mediterranean region.

### Objectives

The project goal is to develop methodologies to improve grain legume plant material of high yield potential, resistant to major diseases and abiotic stresses, suitable for sustainable farming systems. This is achieved by:

- ❑ Evaluation of current and historic legume germplasm for characteristics of importance to sustainable agriculture in the Mediterranean area.
- ❑ Identification of new sources of resistance to major stresses.
- ❑ Genetic studies of desired traits and development of specific markers for pyramiding and rapid screening.
- ❑ Clarification of the epidemiology of major diseases.
- ❑ Development of integrated management strategies.

Legumes

Breeding

Crop protection

Stress resistance

Epidemiology

### Scientific results & innovation potential

- ✓ Improved sustainability can be achieved by identifying and providing germplasm to the legume breeding community that can enable increased productivity with a reduced consumption of natural resources.
- ✓ MEDILEG targets the development of legume pre-breeding germplasm resistant to major crop pathogens specific of the Mediterranean region and abiotic stresses such as drought and salinity and through the refinement of integrated control strategies.
- ✓ The selection of genotypes carrying the desired genes will be greatly facilitated by using molecular markers tightly linked to the desired traits. In this sense, pea is used as a model as the best characterised crop legume.

### Coordinator

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### Partners

- Centre Régional de la Recherche Agronomique de Rabat, Institut National de la Recherche Agronomique (INRA), **MOROCCO**
- UMR IGEPP, Institut National de la Recherche Agronomique (INRA), **FRANCE**
- UMR LEG, Institut National de la Recherche Agronomique (INRA), **FRANCE**
- DISSPAPA Unit, University of Naples Federico II, **ITALY**
- Field Crop Laboratory, Institut National de la Recherche Agronomique de Tunisie (INRAT), **TUNISIA**
- Regional Field Crop Research, **TUNISIA**
- Institut Agronomique et Vétérinaire (IAV) Hassan II, **MOROCCO**
- Plant Cell Biotechnology Laboratory, Instituto de Tecnologia Química e Biológica (ITQB), **PORTUGAL**
- Sakha Agricultural Research Station (SARS), Agricultural Research Center (ARC), **EGYPT**
- École Nationale Supérieure Agronomique (ENSA), **ALGERIA**



## (I) Identification of regional priorities

- All partners prospected and provided the information from their own area.
- Based on this inventory, a relevant collection of 10 genotypes per crop and country was composed to serve as project field testing.

## (II) Studies on stresses

- Adapted varieties as well as germplasm collections from each country were tested, under both field and growth chamber conditions, to identify sources of resistance to major diseases (*Ascochyta* blights, rusts, broomrapes, fusarium wilts and chocolate spot) and drought.
- Stability of the resistance (about 40 entries per crop) was studied in multi-environment experiments.

## (IV) Marker-Assisted Selection

- Standard markers for mapping in pea were developed.
- Development and/or complementation of molecular maps were conducted with SSR (*Simple Sequence Repeats*) and SNP (*Single Nucleotide Polymorphism*) markers on existing RIL (*Recombinant Inbred Lines*) populations. Analysis of QTL (*Quantitative Trait Loci*).
- Assessment of genetic progress through (i) phenotyping; (ii) classification using anonymous SSR markers; (iii) a posteriori control of presence/absence of favourable alleles at QTL.
- Marker-Assisted Breeding: identification of markers linked to QTL of interest that could be used for the simultaneous selection for different stresses.

## (V) Epidemiology

- Development of SSR markers for pathogen population genetic studies.
- Landscape epidemiology study to identify the origin and impact of different primary inoculums on the epidemic development of *Ascochyta* spp.

## (VI) Biological control

- Isolation of phytotoxins produced by fungal pathogens and by root exudates for further chemical characterization.
- Study of the ability to stimulate or inhibit broomrape seed germination.
- Bioassays determination of phytotoxic, antifungal, mycotoxic and allelopathic activities of the pure metabolites isolated.



**Figure 1.** Food legumes studied: lentil, faba bean, chickpea, pea and common bean

## Why collaborating?

- MEDILEG brings together a wide group of cross disciplinary expertise that cannot take place at a National level.
- It adds to the existing legume programs by addressing solutions to the specific Mediterranean constraints for legume production in a collaborative way between research teams across the Mediterranean basin.
- This is achieved through innovative multidisciplinary approaches including the application of biotechnologies (France, Egypt, Portugal, Morocco, Spain), plant breeding (Egypt, France, Portugal, Morocco, Spain, Tunisia), epidemiology (France, Morocco, Spain and Tunisia) and allelopathy (Algeria, Italy, Spain) as the only way to deliver integrated control measures ensuring attractiveness of legume cultivation for farmers, with associated benefits to the rural economy and to the environment, while reducing dependence from imports.

**Figure 2.** Cooperative multidisciplinary initiative to promote legume revalorisation in Mediterranean agriculture



# ACHIEVEMENTS

## (I) Definition of ideotypes

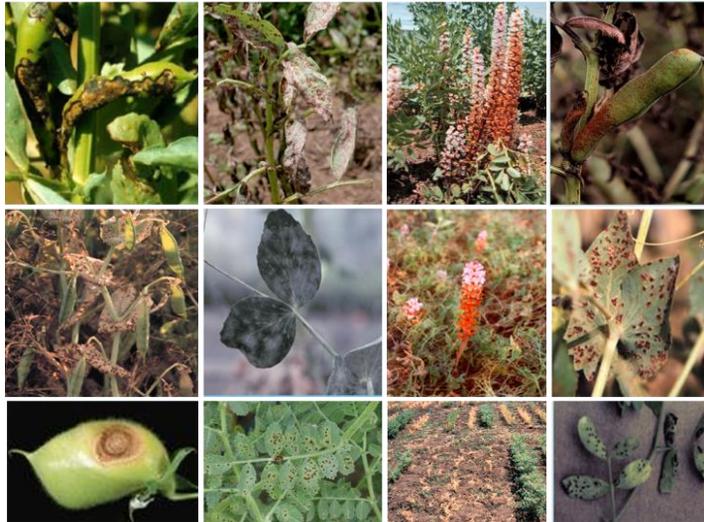
- Inventory of existing data on constraints for legume production were constructed with regard to agronomic performance, resistance, adaptability and nutritional value leading to the definition of desired phenotypes for each crop suitable for each area.

## (II) Studies on stresses

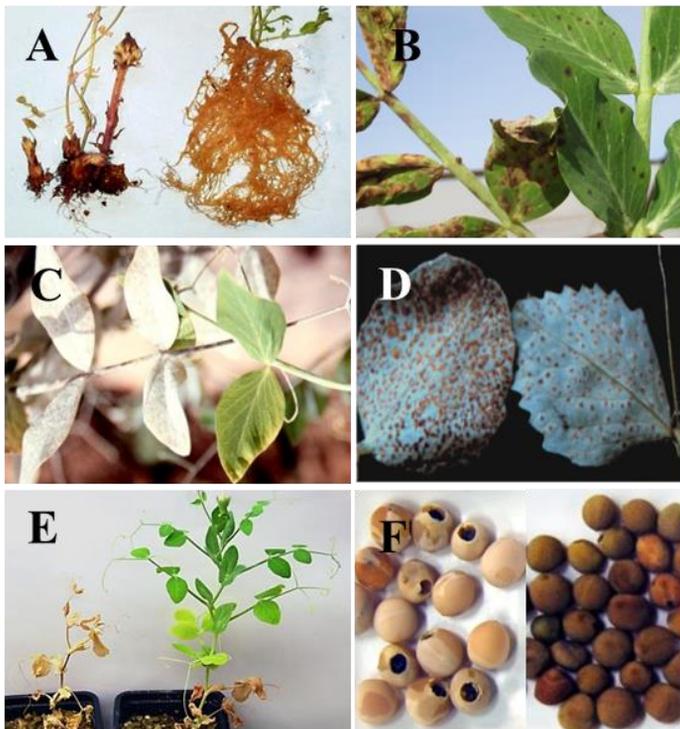
- Reliable screening methods were developed for the most relevant stresses under both field and growth chamber conditions.
- Sources of resistance were identified, resistance mechanisms were characterised at the cellular and physiological level, and the stability of resistance was studied in multilocation experiments.
- The inheritance of drought tolerance was studied in multilocation field trials and laboratory experiments.
- The pathogenic variability among the disease-causing organism was also investigated.

## (IV) Marker-assisted selection

- Genetic markers linked to stresses were identified. RILs segregating for resistances were analysed to test the stability of the putative QTL in different environments and genetic backgrounds.
- Marker-Assisted Breeding: the process of transferring genes and QTL from a donor line to recipient lines in order to obtain genetic material that fit the defined ideotypes was initiated.



**Figure 3.** Some of the stresses covered. 1<sup>st</sup> row: ascochyta, chocolate spot, broomrape and rust on faba bean; 2<sup>nd</sup> row: ascochyta, powdery mildew, broomrape and rust on pea; 3<sup>rd</sup> row: ascochyta, rust and fusarium on chickpea and rust on lentil



**Figure 4.** Symptoms of main pea diseases or pests on susceptible (left) and resistant (right) pea accessions: (A) Broomrape, (B) Ascochyta blight, (C) Powdery mildew, (D) Rust, (E) Fusarium wilt, (F) Weevil

## (V) Epidemiology

- The pattern of genetic variation among populations of pathogens/parasites was analysed.
- A landscape epidemiology study was designed and carried out to identify the origin and the impact of different primary inoculum sources on the epidemic development of *Ascochyta* spp.
- Complementation of resistance with other methods for disease management of legume crops was developed in field trials.

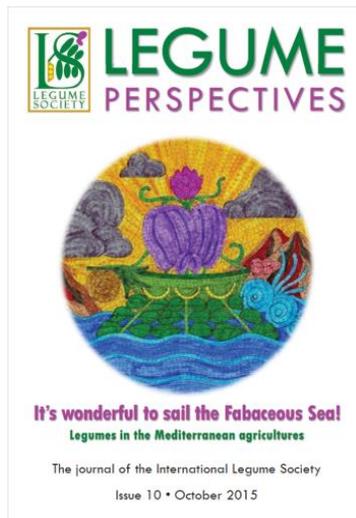
## (VI) Biological control

- Protocols were delivered for the extraction and purification of bioactive metabolites (phytotoxins, herbicides, phytoalexins).
- Structure of new bioactive compounds with original carbon skeleton was determined.
- Phytotoxic, antifungal, mycotoxic and allelopathic activities of the pure metabolites were isolated and characterized.
- Key derivatives for structure confirmation and structure-activity relationships were studied.



## Stakeholder engagement

- The processes developed are designed to have a broad application by ensuring the faster development of more resistant varieties in other crop plants and for other multi-stress conditions, ensuring that scientific results can be fully applied by growers.
- Because of the additional services provided by legume crops, this is an important outcome. Unfortunately, profits from legumes to breeding companies are relatively low, so there are few participants in the sector. Therefore, legume research, except for that on soybean, is mainly carried out by the public sector in most of the world, and particularly in the Mediterranean region. The active involvement of these national agencies ensures proper implementation and dissemination to farmers.



**Figure 5.** Example of joint dissemination product: Issue 10 of Legume Perspectives, jointly with REFORMA

## Next steps

- ❖ Using legume-based cropping systems will make Mediterranean agriculture less dependent on N fertilisers and self-sufficient for protein supplies which will consequently decrease its dependency on imports. Outcomes of MEDILEG will serve to accelerate crop breeding and management. However, an effective Mediterranean network that can fully exploit this should be maintained to ensure improved food and feed security for the Mediterranean region.



**Figure 6.** Example of joint dissemination activity: active participation at the 2016 International Pulses conference in Marrakesh (Morocco), 18-20 April 2016



**Figure 7.** Example of joint dissemination activity: Final project meeting at the 2<sup>nd</sup> International Legume Society Conference in Tróia, Portugal, 11-14 October 2016

## Do you want to know more?

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