Genetic diversity of nodules and of landraces of lupins collected in Morocco over the last five years

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Abstract

In Morocco, many species of Lupins grow spontaneously. Collection of local varieties and some native genetic species and their evaluation allowed us to initiate, in collaboration with INRA-France (Lusignan), a breeding Program of sweet Lupins (*L. albus*) by crossing the best sweet lines from INRA-France selection and some selected Moroccan populations.

Indigenous rhizobial strains associated with native lupin species in Morocco were collected and some phenotypic characteristics and the genetic diversity were studied.The REP-PCR of 159 rhizobial isolates from the nodules of six *Lupinus* species, indicated a high genetic diversity. The strains showed fast growth rates and typical characteristics of the *Rhizobium* genus. Moreover, their good abiotic stress tolerance seems to be close of *Bradyrhizobium*. Most of them showed an interesting biological nitrogen fixation potential. Further characterisation will be useful to study the taxonomic position of these bacteria within the Rhizobiaceae family. Selection of lupinu-rhizobia symbiosis adapted to calcareous alkaline soils will result on the extension of lupins in large areas as an alternative forage legume.

Key Words

Lupinus spp., rhizobia, prospection, evaluation, Morocco.

Introduction

In Morocco, various ranges of wild lupins grow spontaneously in different areas (Atlas and Rif mountains, and coastal regions). Wild lupins cover a wide edapho-climatic range and show a great variability for many agro-morphological characteristics. This genetic potential could be exploited in breeding programs to create adapted cultivars for animal feeding. Wild accessions from Morocco have been previously used in some breeding programs in Australia, such as *L. atlanticus* which is found exclusively in Morocco (Buirchell, 1992)). Currently, the natural habitats of most species in Morocco are submitted to high pressures through the changing in farming practices, overgrazing, drought, urbanisation and desertification. Emphasis should therefore be made on the preservation of the lupins genetic resources by collecting lupine systematically.

In Morocco, only two cultivated varieties are available: the white lupine *L. albus* cv. Multoplupa and the yellow lupine ??Gharb?? a local population of *L.luteus*, which are insufficient to cover a total potential area estimated to be 1 million ha, but which does not exceed 5000 ha per year.

In order to valorise these autochthones resources, many actions on lupin and its microsymbiont *Bradyrhizobium* sp. (*Lupinus*) were carried out.

Research axes

1. Genetic resources: Collect and Evaluation

(a) 1. 1. Plant

To preserve Moroccan genetic resources against genetic erosion, two prospections of lupin were carried out, the first in 1998 in the area of Rabat (The west coast of Morocco), the second in 1999, in the areas of Tangier (the north), Meknes, F?s (the center), and Marrakech (the south) (Lagunes-Espinoza, et.al., 2000).

Collections were made in natural habitats of wild lupins and farmers fields for cultivated lupins. Seeds were also bought in traditional markets.

A total of 31 populations of *L. albus*, 11 populations of *L. luteus*, 24 populations of *L. cosentinii* and six samples of others species were collected. All the populations of white lupin contain bitter seeds, indicating traditional croping use compared to yellow lupin (*L. luteus*) which have sweet seeds.

A breeding program was initiated for the selection of sweet white lupine varieties adapted to the Moroccan pedo-climatic conditions.

The program included the following stages:

1. An analysis of collected traditionally cultivated populations under Moroccan and French conditions,

2. Multi-local evaluation in Morocco of 100 sweet white lupin lines resulting from the selection led in France focusing on the most adapted flowering earliness of the material;

3. The initiation of a varietal creation program, especially by crossing the best sweet lines resulting from French selection and the most productive Moroccan populations.

Components Principal Analysis on the collected populations showed that our genetic material has an intermediate precocity between the spring types and the winter types. It is characterized by a strong vegetative strength.

The weights of thousand seeds are variable within the Moroccan material, but on average definitely higher than the varieties resulting from the French selection. This last character was strongly conditioned by traditional use in the human consumption of these seeds. We can’t identify obvious geographical structuring within the Moroccan populations.

The proportion of pod walls of moroccan populations is 30%. This character is little selected by the medium and human activity indicates that the Moroccan populations probably result from the populations found in the Iberian peninsula, in particular in South-east (Lagunes-Espinoza et al., 2000).

An analysis of the collected populations phenology is coherent with the behavior of all French lines tested in Morocco. The late springs or very early winter types present the best adaptation to the Moroccan p?do-climatic conditions in the various experimentation places.

This analysis of the collected populations allowed us to identify the parents that will be used, in crosses with the best soft lines selected for their flowering date.

Since 2001 have evaluated each year the early generations of these crosses in two locations: Larach (north) and Merchouch (ouest). We are currently in the F4 generation.

(b) 1. 2. Lupin-rhizobia

With the aim of exploiting the natural biodiversity of the bacteria, two prospections to collect *Bradyrhizobium* were carried out in 2001 and 2002. About 200 samples of lupin nodules from four different species: *L Cosentinii GUSS, L angustifolius, L luteus L* and *L atlanticus* were collected. The

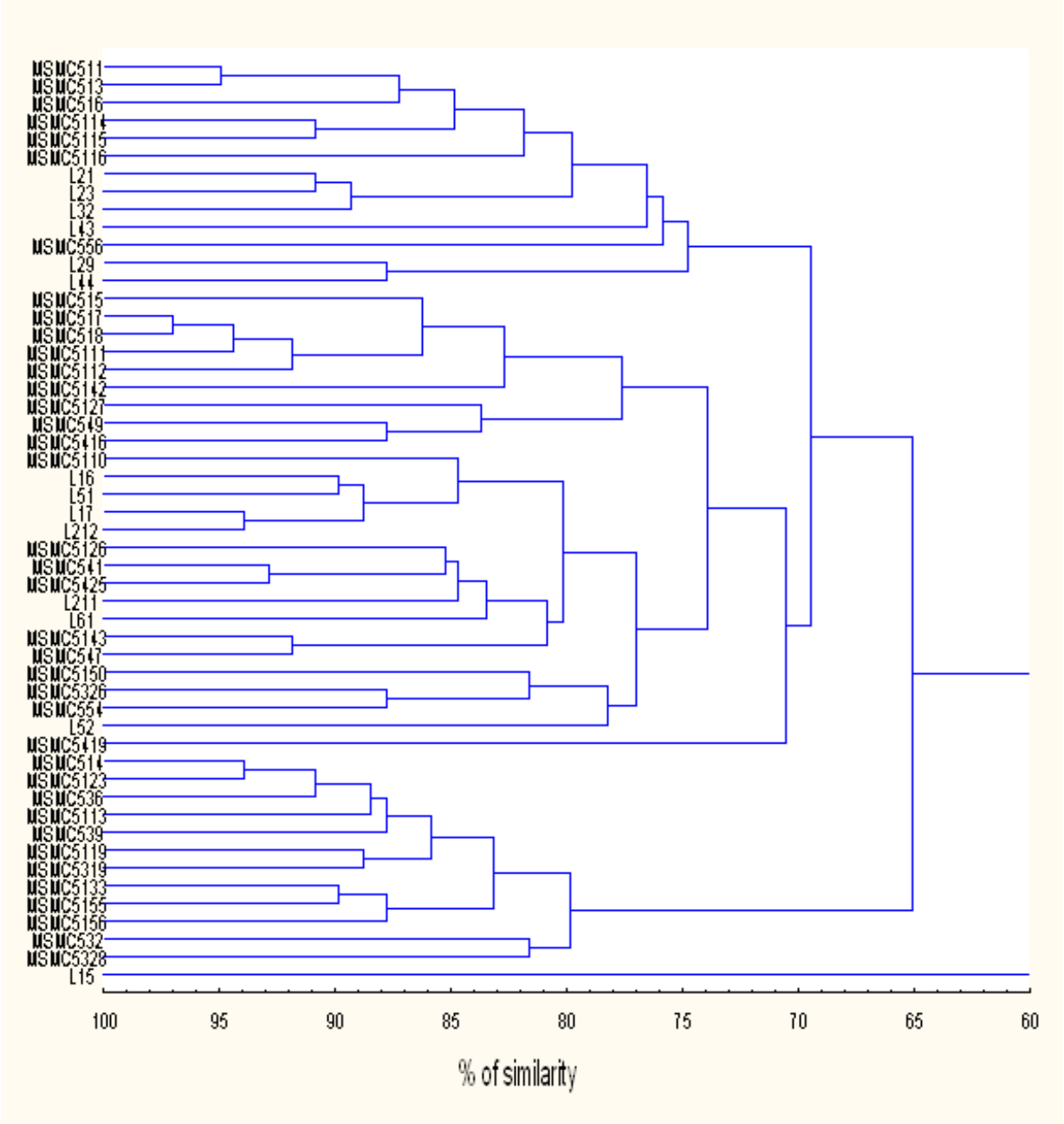
isolation from *L. albus* was done by trapping. A number of 159 strains were isolated from root nodules of the mentioned species.

The genetic diversity of the isolates was assessed by the DNA fingerprinting of the repetitive extragenic palindromic sequences (REP-PCR). The electrophoretic patterns of the PCR products were specific for each strain.

The dendrogram generated from the band pattern analysis showed eight major groups delimited at 70% of similarity. 52 representative strains, selected from these sub-groups, were considered for the phenotypic characterization (Fig. 1).

Based on REP analysis, 52 strains were selected for the physiological characterization. All of the Moroccan lupin isolates were fast growing (G < 3 hours) with 50% acid-producing. These cultural features are typical of the genus *Rhizobium* and do not correlate with previous studies which assigned symbionts of lupines to the genus *Bradyrhizobium.* More than 50% of the isolates were scored to have a positive reaction for urea hydrolysis and nitrate reduction.

Strains were very plastic with a marked tolerance to salt (0.85 to 1.53 M NaCl), pH (3 to 9.5 units) and temperature from 4?C to 42?C. They also showed a large resistance to heavy metals and antibiotics.



Fig?1: Dendrogram of 52 representative strains showing the genetic relationship between the strains based on REP analysis

The following numbers indicating the number of strains
ORIGINE of strains/ host species?:
Code Number MSMC5 and INRAL means lupin species
A number “1” added to code number Number means: *Lupinus luteus* (exemple: MSMC 51– INRA L 1)
A number “2” added to code number Number means: *Lupinus albus* (exemple: MSMC 52– INRA L 2)
A number “3” added to code number Number means: *Lupinus angustifolius* (exemple: MSMC 53– INRA L3)
A number “4” added to code number Number means: *Lupinus cosentinii* (exemple: MSMC 54– INRA L4)
A number “5” added to code number Number means: *Lupinus cosentinii* (exemple: MSMC 54– INRA L4)

A number “5” added to code number Number means: *Lupinus pilosus* (exemple: MSMC 54– INRA L4)
A number “6” added to code number Number means: *Lupinus atlanticus* (exemple: MSMC 54– INRA L4)

Nodulation test indicated that the strains were capable of inducing effective nodule formation on *L. luteus* species, which was used as a host-plant. While efficiencies of strains isolated from *L. luteus* varied considerably: 35% to 87%, most of the strains showed more than 60% N2 fixation efficiencies, relatively to the non-inoculated control fertilized with nitrogen (70 ppm of KNO3).

2. S?lection of lupin-rhizobia adapted to calcareous, alkaline soils

In Morocco the lupine crop is limited to sandy, acidic to neutral soil. However most agricultural rainfed areas have alkaline calcareous soils. In these areas protein sources come from faba bean and vetch. Faba bean is heavily infested whith Orobanche parasite causing yield losses of 15 % at the national level, and 25% to 80% in heavily infested field areas. More over, Vetch is always cultivated as a mixture in a high proportion of cereal crops (Thami Alami et.al., 2002). Under these circumstances lupine crop could be an alternative protein source in such areas. For that lupin and symbiotic bacteria must be selected.

Many trials in the field have been carried out on alkaline calcareous soil (pH KCl, 7.8; CaCO3 total 20%) in Morocco. Tested lupins species were: *L. Luteus* (Moroccan population), *L. albus* cv. Multolupa (INRA Morocco*), L. atlanticus* P 22927 (Australia collected in Morocco) and *L. Cosentinii* cv.Erregula (Australia). Results showed that not all species were nodulated. *L. atlanticus* and *L. cosentinii* grew without any chlorosis. *L.luteus* is more affected than *L.albus*.

The response of the same tested *lupinus* species to inoculation was tested on the same soil in a green house in Rabat (33?58?712 N, 6?51?687 W, 67m) on Alkaline calcareous soil. Four Selected Moroccan autochthones strains of lupinrhizobia were used for inoculation.

The result shows that inoculation had a positive effect on the growth of *lupinus* species (Fig. 2).

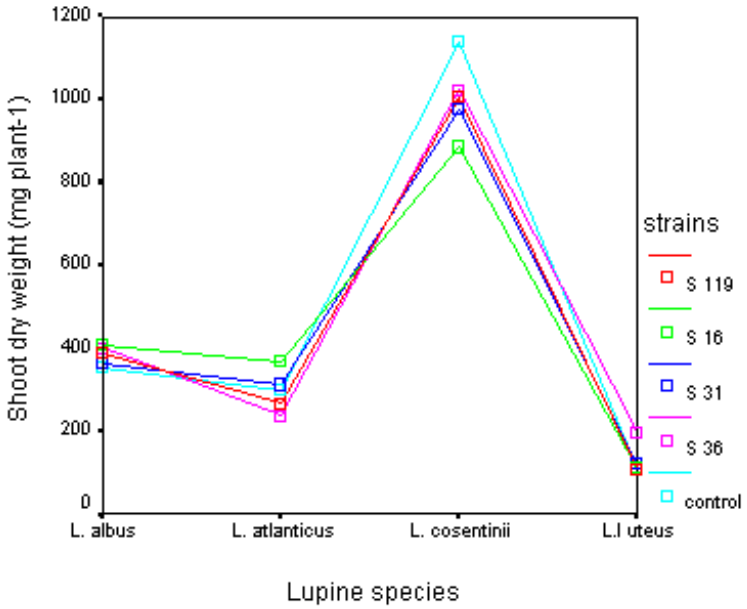


Fig 2: Response of *lupinus spp*. to inoculation

Conclusion and perspectives

Currently there is a gap in research concerning the symbionts of lupin species found in North Africa. In this work we studied some phenotypic characteristics and the genetic diversity of indigenous rhizobial strains associated with native lupin species in Morocco. Study on inoculation of lupins in calcareous alkaline soils permit us to extend the geographical growth area of this crop.

The three lupin species: *L. luteus, L. angustifolius* and *L. cosentinii* could play an important role according to each situation. They could profit from the same strategy of development taking into account the availability of sweet material.

The tolerance of some wild lupin species against some abiotic and biotic stresses in Morocco, such as drought, salinity, orobanche etc.., could be exploited to improve other legumes (sensitive) via gene transfer. In this context, biotechnology tools would be beneficial through plant transformation.

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