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# Alfalfa domestication history, genetic diversity and genetic resources

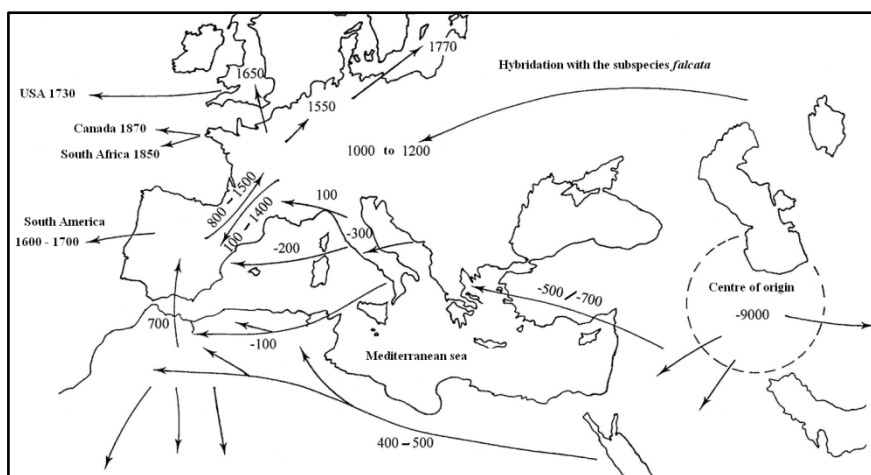
by Jean-Marie PROSPER<sup>1\*</sup>, Eric JENCZEWSKI<sup>2</sup>, Marie-Hélène MULLER<sup>1</sup>, Stéphane FOURTIER<sup>3</sup>, Jean-Paul SAMPOUX<sup>3</sup> and Joëlle RONFORT<sup>1</sup>

**Abstract:** The domestication history of alfalfa is poorly known. Here, we summarize recent results obtained from the investigation of the genetic diversity available in the *Medicago sativa* species complex, using different molecular markers and morphological characterization. We conclude that a large genetic diversity is still available in the wild form of the species, but original populations are restricted to a relatively small geographic area and in some instances submitted to gene flow from the cultivated form. A short description of the European genetic resources is presented.

**Key words:** diffusion routes, gene flow, genetic resources, molecular markers, morphological characterization, wild populations

## Domestication of alfalfa

It is difficult to identify the first traces of alfalfa (*Medicago sativa* L.) domestication. Different authors suggested that alfalfa was cultivated 9,000 years ago in some rare locations in its centre of origin (Near East to Central Asia). Sinskaya in 1950 suggested that its distribution spread throughout the Middle East by 1,000 BC, and from there, to China and India. But actually, we have no element to validate these assumptions. The first written references mentioned the introduction of alfalfa to Greece by the Medes armies, its spread into Italy and then all over the Roman Empire in Europe. During the Middle Ages, the interest in alfalfa regressed considerably in Europe.



**Figure 1.** The different routes and approximate dates of diffusion of cultivated alfalfa from its Centre of origin (the symbol (-) correspond to BC dates)

Alfalfa was reintroduced into Spain through North Africa with the Moors. It then crossed the Pyrenees around the fifteenth century. Thereafter, it was introduced to South America (Mexico, Peru, Chile) by the Spanish in the sixteenth century, and then to the United States through California at the beginning of the nineteenth century as 'Chilean clover'. These major paths of diffusion are summarised, along with a few minor ones, in Fig. 1 (6).

Feral populations of alfalfa are frequent in a variety of open habitats (roadsides, field borders), but wild forms of *M. sativa* subsp. *sativa* are rare, with the exception of the Iberian Peninsula and its centre of diversity. In other west Mediterranean countries (southern France, Italy, Greece, and North Africa), plants of *M. x varia*, are very common as a result of crosses between subsp. *sativa* and subsp. *falcata* populations. The *M. sativa* subsp. *falcata* appears more frequently as a wild species, from the north of the Mediterranean Sea (Bulgaria, Greece) to the northern limits of Russia. It is particularly well adapted to hard winters, as

well as to the hot and dry summers typical of continental climates (6, 7).

## Genetic diversity of the cultivated and wild pools of *Medicago sativa*

The evolutionary history of a wild-cultivated complex is the outcome of different factors, among which are the domestication of the cultivated form, the geographical and demographic expansion of the species, and the gene flow between natural and cultivated populations. To clarify alfalfa's history, we sampled several cultivars and landraces, natural populations of *M. sativa* subsp. *sativa* (diploid and tetraploid), as well as populations of the subsp. *falcata* and other perennial species. A special focus was made on the Spanish wild pool. Molecular markers (RAPD, nuclear DNA sequence polymorphism and mitochondrial DNA variation) and phenotypic characterization were used to measure the differentiation between cultivated and wild types and to quantify the importance of gene flow.

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From all these data, we noted:

(i) the marked originality of the wild pools, with specific characteristics such as prostrate habit, rhizomes, tolerance to drought and grazing (3, 7, 8);

(ii) a significant domestication bottleneck measured on nuclear DNA polymorphism, with a loss of diversity from wild to domesticated material of 30% on average (although more data are needed to strengthen this result). We detected no differentiation between diploid and tetraploid forms (3);

(iii) a high level of mtDNA variation, especially in the wild pool. In the wild pool from the presumed area of origin, the diversity was high but the absence of geographical differentiation in our sample hindered a more precise detection of the centre of domestication (4, 5 and Fig. 2);

(iv) in cultivated alfalfa, the geographical structure of mtDNA and nuclear DNA variation strongly suggests at least two independent routes (north and south) of dissemination from its centre of origin (3, 5);

(v) a specific mitochondrial type in the Spanish wild pool. That suggests an old divergence from the centre of origin and the fact that these populations were established in Spain before the introduction of cultivated alfalfa (5 and Fig. 2);

(vi) an occurrence of crop/wild gene flow in many locations in Spain, together with the persistence of wild Spanish populations showing a great agronomic and morphologic originality (1, 2, 7, 8).

Taken together, these results show that *M. sativa* contains a large genetic diversity and that there is probably much to gain from the study, the conservation and the use of the genetic variability available in the wild fraction of this species.

## Genetic resources and main gene banks

Numerous gene banks in the world exist that concentrate on *Medicago* (National Plant Germplasm System USA, Australian *Medicago* Genetic Resource Centre Australia, ICARDA Syria, Vavilov Institute Russia, etc.). For Europe, genetic resources of perennial *Medicago* species are maintained by many countries. Due to past exchanges of genetic materials among research institutes, accessions such as cultivars and landraces are frequently maintained in several gene banks.

Wild perennial species are generally only maintained by the institute that collected them. They are poorly represented or not represented in germplasm collections, especially those from the centre of origin of

the genus and from Asia. The diploid forms of the *M. sativa* complex species (i.e. subsp. *glomerata* and subsp. *coerulea*) are also under-represented in gene banks.

In order to promote the use and rationalise the maintenance of plant genetic resources in Europe, the European Cooperative Programme for Plant Genetic Resources (ECPGR: <http://www.ecpgr.cgiar.org>) was set up in 1980 and is now coordinated by Bioversity International.

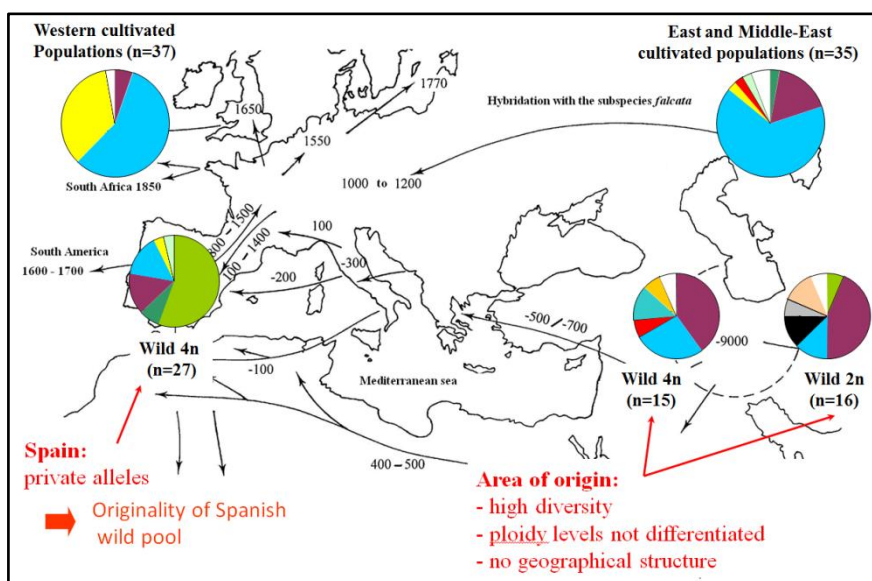
Within ECPGR, a working group on forage species brings together 37 countries. This working group has set up Central Crop Databases (CCDB) for several groups of forage grass and legume species. A part of these CCDB is centralized in the EURISCO database, a web-based catalogue which automatically receives the National Inventories of the gene banks from 42 European countries (<http://eurisco.ecpgr.org>).

The ECPGR Central Crop Database for perennial *Medicago* species references 7874 samples (a third from the Russian Federation) maintained over 25 European national gene banks. Some accessions are present as duplicated samples in several gene banks. A primary holder has been identified for each accession (i.e. a gene bank primarily responsible for the maintenance of the accession). Nineteen species are represented; some of them including several subspecies; but the tetraploid *M. sativa* represents more than 95% of the total. Referenced accessions include cultivars (1920 accessions), landraces (1430 accessions), wild or feral populations (769 accessions) and breeding materials

(1260 accessions). All together, these accessions represent the diversity originating from all parts of Europe. Information about the ECPGR Central Crop Database for perennial *Medicago* species can be obtained from the manager of this database ([http://www.ecpgr.cgiar.org/germplasm\\_data\\_bases/central\\_crop\\_databases/ECCDB\\_List.html](http://www.ecpgr.cgiar.org/germplasm_data_bases/central_crop_databases/ECCDB_List.html)). ■

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**Figure 2. Mitochondrial DNA variation between wild and cultivated pools of *Medicago sativa* in West and East Mediterranean regions. Each mitochondrial haplotype is in a different colour**