Medicago truncatula: a legume model-plant

Huguet T., Prosperi J.M.

in

Genier G. (ed.), Prosperi J.M. (ed.).
The Genus Medicago in the Mediterranean region: Current situation and prospects in research

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 18

1996
pages 171-175

Huguet T., Prosperi J.M. **Medicago truncatula: a legume model-plant.** In : Genier G. (ed.), Prosperi J.M. (ed.). *The Genus Medicago in the Mediterranean region: Current situation and prospects in research* . Zaragoza : CIHEAM, 1996. p. 171-175 (Cahiers Options Méditerranéennes; n. 18)

MEDICAGO TRUNCATULA: A LEGUME MODEL-PLANT

T. Huguet[1] and J.M. Prosperi[2]

ABSTRACT

As most of the legumes, plants belonging to the genus *Medicago* have the ability to associate with soil bacterias (essentially of the genus *Rhizobium*), an important 'plant/soil microorganims' interaction with regard to forage productivity and reduced fertilizer inputs facilities. Due to its high genetic complexity, the cultivated alfalfa (*M.sativa* L.) do not suit to the genetic studies which are required to understand the mechanisms underlying this symbiosis. The model-plant species actually used to develop such studies is the annual, diploid and autogamous species *M.truncatula.* It is shown how, thank to its genetic, biotechnologic, as well as agronomic potential, this species can be used for multidisciplinary approaches (biodiversity, plant-microbe interactions analyses, gene expression, mutagenesis, genetic mapping, agronomic improvement).

Key words: *Medicago,* plant-model, symbiosis, gene mapping, molecular markers, diversity

The genus *Medicago* comprises forage species of high symbiotic nitrogen fixation potential and high protein productivity. The most well known perennial *Medicago* is the cultivated alfalfa (*M.sativa* L.) of world wide economic importance. This species is, together with clovers, the major resource in forage legumes. Alfalfa is cultivated in many places in the world: in the 80th, more than 33 millions of hectares were cultivated mostly in USA (13 millions) and in Europe (8 millions) (Michaud *et al.* 1988). Annual *Medicago* (medics) are all originating from the Mediterranean basin and their agronomical interest rely with their capacity to establish perennial installations well adapted to the variation of climate frequent in the Mediterranean areas.

Plants belonging to the genus *Medicago,* as most of the legumes, have the capacity to associate with soil bacterias, essentially of the genus *Rhizobium,* forming at the surfaceof their roots specialized organs, the so-called nodules, in which bacterias reduce atmospheric nitrogen in ammonia which is, in turn, assimilated by the plant for its growth and development (Long, 1989). The understanding of the mechanisms which rule this symbiosis is a major objective for reducing the inputs of fertilizers and/or to increase the forage productivity, especially regarding marginal lands submitted to drought, salinity or low temperatures. Also, the understanding of symbiosis would allow to control the interactions between plants and soil microorganisms, pathogens or not. Since a simultaneous study of the two symbiotic partners is obligatory, the choice of the plant partner is thus critical because this species would be able to be used in multidisciplinary programs.

Alfalfa (*M.sativa* L.) is autotetraploid, allogamous and show a strong inbreeding depression; this high genetic complexity make genetic studies difficult and has, up to now, limited progress as well in fundamental research programs as in plant breeding. As a consequence, alfalfa cannot be used as model-plant.

Medicago truncatula as a model-plant

M.truncatula is an annual, diploid (2n = 16) and autogamous legume having a relatively small size genome (0.49-0.57 pg/1C, Blondon *et al.*, 1994). It is able to be transformed by *Agrobacterium tumefaciens* and to regenerate *via* somatic embryogenesis (Thomas *et al.*, 1992; Chabaud *et al.*, 1995) giving the opportunity to combine molecular and genetical approaches. In addition, its

---

[1] Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes, CNRS-INRA, BP 27, 31326 Castanet-Tolosan cedex, France.

[2] Laboratoire de Ressources Génétiques et d'Amélioration des Luzernes Méditerranéennes, Station de Génétique et d'Amélioration des Plantes, INRA Montpellier, Domaine de Melgueil, 34130 Mauguio, France.

microsymbiont, *Rhizobium meliloti*, is biochemically and genetically well known: many bacterial mutants are available.

For all these reasons, *M.truncatula* is presently used as a model-plant (Barker *et al.*, 1990) by an increasing number of laboratories working in quite different domains. We are going to summarize some of them.

A. Biodiversity

According to Lesins and Lesins (1979), annual species of *Medicago* derived from perennial ancestors at the end of the Tertiary era. The selfing species *M.truncatula* can be found all around the Mediterranean basin as native populations which have a good tolerance to drought and salinity and grow in a wide range of soils and environmental conditions: 290 polymorphic populations have been sampled by the INRA laboratory of Montpellier since 1985. A high level of variability of quantitative, qualitative and molecular characters can be observed among and within natural populations (Chaulet and Prospéri, 1994; Bonnin *et al.*, 1995). However, relatively few is still known about the biodiversity of these populations regarding biological characters.

B. Plant-Microbe interactions

M.truncatula is nodulated by *Rhizobium meliloti* which is the most studied *Rhizobium* in the world; many mutants are available and the bacterial nodulation factors (sulfated lipooligosaccharides) essential for *Rhizobium*-legume symbiosis have been purified and characterized (Dénarié and Cullimore, 1993). The plant receptors for these *R.meliloti* nodulation factors are under study (Bono *et al.*, 1995).

M.truncatula show a strong cultivar x *Rhizobium* strain specificity (Snyman and Strijdom,1980). About one strain out of three, taken from a laboratory collection of 37 wild-type strains of *R.meliloti*, reveal polymorphism in *M.truncatula* natural populations. The observed polymorphism go from the lack of visible nodulation to the formation of uncompleted, rudimentary nodules. This nodulation polymorphism can also be observed in natural populations. The genetic and physiological basis of this plant and bacterial polymorphisms are not yet known.

It is noteworthy that *M.truncatula* is very sensitive to nitrate: the nodulation by *R.meliloti* and the symbiotic nitrogen fixation are inhibited for concentrations of nitrate of about 1mM (Harper and Gibson,1984).

C. Gene expression

A number of genes related to *Rhizobium*-legume symbiosis have already been isolated and sequenced: leghemoglobin (Gallusci *et al.*, 1991), glutamine synthetase (Stanford *et al.*, 1993), lectines (Bauchrowitz *et al.*, 1992) and nodulins expressed early during symbiosis (Pichon *et al.*, 1992; Crespi *et al.*,1994; Wilson *et al.*, 1994).

The possibility to transform *M.truncatula* using vectors derived from *Agrobacterium tumefaciens* and to regenerate transgenic plants via somatic embryogenesis give the opportunity to study the spatio-temporal expression of genes by testing the expression of constructs associating the promotor of a gene of interest with the coding sequence of a reporter gene (Jefferson *et al.*, 1987). Such method is now routinely used and has allowed to show, in *M.truncatula*, that genes coding for early nodulins are activated after addition of purified nodulation factors (Chabaud *et al.*, 1995).

D. Mutagenesis

Mutants are very important tools to analyse the different steps of a complex developmental process like the *Rhizobium*-legume symbiosis. The variety Jemalong have been mutagenised, using either chemical mutagens or gamma-rays irradiation, in INRA (Dijon, France). The screening is done on the capacity of potential mutants to grow on nitrogen-free mediums using the symbiotic nitrogen fixation as nitrogen source. Plants belonging to the following classes of mutants: non-nodulating (Nod$^-$), nodulating non-fixing (Nod$^+$ Fix$^-$) and supernodulating plants (Nod^{++}) have been isolated and tested (Sagan and Duc, 1995). A mutant deficient for nitrogen fixation was studied in details; it is characterized by the lack of bacteroid development and by defence reactions during *Rhizobium* infection (Bénaben *et al.*, 1995).

E. Genetic map

A genetic map is a powerful tool for understanding the organization and evolution of genomes. 4 F1 hybrids, resulting from crosses between homozygous individuals of 4 populations (1 French, 2 Algerians and 1 variety Jemalong) have been selected to be the starting points of the *M.truncatula* genetic mapping program. Following the method of single-seed-descent, F7 populations of about 200-300 recombinant lines from each F1 plant are expected to be obtained in 1997.

The genetic map of *M.truncatula* is presently under progress using the F2 progeny of two crosses; molecular, symbiotic and morphological markers will be mapped.

a) Molecular markers will comprise RAPD (Williams *et al.*, 1990), CAPS (Koniecny and Ausubel, 1993), isoenzymes and microsatellites. At this moment, 76 RAPD markers have been mapped and 8 linkage groups identified covering 540 cM.

b) The mapping of the genes involved in the *Rhizobium*-legume symbiosis will involve the natural symbiotic polymorphism and the induced mutants (in collaboration with Dr. Duc, Plant Breeding Station, INRA Dijon, France).

c) Agronomically important characters such as nitrogen fixation efficiency, cold tolerance, disease resistance or osmotic stress tolerance will be mapped taking advantage of the high level of polymorphism observed in *M.truncatula* populations.

F. Agronomy

M.truncatula, as all *Medicago* annual species is native from the Mediterranean basin and has an high agronomical potential. It is cultivated to avoid soil erosion, improve soil fertility and as a source of winter forage. Their agronomical interest rely in their capacity to reseed spontaneously from one year to another because of the presence of an high proportion of hard seeds allowing a perennial installation well adapted to the climatically variable Mediterranean environments. *M.truncatula* is one of the species most cultivated especially in Australia. An important effort of selection is conducted for many years in Australia where annual medics are used in the ley-farming system of Cereal-Legume cultivation; several varieties of *M.truncatula* are commercially available. In France, annual medics are used for restoring soil fertility in marginal lands and as a source of winter forage. The selection of annual *Medicago* to introduce them in Mediterranean agriculture is under progress since 1985 at INRA, Montpellier, France (Prospéri, 1989).

M.truncatula: perspectives

Because of its genetic and agronomic potential, *M.truncatula* can be used for multidisciplinary approaches linking quite different scientific and applied domains. In addition, many natural populations show characters of agronomical interest that it should be necessary to study in order to be able to introduce them in cultivated alfalfa using genetic engineering.

REFERENCES

Barker D.G., Bianchi S., Blondon F., Dattée Y., Duc G., Flament P., Gallusci P., Génier G., Guy P., Muel X., Tourneur J., Dénarié J., Huguet T.(1990). *Medicago truncatula,* a model plant for studying the molecular genetics of the *Rhizobium*-legume symbiosis. *Plant Mol. Biol. Rep.*, 8, 40-49.

Bauchrowitz M., Barker D.G., Nadaud I., Rouge P., Lescure B. (1992). Lectin genes from *Medicago truncatula. Plant Mol. Biol.*, 19, 1011-1017.

Bénaben V., Duc G., Lefebvre V., Huguet T. (1995). TE7- An inefficient symbiotic mutant of *Medicago truncatula* Gaertn cv. Jemalong. *Plant Physiology,* 107, 53-62.

Blondon F., Marie D., Brown S., Kondorosi A. (1994). Genome size and base composition in *Medicago sativa* and *M.truncatula* species. *Genome,* 37, 264-270.

Bonnin I., Huguet T., Ghérardi M., Prospéri J.M., Oliviéri I. (1995). High level of polymorphism and spatial structure in a selfing plant species *Medicago truncatula* Gaertn. using RAPDs markers. *American Journal of Botany.* (in press).

Bono J.J., Riond J., Nicolaou K.C., Bockovich N.J., Estevez V.A., Cullimore J.V., Ranjeva R. (1995). Characterization of a binding site for chemically-synthesized lipo-oligosaccharidic NodRm factors in particulate fractions prepared from roots. *Plant J.*, 7, 253-260.

Chabaud M., Larsonnaud C., Marmouget C., Huguet T. (1995). Transformation of Barrel Medic (*Medicago truncatula* Gaertn.) by *Agrobacterium tumefaciens* and regeneration via somatic embryogenesis of transgenic plants with the MtENOD12 nodulin promoter fused to the GUS gene. *Plant Cell Reports* (in press).

Chaulet E., Prospéri J.M. (1995). Genetic diversity of *Medicago truncatula* Gaertn collected in Algeria. in *Proceedings of the 7th Genetic Resources section meeting of Eucarpia.* March 1994, Clermont-Ferrand. Eds INRA.

Crespi M.D., Jurkevitch E. Poiret M., d'Aubenton-Carafa Y., Petrovics G., Kondorosi E., Kondorosi A. (1994). enod40, a gene expressed during nodule organogenesis, codes for a non-translatable RNA involved in plant growth. *The EMBO Journal,* 13, 5099-5112.

Dénarié J., Cullimore J. (1993). Lipo-oligosaccharide nodulation factors: a minireview. New class of signaling molecules mediating recognition and morphogenesis. *Cell,* 74, 951-954.

Gallusci P., Dedieu A., Journet E-P., Huguet T., Barker D.G. (1991). Synchronous expression of laeghemoglobin genes in *Medicago truncatula* during nitrogen-fixing root nodule development and response to exogeneously supplied nitrate. *Plant Mol. Biol.*, 17, 335-349.

Harper J.E., Gibson A.H. (1984). Differential nodulation tolerance to nitrate among legume species. *Crop Science,* 24, 797-801.

Jefferson R.A., Kavanagh T.A., Bevan M.W. (1987). GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *Embo J.*, 6, 3901-3907.

Koniecny A., Ausubel F.M. (1993). A procedure for mapping *Arabidopsis* mutations using codominant ecotype-specific PCR-based markers. *The Plant Journal,* 4, 403-410.

Lesins K.A., Lesins L. (1979). Genus *Medicago.* A taxogenetic study. Dr. W.Junk bv Publishers.

Long S.L. (1989). *Rhizobium*-legume nodulation: life together underground. *Cell,* 56, 203-214.

Michaud R., Lehman W.F., Rumbaugh M.D. (1988). World distribution and historical development. In *Alfalfa and alfalfa improvement.* 25 - 92. Madison (USA). Hanson, A.A., Barnes, D.K., et Hill, R.R. Editeurs, Pichon M. Journet E-P, Dedieu A., de Billy F., Truchet G. Barker D.G. (1992). *Rhizobium meliloti* elicits transient expression of the early nodulin gene ENOD12 in the differentiating root epidermis of transgenic alfalfa. *Plant Cell*, 4, 1199-1211.

Prospéri J.M. (1989). Selection of annual medics for French Mediterranean regions. In *Workshop on introducing the ley farming system to the Mediterranean basin.* 173-191. Perugia (Italy). Eds. S. Christiansen, L. Materon, M. Falcinelli et P. Cocks.

Sagan M., Duc G. (1995). Selection of symbiotic mutants of Medicago truncatula after gamma-ray mutagenesis. In "*Proceedings of the 10th International Congress on Nitrogen Fixation*" Saint Petersburg, Russia.

Snyman C.P., Strijdom B.W. (1980). Symbiotic characteristics of lines and cultivars of *Medicago truncatula* inoculated with strains of *Rhizobium meliloti*. *Phytophylactica*, 12, 173-176.

Stanford A.C., Larsen K., Barker D.G., Cullimore J.V. (1993). Differential expression within the glutamine synthetase gene family of the model legume *Medicago truncatula*. *Plant Physiol.*, 103, 73-81.

Thomas M.R., Rose R.J., Nolan K.E. (1992). Genetic transformation of *Medicago truncatula* using *Agrobacterium* with genetically modified Ri and disarmed Ti plasmids. *Plant Cell Rep.*, 11, 113-117.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A., Tingey S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.*, 18, 6531-6535.

Wilson R.C., Long F., Maruoka E.M., Cooper J.B., (1994). A new proline-rich early nodulin from *Medicago truncatula* is highly expressed in nodule meristematic cells. *Plant Cell* 6, 1265-1275.