Between and within species variation in annual Medicago species

Fyad-Lameche F.Z., Bellatar G., Bouabdallah S., Yahia N.

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 161-169

BETWEEN AND WITHIN SPECIES VARIATION IN ANNUAL *MEDICAGO* SPECIES

F.Z. Fyad-Lameche, G .Bellatar, S. Bouabdallah and N. Yahia[1]

ABSTRACT

Genetic variability of annual *Medicago* species was examined with respect to allozyme, SDS-PAGE seed storage proteins, pollen germination, seed coat hardness, heading date and cold hardiness one hundred accessions of ten species.

The allozyme electrophoretic analysis leads us to the conclusion that all the species studied were very homogenous. Only two cases of within species variation were clearly demonstrated. It concerned LAP system for *M.scutellata* species and the GOT system for *M.polymorpha*. Hypothesis concerning the genetic control of enzymatic systems are formulated. The SDS-PAGE seed storage study showed few polymorphism but the number of bands is relatively high. The patterns were specific for each species, difference is rather qualitative which means that almost all the bands were different from one species to another one. The variation within species concerned minor bands with high molecular weight. Some cases of bands encoded by a single locus have been found.

The data obtained on the pollen grain germination and viability rates showed a wide range of variation at all levels (species, populations or flowers under one plant). The seed coat hardness have been assessed by seed germination rates. Six seed morphological traits (weight, length, color, width, seed coat aspect) suspected to be related to seed coat hardness have been measured and the data obtained showed a wide variation. The variation of heading date have been assessed by a randomised block design. Some growth traits have been measured on the plant for suspected interrelations with heading date.

Key words: *Medicago*, genetic diversity, electrophoresis, seed storage proteins, medics, hard seeds, agronomical traits.

INTRODUCTION

Genetic variability assessment of annual Medicago species was examined with respect to allozyme, SDS-PAGE seed storage proteins, grain pollen germination and viability rates, seed coat hardness, heading date, and cold hardiness variation on ten species and about one hundred accessions.

MATERIALS AND METHODS

1. Plant materiel

A study was performed on an accession on the basis of amount of available seeds.

The allozymes electrophoresis was conducted on the following accessions:

M.truncatula (Tru 309, Tru 697,Tru 764, Tru 763, Tru 642, Tru 663, Tru DZ 26A, Tru 448, Tru 2, Tru DZA-3153-6a, Jemalong), *M.aculeata* (Acu 141, Acu 150, Acu 97, Acu 234, Acu 366, Acu 355, Acu 149), *M.scutellata* (Scu 279, Scu 289, Scu 274, Scu 294, Scu 815, Scu Snail), *M.ciliaris* (M.cil, Cil DZ 13 C) (*M.sativa, M.arabica* (Ara 46 INA), *M.orbicularis* (Orbi DZ 12D, Orbi DZ 19 C, Orbi DZ 26 B, Orbi DZ 23 B, biancae, Orbi 240 INA), *M.polymorpha* (Poly DZ 9A, Poly DZ 15A, Poly DZ 21B (GN), Ply 4).

[1] Laboratoire d'Amélioration des plantes. Université d'Oran-Es-Sénia. Oran-Algérie.

The seed proteins SDS-PAGE concerned the species listed below and their corresponding accessions:

(*M.truncatula, M.scutellata, M.aculeata, M.ciliaris, M.sativa Orca), M.littoralis* (Harbinger), *M.polymorpha, M.orbicularis*, Hybride (LittxTru), *M.laciniata, M.minima, M.rigidula.*

The pollen grain study have been done only on species with long time flowering period, large size flower, easy tripping flower and high grain pollen amounts as *M.truncatula, M.orbicularis, M.polymorpha, M.ciliaris, M.arabica* and *M.rigidula.*

Seed coat hardness have been done on thirty accessions belonging to the following species: *M.polymorpha, M.orbicularis, M.minima, M.rigidula, M.intertexta, M.truncatula, M.ciliaris, M.laciniata, M.rotata, M.tornata* and *M.scutellata.*

Twenty accessions from *M.aculeata, M.polymorpha, M.truncatula* and *M.orbicularis* species have been examined for heading date.

Cold hardiness have been estimated on accessions from *M.truncatula, M.ciliaris, M.aculeata* and *M.polymorpha.*

2. METHODS

a) Allozyme study

The classical electrophoretic methods have been done on the following enzyme systems: (Leuceune-amino-peptidase (E.C.4.11.1.), Glutamine-oxaloacetate transaminase (E.C.2.6.1.1.), Aryl esterase (E.C.3.1.1.2.), Malate Deshydrogenase (E.C.1.1.1.37.), Aconitase (E.C.4.2.1.3.), ß-Amymylase (E.C.3.2.1.2.), Xanthine deshydrogenase (E.C.1.2.1.37.), Phosphoglucomutase (E.C.2.7.5.1.), Phosphoglucoisomerase (E.C.5.3.1.9.), Peroxydase (E.C.1.1.1.17.), Catalase (E.C.1.11.1.6.) which have been analysed on starch and polyacrylamide gel.

b) Seed storage proteins

The SDS-PAGE seed storage protein was performed according to Laemmli (1970).

Protein extraction procedures was performed according to Van Geyt (1986). Four proteins class were examined, total proteins, globulins, prolamins and glutelins.

c) Pollen germination

The pollen grain fertility was performed by in vitro germination and the viability tests were Alexander's and isatin staining methods.

d) Seed coat hardness

The seed coat hardness have been measured by stepped germination tests with repeated accessions control (Australian cultivars) at each essay.

e) Heading date variation

The heading date variation have been measured by a three replicated randomised block design.

f) Cold tolerance

Seedling tolerance to cold acclimatization have been done on seedlings lots with five individuals in each lot. For each accession two lots were examined one cold treated and the other not treated. In one test, all the accessions were represented by two lots of seedlings. Ten similar tests have been done at different times.

3. RESULTS

a) Allozyme variation

The data obtained showed very little polymorphism for all the enzymes studied. We observed often only one phenotype for one species. The populations of the same species having all the same single genotype. The most common situation was one genotype for one species.

In some cases the genetic control of some systems could be guessed through the phenotypes observed. Among all isozymes observed some of them could be used as marker for breeding because of their simple genetic control, their stability and the little number bands pattern.

The interspecies variation was well demonstrated by LAP isozymes where the slower band encoded by one locus, is species specific.

For the other remaining enzymatic systems the variation between species is not so clear.

The variation among species is very scarce and in almost cases all the individuals analysed from different accessions belonging to the same species were similar in their pattern.

Nevertheless some cases of within population variation have been observed.

The first one concerned the *M.scutellata* populations where the LAP1 products had two migration levels, P7 (Scu 274) and P19 (Scu 294) accessions having the higher one while P2 (Scu 279), P4 (Scu 289), and P17 (Scu 815) had the lower one.

The second case concerned G.O.T system in the accession Poly DZ 9A (*M.polymorpha*): Among the four extracts tested on the same gel, three were similar with two bands and the fourth one had two bands too but the slow moving band was the slowest one while the fast band was the most rapid one.

Some hypothesis were proposed concerning genetic control for some system.

LAP bands were coded by two loci, and were monomers.

The G.O.T bands were two loci products, and dimeric enzyme. The three M.D.H fixed bands resolved on starch gel, could be the expression of two duplicated loci perhaps linked, exchanging their allele products to form intergenic dimers. ß-Amylase bands were probably encoded also by two loci, and because of the occurrence of two bands in one of the two zones the enzyme is probably monomeric.

The Xanthine deshydrogenase patterns showed only one band at the same position for all the accessions observed.

Esterase profile were less easy to explain and more than two loci were probably involved in their genetic control.

For PGI, PGM and ACO the most common two banded phenotypes obtained could be du to the expression of two loci rather than one loci, the annual Medicago populations known to be monomorphic rather than polymorphic. In the case of two loci, the phenotypes observed were double homozygous, in the case of one loci the two banded phenotypes were heterozygous, this later proposition being less probable than the former.

b) SDS-PAGE seed storage proteins

In the case of total soluble proteins, the polymorphism even being little, it is nevertheless more evident than in the allozyme case.

The patterns were species specific, each species presented specific profiles. The middle part of the gel electrophoresis was occupied by almost only major bands characterizing each species.

The variation between species is rather qualitative, which means that almost all the bands were different from one species to the other, they were little number of common bands.

But between species closely related the common bands number is rather high. For example *M.truncatula* (Jemalong), *M.littoralis* (Harbinger) and the hybrid (Litt x Tru DZA 3175.2) had almost the same profile, only two or three major bands in the middle of the gel were different, all the others being similar.

The variation within species is less obvious and concerned in almost cases minor bands with high molecular weight. It is also of quantitative rather than qualitative nature.

The genetic variation conclusions were parallel for the different protein classes, the band number, intensity and thickness being different from one protein kind to the other.

As pointed out in literature, species close related present similar seed protein profile (e.g *M.truncatula* and *M.littoralis*).

Some cases of bands with a possible qualitative genetic control have been found:

- one major band with low molecular weight was absent in Harbinger (*M.littoralis*) and present in Jemalong (*M.truncatula*), and in the suspected hybrid (Litt x Tru DZA.3175.2) between the two species.

- In *Poly INA* accession (*M.polymorpha*) twelve seeds examined in the same gel, two major bands located in the middle of the gel presented a qualitative variation. The faster one was present in four seeds, absent in six seeds and the two remaining seeds presented two thin bands. The lower was absent in the four first seeds, present in the five (over six) following seeds and absent in the remaining three seeds.

This variation could be the expression of one locus, were each band is controlled by one allele and each band corresponding to a monomer.

On the globulin fraction gel one major band was present in the Hybride (*Litt x Tru DZA 3175.2),* in *Tru 28 INA* and *Tru DZ 15B* and absent in *Jemalong, Harbinger* and *Tru 448.*

The prolamine and gluteline patterns had less good resolution to be interpreted further.

c) Pollen grain germination

The pollen grain germination and viability rates have been measured on several species. The viability rate was measured by Alexander's method and proline-isatine staining.

The data obtained showed a large range of variation at all levels, among species, within species, within populations, and between flowers in the same plant. Because of the possible quantitative genetic control of this trait this variation could be explainable, by the environmental effect which could emphasize the phenotypic variation.

d) Chromosomic number and stomatic cell guard length

Chromosomic number and stomatic cells guard measurements have been achieved on all the materiel studied previously to verify the ploidy level of the species studied.

e) Seed coat hardness

The seed coat hardness have been assessed by seed germination rate on the following species, each of them represented by several accessions. Six seed morphological traits (weight, length, color, width, seed coat aspect) suspected to be related have been measured on fifty seeds per population.

Thirty seeds from them where randomly sampled and separated in three lots to be submitted (or not) to a treatment before germination.

The first seed lot was immersed in liquid nitrogen, the second one to sulfuric acid and the third served as control.

The experimental design adopted was a block design, with two repetitions.
Stepped germination tests were performed on three accessions and three controls at every essay.

The data obtained showed a large variation, for every morphological traits and for seed coat hardness. The species tested could be classified as follow according to their increasing order of their hard seeds rate (Table 1).

Table 1. Classification of the species according to their hard seeds rate

Species	Number of accessions studied	Order
M.ciliaris	(1)	1
M.minima	(2)	2
M.arabica	(1)	3
M.laciniata	(1)	3
M.orbicularis	(5)	3
M.polymorpha	(6)	4
M.tornata	(1)	5
M.truncatula	(4)	6
M.intertexta	(3)	7
[3]M.rigidula	(3)	8
M.scutellata	(1)	9
M.rotata	(1)	10

The within population variation for each character was important as expected according to the genetic control of such traits.

The correlation analysis between morphological characters and seed coat hardness gave a strong negative correlation between seed weight and seed coat hardness.

SDS-PAGE of seed coat protein did not show any polypeptide whatever was the seed coat removing procedure (after seed imbibition or removing by a razor blade on dry seed).

f) Heading date

The variation of heading date on five species and 25 accessions have been assessed by randomised block design performed at the I.D.G.C. experimental Station (SBA).

Some growth traits have been also measured on the same plant for interrelation with heading date. Only the ration L/l (length on width) of leaflet seems to be related to heading date.

g) Cold hardiness

The variation of cold hardiness have been evaluated by biometrical analysis at the seedling stage. The protein contents in treated and not treated seedlings were compared by SDS-PAGE. Qualitative and quantitative differences in protein profiles between cold treated seedlings and no treated seedlings were analysed.

DISCUSSION

The little polymorphism showed by the electrophoretic data could be explained on one hand by the mating system, in general outcrossing species were more variable than the selfcrossing species.

The selfcrossing species were known to be very homogenous. It also could be due to genetic drift, the repeated sampling could eliminate randomly the rare genes if the sample size is not adequate, the more is the elimination probability, the less is the sample size.

Selection could not account in this case because of the neutral selective nature of allozymes.

The protein variation could be partially adaptive as pointed out by several authors. Nevertheless the seed protein polymorphism remained reduced.

The species and the accessions could be well identified and less costly by this technique.

The quantitative characters as expected were more variable and the within and between species variation was more detectable.

For almost all characters with a quantitative genetic control breeding for increasing or reducing a given phenotypic value would be possible.

These results have not been completely exploited, interpreted and synthesized, and for some parts computing is still on.

The enzyme and protein polymorphism study was supported by a grant from the International Foundation of Science.

REFERENCES

Allot-Deronne M, Blondon F et Sandmeier M, 1986. Marqueurs enzymatiques et état induit de la feuille de *Perilla* ocymoïdes L. C.R. Acad.Sc. Paris, t 303, série III, n° 16, 683-688.

Antikaïnen M and Pihakaski S, 1993. Cold Induced Changes in the Polysome Pattern and Protein Synthesis in Winter Rye (Secale cereale) Leaves. Physiologia Plantarum. 89: 111-116.

Ermittage M.A, 1985. Effect of Leaf number, leaf position and node number on flowering time in hybride *Geranium.* J. Amer. Soc. Hort-Sci. 109 (2): 233-236.

Barnes D.K and Sockness B.A, 1987. Inheritance of red endosperm in alfalfa and its relationship to color, size and viability of seed. Crop. Sci. 27: 75-61.

Berna M.A, Diaz Jo, Ros Barcelo et Merino de Caceres F, 1984. Peroxidase isoenzyme gradients in *Capsicum annum* leaves and their expression in vegetative and flowering plants. Agronomie. (2), 205-210.

Beyaut R, 1988. Comparative study of two techniques for determining the viability of *Vitis vinifera* pollen. Notas technicas. Facultad de Agronomia. n° 5: 4 p.

Bloc D et Gonet J.P;1977. Influence des sommes de température sur la floraison et la maturité du Maïs. Ann. Amélior. plantes, 28 (1), 89-111.

Bosch LL, Casanas F and Nuez F, 1989. Synchronisation of flowering in maïze (*Zea mays* L.) by the genetic System pg11 pg12. Euphytica 41: 129-134.

Boyce K.G and Crawford E.J, 1971. Seed coat impermeability in annual *Medicago* cultivars grown at North-field, South Australia. Aus. Seeds. Res. 1(C): 14-45.

Chançan R; 1994. A time to flower. The Plant Cell. vol 6 (1-3).
Collins W.J and Wilson J.H, 1974. Node of flowering as an index of plant development. Ann. Bot. 38: 175-180.

Collins W.J and Wilson J.H, 1974. The effect of cotyledon excision on reproductive development in Pea (*Pisum sativum* L.). Ann. Bot. 38: 181-188.

Côme D et Semadeni A, 1973. *Dégazage des envelopppes* séminales lors de leur imbibition. III: Application à l'étude de la dureté des graines d'*Hedysarum coronarium* L. Physiol. vég. 11(1): 171-177.

Coulbier Y, 1983. Changes in the electrophoretic patterns of the soluble proteins of Winter wheat and rye following cold acclimation and desiccation stress. Plant Physiol. 71: 400-403.

Crawford E.J, 1977. Seasonal changes in seed coat permeability of 155 lines of *Medicago truncatula* Gaertn under natural field conditions. Aus. Seed. Res. Conf. 1(C): 16-17.

Greppin H and Penel C, 1991. Peroxidase photocontrol and flowering process in Biochemical, Molecular and Physiological aspects of Plant Peroxydases. Lbarzeruski J, Greppin H and Pernel C ed.

Grimm S.S, Jones J.W, Boote K.J and Heskcth D.J, 1993. Parameter estimating for predicting flowering date of Soybean cultivars. Crop. Sc 33: 137-144.

Gupta R.K and Singh S.P, 1990. Genetic analysis of earliness and its inheritance in *Carthamus tinctorius* L. Trans. ISDT, 15: 109-116.

Guasselly Ch., 1978. Observations sur l'utilisation d'un mutant d'amandier à floraison tardive dans un programme d'hybridation. Ann. Amélior. plantes. 28 (6) : 685-695.

Guy C.L, Hummel R.L and Haskell D, 1987. Induction of freezing tolerance in Spinach during cold acclimation. Plant Physiol. 84: 868-871.

Guy C.L, and Haskell D., 1987. Induction of freezing tolerance in Spinach is associated with the synthesis of cold acclimation induced proteins. Plant Physiol. 84: 872-878.

Guy C.L, Haskell D and Yelenosky G., 1988. Changes in freezing tolerance and polypeptide content of Spinach and *citrus* at 5°C. Cryobiology. 25: 264-271.

Guy C.L, and Haskell D., 1989. Preliminary characterization of high molecular mass protein associated with cold acclimation in Spinach. Plant Physiol. Biochem. 27 (5): 777-784.

Guy C.L., 1990. Cold Acclimation and freezing stress tolerance: Role of protein metabolism. Annu. Rev. Plant. Physiol. Plant Mol. Biol. 41: 187-223.

Egley G.H., 1979. Seed Coat impermeability and germination of Showy crotalaria (*Crotalaria spectabilis*) seeds. Weed. Sci.vol 27. n° 4: 355-361.

Hill J and Kowithayakorn L., 1982. A study of influence seed development and some aspects of hard seed content. Seed. Sci. Technol. 10: 179-186.

Holdsworth M., 1956. The concept of minimum leaf number. Journal of Experimental Botany. vol 7, n° 21 p395-409.

Joudrier A., 1985. Le polymorphisme biochimique et l'hérédité des protéines végétales. In "Les protéines végétales" Godon D. coordonateur. Technique et documentation Lavoisier.

Karège F., 1981. L'activité péroxydasique: indicateur de floraison et d'inter-relations organiques chez *Spinacia oleracea*. Thèse Es Science n° 1948, Geneva, 194p.

Kurkela S, Frank M, Heino P, Lang V, and Palva E T., 1988. Cold Induced gene Expression in *Arabidopsis thaliana* L. Plant Cell Reports. 7: 495-498.

Kazan K, Muehlbauer F.J, Weeden N.F and Ladizinsky G., 1993. Inheritance and linkage relationships of morphological and isozyme loci in chickpea (*Cicer arietinum* L.). Theor. Appl. Genet. 86: 417-426.

Ladizinsky G and Hymowitz T., 1979. Seed protein electrophoresis in Taxonomic and Evolutionary Studies. Theor. Appl. Genet. 54: 145-151.

Lesins K.A and Lesins I., 1979. Genus *Medicago* (*Leguminosae*). A taxogenetic study. Dr W. Junk Publishers. The Hague. Ntherlands. p 21-28.

Lin M.S and Nelson R.L., 1988. Effect of plant height and flowering date on seed yield of determinate soybean. Crop. Sci, 28: 218-222.

Malvoisin P., 1984. Organogénèse et croissance du maitre-brin du blé tendre (*Triticum aestivum*) du semis à la floraison. In: Relations observées entre la croissance foliaire et la différenciation des ébauches foliaires ou florales. Agronomie, 14 (b) : 557-564.

Marelin P., 1992. Les maladies physiologiques du froid. In "Les Végétaux et le froid" par Côme D. Hermann ed. Coll. Methodes. pp. 58-105.

Mathison M.J., 1971. Seed coat impermeability attributes in annual *Medicago*. Aus. Seed. Res. Conf. 12-13.

Miège J et Mascherpa J.M., 1979. Etude des surfaces tégumentaires des graines de quelques *Phaseoleae*: Méthodologie. Conservatoire et Jardin Botanique de Genève. 34(1) : 87-97.

Murfet I.C., 1990. Flowering genes in Pea and their use in Breeding. *Pisum* Newsletter. vol. 22: 76-86.

Munoz S, Perez M and Gaballeros., 1986. Phenology a method for predicting and selecting precocious genotypes in *Carica papaya*. Ciencia y técnica en la agricultura, Cítricos y otros Frutales. 9 (4): 133-143.

Maux G.A., 1975. The "Le" locus: Its influence on flowering time. *Pisum* Newsl?", (7): 30-31.

Mohapatra S.S, Pade R.J, and Dhindsa R.S., 1987. Changes in protein patterns and translatable messenger RNA populations during cold acclimation of alfalfa. Plant Physiol. 84: 1172-1176.

Murfet I.C., 1990. Fowering genes in Pea and their use in breeding. Pisum Newsletter. Vol. (22): 76-86.

Nelson R.L., 1988. Response to selection for time of flowering in Soybean. Crop. Sci, 28: 623-626.

Neven L.G, Haskell D.W, Hofiz A, Li Q.B, Guy C.L., 1993. Characterization of a Spinach gene response to Low Temperature and Water Stress. Plant Molecular Biology. 21: 291-305.

Ort D.R, Martino S, Wise R.R, Kent J and Cooper P., 1989. Changes in Protein Synthesis Induced by Chilling and their Influence on the Chilling Sensitivity of Photosynthesis. Plant Physiol. Biochem. 27 (5): 785-793.

Ouellet F, Houde M and Sahran F., 1993. Purification, Characterization and cDNA Cloning of the 200 kDa Protein Induced by Cold Acclimation in wheat. Plant Cell Physiol. 34(1): 59-65.

Paton D.M., 1978. Node of flowering as an index of Plant development; A further examination. Ann. Bot. 42: 1007-1008.

Quiros C.F and Morgan K., 1981. Peroxydase and leucine aminopeptidase in diploid *Medicago* species closly related to Alfalfa. Theor. Appl. Genet. 60: 221-228.

Ramina A, §Hachett P, and Sachs R.M., 1979. Flowering in Bougainvillea. Plant Physiol. 84: 810-813.

Rod Casey, Claire Domoney and Noel Ellis., 1986. Legume storage proteins and their genes. Oxford Surveys of plant molecular and cell Biology. Edited by B.J Miflin. Vol 3: 1-95.

Sahran F and Perras M., 1989. Synthesis of Freezing Tolerance Proteins in Leaves, Crown and Roots During Cold Acclimation of Wheat. Plant Physiol. 89: 577-585.

Salisbury P.A and Halloran G.M., 1983. Inheritance of rate of breakdown of seed coat impermeability in subterranean clover (*Trifolium subterraneum* L.) Euphytica. 32: 807-819.

Thevenot C., 1985. Problèmes physiologiques posés par les obstacles à la germination: Dormance embryonnaire et inhibition tégumentaire. Le sélectionneur français. 35: 13-21.

Tostain S et Riandev M.F., 1985. Polymorphisme et déterminisme génétique des enzymes du Mil pénicillaire (*Pennissetum glaucum*). Etude des malates déshydrogénases. Agronomie 5(3): 227-228.

Paquin R., 1985. Survie à l'hiver des Plantes fourragères et des céréales sous les climats nordiques, en particulier au Quebec : Progrès et Prospectives. Phytoprotection. 66: 105-139.

Volodin V.I and Muzalevskaya R.S., 1988. Method of selecting early forms of plants, mainly *Vicia sativa*. From Referativnyl Zhiernal. 12. 65. 271.

Watts L.E, Stevenson E and Crampton M.J., 1970. Inheritance of flowering time in six Pea cultivars (*Pisum sativum* L.) Euphytica. 19: 405-410.

Yu Y, Bryrne T and Harding J., 1991. Quantitative genetic analysis of flowering time in the Davis population of Gerbera I: Components of genetic variance and the heritability. Euphytica. 53: 19-23.

Yu Y, Bryrne T and Harding J., 1991. Quantitative genetic analysis of flowering time in the Davis population of Gerbera II: Correlation with Yield, flower, quality traits and the efficiency of indirect selection. Euphytica. 70: 97-103.

Zoghlami A., 1988. Etude de la variabilité génétique chez la luzerne *Medicago sativa* L. pour la réponse d'Automne, la sensibilité à l'hiver et le démarrage au Printemps. Thèse de Doctorat 3ème cycle. Institut Agronomique Méditerranéen de Saragosse, 152 p.