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GENETIC DIVERSITY INVENTORY AND VALUATION OF SPONTANEOUS SPECIES BELONGING TO *MEDICAGO* L. GENUS IN TUNISIA

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ABSTRACT

The increasing narrowness of the rural space, the very frequent overgrazing and the effect of human activities lead to a very quick natural vegetation damage and to genetic erosion. This type of regressive evolution has been pointed out in Tunisia. A prospecting program was initiated in 1984 in order to collect species belonging to *Medicago* genus. Their distribution areas linked to rainfall and soil parameters is reported and their agronomic description proposed, some aspects of floral biology and fecundation traits are developed.

The study of six polymorphic loci enable us to specify the natural reproduction biology of *Medicago ciliaris* and *Medicago intertexta*. As far as *Medicago orbicularis* is concerned, electrophoretic variability analysis of eight isoenzymes show them strictly homozygous in twelve populations and confirm the highly autogamous regime of this species; while the same isoenzymatic analysis reveal an important polymorphism for *Medicago polymorpha* and at a lower level for *Medicago truncatula*.

At end, facultative autogamy, as well as aptitude to propagate by vegetative way appears as adaptive ways for the perennial *Medicago sativa* ssp. *tunetana* for which the isoenzymatic polymorphism analysis reveal a continuous variability range within the prospected areas.

Key words: *Medicago*, genetic diversity, isoenzymes, electrophoresis, natural populations

1. INTRODUCTION

Tunisia, well as whole the maghreb countries, had traditionally known pastoralism which was well integrated to rural space. This activity was governed by communal rules which took into account environment potentialities and precariousness. But demographic growth and socio-economic mutations that occurred during this century exert more and more huge pressures on environment and natural vegetation: "it is indeed cultivation which, much more than nomadic life, ruined natural vegetation" (Despois, 1955). The increasing narrowness of the space reserved to course and keeping high sheep population densities in a precarious ecosystem, lead to a very quick natural vegetation damage and to a genetic erosion. Course spaces are hence threatened of becoming desert. A large panel of different arrangements should be taken to cope with this difficult critical situation.

Local phytogenetic resources inventory and safeguard constitute important elements of the solution. Annual species of *Medicago* genus can be used along with the whole autochton flora to improve damaged courses and to substitute fallows by a Medic-cereal biennial rotation. *Medicago sativa* ssp *tunetana*, spontaneous and perennial forms, can, on one hand contribute to improve primary production of courses in semi-arid area, and on the other hand participate to increase the variability range of cultivated lucern.

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PROSPECTING AND DISTRIBUTION AREA

A program was initiated in 1984 in order to prospect and collect species belonging to *Medicago genus*. Prospectings have been achieved at different periods of the year in order to follow the different phenologic stages of plants and to properly determine the species at the botanical level.

Finally, to better surround the largest variability range, various areas distinguished by their vegetation, type of soil climate have been explored. The collect work has been completed by a cotified description of the different characteristics of the medium.

About 160 samples of pods belonging to 17 annual species have been harvested and conserved under appropriate temperature and humidity conditions.

Eleven populations of the perennial species *Medicago sativa* ssp *tunetana*, have been indexed in refuge areas located in the semi-arid bioclimatic stage including its lower and upper variants with fresh winter.

A lining collection has been set up in the Tunis region starting from cuttings derived from different populations of this species.

Study of species distribution as a function of soil types (according to French soil classification) did not reveal this factor as a discriminating element in this distribution. On the contrary, draining quality, soil pH and salinity as well as altitude are factors which act on species distribution.

The different index link species occupying natural areas of variable importance.

M. tornata, M. murex, M. rugosa and *M. sculleta* have limited distribution areas. M. *orbicularis, M. intertexta, M. ciliaris* form a particular group. The distribution area of this group extends from the humid to the semi-arid stage.

A second species group comprising M. *laciniata* and M. *minima* has as distribution area the semi-arid and the saharian stages. M. *polymorpha*, M. *litoralis* cover a wide spectrum of bioclimatic stages going from the humid to the upper arid. M. *truncatula* is found from the humid to the saharian stages.

M. laciniata is adapted to weak rainfall and to arid soils. It is a species characteristic of steppe and hamada dry grounds. M. *litoralis* is adaptable to a wide rain fall range. It is frequent in sandy soils, it is abundant on sandhills. M. *truncatula* is found under all ruinfalls. M. *polymorpha* and M. *ciliaris* tolerate weak soil salinity and draining.

At each bioclimatic stage, a preferential association of annual species exists.

However, the semi-arid stage seems to be at the junction between the whole described species.

REPRODUCTIVE BIOLOGY

Species of *Medicago* L genus belong to "leguminous papillonacae" subfamily. The flower starting mechanism is a biological feature which is typical of *Medicago* L genus. The floral apparatus is favorable to allofecundation mode in perennial species which are classified in the Falcago-Reichenb section (according to Heyn,1963).

However, annual species which are regrouped in the spirocarpos section along with *Medicago orbicularis*, are known to be autogamous. In this case, the flower starting mechanism seems to be maintained at relic state (Heyn, 1963; Lesins and Lesins, 1979).

Drawn like that with its main features the reproduction system used by each of these species may be approached by the floral biology study. Five principal stages which are defined by sizes peculiar to each species can be noticed in floral bud evolution until flower blooming. These stages represent reference states for the whole *Medicago* genus species.

Observation of different stages of flower fertile pieces maturation shows that pollen arrives to maturity from the end of stage 2,whereas ovary reaches maturity and hence receptivity from stage 3.

The early stage from which pollen becomes mature, emphasizes the difficulty of effecting manual castration without injuring the ovary.

The weak successful castration rate in all species of *Medicago* genus in general and more particularly in annual forms, constitutes a big handicap to achieve controlled (intra and inter specific) hybridizations. Floral buds at stage 4 are taken *from M. orbicularis, M. polymorpha, M. truncatula, M. intertexta* and *M. sativa* ssp *tunetana*, taking care of the carina to maintain it closed and avoiding to submit it to shock. Floral buds which are prepared in this way are put on a glass slide in saturated hygrometry conditions. After 48 hours, pistils of *M. orbicularis, M. polymorpha* and *M. truncatula* extend and begin to spiral. This change is a sign indicating that fecundation occurred. After 72 hours fecundated ovules can be distinctly observed.

90 to 100 of untriggered floral buds of *M. orbicularis, M. polymorpha and M. truncatula* give the same response. It clearly appears that these three species do not require releasing of the flower to ensure fecundation. They have a behavior close to cleistogamy. Moreover, their autogamy is confirmed by isoenzymatic polymorphism analysis.

On the contrary, non triggered floral buds of M. *intertexta* do not show any conversion. This species requires flowers releasing to achieve fecundation. This observation has been reported by Heyn (1963).

Annual species can be classified into two big categories taking into account requirement of flower releasing.

1. Cleistogamic species ensure fecundation without flower releasing: e.g*: M. orbicularis, M. polymorpha, M. truncatula...*

2. Species require flower releasing without which neither auto nor allo pollinisations can be successful e.g: M. *intertexta.*

ADAPTATIVE STRATEGIES AND *IN SITU* MAINTENANCE OF ANNUAL *Medicago*

Annual species of Medicago produce at each biological cycle a great amount of pods and seeds by individual.

Hard seeds existence permitted these species to survive some disastrous freaks such as fire and freezing. Moreover, this character confers a good resistance to animal ingestion. Seeds of annual Medicago can hence have great longevity.

Undehiscent pods are the dissemination element of these species. In natural conditions, one seed, rarely two or three, germinate within the pod which remains fixed to the collar of the plant. However, the mean number of seeds by pod in the annual species is clearly superior to the number of germinated grains.

Pods taken in natural state and having given at least one plant, hold viable seeds the germination rate of which is of 90% after mechanical scarification. Time spread germination of seeds belonging to the same pod would avoid a possible elimination under different constraints (particularly climatic) of young plants which would simultaneously emerge.

Seeds of annual Medicago are hard. Their germination rate without scarification reaches 20% after 10 days while scarified seeds of the same age germinate at 95% after 48 H. Recent pods belonging to different species are immersed during five minutes in liquid nitrogen. They are then hidden in compost at one coin of the surface and regularly watered. Depending on the considered species 50 to 100% of the pods give each 3 to 10 young plants. If seeds belonging to the same pod undergo correct scarification they can simultaneously germinate without prejudice of competition phenomena which could subsequently arise. On the contrary, liquid nitrogen untreated pods let young

plants emerge in 30 to 50% of the cases for *M. polymorpha* and *M. truncatula*. Pods which gave at least one young plant have been submitted to an hydrous stress by watering stoppage during two weeks. *M. truncatula* and at lesser degree *M. polymorpha* give response which seems to go in the way of an installation mode adapted to a climate characterized by irregular precipitations and long dryness periods.

This germination mode spread in time allows to maintain, natural populations genetic relatively stable.

If autogamy insures to annual Medicago a better exploitation of environment conditions which are often unpredictable such as rain irregularity, time spread germination of seeds belonging to the same pod, along with their hardness, it confers to annual Medicago the quality of pioneer species and in so far as such, they can colonize various and more or less disrupted habitats.

GENETIC VARIABILITY

1/ *M. ciliaris* and *M. intertexta*

M. intertexta (L) Miller and *M. ciliaris* (L) Krocher present typical morphologic characters: pod shape, black color of the seed and pyramidal form with triangular basis of pollen grains. These species of Intertextae section constitute a well defined group which is distinct from the other annual species of the *Medicago* genus.

Analysis of seven isoenzymatic systems, from the mendelian point of view, allowed to reveal the genetic control of GOT, 6PGD, PGI, LAP and PGM; while two enzymatic systems ICD and MDH proved monomorphic.

The study of six polymorphic loci allowed to specify the natural reproduction biology of these two taxa.

The great homozygous rate observed in M. *ciliaris* is in agreement with its preferential autogamy. However, M. *intertexta* appears to evolve for each of these loci, under panmictic pattern.

On the other hand, some genotype structures found in the two species are in agreement with the possibility of natural hybridization between these two taxa. This presumption of interspecific cross is supported by three arguments: sympatry, flowering synchronogy and great capability of artificial hybridization between the two botanical forms. Hence, *M. ciliaris* and *M. intertexta* constitute a species complex subdivided into two divisions defined essentially by their prevailing natural reproduction system.

2/ *Medicago orbicularis* L.

M. orbicularis diploid annual plant (2n = 16) is characterized by its globrous fruit shape of a big and flat lentil with thin and often undulating sides.

Fruits can be weakly spiralled, flat with nearly equal turns of spirals except the two extremities.

Pods can also be multispiralled with turns of spiral decreasing from the center to the extremities.

Shape variability of the pod and its diameter allow to distinguish botanical varieties.

Electrophoretic variability analysis of eight isoenzymes showed them strictly homozygous in twelve populations from different origins which have been studied. This observation confirms the highly autogamous, indeed cleistogamous regime of M. *orbicularis*. Five isoenzymes: ICD, 6PGD, PGM, PGI and GOT appear strictly monomorphic. On the contrary, MDH, LAP and PER reveal intra and inter population polymorphism.

Study of variability of several morphologic characters is in progress. It would bring other elements on genetic diversity of this species.

3/ *Medicago polymorpha*

The analysis of the morphological variability was carried out in 28 diploid (2n = 16) and natural populations, cultured in the same experimental conditions.

18 morphological parameters related to vegetative and reproductive development of plants were retained to evaluate genetic variability.

The Principal Components Analysis applied to the mean characters, revealed a separation between populations on the plane defined by the two first axes which represented 59% of the variation. The variability range seemed to be continuous on the prospected area and operated according to an East-West gradient.

A great heterogeneity was observed for populations located in the same bioclimatic zone. In the semi-arid zone, the populations situated in the East were less vigorous and more precocious than those located in the West at an altitude of 500-700m. These populations were characterized by small pods with a limited number of seeds.

Populations of humid and sub-humid areas, separated from each other, were distinguished by their great coiled pods.

Isoenzymatic analysis was carried out on 8 populations. The seven enzymatic systems (LAP, GOT, MDH, ICD, 6-PGD, PGI and PGM) revealed a variable polymorphism:

- the PGI and the 6-PGD were monomorphous;

- the GOT and LAP presented 3 and 4 different zymograms, respectively;

- the ICD and PGM systems presented each 11 different profiles;

- the MDH was very polymorphous and showed 13 enzymatic profiles.

The Factorial Correspondence Analysis applied to the electrophoretic band frequency of all the systems revealed an analogous structuration of the populations observed by morphological markers. The populations located at a high altitude were separated from those situated at a low altitude.

4/ *Medicago truncatula*

The analysis was carried out on 11 natural populations: 6 prospected in Tunisia, 5 prospected in Morocco, and three Australian cultivars.

27 morphological markers were measured on the plants at different periods of their development.

The variance analysis for each character revealed great heterogeneity for the populations of the same origin. Significant differences were also revealed for all parameters on the 14 populations analysed together.

The Canonic Discriminant Analysis carried out on all populations showed two population groups on the plane defined by the two first axes (63.25% of the variation):

- the first group, characterized by plants with small pods corresponded to Tunisian populations;

- the second group corresponding to the populations of Morocco was characterized by plants with a great number of flowers per inflorescence and late flowering;

- the cultivars occupied an intermediate position between the two groups.

The isoenzymatic polymorphism analysis for 6 systems (6PGD, ICD, MDH, PGM, LAP and GOT) revealed different variations for each system within populations:

- a monomorphism of ICD;

- MDH and GOT presented low polymorphism;

- 6PGD, PGM and LAP were highly polymorphous.

The Factorial Correspondence Analysis carried on the electrophoretic band frequency showed, on the plane defined by the two first axes(58% of variation), an overlapping of distinction between Tunisian and Moroccan populations. The Australian cultivars constitute a distinct group, well separated from the others.

MEDICAGO SATIVA SSP *TUNETANA*

In *Medicago sativa* ssp *tunetana* non triggered flowers do not show the slightest change which would be indicative of fecundation. Flower releasing ensures stigmatic membrane rupture which is favourable to pollen collection by stigmatic papillas thus allowing its germination. In natural conditions, vector mediated flower triggered necessity generally prevents autopollen to occur alone on the stigma. Performances comparison of 3 pollination ways (controlled autofecundation, controlled crosses and free fecundation) shows significant differences between performances of autofecundation and the other fecundation modes. On the contrary, results of controlled crosses and free fecundations are not significantly different (Abdelkafi, 1992). The weak productivity of auto fecundations as compared with mean performances of the same individuals in crosses, along with similarity of the results obtained in controlled crosses and in free fecundations is in agreement with preferential allogamy in *M. sativa* ssp *tunetana*.

However, this spontaneous plant, such as cultivated lucern to which it is related, accepts autofecundation.

This plant develops an important stolon network (system). This feature gives an excellent adaptation to an environment characterized by hard winters and long dryness periods.

Facultative autogamy as well as aptitude to propagate by vegetative way owing to stolons appears as adaptative ways for the perenniality of the species in areas where climate aridity is increased by anthropic pressures. However, its preferential autogamy allows to maintain its variability whenever natural conditions are favorable.

Genetic determinism research of enzymatic systems on appropriate biological material (autofecundation and controlled crosses lineages) revealed diverse situations. Some systems such as PGI, GOT were shown to be monomorphic. The lack of segregation does not allow any mendelian analysis. Other isoenzymes present profiles comprising several bands (e.g: MDH). The system complexity, far from presenting a favorable situation does not open, in the present state of the studied material, the way to genetic determinism research.

Some systems could be explained only in part (e.g: LAP). But four isoenzymatic systems (ICD, PGM, 6PGD and AAC) have been elucidated as for their genetic determinism.

In most cases and because of the tetraploid level of *M. sativa* ssp *tunetana*, it is difficult to assign a precise genotype to a given profile. Taking into account band intensity would avoid this difficulty. However, this approach often lacks objectivity because of reading mistakes.

Isoenzymatic polymorphism analysis of *Medicago sativa* ssp *tunetana* natural population, revealed a seemingly continuous variability range within the propected area. However, this unbroken polymorphism (*polymorphism continuum*) hides some subdivisions in small point subgroups.

For populations from close geographical origin and similar biotope, a cleavage was observed. The distinction between different regrouping, within the same populations as well as between neighbouring populations would be in agreement with fragmentation of the same original genetic pool under the action of anthropic pressures.

The final state on this genetic erosion is illustrated by isoenzymatic monomorphism of all individuals belonging to Rouhia population which is maintained *in situ* as a "genotypic remainder" by the only way of vegetative propagation.

Over grazing which is the rule in *Medicago sativa* ssp *tunetana* natural areas, does not allow the plant to undergo its vegetative cycle until the end and to reach flowering. This spontaneous and preferentially autogamous plant, is submitted to a strong break against genetic exchange.

Moreover, clearing and cultivation interpose new barriers to genetic mixing while limiting its natural distribution area. Its in situ maintenance is ensured by its powerful stolon system, but its fragmentation into small, isolated and precarious populations, is increased under the action of unconsidered courses exploitation.

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