# Research Activity on Medicago Spp. at the "Istituto Sperimentale per la Colture Foraggere", Lodi-Italy

Rotili P., Berardo N., Gnocchi G., Pecetti L., Piano E., Scotti C.

*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 11-22

# Research activity on *Medicago spp.* at Istituto Sperimentale per la Colture Foraggere Lodi - Italy

**P. Rotili, N. Berardo, G. Gnocchi, L. Pecetti, E. Piano and C. Scotti**[1]


## Abstract

In the last 15 years five new lucerne cultivars were released by ISCF, Lodi. The variety constitution process is characterized by : i) the exploitation of the effects of biological density (competition) in the phenotypic evaluation ; ii) the utilization of a selfing phase in the breeding schemes ; and iii) a variety model based on a little number of partly inbred parental clones from genetically distant populations. In framing breeding procedures, the conceptual guideline of lucerne "stand system" has been elaborated, where plants are the constitutive elements and their spatial organization and degree of interference represent the "structure" of the system. Breeding procedures aim at improving both plants and structure. A plant model has been developed aimed at direct improvement of vigour and enhanced persistence. Development of free-hybrid varieties has been proposed to exploit both additive and heterotic effects. Investigations are in progress to assess the value of different free-hybrid models relative to the corresponding synthetic varieties. Established results suggest that direct selection for protein and fibre content is expected to be ineffective. Indirect selection for quality through physiological characters appears more appropriate. Assessment of lucerne-Rhizobium meliloti relationships have been integrated into the breeding schemes for improvement of plant symbiotic ability. Use of molecular markers has been recently implemented to estimate plant heterozygosity levels with the objective of individuating genotypes which combine high vigour and low heterozygosity. A breeding project aimed at developing creeping-rooted or rhizomatous lucerne varieties suited to grazing has also been activated. This involved the evaluation of morphophysiological traits in wide collections from the *Medicago sativa* complex. Research on annual Medicago spp. involved activities of plant survey and germplasm collection. This activity provided a comprehensive base of genetic diversity and precise information on ecology and distribution of annual medics in relation to environmental variation.


**Key words:** *Medicago sativa*, improvement, methods of selection, molecular markers, tolerance to grazing, genetic diversity

## 1. Introduction

The surface occupied by lucerne (*Medicago sativa*) in Italy correspond more or less to 1 million hectares : 55% in the Po Valley, 35% in Central Italy and 10% in the South. About 600,000 t/year are dehydrated, 15% of which in the last two years was represented by dehydrated hay. The activity of this Institute in Lodi is aimed at the constitution of varieties suitable for the new market demand of dehydrated products (meal and hay). In the last 15 years five new cultivars were registered by ISCF in the National Register of Varieties: Robot, Equipe, Lodi, Iside and Centauro. Four years ago, an important research project was started on perennial lucerne for grazing, to define solutions for a type of agriculture that will reconcile animal production and environmental sustainability. Our activity is conceived as a linear process in which the phases and times of both basic research and technical innovations play a main role in the final product, that is the new cultivar.

## 2. Cutting-type varieties

Our breeding activity is aimed at building varieties for ruminants (cattle and sheep), small monogastrics (poultry, rabbits) and big monogastrics (horses). From a methodological point of view the same breeding scheme is adopted for these different purposes, although selection criteria may

[1] Istituto Sperimentale per le Colture Foraggere, Viale Piacenza, 29, Lodi, Italy.

vary. For instance, in the case of varieties for horses, that need hay with low protein content (15-16%), different rhythms of cutting and model of plant are introduced.

A lucerne variety is based on the following parameters: a) yielding capacity (vigour); b) persistence ; and c) quality.

In the following paragraphs we will discuss the solutions proposed in order to improve these parameters.

## 2.1. Breeding for vigour (P.Rotili, G. Gnocchi, C. Scotti)

The variety constitution process at ISCF is characterized by : 1) the exploitation of biological density (competition) effects in the phenotypic evaluation ; 2) the utilization of a selfing phase ; and 3) a variety model based on a little number of partly inbred, parental clones derived from genetically very different populations.

### *2.1.1. The effects of biological density on the evaluation of the phenotype and estimate of genetic parameters*

It is generally assumed that, the whole yield potential of a plant, is best assessed in non-limiting conditions and, for that reason, spaced plants are usually employed. For the breeder, however, knowledge of yield potential of a lucerne plant in non-limiting conditions is of little value ; on the contrary, it is much more useful to know the yield potential of the lucerne plant in interaction with the other plants, i..e. as an element of the lucerne crop system. In the lucerne stand one limiting factor always operates, i.e. the light. This factor constitutes the main difference between spaced plant and dense sward conditions. On the surface of the lucerne canopy light is never limiting, but when plant height reaches 70-80 cm the intensity of the light decreases to 500-1000 lux at the lower layers with effects on senescence rate, leaf persistence, and stem number at regrowth.

Different experimental conditions yield inconsistent results and, therefore, affect the choice of the plants. Our results show a low correlation (r = 0.4 à 0.6) between spaced plant and dense sward values for dry matter, plant height and number of stems (Rotili and Zannone, 1975). As the genotype ranking can change according to the different densities, it is very important to define the optimum number of plants per m$^2$ to adopt. Furthermore it must be underlined that cutting at 50% blooming does not impair persistence of spaced plants while in dense sward (300 plants/m$^2$) the cutting effect is very selective, with about 20% mortality in the first year (Rotili *et al.*, 1989). As found in different experiments, the estimate of genetic parameters is also affected (Rotili and Zannone, 1975). The value of heritability for dry matter yield and plant height varies with : 1) density (spaced plants or dense sward) ; 2) experimental techniques (pure stand, mixture in alternate rows or in the same row) ; 3) genetic material (clones or progenies) ; and 4) cutting management (cut at 50% flowering or at green bud stage; cut synchronised for the whole material or at a given biological stage for each clone or progeny). Plant density (spaced plants or dense sward) modifies the relative proportion of the different variances (GCA, SCA, Error) for dry matter yield (Rotili, 1979).

However, in some situations the evaluation in spaced plant conditions can be efficient, such as in difficult pedoclimatic environments, where adaptation has overriding importance, and both forage production and, even more, persistence are based on stress resistance. Conversely, in favourable environments, where the resistance factors play a secondary part in comparison with those directly involved in the expression of forage production, the spaced plant procedure is ineffective. So, there are two different types of agriculture demanding two different types of biological "machinery": one has to resist environmental stresses; the other has to exploit at the best the great resources of a "rich" habitat.

### *2.1.2. The lucerne stand system and its structure*

In practice a single plant of lucerne has no interest, because the exploitation concerns not a plant but the whole lucerne stand. We represent the lucerne stand as an open system, where plants are the constitutive elements and their spatial organization and degree of interference produce the "structure" of the system. The structure is, therefore, the morpho-physiological expression of the dynamic relations between plants : each individual of the lucerne stand influences and is influenced at the same time by the others.

The relationships between individuals change either across the phenological phases within each productive cycle or across the subsequent productive cycles (Rotili, 1979). The aim of the breeder is to improve the lucerne stand system. A positive result is possible only if both the plants and the structure are improved. Indeed, an optimal structure of the lucerne crop is necessary but not sufficient in order to secure a high forage yield. The same is true for the plants: good genotypes are necessary but not sufficient.

The relationship existing among the individuals of a lucerne stand can be of the following types: domination (+,-), cooperation (+, +), opposition (-, -), and neutralism (0, 0). Several lucerne populations have been studied in association, following an experimental design defined as "ecological diallel scheme" (Zannone *et al.*, 1983; Zannone, 1985). The results showed that the association effects reflect a situation of domination, generally defined as competition. In the first period of life of the lucerne stand, this situation produces an almost perfect compensation between gains and losses. Afterwards, cumulation of the negative effects of domination leads progressively to the death of the weakest partners. Mortality increases on increasing the degree of genetic heterogeneity of the population (Rotili, 1977).

### 2.1.3. Improvement of the lucerne stand structure

Improving the structure means to modify the relationships between individuals from situations of domination (+, -) towards neutralism (0, 0). Neutralism, indeed, produces the "ideal" structure: a monostratified structure. Some elements producing the "ideal" structure of the lucerne stand system are :

a) total synchrony of individual plants for : i) time of regrowth ; ii) rate of internode elongation ; and iii) time of the bud stage.

b) maximum homogeneity of individual plants for : i) resistance to early cutting (green bud) ; ii) number of stems and length of main stems after each cut; and iii) response to temperature, light and water supply across the different seasons.

To obtain a good lucerne stand cultivars, sufficient homogeneity for the morpho-physiological characters must be used. The most suitable model is a freehybrid variety or a synthetic variety with narrow genetic base (6-8 genetically distant, partly inbred clones).

### 2.1.4. Improvement of the operative model of the plant

The longevity of a lucerne stand is not only the result of an improved structure but it also depends on the degree of resistance of plants to early cutting (green bud stage). While the plants have almost all the same persistence when cut at flowering, they show a large variability in persistence when cut at the green bud stage (Rotili *et al.*, 1989). A selection for both persistence and vigour is possible to constitute cultivars adapted to dehydration.

An operative model for plant phenotype selection at high density and early cutting (green bud stage) in irrigated conditions involves the following characteristics :

1. Morphological characteristics : a) dry weight ; b) stem length at the first reproductive node (on three main stems) ; c) internode number before and after the first reproductive node (on three main stems) ; d) homogeneity of stem length ; e) stem thickness ; f) average internode length up to the first reproductive node; and g) stem number.

2. Ecophysiological characteristics: a) spring growth ; b) response to summer temperatures and water supplies ; c) autumn growth ; d) senescence of leaves ; and e) very early production of the first 6-7 leaves per stem after each cut, combined with low initial stem elongation rate.

The different degree of expression of these characteristics allows the utilization of this model for developing cultivars adapted both to ruminants and monogastrics. All the above-mentioned features account for vigour, quality, and persistence.

The operative model proposed is the result of several experiments concerning the vigour of the whole plant: aerial part and roots. Differences between cultivars are observed for earliness of regrowth, aerial and root biomass (ratio aerial/root biomass) and rate of root biomass recovery after cutting. At every cut, the correlation coefficient between aerial and root dry matter, calculated on individual plant basis for each cultivar, is between 0.80 an 0.90 (Rotili *et al.*, 1989).

### *2.1.5. Main aspects of variety building process*

#### *2.1.5.1. The vigour and its estimation*

The plant model proposed concerns the "direct improvement" of vigour. We use the term vigour as a synonymous of productive value, or of biomass production capability. This definition is appropriate for cultivated plants. On the contrary, for wild populations we consider the vigour as synonymous of reproductive value or fitness.

Vigour is a concept and as concept is not measurable. It can be estimated by measuring different traits; however, the total dry matter yield (result of the activity of the whole genome) can be considered the best estimation of vigour. We propose to estimate vigour in terms of growth rate according to the following formula:

$$\text{Vigour} = \frac{DMYt_1 - DMYt_o}{t_1 - t_o}$$

There are many and different procedures to estimate vigour on the basis of dry matter yield. Among them, the most precise is based on the following rule: the vigour of a genotype in a breeding program must be estimated in pedoclimatic and management conditions as close as possible to those in which the future variety will be utilized, and in the optimal conditions of biological density.

#### *2.1.5.2. The role of selfing*

Tetraploid plants can have five different genetic structures with five levels of heterozygosity: aaaa, monogenic nulliplex or quadruplex (monoallelic) ; abbb, digenic simplex or triplex (diallelic); aabb, digenic duplex (diallelic) ; aabc, trigenic (triallelic) ; abcd, tetragenic (tetrallelic). The vigour of a plant (capability of biomass production) is the result of the direct effects of the alleles and their interactions. Therefore, the breeder's objective is to cumulate in a new variety the highest quality of genes and linkats (additive effects) with the maximum level of heterozygosity. The first objective is the most important and the use of selfing in the breeding scheme is an effective tool to achieve it. Selfing can confer several advantages : i) it is possible to quickly homogenize plant material for physiological characters. Indeed, a great genetic variability for characters such as time and amount of regrowth, growth rate, flowering earliness, negatively affects production, persistence and yield stability. Such variability determines differential responses of the plants to cutting, in relation to their different degree of root reserve recovery at cutting time ; ii) Selfing is the most effective way to concentrate the genetic factors (genes and linkats) promoting vigour. In lucerne, as autotetraploid species, selection is not effective if the level of heterozygosity has not been reduced and unmasking is necessary to assess and select for the quality of genes and linkats. In this way, we were able to improve the breeding value of the parents, i.e. their general combining abilities (Rotili and Zannone, 1974; Rotili, 1976) ; iii) By selfing it is possible to identify plants less sensitive or tolerant to this mating system.

*2.1.5.3. Variety model: synthetic or free-hybrid variety ?*

The vigour of a first generation synthetic (Syn 1) depends on two main components. One, connected to the "in se" value of genes and linkats, has been improved during the selfing phase; the other, related to the level of heterozygosity, depends on the degree of inbreeding of parents and their genetical diversity. What will be the evolution of vigour after three or four generations of multiplication ? The part related to the "in se" value of genes and linkats is virtually the same as in Syn 1. The part connected to the level of heterozygosity depends on: a) the number of parents ; b) their level of inbreeding ; c) their genetic diversity; and d) the effect of stand density in favour of vigorous plants during the generation of multiplication (Rotili *et al.*, 1985). We know that "competition" in favour of allogamy acts at different levels: pollen, embryo, seedling, adult plant. These factors as a whole bring to the conclusion that for the final forage production the level of heterozygosity is higher than expected. The problem whether a free-hybrid variety, in the form proposed at Lodi, is superior or not to the corresponding synthetic is still open (Rotili, 1982; Rotili, 1990; Rotili and Guy, 1991). Figure 1 presents two models of free-hybrid varieties. They are made of two synthetics based on two and four partly inbred parents ($S_2$), multiplied during three generations. The choice of parents must be based on at least two parameters: maximum homogeneity of flowering and maximum genetic diversity. Our most recent data on lucerne show that free-hybrids with four $S_2$ parents outyield by 11%-17% the corresponding synthetics but, as recorded in many experiments, this higher forage yield is accompanied by a lower seed production (Rotili, unpublished data). The limiting factor in this variety model is the poor seed yield obtained by the 2-component parental synthetics. This seems related, at least partly, to the low level of heterozygosity, as demonstrated be the relatively low mean number of alleles per locus (2.69) found in these synthetics (Rotili, 1990 ; Rotili and Guy, 1991). Concerning the advantage of the free-hydrides with eight $S_2$ parents over the corresponding synthetics, experimental data are still not available.

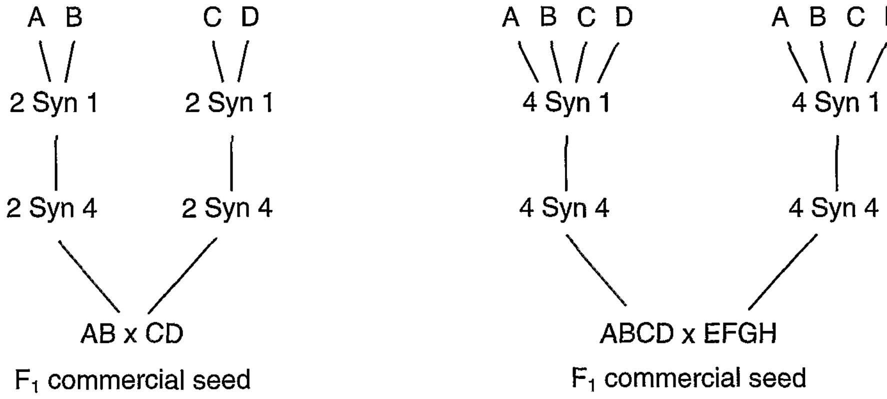


Figure 1. Two models of free-hybrid varieties. The parent components A, B, C, and D are partly inbred clones ($S_2$) deriving from relevant populations with large genetic dicersity

## 2.2. Breeding for quality (P. Rotili, N. Berardo, G. Gnocchi, C. Scotti)

Utilizable variation for crude protein (CP), neutral detergent fibre (NDF), and acid detergent fibre (ADF) has not been found among a range of analysed cultivars (Rotili *et al.*,1992, 1994), leading to the conclusion that it is almost impossible to obtain positive results in the breeding work by direct selection for these traits. Variation is related to the stratigraphy of the lucerne stand and to developmental stage rather than intervarietal differences (Table 1). It seems more efficient to improve qualitative traits by selection for physiological characters such as leaf persistence, onset of senescence and resistance to early cutting regime. However, an early cutting regime leads to a decrease in biomass production and an increase in plant mortality, the latter depending on the fact that at the cutting time not all plants have achieved the maximum recovery of root reserves. Plants which have fully recovered their reserves are at an advantage, while the others undergo a delay in regrowth and development which increases from one cycle to the next. As a consequence of the interference effects, these plants become progressively weaker and eventually die. Our data concerning protein content of the roots show that this trait is severely affected by the cutting regime (Rotili *et al.*, 1989). At the normal cutting regime (50% flowering), the root crude protein content reaches the maximum at the flowering stage and the minimum at the green bud stage. This trend of variation is similar to that of root biomass. Therefore, it seems that the high plant mortality at early cutting regime is mainly due to the progressive decrease of the crude protein root reserves. It must be underlined that crude protein content follows an opposite trend in the aerial part relative to the roots; at flowering it is at the maximum level in the roots and at the minimum in the top-growth. In improving crude protein by indirect ways, i.e. through resistance to frequent cutting, the best moment for cutting lucerne appears to be at 50% blue bud stage rather than at 50% green bud stage, the former reconciling nutritive value, biomass production and persistence.

Table 1. Variation of crude protein (CP) and fibre fractions (NDF,ADF) as determined by the combined effects of stratigraphy and developmental stage in two lucerne varieties

| | LEAVES | | | | | | STEMS | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EQUIPE | | | SEWA | | | EQUIPE | | | SEWA | | |
| | CP | NDF | ADF | CP | NDF | ADF | CP | NDF | ADF | CP | NDF | ADF |
| Cut at 28 cm | | | | | | | | | | | | |
| à-28 cm | 41 | 27 | 15 | 40 | 26 | 15 | 21 | 44 | 36 | 21 | 42 | 34 |
| Cut at 60 cm | | | | | | | | | | | | |
| 0-28 cm | 34 | 28 | 15 | 33 | 28 | 17 | 16 | 54 | 48 | 14 | 57 | 50 |
| 28-60 cm | 40 | 24 | 14 | 39 | 23 | 15 | 22 | 43 | 37 | 21 | 46 | 35 |
| Cut at 50% bloom | | | | | | | | | | | | |
| 0-28 cm | 29 | 23 | 17 | 28 | 26 | 18 | 11 | 63 | 55 | 10 | 65 | 55 |
| 28-60 cm | 33 | 22 | 15 | 30 | 25 | 17 | 12 | 59 | 52 | 12 | 63 | 52 |
| 60 cm TOP | 36 | 17 | 12 | 33 | 22 | 15 | 20 | 42 | 37 | 16 | 49 | 41 |

### 2.3. *Medicago sativa-Rhizobium meliloti* association (C. Scotti)

The study of the association between lucerne and Rh. meliloti has been conducted using the breeding scheme summarized in Figure 2. Some methodological points should be underlined : a) the Rhizobium component is represented by the natural population of the soil; we make the hypothesis that different types of soil carry genetically different Rhizobium populations ; b) symbiotic ability is estimated by nodule fresh weight (NFW) and by the classification of nodules for their developmental stage (size and shape) ; c) nodule biomass is measured in different seasons and productive cycles in order to assess the seasonal variation in symbiotic ability; and d) plant-nodulating strain relationships are studied by means of molecular markers in rhizobia isolated from nodules (Scotti, 1992a).

Divergent selection for high and low NFW applied on 6 lucerne populations and on S1 progenies of the selected plants, followed by selfing of the chosen individuals, has led to a test of comparison of 12 polycross and corresponding $S_1$ families, and of 14 $S_2$ families of contrasting NFW. Nodule biomass was studied at the end of the season (September) and on the following 1st cut (May), on 3-year old plants grown in double-tube plots. High and low NFW families did not differ significantly when considering polycross progenies and differed significantly when considering $S_1$ progenies. Genetically close S2 families with contrasting NFW and comparably high DMY were also obtained. The chosen S2 individuals were crossed and selfed to create new populations with different symbiotic abilities.

As for the role of different types of soil in the symbiotic behaviour of 6 lucerne varieties, preliminary results indicate that while DMY is comparable, significantly lower nodule biomass is observed in a clay soil (38% clay) than in a sandy-loam (6.5% clay). In winter-active varieties the production of nodule biomass in summer is lower than in dormant or intermediate varieties. Interactions variety-soil are significant. Rhizobium strains, from each variety-soil treatment, collected in spring, summer, and autumn are being analyzed by RAPD markers. Established results from molecular markers analysis obtained in preliminary experiments with different lucerne varieties and different soils showed a high genetic diversity within Rhizobium populations of two types of soil (Paffetti *et al.*, 1995). The population structure did not appear to be clonal, but probably freely recombinating with a major differentiation between strains isolated from the two soil types. Other molecular markers were used to validate RAPD results : RFLP of amplified intergenic sequences 16s-23s rDNA genes showed a low degree of polymorphism, while a higher degree was found with RFLP of nod region, located in pSym plasmid. RFLP analysis of nod genes confirmed the genetic difference between the populations of the two soils.

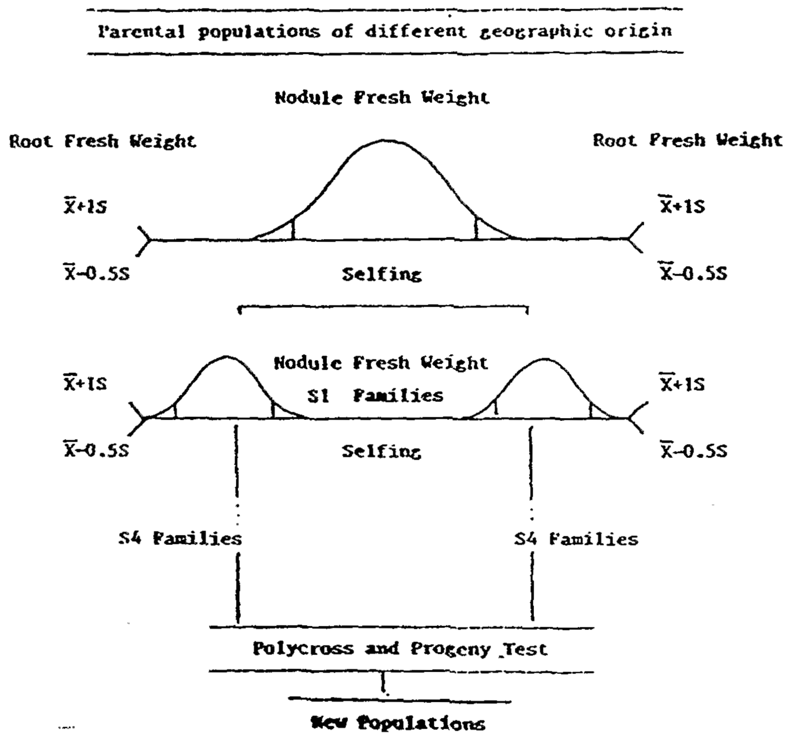


Figure 2. Breeding scheme for symbiotic ability in lucerne.

## 3.- Biotechnologies in plant breeding (C. Scotti)

### 3.1. RFLP markers as a tool to estimate the level of plant heterozygosity

The vigour of an autotetraploid plant is a function of the mean level of heterozygosity and the quality of genes and gene clusters (linkats). The breeding objective is the combination of these two elements at their highest level in a new population. During the selfing phase, RFLP analysis was applied to six $S_0$ mother plants (MP) and to their $S_1$ (15 plants/progeny) and $S_2$ (20 plants/progeny) offsprings in order to estimate their heterozygosity level, with the objective of individuating highly vigourous plants with low average heterozygosity level (Scotti *et al.*, 1994).

The average number of bands for each probe/enzyme combination, used to estimate the degree of heterozygosity, showed a difference among $S_0$ MP that is fairly maintained during selfing generations, though in the presence of an obvious trend of diminution of number of bands due to selfing. $S_2$ plants analysed within each family were chosen for contrasting vigour; nevertheless they did not show a significantly different number of bands, indicating that differences in plant vigour could be due to gene quality rather than to heterozygosity level. $S_2$ plants characterized by means of RFLP analysis were subject to selfing and crossing and the progenies grown in order to verify this result.

Because of their unlimited number, molecular markers can cover the whole genome; in particular, RFLP seems more suitable for this purpose than RAPD, because the latter although easier to handle, is mostly dominant and does not allow to recognize all the heterozygous combinations. On the basis of preliminary results, it seems possible to estimate by RFLP markers the heterozygosity level of lucerne plants and, therefore, to improve the effectiveness of plant breeding procedures in polyploid allogamous species.

### 3.2. Plants from *in vitro* culture

The procedure for the induction of somatic embryogenesis and subsequent propagation was set up by Lupotto (1983) using plants of cv. Robot. The somaclones obtained were propagated by manual crossing and selfing for three cycles and the different generations issued ($S_0$, $S_1$, $S_2$, $S_3$) studied for DMY, earliness and persistence. The somaclones showed a wide range of phenotypic variation associated with a general loss in vigour, fertility (19% of fertile plants in crossed and 14% in selfed somaclones), and persistence. Sexual progenies of somaclones subject to intra-family crossing and selfing showed values of pod fertility always lower than cv. Robot. Inter-family crossing did not improve significantly the number of seeds per pod. As fertility is known to be in relation with the level of heterozygosity in lucerne, we can hypothesize a higher inbreeding coefficient and a reduction of genetic diversity in this material compared to the parental cv. Robot.

An increased range of variation for saponin content and earliness was observed (Scotti, 1992b). A reduction of growth rate was evident in sexual polycross ($S_1$ x $S_1$) and in selfed ($S_1$ and $S_2$) progenies of somaclones (4 cuts/year compared to 6-7 of the "normal" populations); persistence was also lowered.

In this experiment the variation generated during somatic embryogenesis affected negatively vigour and fertility. The severe selection for the capability of sexual reproduction probably counteracted the variability present in somaclones.

## 4.- DEVELOPMENT OF GRAZING-TYPE PERENNIAL LUCERNE (E. Piano, L. Pecetti)

This breeding project, which started in 1991, is related with the demand for sustainable agriculture, environment protection, and diversification of utilization in farming systems. The potential of lucerne as a pasture legume relies on the development of varieties with deep-set crown, associated with creeping-rootedness or rhizomatous habit, characters all contributing to spatial proliferation of the shoots and to persistence under grazing. These characters need to be appropriately combined with good forage quality and productive features (yield potential and seasonal growth patterns) suited to the environments of possible cultivation.

Variation for both productive features and morpho-physiological characters conferring adaptation to grazing has been observed in large collections of the *Medicago sativa* complex (*M. sativa* subsp. *sativa*, subsp. *falcata*, subsp. *varia*, wild "mielga" populations, and materials originated from artificial crosses between different taxa) (Piano *et al.*, 1996). These "germplasm groups" proved rather differentiated for forage yield and other top-growth features. Yield potential decreases in the order: subsp. *sativa* > artificial crosses > subsp. *varia* > "mielga" > subsp. *falcata*.

Also for most morpho-physiological traits and for seasonal growth pattern, "mielga" types resembled the subsp. *falcata* rather than the subsp. *sativa* to which they belong. Although with different frequency, all taxonomic groups included materials potentially suitable for grazing, i.e., with the presence of a deep-set crown. This character appears to be associated with superior yield potential particularly in subsp. varia and in artificial crosses.

In the examined germplasm, deep-crowned types could be categorized, across taxonomic groups, into four broad "top-growth models", morphologically and productively distinct (Figure 3) (Piano *et al.*, 1994a). Shallow-crowned plants could also be categorized into distinct models. Differences among taxonomic groups for yield potential are mainly accounted for by the different frequency of these models in the groups (Pecetti *et al.*, 1995).

In the same germplasm, there was a distinct correspondence between top-growth models and underground morphology, so that selection for the former has also involved indirect selection for the latter. All deep-crown models have shown to be characterized by a branch-rooted system; one of them ($D_2$) has creeping roots, while the other three ($D_1$, $D_3$ and $D_4$) are rhizomatous, with varying underground development and morphology (Piano *et al.*, 1996). A sound result of this germplasm characterization is the very low frequency of creeping-rootedness, even in varieties specifically selected for this trait. The presence of rhizomatous types (i.e., $D_3$ and $D_4$) with remarkable spreading ability and with yield potential and vegetative recovery after winter equal to or higher than that of the creeping type (Table 2), together with the better expressively of the rhizomatous habit, point to the opportunity to pay greater attention to the former types which, in the selection of grazing-tolerant lucerne, have generally been neglected in favour of the creeping model.

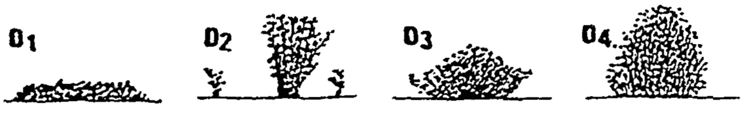


Figure 3. Top-growth models in deep-crowned plants.

Table 2. Mean values in deep-crowned morphological models (D), and in reference shallow-crowned, hay-type models (S)

| | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $S_2$ | $S_3$ |
|---|---|---|---|---|---|---|
| Plant diameter (cm) | 23 a | 22 a | 23 a | 21 a | 13 c | 16 b |
| Early vegetative recovery (1-9) | 3.0 c | 4.5 b | 4.5 b | 5.6 a | 4.6 b | 5.0 ab |
| DMY (g/plant) | 122 c | 197 b | 202 b | 327 a | 192 b | 244 b |

The diversification of growth models, irrespective of their yield potential, has some interest for breeding objectives, as each model could represent a possible ideotype for given environmental situations and/or management systems.

Taxonomic groups and growth models have also been evaluated for nutritive value and presence of antinutritional factors (saponins) (Piano *et al.*, 1994b). The above mentioned morphological plant models suited to grazing can be characterized by qualitative features similar to or even better than those of common hay-type varieties. Rhizomatous types, for instance, tend to have higher protein content, probably in relation to their apparently higher leaf/stem ratio, although some models also show relatively higher NDF values. Of the taxonomic groups, subsp. *falcata* shows relatively higher saponin content, but the intra-specific variation is wide enough for selection to act. "Mielga" populations do not differ from cultivated subsp. sativa types for this character.

On-going activity and future research trends focus on fixing the morphological models in experimental populations, and verify the stability and expressivity of the model per se and of the characters conferring adaptation to grazing at varying conditions of intra- and inter-specific competition and under real grazing. Parallel investigations will be specifically carried out to elucidate on physiological aspects of creepingrootedness and rhizomatous habit, and their genetic control.

## 5.- GERMPLASM COLLECTION OF ANNUAL MEDICS AND RELATED ECOLOGICAL STUDIES (E.Piano)

Research on annual Medicago spp. has involved activities of plant survey (Piano *et al.*, 1982) and germplasm collection in Sardinia and Sicily, conducted either in collaboration with the Western Australian Department of Agriculture (Francis and Piano, unpublished) or within international collecting missions coordinated by the IPGRI (Piano *et al.*, 1991). These survey and collecting missions covered several hundred sites in a wide range of environmental conditions. Assistance was also given to other Australian missions aimed at collecting legume species and/or associated *Rhizobium meliloti* strains.

While selection programs are not being presently carried out at our institute, this significant activity has provided a comprehensive base of genetic diversity for breeding programs conducted elsewhere, and sound information on the ecology and distribution of annual Medicago spp. in relation to edaphic, climatic and biotic variation, the latter also including the effect of grazing (Piano *et al.*, 1982; 1991; Piano, 1987; Piano and Francis, 1992). The acquired evidence of regional patterns of distribution and distinct ecological specialization of several species, in contrast with the more ubiquitous nature of others, point to the importance of enhancing knowledge on species ecology, to adequately plan plant introduction and related selection programs. A major outcome of the ecological studies conducted in Sardinia was the finding of *Medicago* species showing a distinct ability to colonize acid soils, such as M. *murex*. This finding stimulated deep research in Australia, which led to the development of both medic varieties adapted to acid soils and compatible rhizobial strains able to survive in these soils (Howieson and Ewing, 1986), thus providing opportunities for wider utilization of medics in areas where their growth was otherwise limited.

All the collected materials are now stored for long-term conservation and germplasm exchange in the gene banks of Perth and Adelaide, Australia.

## REFERENCES

Howieson J.G., Ewing M.A., 1986. Acid tolerance in the *Rhizobium meliloti - Medicago symbiosis.* Aust. J. of Agric. Res. 37 : 55-64.

Lupotto E., 1983. Propagation of an Embryogenic Culture of *Medicago sativa* L. Z. Pflanzenphysiol. Bd. 111 : 95-104.

Paffetti D., Scotti C., Gnocchi S., Fancelli S., Bazzicalupo M., 1995. Genetic diversity of a *Rhizobium meliloti* population isolated from different *Medicago sativa* varieties in two Northern Italian Soils. Submitted to "Applied and Environmental Microbiology".

Pecetti L., Valentini P., Piano E., 1995. Development of grazing type lucerne: yield variation and relationship with plant morphology in the *Medicago sativa* complex. Cahiers Options Méditerranéennes 12 : 45-48.

Piano E., 1987. Some ecological aspects of the distribution of annual medics and subterranean clovers in Sardinia. In Piano E. ed., Annual Self-reseeding Legumes for Mediterranean Pastures. ISCF, Lodi : 1-7.

Piano E., Berardo N., Pecetti L., Valentini P., Odoardi M., 1994b. Some observations on the qualitative characteristics of grazing type lucerne. In Reheul D. and Ghesquire A. eds., Breeding for quality. Proc. 19th Eucarpia Fodder Crops Meet., Brugge : 107-109.

Piano E., Francis C.M., 1992. The annual species of Medicago in the Mediterranean basin. Ecogeography and related aspects of plant introduction and breeding. In Rotili P., Zannone L. eds., The future of lucerne. Biotechnology Breeding and Variety Constitution. X Int. Conf. EUCARPIA *Medicago* spp. Group, Lodi : 373-385.

Piano E., Pusceddu S., Spanu F., 1991. Observations on the distribution and ecology of annual medics and subterranean clovers in Sicily (Italy). Proc. Fourth Int. Rangel. Congr., Montpellier: 405-409.

Piano E., Sardara M., Porcedu S., 1982. Observations on the distribution and ecology of subterranean clover and other annual legumes in Sardinia. Riv. di Agron. 16 : 273-283.

Piano E., Valentini P., Pecetti L., 1994a. Some observations on the morpho-physiological variation in grazing type lucerne. In Management and breeding of perennial lucerne for diversified purposes. REUR Techn. Series 36, FAO, Rome : 67-69.

Piano E., Valentini P., Pecetti L., Romani M., 1996. Evaluation of a lucerne germplasm collection in relation to traits conferring grazing tolerance. Euphytica, accepted for publication.

Rotili P., 1976. Performance of diallel crosses and second generation synthetics of alfalfa derived from partly inbred parents. I. Forage yield. Crop. Sci. 16 : 247-251.

Rotili P., 1977. Performance of diallel crosses and second generation synthetics of alfalfa derived from partly inbred parents. II. Earliness and mortality. Crop. Sci. 17 : 245-248.

Rotili P., 1979. Contribution à la mise au point d'une méthode de sélection de la luzerne prenant en compte les effets d'interférences entre les individus. I. Etude expérimentale de la structure de la luzernière. Ann. Amélior. Plantes 29 : 353-381.

Rotili P., 1982. Consideration on some aspects concerning the breeding of meadow fodder species. In Annali ISCF, vol. VI, 1979-1982.

Rotili P., 1990. The role of selfing in the synthetic and hybrid varieties constitution in forage crops. In den Nijs A.P.M., Elgersma A. eds., Fodder crops breeding : achievements, novel strategies and biotechnology. 16th Meeting EUCARPIA Fodder Crops
Section, Wageningen: 83-88.

Rotili P., Guy P., 1991. Breeding schemes in autotetraploid forage : recent achievements and new approaches. Proc. XVII Eucarpia Fodder Crop Section Meeting, Alghero : 14-22.

Rotili P., Zannone L., Jacquard P., 1976. Effects of association on the evaluation of luzerne populations. Ann. Amelior. Plantes 26 : 139-155.

Rotili P., Zannone L., Gnocchi G., Proietti S., Scotti C., 1989. Analysis of the aerial part and roots of the lucerne crop to improve the breeding methods. Proc. XVI Int. Grassl. Congr., Nice : 485-486.

Rotili P., Zannone L., 1974. General and specific combining ability in lucerne at different levels of inbreeding and performance of second-generation synthetics measured in competitive conditions. Euphytica 23 : 569-577.

Rotili P., Zannone L., 1975. Principaux aspects d'une méthode de sélection de la luzerne basée sur des dispositifs qui utilisent la concurrence entre les plantes Ann. Amélior. Plantes 25 : 29-49.

Rotili P., Gnocchi G., Scotti C. and Zannone L., 1985. Performance of second and third generation synthetics of lucerne derived from partly inbred parents : forage yield and fertility. In Eucarpia Fodder Crops Section Meeting Report, Svalov.

Rotili P., Valentini P., Berardo N., Zannone L., 1992. Stratigraphical analysis of experimental lucerne meadow. In Rotili P., Zannone L. eds., The future of lucerne. Biotechnology Breeding and Variety Constitution. X Int. Conf. EUCARPIA *Medicago* spp. Group, Lodi : 466-473.

Rotili P., Scotti S., Zannone L., Gnocchi G., 1994. Lucerne stand system: dynamics of forage production, quality and demography; consequences on the variety constitution process. In Management and breeding of perennial lucerne for diversified purposes, REUR Techn. Series 36, FAO, Rome : 54-62.

Scotti C., 1992a. Breeding for the symbiotic ability in *Medicago sativa*. In Rotili P., Zannone L. eds., The future of lucerne. Biotechnology Breeding and Variety Constitution. X Int. Conf. EUCARPIA *Medicago* spp. Group, Lodi : 121-129.

Scotti C., 1992b. Pod fertility at different levels of selfing in lucerne plants derived from *in vitro* culture. In Rotili P., Zannone L. eds., The future of lucerne. Biotechnology Breeding and Variety Constitution. X Int. Conf. EUCARPIA *Medicago* spp. Group, Lodi : 230-235.

Scotti C., Suardi C., Pupilli F., Damiani F., Arcioni S., Rotili P., 1994. Inbreeding depression in *Medicago sativa* : estimation of the different levels of heterozigosity by RFLP. In Management and breeding of perennial lucerne for diversified purposes, REUR, Techn. Series 36, FAO, Rome : 182-185.

Zannone L., 1985. Intraspecific interference in forage crops. Biological density and its implication in the prediction of association performance. Agronomie 5 : 287-296.

Zannone L., Assemat L., Rotili P., Jacquard P., 1983. An experimental study of intraspecific competition within several forage crops. Agronomie 3 : 451-459.