10

Evaluation of Perennial Legumes Collected in Italy: First Year Data

M. A. Pagnotta, S. J. Bennett, P. S. Cocks, M. Ewing, J. Howieson, G. Moore, H. Norman, B. Nutt, C. Porqueddu, L. Russi and R. Snowball

Summary

Ecotypic variation was studied between and within different perennial legume species collected from three Italian regions: Calabria–Sicily, Tuscany–Lathium–Umbria, and Sardinia. The germplasm was evaluated at the University of Tuscia's experimental field in a randomised block design with two replications.

Eighteen morphological characters were scored and analysed using analysis of variance, principal components analysis, and discriminant function analysis. Correlations between the morphological characters and the edaphic factors of the collection sites were calculated. The analyses were run on all the species of the trial, and focused on some species with more accessions, that is *Trifolium pratense*, *Medicago lupulina* and *Lotus corniculatus* group. The major differences were, as expected, between species but there were also some statistical differences between accessions within a species.

Some weak correlations between morphological characters and the environmental conditions of the collection site were observed. Considering all the evaluated collection as a whole, the environmental factor with the highest number of correlations was soil pH: it was correlated to plant structure (petiole and internode length), flowering time and seed production.

Some species, such as *Astragalus monspessulanus*, *Trifolium hybridum* and *Argyrolobium zanonii* did not flower at all. *M. lupulina* was the earliest species to flower and plants from the *Lotus corniculatus* group were the latest, not flowering until June.

Using a discriminant function analysis with all the characters recorded and with the species as a grouping strategy, it was possible to measure the similarity between species. Some species were very similar, for example, the clovers and

the *Lotus corniculatus* group, whereas others were distinctly different, for example, *Psoralea bituminosa*.

Introduction

Over large parts of southern and western Australia, the replacement of Eucalyptus forests with a lay farming system of cereal–annual legumes has led to reduced water usage, which in turn has resulted in rising watertables and with it increased soil salinity. The use of perennial legumes, instead of annuals, that may also be active in the field during the summer could partially resolve the problem in controlling raising watertables and preventing salinisation. Phase-farming with lucerne has been successful in neutral and alkaline soils and even in acid soils where a surface application of lime can raise the soil pH. However, the low soil pH of many Western Australian soils and requirements for a more sustainable strategy suggest the need to investigate the potential of other species for these environments. Some genera that contain species that have the potential to fit into a phase-farming system include: *Lotus, Trifolium, Medicago, Coronilla, Lathyrus, Galega, Anthyllis, Astragalus, Melilotus, Hedysarum, Onobrychis*, and *Dorycnium*. These genera occur widely in the Mediterranean basin, however their ecology and ecogeographic requirements are almost unknown, as are their symbiotic relationship with associated *Rhizobium* species.

Acid soils are not common in the Mediterranean basin where most soils are young and derived from limestone. However they can be found in parts of Italy, Greece, Spain and Portugal, as well as in North Africa.

In order to acquire more information on the ecology of some species of perennial legume and to study their distribution in central and southern Italy, a collaborative project was set up between the Universities of Tuscia, Perugia, Western Australia and Murdoch, the Italian National Council of Research, and Agriculture Western Australia. An assessment of the genetic resources of perennial legume species in central and southern Italy, plus a study of the edaphic requirements and genetic variation of each of the species collected will enable more informed introductions of new species to be made in Australia, as well as potentially increase pasture production in Italy.

The first phase of the project consisted of the identification and ecogeographic survey of about twenty-five sites for each of the three Italian regions selected: Central Italy (Lathium, Umbria and Tuscany); Southern Italy (Calabria and Sicily); and Sardinia (Fig. 10.1; Russi et al. 2003), plus the identification of the perennial legumes found at each site. A collection mission was run in July 1999 to collect seeds of perennial legume species from each of the identified sites (Russi et al. 2003).

An evaluation trial (second phase of the project) is essential to monitor the differences that are present in the collected germplasm, plus it will provide useful information for future germplasm utilisation and collection (Erskine & Williams

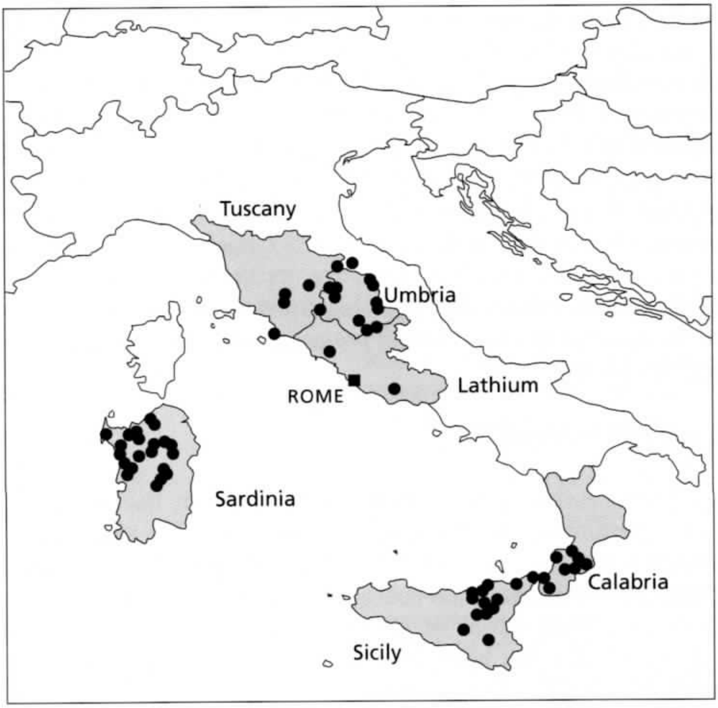

Fig. 10.1: Location of collection sites in Italy in 1999.

1980), even if the differences existing in the environmental characteristics of the three key locations of all the introduction process—collection site, evaluation site and introduction site—are the limit of the evaluation. Hence, it is usual to evaluate the wide adaptation of accessions rather than their specificity to a particular environment. Many breeding programs consider wide adaptation a primary objective, as this quality implies the existence of genes insensitive to change under some environmental conditions. Accessions are then adapted to a wide range of environments, even if the term widely adapted is used mainly in a geographical sense rather than environment sense (Tigerstedt 1994). It should be mentioned that in some cases the farmer's interest, especially in marginal conditions, is to have a constant production; in this case, specific adaptation is more important than a wide one (Ceccarelli 1994). In the present project, one aim of the evaluation is to reduce the germplasm to only the species and accessions that could be of potential interest in southern Australia, as well as measuring adaptation and characterisation of each species. The species selected to be of potential in southern Australia, will then be sent to Australia for further evaluation. It is hypothesised that this evaluation will identify species that are useful in improving the pasture and grazing industry in both Italy and Australia, and that these species could be an important alternative to white clover and lucerne.

For example in the grazing industry of Australia, even if significant improvements are made, white clover has a lack of well-adapted cultivars for the Australian environment (Jahufer et al. 1999; 1996; Mason 1993). A similar study was conducted in Morocco by Beale et al. (1991) on the annual legume species.

The aim of this study was to evaluate the perennial legume species that were collected from southern and central Italy in July 1999 in order to quantify phenological and morphological variation; to relate that variation to the edaphic characteristics of the collection sites of each accession; and to identify promising species and accessions. This paper reports the results of all the species evaluated together, and presents separately the evaluation of three of the more commonly collected species *Trifolium pratense*, *Lotus corniculatus* group, and *Medicago lupulina*.

Materials and Methods

Seeds collected during summer 1999 at three different Italian regions (Fig. 10.1) were germinated in Petri dishes in September 1999 after scarification by sandpaper. The seedlings were transplanted to the greenhouse and then transplanted to the experimental field at the University of Tuscia, Viterbo, Italy (80 km north of Rome, 42° 25′ N, 12° 05′ E) in October 1999. The plants were watered immediately after being transplanted, but were then kept under rainfed conditions without further irrigation.

The trial was set up as a randomised block design with two replications. Each plot of 2 m^2 (1 × 2 m) consisted of a maximum of twelve spaced (50 × 50 cm) plants. Due to low germination rates in some accessions, not all the plots contained the maximum twelve plants. The low germination rate may be a result of having to collect all the seeds from all species and all sites at the same time, even if different species and different sites have different times of maturity. The following species, with the number of accessions in brackets, were evaluated: *Argyrolobium zanonii* (3); *Ononis natrix* (2); *Astragalus monspessulanus* (3); *Ononis* sp. (2); *Coronilla emerus* (2); *Psoralea bituminosa* (11); *Hedysarum coronarium* (3); *Trifolium fragiferum* (3); *Lotus cytisoides* (3); *Trifolium hybridum* (2); *Lotus corniculatus* (22); *Trifolium ochroleucum* (6); *Lotus corniculatus* group (1); *Trifolium pratense* (36); *Medicago lupulina* (15); *Trifolium* sp. (1); *Medicago sativa* (1).

The agronomic and morphological characteristics recorded during the first growing season (1999–2000) are reported in Table 10.1. The season was characterised by a wet autumn and spring, a cold January and a very hot summer with temperatures of 38°C (Fig. 10.2).

Statistical analysis

A fixed effect model of variance of analysis (GLM-ANOVA Procedure) was used to detect the effects of ‘species’, ‘sites’ and ‘blocks’. The analysis of variance was

Table 10.1: Morphological characters scored in the evaluation trial with abbreviations and description.

Character	Abbreviation	Measures
Number of plants on 20.3.2000	Plns 20.3	Number of plants present in each plot on 20 March 2000
Plant width (cm)	Plnwid	Diameter measured in April 2000 on individual plants
Plant height (cm)	Plnlen	Height measured in April 2000 on individual plants
Seed number in 5 legumes	Seednub	Number of seeds in 5 random legumes for each plot
No. of seeds/legume	Seed/leg	Average number of seeds per legume
Dimension (Spring)	Dimnt1	Sliding rule scale on 30 June 2000
Vigour (Spring)	Vig1	Scored on 30 June 2000; 1 = small plant, 5 = large plant
Dimension (Summer)	Dimnt2	Sliding rule scale on 22 August 2000
Vigour (Summer)	Vig2	Scored on 22 August 2000; 1 = small plant, 5 = large plant
Leaf length (mm)	Lflen	At the first flowering node on three plants per plot, on imparipinnate leaf
Leaf width (mm)	Lfwid	At the first flowering node on three plants per plot, on imparipinnate leaf
Petiole length (mm)	Petlen	Measured on three plants per plot from leaflet above
Internode length (mm)	Intnod	Measured on three plants per plot from the upper to the 2nd flowering node
Days to the first flower	Flwstr	Of an individual plant in number of days from 1 May 2000
Days to the 50% flower	Flw50	Of an individual plant in number of days from 1 May 2000
Days to the 100% flower	Flw100	Of an individual plant in number of days from 1 May 2000
End of flowering	EndFlwr	Number of days from 1 May 2000 when all the plant of each plot have no flower
Late re-flowering	LteFlw	Presence of flower (yes or no) on 22 August 2000

also performed considering only the more representative species, one by one; in these cases 'accessions' and 'blocks' were the main factors.

The Principal Component Analysis (PCA) and Discriminant Function Analysis were computed in order to sort the data. PCA reduces the number of variables to a smaller number of unrelated factors, and reports the amount of variance explained by each factor as well as the contribution of the variables to each factor. The discriminant function analysis measures how well it is possible to discriminate between groups, the grouping strategies being specified as both region and species.

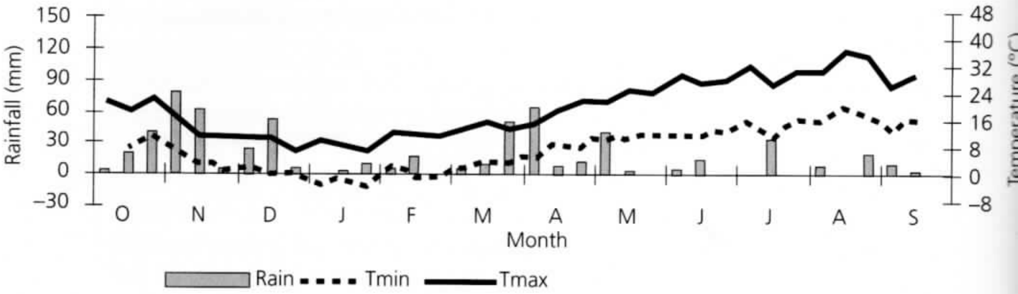

Fig. 10.2: Meteorological data at Viterbo (1999–2000) during the evaluation trial.

'SYSTAT 7 for Windows' and 'BMDP' (Dixon et al. 1990) statistical computer packages were utilised.

Results

Character description

Flowering time ranged over about sixty-four days, however some species (*Astragalus monspessulanus*, *Trifolium hybridum*, *Argyrolobium zanonii*) did not flower during the first year. *M. lupulina* was the earliest species to start and finish flowering, *T. pratense* began flowering mid to end of May, while the *Lotus corniculatus* group was later, beginning in June.

Plant diameter ranged widely both within and between species, the largest species were *T. pratense* (up to 145 cm), *M. lupulina* (up to 137 cm) and *Coronilla emerus* (up to 101 cm wide). The coefficient of variation (CV) was high between species (seventy-one per cent) but less within species (about forty-seven per cent).

On the other hand, plant height did not vary much for prostrate species such as *T. pratense*, *M. lupulina* and *Coronilla emerus*, but for erect species such as *Medicago sativa* and *Psoralea bituminosa* the coefficient of variation was 105 per cent. Some other species such as *T. ochroleucum* and the *Lotus corniculatus* group had a high diversity in plant height with a CV of 151 per cent.

Number of plants present on 20 March 2000 gave an indication of the result of two phenomena: (i) germination rate and (ii) acclimatisation after transplanting. Greatest differences were found to be within species rather than between species, with the number of plants ranging from one to twelve.

Number of seeds per pod was clearly a species specific character, but even within a species it had a coefficient of variability of about forty per cent. A similar situation was found for the number of seeds.

Characters such as leaf length, leaf width, petiole length and internode length were specific for each species. Despite this, variation within species was recorded for all the characters.

Correlations

Tables 10.2 to 10.5 show the significant correlations of the morphological characters recorded at Viterbo in 2000 and the edaphic conditions of the collection sites for all the species evaluated (Table 10.2) and for the three most common species *M. lupulina*, *T. pratense* and the *Lotus corniculatus* group (Tables 10.3 to 10.5) respectively. Soil pH was the environmental factor with the highest

Table 10.2: Statistically significant Pearson's correlations between morphological characters and environmental factors of the collection site.

	Soil pH	Ex-ac[1]	Longitude	Altitude	Surface
Petlen	0.36	0.36	0.40		
Intnod	–0.39	0.36			
Flwstr	0.44	0.40		–0.40	
Flw50	0.42	0.39			
Flw100	0.36	0.36			
Seednub	–0.55	0.56	–0.53		0.43
Seed/leg	–0.57	0.57	–0.54		0.44
Plnwid			–0.40		

[1] Ex-ac = Exchangeable acidity, Surface = Soil type at the soil surface.

Table 10.3: Statistically significant Pearson's correlations between morphological characters and environmental factors of the collection site for *Medicago lupulina*.

1	Plnwid	EndFlwr	Lflen	Lfwid	Seed nub	Seed/ leg	Intnod	Petlen	Dimnt
Gravel			–0.66	–0.65					
Fine-soil			0.66	0.65					
Sand	0.55	–0.41							
Silt		0.50							
Clay	–0.53								
pH				–0.56	–0.62	–0.62	–0.43		
OM							0.49		
N							0.49		
CEC							0.53		
Ex-K								0.47	
Latitude	–0.43	0.50							–0.43
Longitude									0.46
Tmin	0.62	–0.51							
Tmax								0.40	–0.41
Rain					0.45	0.45			
Dayrain					0.48	0.48			
Altitude									–0.55
Aspect	0.51				–0.41	–0.41			
Slope		–0.48							
Habitat									0.41
Grazing									0.40
Surface									–0.54

[1] OM = % organic matter, N = % nitrogen, CEC = electric conductivity, Ex-K = exchangeable potassium, Tmin = minimum temperature, Tmax = maximum temperature, Rain = mean annual rainfall, Dayrain = number of rainy days, Surface = soil type at the soil surface.

Table 10.4: Statistically significant Pearson's correlations between morphological characters and environmental factors of the collection site for *Trifolium pratense*.

	% Phosphate	Latitude	Longitude
Seednub			−0.50
Seed/leg			−0.50
Dimnt	−0.34	0.44	
Lflen	0.37		−0.42
Lfwid	0.51	0.48	

Table 10.5: Statistically significant Pearson's correlations between morphological characters and environmental factors of the collection site for the *Lotus corniculatus* group.

[1]	Plnwid	EndFlwr	Seed nub	Seed/ leg	Dimnt	Lfwid	Petlen	Intnod	Flwstr	Flw50	Flw100
Soil pH	−0.67				0.54		0.72		0.79	0.61	0.50
OM					0.42		0.42	−0.44			
CEC					0.48		0.48	−0.45			
Ex-K							0.49	−0.52			
Latitude			−0.58	−0.58	0.49		0.63			0.43	
Longitude	−0.55				0.57			−0.44	0.59		
Tmin			0.60	0.60	0.49		−0.46	0.50	−0.38	−0.46	
Tmax		0.56			0.41	0.43					
Rain		−0.61				−0.38		−0.43			
Altitude					0.55			−0.40			
Aspect	−0.53		−0.61	−0.61	0.46		0.59		0.52	0.59	0.44
Slope					0.43						
Grazing	0.54					−0.39					
Rock		−0.56	−0.57	−0.57		−0.50					

[1] OM = % organic matter, CEC = Electric conductibility, Ex-K = exchangeable potassium, Tmin = minimum temperature, Tmax = maximum temperature, Rain = mean annual rainfall, Rock = % rocks in soil.

number of correlations if all the species are considered together (Table 10.2), it had an effect on plant structure (petiole and internode length), flowering time and seed production. Looking at the individual species, *Trifolium pratense* had no correlations with soil pH (Table 10.4), altitude was positively related to plant dimension in the *Lotus corniculatus* group, and rainfall affected seed production in *M. lupulina* (Table 10.3) and end of flowering and leaf size in the *Lotus corniculatus* group. The *Lotus corniculatus group* (Table 10.5) had the highest number of significant correlations while *T. pratense* had the lowest. In the *Lotus corniculatus* group the more vigorous plants come from the northern area, from an higher elevation and from more fertile soils; time of flower was related to exposure, with late flowering plants coming from sites with a north-west exposure, higher pH and low minimum temperatures.

Analysis of variance

The analysis of variance shows the presence of highly significant differences between species for all the analysed characters. Differences were found between sites for number of plants on 20 March 2000, first and second plant dimension scores, first and second vigour ratings, leaflet length and width, days to flowering and end of flowering.

The analysis of variance was also run restricting the data, one by one, to the species with higher numbers of accessions (*T. pratense, M. lupulina,* and the *Lotus corniculatus* group). They showed significant differences between accessions for only a limited number of characteristics. These are the seed characters, leaf dimensions and end of flowering in *T. pratense*, and plant and leaf size, and flowering time in the *Lotus corniculatus* group. *M. lupulina* had an highly significant difference between accessions only in the number of plants on 20 March 2000, indicating a very low variability between the different accessions of this species.

Principal components analysis

The first three components of the PCA explained sixty-seven per cent of the variance. The most important characters in the first component were flowering time and the plant dimension characters, in the second component were seed production and plant dimension, and in the third component summer vigour and late flowering (Fig. 10.3).

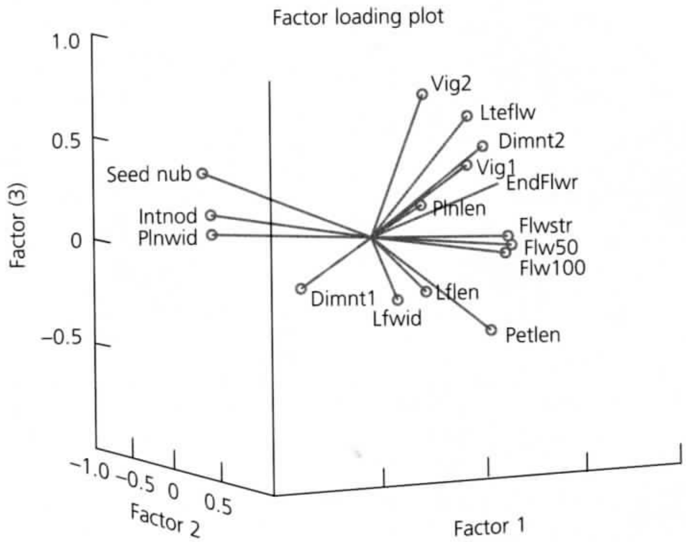

Fig. 10.3: Principal components analysis of evaluated characteristics.

Discriminant analysis

Grouping by region

Using the environmental characters of the sites and regions as the grouping factor, the collection sites were well classified to their region of origin. This included considering Calabria and Sicily as separate regions, with the only exception being that Sicily had one site classified as from Central Italy. Sicily and Calabria were also shown to be close together (Fig. 10.4). All the environmental factors were included in the classification except for percentage clay, fine soil, and nitrogen. Factors one and two were mainly characterised by the soil fertility factors.

However, if the morphological characters evaluated at Viterbo for all the species present in the trial were considered, the sites were not clearly separated into regions, with only just over sixty per cent of the sites correctly classified to their region of origin. This reveals the weak effect of edaphic characters of the regions on the morphological characters recorded.

Restricting the analysis to the species with an high number of accessions (*T. pratense, M. lupulina,* the *L. corniculatus* group and *Psoralea bituminosa*) and merging Calabria and Sicily, the evaluated morphological characters were more successful in discriminating between the regions (Fig. 10.5), with eighty-six per cent of the sites being correctly classified to their region or origin.

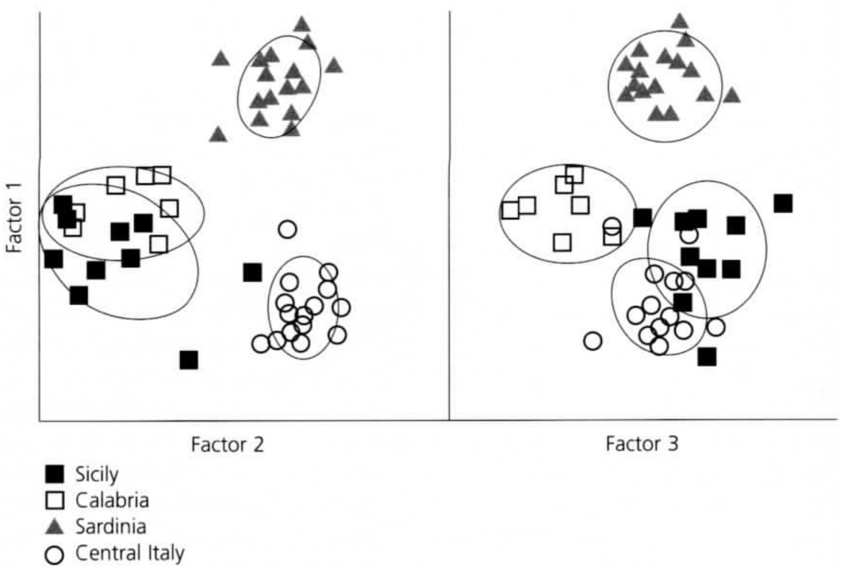

Fig. 10.4: Discriminant analyses using the regions as grouping factor for the edaphic factors of the collection sites.

Grouping by species

When the species was used as the grouping factor and the morphological characters included in the analysis, differences and similarities between the species included in the evaluation were revealed (Fig. 10.6). Some species were shown to be very similar, for example *Trifolium* and *Lotus* species, whereas others were markedly different, for example *Psoralea bituminosa*.

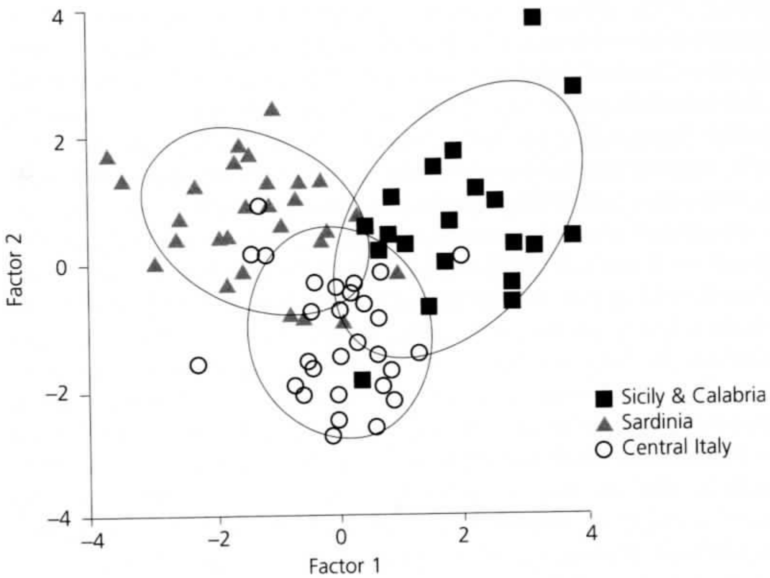

Fig. 10.5: Discriminant analyses restricting the vegetation characteristics data only to the more representative species (*Trifolium pratense*, *Medicago lupulina*, *Lotus* and *Psoralea bituminosa*) grouped on the basis of region of origin.

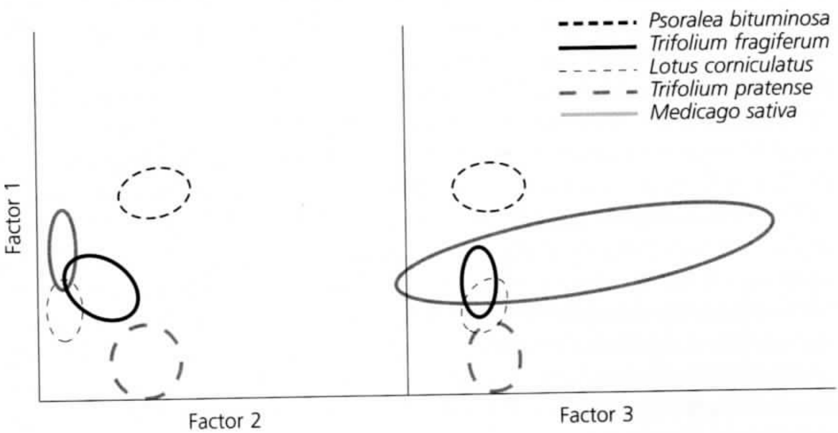

Fig. 10.6: Discriminant analyses of evaluated characteristics using species as the grouping factor.

Discussion

The species analysed in this study were found to show differences in many characters such as flowering time, plant dimension, vigour in summer, and so on. Moreover, some of the species did not flower the first year and there was a wide range in germination vigour. It is suggested that this is due to the fact that the collection was run on a pre-fixed calendar, and that even though latitude was adopted in the collection calendar with the southern sites being visited first, differences in altitude as well as differences between species in seed maturity were not considered. As a result, the seeds were not always collected at the best time. Bennett et al. (1998) faced the same difficulties during a collection in Turkey. They have attributed the lack of some species at a given altitude to the fact that the collection was run too early for seed collection at high elevation sites rather than ascribing it to altitude specifications.

The species that were best adapted to summer growth were the *L. corniculatus* group, as it was late to flower, but also on average was the first to recommence flowering after the hot period even if some accessions had shown poor growth after the summer. As a species it did not have a very high biomass in the first year, and may have suffered more than the other species from transplantation. It is thought that a second year is needed for the plants to attain maximum biomass compared to the other species. *T. pratense* showed good biomass production, which combined with early establishment led to a high biomass in the first year. Despite this, the two species were not shown to be very different by discriminant analysis (Fig. 10.4). By comparison, *Psoralea bituminosa* was different from all the other species evaluated; it showed a very high vigour both in spring and in summer. It has been shown to be a strong species that can survive in dry areas, however there may be a problem with its palatability. Some accessions from France are eaten by sheep while other accessions from north Africa are not touched at all (Gintzburger, pers. comm.). Further research is needed to verify whether the species' forage quality level can be improved, as it would be quite promising in terms of dry matter production and adaptability. *Coronilla emerus* had a good ground cover, but suffered during the dry summer conditions. Only one accession was collected, and hence it is not possible to generalise further. The same should be said about *Hedysarum coronarium* with only three accessions evaluated and with great differences found between accessions. *M. lupulina* established well but flowered very early and was dry during the summer; also it suffered from pod-lodging. This could be an advantage if late developing inflorescences produce smaller seeds than earlier ones as found by Cocks (1990) in annual medics. Since the analysis of variance did not show significant differences between *M. lupulina* accessions, but the coefficient of variation in the individual characters was not very different from the coefficient of variation in other species, it can be concluded that the variability in *M. lupulina* was mainly within accession. The principal components analysis of the morphological characters gives useful information about the relationships between the evaluated characters. For instance, number of days to first flower, fifty per cent of plant flowering and one hundred per cent of plant flowering were three characters that were very time consuming to score as the state of flowering of each plant had to be monitored every day, but were found to be highly related (Fig. 10.3). As a result, recording only one of these three characters could save time and available resources.

Surprisingly the species distribution and the evaluated phenotypic characters showed little relationship to the environmental characters of the collection sites, and even if the three regions were separated by the edaphic characters in the discriminant analysis (Fig. 10.4), they were not if the morphological characters were used. Beale et al. (1991) also found a lack of relationship between the distribution of annual legumes in Morocco and soil nutrient. While Smith et al. (1995) did not find any correlation between flowering time in *Trifolium glomeratum* collected in southern Australia and annual rainfall or growing season length at the collection site, and in general found a weak correlation between plants traits and climatic variables. However, Pagnotta and collaborators (Nevo et al. 1995; Pagnotta et al. 1995; 1997) found a good relationship between edaphic conditions and genetic variability using markers at the DNA level. It is possible that establishment problems in the first year may hide genetic differences in morphological characters that are related to the edaphic conditions of the collection sites. This is a large problem with evaluation trials that take place away from the site of collection, as they do not provide any information on specific adaptability at the site of collection. However when accessions from a number of sites are to be evaluated it is the only feasible method. In the present experiment there were three factors that were likely to have had an impact on the results presented in this paper: (i) the different species had different numbers of accessions so that their contribution in any analysis of a particular character was biased; (ii) only the first year data is presented and some accessions were affected by being transplanted and were not fully established; and (iii) large genetic differences were recorded within an accession which reduced the effect of species differences.

There is little information on the distribution of perennial legumes in general and in southern Italy in particular. This study aimed to record the differences that are present between and within species, and to understand the ecology of some perennial legume species. The study highlights the large genetic diversity both in term of species and variation within species. The results of this study will be used to identify species that are summer active, and therefore have potential for southern Australia. Further evaluation will be undertaken on these species in southern Australia. This collection and subsequent evaluation is also important in preserving the diversity that is currently present in Italy, and in identifying sites where an in situ conservation project would be valuable. This is particularly relevant as some of the collection sites were in natural reserves and protected areas.

References

Beal, P. E., Lahlou, A. & Bounejmate, M. (1991). Distribution of wild annual legume species in Morocco and relationship with soil and climatic factors, *Australian Journal of Agricultural Research*, **42**, 1217–30.

Bennett, S. J., Maxted, N. & Sabanci, C. O. (1998). The ecogeography and collection of grain, forage and pasture legumes in south-west Turkey, *Genetic Resources and Crop Evolution*, **45**, 253–62.

Ceccarelli, S. (1994). Specific adaptation and breeding for marginal conditions, *Euphytica*, **77**, 205–19.

Cocks, P. S. (1990). Dynamics of flower and pod production in annual medics. I. In space plants, *Australian Journal of Agricultural Research*, **41**, 911–21.

Dixon, W. J., Brown, M. B., Engelman, L. & Jennrich, R. I. (1990). *BMDP Statistical Software*, University of California Press, California, 1377.

Erskine, W. & Williams, J. T. (1980). The principles, problems and responsibility of preliminary evaluation of genetic resource samples of seed-propagated crop, *FAO/IBPGR Plant Genetic Resources Newsletter*, **41**, 91–132.

Jahufer, M. Z. Z., Lane, L. A. & Ayres, J. F. (1996). White clover improvement for Australian drylands. I. The White Clover Collection, *Australian Plant Introduction Review*, **26**, 1–5.

Jahufer, M. Z. Z., Cooper, M., Bray, R. A. & Ayres, J. F. (1999). Evaluation of white clover (*Trifolium repens* L.) populations for summer moisture stress adaptation in Australia, *Australian Journal of Agricultural Research*, **50**, 561–74.

Mason, W. (1993). White Clover: a key to increasing milk yields, Dairy research and Development Corporation, Melbourne.

Nevo, E., Pagnotta, M. A., Beiles, A. & Porceddu, E. (1995). Wheat storage proteins: glutenin diversity in wild emmer, *Triticum dicoccoides*, in Israel and Turkey. 3. Environmental correlates and allozymic associations, *Theoretical and Applied Genetics*, **91**, 415–20.

Pagnotta, M. A., Nevo, E., Beiles, A. & Porceddu, E. (1995). Wheat storage proteins: glutenin diversity in wild emmer, *Triticum dicoccoides*, in Israel and Turkey. 2. DNA diversity detected by PCR, *Theoretical and Applied Genetics*, **91**, 409–14.

Pagnotta, M. A., Snaydon, R. W. & Cocks, P. S. (1997). The effects of environmental factors on components and attributes of a Mediterranean grassland, *Journal of Applied Ecology*, **34**, 29–42.

Russi, L., Bennett, S. J., Pagnotta, M. A., Norman, H., Nutt, B. J., Porqueddu, C., Cagiotti, M., Snowball, R. & Moore, G. (2003). Ecogeography of the perennial legume collecting missions in Italy, in *New Perennial Legumes for Sustainable Agriculture*, this volume (ed. S. J. Bennett), UWA Press, Australia.

Smith, F. P., Cocks, P. S. & Ewing, M. A. (1995). Variation in the morphology and flowering time of cluster clover (*Trifolium glomeratum* L.) and its relationship to distribution in Southern Australia, *Australian Journal of Agricultural Research*, **46**, 1027–38.

Tigerstedt, P. M. A. (1994) Adaptation, variation and selection in marginal areas, *Euphytica*, **77**, 171–4.