GENETIC VARIABILITY OF LUCERNE LANDRACES FROM CENTRAL ITALY DETECTED BY RAPD MARKERS

L. Russi, G. Barcaccia, M. Falcinelli and F. Veronesi
Istituto di Miglioramento Genetico Vegetale, University of Perugia (Italy)
Telefax +39 75 5856224 E-mail IMGV@UNIPG.IT

ABSTRACT
With the aim to characterize six lucerne landraces (*Medicago sativa* L.), representing a sample of a collection from central Italy, sixty individuals per landrace were evaluated by screening for RAPD markers with three lucerne-specific primers. Twenty-one amplification products were scored as present or absent across all plants. The dendrogram from mean genetic similarity estimates displayed Casalina alone and the other landraces clustered into one distinct group, showing a single branch point with more than 73% of genetic similarity. The discriminant analysis grouped the landraces in a similar manner. The first function maximally separated the group Grosseto, Gubbio and C. Pieve from Latina and L'Aquila while the second function maximally separated Casalina from the rest of landraces. Overall 56% of individual plants were correctly re-classified into their own groups. Owing to their rather narrow geographic provenance, more primers are needed to increase precision in the estimate of the genetic variability.

KEYWORDS
Lucerne germplasm collection, molecular breeding, genomic polymorphism, cluster analysis, discriminant analysis

INTRODUCTION
In Italy cultivated lucerne (*Medicago sativa* L.) is grown on about one million hectares and represents the most important leguminous forage crop. Because of its relevant agronomic feature in restoring soil structure and fertility and owing to the high energy efficiency of its forage, lucerne stands for low input agricultural systems and occupies a significant economic role in the market of animal food.

In 1995 the Italian National Register of Varieties included 107 cultivars and 14 landraces and in the same year governmental regulations indicated that by year 2002 landraces will be definitely cancelled from it, despite their large use by farmers (70% of the seed market). In the Po Valley (northern Italy), the cultivation area is very large and genetic differences among landraces are expected to be small, while in central Italy the hectares are much less but the variability in soils and climatic conditions have originated many local landraces. The loss of adapted materials is at risk if research institutions would not take care of collecting and conserving this germplasm and, at the same time, evaluate it for agronomic traits and genomic variability.

The genetic diversity present in lucerne populations have been largely detected by isozymes (Quiros and Bauchan, 1988) and RFLP markers (Kidwell *et al*., 1994). The analysis by RAPD markers could also be successfully employed to study the genetic variability within and among lucerne populations.

The present research reports the genetic variability as estimated using RAPD markers within and among lucerne landraces from central Italy and based on individual DNA samples.

MATERIALS AND METHODS
Six landraces from central Italy (Fig. 1) were evaluated at Perugia (43°05'N). Sixty random plants per landrace were used for the analysis of RAPD markers. Total genomic DNA was isolated following the procedure described by Edwards et al. (1991). Primers BY15, NS13, and NS12 were the best from a set of primers selected in previous investigations on the basis of their ability to find homologous binding sites among lucerne genomic templates (Barcaccia, 1994). The polymerase chain reaction and electrophoresis were performed according to the protocol reported in details by Barcaccia (1994). Banding profiles of individual DNA samples were recorded by assigning a number to each polymorphic amplification product. Only intense RAPD bands ranging in size from 0.3 to 2.2 Kb were included in the analysis. Each amplification product was scored as 1 for presence and 0 for absence. Similarity matrices were calculated in all possible pair-wise comparisons between individual plants and between means of landraces using the genetic similarity estimate (GSE) of Dice (1945). The data were also analyzed by a stepwise discriminant procedure and the best predictor markers selected and used to perform a discriminant analysis.

RESULTS AND DISCUSSION
A total of 21 RAPD markers (an average of seven markers per primer) were scored in all landraces. Unique RAPD markers necessary to identify a landrace were not detected, however four different markers resulted monomorphic within a single landrace. On the whole, five amplification products were highly conserved among landraces, being shared from 88.5% to 99.7% of individuals. Most of the amplification products were highly polymorphic, showing a balanced frequency presence among landraces.

The mean GSEs within landraces ranged from 0.690 (Casalina) to 0.777 (Grosseto) and between pair-wise comparisons of landraces from 0.688 (L'Aquila-Casalina) to 0.769 (Grosseto-C. Pieve). The dendrogram of landraces displayed Casalina alone and the rest clustered into one group, which in turn was split into two distinct subgroups; the former included C. Pieve, Grosseto and Gubbio, the latter L'Aquila and Latina (Fig. 2a). The results of the cluster analysis were in agreement with those from discriminant analysis, where the centroids were plotted according to functions 1 and 2 (Fig. 2b). Using 16 out of 21 RAPD markers scored, five discriminant functions were found, resulting all highly significant with the first three functions able to explain as much as 89% of the total variation. Function 1 maximally separated the group Grosseto, Gubbio and C. Pieve from Latina and L'Aquila; the best predictors for discriminating these two groups were BY15(1) and NS12(5). Function 2 maximally separated Casalina from the rest of landraces and the best predictor was NS12(3). Re-substitution results, carried out according to the classification functions, were rather encouraging: overall 56% of individual plants were correctly re-classified into their own groups, with a minimum in Gubbio (47%, due to the closeness of its centroid with Grosseto and C. Pieve), and a maximum in L'Aquila (72%).

The use of RAPD markers with selected ten-mer primers permitted of assaying the levels of genomic relationship within and between lucerne landraces. The analysis based on single plants proved to be essential to estimate the genetic variability between lucerne landraces having a strict geographic origin and to get information sufficient to describe also the level of genetic variability within all lucerne landraces. On the whole, the results obtained suggest that five out of six landraces evaluated here share a large part of the genomic traits. Casalina was clustered apart and this could indicate the presence of a certain amount of unique germplasm.

These preliminary results indicate the need, in presence of germplasm collected from a narrow geographic area, of using other primers in order to have as many RAPD markers as possible and increasing the precision of population characterization by cluster and discriminant analyses. They also suggest that RAPD markers may be a useful tool for the constitution of core collections of allogamous species, such as lucerne, to select the most representative landraces and discard the similar ones.

ACKNOWLEDGMENTS

This research was supported by the Italian Ministry of Agriculture and Forestry, project Foraggicoltura Prativa, subproject Miglioramento Genetico.

REFERENCES

Barcaccia, G. (1994) Development, comparability and potential applications of RAPD markers in genus Medicago. J. Genet. Breed., **48:** 161-168.

Dice, L.R. (1945) Measures of the amount of ecological association between species. Ecology, **26:** 297-302.

Edwards, K., Johnstone, C., and Thompson, C. (1991) A simple and RAPD method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res., **19:** 1349.

Kidwell, K.K., Austin, D.F., and Osborn, T.C. (1994) RFLP evaluation of nine accessions representing the original germplasm sources for North American alfalfa cultivars. Crop Sci., **34:** 230-236.

Quiros, C.F., and Morgan, K. (1981) Peroxidase and leucine-aminopeptidase in diploid Medicago species closely related to alfalfa: Multiple gene loci, multiple allelism, and linkage. Theor. Appl. Genet., **60:** 221-228.

Figure 1

Collection sites of the six lucerne entries extracted from a collection of landraces from central Italy.

Figure 2

(a)

Dendrogram of the six lucerne landraces. Cluster analysis was performed using the mean genetic similarity estimates;

(b)

Centroids relative to the six lucerne landraces plotted according to discriminant functions 1 and 2. Function 1 resulted highly correlated with markers BY15(1) and NS12(5) while function 2 with marker NS12(3).