

# A MULTIVARIATE ASSESSMENT OF VARIATION WITHIN AND AMONG LADINO WHITE CLOVER ECOTYPES

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## ABSTRACT

Some 285 Ladino white clover genotypes selected from four ecotypes were multiplied clonally and evaluated in a replicated pot experiment over one year. Multivariate patterns of variation were depicted by principal component analysis performed on the correlation matrix of five morphophysiological traits, namely petiole length, central leaflet size, head production, number of florets per head and stolon density. Selection was based on high broad sense heritability values of all of these traits. The level of intra-population variation was so high relative to inter-population variation that almost all plant types possible could be found within each ecotype, although at a variable frequency. Genetic distances between populations, computed as unsquared Euclidean distances between mean values of the ecotypes in the space of the significant PC axes, were consistent ( $r = 0.87$ ,  $P < 0.03$ ) with those issued from a previous evaluation in dense, sown plots. The implications of the present findings on collection, preservation and selection activities is discussed.

## KEYWORDS

ecotypes, genetic distance, genetic resources, intra-population variation, Ladino, multivariate analysis, population structure, *Trifolium repens*.

## INTRODUCTION

The improvement of white clover (*Trifolium repens* L.) targeted to intensive, lowland farming has extensively relied on the large-leaved, Ladino type (Williams, 1987). Farm landraces and natural populations of Ladino were collected from their centre of origin, in the Po Valley (northern Italy), and were evaluated in dense, sown plots for several agronomic traits (Annicchiarico, 1993). Multivariate patterns of variation and genetic distances between populations were also assessed (Annicchiarico, 1996). A number of genotypes randomly chosen from four ecotypes were evaluated to investigate the level of variation within populations relative to among populations variation for various individual traits (Annicchiarico and Piano, 1995). The present study adds to the previous findings by providing a multivariate-based comparison of intra- vs. inter-population variation. A second objective of the study was verifying whether the genetic distances between populations estimated from evaluation in dense, sown plots are consistent with genetic distances determined from evaluation of genotypes grown in isolation. The information on the relative extent of variation within and among populations may bring important implications on the strategies for collection, preservation and exploitation for breeding (Frankel and Hawkes, 1975). The repeatability of estimates of genetic distance is a prerequisite for their utilization to define a genetic base for breeding or to establish core collections (Annicchiarico, 1996).

## MATERIALS AND METHODS

With reference to the collection number of the Ladino ecotypes reported by Annicchiarico (1993), 74 genotypes from each of landraces '19' and '20', 72 from landrace '32', and 65 from natural population '24' were evaluated in a pot experiment carried out in 1992 and was designed as a randomised complete block with two replicates. After clonal multiplication of the materials, two cuttings 6 cm long were transplanted into 0.25 m diameter pots with a 0.25 m depth filled with local, sandy-loam soil and placed in open field.

Details about management of the trial and procedures for recording of characters were provided by Annicchiarico and Piano (1995). Multivariate patterns of variation of the genotypes were depicted by a principal components analysis performed on the correlation matrix of five morphophysiological traits, namely petiole length, central leaflet size, number of heads per m<sup>2</sup>, number of florets per head and stolon density. The broad sense heritability of these traits was preliminarily verified. Principal component (PC) axes showing eigenvalues greater than unity were considered significant (Dagnelie, 1975). Mean values of the four populations on PC axes were computed and were used to estimate genetic distances between populations defined as unsquared Euclidean distances in the space of the significant PC axes. The consistency of the six genetic distances, relative to all pair of populations, with those obtained from the previous evaluation of populations in dense, sown plots using the same original variables and statistical procedures (Annicchiarico, 1996) was assessed by simple correlation.

## RESULTS AND DISCUSSION

The broad sense heritability coefficients computed on a genotype mean basis confirmed that a large part of the variation was genetic (Table 1), indicating that the assessment of patterns of variation and the estimates of genetic distance based on phenotypic variation were reliable.

In the principal components analysis, eigenvalues greater than unity were shown by the first three PC axes. PC 1 accounted for 35% of the overall variation and, following its eigenvectors (Table 1), was a positive indicator of leaf lamina and petiole gigantism and head production. PC 2 resumed 26% of variation and was mainly associated to stolon density. PC 3 summarized 20% of variation and was a positive indicator of head production and, to a lesser extent, number of florets per head. The distribution of the genotypes in the space of the first two PC axes is given in Figure 1, in which the mean values of the ecotypes are also reported. There was a trend for the genotypes of ecotype '24', the only natural population, to show lower values on PC 1. The differences between ecotypes were negligible on PC 2, and were small on PC 3 (data not shown). The level of intra-population variation was so high relative to inter-population variation that almost all plant types possible could be found within each ecotype, although at a frequency variable between ecotypes (Figure 1). The present findings, in conjunction with those regarding variation for individual traits (Annicchiarico and Piano, 1995), confirm the high level of intra-population variation already reported by Burdon (1980) and by Caradus and Woodfield (1990) for non-Ladino ecotypes. They also suggest that a wide range of genetic variation can be provided by a relatively low number of collected and/or preserved white clover ecotypes, and that appropriate emphasis should be placed on the evaluation of a high number of genotypes rather than of populations in order to identify superior materials and maximize the selection efficiency.

The six estimates of genetic distances issued from the two evaluation were closely associated ( $r = 0.87$ ,  $P < 0.03$ ), indicating the reliability of their utilization for breeding or germplasm preservation purposes.

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**Table 1**

Broad sense heritability on an entry mean basis ( $h^2$ ) of original variables, and eigenvectors of the first three axes (PC) of a principal components analysis, for 285 Ladino white clover genotypes.

Trait	$h^2 \pm SE$	Eigenvectors		
		PC 1	PC 2	PC 3
Petiole length	$0.76 \pm 0.03$	0.54	0.48	-0.19
Leaflet size	$0.84 \pm 0.02$	0.65	0.00	-0.21
No. heads per m <sup>2</sup>	$0.87 \pm 0.02$	-0.04	0.44	0.81
No. florets per head	$0.88 \pm 0.02$	0.50	-0.20	0.45
Stolon density	$0.73 \pm 0.03$	-0.18	0.73	-0.24

**Figure 1**

Scores on the first two axes (PC) of a principal components analysis of 285 Ladino white clover genotypes from four ecotypes (black point = ecotype '32', white point = '19', white triangle = '20', asterisk = '24'; small symbols = genotypes, large symbols = mean values of populations).

