Analysis of genetic diversity in white clover (*Trifolium repens*) breeding populations using agro-morphological and RAPD markers

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Introduction White clover is an important forage legume for temperate regions, but very little is known about the genetic organisation of its breeding populations. The low amount of variability in the Indian collections of white clover for genetic improvement warrants the introduction of new germplasm and collecting local ecotypes for characterisation, utilisation and conservation. Several molecular techniques have been used for germplasm characterisation, variety identification, marker development and identification, molecular diagnostics, phylogenetic studies and diversity analysis. Because of its simplicity, rapidity and reliability, the RAPD technique has been used extensively for diversity analysis. The present study aims at characterising white clover genotypes of distinct geographical origin using standard descriptors and RAPD markers.

Materials and methods Twenty-eight white clover accessions acquired from Western Regional Plant Station, WSU, Pullman, USA, including some locally adapted populations, were grown in a randomised complete block design. The data on 30 descriptors and descriptor states developed by the IPGRI for white clover were recorded. Cluster analysis was done following Beale (1969). Genomic DNA was also extracted from ten randomly taken plants. DNA was isolated, using the CTAB method of Murray & Thompson (1980). The Numerical Taxonomy System of Multivariate Statistical Program (NTSYS) software package was used for data analysis (Rohlf, 1993). Jaccard's similarity coefficient was used for construction of a dendrogram by the Unweighted Paired Group Method of Arithmetic Averages (UPGMA).

Results The Non Hierarchial Euclidean Cluster analysis grouped accessions into 7 broad clusters, showing a high level of genetic divergence. Clusters 7 and 2 were found to be distantly placed. The accessions clustered arbitrarily with genotypes of the same region were found distributed in more than one cluster, while the genotypes of a heterogeneous region were grouped in the same cluster. The clustering pattern depicted the presence of sufficient genetic diversity and showed the lack of correspondence with geographical affinities of accessions. The morphological characterisation proved useful for cultivar discrimination and diversity analysis and gene bank management for white clover. A local collection RRCP-L-42 was found highly resistant for powdery mildew and clover rot, so that this can be used in resistance breeding. A dendrogram constructed by using molecular data, illustrated that genetic diversity and geographical origin of accessions did not show much correlation. The genotypes of the same regions were distributed in more than one cluster and also the genotypes of heterogeneous regions were grouped in the same clusters. However, one group had all the accessions of local origin. Molecular variation of higher order was resolved in the exotic accessions compared to indigenous germplasm. This suggests the need to introduce exotic germplasm in the Indian white clover gene pool to increase the genetic diversity.

Conclusion The amount and patterns of diversity observed in the white clover accessions can be of value in identifying the populations that parents of synthetic cultivars are derived from and to exploit the variation available in the populations analysed. The characterisation based upon standard descriptors can be of great value in the management of germplasm collections in the gene bank.

References

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