## Changes of Red Clover Variability under Upland Conditions Revealed by Morphological and RAPD Markers

Sonja Grljusic<sup>1</sup>, Snjezana Bolaric<sup>2</sup>, Svetislav Popovic<sup>1</sup>, Marijana Tucak<sup>1</sup> Tihomir Cupic<sup>1</sup> and Vinko Kozumplik<sup>2</sup>

 <sup>1</sup> Department of Forage Crops Breeding and Genetics, Agricultural Institute, Juzno predgrade 17, 31000 Osijek, Croatia
<sup>2</sup> Department of Plant Breeding, Genetics and Biometric, Faculty of Agriculture, University of Zagreb, Svetosimunska cesta 25, 10000 Zagreb, Croatia

Assessment of genetic variability, its partitioning among and within populations and its changes under selection are of concern to plant breeders. Either natural or artificial selection is expected to change gene frequencies in the population under selection pressure. This study was conducted to compare three red clover cultivars (Reichersberger (A), K-17 (SCG), and Croatia (HR)) and their reselections, which survived three years growing in an upland region. The relationship within and between cultivars and reselections was explored. Morphological traits and RAPD markers were used to estimate the variability and changes of variability after natural selection.

The field trial, RCBD with three replications and 200 spaced plants per plot was carried out, at a lowland location. The method used to describe cultivars and reselections morphologically mostly complied with the US protocol of Plant Variety Protection Office for red clover variety description. Types of stem habit, growth type of crown, stem hair, and leaf mark were recorded on each plant. To quantify distance based on morphological characters, type for single plant was marked as 1 and other types of particular character were assigned as 0. Thirty-seven of the oligonucleotide primers were screened. Four of them, with clear, consistent, and highly polymorphic amplified products were used to assay 20 randomly selected plants from each cultivar and reselection. Data were analysed by SAS 6.12, Arlequin 1.1 and NTSYS-pc software.

The proportions of stem hair types were different for all cultivars/reselections investigated. A total of 92 RAPD polymorphic markers were scored. The number of markers per PCR reaction varied from 0 to 17 ranging in size from 300 to 2500 bp for all individuals investigated. There was considerable variation within each cultivar and reselection. The genetic distance between cultivars and reselections based on morphological data ranged from 0,09 to 0,32. Estimated Roger's distance between cultivars and reselections ranged from 0,24 to 0,30. The correlation between matrices was significant at p=0,05. The study has shown that extent of the variability and directions of changes under natural selection were genetically determined. However, further analysis and confirmation of changes are currently underway.