MORPHOLOGICAL AND AGRONOMIC CHARACTERISTICS OF CROATIAN RED CLOVER (*Trifolium pratense* L.) BREEDING POPULATIONS TOLERANT TO ABIOTIC STRESS

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Introduction

Red clover (*Trifolium pretense* L.) is a temperate species originated from Southeast Europe and Asia Minor near the Mediterranean Sea (Taylor and Quesenberry, 1996). It is a short-lived perennial diploid species with seven pairs of chromosomes (Taylor and Quesenberry, 1996), insect pollinated and self incompatible. Red clover can be grown across a wide range of soil types, pH levels, and environmental conditions, and gives good yields in areas not suitable for growing alfalfa (Smith et al., 1985). Knowledge of the amount and distribution of genetic variability within species is crucial for breeders when selecting breeding germplasm (Muntean and Savatti, 2003). Genetic variation is frequently estimated using morphophysiological characters, which often show multigenic inheritance with strong environmental modification (Kölliker et al., 2001).

Tolerance of field crop to abiotic stress, such as drought and cold tolerance (Peter et al., 2006; Pepo et al., 2006; Czővek et al., 2006) is of primary importance for sustainable crop production in stress environment. Tolerance to abiotic stresses in red clover was analysed in few studies (Nowak et al., 1992; Nelke et al., 1999). Red clover is the most important fodder crop in north-western region of Croatia where climate is humid, soils shallow and wet, and vegetation period short (Gagro, 1998). Due to small producing input and multiplied gain, red clover gets more on its importance in sustainable agricultural production where the complete producing cycle tends to be included within the land and facilities of one farm.

Main objective of this research was to estimate variations in morphological and agronomic characteristics among red clover breeding populations tolerant to abiotic stress developed at the Faculty of Agriculture in Zagreb during the last ten years.

Materials and methods

Eleven Croatian red clover breeding populations (Table 1) were included in the study. Commercial cultivar Viola was taken as a standard. The experiment was conducted in the experimental field at the Faculty of Agriculture in Zagreb. Seed was sown in April 2005 in plots. Twelve entries were distributed in randomized block design with four repetitions. Sizes of the experimental plots were $5.25m^2$ and central $3.5m^2$ were considered for evaluation. Soil was not previously fertilized and chemically treated.

Two cuts were performed during the first and three cuts during the second experimental year. Twenty single spaced plants per repetition of each breeding population were used for morphological traits measuring. Phenotypic evaluations of the morphological traits were performed during the flowering stage in June 2006.

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Table 1. Croatian red clover breeding populations included in the study

Entry	Selection type	Entry	Selection type
RD1	no stress	RC7	frost + drought stress
RC2	no stress	RC8	drought stress
RC3	no stress	RC9	drought stress
RC4	no stress	RC10	high altitude
RC5	drought stress	RC11	high altitude
RC6	drought stress	Viola	cultivar

The measuring was performed on two agronomic characteristics (green and dry matter yield), and on 11 morphological traits (length, width and shape of medial leaflet, intensity, position and shape of white marks, length and thickness of stem, number of internodes, density and direction of stem hairs). Analysis of variance (ANOVA) were used for testing differences in agronomic and morphological continued traits. Chi square test (χ^2) was used for ranged traits. Pearson's correlation model was used for identifying correlations between: first year total green matter yield (GMY-05), second year total green matter (GMY-06), total green matter for the two years (GMY-05-06), first year total air dry matter yield (ADMY-05), second year total air dry matter yield (ADMY-06) and total dry matter yield for the two years (ADMY-05-06). Data were analysed using SPSS for Windows v 13.0 (SPSS, 2004).

Results and discussion

Analysis of variance showed significant differences among breeding populations in stem thickness, where breeding populations RC5 and RC6 had significantly thicker stem comparing to control cultivar. RC5 also shown significance to $P<0.01$ compared to the other populations. A significance difference among investigated breeding populations is noticed in medial leaflet width, while leaflet length showed no significance. RC5 had widest medial leaflet ($P<0.01$). Chi square test shown significant differences in qualitative traits among populations: stem hair density and shape of leaf mark to $P<0.01$ and direction of stem hairs ($P<0.05$).

According to ANOVA, air dry matter yield did not differ in populations in the two experimental years. Regarding green matter yield, 11 breeding populations were not significantly differing from standard cultivar in the first year, although several populations had better yield results. Reasonable higher yield had RC5 and RC11. In the second year RC5 and RC6 had significantly higher green matter yield ($P<0.05$) then Viola. Evaluation of these two populations was done in 2005, and continued in 2006 when the drought conditions were emphasized during the June and July which were extremely dry. In this case, the selection for drought tolerance was successful.

Combining the two years, only RC5 produced green matter yield high enough to be statistically significant ($P<0.01$). The RC2 had the lowest yield ($P<0.05$) and it is not suitable for growing in drought environmental conditions. Although not significant RC9, RC10 and RC11 had yields close to the best population (Table 2). RC10 and RC11 are populations developed for freezing tolerance in high altitude conditions. Good results of these two populations in dry years indicate connection between freezing selection and drought tolerance. Blönder et al. (2005) studied spruce progenies and suggested that freezing and drought tolerance were co-occurring traits.

Table 2. Means (dt ha^{-1}), standard deviation and coefficient of variation for total green matter yield for the two experimental years

Entry	Mean	s^2	cv
RD1	1210.71	81.13	6.70
RC2	1042.86	32.47	3.11
RC3	1226.29	122.05	9.95
RC4	1251.64	95.49	7.63
RC5	1425.64	48.43	3.40
RC6	1262.50	86.77	6.87
RC7	1208.93	46.79	3.87
RC8	1201.79	103.08	8.58
RC9	1301.96	164.20	12.61
RC10	1273.21	44.18	3.47
RC11	1312.50	132.14	10.07
Viola	1209.05	117.33	9.70

Freezing tolerance is reported to likely arise in higher plants by adopting drought tolerance mechanisms (Guy, 2003). In our case the similar results were obtained, but in opposite way: by selecting red clover in freezing conditions we were indirectly increasing population drought tolerance.

Table 3. Correlation coefficients between total green matter yield (GMY) and total air dry matter yield (ADMY), for years 2005, 2006 and the two years, in Croatian red clover breeding populations

	GMY-06	ADMY-05	ADMY-06	GMY-05-06	ADMY-05-06
GMY-05	0.36*	0.90**	0.33*	0.76**	0.62**
GMY-06		0.28	0.89**	0.88**	0.67**
ADMY-05			0.30*	0.65**	0.66**
ADMY-06				0.79**	0.75**
GMY-05-06					0.78**

* significant at the 0.05 level, ** significant at the 0.01 level

Pearson's correlation coefficient revealed significance to $P<0.05$ in correlation between total green matter yield in the first and in the second year of vegetation. Similar significance was obtained for air dry matter (Table 3). Muntean and Savatti (2003), according to similar results, suggested that the most productive forms can be selected even in the first year of vegetation.

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Conclusion

Breeding population RC5 arose from this experiment as the most promising breeding population with highest green matter yield and well adapted to growing under drought environmental conditions. Breeding populations RC10 and RC11 were very interesting due to their good yield in freezing conditions they were developed under, but also in drought environmental conditions. Therefore, they might be well adapted to areas with cold winters and dry summers.

References

Blönder C. – Skropa T. – Johnsen Ø. – Polle A. : 2005. Freezing tolerance in two Norway spruce (*Picea abies* [L.] Karst.) progenies is physiologically correlated with drought tolerance - Journal of plant physiology vol. 162 no.5 549-558 pp.

Czövek P. – Király E. – Páldi E. – Molnár I. – Gáspár L. : 2006. Comparative analysis of stress tolerance in *Aegilops* accessions and *Triticum* wheat varieties to detect different drought tolerance strategies - Acta Agronomica Hungarica vol. 54 no. 1 49-60 pp.

Gagro M. : 1998. Industrijsko i krmno bilje - Hrvatsko agronomsko društvo 212-218 pp.

Guy C.L. : 2003. Freezing tolerance of plants: current understanding and selected emerging concepts – Cannadian Journal of Botany vol. 81 no. 12 1216–1223 pp.

Kölliker R. - Jones E.S. - Jahufer M.Z.Z. - Forster J.W. : 2001. Bulked AFLP analysis for the assessment of genetic diversity in white clover (*Trifolium repens* L.) - Euphytica vol. 121 305–315 pp.

Muntean L. – Savatti M. : 2003. Phenotypic correlations between productivity elements of red clover (*Trifolium pratense* L.) - Journal of Central European Agriculture vol. 4 no. 2 185–190 pp.

Nelke M. – Nowak J. – Wright J.M. – McLean N.L. – Laberge S.- Castonguay Y. – Vezina L.P. : 1999. Enhaced expression of a cold-induced gene coding for a glycine-rich protein in regenerative somaclonal variants of red clover (Trifolium pratense L.) – Euphytica vol. 105 211-217 pp.

Nowak J. – Matheson S.L. – McLean N.L. – Havard P. : 1992. Regenerative trait and cold hardiness in highly productive cultivars of alfalfa and red clover – Euphytica vol. 59 189-196 pp.

Pepó P. – Bódi Z. : 2006. Adaptation of maize lines and hybrids to abiotic/biotic stresses - Acta Agronomica Hungarica vol. 54 no. 4 397-403 pp.

Peter R. – Eschholz T.W. – Stamp P – Liedgens M. : 2006. Swiss maize landraces - Early vigour adaptation to cool condition – Acta Agronomica Hungarica vol. 54 no. 3 329-336 pp.

Smith R.R. – Taylor N.L. – Bowley S.R. (1985) : Red clover. In: Taylor N.L. (ed.), Clover Science and Technology. Agron. Monogr. 25. ASA, CSSA,

SPSS (2004) : SPSS for Windows v. 13.0. SPSS Inc.

Taylor N.L. – Quesenberry K.H. (1996) : Red Clover Science. Series: Current Plant Sciences and Biology in Agriculture: 28. Kluwer Academic Publishers, Dordrecht, the Netherlands.