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Agronomic Evaluation and Utilization of Red Clover (*Trifolium pratense* L.) Germplasm

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Abstract

Germplasm collection as a source of variability and genetic diversity must be evaluated for an efficient management and effective utilization. This research was aimed to evaluate the agronomic value of red clover germplasm collection, to group the cultivars and population according to their morpho-agronomical traits, and to select valuable materials for future breeding programs. Thirty red clover cultivars and populations of different geographical origin were included in the study. Investigation was carried out at the experimental field of the Agricultural Institute Osijek in Croatia. The field trial was arranged in a randomized block design with four replications. Each plot included twenty spaced plants (50 x 50 cm) of each cultivar/population. During two consecutive years (2006, 2007) the following data were collected: yields of green mass, dry matter and seed (g/plant), plant height (cm), dry matter content (%), persistence (%), flowering time. The analysis of variance was carried out for all the traits, with the exception of seed yield and flowering time (which were not recorded as replicated data), using PROC GLM of SAS 9.1. Differences among cultivars/populations were highly significant for all analyzed traits. Hierarchical cluster analysis (Ward method) based on the seven morpho-agronomic traits allowed the identification of six groups of red clover cultivars/populations. This research shows that there is a great genetic variability in the investigated germplasm collection. The most promising materials were selected to form a new breeding gene pool that could be helpful for the improvement of our red clover breeding programme.

Keywords: red clover, morpho-agronomic traits, evaluation, cluster analysis, breeding

Introduction

Red clover (*Trifolium pratense* L.) is an important forage legumes widely cultivated in most temperate regions around the world. Its success is based on the following characteristics: high seeding vigor, rapid growth, tolerance to acid and humid conditions, nitrogen fixing ability, and high nutritive value for ruminants (Leto *et al.*, 2004; Sato *et al.*, 2005; Voigt and Mosjidis, 2002). It is a cross-pollinated diploid (2n = 2x = 14) species with a gametophytic self-incompatibility system (Taylor and Quesenberry, 1996). Therefore, red clover populations are heterogeneous and consist of heterozygous genotypes. This results in high levels of genetic variation within and among populations. The breeding development of new red clover cultivars and other genetic similar forage legumes such as alfalfa is a very slow and long-lasting process.

The initial step in the development of a breeding program is to assemble germplasm that may be utilized as a source nursery. Germplasm collection as a source of variability and genetic diversity must be evaluated for an efficient management and effective utilization. Knowledge of the amount and distribution of genetic variability within a species is crucial to the breeders when selecting breeding germplasm (Rosso and Pagano, 2005).

This research was aimed to evaluate the agronomic value of red clover germplasm collection formed by 30 domestic and foreign materials, to group the cultivars and population according to their morpho-agronomical traits, and to select valuable materials for future breeding programs.

Materials and methods

Collection of red clover germplasm

Thirty red clover cultivars and populations (note Pop 1-13) of different geographical origin were evaluated in this study (Tab. 1). Breeding populations Pop 10 and Pop 11 were created after several cycles of phenotypic recurrent selection for disease resistance and winter survival at the Agricultural Institute Osijek. The seed samples of foreign materials were obtained from the following breeding institutions: DLF-Trifolium Denmark, Svalof Weibull AB

Sweden, Deutsche Satveredelung Lippstadt Deutschland Lithuanian Institute of Agriculture and gene bank South Australian *Medicago* Genetic Resource Centre SARDI.

Field trial

The two year (2006-2007 growing seasons) investigation was carried out at the experimental field of the Agricultural Institute Osijek in Osijek, located in the eastern part in Croatia (lat 45032'N, long 18044'E, altitude 90 m). The soil of the experiment site was a eutric cambisol following chemical properties: pH in H_2O = 6.22; pH in 1n KCl = 5.01; hydrolytical acidity 4.14 cmol kg^{-1}; AL-soluble P_2O_5 and K_2O = 19.7 and 25.3 mg 100 g^{-1}, respectively; humus content 2.17%). Average annual temperatures (11.50C and 12.40C for 2006 and 2007, respectively) and total annual rainfall (632.1 mm and 620.9 mm, for 2006 and 2007, respectively) for the studied period was moderately deviated from the 30-years average (10.90C and 654 mm). On 4 April 2006 seeds of each cultivar/population were sown directly into the field. A total of 80 plants per materials was used. The plots consisted of four rows with five plants each and each plot was replicated four times in a randomized complete block design. Plants were spaced 50 cm within and between rows.

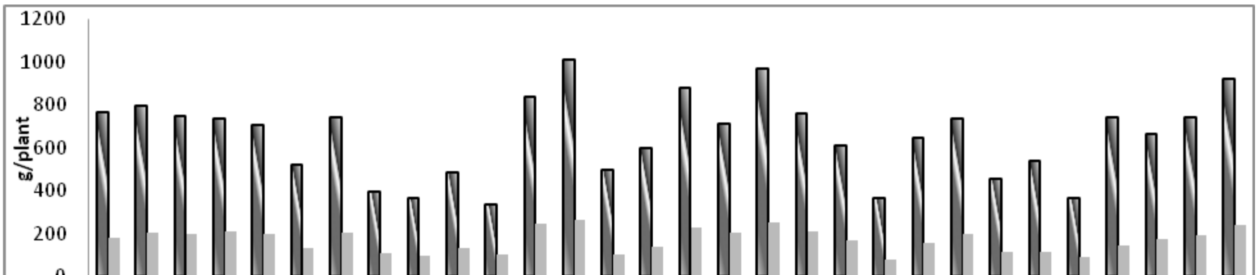
Agronomic evaluation and data analysis

Plots were cut three times in the first growing seasons (15 May, 2 July, 10 September in 2006) and at each cut, data were recorded on all individual plants with respect to green mass yield (GMY, g), plant height (PH, cm) and dry matter yield (DMY, g). To determine dry matter yield the average sample of randomly chosen individual plants of each cultivar/population was taken in all cuts from each row. The samples were weighted, dried at 105°C for 24 h, reweighted to establish dry matter content (DMC, %), and dry matter yield were obtained by calculation. Total yields of green mass and dry matter per plant were determined summing the yields from each of three cuts. The persistence (PER, %) of all cultivar/population was determined by counting the numbers of plants alive in each repetition at the beginning of spring 2007 and calculated as the percentage. Flowering time was observed when three heads per plant had flowered (beginning of June 2007) on 20 randomly chosen individual plants of each cultivar/population, and was scored on a scale (from 1 = very early to 9 = very late) according to UPOV guidelines for the conduct of tests for distinctness, homogeneity and stability (2001). Seed yield per plant (g) was measured after the harvest of 20 individual plants. The plant harvest (August 2007) was performed by hand at full maturity of seeds in the main inflorescence (80-90% of inflorescence per plant are dark brown).

Analysis of variance and Duncan multiple range tests were performed to detect differences among cultivar/population for all traits, with the exception of seed yield and flowering time (which were not recorded as replicated data), using PROC GLM of SAS 9.1. (SAS Institute, 2003). Hierarchical cluster analysis was carried using the Ward method (Ward, 1963).

Results and discussions

Significant differences among cultivars and populations were found for all studied traits. The highest average yields of green mass and dry matter were obtained for the Croatian breeding population Pop 11 (1008.6 and 262.73 g plant-1), and were 52% higher compared with average yields for all materials (Tab. 1, Fig. 1). High yields were obtained by cultivars Temara and Estanzuela 116 and breeding population Pop 10, while significantly lower values were found by Pop 9 (for GMY) and cultivar Kiršinai (for DMY). The average plant height (Tab. 1, Fig. 2) for all cultivars and populations was 53.98 cm, and varied from 32.65 (Pop 7) to 66.69 cm (Astred). The highest dry matter content had population Pop 9 (31.16%), whereas the lowest values were found in cultivar Fanny (20.49%). Winter survival is an important factor for successful red clover forage and seed production depends on its ability to persist for more than one season (Steiner *et al.*, 1997). Croatian breeding population Pop 11 showed the best persistence (95.45% survival plant), which was excepted since this population is obtained by a year-long selection of



	Astred	Redquin	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6	Pop 7	Pop 8	Pop 9	Pop 10	Pop 11	Lone	Raja	Amos	Krano	Temara	Milvus	Pop 12	Kiršinai	Vyliai	Liepsna	Vyčiai	Kamanai	Pallas	Fanny	Nosovskij5	Pop 13	Estanzuela 116
GMY	763	795	750.	735.	704.	518.	739.	394.	364	486.	338.	839.	1009	496.	600.	879.	711.	970.	762.	610.	363.	648	738.	452.	536.	365.	744.	663.	741.	919.
DMY	178.	204.	194.	208.	198.	134.	204	105.	96.8	132.	99.4	244.	262.	103.	136.	224.	204.	249.	207.	168.	75.4	154.	198.	115.	115.	89.2	142.	172.	193.	237.

Fig. 1. GMY and DMY of the cultivars studied

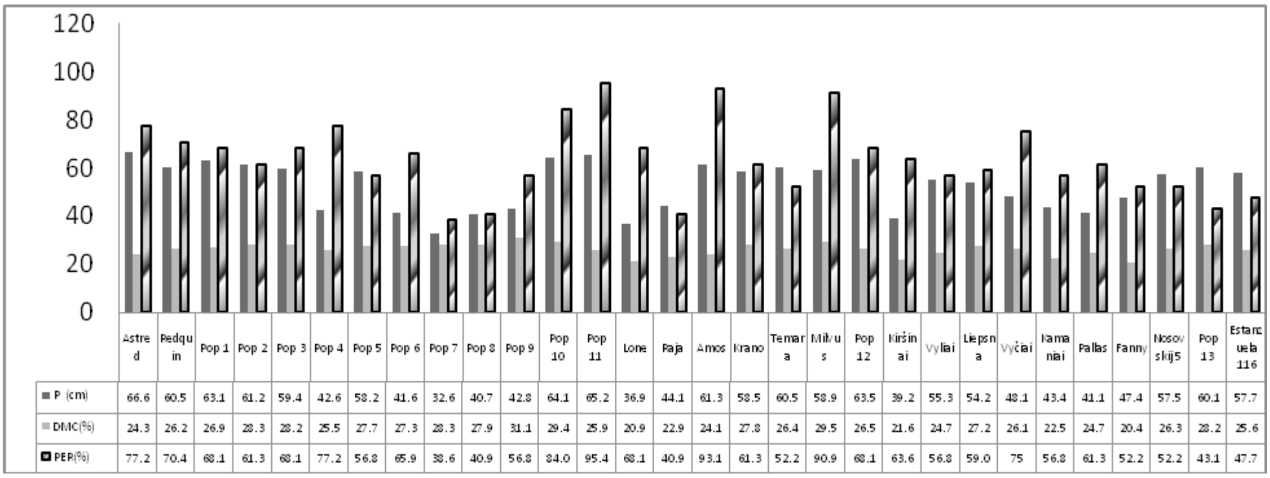


Fig. 2. PH, DMC and PER of the cultivars studied

Tab. 1. Average values and coefficients of variability (CV) of the studied agronomic traits of red clover germplasm collection Osijek, 2006, 2007

Cultivar/ Populatin	Country of origin	GMY	DMY	PH (cm)	DMC (%)	PER (%)
		(g/plant^{-1})				
Astred	Australia	763.0^{a-f}	178.66^{a-g}	66.69^{a}	24.31^{c-f}	77.28^{a-e}
Redquin	Australia	795.0^{a-f}	204.35^{a-e}	60.50^{abc}	26.20^{b-e}	70.46^{a-f}
Pop 1	Australia	750.1^{a-g}	194.87^{a-f}	63.18^{abc}	26.94^{bcd}	68.18^{a-g}
Pop 2	Australia	735.7^{a-g}	208.89^{a-d}	61.28^{abc}	28.33^{abc}	61.37^{d-g}
Pop 3	Australia	704.2^{a-h}	198.73^{a-e}	59.44^{abc}	28.26^{abc}	68.19^{a-g}
Pop 4	Australia	518.7^{d-i}	134.31^{d-h}	42.63^{fg}	25.57^{b-e}	77.27^{a-e}
Pop 5	Australia	739.8^{a-f}	204.04^{a-e}	58.28^{abc}	27.78^{a-d}	56.82^{d-g}
Pop 6	Australia	394.6^{ghi}	105.21^{fgh}	41.62^{fgh}	27.31^{a-d}	65.91^{b-g}
Pop 7	Australia	364.0^{hi}	96.87^{gh}	32.65^{h}	28.31^{abc}	38.64^{g}
Pop 8	Australia	486.4^{e-i}	132.72^{d-h}	40.72^{fgh}	27.91^{a-d}	40.91^{fg}
Pop 9	Afghanistan	338.5^{i}	99.47^{gh}	42.88^{fg}	31.16^{a}	56.82^{d-g}
Pop 10	Croatia	839.1^{a-e}	244.64^{abc}	64.19^{abc}	29.48^{ab}	84.09^{a-d}
Pop 11	Croatia	1008.6^{a}	262.73^{a}	65.28^{ab}	25.98^{b-e}	95.45^{a}
Lone	Denmark	496.5^{e-i}	103.16^{gh}	36.98^{gh}	20.97^{fg}	68.18^{a-g}
Rajah	Denmark	600.1^{c-i}	136.70^{d-h}	44.18^{fg}	22.95^{efg}	40.91^{fg}
Amos	Denmark	879.4^{a-d}	224.54^{a-d}	61.31^{abc}	24.11^{d-g}	93.18^{ab}
Krano	Denmark	711.9^{a-h}	204.29^{a-e}	58.59^{abc}	27.86^{a-d}	61.36^{d-g}
Temara	Germany	970.7^{ab}	249.80^{ab}	60.50^{abc}	26.41^{b-e}	52.27^{efg}
Milvus	Germany	762.3^{a-f}	207.83^{a-d}	58.92^{abc}	29.54^{ab}	90.91^{abc}
Pop 12	Iran	610.4^{c-i}	168.66^{b-g}	63.50^{abc}	26.52^{b-e}	68.18^{a-g}
Kiršinai	Lithuania	363.9^{hi}	75.48^{h}	39.24^{fgh}	21.62^{fg}	63.64^{c-g}
Vyliai	Lithuania	648^{b-i}	154.39^{c-h}	55.36^{b-e}	24.76^{c-f}	56.82^{d-g}
Liepsna	Lithuania	738.3^{a-g}	198.54^{a-e}	54.24^{cde}	27.29^{a-d}	59.09^{d-g}
Vyčiai	Lithuania	452.4^{f-i}	115.21^{e-h}	48.11^{def}	26.14^{b-e}	75.00^{a-e}
Kamaniai	Lithuania	536.9^{d-i}	115.51^{e-h}	43.46^{fg}	22.57^{efg}	56.82^{d-g}
Pallas	Sweden	365.3^{hi}	89.27^{gh}	41.13^{fgh}	24.75^{c-f}	61.39^{d-g}
Fanny	Sweden	744.5^{a-g}	142.70^{d-h}	47.44^{ef}	20.49^{g}	52.27^{efg}
Nosovskij 5	Russia	663.3^{a-i}	172.11^{a-g}	57.50^{a-d}	26.35^{b-e}	52.27^{efg}
Pop 13	USA	741.5^{a-g}	193.35^{a-f}	60.18^{abc}	28.26^{abc}	43.18^{fg}
Estanzuela 116	Uruguay	919.7^{abc}	237.64^{abc}	57.71^{a-d}	25.62^{b-e}	47.73^{efg}
Average		663.36	171.93	53.98	26.12	63.48
CV (%)		58.64	57.88	21.31	6.91	20.81

Averages with the letter in common are not significantly different at $P<0.01$ (Duncan's Multiple Range Test)

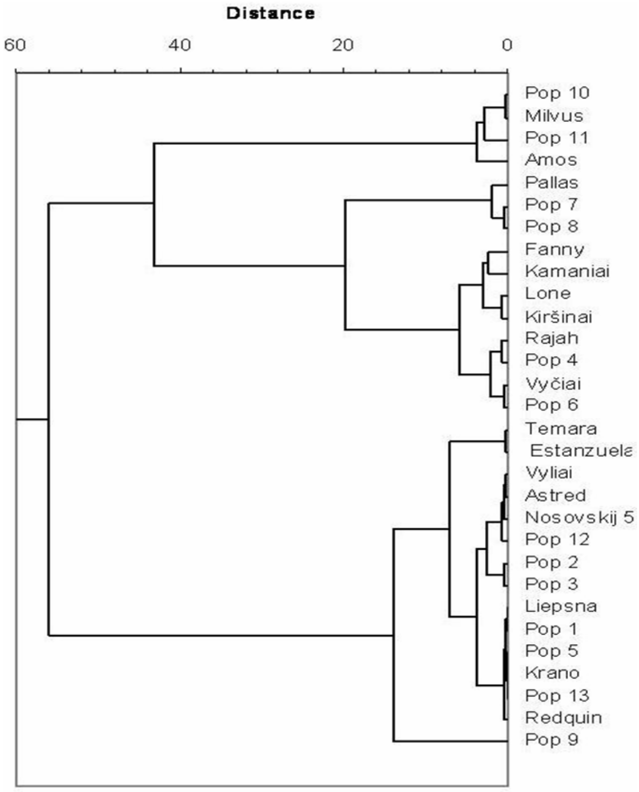


Fig. 3. Dendrogram of 30 red clover cultivars/populations based on 7 morpho-agronomic traits

genotypes tolerance to abiotic stresses. Also, good persistence was approved for cultivars Amos and Milvus (93.18 and 90.91% survival plant). Low persistence was found for Australian populations (Pop 7 and 8) and Denmark cultivar Rajah. The lack of persistence had been recorded in several studies in different countries where red clover is cultivated (Fan *et al.*, 2004; Mela, 2003; Montardo *et al.*, 2003). In addition to genetic basis, the population persistence might be influenced by some other factors, such as illnesses as well as various types of pest causing sudden plant decay. Dias *et al.* (2008) analyzed 58 populations using 21 morphological traits and found relation between high intensity of blooming and low persistence. The high coefficients of variability were recorded among the 30 red clover cultivars and populations for almost all the studied traits (20.81-58.64%) and they indicate the presence of high variability for these traits (Tab. 1). The low coefficient of variability observed for dry matter content (6.91%) suggested that cultivars and populations did not differ much for these traits.

In order to determine the similarities or differences among cultivars/populations a cluster analysis was performed. The statistics of the cluster analysis based on the seven morpho-agronomic traits allowed the identification of six clusters (Fig. 3). Cluster 1 contained four cultivars/populations (Pop 10, Milvus, Pop 11, Amos) characterized by early materials with superior forage and seed yield potential and the best persistence. The second cluster (Pallas, Pop 7, Pop 8) presented materials with the lowest forage yields and persistence, as well as high seed yield capacity. The cluster 3 was composed of eight late to very late cultivars/populations (Fanny, Kamaniai, Lone, Kiršinai, Rajah, Pop 4, Vyčiai, Pop 6) characterized by medium to low forage productivity and modest persistence. Cluster 4 included only two cultivars (Temara, Estanzuela 116) which were characterized by a very good forage yield potential but poor winter survival. Cluster 5 contained the largest number of medium-late cultivars/populations (Vyliai, Astred, Nosovskij 5, Pop 12, Pop 2, Pop 3, Liepsna, Pop 1, Pop 5, Krano, Pop 13, Redquin) characterized by medium seed and forage productivity, and medium to high persistence. Cluster 6 included only population Pop 9 which was characterized by the highest dry matter content and average to lower values for productivity traits and persistence. Similarly, other authors recognized different groups of populations based on the contribution of several morpho-agronomic traits. Roso and Pagano (2005) evaluated 39 introduced and naturalised populations and detected winter forage yield and seed production as the most important traits to explain variation between populations. Tamm and Benber (2006) analyzed 24 tetraploid red clover cultivars originating form 9 European countries by 5 parameters (maturity type; survival; yields of dry matter, seed and crude protein) and identified 5 distinct clusters. Greene *et al.* (2004) studied 33 wild red clover population using 15 morphological traits and found that flowering time and winter survival contributed to group population into three classes

Conclusions

This research shows that a great genetic variability exists in the investigated germplasm collection. The cultivars/populations present in clusters 1 (Amos, Milvus) and 5 (Astred, Redquin, Pop 1, Pop 3, Pop 12) are the most promising materials due to their high yield potential and good persistency. Therefore, those cultivars/populations were selected to form a new breeding gene pool that could be helpful for the improvement of our red clover breeding programme.

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