UDC 575:630
DOI: 10.2298/GENSR1402471P
*Original scientific paper*

# STUDY OF RED CLOVER WILD POPULATIONS FROM THE TERRITORY OF SERBIA FOR THE PURPOSE OF PRE-SELECTION

Mirjana PETROVIĆ[1*], Zora DAJIĆ – STEVANOVIĆ[2], Dejan SOKOLOVIĆ[1], Jasmina RADOVIĆ[1], Jasmina MILENKOVIĆ[1], Jordan MARKOVIĆ[1]

[1]Institute for forage crops, Kruševac, Serbia
[2] Faculty of Agriculture, Universety of Belgrade, Belgrade, Serbia

Petrović M., Z. Dajić – Stevanović, D. Sokolović, J. Radović, J. Milenković, J. Marković (2014): *Study of red clover wild populations from the territory of serbia for the purpose of pre-selection.* Genetika, Vol 46, No. 2, 471-484.

The aim of this paper was to evaluate the agronomic value of red clover (*Trifolium pratense L.*) wild populations. The analyzed material was gathered on the territory of Serbia and it was estimated which populations could be involved in the breeding program. 17 red clover populations were included in the research. Investigation was carried out at the experimental field of the Institute for forage crops, Kruševac, Serbia. The field trial was performed during three years (2008 - 2010) when data about morphological (green mass, dry mater, plant height, number of stems, number of lateral branches, number of internodes, length and width of a middle leaf lamina) and basic chemical parameters (quantity of crude proteins, crude fiber, ash, fat, NFE) were collected. In this paper, results of the research were processed by application of analysis of variance (ANOVA) and multivariation methods. Cluster analysis based on morphological traits resulted in formation of three clusters and the traits with the largest influence on grouping were: green mass, plant height, length and width of a leaf. Principal component method confirmed this separation and resulted in even more clear grouping of populations. Cluster analysis and PCA method were also applied to the traits of dry matter quality, when four clusters were formed, and the traits with the largest influence on grouping were percentages of protein, fat and fiber. Using both set of traits and several types of analyses contribute to grouping of populations which promise most and whose positive traits such as: high green

***Corresponding author:*** Mirjana Petrović, Institute for forage crops, 37251 Globoder, Kruševac, Serbia tel.: +38137 442 590, fax: +38137 441 295, e-mail: mirjana.petrovic@ikbks.com

mass yield, number of stems as well as the percentage of proteins can be combined for the purpose of getting synthetic varieties.

*Key words*: red clover, wild populations, morphological traits, quality of forage, cluster analysis.

## INTRODUCTION

Red clover is one of the most widespread species of *Trifolium* genus in the world. Although red clover has a Mediterranean origin, it is a cosmopolitan species adapted to many edaphic – climatic conditions (TAYLOR and SMITH 1979). Due to its adaptability to the soil of a lower level of acidity in comparison with alfalfa and higher altitude survival, red clover is, besides grasses, a leading forage crop grown in a moderate climate zone (LEE *et al*., 2004). The yield potential of red clover is excellent and some red clover varieties can have higher fodder yield than alfalfa (DROBNA and JANČOVIČ, 2006).

As stated by TAYLOR and QUESENBERRY (1996), creation and breeding of improved varieties, at the global level resulted in decrease of a number of local types and some wild forms, what in its turn led to big erosion of genetic divergence, as opposed to huge variability existing in red clover germplasm. With a lack of wild flora material, a possibility of any kind of improvement or increase of the existing varieties collection is greatly reduced. Since the territory of Serbia is geographically very diverse with lots of regions of the country very weakly used for the purpose of agricultural production, but with well preserved habitats–genetic material of wild clover populations is still very varied. Research into this material forwards choice of material with desirable traits, what is the beginning of a process of breeding (BOLLER *et al.,* 2010).

In order to become at least partially familiar with traits of the examined populations and a degree of the gathered material variability, different research surveys are conducted out of which morphological and chemical analyses are the most frequent ones and they represent the basis for the procedure of selection. Morphological characterization was the first step and continue to be the most important tool in description and classification of genetic resources (RAKONJAC *et al.,* 2014). Both groups of methods are a part of the complex of agronomic traits and their aim is to find the population which will give high yield or which could be exploited for long period under particular agroecological conditions. Various sets of cultivars or populations of red clover were analyzed by using morphological traits (BULINSKA-RADOMSKA, 2000; GREEN et al., 2004). They all pointed out to the large genetic diversity among and within populations.

Aims of this paper are: 1. to analyze variability of red clover collection gathered on the territory of Serbia, based on morphological and chemical methods; 2. to perform grouping of populations in clusters in accordance with their traits in order to describe structure of the collection; 3. a long-term aim is, if desirable traits are discovered in the existing material, to use it in the process of breeding, that is for getting synthetic varieties.

## MATERIALS AND METHODS

The examined material is a part of the collection of seeds of the Institute for forage crops, Kruševac. The gathered material is kept at +4°C in a cold chamber. 17 populations from the existing collection, originating from four regions of Serbia, are included in the analysis.

The field experiment was performed on the experimental field of the Institute for forage crops, Kruševac (altitude 149m, 43°35' 0" N, 21°19' 36" E). The area where the institute is located has characteristics of temperate continental climate, with the average annual temperature of 10.9°C and the average precipitation of 540-820mm.

*Table 1. T. pratense wild populations used for analysis.*

| Abbreviations* | Location | Latitude | Longitude | Altitude (m) |
|---|---|---|---|---|
| CS108 | Nacionalni park | N43°18.38' | E020°52.19' | 1080 |
| CS118 | Nerađe | N43°26.49' | E020°53.00' | 1185 |
| CS146 | Srebrnac | N43°18.92 | E020°51.25' | 1465 |
| CS152 | Rendara | N43°20.17' | E020°51.67' | 1523 |
| CS172 | Konaci | N43°17.41' | E020°48.88' | 1720 |
| ES042 | Boljevac 1 | N43°50.43 | E022°00.21' | 420 |
| ES043 | Čestobrodica 2 | N43°48.15' | E021°45.35' | 425 |
| ES047 | Boljevac 2 | N43°49.83' | E022°03.66' | 470 |
| ES077 | Crni Vrh 1 | N44°08.36' | E021°58.64' | 770 |
| ES086 | Crni Vrh 2 | N44°10.04' | E021°57.03' | 860 |
| RA089 | Osoje | N43°12.12' | E020°17.04' | 890 |
| RO100 | Donja Vapa | N43°17.56' | E020°01.78' | 990 |
| RO116 | Duga Poljana 2 | N43°16.27' | E020°10.69' | 1140 |
| RO123 | Duga Poljana 1 | N43°16.20' | E020°13.41' | 1230 |
| TA055 | Tara 5 | N43°56.05' | E019°34.22' | 550 |
| TA089 | Tara 3 | N43°56.98' | E019°25.18' | 890 |
| TA097 | Tara 2 | N43°54.32' | E019°30.46' | 970 |

* Abbreviation of each localities consist of two parts: letters that present part of territory where population were collected (CS – Central Serbia, ES – Eastern Serbia, RO – Area of Raška, TA - Tara) and three numbers as altitude explanation (for example: 035 - 350m, 123 - 1230m).

The experiment was started at the beginning of October 2008 on the soil of degraded alluvial type with the following characteristics: pH 6.57 in KCl, organic matter content was 2.52%, available P content was 6.60 ppm and available K content was 24.05 ppm. In order to break dormancy of the seeds, their scarification was carried out; the seed was then germinated in plastic trays. When plants reached the stadium of 3-4 permanent leaves, they were moved to the nursery, with 60x60 spacing among individual plants. Plants were harvested in the period of the first cat for two years (2009/10).

Morphological traits which were observed are: green mass yield per plant, dry matter, height of the plant (length of the main stem) - only Height in tables and figures, number of stems per plant (SN), number of lateral branches (NB) (performed on three stems), number of internodes - performed on three stems (NI), length (LL) and width (LW) of the middle lamina of the third leaf viewed from the top of the stem.

Plants were harvested in the period of the first cat during the second and the third year of the research (2009/2010). An average sample of 1 kg of green mass was taken from each population and then dried at 60°C for 48 hours. In this way the dry matter per kilogram of weight of green mass was obtained. Then, basic parametres of forage quality - content of crude proteins, crude fibre, ash, fat and NFE were determined for each population by *Weende* system of analysis. Crude protein was computed indirectly from the amount of total nitrogen, measured by the Kjeldahl method modified by Bremner, multiplied by factor 6.25. Crude fiber was determined by sequently refluxed in dilute base followed by dilute acid. Crude fat was determined by method of Soxlet. The amount of nitrogen free extract in samples was determined as a difference between 1000 and amounts of crude ash, crude protein, crude fiber and crude fat. Chemical features were obtained by analysis of material from the second year of the research.

For each morphological feature (except dry matter), one-way analysis of variance (ANOVA) was used to examine differences in mean values among populations. When critical differences were noted, multiple comparisons were carried out based on LSD test for unequal sample sizes. Differences were considered significant at $p<0.01$. The dendrograms of populations based on morphological and chemical data were constructed using the Euclidean distance and complete linkage clustering method. Principal component analysis (PCA) was applied to search for a general pattern in the measured variables. All statistical analyses were performed using the program STATISTICA 10 (STATSOFT, Inc., Tulsa, OK, USA).

## RESULTS AND DISCUSSION

*Analysis of morphological traits*

Analyses of variance revealed highly significant differences between populations for all analyzed morphological traits.

Results of mean values, coefficient of variance (CV%) and LSD test at $p<0.01$ for each analyzed population are presented in Table 2. We can notice, by observing the results, that there are statistically significant differences between the analyzed populations, and traits which have the largest influence on separation are: plant height, green mass of plant, number of stems, number of lateral branches and number of internodes.

The most variable trait is green mass of plants with CV values ranging from 22% for TA089 population to 81% for RO100 population, what is in line with results obtained by Asci (2011), who, by following a large number of morphological traits on the sample of 47 populations, also noticed mass as the most variable trait. Mean values per population range from 142g to 755g. Population RO100 has the lowest green mass value and ES077 the highest (Table 2).

*Table 2: Average values and coefficients of variability (CV) of 6 characters in 18 Trifolium associations collected in Serbia.*

| Populations | Green mass | | | Plant height | | | Stem number | | | No. of 1. branch. | | | Nr. of internodes | | | Leaf length | | | Leaf width | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | CV% | LSD* | Mean | CV% | LSD | Mean | CV% | LSD | Mean | CV% | LSD | Mean | CV% | LSD | Mean | CV% | LSD | Mean | CV% | LSD |
| CS108 | 272 | 41 | cde | 62.7 | 16 | fgh | 26 | 36 | cde | 4.27 | 21 | bc | 4.13 | 20 | cdefg | 3.14 | 10 | ghij | 1.56 | 17 | bcde |
| CS118 | 255 | 55 | de | 57.7 | 16 | ghi | 31.2 | 39 | bc | 3.47 | 21 | e | 3.4 | 31 | g | 2.76 | 11 | j | 1.26 | 21 | e |
| CS146 | 334 | 38 | cde | 71.7 | 12 | bcdef | 30.9 | 39 | bcd | 4.73 | 19 | b | 5 | 19 | bc | 3.32 | 13 | fgh | 1.69 | 13 | bc |
| CS152 | 253 | 47 | de | 66.7 | 22 | defg | 35.4 | 42 | ab | 4.87 | 27 | b | 4.87 | 30 | bcd | 2.84 | 9 | ij | 1.29 | 22 | e |
| CS172 | 146 | 44 | fg | 53.2 | 19 | hi | 19.5 | 50 | ef | 3.93 | 22 | cde | 4 | 31 | defg | 2.85 | 10 | ij | 1.64 | 16 | bc |
| ES042 | 394 | 42 | c | 79.5 | 11 | ab | 22.9 | 37 | def | 4.73 | 22 | b | 5 | 25 | bc | 3.91 | 16 | a | 2.13 | 15 | a |
| ES043 | 324 | 58 | cde | 76.7 | 14 | abc | 24.5 | 40 | cdef | 4.6 | 27 | bc | 5.07 | 28 | bc | 3.79 | 16 | ac | 1.8 | 15 | b |
| ES047 | 374 | 43 | cd | 79.1 | 15 | ab | 24.9 | 35 | cdef | 4.8 | 23 | b | 5 | 25 | bc | 3.77 | 16 | acd | 1.75 | 25 | bc |
| ES077 | 755 | 23 | a | 82.7 | 7 | a | 41 | 23 | a | 5.6 | 19 | a | 6.07 | 19 | a | 3.45 | 13 | cdefg | 1.74 | 19 | bc |
| ES086 | 610 | 28 | b | 80.5 | 16 | ab | 41.3 | 37 | a | 4.8 | 23 | b | 5.33 | 23 | ab | 3.36 | 15 | defg | 1.59 | 21 | bcd |
| RO089 | 257 | 39 | de | 72.5 | 14 | bcde | 26.8 | 45 | cde | 4.27 | 11 | bc | 4.47 | 14 | bcdef | 3.35 | 13 | efgh | 1.82 | 14 | b |
| RO100 | 142 | 81 | g | 48 | 24 | i | 17.5 | 83 | f | 3.47 | 24 | e | 3.6 | 31 | fg | 3.22 | 16 | fghi | 1.73 | 25 | bc |
| RO116 | 243 | 49 | ef | 65 | 14 | efg | 29.1 | 43 | bcd | 4.4 | 19 | bc | 4.47 | 14 | bcdef | 3.58 | 7 | acdef | 1.74 | 20 | bc |
| RO123 | 339 | 29 | cde | 57.9 | 15 | gh | 41.7 | 33 | a | 4.2 | 18 | bcd | 4.2 | 24 | cdefg | 2.94 | 14 | hij | 1.32 | 17 | de |
| TA055 | 285 | 41 | cde | 75.1 | 16 | abcd | 20.1 | 36 | ef | 3.53 | 18 | de | 3.87 | 19 | efg | 3.76 | 9 | acde | 1.86 | 11 | ab |
| TA089 | 288 | 22 | cde | 67.1 | 10 | cdefg | 31.8 | 27 | bc | 4.8 | 18 | b | 4.73 | 19 | bcde | 3.8 | 14 | ac | 1.86 | 23 | ab |
| TA097 | 244 | 44 | e | 59.5 | 20 | gh | 25 | 30 | cdef | 4.33 | 21 | bc | 4.27 | 19 | cdefg | 3.13 | 12 | ghij | 1.5 | 24 | cde |

*Averages with the letter in common are not significantly different at $p<0.01$ (LSD)

TAYLOR and QUESENBERRY (1996), stem lengths at maturity may vary up to 80cm. In this group of populations, the height ranges from 48cm (RO100) to 82.7cm (ES077) where we can single out one group from East Serbia with very high values: ES086 - 80.5cm, ES077 - 82.7cm, ES042 - 79.5cm and ES047 - 79.1cm. The coefficient of variance for this trait in populations from East Serbia show low to moderate variations (7.4-15.6%) what reflects stability of the trait in this material. Uniform material with higher mean values and lower CV is more suitable for inter population selection.

Since the length of a stem has a positive correlation with mass of a plant as a component of yield, selection is performed with the aim to lengthen plants. One of the most famous and the most successful examples of increase of a stem length was performed in the 80-ies with Kenstar variety, where the length was increased for even 20cm (BOWLEY *et al.,* 1984), from 53 to 73cm.

Number of stems is also one of the significant components of yield. Populations with the highest values are: RO123 - 41.7 (CV-33%), ES086 - 41.3 (CV-37%), ES077 - 41(CV-23%). On the other hand, population RO100 has the lowest mean value of the number of stems - 17.5cm, but with a very high value of CV - 83.4%. Such a high coefficient of variance indicates diversity of material of initial population, which is suitable for improvement of genetic pool and hybridization of genotypes. The obtained results can be considered as very good because majority of them are within the limits of ROSSO and PAGANO (2005) in their study of 39 samples of red clover.

In relation to the number of lateral branches populations are rather uniform, with CV inside populations from 11% - 27%. Number of internodes is a trait which is highly correlated with mass and height, so that population ES077 which has the highest values of mass and height has the largest number of internodes - 6.07. Populations with high values of number of internodes also are: CS146 – 5, ES042 - 5, ES043 - 5.07, ES043 - 5, ES086 - 5.33. LUGIĆ, (1999), while examining varieties K-17 and REINCHERSBERGE, got similar values for the number of internodes in relation to the already mentioned traits. In relation to the values ASCI (2011) got on a large sample of 47 wild populations, all the populations of this collection have higher values of length of a leaf, whereas majority has bigger width of a leaf.

In order to made groups of clusters of similar populations, cluster analysis was applied. Cluster analysis, based on six morphological traits, plus a derived one (dry matter), enabled forming of 3 clusters (Fig. 1). Grouping of populations into clusters based on data obtained by observing morphological traits was also applied in surveys by: DIAS *et al*. (2007), GREEN *et al.* (2004), VYMYSLICKY *et al*. (2012).

We can notice three clusters on the dendrogram. Populations ES077 and ES086 belong to cluster A, populations CS146, RO089, RO116, ES042, ES043, ES047, TA055, TA089 to cluster B, whereas populations CS108, CS172, RO100, TA097, CS118, RO123,CS152, CS035 belong to cluster C.

In order to explain the reasons for grouping like this, principal component analysis (PCA) was applied. PCA graph is shown by the first and the second axis, where the first one enables high degree of separation of 58%, and the second axis separation of 30% (88% in total).

Clusters on the dendrogram and on the PCA graph are marked by the same letters, except from cluster C which is divided in two groups.

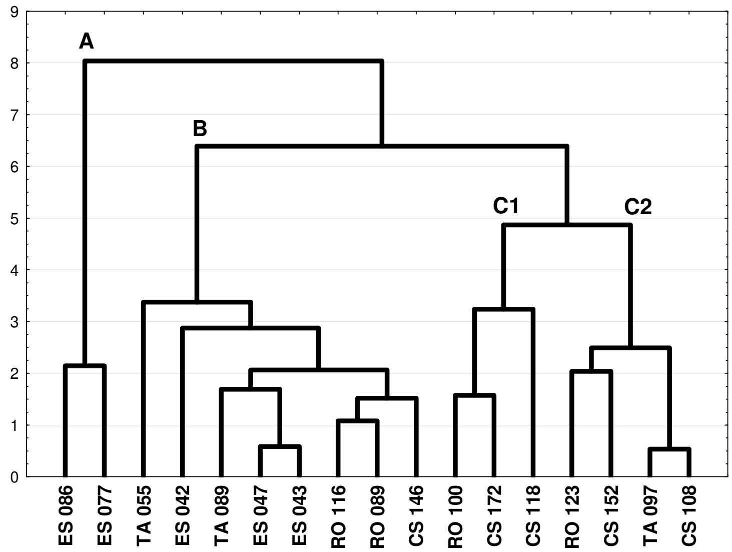


Figure 1: Dendrogram based on the morphological traits of 17 populations of red clover.

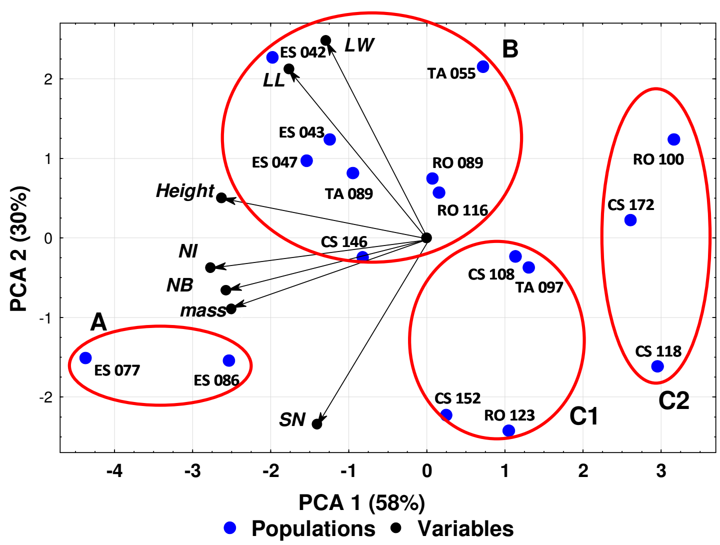


Figure 2. Component loadings for the first of two principal components of PCA of morphological traits. (LL – leaf length, LW – leaf width, NI – number of internodies, NB – number of lateral branches, SN – stem number)

We can notice from Graph 2 that members of cluster A are grouped first of all for their high values of plant height and green mass, and then for the number of stems and also the number of lateral branches. Table 3 shows mean values of all traits for members of the corresponding cluster. It can also be seen from these data (Table 3) that values of traits which mostly influence singling out of cluster A are the highest. Furthermore, it can be concluded, by parallel observation of Graph 2 and Table 3, which led up to formation of other clusters. Cluster B groups populations with increased values of height of a plant, length and width of a leaf. The average height of plants from this cluster is 73.3cm, what is a slightly better value than the one that ASCI (2011) got – for about 13%, while working on collection of 47 wild populations and then LUGIĆ *et al.* (2009) while working on three wild populations (they got the values lower for 29%); LETO *et al.* (2004) - conducted the survey on six varieties and the obtained values were lower for about 19%. Populations of cluster C can be grouped in two groups - C1 and C2. Subcluster C2 (CS108, CS172, RO100, TA097) includes populations with the lowest values of majority of the surveyed parameters. Populations from subcluster C1 also have low values for majority of the surveyed parameters, but what can be noticed about them is a relatively high value for the number of stems (Table 4). There are mostly populations from the altitude of about 1000 m and above within cluster C2, so that it can be concluded that these populations can have difficulties in acclimatization to conditions in plains and that they cannot express or even do not have bigger productive potential. Other two clusters (A and B) mostly consist of populations from lower terrains, so that moving them to field conditions on the altitude of about 150m did not represent such a big shock for them. There are two populations from Crni Vrh in cluster A, and it can be assumed that a slightly bigger quantity of precipitation on this mountain (especially during the winter) in relation to the surrounding area has the influence on good values of majority of the surveyed parameters. There is a possibility in the future that this area is a good source of genetic material of red clover, what should be examined in some of the following research surveys.

*Table 3. Average values of green mass per plant, % of dry metter and dry matter per plant*

| | CS108 | CS118 | CS146 | CS152 | CS172 | ES042 | ES043 | ES047 | ES077 | ES086 | RO089 | RO100 | RO116 | RO123 | TA055 | TA089 | TA097 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Green mass | 272 | 255 | 334 | 253 | 146 | 394 | 324 | 374 | 755 | 610 | 257 | 142 | 243 | 339 | 285 | 288 | 244 |
| Dry matter (%) | 33 | 28 | 23 | 24 | 27 | 25 | 24 | 25 | 25 | 25 | 25 | 24 | 23 | 27 | 24 | 27 | 22 |
| Dry matter (g/plant) | 90 | 71 | 77 | 61 | 39 | 98 | 78 | 94 | 189 | 152 | 64 | 34 | 56 | 92 | 68 | 78 | 54 |

*Table 4. List of mean values of evaluated morphological characters in particular clusters*

| Claster | G. mass | Dry matter | Height | SN | NB | NI | LL | LW |
|---|---|---|---|---|---|---|---|---|
| **A** | **683** | **171** | **81.6** | **41.2** | **5.20** | **5.70** | 3.41 | 1.67 |
| **B** | 313 | 77 | 73.3 | 26.4 | 4.48 | 4.70 | **3.66** | **1.83** |
| **C1** | 277 | 74 | 61.7 | 32.0 | 4.42 | 4.37 | 3.01 | 1.42 |
| **C2** | 181 | 48 | 53.0 | 22.8 | 3.62 | 3.67 | 2.94 | 1.54 |

*Analysis of chemical data*

Red clover is certainly legume which besides alfalfa represents the most significant element of animal feed. It has such a significant position first of all because of high content of protein and good digestibility. One of the main aims in the process of breeding of red clover is quantity of proteins increase (VASILJEVIĆ *et al.,* 1999) and in order to make selection of new varieties, existence of variability in initial material is necessary.

*Table 5. Chemical composition of red clover populations.*

| Population | Ash | Proteines% (per plant) | Fiber | Fat | NFE |
|---|---|---|---|---|---|
| CS108 | 7.98 | 14.60 (13 g) | 35.48 | 1.86 | 40.08 |
| CS118 | 7.70 | 15.96 (11 g) | 37.10 | 2.29 | 36.95 |
| CS146 | 7.66 | 17.51 (13 g) | 28.61 | 1.32 | 44.90 |
| CS152 | 8.68 | 17.05 (10 g) | 31.85 | 2.11 | 40.31 |
| CS172 | 10.55 | 13.90 (5 g) | 33.06 | 1.12 | 41.37 |
| ES042 | 9.44 | 19.54 (19 g) | 26.86 | 2.39 | 41.77 |
| ES043 | 8.81 | 19.09 (15 g) | 24.95 | 1.68 | 45.47 |
| ES047 | 8.31 | 18.98 (18 g) | 32.19 | 2.24 | 38.28 |
| ES077 | 9.47 | 17.48 (33 g) | 31.58 | 2.17 | 39.30 |
| ES086 | 7.03 | 14.40 (22 g) | 38.49 | 1.85 | 38.23 |
| RO089 | 8.05 | 17.21 (11 g) | 35.51 | 1.39 | 37.84 |
| RO100 | 14.5 | 16.82 (6 g) | 27.52 | 1.58 | 39.58 |
| RO116 | 7.74 | 17.19 (10 g) | 27.42 | 1.66 | 45.99 |
| RO123 | 7.45 | 14.21 (13 g) | 38.15 | 1.72 | 38.47 |
| TA055 | 8.49 | 18.04 (12 g) | 32.66 | 2.72 | 38.09 |
| TA089 | 8.88 | 18.66 (15 g) | 30.75 | 2.12 | 39.59 |
| TA097 | 9.02 | 16.34 (9 g) | 31.71 | 1.40 | 41.53 |

Content of crude protein of Serbian varieties ranges from 18-20%, and content of crude fiber was 21-23% (ĐUKIĆ *et al.,* 2010). As stated by ZUK-GOLASZEWSKA *et al*., (2010) content of proteins for varieties of red clover can range even to 21% in dry matter, and percentage of fiber between 18 and 26% of dry matter. Populations which are separated for high content of protein in the range of the already mentioned varieties are: ES042 - 19.54%, ES043 - 19.09%, ES047 - 18.89%, TA055 - 18.04%, TA089 - 18.66%. Two populations are characterized by low level of fiber: ES042 - 26.86%, ES043 - 24.95%, whereas there are populations which have it in very large quantities: ES086 - 38.49%, RO123 - 38.15%. Quantities of ash range from 7.03% for population ES086 to 10.55% CS172, whereas the values TOMIĆ *et al.,* (2005) got range about 7.5% for the surveyed varieties. Values of fat of about 3.97% on the average are also mentioned in the same paper, what is considerably above the values obtained in this paper.

Table 5 shows average values of dry matter per population and average dry matter per plant. Since the yield of dry matter is one of the most significant traits of commercially applicable varieties, it is also important to observe it in a population. As stated by TOMIĆ *et al.* (2005), it is important to study genetic potential of dry matter production because this trait cannot be influenced much in the process of selection and every finding which would make any progress in this direction is important.

By cluster analysis of forage quality features, we got a dendrogram (Fig. 3) where we can notice four clusters. Cluster A contains populations RO089, CS118, RO123, ES086, and CS108. Cluster B contains populations ES042, TA055, TA089, ES047, ES077, and CS152. Cluster C contains populations ES043, RO116 and CS146. Cluster D contains populations RO100, TA097 and CS172.

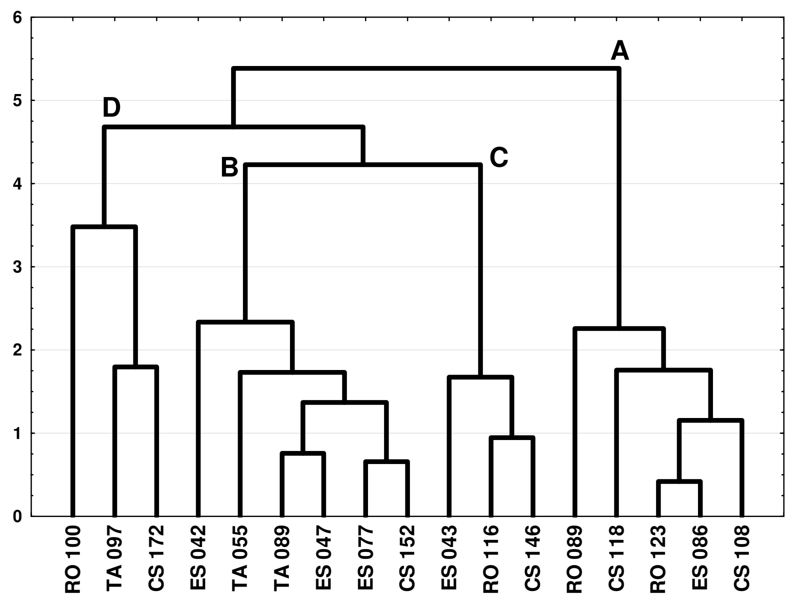


Figure 3. Dendrogram based on the chemical traits of 17 populations of red clover.

In order to explain this arrangement of populations, method of principal component analysis was applied (PCA), where the significance of the surveyed traits for cluster formation was emphasized. The results are presented by the first and the second axis (Fig. 4). The first axis represents 45% of variability, the second 30%; in total 75%.

On Graph 4, we can notice that populations which belong to cluster A have the increased level of fiber. The mean value for fiber in this cluster is 36.9% (Table 6) and this is significantly different from the values obtained by the already mentioned authors, 17.4 - 21.16 % (LUGIĆ et al., 2006) while surveying varieties K-38 and K-39 in field conditions, 20.07% (DROBNA, 2009). Populations which belong to cluster B have the highest contents of fat and proteins. Mean value for this cluster is 18.15% what is a lower value than the values LUGIĆ *et al.,* (2006) got in their papers while surveying material of domestic varieties K-38 and K-39 in field conditions; DROBNA, (2009) while working on six Slovak and Romanian varieties; and better than the values LETO *et al.,* 2004, then Tamm and Bender 2003, as well as VASILJEVIĆ *et al.,* 2011, got while working on varieties. Mean value of fat within this cluster is 2.29%, whereas while working on the variety K-17 DINIĆ *et al*. (2013) got 3.16%. We can notice on Graph 4 that populations belonging to cluster D have the increased level of ash, whereas members of cluster C are singled out for the quantity of NFE matters.

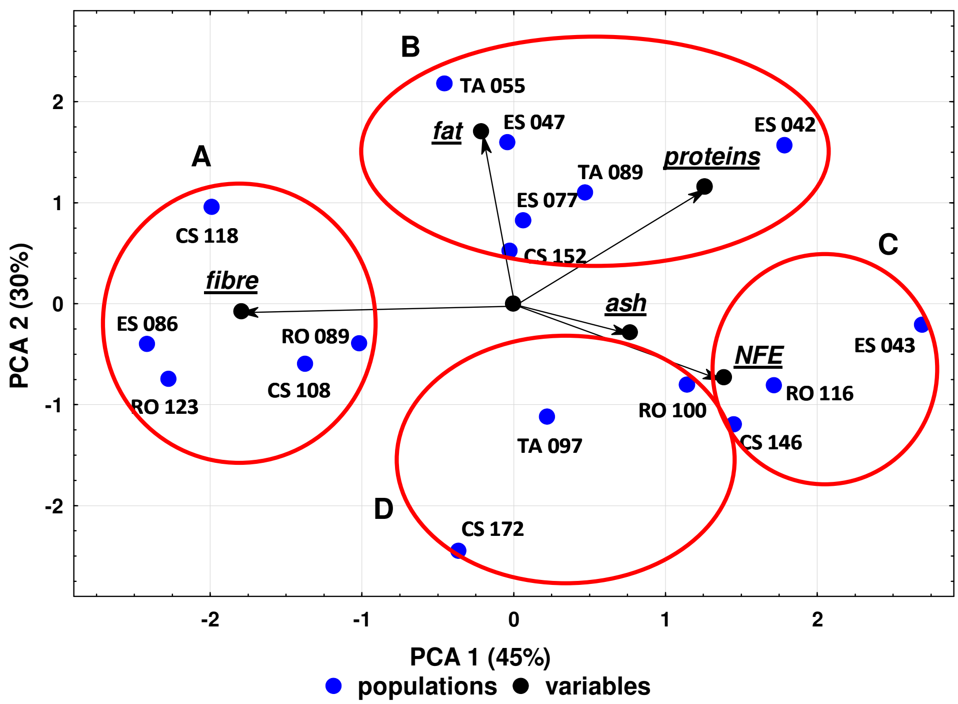


Figure 4. Component loadings for the first of two principal components of PCA of chemical traits.

*Table 6. List of mean values of evaluated chemical characters in particular clusters*

| Claster | Ash | Proteines | Fiber | Ash | NFE |
|---|---|---|---|---|---|
| **A** | 7.6 | 15.3 | **36.9** | 1.82 | 38.3 |
| **B** | 8.9 | **18.3** | 31.0 | **2.29** | 39.6 |
| **C** | 8.1 | 17.9 | 27.0 | 1.55 | **45.5** |
| **D** | **11.4** | 15.7 | 30.8 | 1.37 | 40.8 |

## CONCLUSION

By surveying 17 populations of red clover, we came to the conclusion that there is high variability within the surveyed material. Cluster analysis and a method of principal component analysis enabled grouping of populations according to their features. Separation based on morphological traits led to formation of four clusters; the same number of clusters was formed also by analysis of forage quality traits. It was proved that the gathered material contains populations which are possible to be included in the process of breeding (ES042, ES047, ES077), in order to achieve the main aims in forage production – increase of yield as well as the increase of quantity of crude proteins.

•

## ACKNOWLEDGMENTS

The authors are grateful to the Ministry of education and science of Republic of Serbia for sponsoring of the study within project - TR31057.

Received January 10st, 2014
Accepted May 28th, 2014

## REFERENCES

ASCI, O. O. (2011): Biodiversety in red clover (*Trifolium pratense* L.) collected from Turkey. I: Morpho-agronomic properties. African Journal of Biotechnology, *10(64)*: 14073-14079.

BOLLER, B. and S. L. GREENE (2010): Genetic resources in Boller, B., Posselt, U. K., Veronesi, F., (eds), Fodder crops and amenity grasses - Handbook of plant breeding. Springer.

BULINSKA-RADOMSKA, Z. (2000): Morphological relationships among 15 species of *Trifolium* occurring in Poland, Genet Resour Crop Evol., *47*: 267–272.

DIAS, P.M.B., B. JULIER, J.-P. SAMPOUX, P. BARRE, M. DALL'AGNOL (2008): Genetic diversity in red clover (*Trifolium pratense* L.) revealed by morphological and microsatellite (SSR) markers. Euphytica, *160:* 189–205.

DINIĆ, B., N. ĐORĐEVIĆ, D. TERZIĆ, M. BLAGOJEVIĆ, G. JEVTIĆ (2013): The effect of carbohydrate additive and inoculation on quality of red clover silage. Biotechnology in animal husbandry. *29(1)*: 105-115.

DROBNÁ, J. AND J. JANČOVIČ (2006): Estimation of red clover (*Trifolium pratense* L.) forage quality parameters depending on the variety, cut and growing year. Plant soil environment. *52 (10)*: 468-475.

DROBNA, J. (2009): Yield and forage quality of Romanian red clover (*Trifolium pratense* L.) varieties studied in Slovakia. Not. Bot. Hort. Agrobot. Cluj., *37(1)*: 204-208.

GREENE, S.L., M. GRITSENKO, G. VANDEMARK (2004): Relating morphologic and RAPD marker variation to collection site environment in wild populations of red clover (*Trifolium pratense* L.). Genet Resour Crop Evol., *51*: 643–653.

LETO, J., M. KNEŽEVIĆ, K. BOŠNJAK, D. MAĆEŠIĆ, Z. ŠTAFA, V. KOZUMPLIK (2004): Yield and forage quality of red clover (*Trifolium pratense* L.) cultivars in the lowland and the mountain regions. Plant soil environ., *50(9)*: 391-396.

LUGIĆ, Z., J. RADOVIĆ, D. SOKOLOVIĆ, G. JEFTIĆ (2006): Forage yield and quality of some new red clover (*Trifolium pratense* L.) cultivars in Serbia. Proceedings of XXVI Eucarpia meeting: Breeding and seed production for conventional and organic agriculture. 3rd - 7rd September, Perugia. 179-182.

MARKOVIĆ, J., R. ŠTRBANOVIĆ, M. PETROVIĆ, B. DINIĆ, M. BLAGOJEVIĆ, D. MILIĆ, N. SPASIĆ (2012): Estimation of red clover (*Trifolium pratense* L.) forage quality parameters depending on the cut, stage of growth and cultivar. I međunarodni simpozijum i XVII naučno-stručno savjetovanje agronoma Republike Srpske, 19-22. mart, Trebinje, Bosna i Hercegovina. Agroznanje, *13(1)*: 31-38.

RAKONJAC, V., E. MRATINIĆ, R. JOVKOVIĆ, M. AKŠIĆ FOTRIĆ (2014): Analysis of morphological variability in wild cherry (*Prunus avium* L.) genetic resources from Central Serbia. J. Agr. Sci. Tech., *16*: 151-162.

ROSSO, B. S. AND E. A. PAGANO (2005): Evaluation of introduced and naturalised populations of red clover (*Trifolium pratense* L.) et Pergamino EEA-INTA, Argentina. Genetic resources and crop evolution, *52*: 507-511.

STATSOFT, INC. (2011), STATISTICA (data analysis software system), version 10. www.statsoft.com.

TAYLOR, N. and K. QUESENBERRY (1996): Red clover science, Kluwer Academic Publishers, Dordrecht, The Netherlands.

TAYLOR, N.L. and R. R. SMITH (1979): Red clover breeding and genetics, Adv Agron., *37*:125–155.

TOMIĆ, Z., Z. NEŠIĆ, V. KRNJAJA, M. ŽUJOVIĆ (2005): Parametri produkcije i kvaliteta novih sorti višegodišnjih leguminoza za stočnu hranu. Biotechnology in Animal Husbandry, *21 (3-4)*: 89-96.

VASILJEVIĆ S., D. LUKIĆ, S. KATIĆ (1999): Analiza prinosa i kvaliteta krme sorti crvene deteline. Zbornik radova 2. međunarodne naučne konferencije: Proizvodnja njivskih biljaka na pragu XXI veka. Univerzitet u Novom Sadu, Poljoprivredni fakultet, Novi Sad. 17-18 novembar, 22.

VASILJEVIĆ, S., B. ĆUPINA, Đ. KRSTIĆ, I. PATAKI, S. KATANSKI, B. MILOŠEVIĆ (2011): Sesonal changes of proteins, structural carbohydrats, fats and minerals in herbage dry matter of red clover (*Trifolium pratense* L.). Biotechnology in Animal Husbandry, *27(4)*: 1543-1550

VYMYSLICKY, T., P. SMARDA, J. PELIKAN, T. CHOLASTOVA, J. NEDELNIK, H. MORAVCOVA, R. POKORNY, M. SOLDANOVA, M. POLAKOVA (2012): Evaluation of the Czech core collection of *Trifolium pratense*, including morphological, molecular and phytopathological data. African Journal of Biotechnology, *11(15)*: 3583-3595.

ZUK-GOLASZEWSKA, K., C. PURWIN, B. PYSERA, J. WIERZBOWSKA, J. GOLASZEWSKI (2010): Yields and quality of green forage from red clover di- and tetraploid forms. J. Elementol. *15(4)*: 757-770.

## IZUČAVANJE DIVLJIH POPULACIJA CRVENE DETELINE, SA TERITORIJE SRBIJE U SVRHU PREDSELEKCIJE

Mirjana PETROVIĆ[1*], Zora DAJIĆ – STEVANOVIĆ[2], Dejan SOKOLOVIĆ[1], Jasmina MILENKOVIĆ[1], Jordan MARKOVIĆ[1]

[1] Institut za krmno bilje, Kruševac, Srbija
[2] Poljoprivredni fakultet, Univerzitet u Beogradu, Beograd, Srbija

Izvod

Cilj rada je proceniti agronomsku vrednost kolekcije divljih populacija crvne dateline (*Trifolium pratense* L.). Analizirani materijal prikupljen je na teritoriji Srbije i procenjeno je koje populacije bi mogle ući u program oplemenjivanja. U istraživanje je bilo uključeno 17 populacija crvene deteline. Istraživanje je izvedeno na imanju Instituta za krmno bilje, Kruševac, Srbija. Poljski ogled je trajao tri godine (2008 - 2010); sakupljeni su podaci o morfološkim (zelena masa, suva masa, visina biljke, broj izdanaka, broj bočnih grana, broj internodija, dužina i širina srednje liske) i osnovnim hemijskim parametrima (sadržaj sirovih proteina, sirove celuloze, pepeo, masti, BEM). U radu su rezultati istraživanja obrađeni primenom analize varijanse i multivarijacionim metodama. Klaster analiza bazirana na morfološkim osobinama dovela je do formiranja tri klastera, pri čemu su osobine koje su u najvećoj meri uticale na razdvajanje: zelena masa, visina, dužina i širina lista. Metodom glavne komponente je potvrđeno ovo razdvajanje, i došlo je do još jasnijeg grupisanja populacija. Klaster analiza i PCA metoda su primenjene i na osobine kvaliteta suve materije, pri čemu se izdvojilo četiri klastera, a osobine koje su u najvećoj meri uticale na izdvajanje su procenat proteina, masti i celuloze. Korišćenje obe grupe osobina i nekoliko tipova analiza doprinosi da se izdvoje najperspektivnije populacije a čije pozitivne osobine kao što su: visok prinos zelene mase, broj izdanaka kao i procenat proteina; mogu da se kombinuju u svrhu dobijanja sintetičkih sorti.

Primljeno 10. I. 2014.
Odobreno 28. V 2014.