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Biodiversity of a red clover collection based on morpho-productive traits

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A B S T R A C T

Red clover (*Trifolium pratense* L.) is a meadow and pasture species in natural habitats and also a cultivated species used for animal nutrition. The aim of this research was the assessment of the diversity of 46 red clover accessions based on morpho-productive traits. The traits were investigated according to the UPOV descriptors for red clover – number of internodes, number of branches, stem length, stem thickness, middle leaflet length, middle leaflet width, green matter yield and dry matter yield. The principal components analysis (PCA) explained 74% of the variance of the standardized data and showed relationships between 46 red clover accessions and eight morpho-productive traits, associations among traits and performance of accessions. Among the determined Euclidean distances, the smallest value was obtained for the accessions Rotra and Titus (0.048), the largest value was 1.099 for a pair of NCPGRU2 and Čortanovci accessions, and the average value was 0.380. Two clusters of 46 red clover accessions were separated in the dendrogram based upon UPGMA (Unweighted Pair-Group Method with Arithmetic mean) for eight morpho-productive traits. The first cluster included two subclusters, while the second cluster contained four subclusters. The grouping of the accessions from the red clover collection by the UPGMA cluster analysis can be linked to the geographical origin of the accessions: central and southern Europe for three subclusters and north-eastern Europe for one subcluster.

Keywords: morpho-productive traits, red clover, variability, principal components analysis, cluster analysis.

И З В О Д

Црвена детелина (*Trifolium pratense* L.) јесте ливадско-пашњачка врста на природним staништима, а и култивисана врста која се користи у исхрани животиња. Сврха овог истраживања је била процена диверзитета 46 генотипова црвене детелине на основу морфолошко-продуктивних особина. Проучаване су особине на основу УПОВ дескриптора – броја интернодија, броја грана, дужине стабла, ширине стабла, дужине централне листе, ширине централне листе, приноса зелене масе и приноса суве материје. Анализом главних компоненти (PCA) морфо-продуктивних особина црвене детелине објашњено је 74% варијансе стандардизованих података и приказани су односи 46 генотипова црвене детелине и осам морфолошко-продуктивних особина, повезаност особина и перформанса генотипова. Међу утврђеним Еуклидовим дистанцама, најмања вредност је израчуната за генотипове Rotra и Titus (0,048), највећа је била 1,099 за пар NCPGRU2 и Чортановци, а просечна вредност је износила 0,380. Применом непондерисаног метода парних група са аритметичким просецима (UPGMA), дендрограмом 46 генотипова црвене детелине конструисаним на основу осам морфо-продуктивних особина издвојена су два кластера. Први кластер је садржао два поткластера, док се други кластер састојао од четири поткластера. Груписање генотипова црвене детелине помоћу UPGMA кластер анализе могло се довести у везу са географским пореклом генотипова: централна и јужна Европа за три поткластера и североисточна Европа за један поткластер.

Кључне речи: морфо-продуктивне особине, црвена детелина, варијабилност, анализа главних компоненти, кластер анализа.

1. Introduction

Red clover (*Trifolium pratense* L.) is a broadly distributed and globally significant forage legume grown on approximately 20 million hectares worldwide (Riday, 2010). Before the introduction of mineral nitrogen fertilizers, red clover was of importance in crop rotation and contributed to soil fertility (McKenna et al., 2018). Red clover is able to fix atmospheric nitrogen via symbiosis with *Rhizobium leguminosarum* bv. *trifolii* (Ištvánek et al., 2017) and it reduces the need for synthetic nitrogen fertilizers

(Suwara et al., 2016; Tomić et al., 2012). It is generally used for the production of forage, including green feed, hay and silage. Red clover can be established as a pure stand or in grass/legume mixtures, as a pasture for livestock. The cultivation of mixed grass/legume grasslands has been increasing because of many potential advantages in comparison to monocultures (Arturi et al., 2012). In Serbia, red clover is mostly grown in pure stands on arable land, but it is also seeded in grass mixtures, and occurs to a limited extent in permanent swards (Simić et al., 2013). An important feature of red clover is the presence of the enzymes polyphenol oxidases (PPOs), which reduce both proteolysis and lipolysis during the ensiling of red clover and improve protein absorption efficiency during digestion in ruminants (Hejduk and Knot, 2010). The main limitation of red clover is poor persistence, which is related to high mortality of plants due to a complex of biotic and abiotic factors (Ortega et al., 2014). Being rich in estrogenic isoflavones, red clover also serves as a medicinal plant and a dietary supplement, and can be used to prevent osteoporosis, treat menopausal symptoms, and protect from cardiovascular disease (Du et al., 2013). The main focus of red clover breeding programs oriented to the livestock sub-sector of agriculture has traditionally been on improving forage yield and quality.

Red clover is a diploid (2n=2x=14) species with a genome size of 420 Mb and it has a gametophytic self-incompatibility system which makes it heterozygous and difficult to inbreed without loss of viability and vigor (De Vega et al., 2015). Molecular genetics approaches can theoretically speed up red clover breeding, but red clover's outcrossing nature and high level of heterozygosity make intensive genetic and genomic analyses difficult (Řepková and Nedělník, 2014). Nowadays, besides diploids, there are also tetraploid red clover varieties. Tetraploid varieties are grown mostly in Europe and, although they have high forage yields, compared to diploids, they also have low seed production, accompanied with high seed prices, which limit their widespread use (Sattler et al., 2016).

Genetic variability is a foundation of plant breeding, and genetic resources have a crucial role in broadening the genetic base of plant breeding programs and their associated biotechnology (Rivas et al., 2015). To assess the level of genetic diversity and identify diverse genotypes for developing improved and new varieties, it is necessary to obtain diversity information of the available germplasm, which is the main background for breeders (Gupta et al., 2016). Source material for red clover breeding can be of different origin and includes wild clover populations, landraces, local and foreign commercial varieties, genetic populations, and artificial mutants (Jansone et al., 2014). Data on genetic resources have to be comprehensive and readily available to researchers, in paper and/or electronic form so that samples with desirable traits could be efficiently used in breeding programs (Thormann et al., 2012). Evaluation of genetic resources is based on morphological, phenological and agronomic traits (Pelikán et al., 2014). Although most of the agronomic traits exhibit quantitative variation with a polygenic pattern of inheritance, they are useful traits for the identification of potential divergent heterotic groups for further breeding (Ortiz Ríos, 2015).

The main aims of this study on the red clover collection of the Institute of Field and Vegetable Crops, Novi Sad, were: 1) evaluation of variability among red clover accessions for major morpho-productive traits; 2) estimation of genetic relationships and cluster analysis of red clover accessions based on morpho-productive traits; and 3) assessment of associations among morpho-productive traits and their relation with the red clover accessions.

2. Materials and methods

2.1. Plant material and field trial

Plant material was selected from the collection of the Institute of Field and Vegetable Crops, Novi Sad, and it included 46 diploid and tetraploid red clover accessions, populations and cultivars classified by type of ploidy, from 17 countries (Table 1).

The selected material was studied based on a plant trial sown during the 2011–2012 growing season and laid out in a randomized block design with three replications at a row spacing of 80 × 80 cm, and a planting depth of 2.5 cm. A detailed assessment was performed for the following morpho-productive traits: number of internodes – NOI, number of branches – NOB, stem length – SL, stem thickness – ST, middle leaflet length – MLL, middle leaflet width – MLW, green matter yield – GMY, dry matter yield – DMY, during the first year of the trial. These morpho-productive traits were determined by measuring 30 individual plants (10 plants per replicate) according to the UPOV descriptors (2001). After sowing, during the tillering phase, the stand was thinned to one plant per hill. The soil at the Rimski Šančevi site was chernozem with exceptionally favorable pedological characteristics, which provided optimal soil conditions for the survey. Table 2 presents the agrochemical properties of the Rimski Šančevi soil sampled in 2011, based on data from the Laboratory for Soil and Agroecology of the Institute of Field and Vegetable Crops in Novi Sad. Soil samples were slightly alkaline in reaction, moderately rich in $CaCO_3$, poor in humus, rich in total nitrogen, and with a high content of readily available phosphorus and potassium.

Table 1.
Names, status, ploidy, and origin of the accessions from a red clover collection

Name	Status	Ploidy	Origin
NCPGRU2	Population	2n	Ukraine
NCPGRU3	Population	2n	Ukraine
NCPGRU4	Population	2n	Ukraine
NCPGRU5	Population	2n	Ukraine
Violeta	Cultivar	2n	Bolivia
Nessonas	Cultivar	2n	Greece
Mercury	Cultivar	2n	Belgium
Lemmon	Cultivar	2n	Belgium
SA1	Population	2n	Australia
SA3	Population	2n	Australia
SA4	Population	2n	Australia
BGR1	Population	2n	Romania
BGR2	Population	2n	Romania
BGR3	Population	2n	Romania
Diana	Cultivar	2n	Hungary
Dicar	Cultivar	4n	France
Nemaro	Cultivar	4n	Germany
Una	Cultivar	2n	Serbia
Avala	Cultivar	2n	Serbia
Marina	Cultivar	2n	Serbia
Amos	Cultivar	4n	Denmark
NS-Mlava	Cultivar	2n	Serbia
Italia centrale	Population	2n	Italy
Bolognino	Population	2n	Italy
Marino	Cultivar	2n	Germany
Renova	Cultivar	2n	Switzerland
Titus	Cultivar	4n	Germany
Rotra	Cultivar	4n	Belgium
Kora	Cultivar	2n	Sweden
Vivi	Cultivar	4n	Sweden
Lucrum	Cultivar	2n	Germany
Noe	Cultivar	2n	France
Violetta	Cultivar	2n	Belgium
Britta	Cultivar	2n	Sweden
Krano	Cultivar	2n	Denmark
Triton	Cultivar	4n	Germany
Lutea	Cultivar	2n	Germany
Bjorn	Cultivar	2n	Sweden
Bradlo	Population	2n	Slovakia
Čortanovci	Population	2n	Serbia
89 E-0	Population	2n	Bulgaria
91 E-44	Population	2n	Bulgaria
91 E-63	Population	2n	Bulgaria
Sofia52	Population	2n	Bulgaria
Fertody	Cultivar	2n	Hungary
Quiñequeli	Cultivar	2n	Chile

Table 2.
The agrochemical analysis of the soil at Rimski Šančevi

Depth (cm)	pH (H_2O)	pH (KCl)	N (%)	P_2O_5 (mg 100 g^{-1})	K_2O (mg 100 g^{-1})	$CaCO_3$ (%)	Humus (%)
30	8.25	7.28	0.19	32.4	30.6	2.52	2.58

2.2. Statistical analysis of data

The parameters of descriptive statistics – mean value ($\bar{X}$), standard deviation (σ), the minimum value (Min), the maximum value (Max), and coefficient of phenotypic variation CV_p (%) – were calculated for all measured morpho-productive traits. The significance of differences in the mean values of the measured traits for red clover accessions was determined according to the Tukey test (Table 3).

The cluster analysis was performed based on the mean values of the morpho-productive traits for red clover accessions. To construct dendrograms, an Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) based on the Euclidean matrix was used.

The associations of the examined morpho-productive traits were shown by the Principal Component Analysis (PCA). The PCA method reduced the number of variables to a set of new variables (principal components), thus making it easier to understand the information contained in the measured morpho-productive data. The results of the PCA are graphically presented in a two-dimensional biplot. The statistical analysis of the morpho-productive traits of 46 red clover accessions, including the construction of a distance matrix for the pairs of accessions based on Euclidean distances, the UPGMA hierarchical method of clustering and design of dendrograms, PCA, was performed using R program (R Core Team, 2015).

3. Results and discussions

The mean values of the morpho-productive traits measured in 46 red clover accessions, along with the standard deviation (SD) and the coefficient of variation (CV), are shown in Table 3.

Table 3.
Mean values of morpho-productive traits of 46 accessions from a red clover collection

Genotype	NOI	NOB	SL (cm)	ST (cm)	MLL (mm)	MLW (mm)	GMY (g plant^{-1})	DMY (g plant^{-1})
NCPGRU2	5.45^{cl}	4.13^{e-k}	61.10^{ab}	3.91^{b-h}	40.10^{a}	19.63^{b}	319.00^{a}	60.43^{a}
NCPGRU3	5.86^{b-h}	4.67^{b-g}	41.27^{e-o}	2.63^{mno}	22.67^{pq}	10.83^{jk}	141.67^{d-m}	36.83^{b-m}
NCPGRU4	5.13^{f-o}	4.07^{eik}	38.60^{hip}	2.47^{no}	22.67^{pq}	10.77^{jk}	191.00^{bei}	51.47^{ad}
NCPGRU5	5.88^{b-h}	4.23^{cik}	56.50^{bc}	3.51^{h-l}	31.20^{e-m}	15.23^{c-h}	230.33^{bc}	49.33^{a-h}
Violeta	4.87^{i-o}	3.90^{g-k}	36.00^{lmp}	4.01^{c-h}	32.90^{c-i}	14.70^{deg}	86.00^{k-p}	32.17^{ghn}
Nessonas	4.67^{l-p}	3.67^{jkl}	36.70^{jkp}	3.42^{h-m}	29.03^{h-o}	13.07^{g-j}	88.67^{f-p}	22.60^{lmn}
Mercury	4.89^{g-o}	3.9o^{g-k}	36.10^{lmp}	3.85^{e-l}	27.23^{j-p}	12.40^{g-j}	138.00^{d-n}	36.47^{d-m}
Lemmon	4.95^{h-o}	3.97^{d-k}	38.30^{hip}	3.95^{c-h}	27.60^{k-p}	14.40^{deg}	101.00^{l-p}	27.43^{i-n}
SA1	4.87^{i-o}	3.70^{jkl}	43.27^{e-l}	3.30^{hn}	28.70^{h-o}	15.03^{dei}	118.67^{i-p}	36.43^{d-m}
SA3	3.87^{p}	2.83^{l}	37.63^{gkp}	3.48^{h-m}	38.33^{ab}	23.40^{a}	106.00^{l-p}	35.80^{d-o}
SA4	4.83^{j-p}	4.07^{eik}	39.87^{hip}	3.45^{h-m}	34.77^{b-f}	16.83^{be}	142.67^{d-m}	43.40^{a-i}
BGR1	8.00^{a}	6.60^{a}	59.00^{ab}	4.11^{ah}	26.60^{l-q}	16.20^{c-f}	112.00^{i-p}	34.10^{den}
BGR2	8.10^{a}	4.73^{b-g}	59.03^{ab}	3.68^{h-l}	31.13^{e-l}	16.30^{def}	69.67^{j-p}	24.17^{kmn}
BGR3	8.53^{a}	7.47^{a}	65.27^{a}	4.79^{ad}	27.23^{j-p}	14.63^{deg}	120.67^{eo}	40.23^{b-l}
Diana	5.24^{b-l}	4.23^{cik}	41.27^{e-o}	3.86^{e-l}	28.13^{i-o}	13.67^{f-k}	101.67^{l-p}	26.90^{i-n}
Dicar	4.93^{h-o}	3.90^{g-k}	39.40^{hip}	4.94^{a}	30.43^{f-l}	16.70^{be}	120.33^{i-o}	30.53^{gn}
Nemaro	4.69^{l-p}	3.63^{jkl}	33.40^{np}	3.73^{fhl}	26.20^{lnq}	12.20^{gij}	74.67^{hop}	20.77^{mn}
Una	5.91^{b-h}	4.90^{b-e}	49.13^{cde}	3.55^{h-l}	32.00^{c-k}	13.27^{c-j}	155.33^{c-m}	43.40^{a-i}
Avala	4.77^{l-p}	3.80^{g-k}	41.77^{e-o}	3.85^{e-l}	32.73^{c-i}	14.47^{deg}	117.00^{i-p}	32.30^{cgn}
Marina	5.10^{f-o}	4.07^{eik}	45.27^{e-i}	4.03^{c-h}	32.90^{c-i}	15.27^{c-h}	143.67^{d-m}	37.20^{b-m}
Amos	5.81^{b-j}	4.80^{b-h}	44.40^{e-k}	4.74^{abc}	31.80^{d-k}	16.27^{c-f}	208.33^{bd}	50.87^{acd}
NS-Mlava	5.51^{b-m}	4.50^{b-j}	45.63^{dgi}	3.79^{e-l}	33.53^{b-h}	14.47 ^{deg}	168.33^{c-l}	46.13^{a-g}
Italia Centrale	5.33^{cil}	4.30^{cik}	40.23^{f-p}	3.82^{e-l}	26.77^{l-q}	13.03 $^{g-j}$	175.33^{c-l}	46.70^{adg}
Bolognino	5.37^{cl}	4.33^{bk}	39.40^{hip}	3.93^{c-h}	25.03^{oq}	12.30 $^{g-j}$	151.00^{c-m}	39.67^{b-l}
Marino	5.31^{cil}	4.30^{cik}	38.23^{ip}	3.81^{e-l}	25.30^{nq}	11.90 ^{gj}	80.33^{mop}	23.00^{lmn}
Renova	4.37^{ip}	3.37^{il}	33.67^{op}	3.43^{h-m}	25.07^{oq}	10.83 ^{jk}	47.67^{op}	16.77^{n}
Titus	5.29^{c-l}	4.30^{cik}	40.33^{f-p}	4.30^{afi}	31.00^{e-l}	13.97^{eg}	92.67^{g-p}	25.70^{i-n}
Rotra	5.24^{b-n}	4.10^{e-k}	37.90 ^{gkp}	4.54^{af}	30.80^{e-l}	14.30^{deg}	97.00^{l-p}	26.23^{i-n}
Kora	6.00^{bcf}	5.00^{bce}	48.00^{df}	4.00^{c-h}	36.23^{ad}	14.63^{deg}	200.00^{be}	55.47^{ab}
Vivi	5.97^{b-f}	4.90^{b-e}	44.97^{e-i}	4.78^{ac}	32.43^{c-k}	14.47^{deg}	118.33^{i-p}	41.90^{a-k}
Lucrum	5.57^{b-m}	4.53^{b-j}	41.90^{e-m}	4.22^{a-j}	31.97^{d-k}	13.50^{f-j}	101.33^{l-p}	30.63^{gn}
Noe	4.90^{e-o}	3.90^{g-k}	38.80^{hip}	3.86^{e-l}	30.00^{fno}	12.47^{g-j}	102.00^{l-p}	31.87^{ghn}
Violetta	5.13^{f-o}	4.13^{e-k}	42.53^{e-m}	3.87^{e-l}	32.40^{c-k}	13.30^{f-j}	102.33^{l-p}	33.57^{den}
Britta	4.50^{lop}	3.50^{kl}	46.40^{dh}	4.31^{afi}	35.60^{ae}	17.00^{bd}	112.67^{i-p}	33.10^{dn}
Krano	5.00^{d-o}	4.00^{d-k}	32.67^{p}	4.60^{ae}	33.50^{b-h}	16.77^{be}	97.33^{l-p}	30.37^{gn}
Triton	5.96^{b-f}	4.97^{bce}	46.03^{di}	4.95a	30.10^{fgn}	14.57^{deg}	260.00^{ab}	52.10^{abe}
Lutea	4.48 ^{lp}	3.47^{kl}	34.60^{mp}	3.48^{h-m}	34.43^{b-f}	15.17^{dei}	99.33^{l-p}	27.77^{fn}
Bjorn	5.87^{b-h}	5.03^{bcf}	36.43^{kp}	1.93^{o}	21.97^{q}	10.60^{j}	91.33^{g-p}	30.17^{gn}
Bradlo	6.17^{bc}	5.17^{bc}	40.00^{f-p}	4.03^{c-h}	27.53^{k-p}	12.20^{gij}	77.67^{mop}	30.67^{ghn}
Čortanovci	4.62^{l-p}	3.63^{jkl}	40.03^{f-p}	3.03^{ln}	27.57^{k-p}	13.07^{g-j}	40.33^{p}	17.33^{no}
89 E-0	5.50^{b-m}	4.50^{b-j}	43.50^{e-l}	3.05^{ln}	22.10^{q}	13.60^{f-j}	60.00^{nop}	19.10^{mn}
91 E-44	4.30^{kp}	3.47^{kl}	37.60^{gkp}	3.50^{h-l}	29.23^{h-o}	15.13^{dei}	61.33^{nop}	19.57^{mn}
91 E-63	4.25^{np}	3.37^{il}	33.73^{op}	3.20^{gln}	26.37^{l-q}	13.43^{f-j}	56.00^{op}	18.27^{jno}
Sofia52	5.48^{bco}	4.50^{b-j}	45.43^{e-i}	4.60^{ae}	36.97^{ac}	17.03^{bd}	168.00^{c-l}	51.20^{ad}
Fertody	5.10^{f-o}	4.10^{e-k}	44.77^{e-j}	4.32^{afk}	35.07^{b-g}	16.13^{c-f}	138.33^{d-n}	46.30^{a-g}
Quinekel	6.25^{c}	5.27^{b}	53.57^{bd}	4.60^{ae}	33.57^{b-h}	16.13^{c-f}	162.00^{c-l}	51.83^{abe}
Mean value	5.39	4.3	42.8	3.85	30.2	14.5	124.9	35.2
St. Dev.	0.93	0.8	7.61	0.64	4.32	2.36	56	11.3
CV (%)	17.4	18.7	17.8	16.6	14.3	16.3	44.8	32.2

(NOI – number of internodes, NOB – number of branches, SL – stem length, ST – stem thickness; MLL – middle leaflet length; MLW – middle leaflet width; GMY – green matter yield; DMY – dry matter yield; St. Dev. – standard deviation; CV – coefficient of variation)

The number of internodes ranged from 3.87 (SA3) to 8.53 (BGR3), with the mean value of 5.39. The smallest number of branches was measured for the accession SA3 (2.83), the highest for the accession BGR3 (7.47), and the mean value was 4.3. The accession Krano had the shortest stem length (32.67 cm). The longest stem length was found for the accession BGR3 (65.27 cm). The mean value for stem length was 42.8. Stem thickness ranged from 1.93 cm (Bjorn) to 4.95 cm (Triton), and the mean value was 3.85 cm. The value of middle leaflet length varied from 21.97 cm (Bjorn) to 40.10 cm (NCPGRU2), and the mean value was 30.2 cm. Middle leaflet width was the highest in the accession SA3 (23.40 cm) and the lowest in Bjorn (10.60 cm), with the mean value of 14.5 cm. Green matter yield ranged from 40.33 g for the accession Čortanovci to 319 g for the accession NCPGRU2, and the mean value was 124.9 g. The smallest value of dry matter yield was observed for the accession Renova (16.77 g). The accession NCPGRU2 had the highest value of dry matter yield (60.43 g). The mean value of dry matter yield was 35.2 g. The extent of variation shown through CV was the smallest for MLL (14.3%) and the largest for GMY (44.8%). A medium coefficient of variation (10%–20%) was shown for number of internodes, number of branches, stem length, stem thickness, middle leaflet length, and middle leaflet width, and a large coefficient of variation (>20%) was obtained for green and dry matter yields .

Figure 1 shows a PCA biplot of 46 red clover accessions based on eight morpho-productive traits. The PCA biplot explained 74% of the variance of standardized data. Green and dry matter yields were in a strong positive association, and also positively associated, but to a lesser extent, with stem length and stem thickness. Moreover, green and dry matter yields had a medium to strong positive association with middle leaflet length and middle leaflet width. A moderately strong positive association existed between green and dry matter yields and the number of internodes, while the association between green and dry matter yields and the number of branches was also positive, but less pronounced. Weak positive associations were determined for stem length and middle leaflet length, as well as for stem length and middle leaflet width. Stem diameter and the number of internodes were weakly negatively associated, as were stem diameter and number of branches. Middle leaflet length and middle leaflet width were negatively associated with the number of internodes and the number of branches.

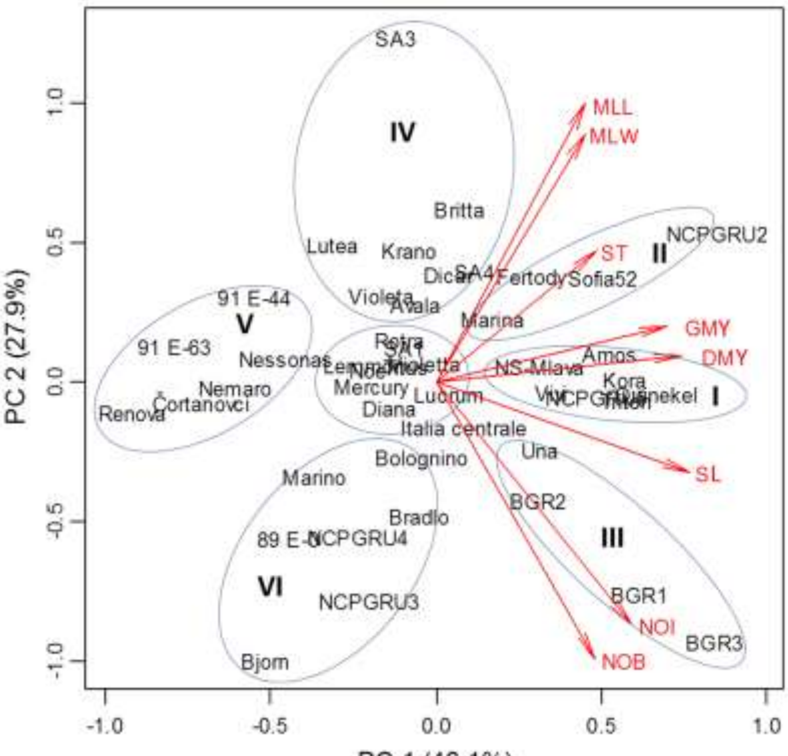

Figure 1. PCA biplot of eight morpho-productive traits measured in red clover accessions (NOI – number of internodes, NOB – number of branches, SL – stem length, ST – stem thickness; MLL – middle leaflet length; MLW – middle leaflet width; GMY – green matter yield; DMY – dry matter yield)

The PCA biplot encompasses 7 groups of accessions from the red clover collection. The first group consists of accessions characterized by high green and dry matter yields , as well as by above-average values of stem thickness, stem length, number of internodes, number of branches, middle leaflet length and middle leaflet width. The accessions of this group are NS-Mlava, Amos, Kora, Vivi, NCPGRU5, Quinekel, and Triton.

The second group comprises accessions that achieved high values of green and dry matter yields, and above-average values of stem thickness, middle leaflet length, and middle leaflet width. The genotypes in this group are Marina, Fertody, Sofia52, and NCPGRU2.

The third group includes the accessions Una, BGR2, BGR1, and BGR3, which achieved high values of green and dry matter yields, and above-average values for the number of internodes, number of branches and stem length.

The fourth group consists of accessions with average values for green matter yield and dry matter yield, above-average values for middle leaflet length and middle leaflet width, and below-average values for number of internodes and number of branches. This group includes Avala, Violeta, Dicar, SA4, Krano, Britta, Lutea and SA3.

The fifth group encompasses accessions with the below-average yields of green and dry matter, and below-average values for stem thickness and stem length. The accessions in this group are Renova, Čortanovci, Nemaro, Nessonas, 91 E-63, and 91 E-44.

The sixth group consists of accessions with below-average values for green and dry matter yields, stem thickness, middle leaflet length and middle leaflet thickness, and above-average values for the number of internodes and number of branches. The genotypes of the sixth group are Bolognino, Marino, Bradlo, 89 E-0, NCPGRU4, NCPGRU3, and Bjorn.

The seventh group comprises accessions that had below-average or average values for most of the observed traits, and they were grouped near the coordinate center of the biplot. This group includes the accessions Rotra, SA1, Lemmon, Titus, Violetta, Noe, Mercury, Lucrum, Diana, and Italia.

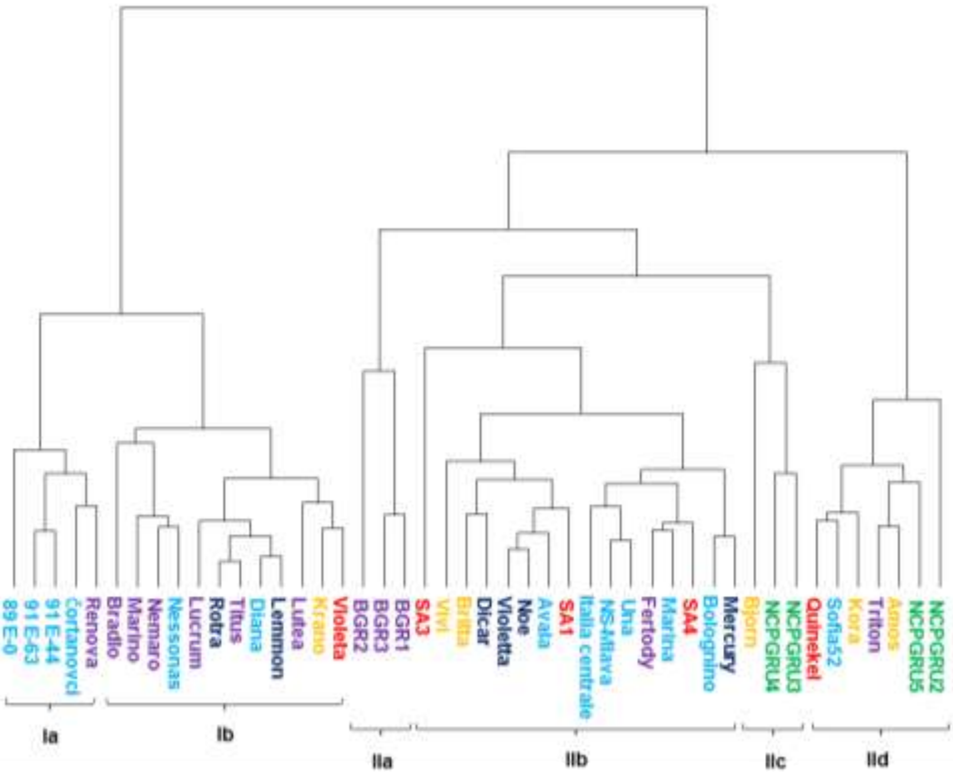

Figure 2. UPGMA dendrogram of red clover accessions based on eight morpho-productive traits (accessions by geographical origin: red – Australia and South America; green – eastern Europe; light blue – southern Europe; purple – Central Europe; yellow – northern Europe; dark blue – western Europe)

The UPGMA dendrogram of 46 red clover accessions, based on eight morpho-productive traits, separated two distinct clusters (Figure 2).

The first cluster includes accessions grouped into two sub-clusters: Ia (Renova, Čortanovci, 91 E-44, 91 E-63, 89 E-0) and Ib (Violeta, Krano, Lutea, Lemmon, Diana, Titus, Rotra, Lucrum , Nessonas, Nemaro, Marino, Bradlo). The second cluster contains accessions grouped into four sub-clusters: IIa (BGR1, BGR2, BGR3), IIb (Mercury, Bolognino, SA4, Marina, Fertody, Una, NS-Mlava, Italia centrale, SA1, Avala, Noe, Violetta, Dicar , Britta, Vivi, SA3), IIc (NCPGRU3, NCPGRU4, Bjorn) and IId (NCPGRU2, NCPGRU5, Amos, Triton, Kora, Sofia52, Quinekel). The grouping of the accessions from the red clover collection by the UPGMA cluster analysis can be linked to the geographical original of the accessions: central and southern Europe for three subclusters and north-eastern Europe for one subcluster.

To assess the genetic distance of 46 red clover accessions based on eight morpho-productive traits, the Euclidean distance (Figure 3) was calculated for the pairs of accessions. The smallest value of the Euclidean distance was determined for the pair of Rotra and Titus accessions (0.048). The largest value of the Euclidean distance was related to the pair of NCPGRU2 and Čortanovci accessions (1.099). The average value of the Euclidean distance of the pairs of genotypes was 0.380.

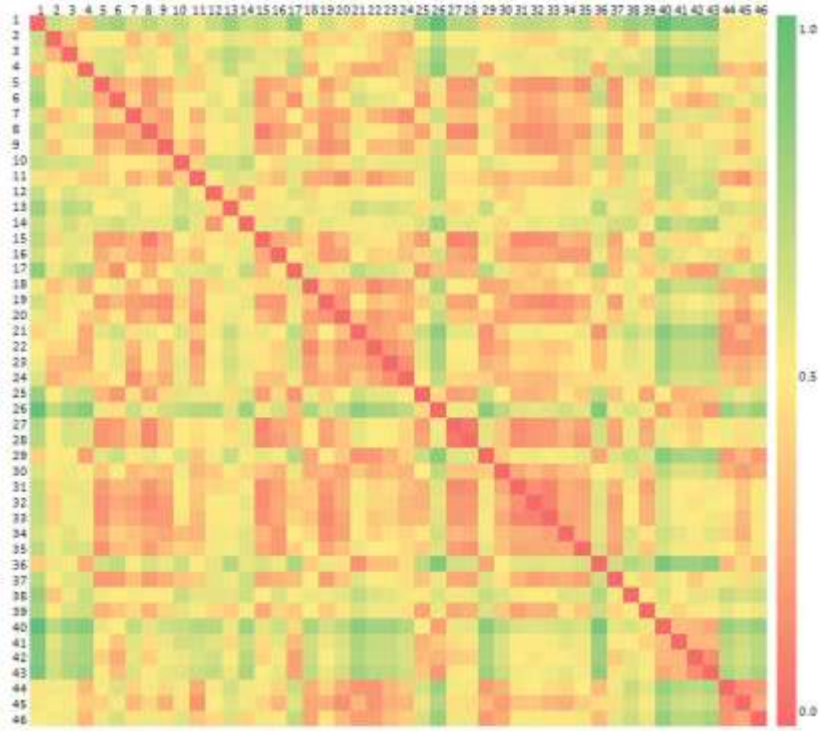

Figure 3. The Euclidean distance matrix for 46 red clover accessions based on eight morpho-productive traits (red clover genotypes are designated by the numbers 1–46, and the Euclidean distance is represented by the color scale, which corresponds to the values 0–1)

Morphological characterization is the first step in the description and classification of genetic resources (Smith and Smith, 1989). Kölliker et al. (2009) emphasized the indispensable role of genetic diversity and detailed characterization within grassland species, concerning the importance of genetic resources for the improvement of agronomically important traits. The present study comprised 46 diploid and tetraploid red clover accessions which were evaluated to provide a comprehensive database for selecting and validating the best accessions of red clover and to identify the most promising material with the best morpho-productive traits for further breeding. The practical implementation of this study would mean to pick out populations with favorable morphological traits and high genetic potential for yield to be included in future programs for the selection and creation of new varieties as well as for the purpose of broadening the genetic base and improving existing cultivars.

Analyzing 48 red clover populations, collected from 20 different locations in the Black Sea Region, Asci (2011) found that the number of internodes ranged from 5.75 to 16.25, more widely than in this study. The reported intervals of variation in stem diameter and middle leaflet length were similar to the ones in our study. The average values of middle leaflet width as well as CV for this trait were higher in this study than those determined by Tucak et al. (2013). Van Minnebruggen et al. (2012) analyzed six red clover genotypes and, based on the number of branches, found that populations were uniform, with CV inside populations ranging from 11% to 27%, while the CV of the same trait in this experiment was 18.7%. Tucak et al. (2009) analyzed 30 red clover cultivars and populations from 11 countries, including two populations from Croatia, and found a high level of variation for green matter yield, as in our study. In our investigation, lower minimum and maximum values were observed for dry matter yield (16.77–60.43 g plant^{-1}) than in Tucak et al. (2009) (99.47 to 142.70 g plant^{-1}). Stem length is a very important yield component. Some researchers (Kuhbanc and Voigtlander 1981; Vasiljević et al., 2006) have found significant correlations between green forage yield and stem length in red clover, which suggests that selection for a longer stem may lead to increased green matter yields. The average stem length for all analyzed cultivars and populations (Tucak et al., 2009) was 53.98 cm and varied from 32.65 to 66.69. Asci (2011) found a wider range of variation for plant height (46.20–92.0 cm) than in our study.

Various authors identified different groups of populations based on the contribution of morpho-agronomic traits. Based on the study of 10 morpho-agronomic traits (flowering time, plant height, stem number per plant, number of nodes, main stem diameter, shape of medial leaflet, density of hairs in the main stem, width of medial leaflet, length of medial leaflet and dry matter yield per plant), the genotypes of red clover studied by Asci (2011) were grouped into eight basic groups, with genotypes from regions close to each other clustered in the same groups. Studying 30 varieties and red clover populations based on seven morpho-agronomic properties (yield of green mass, plant height, the yield of dry matter, dry matter content, persistence, flowering time and seed yield per plant), Tucak et al. (2009) determined the existence of a total of six clusters. They concluded that the most promising materials have high yield potential and good persistence, and are present in two clusters. Rosso and Pagano (2005) stated that the green matter and seed yields of red clover were of highest importance for explaining the variability in 39 introduced and indigenous populations. Based on the study of morphological traits (green matter, dry matter, plant height, number of stems, number of lateral branches, number of internodes, middle leaflet length and middle leaflet width) in 17 wild red clover populations, Petrović et al. (2014) grouped genotypes into three clusters. They found that the traits having the biggest influence on the grouping were: green matter, plant height, and middle leaflet length and width, and determined populations that could be of interest to the process of breeding.

In the principal components analyses of 22 red clover accessions, performed by Pagnotta et al. (2011) on seven statistically significant morpho-physiological traits, the first (PC1) and the second (PC2) axes accounted for 57 and 22 % of the total variation, respectively, 4% more of total variation of standardized data in comparison with our study on eight morpho-productive traits. Trait eigenvectors indicated that PC1 was mainly a positive indicator of erect habit as well as of characteristics contributing to high medium-term forage yield and high seed yield (tall and numerous stems, large leaves, many flowers per inflorescence), reflecting the significant correlations found among these traits. PC2 was mainly a positive indicator of late flowering and a high frequency of plants with leaf marks. Similarly, in our research, PC1 was a positive indicator of green matter yield, dry matter yield, stem thickness, and stem length, and PC2 was a positive indicator of number of internodes, number of branches, middle leaflet length, and middle leaflet width. Petrovic et al. (2014) grouped 9 wild populations of red clover in a PCA biplot in the same cluster B based on increased values of middle leaflet length and middle leaflet width as we did in our study by grouping 8 genotypes in cluster IV and four genotypes in cluster II. The same authors grouped the two wild populations of red clover into cluster A based on higher values of number of branches and number of internodes, similarly as in our research, where four genotypes were grouped in cluster III based on the same two traits.

4. Conclusions

Medium variation was shown for number of internodes, number of branches, stem length, stem thickness, middle leaflet length; and middle leaflet width, and large variation was obtained for green and dry matter yields. The grouping of the cultivars and populations from the red clover collection by the UPGMA cluster analysis encompassed 7 distinct subclusters and can be linked to the geographical origin of the accessions: central and southern Europe for three subclusters and north-eastern Europe for one subcluster. Promising cultivars/populations at the diploid level were separated for further breeding programs (NS-Mlava, Kora, NCPGRU5 and Quinekel) by virtue of their high green and dry matter yields, and above-average values of stem length, number of internodes, number of branches, middle leaflet length and middle leaflet width. These findings could be beneficial and of practical use for red clover growers as well as for the development of new high-yielding varieties in the future breeding process.

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