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VARIATION IN THE DEVELOPMENT OF RED CLOVER (*TRIFOLIUM PRATENSE* L.)

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Abstract: Red clover is an important perennial fodder plant for the production of quality fodder. Since it can be successfully produced even on soils of poorer quality, it is increasingly present in the sowing structure, primarily in hilly and mountainous areas. Trials were carried out on eight genotypes of red clover over several years. There were no significant differences between the average values of stem thickness and plant height of the two first cuttings from the two experimental years. The first cutting of the second year had the highest height (80.0 cm). Genotype 1 had the highest average height (78.0 cm) and leaflet length (45.52 mm). Genotype 6 had the lowest average plant height (74.0 cm), number of stems (7.15), stem thickness (3.31 mm) and leaflet length (36.68 mm). Genotypes 6 and 8 had the widest leaflet in the first cut of the second year of testing (28.94 and 28.93 mm). Aside from plant height, no significant differences were found between the genotypes. Nevertheless, all other examined variables showed statistically significant differences across clover cuttings and genotypes. The average values of the number of stems per plant, the length and width of leaflet in the first clover cut of the first year, and second clover cut of the second year were not statistically significantly different. Red clover is most productive in the first cutting of the second year of life, as confirmed by these investigations, which yielded the greatest values for the majority of the investigated features.

Key words: red clover, cut, genotype, morphological traits, variability.

Introduction

Red clover (*Trifolium pratense* L.) is one of the most important forage legumes alongside alfalfa. It is grown on about 20 million hectares worldwide. In addition to the great importance of clover, a clear trend towards a decrease in areas

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sown with red clover, white clover and trefoil can be observed in the Republic of Srpska, while alfalfa maintains a consistent trend in the sowing structure (Gatarić et al., 2014). In addition to growing red clover in monoculture, it adapts well and is combined in the system of growing grass-clover mixtures (Cnops et al., 2010). Due to the good quality of the forage, red clover favors the nutrition of ruminants (Lee et al., 2009). When grown in grass-clover mixtures and used in the buttoning phase, cheap nutrients are obtained (Karagić et al., 2016). The plant height of red clover is mainly determined by the genetic characteristics of the variety and environmental factors. Leto et al. (1998) determined the highest plant height in the cut that had enough moisture – for the Nada variety of 94.9 cm at the Maksimir locality and 92.44 cm at the Medvednica locality. When measuring the plant height of red clover, it was found that the average height of all tested genotypes was 64.48 cm (Asci, 2011).

Genotype G42 had the lowest plant height (46.20 cm), while genotype G3 had the highest plant height (92.20 cm). When examining the morphological characteristics of diploid and tetraploid varieties of red clover, Muntean (2008) found that in the first year the average plant height of the diploid varieties was 22.96 cm and in the second year it was 49.84 cm, while the tetraploid varieties had an average plant height of 32.6 cm in the first year and 50.3 cm in the second year. In the Republic of Serbia, the average height of a red clover plant is 42.8 cm (Radinović et al., 2022a); in India – 52.0 cm (Verma and Ahmad, 2017).

Popović et al. (2007) tested 16 populations/varieties of red clover and obtained plant heights between 48.97 and 73.99 cm. The phenotypic characterization of 12 populations of red clover resulted in a stem thickness of 4.15–5.04 mm in the second year of life (Primorac et al., 2008). When examining the morphological characteristics of 46 genotypes of red clover, Radinović et al. (2022a) determined an average stem thickness of 3.85 mm. Gatarić et al. (2010) analyzed the number of stems per red clover plant and found that the plants had an average of 8.30 stems per plant in the second year of life, and 9.83 stems per plant in the third year of life. A red clover crop with an area of 1 ha develops leaves with an area of 25 ha (Tatić and Petković, 1998). Red clover produces high yields of green mass and hay, with the first cutting in the second year of life being the most productive (Petković et al., 2021). A higher crude protein content is obtained from the hay of the first cut compared to the second cut from the same year (Petković et al., 2020). In the Republic of Serbia, research was conducted on the correlation between morphological traits, yield and quality of red clover. In the second year, there was the highest correlation between the yield of green mass and the yield of dry matter (Radinović et al., 2022b).

In hilly and mountainous areas and on soils with poor chemical composition, forage is mainly produced from red and white clover, birdsfoot trefoil and sainfoin (Radović et al., 2010). In the examination of eight genotypes of red clover in Banja

Luka, the average annual yield of green mass depended on the genotype and was between 44 and 59 t ha^{-1}, and of hay between 8.6 and 13.3 t ha^{-1} (Petković et al., 2021). In Banja Luka, in the second and third year of using red clover, genotype G1 had the highest yield of hay – 13.7 t ha^{-1} in the first year, and the lowest genotype G2 – 8.3 t ha^{-1} in the second year of testing (Gatarić et al., 2010). In the research of Stevović et al. (2012) in Serbia, the lowest average yield of green mass and hay of red clover was given by the Viola variety, and the highest average yield of green mass of 39.6 t ha^{-1} and hay of 7.18 t ha^{-1} was achieved by the Una variety. The dry matter yield of red clover in the second year of life in Bulgarian conditions is in the interval 16.9–19.7 t ha^{-1} (Mihovsky and Naydenova, 2017). The optimal time for mowing red clover is at the stage when 20% of the inflorescence appears (Katić et al., 2004). Recently, the demand for red clover has increased, which is partly a result of new contemporary trends in plant production, but also the increasing importance of red clover in the production of animal feed and partly due to its use in the pharmaceutical industry. This research aimed to study the morphological characteristics of red clover in the conditions of the mountainous area of the Banja Luka region.

Material and Methods

The experiment was carried out on 13.05.2010 southwest of Banja Luka, in the locality of Dobrnja (N 44°39' E 17°00', 527 m altitude). The morphological characteristics of eight genotypes of red clover were studied in the first cutting of the first year and the first two cuttings of the second year of life. Four prospective genotypes of red clover of domestic origin (G-1, G-2, G-3 and G-4) and four varieties of red clover (G-5 Nike, G-6 Viola, G-7 Kolubara and G-8 Start) were included in the experimental trials. The sowing rate was 17 kg ha^{-1}. Sowing was carried out in four replicates (by hand) at a depth of 1.5–2 cm, and the distance between the rows was 20 cm. The size of the trial plot was 1 x 2 m. Seeds of red clover genotypes from the breeding program of the Agricultural Institute of the Republic of Srpska were used for sowing (G-1, G-2, G-3 and G-4), while the seeds of G-5, G-6, G-7 and G-8 were purchased at retail. The basic soil tillage (plowing) was done in the fall of 2009. The seedbed was prepared on the day of sowing. Pre-sowing fertilization was carried out with 250 kg ha^{-1} NPK 8:20:30. Chemical protection measures against weeds, diseases and harmful insects were not carried out.

Plant height (cm), the number of stems per plant, stem thickness (mm), and length and width of leaflets (mm) were recorded from five plants in the central part of each subplot. The samples for all tests were taken from the middle rows of the plots one day before mowing the green mass in the phenological phase – the beginning of flowering. The results of the biometric measurements were processed

with PC applications for Windows: Statistical Package for Social Sciences and Excel. The results of the studied traits were processed by analysis of variance (ANOVA) using a computer program and the GLM procedure. The Duncan's multiple range test (DMRT) was used to determine the significance of the differences between the examined cuttings and genotypes, and their ranking for the significance level R=0.01. According to this test, average values with the same letter are not statistically different.

Agroecological conditions

The soil in the experimental plot was well supplied with humus (5.2%) and with potassium (30.0 mg K2O in 100 g of soil), whereas the phosphorus content was low (7.0 mg P_2O_5 in 100 g of soil). Soil acidity (pH) in H_2O and KCl, the humus content (%), the phosphorus content (mg P_2O_5/100 g soil) and the potassium content (mg K_2O/100 g soil) were determined by chemical soil analysis. The analyzed parameters were determined using the following methods: total nitrogen in the soil, using the semi-micro-Kjeldahl method, modification according to Bremner (1960), easily accessible phosphorus and potassium in the soil, using the AL-method according to Egner et al. (1960) soil reaction (pH), using the potentiometric method.

Meteorological data: Mean monthly air temperatures and precipitation amounts were taken from the Hydrometeorological Institute of the Republic of Srpska, from the nearest measuring station in Banja Luka, which is located in the village of Lazarevo ($44^0$80'806" N, $17^0$21'278" E and 150 m altitude), about 30 km from the location of the experiment. In both years of testing, mean monthly temperatures were higher than the multi-year average (Table 1).

Table 1. Monthly and annual total precipitation sums (mm), mean monthly and annual air temperatures (ºC) in 2010 and 2011 and multi-year averages of precipitation sums and mean temperatures (1961–2010) for the location of Banja Luka (Republican Hydrometeorological Institute).

	Month												Total/ average
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
P10	132.2	101.6	113.8	71.1	148	234.6	66.3	87.0	196	83.8	73.8	87.6	1395.8
P11	51.6	29.3	34.2	37.7	62.6	37.0	112.7	8.9	26.3	62.1	5.1	120.7	588.2
AP	70.4	62.8	80.1	88.7	94.4	113.2	91.8	86.1	94.5	78.4	94.6	90.9	1044.3
T10	0.2	2.4	7.5	12.0	16.5	20.3	23.1	21.8	15.7	9.4	8.9	1.5	11.6
T11	1.9	1.7	7.1	13.0	16.0	21.2	23.1	23.7	20.2	11.0	3.1	3.9	12.2
AT	0.1	2.0	6.5	11.1	16.1	19.5	21.2	20.7	16.1	11.2	6.3	1.4	11.0

P10 – monthly and annual sum of precipitation for 2010; T10 – average monthly and annual temperature for 2010; P11 – monthly and annual sum of precipitation for 2011; T11 – average monthly and annual temperature for 2011; AP – multi-year average of precipitation sums (1961–2010); AT – multi-year averages of mean temperatures (1961–2010).

In both years of testing, the mean monthly temperatures were higher than the multi-year average (Table 1). In both years, the mean monthly temperatures in June, July and August were higher than the long-term average for these months. The first year of testing was characterized by 351.5 mm more and the second with 456.1 mm less precipitation compared to the multi-year average (1961–2010). In the first year compared to the second year, there was a higher amount of precipitation in the amount of 807.6 mm (Table 1). The amount of precipitation in the first year affected the production of tall plants with thicker stems in the first cutting of the first year. During the vegetation period, the highest amount of precipitation in 2010 was in the month of June, 234.6 mm, while in the vegetation period of 2011, the highest amount of precipitation was in July 112.7 mm, however, a small amount of precipitation was recorded in June – 37.0 mm. The low amount of precipitation and high average monthly temperatures in June 2011 caused a lack of moisture and less favorable conditions for the growth and development of the red clover plants of the second crop in that year.

Results and Discussion

The cut had a statistically highly significant influence on all investigated traits (Table 2). Genotype had a statistically significant effect on stem thickness and leaf width, and a highly significant influence on the number of stems per plant and length of leaf. The interaction of cut x genotype had a statistically significant effect on leaflet length.

Table 2. Analysis of variance for plant height, number of stems per plant, stem thickness, leaf length, leaf width of the eight tested genotypes of red clover.

Source of variation	Traits				
	Plant height	Number of stems per plant	Stem thickness	Leaf length	Leaf width
Cut	**	**	**	**	**
Genotype	ns	**	*	**	*
Cut x genotype	ns	ns	ns	*	ns
Error	1460	37.5	4,30	937	354
Total	565093	6471.7	1228.9	181688	56185

* $p<0.05$; ** $p<0.01$; ns – not significant.

The average values of the investigated traits for the first cutting of the second year were statistically significantly higher than the values obtained for the second cutting of the same year (Table 3). There were no significant differences between the average values of all genotypes for plant height and stem thickness between the

first cuttings of the two years. Still, the average values of these two traits were significantly higher in the first cutting of the first year than in the second cutting of the second year. Except for plant height, significant differences were found between the mean values of the genotypes for all investigated traits.

The average values of traits by cut showed that the lowest average plant height, number of stems, stem thickness and leaflet width were obtained in the second cut of the second year of research and the life span of the red clover. Except for leaflet width, genotype 6 had the lowest average values of the tested traits. Genotype 7 obtained the highest average number of stems per plant (8.52), but also the leaflet with the smallest width (21.88 mm). Genotype 1 had the highest average plant height (78.0 cm) and leaflet length (45.52 mm), and genotype 4 had the highest average leaflet width (25.19 mm). In these studies, the average plant height by genotype ranged from 74.0 to 78.0 cm. Popović et al. (2007) examined 16 populations/varieties of red clover when they determined plant height in an interval from 48.97 to 73.99 cm. In these experiments, the plant height was determined in a larger interval (46.20–92.20 cm), but with a smaller average value (64.50) (Asci, 2011). The average plant height of red clover collections from the northwestern Himalayas is 52.0 cm (Verma and Ahmad, 2017).

The average plant height was 77.0 cm. A lower average height was determined by Leto et al. (1998), in whose tests the average plant height at a site in a lowland area was 58.93 cm, and at a site at a higher altitude – 64.0 cm. Tucak et al. (2016) determined the plant height of red clover in an interval of 59.38–78.66 cm in the second year of the experiment. Radinović et al. (2022a) presented the results of their studies on the morphological characteristics of red clover, in which the average plant height was 42.8 cm.

Statistically significant differences were found between cuts and genotypes for the trait number of stems per plant. The highest average number of stems per plant (10.0) was obtained in the first cutting of the second year, and the lowest (7.04) in the second cutting of the same year. The reason for the lower number of stems per plant in the second cutting is the tendency of red clover to produce higher yields of green mass and hay in the first cutting compared to subsequent cuttings in the same year. The differences in the average values of stem thickness between genotypes and cut were statistically significant. In addition to the genetic predisposition of red clover, sufficient moisture during the vegetation period of this section also affected the achievement of a high average stem thickness value in the first section of the second year.

Table 3. Average values of plant height (cm), number of stems, stem thickness (mm), leaf length (mm) and leaf width (mm) in eight genotypes of red clover in a two-year period.

	Cut				
Genotype	First 2010	First 2011	Second 2011	Average by genotypes	CV (%)
			Plant height (cm)		
G-1	80	82	73	78[a]	6.18
G-2	79	81	70	77[a]	8.01
G-3	80	82	71	77[a]	7.48
G-4	80	81	70	77[a]	10.19
G-5	80	77	70	76[a]	8.69
G-6	75	78	69	74[a]	6.19
G-7	78	80	72	76[a]	8.06
G-8	78	79	72	76[a]	6.85
Average per cut	79[a]	80[a]	71[b]	77	
			Number of stems per plant		
G-1	7.15	10.35	7.35	8.28[a]	20.21
G-2	7.45	10.00	7.35	8.27[a]	17.82
G-3	7.55	10.40	6.55	8.17[a]	23.44
G-4	7.45	10.20	6.80	8.15[a]	20.16
G-5	7.00	10.30	7.25	8.18[a]	20.30
G-6	6.20	8.90	6.35	7.15[b]	19.38
G-7	7.90	10.10	7.55	8.52[a]	15.56
G-8	6.30	9.75	7.15	7.73[ab]	21.45
Average per cut	7.13[b]	10,00[a]	7.04[b]	8.06	
			Stem thickness (mm)		
G-1	3.87	3.79	3.24	3.63[a]	9.72
G-2	3.89	3.71	3.17	3.59[ab]	11.60
G-3	3.91	3.67	3.16	3.58[ab]	11.33
G-4	3.78	3.60	3.55	3.65[a]	4.91
G-5	3.75	3.77	3.50	3.67[a]	4.61
G-6	3.44	3.39	3.10	3.31[b]	9.03
G-7	3.67	3.65	3.16	3.49[ab]	10.26
G-8	3.88	3.65	3.17	3.57[ab]	10.99
Average per cut	3.77[a]	3.65[a]	3.26[b]	3.56	
			Leaf length (mm)		
G-1	46.60	48.20	41.77	45.52[a]	2.35
G-2	45.67	44.72	38.08	42.83[a]	2.24
G-3	43.75	46.78	43.75	44.76[a]	1.65
G-4	44.45	48.03	42.91	45.13[a]	1.61
G-5	43.26	46.85	42.70	44.27[a]	1.87
G-6	31.26	45.47	33.30	36.68[b]	3.34
G-7	40.35	47.93	40.88	43.05[a]	2.17
G-8	40.20	46.22	43.35	43.26[a]	1.61
Average per cut	41.94[b]	46.78[a]	40.84[b]	43.19	
			Leaf width (mm)		
G-1	23.11	27.55	22.29	24.32[ab]	11.89
G-2	21.73	25.29	22.61	23.21[ab]	10.25
G-3	22.98	25.66	23.14	23.93[ab]	9.36
G-4	22.82	28.28	24.46	25.19[a]	12.93
G-5	23.09	26.60	25.55	25.08[a]	11.34
G-6	22.21	28.94	20.18	23.77[ab]	18.82
G-7	19.98	24.22	21.44	21.88[b]	10.10
G-8	21.76	28.93	22.74	24.47[ab]	16.99
Average per cut	22.21[b]	26.93[a]	22.80[b]	23.98	

CV = coefficients of variation; Values marked with the same letter are not statistically significantly different at the p=0.01 level (Duncan's multiple range test).

Jakešova et al. (2011) determined a stem thickness of 4.53 mm when investigating the morphological characteristics of red clover. Radinović et al. (2022a) obtained an average thickness of red clover stem of 3.85 mm. Primorac et al. (2008) measured the mean leaflet in the second year of life in three cuttings of 12 populations of red clover, and found it to be between 42.31 and 48.51 mm. In our study, the average length of leaflets (average in three sections) was in the indicated interval in all other genotypes except genotype 6, so it can be said that the data obtained in this work are consistent with the above results. When examining the variability of leaflet length of three genotypes of red clover in the Manjača region, an average length of 4.10 cm in the second year of use and 4.03 cm in the third year of use was determined (Gatarić et al., 2010). Compared to the data presented in this paper, all genotypes, except genotype 6, showed a higher average leaflet length.

When describing the morphological and biological properties of the red clover variety Viva, Popović et al. (2011) state that the leaf is trifoliate, and the leaflets have an elongated shape with a length in the interval 4.6–5.5 cm. Primorac et al. (2008) examined the middle leaflet in the second year of use of 12 populations of red clover, and determined its average width from 21.62 to 27.74 mm. Comparing the average leaflet width obtained in this work, it can be seen that all genotypes had average leaflet widths in the specified interval. Compared to the data of Gatarić et al. (2010), who found that the average width of the leaflets in two years was 2.10 and 2.21 cm, in this work, all genotypes showed a larger average width of the leaflet. Popović et al. (2011) state that the leaf width of the red clover variety Viva is 1.6–2.6 cm. The average value of the length and width of the leaflet in this paper was higher compared to the data for the average leaflet of the red clover presented by Radinović et al. (2022a). In this paper, the data for the width and length of the leaflets had smaller values compared to the data for the middle leaflet of red clover determined by Grljušić et al. (2006), in whose study the average length was 5.30 cm and the width was 2.94 cm.

Conclusion

The studied red clover genotypes showed significant variability for all traits. In the first year (the first cutting in the year of sowing), high values of the tested traits were obtained. For good growth and development in that cutting, a large contribution was made by the available sufficient amount of moisture. The values of the height of the plant, the number of stems per plant, the thickness of the stem, the length and the width of the leaf were smaller in the second cut compared to the first cut in the same year, thereby confirming the biological characteristic of red clover that it gives lower values in that cut. Unfavorable weather conditions in June also had an impact on obtaining lower values of the tested traits in the second

section of the second year. The researched genotypes represent good material for growing red clover with high values of the examined traits in the locality of the research, as well as in other localities characterized by similar conditions.

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VARIRANJE RAZVIĆA CRVENE DJETELINE (*TRIFOLIUM PRATENSE* L.)

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R e z i m e

Crvena djetelina je značajna višegodišnja krmna biljka za proizvodnju kvalitetne stočne hrane. Zbog mogućnosti uspijevanja na zemljištima lošijeg kvaliteta, sve više je zastupljena u setvenoj strukturi prvenstveno u brdsko-planinskim područjima. Eksperimentalna ispitivanja su realizovana na osam genotipova crvene djeteline u višegodišnjem periodu. Između prosječnih vrijednosti debljine stabla i visine biljke dva prva otkosa iz dvije godine ispitivanja nije bilo značajnih razlika, a najveću visinu je imao prvi otkos druge godine (80,0 cm). Genotip 1 je imalo najveću prosječnu visinu (78,0 cm) i dužinu liske (45,52 mm). Genotip 6 je imao najmanju prosječnu visinu biljke (74,0 cm), broj stabala (7,15), debljinu stabla (3,31 mm) i dužinu liske (36,68 mm). Genotipovi 6 i 8 su imali najširu lisku u prvom otkosu druge godine ispitivanja (28,94 mm i 28,93 mm). Izuzev osobine visina biljke kod koje nisu dobijene značajne razlike između genotipova, za sve ostale ispitivane osobine između otkosa i genotipova dobijene su statistički značajne razlike. Prosečne vrednosti broja stabala po biljci, dužine i širine lista u prvom otkosu prve i drugom otkosu druge godine nisu se statistički značajno razlikovale. Crvena djetelina je najproduktivnija u prvom otkosu druge godine života, i to se potvrdilo i u ovim istraživanjima u kojima su najveće vrijednosti većine ispitivanih osobina dobijene u tom otkosu.

Ključne reči: crvena djetelina, otkos, genotip, morfološke osobine, varijabilnost.

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