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# Variation in yield, forage quality and morphological traits of red clover (*Trifolium pratense* L.) breeding populations and cultivars

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## Abstract

The main objectives of this study were 1) to estimate variation in yield, forage quality and morphological traits of newly developed red clover (*Trifolium pratense* L.) breeding populations of different origin, 2) to compare their yield potential with foreign cultivars, 3) to determine the relationship between studied traits and 4) to identify and select the most promising populations for future breeding work. Eight red clover breeding populations of different origin (OPCD-2, RCOP-1,3,4,5,6,7,8 created within the framework of the forage crops breeding program at the Agricultural Institute Osijek, Croatia) and eight foreign cultivars ('Liepsna', 'Kamaniai', 'Nosovskij 5', 'Pallas', 'Astred', 'Redquin', 'Milvus', 'Amos') were evaluated for thirteen morpho-agronomic and forage quality traits. Field experiment was designed as a randomised complete block with three replications over two consecutive years (2008 and 2009) at the Agricultural Institute Osijek, Croatia. The studied breeding populations/cultivars of red clover significantly varied in yields of green mass and dry matter, plant height, number of internodes, density of hairs, width and length of central leaflet, shape of medial leaflet, leaf to stem ratio, content of crude protein and relative feed value. Croatian breeding populations RCOP-3, OPCD-2 and RCOP-1 had significantly higher total yields of green mass (from 98.50 to 111.82 t $ha^{-1}$) and dry matter (from 22.61 to 25.64 t $ha^{-1}$) compared to the observed yields of other populations and foreign cultivars of red clover, which indicates their high genetic potential for yield. Besides the high forage yield, those breeding populations achieved favourable values of quality and other analyzed morphological traits, and they are representing valuable germplasm for further selection work and improving of our breeding program and/or application for plant cultivar registration. Positive correlations were found between forage yields and plant height, number of internodes and between width and length of central leaflet, and these properties were positively correlated with each other. Most quality traits were in negative correlation with yield and other observed morpho-agronomic traits.

Key words: correlation, forage quality, morphological traits, *Trifolium pratense*, variation, yields potential.

## Introduction

Red clover (*Trifolium pratense* L.) is one of the most important forage legumes and a valuable component of permanent grassland as well as grass-clover mixtures in temperate regions (Herrmann et al., 2008). Red clover is adapted to a wide range of soil types, environmental conditions, fertility levels, use patterns and management (Drobna, 2009). It is of high ecological value due to its ability to fix atmospheric nitrogen, allowing a reduction in the use of nitrogenous fertilizer (Abberton, Marshall, 2005). The usual stand life for red clover production is no longer than three years, with few cuts and in one growing. Under ecological conditions of West Croatia had achieved average annual forage yield from 9.18 to 10.8 t $ha^{-1}$ (Leto et al., 2004). It is a perennial, diploid ($2n = 2x = 14$) and outcrossing species with a gametophytic self-incompatibility system (Taylor, Quesenberry, 1996). Red clover, as well as other forage crops', breeding has traditionally focused on increasing animal productivity by providing high yielding, persistent forages resistant to a range of pests and diseases. Generally red clover breeding programs are based on mass phenotypic or recurrent selection, and therefore the cultivars produced are heterogeneous with highly heterozygous individuals (Berzina et al., 2008; Tucak et al., 2009). Forage yield genetic gains per year for red clover have been similar to reported gains seen in alfalfa and other forage legumes with similar breeding methodology. The estimates of genetic gain in red clover forage yield varied from 0.21%

to 1.39% per year (Riday, 2010). Compared with other crops, such as maize, slow yield gains in perennial forage legumes have been attributed to multiyear selection cycles, lack of progeny testing, underuse of nonadditive genetic interactions, and focus of breeding programs on a wide range of economically important traits, such as disease or insect resistance and forage quality, many of which are not specifically correlated or may be negatively correlated with the forage yield (Brummer, 1999; Casler, Brummer, 2008; Riday, Krohn, 2010).

Use of genetically variable and divergent genotypes' favourable values of desirable traits during the selection process is a prerequisite for successful red clover breeding (Popović et al., 2007).

The main objectives of this study were 1) to estimate variation in yield, forage quality and morphological traits of newly developed red clover breeding populations of different origin, 2) to compare their yield potential with foreign cultivars, 3) to determine relationship between studied traits and 4) to identify and select the most promising populations for future breeding work.

## Materials and methods

The morpho-agronomic and forage quality traits of eight red clover (*Trifolium pratense* L.) breeding populations of different origin (OPCD-2, RCOP-1,3,4,5,6,7,8 created within the framework of the forage crops breeding program at the Agricultural Institute Osijek, Croatia) and eight foreign cultivars were evaluated in this study (Table 3). Red clover breeding populations were derived from the local eastern Croatian population and introduced foreign materials by five cycles of phenotypic selection for forage yield potential and persistency. The research was conducted during two consecutive years (2008 and 2009) at the experimental field of the Agricultural Institute Osijek at a lowland location in the eastern part of Croatia (lat. 45°32′ N, long. 18°44′ E, alt. 90 m). The soil type of the experimental site was *Eutric Cambisol* (*CMeu*), characterised by silt clay-loam texture (Soil Survey Division Staff, 1993). In both years, the average monthly temperatures during the growing season of red clover were similar and they were not significantly deviated from the long term average temperatures, while there were significant variations in the precipitation (Table 1). In 2009, the total amount of precipitation was 287.1 mm, which was 38.62% less than the average amount of rainfall in long-term period (467.8 mm), while in 2008 the total amount of precipitation was 504.3 mm, which was 7.80% more than in long-term period. A significant lack of rainfall in 2009 did not significantly affect the growth and development of red clover, which is likely to be associated with deep and well developed root system.

***Table 1.*** Average monthly temperatures and total precipitation during the growing seasons for the experimental yeas and for the average in long-term period (1971–2000) at the location Osijek, Croatia

| Month | Average air temperature °C | | | Total precipitation mm | | |
|---|---|---|---|---|---|---|
| | 2008 | 2009 | LTA | 2008 | 2009 | LTA |
| March | 7.5 | 6.8 | 6.4 | 82.4 | 26.5 | 40.5 |
| April | 12.5 | 14.6 | 11.2 | 48.8 | 18.7 | 51.0 |
| May | 18.1 | 18.3 | 16.7 | 66.9 | 39.4 | 59.2 |
| June | 21.5 | 19.2 | 19.6 | 76.3 | 62.8 | 82.0 |
| July | 21.9 | 23.2 | 21.3 | 67.6 | 13.8 | 66.3 |
| August | 21.8 | 22.9 | 20.8 | 46.2 | 60.6 | 61.9 |
| September | 15.7 | 19.1 | 16.5 | 86.3 | 10.0 | 51.0 |
| October | 13.0 | 11.5 | 11.1 | 29.8 | 55.3 | 55.9 |
| Average/total* | 16.5 | 16.9 | 15.4 | 504.3* | 287.1* | 467.8* |

*Total precipitation, LTA – long-term period average

On March 17th 2008, the red clover breeding populations/cultivars were hand sown in rows spaced at a distance of 20 cm at a seed rate of 20 kg $ha^{-1}$ (12 g $plot^{-1}$) after the standard germination test of all breeding populations/cultivars. The size of plots was 6 $m^2$, they consisted of five rows, each six m in length. The experimental design was randomised complete block with three replications. Irrigation, fertilization or weed/pest/disease protection were not applied after sowing and cutting. The plots were cut at full bloom stage three times per growing season (27 June, 13 August, 28 October in 2008 and 18 May, 6 July, 28 August in 2009) using a forage plot harvester "Hege Model 212" ("Wintersteiger AG", Germany) and weighed to within ± 0.1 kg. At each cutting in both growing seasons green mass and dry matter yields (GMY and DMY) per plot and plant height (PH measured before cut on the ground to the top of the inflorescence on five randomly selected stems of each population/cultivar in all plots, and expressed as a two year average height in cm) were measured. Before each cutting to determine the dry matter yield fresh forage samples (approximately 500 g) were taken randomly from each plot of all populations/cultivars. After weighing, the samples were dried at 105°C to a constant weight. The dry matter yield was calculated as dry matter content × green mass yield/100. In the period of two years yields of green mass and dry matter for each plot were determined by summing the biomass yields from each cut of both growing seasons and calculated in t $ha^{-1}$.

Phenotypic evaluations of the morphological traits such as number of internodes (NI), density of hairs (DH), width and length of central leaflet (WCL and LCL, cm) and shape of medial leaflet (SML) were performed according to the UPOV guidelines for the conduct of

tests for distinctness, uniformity and stability of red clover (UPOV/TG/5/7, 2001) in the second cut of the second growing season. All these traits were assessed at the full flowering stage (14 days after three heads of a plant had begun to flower) on thirty randomly selected stems of each population/cultivar. In the same cut, forage samples of 1 kg were randomly taken in the middle rows from each plot of all populations/cultivars for leaf to stem ratio (LSR) and forage quality traits determination. In those samples leaves and stems were hand separated to calculate LSR, after that the same samples were oven-dried at 60°C for 48 hours and prepared for chemical analyses. The contents of crude protein (CP) and neutral and acid detergent fibre (NDF, ADF) in dry matter were determined according to standard methods (AOAC, 1995), relative feed value (RFV) was calculated from the estimates of dry matter digestibility (DMD) and dry matter intake (DMI) according to the following equations adapted from common formulas for forages (Schroeder, 1994): RFV = (%DDM × %DMI) / 1.29, DDM = 88.9 − (0.779 × %ADF), DMI = 120 / %NDF. Standards for RFV as a criterion to grade hay have been proposed by the Hay Marketing Task Force of the American Forage and Grassland Council (Rohweder et al., 1987), and are presented in Table 2.

***Table 2.*** Quality standards of legume, grass and legume-grass mixture

| Quality standard[a] | CP, % of DM | ADF, % of DM | NDF, % of DM | RFV |
|---|---|---|---|---|
| Prime | >19 | <31 | <40 | >151 |
| Premium (1) | 17–19 | 31–35 | 40–46 | 151–125 |
| Good (2) | 14–16 | 36–40 | 47–53 | 124–103 |
| Fair (3) | 11–13 | 41–42 | 54–60 | 102–87 |
| Poor (4) | 8–10 | 43–45 | 61–65 | 86–75 |
| Reject (5) | <8 | >45 | >65 | <75 |

*Note.* [a] – standard assigned by Hay Marketing Task Force of the American Forage and Grassland Council; CP – crude protein, DM – dry matter; RFV – relative feed value, reference hay of 100 RFV contains 41% ADF (acid detergent fibre) and 53% NDF (neutral detergent fibre).

All collected data were processed by the analysis of variance (*ANOVA*) applying the least significant differences (LSD) test using the general linear model (GLM) procedure of *SAS STAT 9.1.3.* software (2002–2003). Spearman correlation coefficients were calculated to determine the relationships between the studied traits using the *SPEARMAN* option with *PROC CORR* of *SAS.*

## Results and discussion

Analysis of variance for the observed morpho-agronomic and forage quality traits showed significant differences between breeding populations/cultivars of red clover for all properties, except for the contents of neutral and acid detergent fibre in dry matter (Tables 3 and 4; Fig.). Croatian breeding population RCOP-3 had the highest overall yields of green mass and dry matter (111.82 and 25.64 t $ha^{-1}$), which was not significantly higher in comparison to the yields of populations OPCD-2 (98.55 and 24.41 t $ha^{-1}$), RCOP-1 (98.50 and 22.61 t $ha^{-1}$) and RCOP-8 for dry matter yield (21.18 t $ha^{-1}$) and the Danish cultivar 'Amos' for green mass yield (94.90 t $ha^{-1}$) and the Australian cultivar 'Astred' for dry matter yield (20.86 t $ha^{-1}$). Lithuanian cultivar 'Kamaniai' and Swedish cultivar 'Pallas' had the lowest yields in this research. Average yield of all breeding populations/cultivars was 84.26 t $ha^{-1}$ for GMY and 19.29 t $ha^{-1}$ for DMY (Table 3). Most Croatian breeding population achieved higher yields, up to 30.90% for GMY in RCOP-3 population, compared to an average yield of all populations/cultivars. Foreign cultivars in most cases achieved lower yields compared to the national populations, which may be due to low genetic potential for yield and/or lower persistence and adaptability of these cultivars to the agroecological conditions of the testing area. The result obtained in this study is consistent with research of Vasiljević et al. (2010) who examined the potential fertility of domestic and foreign varieties of red clover and confirmed the value of the local materials, and given a two-year total yields (from 116.1 to 120.1 t $ha^{-1}$ for GMY and from 24.9 to 26.3 t $ha^{-1}$ for DMY) were similar to those of local populations in this study. Research of the number of authors have confirmed the value of adapted local populations of red clover by the fact that they can produce increased forage yield in comparison to the materials from other geographical areas, and their importance was emphasized as potential source of traits that can be used for breeding purposes, such as tolerance to various abiotic stresses (Herrmann et al., 2003; Kölliker et al., 2003; Boller et al., 2004; Dujmović-Purgar et al., 2009). Plant height is one of the most important yield components and is often used in breeding as a criterion in the selection of superior genotypes to increase yields. The average height of plants of all populations/cultivars was 64.79 cm (Table 3).

The highest plant height (70.80 cm) was determined for the population RCOP-1, while the lowest value of this trait was recorded for the cultivar 'Kamaniai' (50.80 cm). Plant height in the population RCOP-1 was not statistically significantly greater than that of plants in many of the observed breeding populations and foreign cultivars (RCOP-3, OPCD-2, RCOP-8,6,5, 'Amos', 'Liepsna', RCOP-4, RCOP-7, 'Nosovskij 5', 'Milvus').

Variations in plant height between the studied populations/cultivars of red clover in this study are consistent with those obtained by Asci (2011) and higher values of plant height have been reported by Bukvić et al. (2008), Vilčinskas and Dabkevičienė (2009) and Pagnotta et al. (2011), which may be explained by the

***Table 3.*** Total yields of green mass (GMY) and dry matter (DMY), average values for plant height (PH) of the tested red clover populations/cultivars during the two experimental years (2008 and 2009)

| Populations/ cultivars | Ploidy | Country of origin | GMY t ha$^{-1}$ | DMY t ha$^{-1}$ | PH cm |
|---|---|---|---|---|---|
| OPCD-2 | 2× | Croatia | 98.55$^{AB}$ | 24.41$^{AB}$ | 68.60$^{AB}$ |
| RCOP-1 | 2× | Croatia | 98.50$^{AB}$ | 22.61$^{ABC}$ | 70.80$^{A}$ |
| RCOP-3 | 2× | Croatia | 111.82$^{A}$ | 25.64$^{A}$ | 70.20$^{A}$ |
| RCOP-4 | 2× | Croatia | 82.96$^{B-F}$ | 20.20$^{BCD}$ | 67.74$^{AB}$ |
| RCOP-5 | 2× | Croatia | 76.87$^{C-F}$ | 17.84$^{CDEF}$ | 68.20$^{AB}$ |
| RCOP-6 | 2× | Croatia | 86.13$^{BCDE}$ | 20.30$^{BCD}$ | 68.33$^{AB}$ |
| RCOP-7 | 2× | Croatia | 88.07$^{BCDE}$ | 20.22$^{BCD}$ | 67.20$^{ABC}$ |
| RCOP-8 | 2× | Croatia | 90.62$^{BCD}$ | 21.18$^{ABCD}$ | 68.40$^{AB}$ |
| 'Liepsna' | 2× | Lithuania | 78.56$^{C-F}$ | 18.35$^{CDEF}$ | 67.73$^{AB}$ |
| 'Kamaniai' | 2× | Lithuania | 65.69$^{F}$ | 13.61$^{F}$ | 50.80$^{E}$ |
| 'Nosovskij 5' | 2× | Russia | 78.18$^{C-F}$ | 17.33$^{DEF}$ | 63.60$^{ABC}$ |
| 'Pallas' | 2× | Sweden | 69.45$^{EF}$ | 14.32$^{EF}$ | 53.40$^{DE}$ |
| 'Astred' | 2× | Australia | 82.82$^{B-F}$ | 20.86$^{ABCD}$ | 59.13$^{CD}$ |
| 'Redquin' | 2× | Australia | 71.49$^{D-F}$ | 16.42$^{DEF}$ | 61.20$^{BCD}$ |
| 'Milvus' | 2× | Germany | 73.60$^{D-F}$ | 16.45$^{DEF}$ | 63.40$^{ABC}$ |
| 'Amos' | 4× | Denmark | 94.90$^{ABC}$ | 18.84$^{CDE}$ | 68.00$^{AB}$ |
| Average | | | 84.26 | 19.29 | 64.79 |
| CV % | | | 10.37 | 11.05 | 5.57 |
| $LSD_{0.05}$ | | | 14.57 | 3.55 | 6.02 |
| $LSD_{0.01}$ | | | 19.63 | 4.78 | 8.11 |

*Note.* Values followed by the same letter are not significantly different at the $P \leq 0.05$.

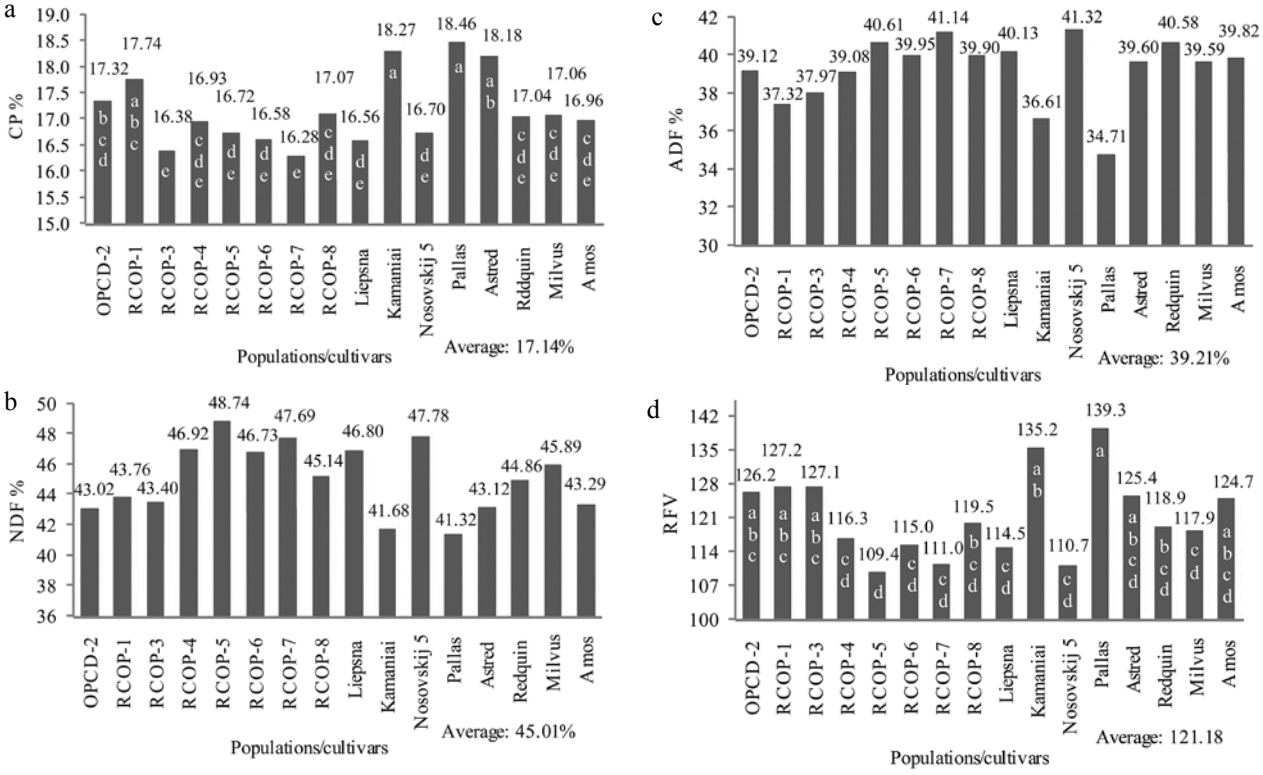
genetic diversity of materials, but differences may be also the result of other factors such as time of mowing, planting density and spacing, ploidy, the reaction of the studied materials to climate and soil conditions of the experimental area, etc. High values of plant height and yield of Danish cultivar 'Amos' were probably related to its ploidy level because compared to diploid, tetraploid cultivars are more robust, have thicker stems, larger leaves and therefore produce higher yields. The number of internodes per stem ranged from 4.80 (cvs 'Redquin' and 'Kamaniai') to 6.00 (population RCOP-8), and the average value for all populations/cultivars was 5.26 stem$^{-1}$ NI (Table 4). Most Croatian breeding populations had a greater number of internodes per stem, which thus were shorter, compared to the other studied cultivars. This result is extremely important because of the possibility of improving the quality of roughage in our breeding program, because numerous studies on various forage crops confirmed that the morphological modifications of the stem (a larger number of shorter internodes) are associated with increasing leaf mass and resistance to lodging that can indirectly affect forage quality. Variations in the number of internodes per stem between the studied red clover populations/cultivars identified in this study are similar to those obtained by Vasiljević et al. (2000) and Muntean (2006). The largest stem hairiness was recorded in the Australian cultivar 'Redquin', while the expression of this trait was the weakest in the population RCOP-5. Increased stem hairiness, especially of foreign varieties, in addition to genetic differences between the studied materials can be associated with their reaction to stressful conditions due to extreme drought during the growing period of red clover in the second year of this research. The role of hair on the leaf and/or stem in protecting plants from stressful growth conditions and attacks of various pests has been confirmed in numerous studies involving various plant species. Danish cultivar 'Amos' had the biggest leaf (the largest width and length of central leaflet, 2.43 and 4.58 cm), very round, which was expected because it is a tetraploid cultivar and it agreed with the results of Jake ova et al. (2011). Most Croatian breeding populations had medium long to long and medium broad to broad central leaflet of the trifoliate leaf shape with highly elongated leaves in populations RCOP-3,5,8 and round shape leaves in the populations RCOP-6 and OPCD-2 (Table 4).

The best quality properties in this study were observed in cvs 'Kamaniai', 'Pallas' and 'Astred' (Table 4 and Fig.). This means that these cultivars had good leaf to stem ratio (the best in the cv. 'Kamaniai' 1.025), a high protein content (the highest in the cultivar 'Pallas' 18.46%) and low content of neutral and acid detergent fibre (the lowest in the cultivar 'Pallas' 41.32% NDF and 34.71% ADF). In accordance with the presented results, it was expected that this cultivar had high relative feed value (cv. 'Pallas' had the highest 139.3), which by the legume, grass and legume-grass mixture quality standards classified it as high-quality cultivar (RFV – 125–151) (Table 2). These high quality cultivars had the lowest forage yield, which confirms the existence of negative correlations between the yield and quality, and indicates the complexity of breeding on quality traits. The obtained result is consistent with that of the authors who studied the correlation between yield and yield components and quality traits in red clover (Drobna, 2009; Vilčinskas,

***Table 4.*** Average values for number of internodes (NI), density of hairs (DH), width and length of central leaflet (WCL and LCL), shape of medial leaflet (SML) and leaf to stem ratio (LSR) in the second cut of the second experimental year

| Populations/ cultivars | NI stem$^{-1}$ | DH (1 – very low, 5 – high) | WCL cm | LCL cm | SML (1 – elongated, 3 – rounded) | LSR |
|---|---|---|---|---|---|---|
| OPCD-2 | 5.46$^{ABC}$ | 3.40$^{ABC}$ | 2.10$^{ABC}$ | 4.30$^{ABC}$ | 1.80$^{BC}$ | 0.835$^{C-F}$ |
| RCOP-1 | 5.53$^{AB}$ | 3.40$^{ABC}$ | 1.91$^{BC}$ | 4.12$^{ABCD}$ | 1.53$^{BCD}$ | 0.839$^{C-F}$ |
| RCOP-3 | 5.53$^{AB}$ | 3.13$^{BCD}$ | 1.98$^{ABC}$ | 4.32$^{AB}$ | 1.46$^{BCD}$ | 0.901$^{ABCD}$ |
| RCOP-4 | 5.60$^{AB}$ | 3.26$^{ABCD}$ | 1.93$^{BC}$ | 3.94$^{BCDE}$ | 1.73$^{BC}$ | 0.872$^{B-F}$ |
| RCOP-5 | 5.27$^{BCD}$ | 1.80$^{E}$ | 1.79$^{BC}$ | 4.07$^{A-E}$ | 1.33$^{CD}$ | 0.876$^{BCDE}$ |
| RCOP-6 | 5.07$^{BCD}$ | 2.20$^{DE}$ | 2.14$^{AB}$ | 4.04$^{A-E}$ | 1.93$^{AB}$ | 0.812$^{D-F}$ |
| RCOP-7 | 5.26$^{BCD}$ | 3.00 $^{CD}$ | 1.99$^{ABC}$ | 4.06$^{A-E}$ | 1.73$^{BC}$ | 0.746$^{F}$ |
| RCOP-8 | 6.00$^{A}$ | 3.40$^{ABC}$ | 1.88$^{BC}$ | 4.19$^{ABCD}$ | 1.20$^{D}$ | 0.951$^{ABC}$ |
| ‘Liepsna’ | 5.06$^{BCD}$ | 3.26$^{ABCD}$ | 1.73$^{BC}$ | 3.75$^{CDE}$ | 1.53$^{BCD}$ | 0.794$^{D-F}$ |
| ‘Kamaniai’ | 4.80$^{D}$ | 2.73$^{CDE}$ | 1.68$^{C}$ | 3.56$^{E}$ | 1.60$^{BCD}$ | 1.025$^{A}$ |
| ‘Nosovskij 5’ | 5.33$^{BCD}$ | 3.40$^{ABC}$ | 1.83$^{BC}$ | 3.92$^{BCDE}$ | 1.46$^{BCD}$ | 0.896$^{BCD}$ |
| ‘Pallas’ | 5.00$^{BCD}$ | 3.00$^{CD}$ | 1.82$^{BC}$ | 3.70$^{DE}$ | 1.66$^{BCD}$ | 0.829$^{C-F}$ |
| ‘Astred’ | 4.86$^{CD}$ | 4.20$^{AB}$ | 1.78$^{BC}$ | 3.53$^{E}$ | 1.73$^{BC}$ | 0.977$^{AB}$ |
| ‘Redquin’ | 4.80$^{D}$ | 4.33$^{A}$ | 1.84$^{BC}$ | 3.72$^{DE}$ | 1.66$^{BCD}$ | 0.990$^{AB}$ |
| ‘Milvus’ | 5.20$^{BCD}$ | 3.26$^{ABCD}$ | 1.90$^{BC}$ | 4.05$^{A-E}$ | 1.53$^{BCD}$ | 0.870$^{B-F}$ |
| ‘Amos’ | 5.33$^{BCD}$ | 3.53$^{ABC}$ | 2.43$^{A}$ | 4.58$^{A}$ | 2.33$^{A}$ | 0.760$^{EF}$ |
| Average | 5.26 | 3.20 | 1.92 | 3.99 | 1.64 | 0.873 |
| CV % | 7.24 | 22.12 | 10.42 | 8.22 | 13.04 | 6.50 |
| $LSD_{0.05}$ | 0.63 | 1.18 | 0.33 | 0.54 | 0.35 | 0.094 |
| $LSD_{0.01}$ | | | 0.45 | | 0.48 | 0.127 |

*Note.* Values followed by the same letter are not significantly different at the $P \leq 0.05$.




CP – crude protein, NDF – neutral detergent fibre, ADF – acid detergent fibre, RFV – relative feed value

***Figure.*** Quality properties of investigated red clover populations/cultivars, values followed by the same letter are not significantly different at the $P \leq 0.05$

Dabkevičienė, 2009; Lugić et al., 2010). In the Croatian material the best values of quality traits were recorded in breeding populations RCOP-1, 3 and OPCD-2, classified as high-quality cultivars, based on RFV.

In plant breeding, it is important to know the correlations between the various traits, because intense selection on one trait can affect other traits in both the desired (by improving the properties of interest) and in the undesired direction. Knowledge about the strength and direction of the association between the traits is particularly important for the traits of low genetic variability, where the genetic progress can be achieved by indirect selection. Table 5 shows the linear correlation by Spearman coefficient and the level of significance for the analysed traits.

***Table 5.*** Spearman correlation coefficients between 13 morpho-agronomic and forage quality traits of red clover populations/cultivars

| | GMY | DMY | PH | NI | DH | WCL | LCL | SML | LSR | CP | NDF | ADF | RFV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | – | 0.93** | 0.85** | 0.74** | 0.23 | 0.72** | 0.79** | 0.16 | −0.09 | −0.31 | −0.03 | −0.05 | 0.10 |
| 2 | | – | 0.81** | 0.65** | 0.20 | 0.54* | 0.62** | 0.07 | 0.09 | −0.22 | −0.03 | −0.08 | 0.12 |
| 3 | | | – | 0.79** | 0.02 | 0.60** | 0.84** | −0.13 | 0.01 | −0.34 | 0.18 | 0.02 | −0.04 |
| 4 | | | | – | 0.10 | 0.52* | 0.77** | −0.25 | 0.02 | −0.23 | 0.26 | −0.06 | −0.07 |
| 5 | | | | | – | 0.08 | 0.04 | 0.14 | 0.41 | 0.27 | −0.24 | 0.09 | 0.17 |
| 6 | | | | | | – | 0.72** | 0.51* | −0.49 | −0.35 | 0.04 | 0.02 | −0.03 |
| 7 | | | | | | | – | −0.06 | −0.23 | −0.33 | 0.10 | 0.03 | −0.03 |
| 8 | | | | | | | | – | −0.53* | 0.07 | −0.32 | −0.11 | 0.14 |
| 9 | | | | | | | | | – | −0.09 | 0.20 | 0.24 | −0.19 |
| 10 | | | | | | | | | | – | −0.73** | −0.64** | 0.72** |
| 11 | | | | | | | | | | | – | 0.76** | −0.94** |
| 12 | | | | | | | | | | | | – | −0.87** |
| 13 | | | | | | | | | | | | | – |

*Notes.* For explanations of abbreviations see Tables 3 and 4 and Figure. * – significant at the 0.05 probability level, ** – significant at the 0.01 probability level.

Positive correlations were found between the yield of green mass and dry matter yield (0.93**), yield of green mass and dry matter with the plant height (0.85**, 0.81**), number of internodes (0.74**, 0.65**) and width and length of central leaflet (0.72**, 0.54*, 0.79**, 0.62**). Plant height was in a significant positive correlation with the number of internodes (0.79**) and with the width and length of central leaflet (0.60**, 0.84**). Positive correlations were found between width and length of central leaflet (0.72**), width of central leaflet and shape of medial leaflet (0.51*) and between the number of internodes and width and length of central leaflet (0.52*, 0.77**). Most forage quality traits were negatively correlated to other observed morpho-agronomic traits. The protein content was significantly negatively correlated with the content of neutral and acid detergent fibre (−0.73**, −0.64**) and positively associated with a relative feed value (0.72**), relative feed value was in a negative correlation with related content of neutral and acid detergent fibre (−0.94**, −0.87**), while the content of neutral and acid detergent fibre was in positive correlation (0.76**).

## Conclusions

1. Significant differences were found among the breeding populations/cultivars of red clover in all the morpho-agronomic and forage quality characteristics except for the contents of neutral and acid detergent fibre in dry matter.

2. Croatian breeding populations RCOP-3, OPCD-2 and RCOP-1 had significantly higher yields of green mass and dry matter yields compared to the other studied populations and foreign cultivars of red clover, which indicates their high genetic potential for yield.

3. Besides the high forage yield, those populations exhibited favourable values of quality and other analyzed morphological traits, and they are representing valuable germplasm for further selection work and improvement of our breeding program and/or application for plant cultivar registration.

4. Positive correlations were found between forage yields and plant height, number of internodes and length and width of central leaflet, and these properties were in positive correlation with each other. Most quality traits were in negative correlation with yield-related and other observed morpho-agronomic traits.

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# Raudonojo dobilo (*Trifolium pratense* L.) selekcinių populiacijų ir veislių derlingumas, pašaro kokybės bei morfologinių požymių įvairavimas

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## Santrauka

Tyrimo tikslai: 1) įvertinti naujų skirtingos kilmės raudonojo dobilo (*Trifolium pratense* L.) selekcinių populiacijų derlingumo, pašaro kokybės ir morfologinių požymių įvairavimą, 2) palyginti jų ir užsienietiškų veislių derliaus potencialą, 3) nustatyti tirtų požymių tarpusavio ryšius ir 4) atrinkti perspektyviausias populiacijas tolesniam selekciniam darbui. Raudonojo dobilo aštuonios skirtingos kilmės populiacijos (OPCD-2, RCOP-1,3,4,5,6,7,8, išvestos Osijek žemės ūkio institute Kroatijoje vykdant pašarinių augalų selekcinę programą) ir aštuonios užsienietiškos veislės (‘Liepsna’, ‘Kamaniai’, ‘Nosovskij 5’, ‘Pallas’, ‘Astred’, ‘Redquin’, ‘Milvus’, ‘Amos’) vertintos pagal trylika morfologinių, agronominių požymių ir pašaro kokybės savybių. Bandymų laukeliai buvo išdėstyti atsitiktine tvarka trimis pakartojimais, bandymas vykdytas dvejus (2008 ir 2009) metus Osijek žemės ūkio institute Kroatijoje. Tirtos raudonojo dobilo selekcinės populiacijos ir veislės smarkiai skyrėsi žalios masės ir sausųjų medžiagų derliumi, augalų aukščiu, tarpubamblių skaičiumi, plaukelių tankiu, centrinio lapelio pločiu bei ilgiu, vidurinio lapelio forma, lapo ir stiebo santykiu, žalių baltymų kiekiu ir santykine pašarine verte. Palyginus su kitomis raudonojo dobilo populiacijomis ir užsienietiškomis veislėmis, Kroatijoje išvestos populiacijos RCOP-3, OPCD-2 bei RCOP-1 išaugino gerokai didesnį bendrą žalios masės (nuo 98,50 iki 111,82 t $ha^{-1}$) ir sausųjų medžiagų (nuo 22,61 iki 25,64 t $ha^{-1}$) derlių, o tai patvirtina jų didelį genetinį derliaus potencialą. Šios populiacijos ne tik davė didelį žolės derlių, bet ir atskleidė gerus morfologinius požymius, todėl yra vertinga pradinė selekcinė medžiaga, tinkama naujoms veislėms registruoti. Nustatytos teigiamos koreliacijos tarp žolės derliaus ir augalų aukščio, tarpubamblių skaičiaus ir centrinio lapelio pločio bei ilgio; šie požymiai teigiamai koreliavo vienas su kitu. Dauguma kokybės savybių neigiamai koreliavo su derliumi ir kitais tyrinėtais morfologiniais bei agronominiais požymiais.

Reikšminiai žodžiai: derliaus potencialas, kitimas, koreliacija, morfologiniai požymiai, pašaro kokybė, *Trifolium pratense*.