

Evaluation of a diverse collection of red clover for forage quality and antioxidant activity

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Description of the subject. Red clover is an important forage legume and a rich source of high quality forage for livestock feed. This study assesses of a diverse red clover collection for agronomic value, forage quality and antioxidant activity in relation to status (cultivar vs natural population) and ploidy level (diploid or tetraploid) for the purpose of diversity study and for identification of potential heterotic groups and classification of accessions according to the results of analyses.

Objectives. The aims of this research were to: i) explore agronomic traits, forage quality, and antioxidant activity in relation to status and ploidy level; ii) assess trait associations and the possibility of indirect selection; iii) cluster red clover accessions with regard to forage quality and antioxidant activity.

Method. Red clover was represented by 46 accessions, the cultivars and natural populations of diploid (2n) and tetraploid (4n) ploidy levels from 17 countries, which were collected and preserved in the Institute of Field and Vegetable Crops in Novi Sad, Serbia. The following traits were determined from the two-year field trial at Rimski Šančevi, Serbia: plant height (PH), internodes number (IN), green mass yield (GMY), dry matter yield (DMY), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV) and antioxidant activity. All accessions were characterized in the second cut of the second year of life when 20-25% of flowers appeared.

Results. The cultivars had higher values for PH, IN, GMY, DMY, DDM, DMI, and RFV. The tetraploid accessions had higher values for IN, GMY, DMY, CP, NDF and DDM. The natural populations and diploid accessions had 39.9% and 21.9% smaller antioxidant capacity, respectively. The antioxidant capacity was positively associated with RFV, DDM, DMI, PH, IN, GMY and DMY, but negatively with ADF and NDF.

Conclusions. The grouping of red clover accessions based on forage quality parameters and antioxidant activity was represented by five clusters. High-quality cultivars had a shorter length of internodes and a good leaf to stem ratio with a high leaf proportion.

Keywords. *Trifolium pratense* L., nutritive value, antioxidant properties, agricultural productivity, breeding programmes, diploidy, polyploidy.

Évaluation d'une collection diversifiée de trèfle rouge pour la qualité du fourrage et l'activité antioxydante

Description du sujet. Le trèfle violet est une légumineuse fourragère importante et une riche source de fourrage de haute qualité pour l'alimentation du bétail. Cette étude évalue une collection diversifiée de trèfle violet pour la valeur agronomique, la qualité du fourrage et l'activité antioxydante en relation avec le statut (cultivar vs population naturelle) et le niveau de ploïdie (diploïde ou tétraploïde) aux fins d'étude de diversité et d'identification de groupes hétérotiques potentiels et de classification des accessions en fonction des résultats des analyses.

Objectifs. Les buts de cette recherche étaient : i) d'explorer les caractéristiques agronomiques, la qualité du fourrage et l'activité antioxydante en relation avec le statut et le niveau de ploïdie ; ii) d'évaluer les associations de caractéristiques et la possibilité de sélection indirecte ; iii) de regrouper les accessions de trèfle violet en ce qui concerne la qualité du fourrage et l'activité antioxydante.

Méthode. Le trèfle violet était représenté par 46 accessions, les cultivars et populations naturelles de niveaux de ploïdie diploïde (2n) et tétraploïde (4n) originaires de 17 pays qui ont été collectés et conservés à l'Institut des grandes cultures et des

cultures maraîchères de Novi Sad, Serbie. Les caractéristiques suivantes ont été définies sur la base d'un essai bisannuel sur un champ dans la localité de Rimski Sancevi en Serbie : hauteur de la plante (PH), nombre d'entre-nœuds (IN), rendement en masse verte (GMY), rendement en matière sèche (DMY), protéine brute (CP), fibre détergente acide (ADF), fibre détergente neutre (NDF), matière sèche digestible (DDM), apport en matière sèche (DMI), valeur alimentaire relative (RFV) et activité antioxydante. Toutes les accessions ont été caractérisées à la deuxième coupe de la deuxième année de vie lorsque 20 à 25 % des fleurs sont apparues.

Résultats. Les cultivars avaient des valeurs plus élevées pour PH, IN, GMY, DMY, DDM, DMI et RFV. Les accessions 4n avaient des valeurs plus élevées pour IN, GMY, DMY, CP, NDF et DDM. Les populations et les accessions 2n avaient une capacité antioxydante inférieure de 39,9 % et 21,9 %, respectivement. La capacité antioxydante était positivement associée à RFV, DDM, DMI, PH, IN, GMY et DMY, mais négativement à ADF et NDF.

Conclusions. Le regroupement des accessions de trèfle violet sur la base des paramètres de qualité du fourrage et de l'activité antioxydante a été représenté par cinq groupes. Les cultivars de haute qualité avaient une longueur d'entre-nœuds plus courte et un bon rapport feuille/tige avec une proportion élevée de feuilles.

Mots-clés. *Trifolium pratense* L., valeur nutritive, propriété antioxydante, productivité agricole, programme d'amélioration, diploïdie, polyplôidie

1. INTRODUCTION

Red clover (*Trifolium pratense* L.) is grown for animal fodder via grazing, hay or silage (Watson & Stoddard, 2017), and can improve livestock performance via the superior nutritive value of grass plus clover forages versus grass alone (Ciaran & Ratnieks, 2021). The importance of red clover, like other forage legumes, is reflected in providing significant nitrogen (N) input via symbiosis through root nodule bacteria, *Rhizobium leguminosarum* cv. *trifolii*, therefore the need for N fertilizers diminishes in grassland systems containing red clover.

Red clover is naturally a diploid ($2n = 2x = 14$) species, with gametophytic self-incompatibility, *i.e.* it needs to be cross-fertilized before it can set seed. Modern autotetraploid red clover cultivars ($2n = 4x = 28$) have been developed from diploid genotypes through chromosome doubling (Taylor & Quesenberry, 1996).

Cultivated types of red clover have a similar level of genetic diversity as wild accessions, implying that modern red clover breeding programs did not negatively affect genetic diversity or population structure (Osterman et al., 2021). Red clover populations are heterogeneous, and showed a higher level of intra-population phenotypic and genotypic variation than inter-population variation.

The major breeding goals for red clover are forage yield, persistence, and resistance to economically important diseases, but an increase in forage quality, most importantly the crude protein content, remains an important objective of red clover breeding (Petrauskas et al., 2018). Red clover is wealthy in protein and minerals and is characterized by a high intake rate by ruminants (Frankow-Lindberg, 2017). The crude protein content in red clover amounts to about 18% and remains stable during the growing season (Vleugels et al., 2019). Taylor & Quesenberry (1996) claimed

that the content of crude protein and *in vitro* dry matter digestibility are the two most reliable quality characteristics. Their value decreases with age because of a decreasing portion of leaves in relation to stems and the process of lignification. The optimum time for red clover cutting, in terms of both, forage quality and total yields, is the stage when 20–25% of flowers are in bloom (Wiersma et al., 1998). At that stage, the protein content ranges from 16 to 18%. Other parameters, such as acid detergent fiber content, neutral detergent fiber content, digestible dry matter, dry matter intake, relative feed value and antioxidant activity, are important criteria for determining forage quality in animal feeding (Sousa et al., 2020). In recent years, the use of natural antioxidants found in red clover has attracted interest due to their presumed therapeutic value (Vlaisavljevic et al., 2014).

The aims of this research were to:

- explore a diverse red clover collection in relation to status and ploidy level for agronomic traits, forage quality parameters, and antioxidant activity;
- assess associations among studied traits and the possibility of conducting indirect selection;
- cluster red clover accessions regarding forage quality and antioxidant activity.

2. MATERIALS AND METHODS

2.1. Plant material, field trial and soil samples analysis

The collection of red clover (*Trifolium pratense* L.) germplasm for examination in this research was represented by 46 accessions. These 46 accessions were the cultivars and natural populations of diploid (2n) and tetraploid (4n) ploidy levels from 17 countries, and were collected and preserved in the Institute of Field and Vegetable Crops in Novi Sad, Serbia (Table 1).

Table 1. Accession names, origin/donors, variety type, ploidy level, agronomic traits, forage quality parameters, and antioxidant activity for all accessions studied — *Nom, origine/donneurs, statut des accessions, niveau de ploïdie, caractères agronomiques, paramètres de qualité du fourrage et activité antioxydante pour toutes les accessions évaluées.*

Accessions	Origin/ Donors	Variety type	Ploidy level	PH (cm)	IN	GMV (g-plant ⁻¹)	DMY (g-plant ⁻¹)	CP (%)	ADF (%)	NDF (%)	DDM (%)	DMI (%)	RFV (%)	DPPHIC ₅₀ (µg·ml ⁻¹)
89 E-0	Bulgaria/IPGR	natural population	2n	37.2	5.4	247.7	77.5	18.1	42.6	55.5	55.7	2.2	93.3	52.2
91 E-44	Bulgaria/IPGR	natural population	2n	30.5	4.8	114.0	35.2	16.8	38.3	45.8	59.0	2.6	119.7	45.2
91 E-63	Bulgaria/IPGR	natural population	2n	26.8	4.6	106.0	33.9	16.2	34.6	45.2	61.9	2.6	127.3	10.7
Amos	Denmark/DLF	cultivar	4n	46.5	6.2	279.0	93.4	15.4	32.4	47.0	63.7	2.6	126.1	6.4
Avala	Serbia/IFVCNS	cultivar	2n	44.4	6.2	306.0	114.2	15.2	35.0	48.1	61.6	2.5	119.1	29.4
BGR1	Romania/BRGV	natural population	2n	39.7	4.9	225.0	61.1	15.4	38.9	48.1	58.6	2.5	113.4	120.0
BGR2	Romania/BRGV	natural population	2n	45.5	4.9	225.0	60.8	14.6	39.8	53.6	57.9	2.2	99.9	103.8
BGR3	Romania/BRGV	natural population	2n	40.5	4.7	220.0	57.2	17.9	36.6	45.6	60.4	2.6	123.1	12.4
Bjorn	Sweden/IPK	cultivar	2n	37.1	6.0	98.3	42.7	15.5	39.4	49.0	58.2	2.4	110.6	11.4
Bolognino	Italy/CREA	natural population	2n	37.7	6.0	174.3	60.8	17.2	28.9	43.1	66.4	2.8	143.4	26.0
Bradlo	Slovakia/RIPP	natural population	2n	44.7	7.4	162.0	67.9	14.7	27.8	45.6	67.3	2.6	137.2	54.7
Britta	Sweden/IPK	cultivar	2n	45.7	7.3	230.7	77.5	16.4	36.9	44.1	60.1	2.7	126.8	35.4
Čortanovci	Serbia/IFVCNS	natural population	2n	38.3	4.6	111.3	39.7	16.4	34.9	48.2	61.7	2.5	118.9	29.0
Diana	Croatia/AIO	cultivar	2n	49.3	6.5	301.0	88.7	17.5	41.0	48.1	57.6	2.5	110.1	10.4
Dicar	France/INRA	cultivar	4n	44.4	5.7	298.7	84.7	14.9	41.3	50.8	56.7	2.4	103.7	13.5
Fertody	Hungary/USDA/ARS WRPIs	cultivar	2n	41.4	5.7	210.0	63.0	16.8	30.6	45.6	65.1	2.6	132.9	57.2
Italia centrale	Italy/CREA	natural population	2n	34.5	5.7	188.3	62.9	17.8	33.9	48.0	62.6	2.5	121.1	25.6
Kora	Sweden/IPK	cultivar	2n	47.4	8.7	180.0	57.6	14.4	37.5	45.9	59.7	2.6	121.1	39.4
Krano	Denmark/IPK	cultivar	2n	37.8	7.7	189.0	66.9	14.4	34.2	47.4	62.3	2.5	122.2	25.8
Lemmon	Belgium/ILVO	cultivar	2n	38.8	5.5	196.7	55.0	16.0	32.4	45.8	63.7	2.6	129.4	21.6
Lucrum	Germany/IPK	cultivar	2n	37.6	6.3	158.0	56.6	16.4	29.4	46.8	66.0	2.6	131.3	30.4
Lutea	Germany/IPK	cultivar	2n	39.5	5.9	167.7	55.6	17.2	34.2	43.4	62.3	2.8	133.5	20.5
Marina	Serbia/IFVCNS	cultivar	2n	43.7	6.1	274.0	101.4	17.1	28.5	46.0	66.7	2.6	134.8	9.6
Marino	Germany/IPK	cultivar	2n	40.9	6.0	197.0	61.9	15.0	34.9	49.2	61.7	2.4	112.9	8.3
Mercury	Belgium/ILVO	cultivar	2n	39.9	5.9	195.3	67.2	13.7	38.9	52.5	58.6	2.3	103.8	50.9
NCPGRU2	Ukraine/ NCPGRU	natural population	2n	43.5	5.8	178.3	49.9	15.1	37.2	53.4	59.9	2.2	104.2	22.9

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Table 1 (continued). Accession names, origin/donors, variety type, ploidy level, agronomic traits, forage quality parameters, and antioxidant activity for all accessions studied — *Nom, origine/donneurs, statut des accessions, niveau de ploïdité, caractères agronomiques, paramètres de qualité du fourrage et activité antioxydante pour toutes les accessions évaluées.*

Accessions	Origin/ Donors	Variety type	Ploidy level	PH (cm)	IN	GMY (g·plant ⁻¹)	DMY (g·plant ⁻¹)	CP (%)	ADF (%)	NDF (%)	DDM (%)	DMI (%)	RFV (%)	DPPH IC ₅₀ (µg·ml ⁻¹)
NCPGRU3	Ukraine/NCPGRU	natural population	2n	41.7	6.0	165.0	43.0	16.1	37.9	45.3	59.4	2.6	122.0	23.4
NCPGRU4	Ukraine/NCPGRU	natural population	2n	43.1	5.9	253.3	68.4	16.3	30.2	45.2	65.3	2.6	131.7	90.2
NCPGRU5	Ukraine/NCPGRU	natural population	2n	45.2	5.5	210.7	44.3	16.2	35.6	47.6	61.2	2.5	119.7	45.5
Nemaro	Germany/SZS	cultivar	4n	36.2	5.1	176.0	54.8	16.4	33.7	47.8	62.6	2.5	121.9	12.0
Nessonas	Greece/NAGREF	cultivar	2n	42.4	6.3	188.7	62.5	17.0	32.2	46.3	63.9	2.6	128.3	50.6
Noe	France/IPK	cultivar	2n	39	5.9	169.7	54.4	15.7	33.8	53.7	62.6	2.2	108.5	24.8
NS-Mlava	Serbia/IFVCNS	cultivar	2n	50.2	6.3	348.0	69.0	15.7	41.5	46.7	56.6	2.6	112.8	20.5
Quinekeli	Chile/USDA/ARS WRPIS	cultivar	2n	45.8	6.9	132.0	43.6	17.3	36.4	44.0	60.5	2.7	127.8	17.3
Renova	Switzerland/IPK	cultivar	2n	38.4	5.6	143.7	48.6	16.4	35.3	49.9	61.4	2.4	114.5	15.2
Rotra	Belgium/IPK	cultivar	4n	40.6	5.9	141.0	49.3	17.7	33.4	47.6	62.9	2.5	122.9	28.5
SA1	Australia/AMGRC	natural population	2n	41.5	4.6	205.7	67.8	17.3	35.1	48.4	61.6	2.5	118.5	53.6
SA3	Australia/AMGRC	natural population	2n	28.1	3.3	75.3	23.3	16.8	42.0	51.1	56.2	2.4	102.3	26.8
SA4	Australia/AMGRC	natural population	2n	29.4	3.7	107.0	23.8	16.9	37.6	47.3	59.6	2.5	117.1	67.1
Sofia52	Bulgaria/IPGR	natural population	2n	31.9	4.8	164.3	54.1	16.6	42.6	47.8	55.7	2.5	108.5	28.6
Titus	Germany/SZS	cultivar	4n	40.1	6.1	163.7	51.4	17.4	41.2	50.2	56.8	2.4	105.3	16.1
Triton	Germany/IPK	cultivar	4n	28.9	6.7	230.0	59.8	19.2	31.2	44.7	64.6	2.7	134.4	22.6
Una	Serbia/IFVCNS	cultivar	2n	47.8	6.4	347.0	130.8	15.7	35.6	45.6	61.1	2.6	124.6	10.3
Violeta	Bolivia/UMSS, FCA	cultivar	2n	42.4	5.5	208.7	68.1	16.5	35.9	45.2	60.9	2.7	125.4	83.6
Violetta	Belgium/IPK	cultivar	2n	40.1	5.9	203.7	72.5	15.2	35.0	43.6	61.6	2.8	131.4	23.6
Vivi	Sweden/NGB	cultivar	4n	36.7	6.0	132.0	49.0	13.7	30.9	48.2	64.8	2.5	125.1	101.9
Mean	-	-	-	40.1	5.8	195.5	61.6	16.2	35.6	47.6	60.1	2.5	120.1	35.5
Min	-	-	-	26.8	3.3	75.3	23.3	13.7	27.8	43.1	56.8	2.2	93.3	6.4
Max	-	-	-	50.2	8.7	348.0	130.8	19.2	42.6	55.5	67.3	2.8	143.4	120.0
SD	-	-	-	5.6	1.0	63.3	20.9	1.2	3.9	2.9	3.04	0.1	11.1	27.5
CV (%)	-	-	-	13.9	16.6	32.4	33.9	7.3	11.0	6.1	5.0	5.9	9.2	77.3

NCPGRU: National Center for Plant Genetic Resources of Ukraine; UMSS, FCA: Universidad Mayor de san Simon, Facultad de Ciencias Agrícolas, Bolivia; NAGREF: National Agricultural Research Foundation, Greece; ILVO: Institute for agricultural, fisheries and food research, Belgium; AMGRC: Australian Medicago Genetic Resources Centre; BRGV: Banca de Resurse Genetice Vegetale Suceava, Romania; AIO: Agricultural Institute Osijek, Croatia; SZS: Saatucht Steinach GmbH, Germany; IFVCNS: Institute of Field and Vegetable Crops Novi Sad, Serbia; DLF: seed company, Denmark; CREA: Council for Agricultural Research and Economics, Italy; IPK: Institut für Pflanzen-genetik und Kulturpflanzenforschung, Gatersleben, Germany; NGB: Nordic Gene Bank, Sweden; RIPP: Research Institute of Plant Production, Piešťany, Slovakia; IPGR: Institute of Plant Genetic Resources, Sadovo, Bulgaria; WRPIS: Western Regional Plant Introduction Station, Washington, United States.

The selected plant material was studied based on the field trial sown at the beginning of April during the 2011-2012 vegetation season at the location Rimski Šančevi, Novi Sad, Serbia. The experiment was laid out in a randomized block design with three replications. Each replication consisted of 46 elementary plots of 8 m² area with row spacing measuring 80 × 80 cm with row depth of 2.5 cm, and the distances between blocks, 1 m. No chemical fertilizers, pesticides, nor irrigation were applied at the trial site. Hand weeding was carried out when necessary during the course of the experiment. For the analysis of quality parameters, plant samples were obtained from the second cut of the second growing season at the beginning of July when approximately 25% of flowers appeared (7 days after three heads of a plant had begun to flower). Each red clover accession was represented by a sample consisting of 10 plants per replication.

The soil at Rimski Šančevi is the chernozem type with favorable pedological characteristics. **Table 2** presents the agrochemical properties of the soil at the site Rimski Šančevi, based on the data from the Laboratory for Soil and Agroecology of the Institute of Field and Vegetable Crops in Novi Sad. The total content of nitrogen was determined according to AOAC 972.43 (AOAC, 2006) by a CHNS analyzer. Assessment of easily accessible P₂O₅ in the soil was performed with the AL method DM 8/1-3-020 (JDPZ, 1966). The K₂O content was specified according to AL method DM 8/1-3-090 (JDPZ, 1966). Volumetric determination of free calcium carbonate (CaCO₃) was performed according to the DM 8/1-3-016 method (Hadzic et al., 2004). The organic matter content was estimated by the method of Tjurin, DM 8/1-3-017 (JDPZ, 1966). The soil samples were slightly alkaline, with a medium content of CaCO₃, poor in organic matter, rich in total nitrogen, with high content of easily accessible phosphorus and potassium, which are of particular importance for red clover.

2.2. Analysis of agronomic traits, forage quality parameters and antioxidant activity of red clover accessions

The evaluation of the investigated agronomic traits (plant height-PH, internodes number per stem-IN, green mass yield-GMY, dry matter yield-DMY) was performed on plant material from the second cut of

the second year of red clover life when about 25% of the flowers appeared (early July). Each accession was analyzed for agronomic traits on 30 individual representative plants. Dry matter yield was determined after drying the plant samples at 105 °C until the samples were completely dry.

For the analysis of quality parameters, plant samples were obtained from the second cut of the second growing season, when approximately 25% of the flowers appeared (7 days after three heads of a plant had begun to flower). For each of the examined red clover accessions, three average bulk green mass samples were taken and dried at 60 °C for 48 h. Each bulk sample contained 10 single plants. The official method that was used for the evaluation of crude protein content in red clover accessions was that of the AOAC (1990). Determination of acid detergent fiber (ADF) content in plant material was performed according to Van Soest (1963). Determination of the content of neutral detergent fibers (NDF) in plant material was accomplished using the Van Soest & Wine (1967) method. Relative feed value (RFV) was calculated from the estimates of digestibility of dry matter (DDM) and dry matter intake (DMI) according to the following equations adapted from common formulas for forages (Schroeder, 1994):

$$RFV = \frac{(\%DDM \times \%DMI)}{1.29}$$

$$DMI = \frac{120}{\%NDF}$$

$$DDM = 88.9 - (0.779 \times \%ADF)$$

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., 1978) and presented as the quality standards of legume, grass and legume-grass mixtures in **supplementary material**.

The antioxidant activity of red clover was determined by using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay by a method with suitable modifications upon Kumarasamy et al. (2007). The final results are presented as IC₅₀ values, *i.e.* the concentration of the studied extracts that causes a

Table 2. The agro-chemical soil analysis of the Rimski Šančevi location — *L'analyse agrochimique du sol du site de Rimski Šančevi*.

Depth (cm)	pH (H ₂ O)	pH (KCl)	N (%)	P ₂ O ₅ (mg·100 g of soil ⁻¹)	K ₂ O (mg·100 g of soil ⁻¹)	CaCO ₃ (%)	Organic matter (%)
30	8.25	7.28	0.19	32.4	30.6	2.52	2.58

decrease in the initial DPPH concentration by 50%. The synthetic antioxidants used as reference standards were butylated hydroxytoluene-BHT ($IC_{50} = 11.3 \mu\text{g}\cdot\text{ml}^{-1}$) and butylated hydroxyanisole-BHA ($IC_{50} = 10.9 \mu\text{g}\cdot\text{ml}^{-1}$). The percentage inhibition was calculated using the equation:

$$\text{Inhibition \%} = \frac{(\text{A of control} - \text{A of sample})}{\text{A of control}} 100.$$

where A is the antioxidant activity.

The principal component analysis (PCA) was performed using the R program (R Core Team, 2017).

3. RESULTS

The parameters of descriptive statistics are shown for agronomic traits, forage quality parameters, and antioxidant activity across all investigated accessions of red clover in **table 1** and across cultivars, natural populations, accessions with diploidy, and accessions with tetraploidy in **table 3**.

3.1. Agronomic traits of red clover accessions

The plant height of red clover accessions ranged from 26.8 cm in the 91 E-63 natural population from Bulgaria to 50.2 cm in the cultivar NS-Mlava from Serbia, with a mean value of 40.1 cm, and CV of 13.9%. The mean value and CV for PH were 41.5 cm and 11.2% for cultivars, 37.8 cm and 16.3% for natural populations, 40.2 cm and 13.8% for diploid accessions, and 39.1 cm and 15.0% for tetraploid accessions. The internode number per stem for red clover accessions ranged from 3.3 (natural population SA3 from Australia) to 8.7 (cultivar Kora from Sweden), and the mean value and CV for all analyzed accessions of red clover were 5.8 and 16.6%, respectively. The mean value and CV for IN were 6.2 and 11.7% for cultivars, 5.2 and 18.0% for natural populations, 5.8 and 17.8% for diploid accessions, and 6.0 and 8.2% for tetraploid accessions. The two cultivars NS-Mlava and Una from Serbia achieved maximum values for green mass yield (348 and 347 $\text{g}\cdot\text{plant}^{-1}$, respectively), whereas the SA3 natural population from Australia had a minimum value of 75.3 $\text{g}\cdot\text{plant}^{-1}$, and the mean value and CV across all investigated accessions of red clover were 195.5 $\text{g}\cdot\text{plant}^{-1}$ and 32.4%, respectively. The mean value and CV for GMY were 209.5 $\text{g}\cdot\text{plant}^{-1}$ and 31.6% for cultivars, 174.1 $\text{g}\cdot\text{plant}^{-1}$ and 30.6% for natural populations, 194.3 $\text{g}\cdot\text{plant}^{-1}$ and 32.7% for diploid accessions, and 202.9 $\text{g}\cdot\text{plant}^{-1}$ and 33% for tetraploid accessions. The dry matter yield of cultivar Una was the maximum value (130.8 $\text{g}\cdot\text{plant}^{-1}$), the lowest DMY value was obtained in natural population SA3 from Australia

(23.3 $\text{g}\cdot\text{plant}^{-1}$), and the mean value and CV across all accessions of red clover were 61.6 $\text{g}\cdot\text{plant}^{-1}$ and 33.9%, respectively. The mean value and CV for DMY were 67.9 $\text{g}\cdot\text{plant}^{-1}$ and 31.5% for cultivars, 51.8 $\text{g}\cdot\text{plant}^{-1}$ and 30.8% for natural populations, 61.3 $\text{g}\cdot\text{plant}^{-1}$ and 35.0% for diploid accessions, and 63.2 $\text{g}\cdot\text{plant}^{-1}$ and 28.8% for tetraploid accessions. Cultivars had higher levels of PH, IN, GMY, and DMY in comparison to natural populations, whereas tetraploid accessions showed medium values for all examined agronomic traits in comparison to diploid accessions.

3.2. Forage quality parameters of red clover accessions

The lowest value of crude protein content was measured for the diploid cultivar Mercury from Belgium (13.7%), the highest value was obtained for the tetraploid cultivar Triton from Germany (19.2%), and the mean value for CP for all analyzed accessions with CV was 16.2% and 7.3%, respectively. The mean value and CV for CP were 16.1% and 7.9% for cultivars, 16.5% and 6.2% for natural populations, 16.2% and 6.5% for diploid accessions, and 16.4% and 11.4% for tetraploid accessions, respectively. The acid detergent fiber content ranged from 27.8% (Bradlo natural population from Slovakia) to 42.6% (89 E-0 natural population from Bulgaria), with a mean value and CV across all investigated red clover accessions of 35.6% and 11.0%, respectively. The mean value and CV for ADF were 35.1% and 10.4% for cultivars, 36.4% and 11.8% for natural populations, 35.7% and 10.8% for diploid accessions, and 34.9% and 12.9% for tetraploid accessions, respectively. The neutral detergent fiber content ranged from 43.1% (Bolognino natural population from Italy) to 55.5% (89 E-0 natural population from Bulgaria), with a mean value and CV across all investigated red clover accessions of 47.6% and 6.1%, respectively. The mean value and CV for NDF were 47.3% and 5.4% for cultivars, 48.0% and 7.0% for natural populations, 47.5% and 6.4% for diploid accessions, and 48.0% and 4.2% for tetraploid accessions, respectively. The relative feed value for red clover accessions ranged from 93.3% (89 E-0 natural population from Bulgaria) to 143.4% (Bolognino natural population from Italy) and the mean value and CV for all analysed accessions of red clover were 120.1% and 9.2%, respectively. The mean value and CV for RFV were 121.5% and 8.0% for cultivars, 117.8% and 11.0% for natural populations, 120.1% and 9.3% for diploid accessions, and 119.9% and 9.4% for tetraploid accessions, respectively. The best quality properties in this study were observed in the tetraploid cultivar Triton (Germany), diploid cultivar Lutea (Germany), diploid cultivar Marina (Serbia), diploid

Table 3. The parameters of descriptive statistics for agronomic traits, forage quality parameters and antioxidant activity for cultivars, natural populations, for accessions of diploid and tetraploid ploidy level — *Les paramètres de statistiques descriptives pour les caractères agronomiques, les paramètres de qualité fourragère et l'activité antioxydante pour les cultivars, les populations naturelles, pour les accessions de niveau de ploïdie diploïde et tétraploïde.*

Trait	Cultivars			Natural populations			Diploid accessions			Tetraploid accessions		
	Mean ± SD	CV	n	Mean ± SD	CV	n	Mean ± SD	CV	n	Mean ± SD	CV	n
PH (cm)	41.5 ± 4.7	11.2	28	37.8 ± 6.2	16.3	18	40.2 ± 5.6	13.8	39	39.1 ± 5.8	15.0	7
IN	6.2 ± 0.7	11.7	28	5.2 ± 0.9	18.0	18	5.8 ± 1.0	17.8	39	6.0 ± 0.5	8.2	7
GMY (g·plant ⁻¹)	209.5 ± 66.2	31.6	28	174.1 ± 53.3	30.6	18	194.3 ± 63.5	32.7	39	202.9 ± 66.9	33.0	7
DMY (g·plant ⁻¹)	67.9 ± 21.4	31.5	28	51.8 ± 16.0	30.8	18	61.3 ± 21.5	35.0	39	63.2 ± 18.2	28.8	7
CP (%)	16.1 ± 1.3	7.9	28	16.5 ± 1.0	6.2	18	16.2 ± 1.1	6.5	39	16.4 ± 1.8	11.4	7
ADF (%)	35.1 ± 3.6	10.4	28	36.4 ± 4.3	11.8	18	35.7 ± 3.9	10.8	39	34.9 ± 4.5	12.8	7
NDF (%)	47.3 ± 2.6	5.4	28	48.0 ± 3.4	7.0	18	47.5 ± 3.0	6.4	39	48.0 ± 2.0	4.2	7
DDM (%)	61.6 ± 2.8	4.6	28	60.6 ± 3.4	5.6	18	61.1 ± 3.0	4.9	39	61.7 ± 3.5	5.6	7
DMI (%)	2.6 ± 0.1	5.4	28	2.5 ± 0.2	6.8	18	2.5 ± 0.2	5.9	39	2.5 ± 0.1	4.4	7
RFV (%)	121.5 ± 9.8	8.0	28	117.8 ± 12.9	11.0	18	120.1 ± 11.2	9.3	39	119.9 ± 11.3	9.4	7
DPPH IC ₅₀ (μg·ml ⁻¹)	28.5 ± 22.6	79.4	28	46.6 ± 31.2	67.1	18	36.8 ± 26.7	72.5	39	28.7 ± 33.1	115.2	7

SD: standard deviation — *écart-type*; CV: coefficient of variation — *coefficient de variation*; PH: plant height — *hauteur de la plante*; IN: internodes number — *nombre d'entre-nœuds*; GMY: green mass yield — *rendement en matière verte*; DMY: dry matter yield — *rendement en matière sèche*; CP: crude protein content — *teneur en protéines brutes*; ADF: acid detergent fiber content — *teneur en fibres détergentes neutres*; NDF: neutral detergent fiber content — *teneur en fibres détergentes neutres*; DDM: dry matter digestibility — *digestibilité de la matière sèche*; DMI: dry matter intake — *apport en matière sèche*; RFV: relative feed value — *valeur d'alimentation relative*; DPPH: antioxidant activity — *activité antioxydante*.

cultivar Nessonas (Greece) and diploid natural population Bolognino (Italy).

These red clover accessions had high relative feed value (125-151%), which by quality standards classified it as premium-quality cultivars/population (**Supplementary material**). Generally, these high-quality cultivars had a shorter length of internodes and a good leaf to stem ratio with a high leaf proportion. Cultivars had medium values for RFV and ADF in comparison to natural populations which showed extreme values (maximum and minimum), whereas tetraploid accessions had values for CP more moved toward extreme values (maximum and minimum) and medium values for other traits in comparison to diploid accessions.

3.3. Antioxidant activity of red clover accessions

Values of antioxidant activity for all red clover accessions investigated in this study ranged from 6.4 μg·ml⁻¹ to 120.0 μg·ml⁻¹, and the mean value and CV for all analyzed accessions of red clover were 35.5 μg·ml⁻¹ and 77.3%, respectively. Extracts of five red clover cultivars (Amos, Marino, Marina, Una and Diana) and one natural population 91 E-63 showed antioxidant activity greater than the capacity of synthetic antioxidants used as reference standards. A higher ability to neutralize DPPH radicals than synthetic antioxidants was obtained by cultivars Amos from Denmark (IC₅₀ = 6.4 μg·ml⁻¹), Marino from Germany (IC₅₀ = 8.3 μg·ml⁻¹), Marina from Serbia (IC₅₀ = 9.6 μg·ml⁻¹), Una from Serbia (IC₅₀ = 10.3 μg·ml⁻¹) and Diana from Croatia (IC₅₀ = 10.4 μg·ml⁻¹), and one natural population 91 E-63 from Bulgaria (IC₅₀ = 10.72 μg·ml⁻¹). The lowest ability to neutralize DPPH radicals was obtained by extracts of two natural populations BGR1 (IC₅₀ = 120.0 μg·ml⁻¹) and BGR2 (IC₅₀ = 103.8 μg·ml⁻¹) from Romania. The mean value and CV for antioxidant activity were 28.5 μg·ml⁻¹ and 79.4% for cultivars, 46.6 μg·ml⁻¹ and 67.1% for natural populations, 36.8 μg·ml⁻¹ and 72.5% for diploid accessions, and 28.7 μg·ml⁻¹ and 115.2% for tetraploid accessions. The better antioxidant activity assessed by the DPPH assay was shown for cultivars in comparison to natural populations, whereas tetraploid accessions had values

for antioxidant activity assessed by the DPPH assay that moved toward extreme values (maximum and minimum) in comparison to diploid accessions.

3.4. Analysis of the main components (PCA) of the red clover agronomic properties, forage quality parameters and antioxidant activity

Based on the tested accessions from the red clover collection for agronomic properties, forage quality parameters and antioxidant activity, the PCA biplot explained 62.7% of the total variation showing that PH, IN, GMY, and DMY were positively associated for cultivars *vs* natural populations view (**Figure 1a**), and for diploid accessions *vs* tetraploid accessions view (**Figure 1b**). RFV, DMI and DDM were positively associated for cultivars *vs* natural populations view (**Figure 1a**), and for diploid accessions *vs* tetraploid accessions view (**Figure 1b**), and also ADF and NDF. The RFV, DMI and DDM were negatively correlated with ADF and NDF and also with PH, IN, DMY (**Figures 1a** and **1b**). Additionally, for the interpretation of the graphics, it is important to note that DPPH values and antioxidant capacity of extracts from red clover genotypes are in inverse proportion. The DPPH antioxidant activity was strongly negatively associated with RFV, DDM, DMI and with PH, IN, GMY, DMY, but the antioxidant capacity of red clover accessions was positively associated with RFV, DDM, DMI, PH, IN, GMY, DMY, but negatively associated with ADF and NDF (**Figures 1a** and **1b**). Trait eigenvectors indicated that PC1 was mainly a positive indicator of RFV, ADF, and NDF. PC2 was mainly a positive indicator of GMY and DMY. The 11 populations were positioned on the PCA biplot sectors with negative PC1 values, and seven populations on the PCA biplot sectors with positive PC1 values (**Figure 1a**). Generally, cultivars exhibited better agronomic properties, RFV, DDM, DMI and higher antioxidant activity in comparison to natural populations (**Figure 1a**). The five tetraploid accessions of red clover were positioned on the PCA biplot sectors with positive PC1 values, and two tetraploid accessions (Dicar, Titus) on the PCA biplot sectors with negative PC1 values (**Figure 1b**). The Amos tetraploid cultivar had exceptional agronomic properties and antioxidant capacity, and the four tetraploid accessions had excellent RFV, DDM, DMI, and CP values (**Figure 1b**).

3.5. Grouping of the red clover accessions based on forage quality parameters and antioxidant activity, and associations among traits

Based on the tested accessions from the red clover collection for forage quality parameters and antioxidant activity, a PCA biplot with 76% of total variation

showed that RFV, DMI and DDM were positively associated (**Figure 2b**). These three traits were negatively correlated with ADF and NDF (**Figure 2b**). A medium-strong correlation existed between ADF and NDF, and between DDM and DMI (**Figure 2a**). CP and DMI were strongly negatively associated with DPPH antioxidant activity, whereas RFV showed a slightly negative association with DPPH antioxidant activity (**Figures 2a** and **2b**). The PCA biplot representing red clover accessions measured for seven traits (CP, DMI, RFV, DDM, DPPH, NDF, and ADF) displayed five groups with 45 accessions, and one accession that was separated from others (Vivi), and that could not be included in any of these five groups (**Figure 2b**). The first group is characterized by high levels of CP and high antioxidant capacity. The accessions of this group also have above-average DMI and RFV. The DDM is above average except for BGR3, 91 E-44, and NCPGRU3. Group I included BGR3, 91 E-44, NCPGRU3, Italia centrale, Rotra, Una, 91 E-63, Britta, Quinekeli, Lutea, and Triton. The second group comprised accessions that have high levels of RFV, DMI, and DDM. They show low antioxidant capacity except for Violetta and Bolognino which had above-average antioxidant capacity. Here are following accessions Violetta, Bolognino, Lemmon, Marina, Violeta, Amos, Nessonas, Fertody, Lucrum, NCPGRU4, and Bradlo. The third group encompassed accessions with low values of antioxidant capacity, CP, RFV, DMI, and DDM and with above-average negative parameters of ADF and NDF. The accessions belonging to this group are NCPGRU2, Noe, BGR1, Mercury, and BGR2. The fourth group is a group of accessions that had low values of RFV, DMI, and DDM, and above average and high values of ADF and NDF. Their antioxidant capacity is above average and good. Accessions of this group are Dicar, 89 E-0, SA3, Bjorn, NS-Mlava, Sofia 52, Titus and Diana. The fifth group is a group of accessions that had mostly average values of investigated traits and among them were Nemaro, Čortanovci, NCPGRU5, SA4, SA1, Renova, Marino, Kora, Avala, and Krano.

4. DISCUSSION

The cultivars of red clover studied herein had higher values for PH, IN, GMY, and DMY than natural populations, and tetraploid accessions had higher values for IN, GMY, and DMY than diploid accessions which is in accordance with the research of Naydenova & Vasileva (2019). Compared to the diploid type, tetraploid red clover is more productive with larger leaves, larger flower heads, thicker stems, and larger seeds (Amdahl et al., 2017). Tetraploid red clover cultivars also have increased hardiness which

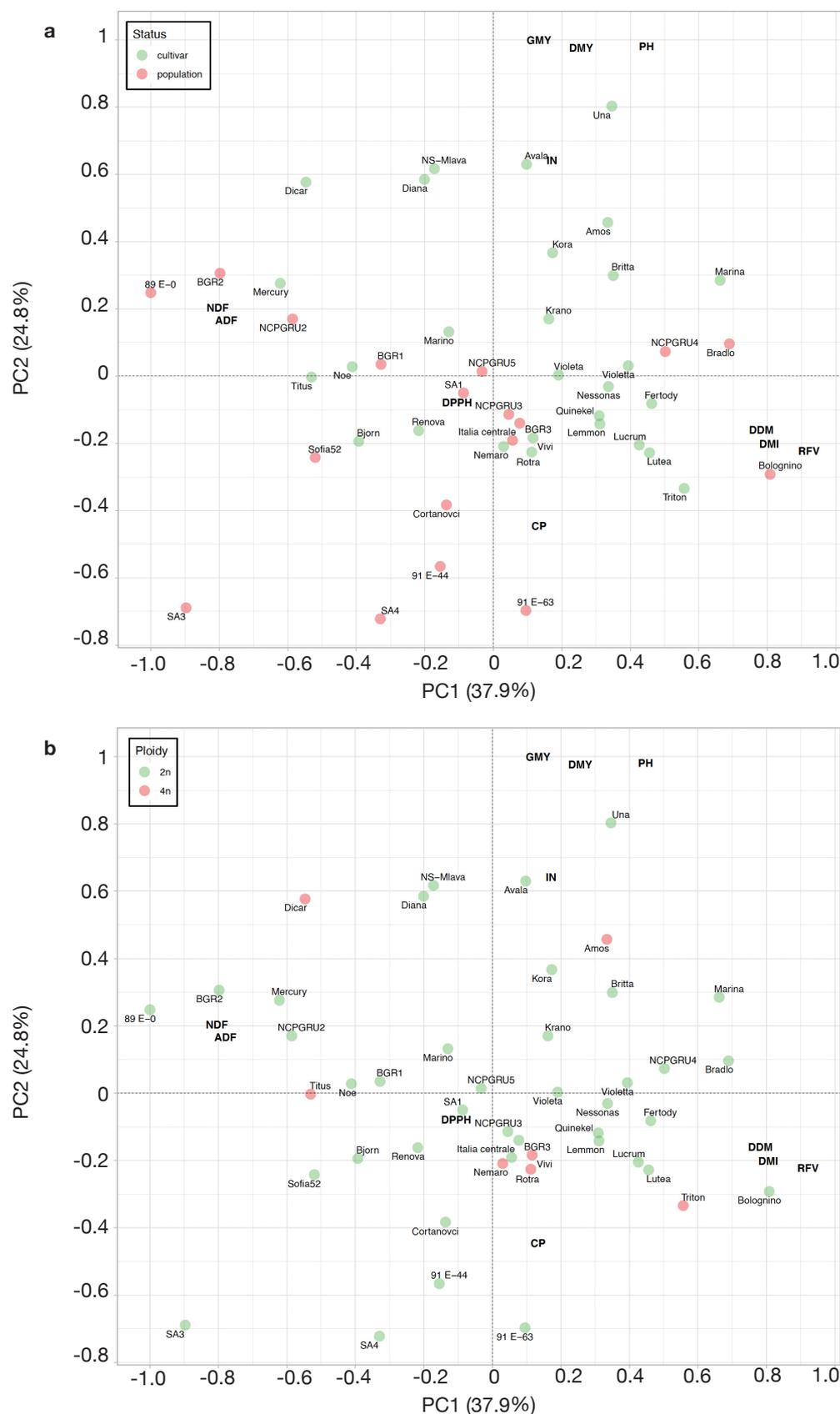


Figure 1. Principal component analysis (PCA) biplot for examined traits by (a) status of accessions, (b) ploidy of accessions — *Biplot d'analyse en composantes principales (ACP) pour les caractères examinés par (a) statut des accessions, (b) ploïdie des accessions.*

NDF, ADF, DDM, DMI, RFV, CP, DPPH, IN, PH, GMY, DMY: see **table 3** — voir **tableau 3**.

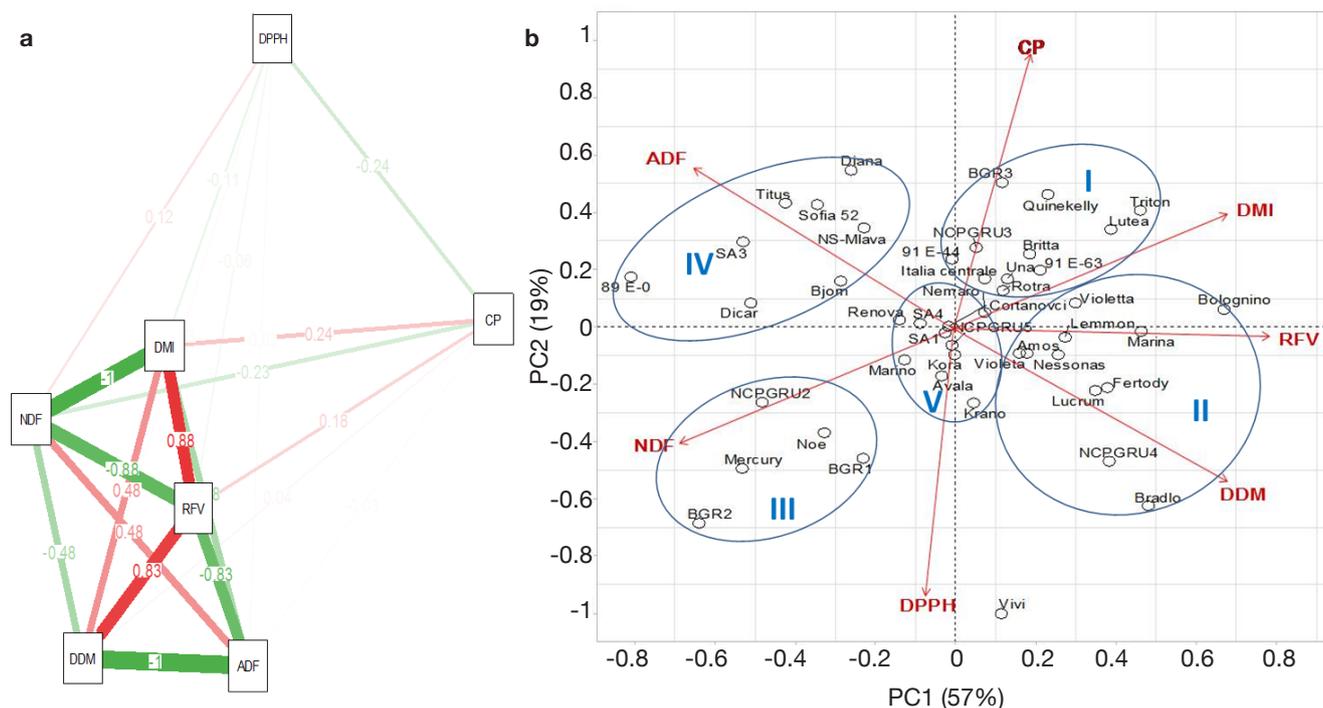


Figure 2. a. The Pearson correlations among forage quality parameters and antioxidant activity — *les corrélations de Pearson entre les paramètres de qualité du fourrage et l'activité antioxydante*; b. Principal component analysis (PCA) biplot for forage quality parameters and antioxidant activity — *biplot d'analyse en composantes principales (ACP) pour les paramètres de qualité du fourrage et l'activité antioxydante*.

NDF, ADF, DDM, DMI, RFV, CP, DPPH: see **table 3** — *voir tableau 3*.

coincides with their evolutionary connection to adverse environments, higher levels of biomass accumulation due to relatively larger polyploid cells, increased resistance to diseases, and increased persistence. However, despite their superior advantages in meeting the primary breeding goals, the seed yield of tetraploid red clover is substantially lower than that of diploid red clover (McKenna, 2017; Jing & Boelt, 2021). The most variable agronomic trait for red clover accessions from our research measured with CV was DMY (33.9%), followed by GMY (32.4%). Natural populations had 31.1% and 35% larger CVs for PH and IN, respectively, in comparison to cultivars. Additionally, diploid accessions of red clover had 53.9% and 17.8% higher CVs for IN and DMY, respectively, in comparison to tetraploid accessions. Foreign red clover accessions in most cases achieved lower forage yields than material originating from Serbia and Croatia, which may be due to the lower adaptability of these cultivars to the agroecological conditions of the testing area. Tucak et al. (2009) analyzed 30 accessions of red clover that originated from 11 countries including two populations from Croatia and found greater variation for the GMY of 58.6% (CV) with a greater mean of 663.4 g·plant⁻¹. The highest average values of green mass and dry

matter yields were recorded in one local population from Croatia (green mass yield 52% higher than the average). Well adapted populations of red clover are able to produce higher forage yields than material from other geographical areas, and their importance has been emphasized as a 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; Tucak et al., 2013). Smaller mean values and CVs for the green mass yield (84.3 t·ha⁻¹, 10.4%) and dry mass yield (19.3 t·ha⁻¹, 11.0%) were obtained for 16 red clover cultivars during the two experimental years in the research of Tucak et al. (2013). Hejduk & Knot (2010) demonstrated that forage from tetraploid varieties contained significantly more water in both harvest years. Plant height, as one of the most important yield components, is a major selection criterion in breeding red clover for increased forage yield. The significant high positive correlations between green mass yield and plant height in red clover, suggests that breeding for a longer stem may lead to an increased forage yield (Tucak et al., 2013). The average plant height of all populations/cultivars was 40.06 cm in our study. According to Asci (2011), a large difference between the minimum and maximum

values of plant height (36 cm) in 48 studied accessions was identified with a CV of 19.8%. Tucak et al. (2009) determined the average plant height in analyzed red clover cultivars and populations of 54.0 cm, and the variation was in the range of 32.6-66.7 cm. Tucak et al. (2013) observed a mean value of 64.8 cm for plant height with a CV of 5.6% for 16 red clover cultivars/populations, all diploid, except the tetraploid cultivar Amos. Analyzing 48 accessions of red clover collected from 20 different sites in the Black Sea region, Ascii (2011) found that the internodes number varied over a wider range than in this study, from 5.8 to 16.2, with a CV of 22.4%. The number of internodes per stem ranged from 4.8 to 6.0 and the average value for all populations/cultivars was 5.3-stem⁻¹, with a CV of 7.2% (Tucak et al., 2013), which was a smaller range mean and CV than in our study. A larger number of shorter internodes increases leaf mass and resistance to lodging, indirectly affecting forage quality. Variations in the number of internodes per stem in this study between the tetraploid red clover populations/cultivars are of a smaller range than the interval 2.6-6.5 obtained in the research of Muntean (2006) on 22 red clover cultivars of tetraploid ploidy status. A comparison of the tetraploid cultivars from the point of view of their origin area shows that the Central-European ones have the most internodes/stem, being closely followed in this respect by the Asian and Northern-European ones (Muntean, 2006).

The natural populations of red clover from our research had higher values for CP, ADF, and NDF than cultivars, whereas cultivars achieved better values for DDM, DMI, and RFV than natural populations. The tetraploid accessions had higher CP content, NDF content, and DDM than diploid accessions, which had higher values for ADF, DMI, and RFV. The most variable trait among forage quality parameters in terms of CV was ADF content (11%) and RFV (9.2%), whereas the least variable parameter was DDM (5.0%). The differences in the coefficient of variation for forage quality parameters between cultivars and natural populations were not pronounced, but differences between diploid and tetraploid accessions were obvious for CP and NDF. The tetraploid accessions varied 42.7% more than diploid accessions for CP, whereas diploid accessions varied 33.4% more for NDF than tetraploid accessions. The quality traits of perennial legumes are very complex, and their expression is influenced by, in addition to genetic factors, environmental factors (especially drought through direct and indirect effects on plant morphology and physiology) and applied management (cutting frequency, plant maturity stage at cutting time, and cutting height) (Tucak et al., 2021). One of the main goals in red clover breeding for the improvement of forage quality is increasing protein content and fiber

digestibility and Tucak et al. (2021) assessed CP to be in the range 18.6%-21.9%, NDF 40.5%-47%, ADF 24.6%-31.6% and RFV 130-155% for one cultivar and 19 elite breeding populations. The content of crude protein in Serbian populations ranged from 18-20% (Petrovic et al., 2014). As stated by Zuk-Golaszewska et al. (2010), the content of proteins in varieties of red clover can range to 21% in dry matter. Low amounts of non-digestible cellulose fibers (NDF and ADF) lead to the enhancement of the feeding value of perennial forage legumes such as alfalfa and red clover for livestock by improving forage fiber digestibility, and increasing harvest management flexibility (Grev et al., 2017; Jungers et al., 2020). Selection for reduced fiber concentration (NDF, ADF) might be successful, but this approach can reduce herbage yield (negative correlations with DM yield). An alternative is to increase the digestibility of the fiber. Since the three fiber components (hemicellulose, cellulose, and lignin) have different digestibility, a decrease in less digestible fiber components or an increase in more digestible fiber components should increase herbage DM digestibility (Bélanger & Tremblay, 2010). The decrease in digestibility after red clover budding is a result of increased lignin content and a drop in the digestibility of non-starch polysaccharides. In this context, the relation between structural and non-structural carbohydrates is especially important from the perspective of ruminant nutrition.

A higher DPPH value means that a higher concentration of plant extract is required to reduce 50% DPPH free radical, and it is valid for IC₅₀ values. The natural populations of red clover from our research had an unfavorable 39.9% higher antioxidant activity measured by the DPPH assay than cultivars, and diploid accessions had an unfavorable 21.9% higher antioxidant activity than tetraploid accessions. The variation in antioxidant activity measured by the DPPH assay in terms of CV was very high (77.3%) for all accessions of red clover, 37% larger in tetraploid accessions than in diploid accessions, and 15.6% larger in cultivars than in natural populations. Horvat et al. (2020) investigated 29 accessions of diploid ploidy level-cultivars, breeding populations, and local populations and found that DPPH radical scavenging varied from 31.5% to 63.1% and that four breeding populations and one local population had the most pronounced ability to scavenge DPPH free radicals, in contrast to our study. In addition to genetic factors, there are other factors that influence differences in the antioxidant capacity of red clover accessions, which are primarily geographical origin and climate (Vlaisavljević et al., 2014; Esmaelli et al., 2015). The antioxidant activity of red clover extracts is due to the presence of various phenolic components, especially isoflavonoids, of which genistein has been shown to be

a highly active antioxidant (Kučukboyaci et al., 2013).

From the plant breeding aspect, it is important to assess the correlations between the various traits, because direct selection on one trait can affect other traits in both the desired and undesired directions, which is considered indirect selection. Tucak et al. (2013) found highly significant ($p < 0.01$) coefficients of correlations for the following pairs of traits: GMY-DMY (0.93), GMY-PH (0.85), DMY-PH (0.81), GMY-NI (0.74), DMY-NI (0.65), PH-NI (0.79), CP-NDF (-0.73), CP-ADF (-0.64), CP-RFV (0.72), RFV-NDF (-0.94), RFV-ADF (-0.87), and NDF-ADF (0.76), which was consistent with our research for all pairs of traits. Petrovic et al. (2014) conducted a principal component analysis for the eight morphological traits of 17 wild red clover populations from Serbia and noticed strong associations between NI, GMY, and PH and clustered accessions in the same group on the bases of green mass yield, plant height, medium lamina length and medium lamina diameter. The high quality red clover accessions from our study had a low forage yield, which confirms the existence of negative correlations between 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; Lugić et al., 2010). Reiné et al. (2020) examined the nutritional quality of plant species in Pyrenean hay meadows of high diversity and obtained negative associations between CP and ADF, and CP and NDF, and positive associations between NDF and ADF. In our study the association between CP and ADF was positive. The grouping of the 46 red clover accessions in this study based on forage quality parameters and antioxidant activity was represented by five clusters (I-V). Regarding the standard assigned by the Hay Marketing Task Force of the American Forage and Grassland Council, the premium-quality accessions (with favorable values of CP, ADF, NDF, RFV) in this study were shown to be the Triton and Lutea cultivars belonging to group I with better CP, and the Marina and Nessonas cultivars and population Bolognino belonging to cluster II with better RFV, suggesting two potential heterotic groups for breeding. The best quality accessions in terms of antioxidant activity belong to different clusters: cultivar Una and natural population 91 E-63 to group I, cultivars Amos and Marina to group II, cultivar Diana to group IV, and cultivar Marino to group V, showing more dispersive behavior across clusters in regard to other measured traits. The seven cultivars of tetraploid ploidy were not in the same cluster (Rotra and Triton-cluster I, Amos-cluster II, Dicar and Titus-cluster III, Nemaro-cluster V, Vivi-not included in any of these five groups). Among the 18 natural populations and 28 cultivars of red clover

examined, the highest number of natural populations per group (5) and the highest number of cultivars per group (9) were grouped in cluster I (favorable values of CP, DMI, NDF, antioxidant capacity).

5. CONCLUSIONS

Cultivars had higher values for plant height, internodes number, green mass yield, dry matter yield, digestible dry matter, dry matter intake, and relative feed value than natural populations, which had higher values for crude protein, acid detergent fiber, and neutral detergent fiber. The tetraploid accessions had higher values for internodes number, green mass yield, dry matter yield, crude protein, neutral detergent fiber, and digestible dry matter than diploid accessions, which had higher values for acid detergent fiber, dry matter intake, and relative feed value. The antioxidant capacity of red clover accessions was positively associated with relative feed value, digestible dry matter, dry matter intake, plant height, internodes number, green mass yield, and dry matter yield but negatively associated with acid detergent fiber and neutral detergent fiber. Extracts of five red clover cultivars (Amos, Marino, Marina, Una and Diana) and of one natural population 91 E-63 showed antioxidant activity greater than the capacity of synthetic antioxidants used as reference standards. The grouping of the 46 red clover accessions in this study based on forage quality parameters and antioxidant activity was represented by five clusters. Cultivars originating from Serbia had significantly higher dry matter yields, which confirmed their better adaptability and higher genetic potential for yield at the trial site compared to the remaining 43 accessions. Generally, high forage quality cultivars had a shorter length of internodes and a good leaf to stem ratio with high leaf proportion.

Abbreviations

ADF: acid detergent fiber
 BHA: butylated hydroxyanisole
 BHT: butylated hydroxytoluene
 CP: crude protein
 CV: coefficient of variation
 DDM: digestible dry matter
 DMI: dry matter intake
 DMY: dry matter yield
 DPPH: 2,2-diphenyl-1-picrylhydrazyl
 GMY: green mass yield
 IN: internodes number
 NDF: neutral detergent fiber
 PCA: principal component analysis
 PH: plant height

RFV: relative feed value

SD: standard deviation

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