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Cultivars Ploidy Influence on Variability of Some Red Clover Traits Important for Agriculture and Medicinal Purpose

Alexandru D. COSTIN[1*], Leon MUNTEAN[1], Andreea D. ONA[1,2], Mihai C. POPA[1]

[1] Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăștur 3-5, 400372, Cluj-Napoca, Romania
[2] College of Agricultural and Environmental Sciences, University of Georgia, 147 Cedar Street, 30602, Athens, Georgia, United States
** Corresponding author: A.D. Costin e-mail: alexdan.costin@gmail.com*

RESEARCH ARTICLE

Abstract

Red clover also known as *Trifolium pratense* L., is a perennial allogame plant belonging to *Trifolium* genus and *Fabaceae* family. Besides its benefits in animal feed and soil improvement, red clover was studied as well for its medicinal properties, being used in different forms like essential oil, tea, pills and other. This study aimed to perform a comparative analysis on some diploid and tetraploid red clover cultivars regarding morphological and biochemical traits. The study was carried out between 2022 and 2023 on 90 red clover cultivars, divided into two samples represented by diploids and tetraploids. Tetraploids were superior to diploids in the 2nd year and inferior in the 3rd year. Regarding the polyphenols content, diploids showed higher values than tetraploids. Between diploids and tetraploids, there was a significant difference in the number of inflorescences/50g and inflorescence weight from the 3rd year.

Keywords: diploid, perennial, red clover cultivars, tetraploid.

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INTRODUCTION

Red clover also known as *Trifolium pratense* L., is a perennial plant belonging to the *Trifolium* genus and *Fabaceae* family (Taylor and Smith, 1980). *Trifolium* genus contains more than 200 species and present importance due to its wide range of uses (Booth et al., 2006). This species grows wildly in temperate and subtropical region throughout Asia and Europe, being introduced even in North America (Zielinska et al., 2012; Vlaisavljević et al., 2017). It's an allogame plant that present a higher variability due to its heterozygosity (Muntean, 2006). Red clover is well known for its medicinal properties. It contains significant amounts of flavonoids, phytoestrogens, isoflavonoids, lignans, coumestans (Bou et al., 2003; Romm et al., 2010) and other substances like salicylates, glycosides, sitosterols, cyanogenic carbohydrates, coumarins, saponins, vitamins, minerals, fatty acids, starch and volatile oil (Romm et al., 2010; Zielinska et al., 2012). These substances can be found in different plant parts (for example isoflavones can be found in all aerial plant parts in different quantities) (Table 1) and can be used in medicine for various ailments, in different forms as essential oil, ointment, tea, pills and other. The essential oil obtained from red clover can be used by people both internally and externally, the ointment (medicinal ointment) can be used in skin disorders treatment and as for the tea and pills, they can be used internally for many severe medical conditions. This plant has adapted to different pH levels,

soil types and other environmental conditions. These characteristics allowed red clover to retain besides its medicinal properties, the usefulness for pasture, hay, silage, (Taylor and Smith, 1980) and soil improvement due to its capacity to form symbiosis with root nodule bacteria (Høgh-Jensen et al., 2004; Hatch et al., 2007). Regarding red clover crop, the breeding programs aimed to obtain crops with a higher production (both as animal feed and biochemical content), resistance to environmental factors stress and improved qualitative characters. It has two forms: diploid (2n=2x=14 chromosomes) (Trněný et al., 2019; Vasiljević et al., 2022; Petrauskas et al., 2023) and tetraploid (2n=4x=28 chromosomes) (Amdahl et al., 2016; Egan et al., 2021). Important characteristics of *Trifolium pratense* like productivity or resistance to different factors, are influenced by a series of physiological and morphological traits, these traits being influenced by environmental factors and genetics (Muntean, 2006). From morphologically perspective, tetraploid cultivars shoved a difference in size and sturdiness compared to diploid cultivars (Muntean, 2008). Tetraploid cultivars presented a higher height, round thick leaves and rougher stems, longer floral tube and bigger flower-heads (Julen, 1959, cited by Muntean, 2008). Another characteristic of this species with importance in biochemical content was represented by perenniality (the capacity to be productive for more than 2 year). This study aimed to perform a comparative analysis on some diploid and tetraploid red clover cultivars, regarding morphological and biochemical traits.

Table 1. Isoflavone quantity in different aerial plant parts (mg/g DM)

Aerial plant part	**Mean** ± SD	Min	Max
Stems	**3.32** ± 1.34	1.52	4.74
Leaves	**2.74** ± 1.39	1.11	4.29
Flowers	**2.22** ± 1.36	0.99	4.17

Note: DM– dried matter; Values represented as Mean ± SD (Standard Deviation) (adapted: Butkutė et al., 2014)

MATERIALS AND METHODS

Plant material and study site characteristics

The study was carried out in the pedoclimatic conditions from UASVM Cluj-Napoca Agrobotanical Garden between 2022 (2nd year of vegetation) – 2023 (3rd year of vegetation) with an annual temperature of 10.6ºC and the total annual rainfall of 533.4 mm for 2022, respectively 9.3ºC and 2832.0 mm for 2023 (the data were available till September 2023 from UASVM weather station). In the study 90 red clover cultivars were used, divided into two samples represented by 20 tetraploids (Table 2) and 70 diploids (Table 3) from UASVM Cluj-Napoca collection.

Table 2. List of tetraploid cultivars (4n)

No.	Cultivar	Seeds' country of origin
1	Amos*	Czech Republic
2	Beskyd	Czech Republic
3	Bivoj*	Czech Republic
4	Dolina	Czech Republic
5	Ilte*	Estonia
6	Magura	Slovakia
7	Monsun	Germany
8	Nodula	Czech Republic
9	Poljanka*	Slovenia
10	Rezista	Czech Republic
11	Sadunai*	Lithuania
12	Sigord	Slovakia
13	Tedi*	France
14	Titus	Germany
15	Tornado	Germany
16	Vesna	Czech Republic
17	Lars	Norway
18	Lasang	Norway
19	Legato*	Norway
20	Linus	Norway

Note: * tetraploid cultivars with the highest polyphenols content (4n)

Table 3. List of diploid cultivars (2n)

No.	Cultivar	Seeds' country of origin	No.	Cultivar	Seeds' country of origin
1	AberChianti	UK	36	Marga Liv	Romania
2	AberClaret*	UK	37	Rotrif	Romania
3	Arimaiciai*	Lithuania	38	Livada Sara*	Romania
4	Callisto*	Denmark	39	Tinu Liv	Romania
5	Callisto*	Czech Republic	40	Livada Ralu	Romania
6	Corvus*	Switzerland	41	Flora	Romania
7	Diadem	France	42	Verdi	France
8	Dimanche	France	43	Sapporo	France
9	Diplo*	France	44	Britta	Sweden
10	Diplomat	Germany	45	Niderheicher	Germany
11	Discovery	France	46	Oden Walder	Germany
12	Dizstende*	Latvia	47	Segur	France
13	Kindia*	France	48	Noe	France
14	Lemmon	Belgium	49	Taşnad	Romania
15	Manuela	Slovakia	50	GKT Junior	Hungary
16	Marieta	Slovakia	51	Granta	UK
17	Mercury	Belgium	52	Leliceni	Romania
18	Merian	Belgium	53	Sepia	USA
19	Merviot	Belgium	54	Violeta	France
20	Merviot	Belgium (Different centre)	55	Marino	France
21	Metis	Denmark	56	Justin	France
22	Montcalme	France	57	Reichersberger	Austria
23	Nemaro	Germany	58	Gloria Mestnaia	Ukraine
24	Pavo	Switzerland	59	Mistral	France
25	Podjavorina*	Slovakia	60	Otawa	Canada
26	Radviliai*	Lithuania	61	Palna	France
27	Raunis	Latvia	62	Marcom	France
28	Sinope	Denmark	63	Aita Mare	Romania
29	Slatina	Slovakia	64	Mestecăniş	Romania
30	Suez	Denmark	65	Essi	Sweden
31	Violetta R.v.P.	Belgium	66	Tabor	Czech Republic
32	Vyciai*	Lithuania	67	Dipper	France
33	Gandalf*	Norway	68	Kuhn	Netherlands
34	L.E. 116	Uruguay	69	Lucrum	France
35	David Liv	Romania	70	Kornicevskij	Russia

Note: * diploid cultivars with the highest polyphenols content (2n)

Study methods and traits analysis

Each cultivar was sown in three repetitions in a single row block method, with 7 m length rows (equivalent to 1 m^2 surface) and a distance between rows of 20 cm. The observed aspects were the plant height (cm), the leaflet length and width (cm), these being expressed in centimeters using a ruler in the field; the green matter weight (t/ha) which was determined as well in the field as weight in kg/m^2 using a hand scale and was transformed and reported as t/ha; the number of ramifications/stem and the number of stems/plant which were determined individually on the plant in the field; the number of inflorescences/50g and inflorescence weight (g) which were determined using a normal scale in laboratory.

Phytochemical analysis

Extraction of phenolic compounds

For extraction, 0.5 g of fresh crushed inflorescence (harvested in the 2nd year of vegetation) were mixed with 10 ml

of methanol and vortexed for 5 min at the Heidolph Reax top vortex, sonicated for 15 min in the sonication bath and centrifuged at 10,000 rpm for 10 min and T = 240˚C at centrifuge Eppendorf AG 5804 Elmasonic E 15H. The above operations were repeated until the sample was discoloured. Among all 90 red clover studied cultivars, the first 20 (both diploids and tetraploids) that presented in general the highest polyphenols content, were used for LC-MS analysis. These cultivars were noted with * în Table 2 and Table 3. The total polyphenol content was determined for all samples, using Folin-Ciocâlteu method. From each sample, 25 µl of extract were used, to which 120 µl of Folin reagent were added and then it was left to rest for 5 minutes in a place without light. After 5 minutes, 340 µl of Na_2CO_3 were added, and it was left again in rest for another 30 minutes. After 30 minutes, 1800 µl of H_2O were added, and the results were read using a spectrophotometer at a wavelength of λ = 750. The supernatant was filtered through a 0.45 µm Chromafil Xtra nylon filter and 20 µl were injected into the HPLC system.

LC-MS analysis

An Agilent 1200 HPLC system equipped with a quaternary pump, an autosampler, solvent degasser and UV-Vis detector with photodiode (DAD), coupled with a quadrupole mass detector (MS) Agilent model 6110 (Agilent Technologies, CA, USA) was used. Compounds separation was performed on a Kinetex XB C18 column, 4.6x150 mm, with 5 µm particles (Phenomenex, USA), using the mobile phases (A) 0.1% acetic acid + water and (B) 0.1% acetic acid + acetonitrile, at a temperature of 25˚C for 30 minutes, with 0.5 ml/min flow rate. Gradient (expressed in % B): first 0 min at 5% B; then 0-2 min at 5% B; and forward, 2-18 min, at 5%-40% B; 18-20 min at 40%-90% B; 20-24 min at 90% B; 24-25 min at 90%-5% B and 25-30 min at 5% B. In all peaks, the spectral values were recorded in the 200-600 nm range. At the wavelength of λ = 280 and 340 nm, the chromatograms were recorded. The full scan ESI positive ionization mode was used for MS, in the following working conditions: temperature 350˚C, capillary voltage 3000 V, nitrogen flow of 7 l/min and m/z 120-1200. ChemStation software (Agilent Technologies, CA, USA), version B.02.01-SR2 was used for the results interpretation and data acquisition. Compounds quantification was done using gallic acid and rutin standard curve.

Statistical analysis

The results were obtained using R4.3.1 statistical software (R Core Team, 2023) and were statistically interpreted using 2 independent sample Student t-test with equal variances and Welch Two Sample t-test for normal distributed data and Mann Whitney U test for non-normal distributed data. To check data normality, descriptive statistics, Kolmogorov–Smirnov test and QQ plot (quantile-quantile) was used. The results were presented as median with interquartile interval [IQR] for both variables that presented departures from Gaussian distribution and normal distributed variables (since one normality assumption is that the mean is equal to median and mode value). To estimate the studied traits variability, the variability coefficient was calculated (CV%). The values for variability coefficient were interpreted according to data from literature. For a variability coefficient value less than 10%, variability is considered low, for a value between 10% and 20%, variability is considered average, and for a value greater than 20%, variability is considered high (Ceapoiu, 1968).

RESULTS AND DISCUSSIONS

Plant morphology analysis

The plant height

Tetraploids were higher than diploids in the 2nd year and shorter than diploids in the 3rd year. Similar values for the 2nd year, were obtained by Muntean (2008). Regarding ploidy, there was no significant difference in plant height between diploids and tetraploids in both years (p>0.05, Mann Whitney U, 2nd year and p>0.05, 2 independent sample Student t-test with equal variances, 3rd year). The variability among cultivars was average for both tetraploids and diploids in the 3rd year, which recommends the studied material to be used for breeding programs towards this trait. Both diploids and tetraploids were higher in the 2nd year than in the 3rd year (Table 4). Diploids presented a better plant height stability than tetraploids over time.

Table 4. The plant height (cm)

Year	Cultivars	Median [IQR]	Variation limits		CV%	p-value
			Min	Max		
2nd	Diploids	90.45 [87.83; 93.98]	74.30	101.60	5.64	0.269
	Tetraploids	91.40 [89.10; 94.68]	87.40	101.20	4.79	
3rd	Diploids	56.80 [50.03; 65.26]	31.75	77.85	18.09	0.394
	Tetraploids	53.53 [50.28; 57.64]	43.85	72.30	14.36	

The leaflet length

Tetraploids presented a lower leaflet length than diploids in both years. Regarding ploidy, there was no significant difference in leaflet length between diploids and tetraploids in both years (p>0.05, 2 independent sample Student t-test with equal variances, 2nd year and p>0.05, 2 independent sample Student t-test with equal variances, 3rd year). The variability among cultivars was lower for both tetraploids and diploids, which not recommends the studied material to be used for breeding programs towards this trait. Both diploids and tetraploids presented a lower leaflet length in the 2nd year than in the 3rd year (Table 5). Diploids presented a better leaflet length stability than diploids over time.

Table 5. The leaflet length (cm)

Year	Cultivars	Median [IQR]	Variation limits		CV%	p-value
			Min	Max		
2nd	Diploids	4.90 [4.63; 5.15]	4.10	5.70	7.30	0.825
	Tetraploids	4.89 [4.70; 5.03]	4.16	5.87	7.96	
3rd	Diploids	5.11 [4.83; 5.34]	4.42	6.05	7.29	0.721
	Tetraploids	5.09 [4.93; 5.27]	4.47	5.94	6.82	

The leaflet width

Tetraploids presented a lower leaflet width than diploids in both years. Regarding ploidy, there was no significant difference in leaflet width between diploids and tetraploids in both years (p>0.05, Mann Whitney U, 2nd year and p>0.05, 2 independent sample Student t-test with equal variances, 3rd year). The variability among cultivars was average for both tetraploids and diploids, which recommends the studied material to be used for breeding programs towards this trait. Both diploids and tetraploids presented a lower leaflet width in the 2nd year than in the 3rd year (Table 6). Diploids presented a better leaflet width stability than tetraploids over time.

Table 6. The leaflet width (cm)

Year	Cultivars	Median [IQR]	Variation limits		CV%	p-value
			Min	Max		
2nd	Diploids	2.87 [2.53; 3.05]	1.89	3.51	13.23	0.372
	Tetraploids	2.80 [2.43; 2.97]	1.78	3.63	18.61	
3rd	Diploids	3.15 [2.84; 3.33]	2.32	3.72	11.84	0.155
	Tetraploids	3.00 [2.60; 3.19]	2.20	3.74	14.89	

The green matter weight

Tetraploids presented a higher green matter weight than diploids in the 2nd year and a lower green matter weight than diploids in the 3rd year. Regarding ploidy, there was no significant difference in green matter weight between diploids and tetraploids on both years (p>0.05, Welch Two Sample t-test, 2nd year and p>0.05, 2 independent sample Student t-test with equal variances, 3rd year). The variability among cultivars was high for both tetraploids and diploids, which recommends the studied material to be used for breeding programs towards this trait. Both diploids and tetraploids presented a higher green matter weight in the 2nd year than in the 3rd year (Table 7). Diploids presented a better green matter weight stability than tetraploids over time. Similar values were obtained by Liatukas and Bukauskaitë (2012) and by Naydenova and Vasileva (2019).

Table 7. The green matter weight (t/ha)

Year	Cultivars	Median [IQR]	Variation limits		CV%	p-value
			Min	Max		
2nd	Diploids	94.40 [82.65; 106.69]	38.05	136.70	19.39	0.952
	Tetraploids	95.63 [77.28; 116.00]	26.00	134.65	29.94	
3rd	Diploids	24.80 [21.08; 28.66]	8.80	38.30	25.35	0.286
	Tetraploids	23.43 [16.56; 29.69]	11.10	36.60	35.59	

The number of stems/plant

Tetraploids presented a higher number of stems/plant than diploids in both years. Regarding ploidy, there was no significant difference in number of stems/plant between diploids and tetraploids in both years (p>0.05, Welch Two Sample t-test, 2nd year and p>0.05, Mann Whitney U, 3rd year). The variability among cultivars was lower for

both tetraploids and diploids, which not recommends the studied material to be used for breeding programs towards this trait. Both diploids and tetraploids presented a higher number of stems/plant in the 2nd year than in the 3rd year (Table 8). Tetraploids presented a better number of stems/plant stability than tetraploids over time.

Table 8. The number of stems/plant

Year	Cultivars	Median [IQR]	Variation limits		CV%	p-value
			Min	Max		
2nd	Diploids	10.10 [9.60; 10.60]	8.60	11.50	6.62	0.727
	Tetraploids	10.15 [9.88; 10.30]	9.50	11.20	4.16	
3rd	Diploids	9.40 [9.10; 10.08]	8.30	10.90	6.22	0.896
	Tetraploids	9.45 [9.18; 9.90]	8.10	10.20	5.35	

The number of ramifications/stem

Tetraploids presented a higher number of ramifications/stem than diploids in the 2nd year and a lower number of ramifications/stem than diploids in the 3rd year. Similar values for the 2nd year, were obtained by Muntean (2008). Regarding ploidy, there was no significant difference in number of ramifications/stem between diploids and tetraploids in both years ($p>0.05$, Mann Whitney U, 2nd year and $p>0.05$, Mann Whitney U, 3rd year). The variability among cultivars was lower for both tetraploids and diploids, which not recommends the studied material to be used for breeding programs towards this trait. Both diploids and tetraploids presented a higher number of ramifications/stem in the 2nd year than in the 3rd year (Table 9). Diploids presented a better number of ramifications/stem stability than tetraploids over time.

Table 9. The number of ramifications/stem

Year	Cultivars	Median [IQR]	Variation limits		CV%	p-value
			Min	Max		
2nd	Diploids	2.97 [2.91; 3.00]	2.50	3.23	3.55	0.330
	Tetraploids	2.98 [2.93; 3.03]	2.85	3.15	2.68	
3rd	Diploids	2.63 [2.55; 2.75]	2.33	3.10	6.78	0.261
	Tetraploids	2.56 [2.50; 2.76]	2.38	2.98	6.92	

The number of inflorescences/50g

Tetraploids presented a higher number of inflorescences/50g than diploids in the 2nd year and a lower number of inflorescence/50g than diploids in the 3rd year. Regarding ploidy, there was a significant difference in number of inflorescence/50g between diploids and tetraploids in the 3rd year ($p>0.05$, Mann Whitney U, 2nd year and $p<0.05$, Mann Whitney U, 3rd year). The variability among cultivars was higher for both tetraploids and diploids, which recommends the studied material to be used for breeding programs towards this trait. Diploids presented a lower number of inflorescences/50g in the 2nd year than in the 3rd year and tetraploids presented a higher number of inflorescences/50g in the 2nd year than in the 3rd year (Table 10). Diploids presented a better number of inflorescences/50g stability than tetraploids over time.

Table 10. The number of inflorescences/50g

Year	Cultivars	Median [IQR]	Variation limits		CV%	p-value
			Min	Max		
2nd	Diploids	94 [86.00; 107.50]	64.00	190.00	28.06	0.409
	Tetraploids	109 [76.25; 131.00]	56.00	223.00	39.81	
3rd	Diploids	100 [83.00; 100.00]	71.00	167.00	18.44	0.001
	Tetraploids	77 [69.00; 100.00]	56.00	167.00	32.66	

The inflorescence weight

Tetraploids presented a lower inflorescence weight than diploids in the 2nd year and higher than diploids inflorescence weight in the 3rd year. Regarding ploidy, there was a significant difference in inflorescence weight between diploids and tetraploids in the 3rd year ($p>0.05$, Welch Two Sample t-test, 2nd year and $p<0.05$, Mann Whitney U, 3rd year). The variability among cultivars was higher for both tetraploids and diploids, which recommends the studied material to be used for breeding programs towards this trait. Diploids presented a higher

inflorescence weight in the 2nd year than in the 3rd year, and tetraploids presented a lower inflorescence weight in the 2nd year than in the 3rd year (Table 11). Tetraploids presented a better inflorescence weight stability than diploids over time.

Table 11. The inflorescence weight (g)

Year	Cultivars	Median [IQR]	Variation limits		CV%	p-value
			Min	Max		
2nd	Diploids	0.53 [0.47; 0.58]	0.26	0.78	22.34	0.920
	Tetraploids	0.46 [0.38; 0.66]	0.20	0.90	37.11	
3rd	Diploids	0.50 [0.50; 0.60]	0.30	0.70	15.87	0.001
	Tetraploids	0.65 [0.50; 0.73]	0.30	0.90	25.92	

Chemical analysis

The total phenolic compounds

The first 20 red clover cultivars (both diploids and tetraploids) that presented the highest content in total polyphenols among the 90 analyzed cultivars (noted with * in Table 1 and Table 2), were further tested by LC-MS analysis. Diploids presented a higher phenolic compounds quantity than tetraploids. Regarding ploidy, there was no significant difference between diploids and tetraploids ($p<0.05$, 2 independent sample Student t-test with equal variances). The variability among cultivars was average for both tetraploids and diploids, which recommends the studied material to be used for breeding programs towards this trait (Table 12).

Table 12. Total phenolic compounds (mg/g)

Cultivars	Median [IQR]	Variation limits		CV%	p-value
		Min	Max		
Diploids	**24.34 [22.16; 25.52]**	16.58	31.59	17.38	0.469
Tetraploids	**23.21 [20.06; 24.06]**	18.18	27.65	14.41	

In addition to the total amount of phenolic compounds, several compounds were determined individually (Table 13).

Table 13. Phenolic compounds in diploids and tetraploids red clover cultivars (mg/g)

Peak	Phenolic compound	Subclass	Cultivars	
			Diploids	Tetraploids
1	3-Hydroxybenzoic acid	Hydroxybenzoic acid	0.16 [0.14; 0.21]	0.25 [0.22; 0.28]
2	2-Hydroxybenzoic acid	Hydroxybenzoic acid	0.37 [0.22; 0.54]	0.65 [0.50; 0.75]
3	Daidzein-glucosides	Isoflavonoid	0.10 [0.09; 0.11]	0.10 [0.10; 0.11]
4	Genistein-malonyl-glucosides	Isoflavonoid	0.07 [0.00; 0.10]	0.05 [0.00; 0.06]
5	Quercetin-galactosides	Flavonols	2.98 [1.33; 3.24]	2.18 [1.66; 2.78]
6	Kaempferol-galactosides	Flavonols	3.05 [2.70; 3.71]	2.42 [1.99; 2.80]
7	Kaempferol-rhamnosyl-xylosyl-glucoside	Flavonols	2.21 [1.78; 2.41]	1.90 [1.68; 2.42]
8	Kaempferol-glucosides	Flavonols	0.78 [0.56; 1.10]	0.83 [0.76; 1.35]
9	Quercetin-rhamnoside	Flavonols	0.71 [0.57; 0.84]	0.63 [0.58; 0.89]
10	Quercetin-malonyl-glucosides	Flavonols	3.12 [2.39; 3.44]	2.22 [2.00; 3.17]
11	Formononetin-glucosides	Isoflavonoid	0.44 [0.40; 0.45]	0.49 [0.47; 0.58]
12	Kaempferol-malonyl-glucosides	Flavonols	2.59 [2.33; 3.17]	2.61 [2.20; 2.72]
13	Kaempferol-xylosyl-glucosides	Flavonols	1.84 [1.65; 2.24]	1.38 [1.34; 1.94]
14	Kaempferol-diglucosides	Flavonols	0.82 [0.66; 1.38]	0.78 [0.67; 0.93]
15	Biochanin A-malonyl-glucoside	Isoflavonoid	0.31 [0.26; 0.48]	0.50 [0.46; 0.65]
16	Quercetin	Flavonols	0.33 [0.14; 0.38]	0.29 [0.20; 0.34]
17	Biochanin-glucosides	Isoflavonoid	0.13 [0.09; 0.27]	0.36 [0.22; 0.47]
18	Prunetin-glucosides	Isoflavonoid	0.25 [0.24; 0.34]	0.52 [0.21; 0.73]
19	Genistein	Isoflavonoid	0.43 [0.27; 0.60]	0.52 [0.34; 0.63]
20	Kaempferol	Flavonols	0.26 [0.17; 0.44]	0.29 [0.22; 0.30]
21	Formononetin	Isoflavonoid	0.91 [0.70; 1.43]	1.10 [0.97; 1.12]
22	Biochanin A	Isoflavonoid	1.21 [0.92; 1.56]	1.46 [1.42; 1.74]

Note: Values represented as Median and quartile interval [IQR]

Each phenolic compound was then grouped according to cultivars ploidy and described as median and quartile range (IQR). According to obtained data, diploids presented higher values than tetraploids for Genistein-malonyl-glucosides, Quercetin-galactosides, Kaempferol-galactosides, Kaempferol-rhamnosyl-xylosyl-glucoside, Quercetin-rhamnoside, Quercetin-malonyl-glucosides, Kaempferol-xylosyl-glucosides Kaempferol-diglucosides and Quercetin (Table 13),

Compared to diploids, tetraploids cultivars presented higher values for 3-Hydroxybenzoic acid, 2-Hydroxybenzoic acid, Kaempferol-glucosides, Formononetin-glucosides, Kaempferol-malonyl-glucosides, Biochanin A-malonyl-glucoside, Biochanin-glucosides, Prunetin-glucosides, Genistein, Kaempferol, Formononetin and Biochanin A. Regarding Daidzein-glucosides quantities, both diploid and tetraploid cultivars presented the same amount.

Following the analyses, 2 chromatograms (Figure 1 and Figure 2) were generated with different reading frequencies (at 280 nm and 340 nm) for one of the cultivars with a high content of polyphenols (diploid).

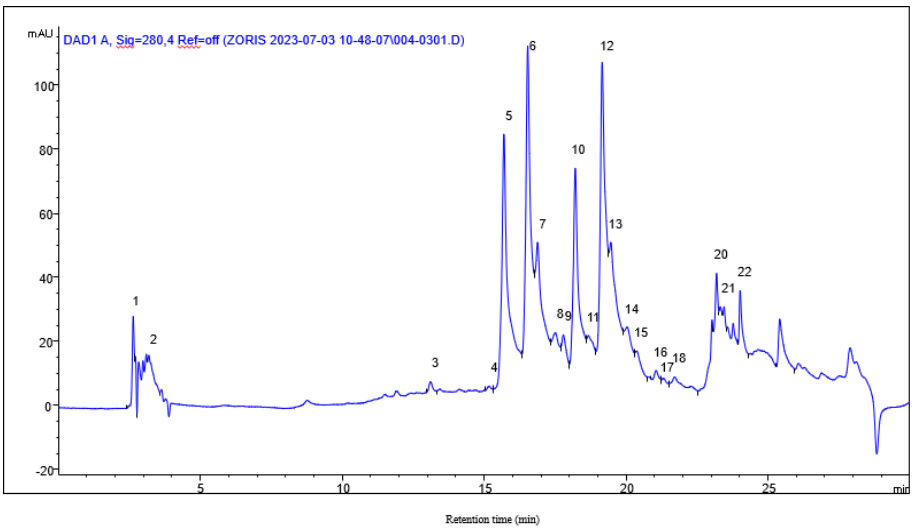

Figure 1. Clover sample chromatogram at 280 nm

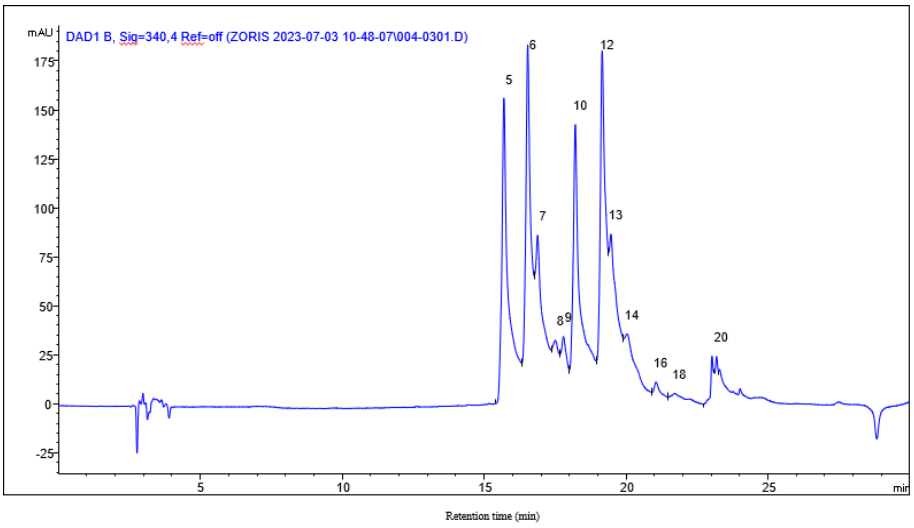

Figure 2. Clover sample chromatogram at 340 nm

CONCLUSIONS

This study show that tetraploids are superior to diploids regarding the plant height, the green matter weight, the number of stems/plant, the number of ramifications/stem and the number of inflorescences/50g in the 2nd year of vegetation, traits that can influence the plant biochemical compounds quantity used for both agriculture and medicine. Regarding the number of inflorescences/50g and inflorescence weight from the 3rd year, the differences between tetraploids and diploids were significant, so these aspects should be considered for breeding programs. The diploids presented better results than tetraploids in majority of morphological traits from the 3rd year, this

thing shoving that diploids have a better stability in time, so they must be studied more for this characteristic that can influence the plant biochemical compounds quantity used for both agriculture and medicine. Although there was no significant difference in the total amount of phenolic compounds between diploids and tetraploids, diploids performed better than tetraploids in the 2nd year of vegetation in terms of these compounds. Since tetraploids present better results in the 2nd year and diploids in the 3rd year, the most productive of each group, can be used in breeding programs as parents, aiming to obtain new and more productive cultivars.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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