THE BIODIVERSITY, THE RELATIVE SHARE AND SOME FORAGE QUALITY TRAITS OF CLOVER SPECIES (*Trifolium* spp.) IN THE GRASSLANDS OF THE CENTRAL NORTHERN BULGARIA

Galina NAYDENOVA[*], Tatyana BOZHANSKA[**]

[*]*Experimental Station on Soybean, 61 "Ruski" Str., 5200 Pavlikeni, Agricultural academy, Sofia, Bulgaria*
[**]*Research Institute of Mountain Stockbreeding and Agriculture, 281" V. Levski" Str., 5600 Troyan, Agricultural academy, Sofia, Bulgaria*
Correspondence author: Galina Naydenova, Experimental Station on Soybean, 61 "Ruski" Str., 5200 Pavlikeni, Agricultural academy, Sofia, Bulgaria, email: gmvg@abv.bg

Abstract. The study was conducted in the period 2017-2019, in the foothills of Central Northern Bulgaria, on three types of grasslands - *Chrysopogon gryllus* meadow, Genista-pseudo sheep's fescue (*Festuca ovina - Festuca valesiaca*) pasture and Bermuda grass-ryegrass (*Cynodon dactylon - Lolium perenne*) pasture, typical of meadows and pastures in Bulgaria. The relative share of clover species in grassland was assessed at different altitudes (134 m, 385 m and 456 m above sea level), and the chemical composition of dry fodder matter was analyzed. A total of 14 species of genus *Trifolium* were identified in the most common types of meadows and pastures in the Central Northern Bulgaria. The mountain and plain grasslands have specific dominant clover, which is associated by the different physiological, morphological and biological profiles of the identified species. For the mountainous region these were *T. pannonicum*, *T. medium* and *T. incarnatum*, and for the plain - *T. echinatum*, *T. campestre*. Species diversity and relative share are more related to the use of grasslands and edaphic factors than to climatic ones. It is important to note that the biodiversity inherent in genus *Trifolium* in terms of the life cycle of the species, as well as in terms of reproduction system, is equally represented in the different types of grasslands. The high natural distribution, high protein and balanced fiber and macromineral content of *T. incarnatum* in the mountain meadow and *T. echinatum* in the plain pasture, suggests the potential for inclusion of these species by resowing in the functional group of legumes in different types of grasslands.

Keywords: *Trifolium* spp.; clovers, biodiversity; forage quality.

INTRODUCTION

In the natural grasslands of Bulgaria there are about 100 species of legumes with good forage qualities [36]. The largest number of them belong to genus *Trifolium* L.. Results related to the share of clover species of forage importance in natural and semi-natural grasslands in Bulgaria were published before [11, 30, 40, 43]. These studies concern floristic regions, located in Southern Bulgaria and differ significantly from those in the northern part of Bulgaria. In Northern Bulgaria there is also a great variety of climatic and edaphic conditions. In the foothills, the average annual precipitation and precipitation for the growing season are 241 and 200 mm higher, respectively, than in the hilly and plain areas [28]. There is also significant variation in terms of phosphorus content in the soil and soil acidity. This affects both the distribution of individual clover species depending on their physiological characteristics and causes significant intra- and interpopulation genetic / phenotypic variability within the species.

Changes in climatic and anthropogenic conditions within each physical-geographical area are continuous. This causes significant variation in the floristic composition, ecology and syntaxonomy of natural and semi-natural grasslands [5, 41]. The study of these processes provides new information about the importance of legumes and in particular clover in terms of the functioning and conservation of meadow ecosystems. Genetic and species diversity are the main structural components of biological diversity [8]. In this aspect, the study of clover in natural and semi-natural grasslands is important for monitoring and assessment of ecological processes in meadow ecosystems. Local populations of clover species with specific adaptation to the conditions of the individual habitats and respectively specific morphological, biological and forage profile can also be defined as an important element of biodiversity.

The biological and forage characteristics of wild clover are of interest because of the possibility that these species can be used as an alternative to red (*T. pratense* L.) and white clover (*T. repens* L.) due to their higher tolerance to stress [20, 21, 27]. Genus *Trifolium* includes more than 20 clover species with potential for economically significant legume components of perennial or annual grass (*Poaceae* sp.) mixtures [14].

The aim of the present study was to determine the biodiversity and the relative share of clover species in grasslands, typical for the Central Northern Bulgaria.

MATERIALS AND METHODS

In the hilly and foothill area of the Central Northern Bulgaria, at different altitudes, grasslands have been selected, representative of the most common, according to type, meadows and pastures in the area, namely:

1. *Chrysopogon gryllus* meadow is located in the area of Krapets dam (43°03′57″ N; 24°52′19″ E), 456 m above sea level, with an area of 10 ha, northeastern exposure at a slope about 10°. The soils are gray luvisols (FAO) with a very low supply of absorbable phosphorus (1.8 mg/100g soil) and weak of absorbable potassium (7.9 mg/100g soil) and mobile forms of nitrogen (8.6 mg/100g soil). The reaction of the soil is acidic (pH 4.3). The use of the meadow is combined - the first growth is mowed for hay (at the end of June), the second is used for grazing, in conditions of low loading;

2. Genista-pseudo sheep's fescue (*Festuca ovina - Festuca valesiaca*) pasture is located in the area of the village of Kramolin (43°08′N; 25°05′E), 385 m above sea level, with an area of 20 ha at a slope of about 15º, the grassland is not used. The soils are gray luvisols (FAO). The soil reaction is acidic (pH 5.2), the humus content is 2.3%, the stock with mobile phosphorus (2.5 mg/100g), and nitrogen (11.2 mg/100g) is low and with potassium is good (54.4 mg/100g).

3. Bermuda grass-ryegrass (*Cynodon dactylon - Lolium perenne*) pasture is located in a hilly area, municipal common pasture of the town of Pavlikeni (43°15′04″ N; 25°18′37″ E), 134 m above sea level, with an area of 10 ha, flat terrain, on leached chernozem soil-type. The soil reaction is neutral (pH 7.05), the humus content is 3-4%, the stock with mobile phosphorus (5.3 mg/100g), and nitrogen (23.0 mg/100g) is medium and with potassium is good (48.4 mg/100g).

During 2017-2019 and months May-June on the territory of study area the relative share of clover species in grassland was assessed by 10 strip transects (10/0.5 l. m) on the Uranov occurrence scale [35]. Scores are determined by the number of transects in which the species occurs as follows:

Species share in the grassland	Score
species present with some individuals	+
5% (present in 2 of 10 transects)	1
5-25% (present in 2-4 of 10 transects)	2
25-50% (present in 4-8 of 10 transects)	3
50-75% (present in 6-8 of 10 transects)	4
75-100% (present in 8-10 of 10 transects)	5

The areas in which the studied grasslands are located differ both in edaphic and climatic conditions. For the study period (2017-2019) the precipitation for the growing season in the mountainous region are from 221 to 277 mm more than those in the flat region - Table 1. The monthly rainfall in May, June and July were also significantly higher for the mountainous area.

Above-ground biomass of clovers, with a reported score for share in grassland above `2`, has been analyzed to determine the basic chemical composition. The samples were taken in the phenophase of bud formation and flowering of the first regrowth in the third experimental year (2019), according to the results of the species from the previous two years. The forage mass was dried at 60°C to constant weight and ground to a particle size of up to 1 mm. The chemical composition of the dry forage matter was analyzed, which includes: Crude protein (CP, %) according to *Kjeldahl*, as a percentage of dry matter (%, DM); Crude fiber (CF,%) according to AOAC [3]; Crude ash (CA,%) according to AOAC [2]; Ca (%) and P (%) contents were carried out by the procedures of AOAC [3]; Condensed tannins by the butanol HCl method (38).

RESULTS

The largest number of clover species (11 species) were observed in *Chrysopogon gryllus* meadow. Five of them were perennial and six - annual. Hungarian (*T. pannonicum* Jacq.), zigzag (*T. medium* L.) and red clovers (*T. pratense* L.) had a higher average share among the perennial species in the grassland. Crimson (*T. incarnatum* L.), field (*T. campestre* Schreb.) and hare's-foot clovers (*T. arvense* L.) were the annual species - Table 2.

Opposite results were found in terms of the number of species in Genista-pseudo sheep's fescue pasture (Table 3). Only the perennial species of white (*T. repens* L.) and red clover (*T. pratense* L.) were observed during the three experimental years. Their share in grassland is about 5%.

Seven species of clovers were found in Bermuda grass-ryegrass pasture, located at the lowest altitude. Three of them were perennial [red clover, white clover, strawberry clover *(T. fragiferum* L.)] and four were annuals [Prickly clover (*T. echinatum* M. Bieb.), field clover (*T. campestre* Schreb.), Persian clover (*T. resupinatum* L.), scukling clover (*T. dubium* Sibth.)] - Table 4. Prickly clover had the highest relative share (about 25%) in the grassland. Strawberry clover and Persian clover varied greatly from year to year.

The highest average score for the relative share of clovers was found in Bermuda grass-ryegrass pasture ("1.76") compared to "1.33" for *Chrysopogon gryllus* meadow and "1.10" for Genista-pseudo sheep's fescue pasture.

The highest share of clovers was in July in the first experimental year (2017) in the observed grasslands, while for 2018 and 2019 in June.

The ratio between the average scores for the share of perennial and annual species shows a slight predominance in favour of perennial clover, both in the mountain meadow (1.36 / 1.30) and in the plain pasture (1.86 / 1.67).

Significant differences in the main chemical composition of aboveground biomass were observed among the analyzed species (Tables 5 and 6).

The highest crude protein content in the dry matter of clovers was found in perennial species zigzag clover (*T. medium* L.) (18.08%) and alsike clover (*T. hybridum* L.) (17.99%) in *Chrysopogon gryllus* meadow. The lowest values of this qualitative indicator were found in the annual *T. pallidum* Waldst. & Kit.(11.89%). Species in the grassland showed significant differences in crude fiber content. Its values varied from 16.67% (alsike clover) to 34.19% (zigzag clover) with a difference of 17.52 percentage units. The quantitative ratio of crude protein / crude fiber, assessed as the main indicator of quality and nutritional value of grass biomass, also variedwidely from 1 / 0.93 (alsike clover) to 1 / 2.63 (*T. pallidum* Waldst. & Kit.). The most favourable ratio of both fractions in *Chrysopogon gryllus* meadow was observed in crimson clover (1 / 1.35) and scukling clover (1 / 1.42).

Table 1. Monthly precipitation (mm) for the vegetation period (April-October) during the experimental years (mm)

Experimental years	Above sea level	IV	V	VI	VII	VIII	IX	X	Rainfall amount in April-October
2017	520 m	90.4	133.1	113.2	186.6	13.2	38.9	126.3	701.7
	144 m	58.5	82.4	37.8	98.9	8.0	21.5	117.5	424.6
2018	520 m	22.8	82.5	174.3	241.1	9.4	30.0	56.2	616.3
	144 m	10.3	44.3	134.6	126.6	12.6	49.2	17.6	395.2
2019	520 m	106.9	82.4	234.6	106.7	37.7	21.9	48.0	638.2
	144 m	94.4	41.8	149.0	77.8	8.6	2.4	13.0	387.0

Table 2. Species of Genus *Trifolium* in *Chrysopogon gryllus* meadow

Species	2017			2018			2019			Mean	CV, %
	May	June	July	May	June	July	May	June	July		
Trifolium repens L.	1	1	+	1	1	1	1	2	1	1.1	35
Trifolium pratense L.	1	2	+	1	2	1	1	2	1	1.4	52
Trifolium hybridum L.	1	1	+	1	1	1	1	2	2	1.3	46
Trifolium medium L.	1	2	+	1	2	2	1	1	1	1.4	52
Trifolium pannonicum Jacq.	1	2	2	1	2	2	1	1	2	1.6	53
Trifolium pallidum Waldst.& Kit.	2	1	1	2	2	1	1	1	1	1.3	50
Trifolium incarnatum L.	2	2	+	2	1	1	2	2	-	1.7	53
Trifolium dubium Sibth.	1	1	+	+	+	1	1	1	1	1.0	0
Trifolium campestre Schreb.	1	1	2	+	2	1	2	2	1	1.4	53
Trifolium arvense L.	1	1	+	1	1	-	1	1	-	1.4	0
Trifolium striatum L.	1	1	+	-	-	1	-	-	+	1.0	0
Mean	1.2	1.4	1.7	1.3	1.5	1.2	1.2	1.5	1.3	**1.33**	**35.8**

Table 3. Species of Genus *Trifolium* in Genista-pseudo sheep's fescue pasture

Species	2017			2018			2019			Mean	CV, %
	May	June	July	May	June	July	May	June	July		
Trifolium repens L.	1	1	1	1	1	1	1	2	1	1.1	30
Trifolium pratense L.	1	1	1	1	1	1	1	2	1	1.1	30
Mean	1.0	1.0	1.0	1.0	1.0	1.0	1.5	2.0	1.0	**1.10**	**30.0**

Table 4. Species of genus *Trifolium* in Bermuda grass-ryegrass pasture

Species	2017			2018			2019			Mean	CV, %
	May	June	July	May	June	July	May	June	July		
Trifolium repens L.	1	1	1	3	2	1	2	2	3	1.8	83
Trifolium pratense L.	1	1	1	3	2	1	3	2	2	1.8	83
Trifolium fragiferum L.	1	+	+	2	+	+	1	3	3	2.0	100
Trifolium echinatum M. Bieb.	1	2	3	2	3	3	2	3	3	2.4	73
Trifolium campestre Schreb.		2	2	2	3	2		2	2	2.1	38
Trifolium dubium Sibth.	1	1	+	2	-	1	1	+	1	1.2	0
Trifolium resupinatum L.	+	-	-	1	-	-	1	1	-	1.0	0
Mean	1.0	1.4	1.8	2.1	2.5	1.6	1.7	2.2	2.3	**1.76**	**53.9**

Table 5. Basic chemical composition of species in genus *Trifolium* in *Chrysopogon gryllus* meadow

Species / Indicator	*Trifolium hybridum* L.	*Trifolium incarnatum* L.	*Trifolium medium* L.	*Trifolium pallidum* Waldst.& Kit.	*Trifolium campestre* Schreb.	*Trifolium dubium* Sibth.
Crude protein (CP), %	17.99	15.62	18.08	11.89	12.26	17.51
Crude fibers (CF), %	16.67	21.16	34.19	31.31	30.13	24.81
CP / CF	1 / 0.93	1 / 1.35	1 / 1.89	1 / 2.63	1 / 2.46	1 / 1.42
Ca, %	1.66	1.41	2.10	1.37	0.90	1.19
P, %	0.47	0.43	0.21	0.32	0.37	0.45
Ca / P	3.53 / 1	3.28/ 1	10.00 /1	4.28 / 1	2.43 / 1	2.64 / 1
Condensed tannins, %	1.52	1.53	2.37	1.53	3.12	1.33

Table 6. Basic chemical composition of species in genus *Trifolium* in Bermuda grass-ryegrass pasture

Species / Indicator	*Trifolium fragiferum* L.	*Trifolium echinatum* M. Bieb.	*Trifolium campestre* Schreb.
Crude protein, %	21.61	16.80	12.70
Crude fibers, %	26.47	23.38	28.20
CP/CF	1 / 1.22	1 / 1.39	1 / 2.22
Ca, %	2.77	1.62	1.02
P, %	0.22	0.57	0.55
Ca / P	12.59 / 1	2.84 / 1	1.85 / 1
Condensed tanins, %	2.34	1.88	3.31

The content of Ca was in the range of 0.90 (field clover) - 2.10% (zigzag clover), and that of phosphorus from 0.21 (zigzag clover) to 0.47% (alsike clover).

The highest content of crude protein was found in the plain pasture with strawberry clover (*T. fragiferum* L.) (21.61%) and Prickly clover (*T. echinatum* M. Bieb.) (16.80%), as well as with optimal values of protein / fiber ratio (1 / 1.22 and 1 / 1.39 respectively). The species of clover analyzed in this grassland have less pronounced variability in crude fiber content. In contrast, the interspecific differences in the amount of macrominerals are more pronounced than those in *Chrysopogon gryllus* meadow. The content of Ca and P was from 1.02 (field clover) to 2.77% (strawberry clover) and from 0.22 (zigzag clover) to 0.57% (alsike clover), respectively.

The biomass of field clover (*T. campestre* Schreb.) (found in the plains and mountains) had the highest content of condensed tannins (3.31-3.12%). For the species, differences were observed in favour of the populations from the plain pasture in the content of calcium (by 11.4%) and phosphorus (by 32.4%).

DISCUSSION

The results observed are primarily related to the ecological characteristics of the species. Red (*T. pratense* L.) and white clovers (*T. repens* L.) are highly adaptable [9, 32], which explains their presence in all observed grasslands, regardless of differences in edaphic and climatic conditions. At the same time, the share of these two species is markedly higher in the plain pasture, which can be explained by higher levels of phosphorus content in the soil and acidity close to pH = 5-6, indicated as optimal for these species [7]. Hare's-foot clover (*T. arvense* L.), knotted clover (*T. striatum* L.) and scukling clover (*T. dubium* Sibth.) were found in *Chrysopogon gryllus* meadow, which according to Maxwell et al. [25] surpass white clover in yield and share in pasture grasslands on poor phosphorus and acid soils. Under such soil conditions and in mixed grasslands, white clover has low competitiveness (18), which may be related to the lower presence of the species observed in Chrysopogon gryllus meadow, located on light gray soils. According to results for the share by years, field clover (*T. campestre* Schreb.) registered high adaptability in the mountain meadow and the plain pasture. Scoppola et al. [32] also reported that this species is currently among the most widespread clover species in southern Europe.

The greater species diversity of *Chrysopogon gryllus* meadow, developed on poorer soils, is in line with the studies of Plantureux et al. [31].

In a number of studies, the annual and seasonal fluctuations in the share of clover in natural and semi-natural grasslands are explained by differences in rainfall [9, 15, 25]. According to the current results, this connection is direct, but only for the plain pasture grassland. At the same time, in habitats with a very strong variation of moisture supply (such as Genista-pseudo sheep's fescue pasture), clover has no role as an element of the functional group of legumes. This grassland is developed on a sloping southern terrain with rapid runoff of surface water. This reduces the possibility of soil moisture and water retention, which explains the small number of identified species of clover, as well as their stable low relative share. The niche of legume components in the grassland is occupied by species indicative of dry habitats - *Astragalus onobrychis* L., *Medicago minima* (L.) Bartal, *Onobrychis alba* (W. et K.) Desv.

The comparison of the results shows that the share of species is also influenced by the specific ways of using the grassland in the present study. Haymaking results in significantly greater species diversity [13], which was in the *Chrysopogon gryllus* meadow in the present study too. White clover (*T. repens* L.) had also a lower share in *Chrysopogon gryllus* meadow compared to red clover (*T. pratense* L.), while both species had an equal share in Bermuda grass-ryegrass pasture. This may be related to the intensive use of the pasture, which affirms white clover because of its pasture persistence and open reproductive system [37]. The high share of strawberry clover (*T. fragiferum* L.) in the grassland can also be attributed to grazing tolerance based on a vegetative system of reproduction, as in the case of white clover. The effect of the way of using the grasslands can be connected with the more stable share of field clover (*T. campestre* Schreb.) in the plain pasture in terms of years and seasons, compared to the mountainous *Chrysopogon gryllus* meadow. This species has a rapid and vigorous growth after grazing and repeated seed formation, which maintain the populations by self-sowing. According to Ghassali et al. [10] seeds of this species have low digestibility when ingested by animals, which also maintains the spread of field clover in the grassland. In the study by Seydosoglu et al. [33] this annual species has the highest relative share (11.48%) among six clovers found in dry pasture with moderate load.

In contrast, the late hay use of *Chrysopogon gryllus* meadow is a factor determining the observed high share of crimson clover (*T. incarnatum* L.) in the composition of the grassland. The species is autogamic, with very early development during the vegetation season, which allows its self-sowing before or during mowing. Zigzag clover (*T. medium* L.) also responds with a higher share in grassland in late haymaking or if it is not mowed annually [19], which is also observed in the present study.

According to Li et al. [24], grazing use favours small-seeded, low-growing species, which is confirmed by the present results. The largest share of clovers with upright growth habitus [(Hungarian (*T. pannonicum* Jacq.), zigzag (*T. medium* L.), red (*T. pratense L.*) and crimson clovers (*T. incarnatum* L.)] was found in *Chrysopogon gryllus* meadow, while in the plain pasture predominated clovers that formed grassland

with small height [field clover (*T. campestre* Schreb.) and Prickly clover (*T. echinatum* M. Bieb.)].

In dry habitats, genus *Trifolium* is thought to be predominantly represented by annual autogamous species because stress conditions reinforce this reproductive system [29]. In the present study, almost equal share of:

- annuals compared to perennial clover;
- allogamic versus autogamic species;
- species with a closed system of reproduction (only with seeds) compared to those with an open (simultaneously with seeds and stolons or rhizomes - white (*T. repens* L.), strawberry (*T. fragiferum* L.), and zigzag clovers (*T. medium* L.).

The large natural distribution of some of the observed species of clover [Hungarian (*T. pannonicum* Jacq.), crimson (*T. incarnatum* L.), Prickly (*T. echinatum* M. Bieb.) and field (*T. campestre* Schreb.)] implies excellent adaptability and therefore potential for inclusion (by artificial sowing) in the functional group of legumes in different types of grasslands. Results for the introduction of Hungarian clover (*T. pannonicum* Jacq.) in semi-natural grasslands in Western Siberia, as a high-yielding, long-lasting species, resistant to diseases and pests are presented by Gripas [12], Krasnoperov [21] and Bogolyubova [6]. Prickly clover (*T. echinatum* M. Bieb.) and field clover (*T. campestre* Schreb.) have been identified by Abbasi et al. [1] as short-lived drought-resistant species with forage potential in artificial sowing after a study in different climatic regions of Iran.

Grass communities respond to changes in the environment both through species turnover and through intraspecies biological and morphological changes [42]. A difference in the biological characteristics of the populations with which the individual species are represented in the studied grasslands is also observed in the present study. The highest share of red clover in Bermuda grass-ryegrass pasture was in May, and in the grassland of *Chrysopogon gryllus* meadow in June and during the three years of research. These results correspond to other Bulgarian studies, according to which in the plains and hilly regions of Northern Bulgaria red clover (*T. pratense* L.) is represented by early-flowering, fast-growing genotypes that have their maximum development in May [28].

For some of the identified species [crimson clover (*T. incarnatum* L.) Prickly clover (*T. echinatum* M. Bieb.) and field clover (*T. campestre* Schreb.)] the share in grassland was over 25% in the individual reports. Based on this, they significantly affect the biological and forage value, as well as the ecology of grasslands. The balanced basic chemical composition is of key importance for determining the potential of wild clover species and for their use in artificial sowing under the conditions of Northern Bulgaria.

The differences in chemical composition are a function of the specific characteristics of legumes regarding the extraction and accumulation of nutrients from the soil and from their nitrogen-fixing activity. The morphological composition of the formed biomass of each species also leads to significant differences in the amount of crude protein and fiber [39]. Evaluated by the main criteria for feed quality (protein content and quantitative ratio of crude protein / crude fiber), the species strawberry clover (*T. fragiferum* L.), crimson clover (*T. incarnatum* L.) and Prickly clover (*T. echinatum* M. Bieb.) are compared with alfalfa, which is used as a benchmark for feed quality - 1:1.22 [34]. The high content of fiber components observed in the present study in zigzag clovers (*T. medium* L.) and field clover (*T. campestre* Schreb.) suggests a higher share of stem fraction in the formed biomass and lower digestibility compared to cultivated clover species.

The mineral content of meadow grasses depends on the presence of minerals in the soil, climatic conditions, and phenological development [22]. High content of Ca was observed for all analyzed species, which is characteristic for all clovers, with the exception of field clover, which leads to significantly higher than optimal values for Ca / P ratio. Mineral imbalance with potentially adverse effect on metabolic processes in ruminants [16] was observed for *T. medium* L., *T. pallidum* Waldst. & Kit. and *T. fragiferum* L.

A higher soil phosphorus content can also explain the increased content of the element in the biomass of clover from the plains. An exception was observed for strawberry clover (*T. fragiferum* L.). At the same time, high values of P content have also been observed for values from *Chrysopogon gryllus* meadow (*T. incarnatum* L., *T. hybridum* L., *T. dubium* Sibth.) which may be associated with genetic adaptation to a deficiency of this macronutrient in the soil. The high content of crude protein in these species is also evidence of good nitrogen fixation in acidic soils with insufficient absorption of phosphorus.

The protein nutrition of the fodder was assessed by the protein digested in the small intestine. In this regard, the content of secondary metabolites and in particular condensed tannins has a clear biological activity associated with the prevention of swelling in ruminants. Field clover (3.12-3.31%) had an optimal tannin content among all analyzed species.

REFERENCE

[1] Abbasi, M.R., Hassanzadeh, A., Mahdipour, A., Anahid, S., Safari, S., (2019): Forage yield in some Iranian wild *Trifolium* genetic resources under different climatic and irrigation conditions. Journal of Agricultural Science and Technology, 21(4): 993-1004.

[2] AOAC, (1990): Official Methods of Analysis of AOAC International. 15th Edition. 1990, Association of Official Analytical Chemists, USA. Ed.: K. Helrich, Animal Feed, pp. 69-84.

[3] AOAS, (2016): Official Methods of Analysis of AOAC International, 20th Edition, 2016, Maryland, USA, Ed.: Dr. George W. Latimer, Jr., 4. Animal Feed, pp. 1-44

[4] Ates, E., (2011): Some chemical and morphological properties of five clover species (*Trifolium* sp.) at different aspect of pasture in Belovets village (Razgrad),

Bulgaria. International Journal of Plant Production, 5(3): 255-262.

[5] Berauer, B.J., Wilfahrt, P.A., Arfin-Khan, M.A., Eibes, P., Von Heßberg, A., Ingrisch, J., Schloter, M., Schuchardt, M.A., Jentsch, A., (2019): Low resistance of montane and alpine grasslands to abrupt changes in temperature and precipitation regimes. Arctic, Antarctic, and Alpine Research, 51(1): 215-231.

[6] Bogolyubova, E.V., (2019): Invasive activity of *Trifolium pannonicum* Jacq. in the forest-steppe of the Priobya region. Problems of botany of Southern Siberia and Mongolia, 1(18): 206-209.

[7] Caddel, J.L., Zhang, H., Wise, K., (2004): Responses of alfalfa, red clover, and white clover to soil pH and lime treatments. Forage and Grazinglands, 2(1): 1-8.

[8] Chipev, N., (2002): Biological diversity - an evolving concept. Journal of the Bulgarian Academy of Sciences, 4: 6-11.

[9] Dujmović Purgar, D., Sraka, M., Gunjača, J., Jelaska, S.D., Škvorc, Ž., Bolarić, S., (2020): Ecogeographical characteristics of red clover local population sites in north-western part of Croatia. Journal of Central European Agriculture, 21(1): 135-150.

[10] Ghassali, F., Osman, A.E., Cocks, P.S., (1998): Rehabilitation of degraded grasslands in north Syria: the use of Awassi sheep to disperse the seeds of annual pasture legumes. Experimental Agriculture, 34(4): 391-405.

[11] Goranova, V., Vassilev, K., Pedashenko, H., (2013): Vascular flora of the valley of Mesta river floristic region, SW Bulgaria. Phytologia Balcanica, 19(1): 89-114.

[12] Gripas, M.N., Arzamasova, E.G., Popova, E.V., (2017): Introduction of Pannonian clover (*Trifolium pannonicum* Jacg.) in the conditions of the European northeast of Russia. pp. 108-110. In Current and new directions in selection and seed production of agricultural crops. Reports of scientific session, Vladikavkaz, February 18, 2017.

[13] Hansson, M., Fogelfors, H., (2000): Management of a semi-natural grassland; results from a 15-year-old experiment in southern Sweden. Journal of vegetation science, 11(1): 31-38.

[14] Hirano, R., (2005): Ecogeographic and genetic survey of white clover (*Trifolium repens* L.) on St Kilda. School of Biosciences University of Birmingham Birmingham, UK, pp. 26-78.

[15] Hossain, M.L., Beierkuhnlein, C., (2018): Enhanced aboveground biomass by increased precipitation in a central European grassland. Ecological Processes, 7(1): 37.

[16] Hovi, M., Sundrum, A., Thamsborg, S.M., (2003): Animal health and welfare in organic livestock production in Europe: current state and future challenges. Livestock Production Science, 80: 41-53.

[17] Janssens, F., Peeters, A., Tallowin, J.R.B., Bakker, J.P., Bekker, R.M., Fillat, F., Oomes, M.J.M., (1998): Relationship between soil chemical factors and grassland diversity. Plant and soil, 202(1): 69-78.

[18] Jouany, C., Cruz, P., Petibon, P., Duru, M., (2004): Diagnosing phosphorus status of natural grassland in the presence of white clover. European Journal of Agronomy, 21(3): 273-285.

[19] Köhler, B., Gigon, A., Edwards, P.J., Krüsi, B., Langenauer, R., Lüscher, A., Ryser, P., (2005): Changes in the species composition and conservation value of limestone grasslands in Northern Switzerland after 22 years of contrasting managements. Perspectives in Plant Ecology, Evolution and Systematics, 7(1): 51-67.

[20] Komainda, M., Küchenmeister, K., Küchenmeister, F., Breitsameter, L., Wrage-Mönnig, N., Kayser, M., Isselstein, J., (2019): Forage legumes for future dry climates: Lower relative biomass losses of minor forage legumes compared to *Trifolium repens* under conditions of periodic drought stress. Journal of Agronomy and Crop Science, 205(5): 460-469.

[21] Krasnoperov, A.G., Buyankin, N.I., Chekster, N.Y., (2018): New clover species (*Trifolium pannonicum* Jacq.) for feed production in the Kaliningrad region. Fodder Production, 7: 25-31.

[22] Kuusela, E., (2006): Annual and seasonal changes in mineral contents (Ca, Mg, P, K and Na) of grazed clover-grass mixtures in organic farming. Agricultural and Food Science, 15(1): 23-34.

[23] Lepš, J., Šmilauerová, M., Šmilauer, P., (2019): Competition among functional groups increases asynchrony of their temporal fluctuations in a temperate grassland. Journal of Vegetation Science, 30(6): 1068-1077.

[24] Li, W., Tian, F.P., Ren, Z.W., Huang, H.Z., Zhang, Z.N., (2013): Effects of grazing and fertilization on the relationship between species abundance and functional traits in an alpine meadow community on the Tibetan Plateau. Nordic Journal of Botany, 31(2): 247-255.

[25] Maxwell, T.M., Moir, J.L., Edwards, G.R., (2016): Grazing and soil fertility effect on naturalized annual clover species in New Zealand high country. Rangeland ecology & management, 69(6): 444-448.

[26] Maxwell, T.M.R., Moir, J.L., Edwards, G.R., (2012): Sulphur and lime response of four adventive annual clovers grown in a New Zealand high country soil under glasshouse conditions. New Zealand Journal of Agricultural Research, 55: 47-62.

[27] Moir, J., Jordan, P., Moot, D., Lucas, R., (2016): Phosphorus response and optimum pH ranges of twelve pasture legumes grown in an acid upland New Zealand soil under glasshouse conditions. Journal of soil science and plant nutrition, 16(2): 438-460.

[28] Naydenova, G., Vasileva, V., (2019): Comparative evaluation of diploid and tetraploid red clover genotypes in a flat area of Northern Bulgaria. Journal of Central European Agriculture, 20(3): 919-927.

[29] Nosrati, H., Feizi, M.H., Razban-Haghighi, A., Seyed-Tarrah, S., (2015): Impact of life history on genetic variation in *Trifolium* (*Fabaceae*) estimated by ISSR. Environmental and Experimental Biology, 13: 83-88.

[30] Pederson, G.A., Quesenberry, K.H., Smith, G.R., Guteva, Y.K., (1999): Collection of *Trifolium* sp. and other forage legumes in Bulgaria. Genetic Resources and Crop Evolution, 46(4): 325-330.

[31] Plantureux, S., Peeters, A., McCracken, D., (2005): Biodiversity in intensive grasslands: Effect of management, improvement and challenges. Agronomy research, 3(2): 153-164.

[32] Scoppola, A., Tirado, J.L., Gutiérrez, F.M., Magrini, S., (2018): The genus *Trifolium* (*Fabaceae*) in south Europe: a critical review on species richness and distribution. Nordic Journal of Botany, 36(1_2), njb-01723.

[33] Seydosoglu, S., Cacan, E., Sevilmis, U., (2019): Determination of botanical composition, yield and pasture quality ratings of infertile pastures in kozluk district of Batman province of Turkey. Fresenius environmental bulletin, 28(4A): 3388-3394.

[34] Stanisavljević, R., Milenković, J., Đokić, D., Štrabanović, R., Vasić, T., (2008): Yield, yield components and forage quality of alfalfa varieties and their correlation dependence. Journal of Mountain Agriculture on the Balkans, 11(5): 896-908.
[35] Stoyanov, K., (2013): Lecture notes on phytocenology. Agricultural University Plovdiv, Academic Press. Version on-line: https://botanica.gallery/wp/
[36] Stoyanov, K., Naydenova, G., Yancheva, Ch., (2018): Atlas Forage leguminous plants in Bulgaria. Agricultural University Plovdiv Academic Press. Version on-line ISBN 978-954-517-268-7. https://botanica.gallery/wp/archives/19645.
[37] Taylor, N., (2008): A century of clover breeding developments in the United States. Crop Science, 48: 1-13.
[38] Terrill, T.H., Rowan, A.M., Douglas, G.B., Barry, T.N., (1992): Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. Journal of the Science of Food and Agriculture, 58(3): 321-329.
[39] Uslu, O., Kurt, O., Kaya, E., Kamalak, A., (2018): Effect of species on chemical composition, metabolizable energy, organic matter digestibility and methane production of some legume plants grown in Turkey. Journal of applied animal research, 46(1): 1158-1161.
[40] Vateva, V., Stoeva, K., Pavlov, D., (2014): Botanical composition of the main pasture types in Sakar and the Strandja region. Agricultural Science & Technology, 6(1): 72-75.
[41] Velev, N., Vassilev, K., (2014): Management regimes within syntaxa of semi-natural grasslands in West Bulgaria. Hacquetia, 13(1): 191-204.
[42] Volf, M., Redmond, C., Albert, Á.J., Le Bagousse-Pinguet, Y., Biella, P., Götzenberger, L., Šebelíková, L., (2016): Effects of long-and short-term management on the functional structure of meadows through species turnover and intraspecific trait variability. Oecologia, 180(4): 941-950.
[43] Yancheva, Ch., (1986): Study of annual clovers in meadows, pastures and natural grassland in Strandzha mountain. PhD Thesis, Agricultural University – Plovdiv.

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