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WILD ALFALFA IN THE SEMI-NATURAL GRASSLANDS OF CENTRAL NORTHERN BULGARIA

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Abstract

The study was conducted in the Central Northern Bulgaria in order to establish some biological, morphological and qualitative characteristics of wild species of genus Medicago. Medicago arabica (annual species) and Medicago falcata (perennial species) have the highest share in grassland, with significant seasonal productivity and feed quality. Medicago arabica dominates in spring and Medicago falcata in summer. Medicago falcata fodder is the richest in protein (25.32%) and has the most favourable ratio of crude protein and crude fiber. Compared to other short-lived species, Medicago falcata has lower levels of minerals and calcium. The presence of the fast-growing, drought-resistant species Medicago minima and Medicago polymorpha may be associated with the observed spring and late droughts in the study areas. The concentration of crude fiber (38.39%), crude fat (4.94%), acid-detergent lignin (10.98%), calcium (2.97%) and phosphorus (0.33%) is predominant in the biomass of Medicago polymorpha. The dry matter of the species has the lowest in vitro digestibility (80.71%) and hemicellulose concentration (4.61%). Medicago arabica has the lowest content of crude fiber (25.09%) and the highest of crude ash (13.45%), and acid-detergent fiber (27.90%). Compared to Medicago polymorpha (38.39%), the fiber fraction in the composition of the species is 53.0% lower. Medicago minima and Medicago lupulina registered an insignificant difference in the values of the indicators characterizing the fiber components of the cell walls and in vitro digestibility of the dry matter. The species have a low content of ADL (6.24-6.26%) and the highest digestibility of dry matter (84.39-84.41%). Medicago minima has the lowest value for the amount of crude protein (19.86%) and the highest for the content of hemicellulose (11.82%).

Key words*: Medicago spp., morphological characteristics, chemical composition.*

INTRODUCTION

Bulgaria is extremely rich in local plant material of legume forage grasses, including alfalfa. This implies excellent adaptability and potential for the use of these species as forage crops to improve soil fertility. Wild forage grasses provide a fuller realization of the ecological resources in the semi-natural grasslands, and the monitoring of their share is an important indicator for the change of the habitats and the functioning of the meadow ecosystems.

Alfalfa fields are highly productive and important contributors to the biodiversity of agricultural systems (Putnam et al., 2001; Georgieva, N., & Nikolova, 2015; Marinova et al., 2019; Vasileva et al., 2020). The best known member of the genus *Medicago* is *Medicago sativa* (an important forage crop) with superior traits such as cold resistance, salt tolerance, wide adaptability, high yield, good herbage quality, resistance to frequent cutting, good persistence, soil amelioration, and economic benefit (Veronesi et al., 2010; Shi et al., 2017). *Medicago sativa* achieves a high rooting rate in deep and hard soil layers, forms soil macropores that improve soil aeration and structure (Peoples et al., 2009), and *Medicago polimorpha* and *Medicago intertexta* species are drought tolerant, making them suitable for growing in dry and semi-dry areas (Bhandaria et al., 2020). *Medicago falcata* is one of the stress-tolerant candidate leguminous species and is able to fix atmospheric nitrogen. This ability allows leguminous plants to grow in nitrogen deficient soils. *Medicago species* synthesize a variety of bioactive natural products that are used to engage into symbiotic interactions but also serve to deter pathogens and herbivores (Gholami et al., 2014). The genus *Medicago* is an important socio-economic resource that offer novel genetic diversity required to maintain future food security and the changing climate conditions (Kell et al., 2008; Desheva et al., 2016;

Marinova, 2020a, b). The significance of wild alfalfa as a model plant for genomic research and, accordingly, for the identification of genes associated with stress resistance in the most important grass-forage crops (Iantcheva & Revalska, 2018) is of great importance.

The objective of the present study was to determine the share, main biological and morphological characteristics, as well as the quality of forage biomass of genus *Medicago*, found in mesophytic grassland, typical for the plains and hilly regions of Central Northern Bulgaria.

MATERIALS AND METHODS

The studied mesophytic grass cover is predominantly used for unregulated grazing. It is located in a hilly region (43°15′04″N; 25°18′37″E), with an average altitude of 112–134 m, near the Rositsa river valley. The climate is continental, the average annual rainfall is 544 mm, and the average rainfall during vegetation is 367 mm, with high annual fluctuations. For the study period (2018-2020) the share for the vegetation season in the region are shown in Figure 1. Spring droughts, as well as late summer and autumn droughts, were observed in all three experimental years.

The soil reaction is neutral (pH 7.05), the humus content is 3-4%, the level of mobile phosphorus (5.3 mg/100 g), and nitrogen (23.0 mg/100 g) is medium and with potassium is good (48.4 mg/100 g).

During 2018-2020 on months April - July on the territory of study area the relative share of *Medicago* in grassland was assessed by 10 strip transects (10/0.5 m) on the Uranov occurrence scale (Stoyanov, 2013). Scores are determined by the number of transects in which the species occurs (Table 1).

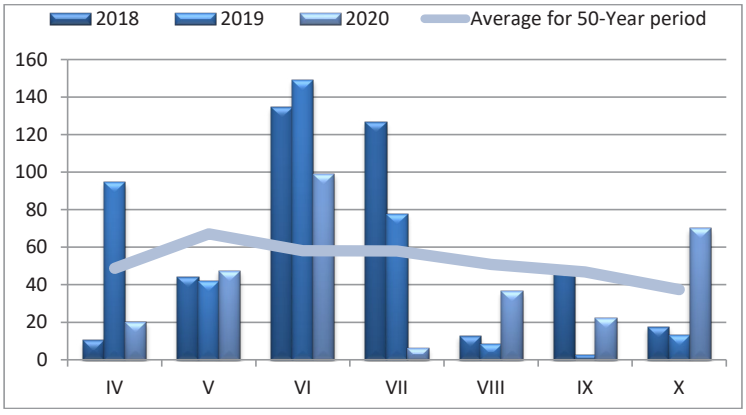

Figure 1. Monthly precipitation (mm) for the vegetation period (April-October) during the experimental years (mm)

Table 1. Scores are determined by the number of transects in which the species occurs

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

To monitor the indicators, such as data and synchronization of blossoming, height/ length of generative stems (cm), dry matter content, as well as to assess the forage quality of biomass from the identified species the samples were taken in the phenophase of bud formation and flowering of the first regrowth in the third experimental year (2020). 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 (2016); Crude ash (CA, %) according to AOAC (1990); Crude fat (Cft, %) by extraction in a *Soxhlet* type extractor and drying in a laboratory oven at 95°C to constant weight (according to BDS/ISO-6492); Ca (%) and P (%) contents were carried out by the procedures of AOAC (2016). The fibrous structural elements in the plant cell are analyzed in laboratory: Neutral Detergent Fibers (NDF, %); Acid detergent fiber (ADF, %) and Acid detergent lignin (ADL, %) by the Van Soest & Robertson (1979) detergent assay and *in vitro* dry matter digestibility (IVDMD) according to a two-way pepsin-cellulase method of Aufrere (1982). The polyosides are empirically calculated: Hemicellulose (%) = NDF - ADF and Cellulose (%) = ADF - ADL. The lignification degree is expressed as the percentage of ADL and NDF.

RESULTS AND DISCUSSIONS

The following 4 short-lived alfalfa species were identified in the grassland: *M. arabica, M. minima, M. polymorpha* and *M. lupulina*. Two medium-long-term species were observed: *M. falcata* and *M. sativa*.

For the study period, the relative share in grassland of *M. arabica* and *M. falcata* was the highest (Table 2). Both species had equal average scores, with *M. arabica* being the dominant legume species in spring and *M. falcata* in summer, with a corresponding relative share in the grassland of about 25%. Regarding the share of both species, the highest seasonal and annual fluctuations were observed (SD = 0.98 and SD = 0.97). The annual species *M. polymorpha* is characterized by the most stable share in grassland over the years.

Table 2. Monthly and average scores for the share of Species of Genus *Medicago* in grassland studies

Species	2018 yr				2019 yr				2020 yr				Mean	SD
	IV	V	VI	VII	IV	V	VI	VII	IV	V	VI	VII		
M. arabica	3	3	-	-	3	2	-	-	1	1	-	-	2.17	0.98
M. minima	2	2	-	-	1	1	-	-	-	1	2	-	1.50	0.55
M. polymorpha	2	2	-	-	1	2	-	-	2	2	-	-	1.83	0.41
M. lupulina	1	2	1	-	1	2	1	-	1	2	2	-	1.44	0.53
M. falcata	-	1	3	2	-	1	3	3	-	1	3	3	2.22	0.97
M. sativa	-	1	1	1	-	1	2	2	-	1	1	1	1.22	0.44
	2.0	1.8	1.7	1.5	1.5	1.5	2.0	2.5	1.3	1.3	2.0	2.0		

The most early-ripening species among all established is *M. minima* (small seeds) - Table 3. The annual species enters the bud-formation-blossoming period in the last ten days of April, and in mid-May in the phenophase of pod formation.

M. minima has a spreading habitus and short generative stems, due to which its forage potential is insignificant, despite the distinct share of the species in the grassland and the established very high dry matter content during spring growth. The species is of interest as an early and fast-growing legume component of grassland, with a seasonal role in trophic relationships of the ecosystem (Zhang et al., 2013; Song et al., 2017).

The other two annual species, such as *M. arabica* and *M. polymorpha* are equal both in phenological development and in dry matter content in their fresh biomass.

M. lupulina is a biennial species and has the slowest development among the established short-lived alfalfa.

It is characterized by a spreading habitus and high values in terms of dry matter content in the studied grassland. The perennial species *M. falcata* is characterized by the latest and slowest development, as it entered the bud-formation period-beginning of blossoming in the period 5-15 July. It formed medium-tall, semi-spreading plants. It is characterized by uneven growth of generative stems and consequently with prolonged blossoming (until the end of July).

Table 3. Biological and morphological characteristics of the identified species of Genus *Medicago*

Species	Beginning of blossoming	Height/length of generative stems, cm	Growing habitus	Dry matter content, %
M. arabica	1-10 V	55.4	semi-spreading	19.75
M. minima	22-30 IV	25.7	spreading	28.57
M. polymorpha	1-10 V	32.5	semi-upright	19.45
M. lupulina	20-30 V	45.6	spreading	26.07
M. falcata	5-15 VI	58.6	semi-spreading	24.25
M. sativa	5-15 V	75.4	upright	23.40

The dry matter content in the beginning of blossoming was high (24.25%). These biological features determine the pasture suitability of the species and the interest in it for the improvement of cultivated alfalfa through interspecific hybridization (Riday & Brummer, 2006).

The presented results for the share of the species can be related to the specific way of using the grassland for the present study. In Bulgaria, mesophytic meadows located near settlements are used for unregulated grazing, and are often overgrazed. This establishes species with high grazing sustainability, such as *M. arabica* and *M. falcata*, as well as the fast-growing species of *M. minima*, which maintains its populations by self-sowing (early summer). According to Li et al. (2013) pasture use favors low-growing or semi-spreading species, which is confirmed by our results. The effect of the way the grassland is used can be related to the low share of the cultivated species of alfalfa, which is characterized by low grazing sustainability and upright growing habitus.

Medicago minima resides in various climatic environments, namely semi-arid and Mediterranean climates, and they are the most abundant annual *Medicago* plants (Kabtni et al., 2020). *Medicago polymorpha* also shows drought tolerance (Kirilov et al., 2016). Drought resistance may be associated with higher scores of these species for grassland share in the first and third experimental years, when severe spring droughts were observed.

Among the short-lived alfalfa species, *M. polymorpha* is characterized by the highest content of crude protein, crude fiber, cude fat, calcium and phosphorus (Table 4). The excess in the values of the indicated indicators in percentage units was respectively from 0.7 to 2.9 (for CP), from 7.1 to 13.3 (for CFr), from 0.91 to 1.52 (for CF), from 0.05 to 0.71 (for Ca) and from 0.12 to 0.19 (for P). Kamali et al. (2020) also define *M. polymorpha* as good enough for grazing specially at vegetative and blossoming stages. NDF and ADF content of plants increased with the growth but their CP, Ash, P, K, Cu, DMD, OMD, DOMD content and Ca decreased. The quantitative ratio of crude protein/crude fiber, assessed as the main indicator of quality and nutritional value of grass biomass in the studied short-lived wild species varied from 1/1.21 to 1/1.62. The most favorable ratio was observed for *M. arabica* (1/1.21). These results support the finding by Genç Lermi and Palta (2016) that the species have high agronomic potential than other annual legumes. *M. arabica* can fix nitrogen about 12 kg da^{-1} per year, improve soil properties, to assist feed requirements and to be used land as alternative legumes.

Table 4. Basic chemical composition (%) of Species in Genus *Medicago*

Species Indicator	*M. arabica*	*M. minima*	*M. polymorpha*	*M. lupulina*	*M. falcata*
Crude protein (CP)	20.78	19.86	23.66	22.94	25.32
Crude fiber (CF)	25.09	30.76	38.39	31.34	29.53
CP / CF	1 / 1.21	1 / 1.55	1 / 1.62	1 / 1.37	1 / 1.17
Calcium (Ca)	2.75	2.92	2.97	2.26	2.24
Phosphorus (P)	0.14	0.16	0.33	0.21	0.21
Ca / P	19.6 / 1	18.3 / 1	9.0 / 1	10.8 / 1	10.7 / 1
Crude ash (CA)	13.45	9.53	11.89	10.45	9.08
Crude fat (Cft)	3.42	3.89	4.94	4.03	3.97

For the perennial species *M. falcata*, an optimal value of the commented indicator (1/1.17) was also observed, which defines the species as a very valuable grazing component of the grassland in the summer months.

The dry matter of the perennial species *M. falcata* has the highest concentration of crude protein, as well as the most favorable ratio for the content of crude protein and crude fiber. Compared to short-lived species, *M. falcata* has a lower content of minerals and calcium.

For all analyzed species, a high content of Ca was observed, which leads to significantly higher values than the optimal ones for the ratio of the two macroelements (Ca/P). They ranged from 9.0/1 (*M. polymorpha*) to 19.6/1 (*M. arabica*). *M. arabica* can be mentioned as the species with the largest mineral imbalance and respectively with potential adverse effect on metabolic processes in ruminants (Hovi et al., 2003), given its higher share in grassland in spring and its higher forage productivity.

Cell wall fiber components and *in vitro* dry matter digestibility of species in genus *Medicago*

The content of neutral-detergent fibers (an indicator that makes up the total content of the fiber components of the cell walls) and acid-detergent fibers (ligno-cellulose complex, which determines the digestibility of animal fodder) varies from 32.10% (*M. polymorpha*) to 37.58% (*M. minima*) at an average value of 35.27% and from 25.02% (*M. falcata*) to 27.90% (*M. arabica*) at an average value of 26.42% (Table 5). The results obtained by us regarding the content of acid detergent fiber (27.90%) and neutral detergent fiber (36.84%) in the dry matter of *Medicago arabica* correspond to those established by Aydın et al. (2010), namely - 15.47-29.0% (CP), 22.60-32.93% (ADF) and 30.54-46.39% (NDF).

The high concentration of the lignin fraction (10.98%) in the dry matter of *M. polymorpha* is associated with lower digestibility (80.71%) of the biomass of the species compared to that of other alfalfa species.

The species *M. minima* and *M. lupulina* are with the lowest degree of lignification (coefficient = 16.66-17.30) (Figure 2) and with the lowest content of acid-detergent lignin (6.24-6.26%). Thus, they registered 2.0% higher *in vitro* digestibility of dry matter compared to the average value of the indicator (82.75%) in the studied species.

The biological value of the fodder mass largely depends on the content of fully digestible polyoside hemicellulose and incompletely digestible cellulose. In the studied wild alfalfa species, the concentration of hemicellulose in the dry matter ranged from 4.61% (*M. polymorpha*) to 11.82% (*M. minima*).

Empirically calculated values of the cellulose fraction ranged from 16.08% (*M. falcata*) to 19.68% (*M. lupulina*). The highest degree of lignification (coefficient = 34.21) was the grass mass of *M. polymorpha*, followed by that of *M. falcata* (coefficient = 26.52) and *M. arabica* (coefficient = 25.22).

Table 5. Basic structural fiber components of cell walls and *in vitro* digestibility of dry matter (%) of species of genus *Medicago*

Species Indicator	*M. arabica*	*M. minima*	*M. polymorpha*	*M. lupulina*	*M. falcata*	*Mean±Sx*	*SD*
NDF	36.84	37.58	32.10	36.08	33.73	***35.27±1.02***	***2.3***
ADF	27.90	25.76	27.49	25.92	25.02	***26.42±0.55***	***1.2***
ADL	9.29	6.26	10.98	6.24	8.95	***8.34±0.92***	***2.1***
Hemicell.	8.95	11.82	4.61	10.16	8.71	***8.85±1.19***	***2.7***
Cellulose	18.61	19.50	16.51	19.68	16.08	***18.85±0.75***	***1.7***
IVDMD	82.03	84.39	80.71	84.41	82.20	***82.75±0.72***	***1.6***
Mean±Sx	***30.60±11.19***	***30.89±11.60***	***28.73±11.20***	***30.42±11.60***	***29.12±11.33***		
SD	***27.4***	***28.4***	***27.4***	***28.6***	***27.7***		

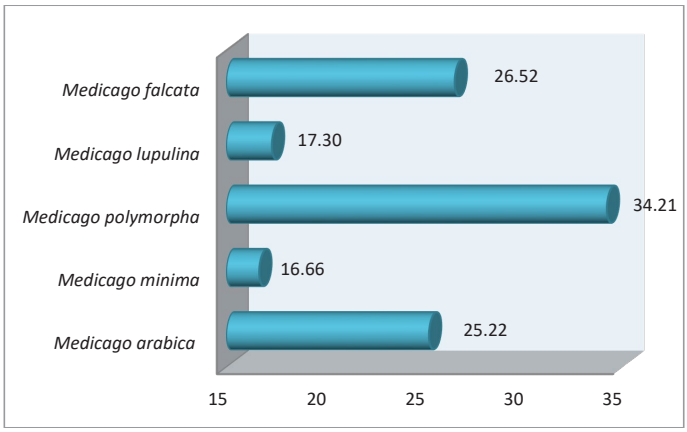

Figure 2. Lignification degree of forage biomass of species in genus *Medicago* (coefficient)

CONCLUSIONS

Among the six wild alfalfa species identified, the annual species *M. arabica* and the perennial *M. falcata* had the highest share, respectively significance for seasonal productivity and forage quality of the studied mesophytic grassland. *M. arabica* was the dominant legume species in spring and *M. falcata* in summer. The share of fast-growing, drought-resistant species, such as *M. minima* and *M. polymorpha,* may be associated with the observed spring and late droughts in the study area.

The dry matter of *M. falcata* had the highest concentration of crude protein (25.32%), as well as the most favourable ratio for the content of crude protein and crude fiber (1/ 1.17). Compared to other short-lived species, *M. falcata* had a lower content of minerals (9.08%) and calcium (2.24%).

The dry fodder mass of *M. polymorpha* was the richest in crude fiber (38.39%), crude fat (4.94%), acid-detergent lignin (10.98%), calcium (2.97%) and phosphorus (0.33%), and demonstrated the most favourable ratio between the amount of the two macroelements (9.0/1). The dry matter of that short-lived species had the lowest *in vitro* digestibility (80.71%) and hemicellulose concentration (4.61%).

M. arabica had the lowest content of crude fiber (25.09%) and the highest concentration of minerals (13.45%), and acid-detergent fiber (27.90%) - a factor that determines the relatively lower digestibility of 0.9% compared to the average and by 2.9% compared to the highest value of the indicator) of the dry matter. Compared to *M. polymorpha*, the fiber fraction in the composition of *M. arabica* was 53.0% lower.

The difference in the results of the analyzed and empirically determined biochemical parameters (fiber components of the cell walls and *in vitro* digestibility of dry matter) in *M. minima* and *M. lupulina* was insignificant. The studied species registered low content of ADL (6.24-6.26%) and highest digestibility of dry matter (84.39-84.41%). *M. minima* was the species with the lowest value in terms of crude protein (19.86%) and the highest in terms of hemicellulose (11.82%).

REFERENCES

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.

AOAC (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

Aufrere, J. (1982). Etude de la prevision de la digestibilite de la fourrages par une method enzymatic. *Annales de Zootechnie*, *31*(2), 111–130.

Aydın İ., Kutbay, H. G., Seyis, F., Uzun, F., & Sürmen, M. (2010). Determination of phenological, morphological and agronomic characteristics of some annual medic species TOVAG Project No: 107 O 087.

Bhandaria, B. K., Westa, C. B., Acosta-Martinez, V. (2020). Assessing the role of interseeding alfalfa into grass on improving pasture soil health in semi-arid

Texas High Plains. *Applied Soil Ecology*, *174*. 103399. https://doi.org/10.1016/j.apsoil.2019.103399

Desheva, G., Uzundzhalieva, K., & Stoyanova, S. (2016). Ex situ and in vivo conservation and utilization of crop wild relatives in Bulgarian National Genebank. *Phytologia Balcanica*, *22*(2), 233–241.

Dewhurst, R. J., Delaby, L., Moloney, A., Boland, T., & Lewis, E. (2009). Nutritive value of forage legumes used for grazing and silage. *Irish J. Agric. Food Res.*, *48*. 167–187. www.jstor.org/stable/20720367.

Genç Lermi, A. & Palta, Ş. (2016). Determination of Some Morphologic Properties of Medicago arabica L. (Huds) within Ecology of Bartın Province. International Forestry Symposium (IFS 2016), 07-10 December 2016, Kastamonu/TURKEY, Proceedings Faculty of Forestry, 177–184.

Georgieva, N., & Nikolova, I. (2015). Stem formation at alfalfa varieties and correlative dependences with some main parameters. *Journal of Central European Agriculture*, *16*(2), 89–98.

Gholami, A., De Geyter, N., Pollier, J., Goormachtig, S., & Goossens, A. (2014). Natural product biosynthesis in Medicago species. *Nat. Prod. Rep.*, *31*. 356–380. https://doi.org/10.1039/C3NP70104B

Hamachera, M., Malischa, C. S., Reinscha, T., Taubea, F., & Loges, R. (2021). Evaluation of yield formation and nutritive value of forage legumes and herbs with potential for diverse grasslands due to their concentration in plant specialized metabolites. *European Journal of Agronomy, 128*. 126307. https://doi.org/10.1016/j.eja.2021.126307

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.

Iantcheva, A., Revalska, M. (2018). Early events during the induction of somatic embryogenesis in genera Medicago. *Bulgarian Journal of Agricultural Science*, *24*(6), 1042–1052.

Kabtni, S., Sdouga, D., Rebey, I. B., Save, M., Trifi-Farah, N., Fauconnier, M-L., & Marghali, S. (2020). Influence of climate variation on phenolic composition and antioxidant capacity of *Medicago minima* populations. *Scientific Reports*, *10*. 8293. https://doi.org/10.1038/s41598-020-65160-4

Kamali, A., Dashtizadeh, M., Kabiri, Fard, A., Khaj, H., Sadeghi, S. A., Sadeghi, M. (2020). Growth stages effect on chemical composition and digestibility of *Medicago polymorpha* and *Malva parviflora* case of Bushehr province. Animal Science Research Department, Bushehr Agricultural and Natural Resources Research and Education Center, AREEO, Bushehr, 14, 3, 479–489.

Kell, S., Knüpffer, H., Jury, S., Ford-Lloyd, B., & Maxted, N. (2008). Crops and wild relatives of the Euro-Mediterranean region: mak-ing and using a conservation catalogue. In: Maxted, N., Ford-Lloyd, B.V., Kell, S.P., Iriondo, J., Dulloo, E. & Turok, J. (eds), Crop Wild Relative Conservation and Use. CAB International, Wallingford, UK., pp. 69–109.

Kirilov, A., Stoycheva, I., Vasileva, V. (2016). Appetite of annual and perennial legumes. *Bulgarian Journal of Animal Husbundry*, *LIII*(1-2), 66–71.

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.

Marinova, D. 2020b. Assessment of variability and phenotypic correlations between important agronomic and morphological traits in full-sibs alfalfa progenies. *Journal of Mountain Agriculture on the Balkans*, *23*(6), 121–138.

Marinova, D. 2020a. Assessment of Alfalfa Breeding Accessions (*Medicago sativa* L.) Based on Main Agronomic Characteristics. *Journal of Mountain Agriculture on the Balkans*, *23*(5), 47–63.

Marinova, D., Soiyanova, Sv., & Petrova, I. (2019). Study of the Effect of Biostimulants Application on Green Mass and Dry Matter Yield in Alfalfa. *Journal of Mountain Agriculture on the Balkans*, *22* (3), 64–80. http://rimsa.eu/images/forage_production_vol_22-3_2019.pdf

Nikolova, I. (2019). I mportant insect pests in Madicago sativa L. in Bulgaria. *Asian Journal of Research and Review in Agriculture*, *1*(1), 8–24. Article no.AJRRA.59

Peoples, M. B., Brockwell, J., Herridge, D. F., Rochester, I. J., Alves, B. J. R., Urquiaga, S., Boddey, R. M., Dakora, F. D., Bhattarai, S., Maskey, S. L, Sampet, C., Rerkasem, B., Khan, D. F., Hauggaard-Nielsen, H., & Jensen, E. S. (2009). The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. *Symbiosis*, *48*. 1–17

Putnam, D. H, Russelle, M., Orloff, S., Kuhn, J., Fitzhugh, L., Godfrey, L., Kiess, A., & Long, R. F. (2001). Alfalfa, Wildlife and the Environment. The importance and benefits of alfalfa in the 21st century. friendly and informative guide to alfalfa, the queen of forages. California Alfalfa and Forage Association, 24.

Riday, H., & Brummer, E. C. (2006): Persistence and yield stability of intersubspecific alfalfa hybrids. *Crop Sci. 46*(3): 1058–1063. doi:10.2135/cropsci2005.0272

Shi S., Nan, L., Smith, K. F. (2017). The Current Status, Problems, and Prospects of Alfalfa (*Medicago sativa* L.) Breeding in China. *Agronomy*, *7*(1) 1–11. doi:10.3390/agronomy7010001

Song, J., Hui, Y., Yu, C. D., Zhang, Q., Zhou, Y. Q., Li, Y., Liu, X. H., Zhu, L. L., Hui, D. F., & Wan, S. Q. (2017). Carbon balance under four double-season cropping systems in North China Plain. *Plant Soil*, *421*. 319–336.

Stoyanov, K. (2013). Lecture notes on phytocenology. Agricultural University Plovdiv, Academic Press.

Stoyanov, K., Naidenova, G., & Yancheva, H. (2018). Atlas Fodder legumes in Bulgaria. Academic Publishing House of the Agricultural University, Plovdiv.

Van Soest, P. J.q., Robertson, J. B. (1979). Systems of analysis evaluating fibrous feeds. Cornell University-lthaca, N.Y.

Vasileva, V., Vasilev, E., Dražić, G., & Vučković, S. (2020). New technologies in forage production and environment protection. IRASA Second International Scientific Conference Science, Education, Technology and Innovation - SETI II, Belgrade, October 2-3, 2020, *Book of Proceedings*, 53–61.

Veronesi, F., Brummer, E. C., & Huyghe, C. (2010). Alfalfa. In: Boller B, Posselt UK, Veronesi F (Eds). Fodder crops and amenity grasses. Series: Handbook of Plant Breeding. Springer, New York, USA pp 395–437.

Zhang, Y., Ren, J., Wang, M., Yang, G. (2013). Discussion on the position and development distribution of forage industry in China's agricultural industry structure. *J. Agric. Sci. Technol.*, *15*. 61–71.