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Original Article

Evaluation of Alfalfa Swards in the Conditions of Middle Balkan Mountains

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Abstract

One of the most effective measures used to enhance natural grasslands in mountain and semi-mountain conditions is the inclusion of species such as alfalfa which is characterized by increased productivity, stability and adaptability. Thus, a comparative study of 3 varieties ('Pleven 6', 'Prista 2', 'Multifoliate') and 2 local populations (LP1, LP2) of alfalfa was conducted in the semi-mountain conditions of the Middle Balkan Mountains. The complex evaluation, comprising productivity and parameters of stability and adaptability, determined the sward of local population LP1, followed by varieties 'Prista 2' and 'Pleven 6' as the most suitable for cultivation in the Middle Balkan Mountains. They can be used successfully to enhance the composition of natural grasslands. The swards of species, such as birdsfoot trefoil and red clover, which were traditionally grown and typical for the Middle Balkan Mountains, were characterized by lower productivity, stability and adaptability respectively.

Keywords: alfalfa; adaptability; evaluation; populations; productivity; stability; varieties

Introduction

Meadows and pastures are one of the main sources of grass forages. Over the last 100 years, a dramatic decline was observed of the natural and semi-natural grasslands in Europe, even though efforts have been directed to maintaining and enhancement of these areas (Poschlod and Bonn, 1998; Eriksson *et al*., 2002). According to statistical data, permanent grasslands in Bulgaria occupy about 13 million decares (Eurostat Statistics, 2018). Their meaning is particularly important in the mountain and semi-mountain regions where they provide a major part of the hay and grazing for sheep and cattle. Potential opportunities of meadows and pastures are not yet fully utilized. One of reasons for the low average yields (200 kg/dka) is that systematic care is not taken to improve and use them properly (Terziev, 2008).

Measures used to enhance or restore natural grasslands include weed control, introducing new plant species, fertilization, drainage of over-wetted areas, etc. (Walker *et al*., 2004; Terziev, 2008; Dicks *et al*., 2017). One of the most effective measures is the inclusion of species that are characterized by increased nutritional value, productivity and stability. Alfalfa (*Medicago sativa* L.) meets these requirements to a very large extent. According to Veronesi *et al*. (2010), it is the most important and high-yielding crop, whose excellent nutritional value makes it ideal for dairy farming.

However, it is necessary to be chosen varieties or local populations to realize their potential under the soil and climatic conditions of a given region. The region of Middle Balkan Mountains is characterized by unpredictable climatic conditions during the vegetation period and pseudopodzolic soils with increased acidity and aluminity toxicity (Mitev and Belperchinov, 2000). Thus, for the improvement of natural grasslands should be used alfalfa accessions with high resistance to stress factors. In addition, since the choice and inclusion of alfalfa in order to improve the grassland composition in the Middle Balkan Mountains is not an objective in itself, a comparison is made with the traditional leguminous species such as birdsfoot trefoil (Churkova, 2007) and red clover (Mihovski and Yancheva, 1998).

The aim of the present experiment was to evaluate (according to basic indicators and parameters) alfalfa varieties and populations under the ecological conditions of Central Balkan Mountains and to determine suitable ones for enhancing the composition of natural grasslands.

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Materials and Methods

The study was conducted in the Research Institute of Mountain Stockbreeding and Agriculture (Troyan, Bulgaria) during the period 2010-2013, by a randomized block method. The following alfalfa (*Medicago sativa* L.) swards (varieties and local population) were the subject of study: variety 'Pleven 6' (V1) - standard, variety 'Prista 2' (V2), variety 'Multifoliate' (V3), local population LP1 (V4) and local population LP1 (V5). As it was mentioned above, because the choice of species was not an end in itself, alfalfa was compared with traditional leguminous species - birdsfoot trefoil (local population) (V6) and red clover (local population) (V7).

Soil was pseudopodzolic soil, with pH $_{/KCl/}$ 4.7. Soil preparation included autumn plowing (18-20 cm) and spring cultivation, which brought the soil to a garden state. Sowing was conducted manually, with a rate of 800 seeds per m^2. Plants were grown in organic farming conditions and no pesticides or fertilizers were used during the vegetation period. All swards were cut at the flowering stage. Dry biomass yield was recorded. The precipitation amount during the study period was 749 mm per year compared to 734 mm per year during the previous 30-year period.

All obtained data were processed by two-factor analysis of variance for determination of the impact of sward (V), environment (E) (the year) and their interaction (V × E). The evaluation of the ecological stability of the tested swards was done through application of the following methods: regression analysis (according to Eberhart and Russell, 1966) in which the regression coefficient (bi) and the variance of deviations from regression (Si^2) were determined; analysis of variance - in which the index W_i (according to Annicchiarico, 1992) was calculated; and nonparametric analysis - by using Pi parameter (Lin and Binns, 1988). The total adaptability (Valchinkov, 1990) of studied swards was calculated. GGE biplot analysis was performed with the software product of Yan (2002). All experimental data were processed statistically by using the computer software GENES 2009.7.0 for Windows XP (Cruz, 2009).

Results

Productivity of the swards

The results of 4-year study period showed that the alfalfa sward of variety-standard 'Pleven 6' (V1) demonstrated the highest yield of dry biomass (11.10 t ha^{-1}) (Fig. 1). The local alfalfa population LP1 (V4) slightly retreated regarding productivity to 'Pleven 6' (by 5.1%) and exceeded variety 'Pliska 2' (V2) by 9.0%, but under statistically nonsignificant differences among the three swards. The lowest-productive alfalfa sward was that of the local population LP2 (V5), whose dry matter yield was on average with 23.0% below the yield, formed from 'Pleven 6'. Trends in alfalfa feed yields, averaged over the period, were generally identical and except for the 1st experimental year, were also observed during the individual years of the survey.

On average, for the experimental period, the local population of birdsfoot trefoil (V6) formed a yield close to that of the alfalfa swards of 'Prista 2' and 'Multifoliate', but significantly lower than the yield of 'Pleven 6' and LP1. All swards realized higher yields than the local population of red clover (V7), with differences from 29.5 to 68.2%.

Analysis of variance

In the conducted study was found an essential difference between the tested variants in regard to the complex indicator of dry mass productivity, as the variation was a result of the influence of the main factors: environment (year), sward (variant) and their interaction (Table 1). According to the results obtained of two-factor dispersion analysis, the impact of the environment (88.09%) was dominant, which is why the variants showed a considerable variation of the yield by years (Fig. 2). The second factor (swards) had a definitely smaller share in the variance of the indicator (5.22%), but nevertheless, the variants significantly differed from each other. The presence of significant interaction between the two main factors (year × sward, 6.69%) allowed an assessment of the manifestations of stability and adaptability of the studied indicator.

Stability of dry matter yield

The calculated stability parameters for each sward are presented in Table 2. Considerably lower magnitude of the interaction year × sward supposed a greater stability of the variants regarding the studied trait. Alfalfa swards of variety 'Multifoliate', populations LP1 and LP2, as well as red clover population, had a coefficient of regression bi < 1, which characterizes them as suitable for growing under more unfavorable conditions. Under such conditions, they could realize more stable yields.

The highest-productive variants (the standard 'Pleven 6'), as well as 'Prista 2', which was also characterized by a high yield of feed, had a linear regression coefficient very close to 1 (bi = 1.0061 and bi = 1.0319, respectively). The values of the variances of regression (Sd2) were statistically significant at a high probability level and, although 'Pleven 6' had a higher yield, the stability of 'Prista 2' was greater due to the weaker scattering (Sd2 = 4,415.4866). Also deserving attention was the local LP1 population, which was characterized by high yield, coefficient of ecological stability less than 1 and parameter Sd2 = 1,038.0859 (Table 2). Stability parameters of Eberhart and Russel (1966) and Annicchiarico (1992) determined the local birdsfoot trefoil population as ecologically unstable (bi > 1 and Sd2 = 22,331.4006). This is a variant with a strongly predictable response to the environmental conditions. Under favorable conditions, it could provide high yields.

The parameter Pi (Lin and Binns, 1988) and the rank method were used for complex evaluation of the average level of manifestation of the indicator and its stability. Ranking through this parameter is an appropriate way of estimating plant material with respect to the stability of a given indicator under limited environmental conditions. Stands of 'Prista 2' (Pi = 1) and LP1 (Pi = 2) were distinguished with the highest level of stability and they also realized productivity close to that of the standard. The last three positions were occupied by the lowest-yielding variants: the alfalfa stands of LP2 (Pi = 5) and 'Multifoliate' (Pi = 7) and the red clover population (Pi = 6). They can be defined as stable, but with a low adaptive capability. In a similar way, they were evaluated by the parameter Wi (Annicchiarico, 1992) especially the alfalfa swards of LP2 and 'Multifoliate' (Table 2).

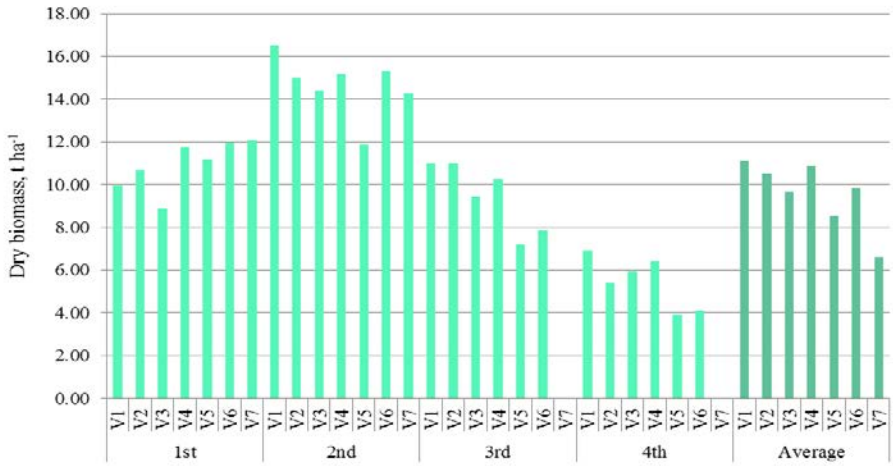

Fig. 1. Dry biomass productivity in different legume swards during 4-year growing period. Variants: V1-'Pleven 6', V2-'Prista 2', V3-'Multifoliate', V4-alfalfa local population LP1, V5-alfalfa local population LP2, V6-local population of birdsfoot trefoil, V7-local population of red clover; 1st, 2nd, 3rd, 4th – year of growing; cultivation of red clover during the 3rd experimental year was ceased due to the strong decrease of stand density

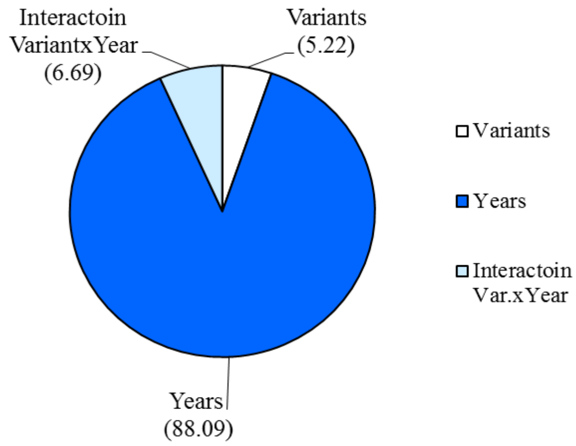

Fig. 2. Influence of factors (%)

Table 1. Analysis of variance in regard to productivity of the studied swards

Sources of variation	Degrees of freedom	Sum of squares	Mean square	Significance
Environment (Year)	3	9,146,457.81	304,8819.27	**
Variant (Sward)	6	542,410.59	90,401.77	**
Interaction Variant × Environment	18	694,221.1	38,567.84	**
Environment/Variant	21	9,840,678.91	468,603.76	**
Env/Variant 1	3	1,449,918.2	483,306.07	**
Env/Variant 2	3	1,403,464.84	467,821.61	**
Env/Variant 3	3	1,104,814.23	368,271.41	**
Env/Variant 4	3	1,188,205.52	396,068.51	**
Env/Variant 5	3	1,247,131.34	415,710.45	**
Env/Variant 6	3	2,146,337.66	715,445.89	**
Env/Variant 7	3	1,300,807.12	433,602.37	**
Residue	27			

Significant at P = 0.01 (**)
V1-'Pleven 6', V2-'Prista 2', V3-'Multifoliate', V4-alfalfa local population LP1, V5-alfalfa local population LP2, V6-local population of birdsfoot trefoil, V7-local population of red clover

Table 2. Stability parameters of dry mass yield

Variants	Eberhart and Russel (1966)		Annicchiarico (1992) (Wi)			Lin and Binns (1988)
	bi	Sd^2	general	unfavorable	favorable	Pi
V1	1.0061**	23,868.4604**	100.2526	110.5854	91.5143	4
V2	1.0319**	4,415.4866**	98.6328	99.4346	97.8083	1
V3	0.8872**	14,483.3522**	87.9567	94.9240	81.5274	7
V4	0.9565**	1,038.0859**	103.1278	102.9462	103.6521	2
V5	0.9102**	29,852.923**	72.1163	71.7770	81.6740	5
V6	1.2476**	22,331.4006**	80.1897	75.3848	104.6375	3
V7	0.9605**	38,531.4388**	99.3766	100.6503	97.7280	6

Significant at P = 0.01 (**)
V1-'Pleven 6', V2-'Prista 2', V3-'Multifoliate', V4-alfalfa local population LP1, V5-alfalfa local population LP2, V6-local population of birdsfoot trefoil, V7-local population of red clover

Adaptive ability

Adaptability is determined by the ability of a species/population to form high yields under different environmental conditions. To estimate the adaptability in the conditions of the present study, the coefficient of general adaptability was calculated according to the values of which their productive capabilities can be estimated (Fig. 3). During the 4-years experimental period, all alfalfa swards (excluding the local population LP2) had positive coefficients of general adaptability, as two of them (LP1 and 'Pleven 6') were distinguished with values above 0.100. With the lowest and negative values were the populations of red clover and birdsfoot trefoil. According to their adaptive ability, varieties and populations can be ranked in the following order: LP1 > 'Pleven 6' > 'Multifoliate' > 'Prista 2' > LP2 > birdsfoot trefoil > red clover.

It should be noted that variants with high adaptability represent "a value" only in case of a low variability of their yield under different conditions. That was observed in the greatest extent for 'Prista 2' and LP1, whose high productivity was combined with relatively low variability (respectively high stability) and high adaptability.

GGE biplot analysis

Biplot analysis provides valuable information on productivity as it takes into account the variability of the indicator against the background of complex interactions with environmental conditions, which in turn reveals additional possibilities for estimating and predicting yields. According to GGE biplot analysis (Yan and Holland, 2010), the more productive variant correlates with higher PC1 values and with higher stability when PC2 values are situated closest to zero. The two principal components comprised 93.3% of the dispersion of the trial (Fig. 4). The polygon was located at the most distant points (swards) from the beginning of the coordinate system. In regard to the productivity, they were arranged in the following order: 'Pleven 6' > alfalfa local population LP1 > 'Prista 2' > local population of birdsfoot trefoil > 'Multifoliate' > alfalfa local population LP2 > local population of red clover. The GGE biplot polygon also showed which swards were stable in the respective environment of cultivation. In this respect, LP1 and 'Prista 2' can be identified as closest to the ideal variant, as they demonstrated a relatively high and stable yield. The standard ('Pleven 6') was considerably further from the center and the swards of LP1 and 'Prista 2', which, as a whole, performed better.

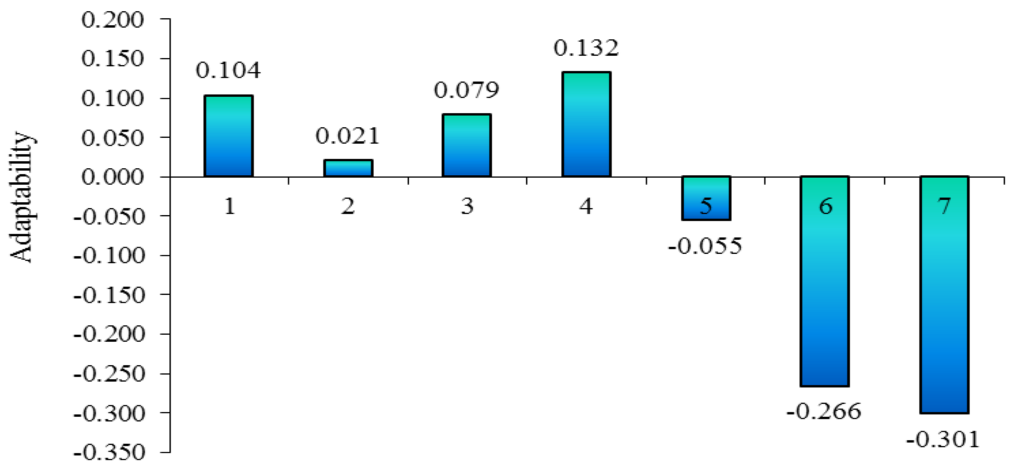

Fig. 3. Total adaptive ability (A) (Valchinkov, 1990) of the studied swards; 1-'Pleven 6', 2-'Prista 2', 3-'Multifoliate', 4-alfalfa local population LP1, 5-alfalfa local population LP2, 6-local population of birdsfoot trefoil, 7-local population of red clover

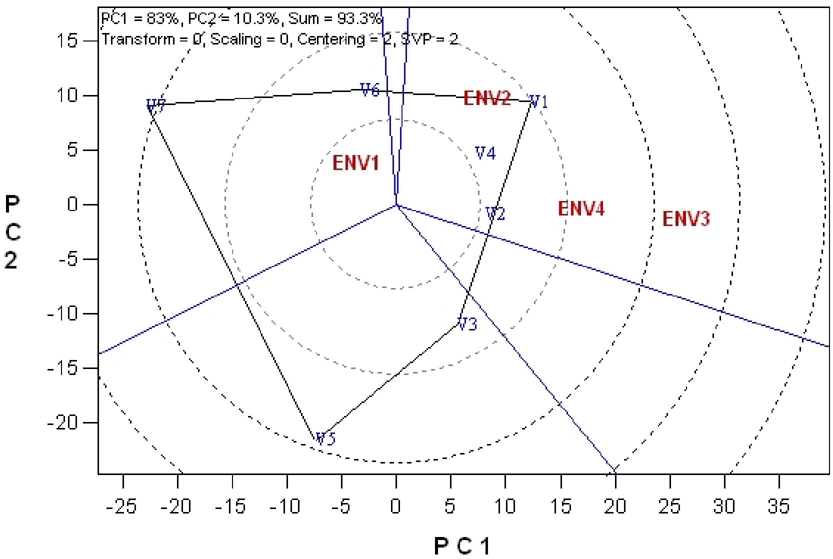

Fig. 4. GGE biplot analysis; V1-'Pleven 6', V2-'Prista 2', V3-'Multifoliate', V4-alfalfa local population LP1, V5-alfalfa local population LP2, V6-local population of birdsfoot trefoil, V7-local population of red clover

Discussion

In organizing livestock farms in the semi-mountain and mountain areas, as well as in the production of organic products, the use, along with maintenance and improvement of natural meadows and pastures have important implications for the provision of high-quality grass forage (Terziev, 2008). Moreover, they have a considerable cultural importance and are more aesthetically pleasing than the modern farm grasslands, add color and visual diversity to the landscape and contribute to the unique character of the local nature (Liley, 2018).

Improving the grassland composition is a very difficult process. This is an expensive measure and the first step towards success is to enhance the composition with species with the right characteristics (Christian and Peel, 2011). Legumes are mainly recommended, including species as alfalfa, birdsfoot trefoil and sainfoin, which increase the productivity and quality of the feed (Stoyanov, 2013). But the specific conditions of the area in which the current experiment was carried out (low autumn-winter temperatures, soils with increased acidity and aluminum toxicity) required pre-selection and assessment of alfalfa accessions to determine the most suitable one for the region. Such detailed information regarding what species, varieties, ecotypes, etc. are suitable for enhancing the grassland composition in the specific conditions of a given region, is almost absent in the scientific literature.

Several studies (Julier and Huyghe, 1997; Julier *et al.*, 2000; Annicchiarico, 2006) indicated a strong variation among alfalfa varieties and local populations concerning basic parameters (fresh mass yield, dry mass yield, stems formation, etc.). Comparing the productivity of 25 populations and 11 alfalfa varieties in North Africa, Tunisia, Benabderrahim *et al.* (2015) found that the fresh mass yield of the varieties exceeded that of local populations by 61 to 76% on average. Opposite results were found by Zeinab *et al.* (2014) in a study of 8 local populations and 2 alfalfa variety in Giza, Egypt. The authors reported that the local forms 'Ismaelia' and 'Siwa-1' had the best performance in terms of yield and main agronomic traits compared with the others. In the conditions of this experiment, the local population LP1 formed yield that exceeded the average yield of the three studied varieties by 4.4% and that of LP2 was lower by18.0%.

According to Shi *et al.* (2017), although local ecotypes and populations usually demonstrated lower productivity, they were characterized by increased pest resistance, wide adaptability and yield stability. In this regard, Julier *et al.* (2010) recommended populations originating in Southern Europe, which were characterized by a greater adaptability. Bolaños *et al.* (2000) recommended a local ecotype called 'Gabssia', which had a long history of cultivation worldwide and has been shown a wide range of adaptation to different environmental conditions. Similar characteristics in the present study were demonstrated by the local population LP1, which did not differ in terms of productivity from the standard variety, but had a higher adaptability and stability in some of the parameters.

Conclusions

The comparative study of alfalfa swards, subjects of the present study, under the ecological conditions of Middle Balkan Mountains, showed high productivity in varieties 'Pleven 6' and 'Pliska 2', as well as local population LP1. The values of the regression coefficient, variance of regression and parameter Pi determined a higher level of stability in 'Prista 2' and LP1, while regarding the total adaptive ability the highest adaptability was within LP1. The complex evaluation, comprising productivity, and parameters of stability and adaptability, determined the sward of local population LP1, followed by varieties 'Prista 2' and 'Pleven 6', as the most suitable for cultivation in the Middle Balkan Mountains. They can be used successfully to enhance the composition of natural grasslands. The swards of species, such as birdsfoot trefoil and red clover, which were traditionally grown and typical for the Middle Balkan Mountains, were characterized by lower productivity, stability and adaptability.

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