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Productivity and stability of grasses in the Middle Balkan Mountains region

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Abstract

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Perennial grasses have got greater attention in the past decade due to their potential to be productive over several years and their positive impacts on the environment. A great resource in this regard are the native species, which are well adapted to the specific conditions of the environment, but are often underestimated and irrationally used. The objects of study in the present research were 8 grasses (*Festuca rubra*, *Lolium perenne*, *Dactilis glomerata*, *Arrhenatherum elatius*, *Festuca arundinacea*, *Briza maxima*, *Trisetum flavescens*, *Agrostis alba*), typical for the Middle Balkan Mountains region, in order to assess their productivity and stability. The two-factor analysis of variance in terms of dry mass yield revealed significant influence of the factors of species, environment, and their interaction. During the 9-year experimental period, the highest yield was demonstrated by *F. rubra* (5.75 t ha^{-1}), followed by *F. arundinacea* (5.54 t ha^{-1}) and *A. elatius* (5.43 t ha^{-1}). The stability parameters, calculated by the methods of regression analysis defined as stable the species of *A. elatius* and *A. alba*, and those, calculated by variance and non-parametric analyzes – *F. rubra*, *D. glomerata* and *F. arundinacea*. Differences in the stability assessment of the individual parameters are due to the fact that the different methods are based on different concepts of stability. The GGE biplot analysis, which integratedly assesses the species for their productivity and stability, identified *F. arundinacea* and *A. elatius* as the most favorable combining of these indicators. *Trisetum flavescens* was also characterized by good stability and average productivity. These species are suitable for cultivation in a wide range of environmental conditions.

Keywords: grasses; stability; adaptability; grain mass yield

Introduction

Perennial grasses have got greater attention in the past decade due to their potential to be productive over several years and their positive impacts on the environment (Cattani, 2017; Crews et al., 2017). Compared to the annual species, the potential environmental advantages of these perennial crop systems include greater ability for carbon storage, higher utilization efficiency of the water and better nutrients management (Cattani et al., 2018; De Oliveira et al., 2018). Some researchers point, out as benefits of the perennial crops, the longer growing season, providing constant soil cover, deeper root systems, and up to five times greater potential for water retention from rainfall (Huggins et al., 2001; Zhang et al., 2011). As a result, perennial crops are extremely competitive in irregular rainfall pattern, as well as in a shortage of moisture. The greater root mass decreases the possibility of erosion (Gomiero et al., 2011) and soil salinization (Ward et al., 2006), supports higher soil carbon sequestration potential, and secures more efficient nutrients use (Crews, 2005; Cox et al., 2006; Pimentel et al., 2012). Last, but not least, perennial crop systems provide suitable conditions for the application of organic farming systems (Rasche et al., 2017). One of its main principles is species choice, in which the use of non-renewable and external resources was minimized.

Perennial grasses are considered as one of the most important sources of inexpensive feed for animals (Cotigă, 2010; Maruşca et al., 2014; Bancivanji et al., 2014). They are characterized by high productive potential, ecological plasticity, adaptability and energy value of the feed (Kostov & Pavlov, 2001). Stress-tolerant feed resources are increasingly needed for the environmental sustainability of extensive livestock systems, as well as in global warming conditions (Annicchiarico et al., 2011). A great resource in this regard are the native species, which are well adapted to the specific conditions of the environment, but are often underestimated and irrationally used. The efficient use of species of local origin, which are typical for the specific region, is the key to sustainable and successful agricultural production (Akdeniz et al., 2019).

The aim of the study was to make an ecological assessment involving dry mass productivity and stability of perennial grasses typical for the Middle Balkan Mountains region.

Material and Methods

The experimental activity was carried out in the Research Institute of Mountain Stockbreeding and Agriculture (Troyan), during the period 2011-2019, with the following variants:1 – *Festuca rubra* L., 2 – *Lolium perenne* L., 3 – *Dactilis glomerata* L., 4 – *Arrhenatherum elatius* P.B., 5 – *Festuca arundinacea* Schreb., 6 – *Briza maxima* L., 7 – *Trisetum flavescens* L., 8 – *Agrostis alba* L. All species are typical for the Middle Balkan Mountains region. Sowing is manual, with a rate of 800 seeds per m^2. Seeds from local populations were used. The soil was pseudopodzolic. The tillage included plowing and pre-sowing tillage. It was used the block method, in 4-fold repetition of the variants. Swards were harvested at the heading stage.

For statistical data processing, analysis of variance was used to determine the influence of swards, environments (years), and their interaction. The stability of the studied variants was evaluated using the following analyzes, parameters and methods: 1) *regression analysis* – according to Eberhart and Russell, where the regression coefficient (bi) and the variance of the deviations from regression (Si^2) were calculated; according to Tai (1979), in which the parameters a_i (linear response to the environmental effects) and λ_i (deviation from the linear response) were determined; 2) *variance analysis* – through ecovalence (W^2) according to Wricke (1965); mean variance component (PP) according to Plaisted & Peterson (1979); stability variances (σ^2) (Shukla, 1972) and (W_i) (Annicchiarico, 1992); and 3) *non-parametric analysis* – by the followingparameters: „PI“ (Lin & Binns, 1986); $S^{(1)}$, $S^{(2)}$, $S^{(3)}$, $S^{(6)}$ according to Huhn (1990) and Nassar & Huhn (1987); $NP^{(1)}$, $NP^{(2)}$, $NP^{(3)}$, $NP^{(4)}$ (Thennarasu, 1995) and „KR“ (Kang, 1988). Plaisted & Peterson's (1979) mean variance component (PP) was a measure for the contribution of a sward to the G × E interaction and was determined from a total through "*pair-wise"* analysis. According to Annicchiarico's method (1992), a reliability index (W_i) was calculated, which estimates the probability of a sward to show a performance below the environmental average or below any standard used.

The coefficient of total adaptability "A" was calculated by the method of Valchinkov (1990). A model of GGE biplot (Yan, 2001) was done, which uses the values of the first two main components (PC1 and PC2). The software products MS Excel (2003), statistical program Stabilitysoft and GENES 2009.7.0 for Windows XP (Cruz, 2009) were used in the processing of the experimental data.

Results and Discussion

One of the possibilities for assessing the tolerance of a plant population to environmental conditions is the formed yield. The basis of its realization is the genotype-environment relationship.

Dispersion analysis

Table 1 presents the results of the two-factor dispersion analysis of dry matter yield over the years of the study. It was established significant influence of the factors – species, year of cultivation and the interaction between them, on the trait manifestation. The variance analysis of the yield showed that the environmental conditions had a dominant influence on its variation of 64.22%. The influence strength of the type of grass on the trait variability was considerably less expressed (7.86%).

When assessing the stability and adaptability of the species, the presence of significant interaction between the studied species and the environment, in which they are grown is of special importance for the reliability of the obtained results. In the present study, the influence of genotype-environment interaction factor represented 27.92% of the total variation (Figure 1). This influence was significant, which allows the assessment of the ecological stability of the species in terms of productivity.

Productivity

Determinants for the productivity of fodder crops are the cultural practice, species, cultivar and environment (Akdeniz et al., 2018). The average dry mass yields of the studied

Table 1. Analysis of variance regarding dry mass productivity in meadow grasses for the period 2011–2019

Source of variation	Degree of freedom	Sum os squares	Mean swuares	Probability
Environment (years)	8	726.11	90.76	**
Genotype (grasses)	7	88.83	12.69	**
Interaction G × E	56	315.67	5.64	**
Env/Gen	64	10417.78	16.28	**
Env/Gen 1	8	144. 0	18.0	**
Env/Gen 2	8	40. 0	5.0	**
Env/Gen- 3	8	96.89	12.11	**
Env/Gen 4	8	224.88	28.11	**
Env/Gen 5	8	192. 00	24. 0	**
Env/Gen 6	8	43.56	5.44	**
Env/Gen 7	8	118.22	14.78	**
Env/Gen 8	8	182.22	22.78	**
Residuo	71			

Gen 1 – *Festuca rubra,* Gen 2 – *Lolium perenne*, Gen 3 – *Dactilis glomerata*, Gen 4 – *Arrhenatherum elatius*, Gen 5 – *Festuca arundinacea*, Gen 6 – *Briza maxima*, Gen 7 – *Trisetum flavescens* L., Gen 8 – *Agrostis alba* L. Significant at P = 0.05 (*), ** P = 0.01(**)

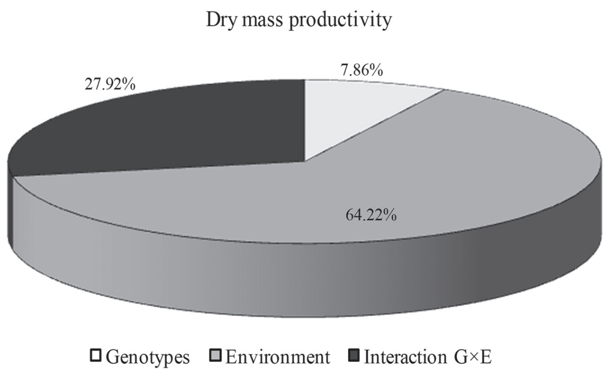

Fig. 1. Influence of the studied factors regarding productivity

plant species in the Middle Balkan Mountains region for a nine-year experimental period varied from 4.08 to 5.75 t ha^{-1}. Significantly, the highest yield was realized by *F. rubra*, followed by *F. arundinacea* (5.54 t ha^{-1}) and *A. elatius* (5.43 t ha-1), with a non-significant difference between them. The great potential of Festuca species as fodder and pasture species has been established by a number of researchers (Akdeniz et al., 2019; Bozhanska, 2019). In a two-year comparative study of eight grasses in the region of Igdir (Turkey), Akdeniz et al. (2019) indicated that *F. arundinacea* and *F. rubra* were the most productive, with 8.36 and 4.93 t ha^{-1} dry mass, respectively. It should be noted that *F. rubra* is the major structural component in the meadows and pastures of the Middle Balkan Mountains, and its participation in grasslands often reaches 88-98% (Mitev, 1997; Mitev et al., 2013). A dry mass yield of *F. rubra*, similar to that obtained in the present study, was reported by Rusinovci et al. (2016) – 4.65 t ha^{-1} in the area of Prishtina (Kosovo), Emelyanova (2013) – 4.4 t ha^{-1} along the Lena River (Russia) and Kacorzyk et al. (2013) – mm 4 t ha^{-1} in Krakow (Poland). According to Maruska et al. (2016), however, in the Brasov region (Romania), F. rubra showed twice the yield (8-9 t ha^{-1}). Regarding the *F. arundinacea*, a number of researchers defined it as an invasive species with great productive potential. For a three-year experimental period, Frydrych et al. (2020) reported an average dry biomass yield of 6.56 t ha^{-1} in the Beskydy region (Czech Republic), Wellie-Stephan (1998) – from 11.4 to 13.1 t ha^{-1} in Germany, Kryzeviciene (2005) – approximately 7 t ha^{-1} in poorer soils in Lithuania, Tîtei et al. (2019) – between 7.96 and 8.98 t ha^{-1} in the Chişinău region (Moldova). According to Koc et al. (2004), in Turkey, the hay yield in pure stands of *F. arundinacea* varied depending on the location, from 3.7 to 11.6 t ha^{-1}. Skládanka et al. (2010) identified *A. elatius* as a species very suitable for Central European conditions, and Glukhov et al. (2011) recommended it for the steppe regions of Ukraine, where the average dry matter productivity was 3.8 to 4.1 t ha^{-1}. Considerably higher yield was demonstrated by *A. elatius* in the Krakow region (Poland) – 8.5 t ha^{-1} (Kacorzyk et al., 2013). These data confirm the results of the analysis of variance, namely the dominant influence of the environment on the species manifestations.

The lowest productivity in the experimental conditions was demonstrated by *L. perenne* and *D. glomerata*, on average by 29.1 and 20.4% compared to *F. rubra*. The other three species (*A. alba, B. maxima, Tr. flavescens*) occupied an intermediate position, with yields close to the average for the experiment (5.11 t ha^{-1}).

Regarding the realized yields in the individual experimental years, it should be noted that there were no one-way dependencies. Most of the species (*A. elatius, F. arundinacea, B. maxima*, *Tr. flavescens, D.s glomerata*) showed the highest yield in 2013. *F. rubra* and *A. alba* revealed their best productive potential in 2012, and *L. perenne* – in 2011. In general, in the second (2012) and third experimental year (2013), plant swards demonstrated considerably higher yields, on average, by 67.4% compared to the average for the period 2011-2019.

Botanical composition

On average, for the 9-year experimental period, the share of the studied species in the swards varied from 24.3 to 66.4%. Four of them (*F. arundinacea, F. rubra*, *B. maxima, A. alba*)

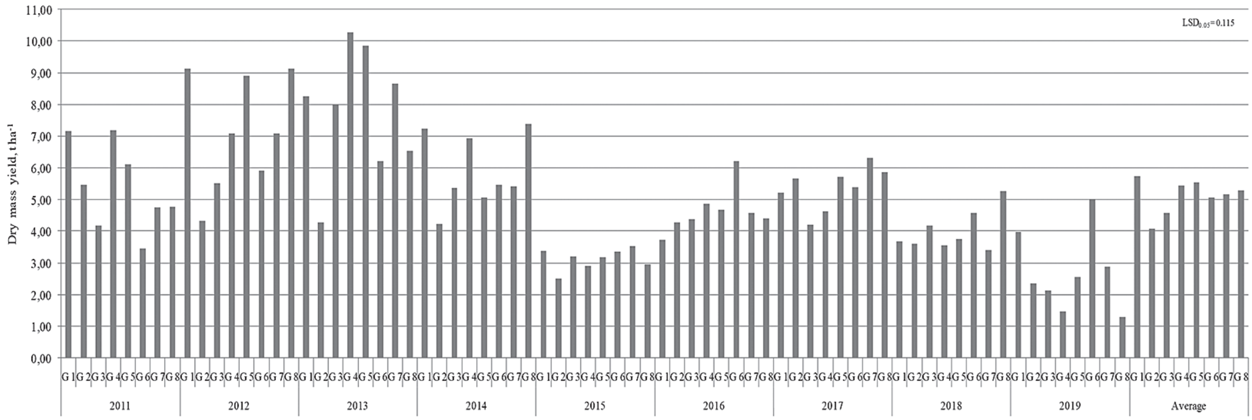

Fig. 2. Productivity of meadow grasses (dry mass t ha^{-1}) during the period 2011-2019
G1 – *Festuca rubra*, G2 – *Lolium perenne* L.), G3 – *Dactilis glomerata*, G4 – *Arrhenatherum elatius*, G5 – *Festuca arundinacea*, G6 – *Briza maxima,* G7 – *Trisetum flavescens*, G8 – *Agrostis alba*

were with a dominant participation (over 50%) determined by both their high competitiveness and their unassuming to environmental conditions, established by other authors (Kostov & Pavlov, 2001). In addition to these advantages is their greater longevity. In contrast, with a percentage share of less than 30%, respectively with a high share of weeds in their swards, were *L. perenne* and *D. glomerata*. They had less competitiveness and less longevity. *A. elatius* and *T. flavescens* occupied an intermediate position (Figure 3).

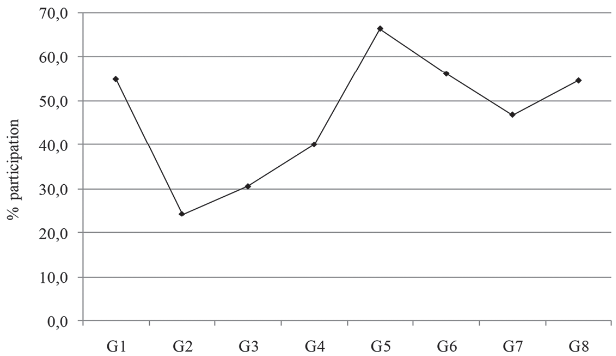

Fig. 3. Percentage participation of the species in sward (average for the period)
G1 – *Festuca rubra*, G2 – *Lolium perenne* L.), G3 – *Dactilis glomerata*, G4 – *Arrhenatherum elatius*, G5 – *Festuca arundinacea*, G6 – *Briza maxima,* G7 – *Trisetum flavescens*, G8 – *Agrostis alba*

Ecological stability

According to Hristov et al. (1997), a sufficiently true assessment of the ecological stability of the species can be obtained from a smaller number of experimental years, but a minimum of 9-12 years is required for an accurate assessment. Several statistical methods have been developed to determine the degree of environmental influence on yield, to distinguish genotypes, in which this influence is minimal and to predict their phenotypic response to changes in environmental conditions. The most common methods included linear regression analysis, nonlinear regression analysis, multivariate analysis, and nonparametric statistics (Mortazavian et al., 2014; Solonechnyi et al., 2015).

The genotype-environment interaction is equivalent of genotypic variation on phenotypic plasticity. This property is expressed by the regression coefficient bi. When presenting the parameters characterizing the ecological stability of the individual grasses, some differences were observed. With high responsiveness, according to the criteria of Eberhart & Russell (1966) and Tai (1979), were *F. arundinacea* (bi = 1.62) and *T. flavescens* (bi = 1.32) (Table 2a). Species with $Si^2 = 0$ would be the most stable and most desirable, while those with $Si^2 > 0$ would show lower stability in all environments. The results analysis defined the species of *D. glomerata* as the most stable in this respect (Si2 = 1.98). *L.perenne* and *B. maxima*, which have regression coefficient "bi" of 0.30 and 0.54, respectively, are outlined to be definitely stable. The species *A. elatius* (bi = 1.07) and *A. alba* (bi = 1.06) are distinguished by the most favorable combination – high dry mass yield and good adaptive ability.

According to the parametric assessment of stability and the mean variance component PP (Plaisted & Peterson, 1959), species that show a lower value for 'PP' are considered more stable. In the present study, these were *F. rubra* and *D. glomerata*. The ecovalence (W^2) (Wricke, 1965) and stability variance (σ^2) (Shukla, 1972) also determined *D.*

Table 2a. Stability parameters of the grasses studied in the Middle Balkan Mountains conditions

Species	Eberhart and Russell (1966)		Tai (1979)		Shukla (1972)	Plaisted & Peterson (1959)	Wricke (1965)	Annicc Hiarico (1992)
	bi	Si^2	ai	λi	$σ^2$	PP	W^2	Wi
Festuca rubra	1.17	4.23**	1.17	17.93	1.13	6.607	142.35	98.33
Lolium perenne	0.30**	6.27**	0.30	25.99	2.11	12.232	457.35	79.63
Dactilis glomerata	0.93	1.98**	0.93	8.91	0.37	5.232	65.35	91.43
Arrhenatherum elatius	1.07	5.84**	1.07	24.38	1.81	7.155	173.01	92.57
Festuca arundinacea	1.62**	3.44**	1.62	14.69	0.99	9.710	316.13	100.48
Briza maxima	0.54**	6.87**	0.54	28.44	2.44	9.708	316.01	85.18
Trisetum flavescens	1.32**	4.45**	1.32	18.79	0.25	7.429	188.35	86.69
Agrostis alba	1.06	9.50**	1.06	38.98	1.73	8.970	274.68	97.57

Significant at P = 0.05 (*), ** P = 0.01(**)

glomerata as the most stable. According to the method of Annicchiarico (1992) and the parameter W_i, *F. rubra* and *F. arundinacea* were in the most favorable position. The same species also had a high dry matter yield.

According to the obtained results for dry mass yield, the calculated non-parametric stability indicators according to the coefficients of Huhn (1990) and Nassar & Huhn (1987), and the total adaptability, the species can be classified into different groups (Table 2b). *F. arundinacea* was highly productive and highly stable in unfavorable weather conditions. *D. glomerata* was also very stable, but with an unsatisfactory dry matter yield. In contrast, *A. elatius* was distinguished with high productive potential, but it performed sensitivity to worsening weather conditions and low adaptability. *F. rubra* had similar characteristics, but its adaptability was higher. *Tr. flavescens* and *A. alba* were characterized by medium high yield and medium to low stability and adaptability. The calculated coefficients of Huhn (1990) and Nassar & Huhn (1987) in the representatives of the group of low-yielding grasses (*L. perenne* and *B. maxima*) had high values i. e. the species appeared to be highly unstable.

The analysis, according to Lin & Binns (1988) and the parameters of Thennarasu (1995), place *F. rubra* and *F. arundinacea* in the group of stable high-yielding species. Differences observed in the stability assessment of the individual parameters are due to the fact that the different methods are based on different concepts of stability.

The total adaptability index „A“ (Valchinkov, 1990) puts *F. rubra* and *A. alba* species in a group, combining good adaptability with higher than average dry matter yield. *A. elatius*, which is highly productive and moderately adaptable, can also be included in this group. With the strongest adaptability was *L. perenne* but, it had the lowest productivity. The last species, according to the parameter „KR“ (Kang, 1988), was also defined as the most stable.

GGE biplot analysis

According to Farshadfar (2008), stability is not the only parameter for assessing plant populations, as stable species often have low productivity. Other approaches are needed, which include an integrated assessment of yield and stability in an index. GGE biplot analysis characterizes grass species

Table 2b. Stability parameters of the grasses studied in the Middle Balkan Mountains conditions

Species	Huhn (1990), Nassar & Huhn (1987)				Thennarasu (1995)				Lin & Binns (1988)	Kang (1988)	Valchinkov (1990)
	$S^{(1)}$	$S^{(2)}$	$S^{(3)}$	$S^{(6)}$	$NP^{(1)}$	$NP^{(2)}$	$NP^{(3)}$	$NP^{(4)}$	Pi	KR	A
Festuca rubra	2.44	5.00	7.50	3.13	2.33	0.25	0.43	0.46	6.06	5.0	1.13
Lolium perenne	2.00	3.03	9.48	5.30	2.22	1.48	1.04	0.78	33.61	15.0	1.43
Dactilis glomerata	1.83	2.44	6.07	3.31	1.33	0.67	0.51	0.57	14.83	9.0	0.84
Arrhenatherum elatius	3.11	7.28	12.78	4.73	2.56	0.41	0.60	0.68	11.28	9.0	0.84
Festuca arundinacea	1.61	2.19	3.36	1.87	1.56	0.28	0.36	0.31	3.28	5.0	0.69
Briza maxima	3.11	6.61	10.82	3.91	1.89	0.35	0.48	0.64	23.11	14.0	0.94
Trisetum flavescens	2.67	5.11	8.00	2.96	1.11	0.28	0.26	0.52	14.17	6.0	0.55
Agrostis alba	3.11	7.11	11.13	4.13	2.67	0.35	0.53	0.61	8.67	9.0	0.96

by combining average yield and stability. In the presented graph (Figure 4), the first two components (PCA1 and PCA2) of the GGE biplot model explain 81.2% of the genotype-environment interaction. Species, located to the right of the center have higher average productivity, and on the left – lower productivity. The remoteness of the species from the abscissa along the ordinate in both directions characterizes the yield variability concerning its expected level of performance in specific environments (years). The closer the species are to the abscissa, the more stable they are, and vice versa, the farther away they are, the greater their variability.

The data analysis presented in Figure 4 showed that *F. rubra*, *F. arundinacea* and *A. elatius* are characterized by maximum productivity. The projection of these species along the ordinate axis defined the first species as unstable and the other two as showing high stability. *T. flavescens* and *D. glomerata* also had good stability, and their yield was close to or below the average for the studied grasses. *L. perenne* and *B. maxima* were characterized by a yield lower than the average for the experiment and can definitely be referred to highly variable species. With similar variability was *A. alba*, but it formed considerably higher dry mass yield compared to them.

The projection of each environment on the bipolar plane is a measure for its usefulness in selecting suitable species for the mega-environment. Thus, the usefulness of the nine environments (years) can be classified as follows: VII > V > VIII > VI > IV > IX > I > II > III. Environment "III" (2013) was the least representative. It was not preferable when choosing suitable plant species but can be useful in the rejection of unstable genotypes.

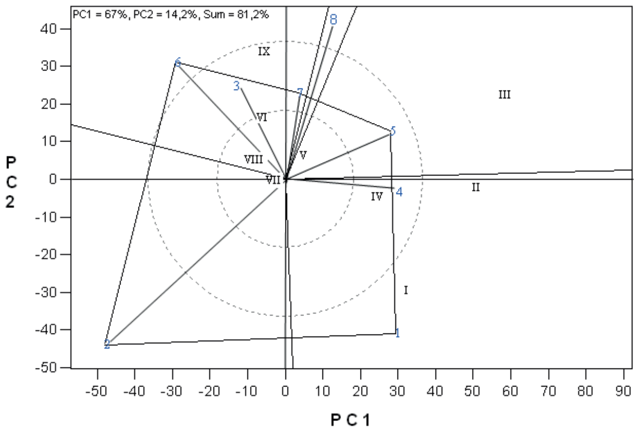

Fig. 4. GGE-biplot analysis in the meadow grasses studied

1 – *Festuca rubra*, 2 – *Lolium perenne* L., 3 – *Dactilis glomerata*, 4 – *Arrhenatherum elatius*, 5 – *Festuca arundinacea*, 6 – *Briza maxima,* 7 – *Trisetum flavescens*, 8 – *Agrostis alba*

Conclusions

The results of the present study showed that considerable variability exists among the eight grasses studied concerning their stability and productive potential in the Middle Balkan Mountains conditions.

The two-factor analysis of variance in terms of dry mass yield revealed significant influence of the factors of species, environment, and their interaction. During the 9-year experimental period, the highest yield was demonstrated by *Festuca rubra* (5.75 t ha^{-1}), followed by *Festuca arundinacea* (5.54 t ha^{-1}) and *Arrhenatherum elatius* (5.43 t ha^{-1}).

The stability parameters calculated by the methods of regression analysis defined as stable the species of *A. elatius* and *Agrostis alba*, and those calculated by variance and non-parametric analyzes – *F. rubra*, *Dactilis glomerata* and *F. arundinacea*. Differences in the stability assessment of the individual parameters are due to the fact that the different methods are based on different concepts of stability.

The GGE biplot analysis, which integratedly assesses the species for their productivity and stability, identified *F. arundinacea* and *A. elatius* as the most favorable combining of these indicators. *Trisetum flavescens* was also characterized by good stability and average productivity. These species are suitable for cultivation in a wide range of environmental conditions.

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