



PRODUCTIVITY AND STABILITY OF FOOTHILL MEADOW SPECIES IN THE BALKAN MOUNTAINS CONDITIONS

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ABSTRACT. The creation of grasslands with the participation of species of local origin, suitable for the climatic conditions of the region and with increased productivity and stability of yield is an important condition for ensuring sustainable or organic feed production. For ecological assessment of meadow species in the semi-mountainous regions of Balkan Mountains according to indicators and parameters related to productivity and stability, an experiment is carried out in the period 2011–2019 in the following variation: *Festuca rubra* L., *Lolium perenne* L., *Dactylis glomerata* L., *Arrhenatherum elatius* P.B., *Festuca arundinacea* Schreb., *Briza maxima* L., *Trisetum flavescens* L., *Agrostis alba* L. The experiment is performed by the block method, and the methods of regression, variance and nonparametric analysis are used to assess the stability. According to the values of most of the calculated stability parameters (b_i , Si_2 , λ_i , σ_{2i} , PP , W_2 , $S^{(3)}$, $S^{(6)}$, $NP^{(1)}$) *Dactylis glomerata* shows good ecological stability. Complex evaluation by GGE biplot analysis identifies *Festuca rubra* L. as a species that favourably combines high productivity with relative stability. *Dactylis glomerata* and French ryegrass are characterized by high stability and yield close to the average for the group. These species are suitable for growing in a wide range of environmental conditions.

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Introduction

The irrational use and management of natural resources, as well as non-compliance with the biological characteristics of individual species by their adaptability, is one of the causes of the crisis in modern agriculture. Its authorization is related to the application of methods for ecological assessment of adaptability and stability of species and scientifically based organization of production (Korzun, Brujlo, 2011; Rybas, 2016). The basis of the adaptive potential is the variability, characterized by the terms stability and plasticity (Dragavtcev, 2000).

When creating a grassland, the ecological conditions of the environment are decisive for the choice of species. Under extreme environmental conditions that occur during the growing season, some of them show greater compensatory capabilities and plasticity than others (Dimova *et al.*, 2006). Studies by several authors (Chourkova, 2007; Naydenova, Mitev, 2010; Mitev *et*

al., 2013) show that in mountainous and semi-mountainous areas, the creation of grasslands from species that do not meet local habitats leads to their rapid degradation. Those of local origin can form stable, long-lasting and highly productive grasslands in specific environmental conditions. In addition, the use of species suitable for the respective climatic conditions and with increased yield stability is an important condition in ensuring sustainable or organic feed production (agriculture) (Uhr, Ivanov, 2015; Shamsutdinov *et al.*, 2016).

The study aims to assess meadow species by indicators and parameters related to productivity and stability in the semi-mountainous regions in the Balkan Mountains conditions.

Material and Methods

In the conditions of the present experiment, the created grass stands included cereal meadow species, which are typical for the semi-mountainous regions of



the Central Balkan Mountains (Totev, 1984; Totev, Valkov, 1988). The experimental activity was carried out in the Research Institute of Mountain Stockbreeding and Agriculture (RIMSA) (Trojan) in the period 2011–2019, with the following variants: V1 – *Festuca rubra* L., V2 – *Lolium perenne* L., V3 – *Dactylis glomerata* L., V4 – *Arrhenatherum elatius* P.B., V5 – *Festuca arundinacea* Schreb., V6 – *Briza maxima* L., V7 – *Trisetum flavescens* L., V8 – *Agrostis alba* L. The stands investigated in this study were situated at an altitude of 430 m.

Meteorological conditions during the 9-year experimental period were characterized by an average daily temperature of 11 °C and a precipitation sum of 858 mm. In general, the amount of precipitation in individual years was close to the average for the experimental period, except for the years 2012 and 2013, when the amount of precipitation averaged up to 35% higher, which corresponds to the more biomass formation. The soil type was Planosol. Tillage included ploughing and pre-sowing tillage, which brings it to a garden condition. Sowing was manual, with a rate of 800 seeds m⁻². All seeds were from local populations. The experiment was based on the block method, in the 4-fold repetition of the variants. The grasses were cut in the phase of complete earing and fresh mass yield was reported.

For statistical data processing Statgraphics Plus Version 2.1, analysis of variance was used to determine the influence of grassland, environment (year) and their interaction. The stability of the variants was assessed by the following analyzes and parameters: regression analysis – in which the regression coefficient (bi) and variance of regression deviation (Si²) were calculated (according to Eberhart, Russell, 1966), the parameters ai (linear response to environmental effects) and λi (deviation from the linear response) (according to Tai, 1979); variance analysis – by the parameter σ²i by Shukla (1972), mean-variance component (PP) according to Plaisted and Peterson (1959), ecovalence (W2) according to Wricke (1965) and Annicchiarico (2002); and nonparametric analysis – through the parameters: "PI" of Lin and Binns (1988); S⁽¹⁾, S⁽²⁾, S⁽³⁾, S⁽⁶⁾ by Huhn (1990) and Nassar and Huhn (1987); NP⁽¹⁾, NP⁽²⁾, NP⁽³⁾, NP⁽⁴⁾ by Thennarasu (1995) and "KR" Kang (1988). The mean component of variation (PP) of Plaisted and Peterson (1959) was a measure of the contribution of the variant/grassland to its interaction with the environment and was calculated from the total by pair-wise analysis. The Annicchiarico method offers a reliability index (Wi), which was used in the present study to assess the probability that a grassland will perform 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 was made according to the method of Yan (2002), which used the values of the first two main components (PC1 and PC2).

Results and Discussion

Dispersion analysis

The analysis of the variance in terms of fresh mass yield (t ha⁻¹) shows that the effect of all three factors year (environment), meadow species (grassland) and the interaction between them is statistically significant at a high level (Table 1). The environmental factor has the strongest influence on productivity – 94.71% (Fig. 1), which is why meadow species show a significant variation in yield by year. The strength of the influence of the interaction environment × grassland is significantly lower (4.10%) than that of the environment and slightly exceeds the influence of the grass factor (1.19%).

Table 1. Analysis of variance for green mass productivity in meadow species

Source of variation	DF	Sum of Squares	Mean Square	Significance
Environment (year)	8	44641.111	558.0139	**
Species	7	561.7778	80.254	**
Species × environment	56	1933.2222	34.5218	**
Env./Gen.	64	6397.3333	99.9583	**
Env./Species 1 – <i>Festuca rubra</i> L.	8	894.2222	111.7778	**
Env./Species 2 – <i>Lolium perenne</i> L.	8	232.00	29.00	**
Env./Species 3 – <i>Dactylis glomerata</i> L.	8	542.2222	67.7778	**
Env./Species 4 – <i>Arrhenatherum elatius</i> P.B.	8	803.5556	100.4444	**
Env./Species 5 – <i>Festuca arundinacea</i> Schreb.	8	1563.5556	195.4444	**
Env./Species 6 – <i>Briza maxima</i> L.	8	363.5556	45.4444	**
Env./Species 7 – <i>Trisetum flavescens</i> L.	8	1102.2222	137.7778	**
Env./Species 8 – <i>Agrostis alba</i> L.	8	896.00	112.00	**
Residue	71			

Significance – * P < 0.05, ** P < 0.01

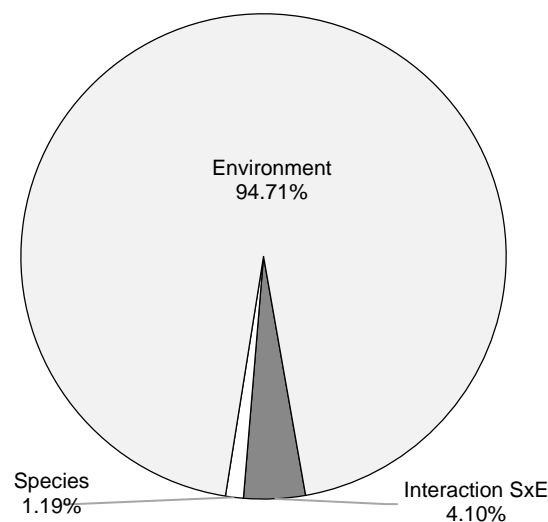


Figure 1. Influence of environmental factors, species and their interaction in terms of green mass yield in the studied meadow grasses

Productivity

The data on the yield obtained from the studied species (Fig. 2) in the period 2011–2019 vary widely, from 10.5 to 33.0 t ha⁻¹ fresh mass. The average values for the period of this trait determine as the most productive species *Festuca arundinacea* Schreb. (21.0 t ha⁻¹), *Agrostis alba* L. (20.0 t ha⁻¹) and *Festuca rubra* L. (19.9 t ha⁻¹). High productivity is demonstrated by *Festuca arundinacea* Schreb. in 2012 and 2013 with an average yield of 31.0–33.0 t ha⁻¹, followed by *Agrostis alba* L. with 30.0 t ha⁻¹ in 2012, and *Arrhenatherum elatius* P.B. and *Trisetum flavescens* L. by 29.5 and 29.0 t ha⁻¹ in 2013, respectively. It should be noted that in the individual experimental years, there is no strictly defined one-way in the data on the yield of the fresh mass of the studied plant species. However, *Festuca rubra* L. and *Festuca arundinacea* Schreb. are of some interest, as they show high yields during half of the 9-year experimental period. They are suitable components in the creation of artificial grasslands in the foothills in the Balkan Mountains conditions. We have also reported high productivity and adaptability of *Festuca rubra* L. in the foothills in the Balkan Mountains conditions in our previous studies (Georgieva *et al.*, 2018). The high productive potential of *Festuca arundinacea* Schreb. has been

established by a number of researchers in different geographical areas. Titei *et al.* (2019) indicate the yield of green mass of different varieties and *Festuca arundinacea* Schreb., between 30.83 and 36.96 t ha⁻¹ in the region of Chişinău (Moldova), Dronova *et al.* (2016) – from 24.3 to 32.5 t ha⁻¹ in irrigation in the region of Volgograd (Russia), a Frydrych *et al.* (2020) – between 15.96 and 19.61 t ha⁻¹ in the Beskydy region, Czech Republic.

Arrhenatherum elatius P.B., *Dactylis glomerata* L. and *Trisetum flavescens* L. have relatively limited productive possibilities during the experimental period, whose yields on average for the period vary in the range 18.0–18.7 t ha⁻¹ statistically proven lowest yields are formed by *Lolium perenne* L. and *Briza maxima* L., on average by 16.9 and 11.6% below the group average. *Trisetum flavescens* L. and *Briza maxima* L. are species that are less studied in our country.

Although with lower productivity, they are defined as valuable forage species, are preferred by animals (Sanz *et al.*, 2011; Macháč, 2014; Zueva, Tsipchenko, 2018) and are suitable for inclusion in grasslands in semi-mountainous and mountainous areas (Sackl *et al.*, 2012).

The proven influence of the environmental factor (year) on the variation of the yield of meadow grasses gives grounds to evaluate their ecological stability.

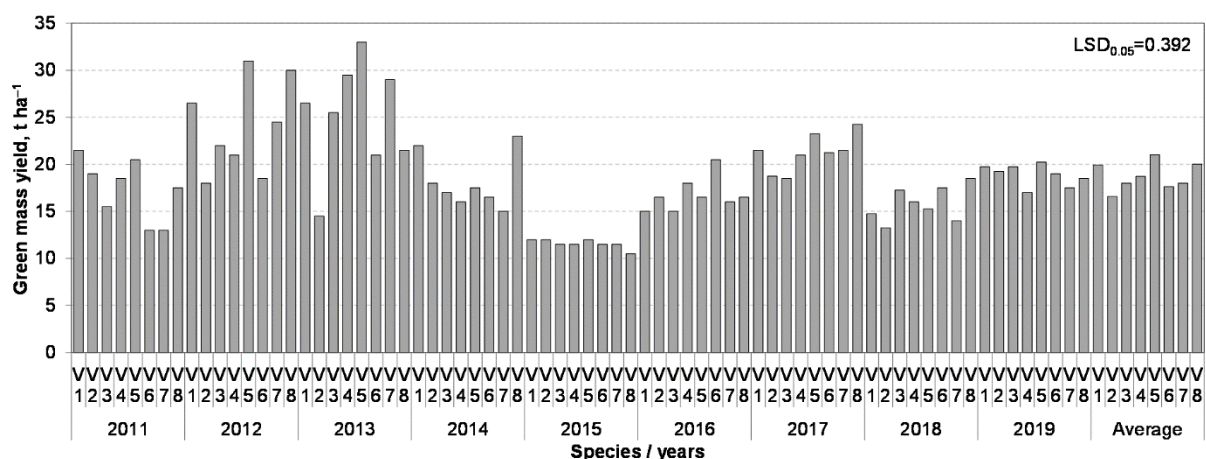


Figure 2. Productivity of meadow grasses (green mass t ha⁻¹) during the period 2011–2019 (V1 – *Festuca rubra* L.; V2 – *Lolium perenne* L.; V3 – *Dactylis glomerata* L.; V4 – *Arrhenatherum elatius* P.B.; V5 – *Festuca arundinacea* Schreb.; V6 – *Briza maxima* L.; V7 – *Trisetum flavescens* L.; V8 – *Agrostis alba* L.)

Ecological stability

The phenotypic stability of plants can be divided into two main types, stability in a biological and agronomic sense. Resistance in the biological sense refers to the ability of a plant population to maintain relative constancy (homeostasis) at the level of the trait in different environments, with low variation between them. Stability in the agronomic sense shows that the same population responds positively to improvements in growing conditions and can perform well above average in different areas (Sabaghnia *et al.*, 2015). In the conditions of the present experiment, to clearly differentiate meadow grasses by ecological stability, three types of parameters were used (Tables 2 and 3). Of the studied plant species, the *Dactylis glomerata* L.

showed relatively good stability, judging by the coefficient of linear regression ($b_i = 0.93$). The values of the stability variant according to the regression analysis, as well as those of most indicators in the other two types of analyzes (λ_i , σ_{2i} , PP, W2, $S^{(3)}$, $S^{(6)}$, $NP^{(1)}$), are low and mathematical proof, which suggests that it has a definite advantage over other grasses. The linear regression coefficient characterized as stable *Lolium perenne* L. ($b_i = 0.30$) and *Briza maxima* L. ($b_i = 0.54$). The same species received different scores according to the values of the parameters of Plaisted and Peterson (1959), Wricke (1965), Annicchiarico (2002) and Lin and Binns (1988). The established differences in the assessment of the stability of the individual parameters are because the various methods are based on different

concepts of stability. For the study period, *Festuca arundinacea* Schreb. (bi = 1.62), *Trisetum flavescens* L. (bi = 1.32) and *Festuca rubra* L. (bi = 1.17) are characterized by instability, which under the same growing conditions show more pronounced responsiveness.

Table 2. Parameters of phenotypic stability of meadow grasses by Green mass yield

Type	Eberhart and Russell (1966)		Tai (1979)		Shukla (1972)	Plaisted and Peterson (1959)	Wricke (1965)	Annicchiarico (2002)
	bi	Si ²	ai	λi	σ ² i	PP	W ²	Wi
<i>Festuca rubra</i> L.	1.17	4.23**	1.17	17.93	4.10	6.607	142.35	98.33
<i>Lolium perenne</i> L.	0.30**	6.27**	0.30	25.99	18.72	12.232	457.35	79.63
<i>Dactylis glomerata</i> L.	0.93	1.98**	0.93	8.91	1.43	5.232	65.35	91.43
<i>Arrhenatherum elatius</i> P.B.	1.07	5.84**	1.07	24.38	5.13	7.155	173.01	92.57
<i>Festuca arundinacea</i> Schreb.	1.62**	3.44**	1.62	14.69	12.33	9.710	316.13	100.48
<i>Briza maxima</i> L.	0.54**	6.87**	0.54	28.44	10.73	9.708	316.01	85.18
<i>Trisetum flavescens</i> L.	1.32**	4.45**	1.32	18.79	6.06	7.429	188.35	86.69
<i>Agrostis alba</i> L.	1.06	9.50**	1.06	38.98	11.22	8.970	274.68	97.57

Significant at P = 0.05 – *; P = 0.01 – **

Table 3. Non-parametric indicators for stability and adaptability in meadow species

Species	Huhn (1990); Nassar and Huhn(1987)				Thennarasu (1995)				Lin and 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
<i>Festuca rubra</i> L.	2.39	4.44	6.81	2.64	2.00	0.26	0.41	0.46	6.06	5.00	5.88
<i>Lolium perenne</i> L.	2.67	5.28	11.88	5.19	2.44	0.69	0.74	0.75	33.61	16.00	3.83
<i>Dactylis glomerata</i> L.	2.22	3.53	8.19	3.94	1.22	0.50	0.49	0.65	14.83	6.00	4.36
<i>Arrhenatherum elatius</i> P.B.	2.61	4.86	10.00	4.34	1.33	0.40	0.42	0.67	11.28	7.00	5.54
<i>Festuca arundinacea</i> Schreb.	1.94	2.75	3.47	2.00	2.11	0.33	0.38	0.31	3.28	8.00	5.19
<i>Briza maxima</i> L.	2.72	5.75	12.55	4.55	2.33	0.89	0.69	0.74	23.11	12.00	4.21
<i>Trisetum flavescens</i> L.	2.17	3.50	9.33	4.67	1.56	0.74	0.63	0.72	14.17	10.00	4.83
<i>Agrostis alba</i> L.	3.11	7.11	11.13	4.13	2.78	0.35	0.54	0.61	8.67	8.00	5.09

In general, the significantly greater influence of the environment-grassland interaction factor compared to the influence of the grassland factor implies a greater instability of the studied meadow species in terms of yield. This is confirmed by the calculated values of the parameter Si², especially for the white vole (9.50). In this type, there is an instability of linear type with a proven value of Si². The instability is due to the significant differences in yields between the individual experimental years, as obviously the weather conditions have a strong influence.

The nonparametric coefficients for determining the phenotypic stability are presented in Table 3. The first two nonparametric coefficients for assessing the phenotypic stability of Nassar and Huehn (1987) – Si (1) and Si (2) identify the most stable species of *Festuca arundinacea* Schreb. and *Trisetum flavescens* L., and Si (3) and Si (6) define the representatives of the *Festuca* species as stable. Plant populations that show a lower value of Thennarasu (1995) parameters are considered the most stable. As such, according to Npi (1), *Dactylis glomerata* L. and *Arrhenatherum elatius* P.B. are the most stable, while the other coefficients Npi (2), Npi (3) and Npi (4) determine *Festuca arundinacea* Schreb. as the most stable. Stress-tolerant feed species are increasingly needed for the ecological and economic sustainability of extensive livestock systems (Annicchiarico *et al.*, 2002), as well as in global warming conditions (Katova, 2008).

Rank Sum (KR) Kang (1988) uses both yield and 'σi²' as selection criteria. Here the species of *Lolium perenne* L. (16), *Briza maxima* L. (12) and *Trisetum flavescens* L. (10) are characterized by the highest stability,

which is distinguished by yields around and below the average in the totality of the studied plant species. According to the "KR" parameter, the fourth and fifth positions are occupied by the species *Festuca arundinacea* Schreb. and *Agrostis alba* L., which are less ecologically stable but have the highest yield of green mass.

The coefficient of general adaptability gives a lower estimate of the species of *Lolium perenne* L. (A = 3.83) and *Briza maxima* L. (A = 4.21) for the experimental conditions. From the point of view of this coefficient, *Festuca rubra* L. and *Arrhenatherum elatius* P.B. receive high marks. They could also be grown in less favourable environmental conditions, as they combine good productivity with greater adaptability.

GGE biplot analysis

According to Farshadfar (2008), stability is not a sufficient criterion for assessing plant populations, as stable species are often low yielding. Therefore, other approaches are needed, which include an integrated assessment of yield and stability in an index. The GGE biplot is a complex analysis system according to which most of the aspects of the genotype-environment interaction, presented in tabular form, can be represented graphically. In this way, the visual assessment of the species and the identification of the "mega" environment are significantly simplified. The first two main components (PC1 and PC2) can be easily plotted on a two-dimensional graph so that the interaction between each species and the defined environment can be visualized. The GGE biplot allows for visual ranking

of species through their productivity and stability in several environments. The data show that PC1 and PC2 represent 89.8% of the total variability that results from the interaction of the species with the environment (Fig. 3). Only the first two main components are presented, as this model emphasizes the best-observed patterns and levels of unnecessary data. In Figure 3, the abscissa "X", or the productivity line, passes through the beginning of the coordinate system of the biplot with an arrow indicating the positive end of the axis. The "Y" axis (stability axis) passes through the beginning of the biplot, perpendicular to the "X" axis. Thus, the average yield of genotypes is estimated by the projection of their markers on the "X" axis, and the stability – by the projection on the "Y" axis. The axis with the arrow marked with a circle passing through the centre of the coordinate system represents the average value of the species on the studied feature. From left to right, the species are classified by average yield. In the vertical direction, the mean value for the abscissa intersects the mean value for the ordinate. The intersection point at the same time represents the average yield. According to the graphic image, the meadow species *Festuca arundinacea* Schreb., *Agrostis alba* L. and *Festuca rubra* L. are characterized by the highest productivity, and *Briza maxima* L. and *Lolium perenne* L., respectively, with the lowest. The species of *Lolium perenne* L. and *Agrostis alba* L. are characterized by the greatest variability of yield, while *Dactylis glomerata* L., *Festuca rubra* L., *Arrhenatherum elatius* P.B. show significant stability of the trait. The position of *Festuca rubra* L. in the figure defines it as a favourable combination of high productivity with relative stability. Ivanov *et al.* (2018) point out that simultaneous assessment of yield and stability provides important information about the value of a population as it is based on the reliability of differences in yield and the variant of interaction with the environment. In this way, a generalized assessment is obtained, arranging the populations and species in descending order according to their economic value.

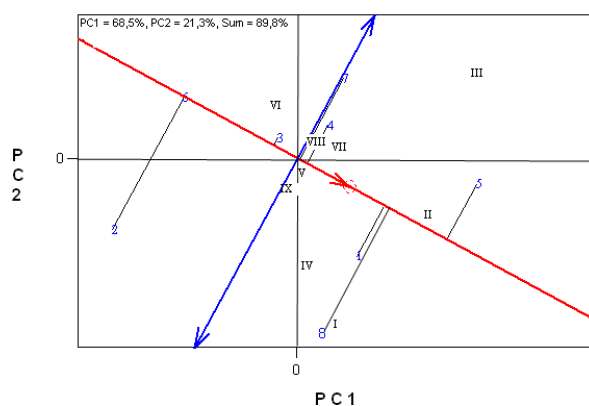


Figure 3. GGE biplot analysis for Green mass yield of meadow grass plant populations (1 – *Festuca rubra* L.; 2 – *Lolium perenne* L.; 3 – *Dactylis glomerata* L.; 4 – *Arrhenatherum elatius* P.B.; 5 – *Festuca arundinacea* Schreb.; 6 – *Briza maxima* L.; 7 – *Trisetum flavescens* L.; 8 – *Agrostis alba* L.; I–IX – years (environments) of cultivation from 2011 to 2019)

Conclusion

Through the analysis of the variant, a proven influence of the factors environment, type and type-environment interaction has been established. The environment has the strongest influence on the yield of fresh mass – 94.71% of the total variation. The influence of the species-environment interaction (4.10%) and the species (1.19%) is significantly weaker.

The assessment of the stability of the studied eight species of meadow grasses in the foothills in the Balkan Mountains conditions by the methods of regression, variance and nonparametric analysis is not one-way as it is based on different concepts of stability. According to the values of most of the calculated stability parameters (b_i , S_i^2 , λ_i , σ^2_i , PP, W2, $S^{(3)}$, $S^{(6)}$, NP⁽¹⁾) *Dactylis glomerata* L. shows good ecological stability. Positive assessments can also be given to the low-yielding species of *Lolium perenne* L. ($b_i = 0.30$, 16.6 t ha⁻¹) and *Briza maxima* L. ($b_i = 0.54$, 17.60 t ha⁻¹). The most unstable, but with a high yield of fresh mass is *Festuca arundinacea* Schreb. ($b_i = 1.62$; 21.00 t ha⁻¹).

Complex evaluation by GGE biplot analysis identifies *Festuca rubra* L. as a species that favourably combines high productivity with relative stability. *Dactylis glomerata* L. and *Arrhenatherum elatius* P.B. are characterized by high stability and yield close to the average for the group. These species are suitable for growing in a wide range of environmental conditions.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions

NG – critical revision and approval of the final manuscript;
VK – analysis and interpretation;
DM – study conception, design and acquisition of data;
IS – drafting of the manuscript.

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