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Original scientific paper

GENETIC VARIABILITY OF SEED YIELD AND SEED YIELD COMPONENTS OF AUTOCHTHONOUS *Lolium perenne* L. POPULATIONS

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The aim of this study was to examine genetic variability, heritability and correlation of seed yield components and seed yield of progenies of autochthonous populations and cultivars of perennial ryegrass, and that on the basis of these results distinguish genotypes that would be later used in the creation of new local cultivars of perennial ryegrass. Research was carried out on experimental fields and laboratories of the Agricultural Institute of Republic of Srpska in Banja Luka in 2007. and 2008., on 6 natural populations of perennial ryegrass collected in Bosnia and Herzegovina and two cultivars, *Maja* and *Calibra* as standard. Following parameters were analyzed: number of generative tillers per plant, time of flowering, spike length, number of spikelets per spike, seed yield per spike and plant and 1000 seed weight.

Analysis of the results of the number of generative tillers per plant indicates a statistically significant difference in the studied populations and cultivars of perennial ryegrass. The highest average number of tillers per plant was found in the cultivar *Maja* (193.8), and the least number was detected in population *Dragočaj* (78.9), so statistical differences were highly significant. The longest period to beginning of flowering was determined in cultivar *Calibra* (56.5 days) and the shortest in population *Laminci* (43 days). The average length of spike of perennial ryegrass in the studied population was 22.78 cm and was significantly lower than cultivar *Maja*. Statistically significant differences between populations and variety *Maja* in the number of spikelets per spike were detected. The average seed yield per spike of studied populations was in level with cultivar *Maja* and 37.5% higher compared with variety *Calibra*. In the studied

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populations of perennial ryegrass seed yield ranged from 5.21 g (*Dragočaj* population) to 15.40 g (*Kupres* population). Weight of 1000 seeds was highest in the variety *Calibra* (2.60 g) and lowest in population *Maglajani* (1.94 g).
Proportion of genetic to phenotypic variance for time of flowering, the number of generative tillers, seed yield per spike and plant and 1000 seed weight indicate that the variability of these traits in this collection of genotypes, largely derived from plant genotype.
In this investigation, the presence of a highly significant positive genetic correlation was found between time of flowering and spike length (0.98), time of flowering and 1000 seed weight (0.97), number of generative tillers and seed yield per plant (0.91) and spike length and 1000 seed weight (0.98).
Key words: perennial ryegrass, population, cultivar, seed yield, seed yield components

INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.) is one of the most important perennial cool-season forage grasses. Perennial ryegrass is widespread in the Republic of Srpska and BiH and it is known by the popular names common ryegrass, lea grass or English grass. It is highly productive (SOKOLOVIC *et al*., 2010), with the higher nutritive value compared with other forage grasses, adapted for grazing and frequent mowing (PEETERS, 2004). In natural habitats usually grows on the fields, in gardens, roadsides, pastures and meadows. Perennial ryegrass is particularly adapted to dry to moderately fertile soil moist, with different pH values, although in the local agro-ecological conditions of Republic of Srpska or Bosnia and Herzegovina, it is prevalent on poor acid soils where there is a free ecological niche.

Perennial ryegrass breeders driven by market demands focus their interest to production of cultivars with high, stable yield and good quality dry matter, improved persistency, tolerant to drought (SOKOLOVIC *et al.,* 2012), frost and other stressful environmental conditions, with different maturity. But also there is one very important trait in breeding which is not usually essential to farmers as end users of grasses. This is seed yield. Without good seed yield and quality it is impossible to multiply and produce enough amount of cultivar seed for market (SIMIC *et al.,* 2010).

According to that, initial breeding material must be heterogeneous, with a range of different genotypes. Wild autochthonous populations usually show great adaptability and are acclimatized on local agro-ecological conditions. In this article the diversity in a collection of perennial ryegrass autochthonous genotypes has been investigated at the beginning of the breeding process.

For successful breeding, and the creation of new varieties of perennial ryegrass, one of the basic conditions is that there is large variability of the starting material (SOKOLOVIC *et al.,* 2003; 2004). Traits of perennial ryegrass such as heading date, spike length, number of spikelets per spike and leaf width usually show high variability and heritability and therefore their breeding is promising (SOKOLOVIC, 2006). However, in order to improve mentioned traits in the selection process, they should be favorably correlated with seed yield (ELGERSMA, 1990).

The aim of this study was to examine genetic variability, heritability and correlation of seed yield components and seed yield of progenies of autochthonous populations from Republic of Srpska and cultivars of perennial ryegrass in order to use these results to distinguish genotypes that would be later used in the creation of new local varieties of perennial ryegrass.

MATERIALS AND METHODS

Investigation of seed yield and seed yield components of autochthonous populations and cultivars of perennial ryegrass was carried out on experimental fields and laboratories of the Agricultural Institute of Republic of Srpska in Banja Luka in 2007. and 2008.. Investigated genotypes were six native populations of perennial ryegrass collected in Bosnia and Herzegovina (Tab. 1) and two varieties Maya (Poland, 1987) and Calibra (DLF, Denmark, 1998).

Table 1 Geographical origin of collected autochthonous populations of perennial ryegrass

Populations	Locality	Latitude and longitude	Altitude (m)	Habitat
Dragočaj	Stranjani	N 44^0 50' E 17^0 07'	240.5	Garden, orchard
Kosjerovo	Lijevče polje - channel	N 44^0 57' E 17^0 22'	108.5	Next to field road
Laminci	Lijevče polje - lakes	N 45^0 06' E 17^0 13'	91.8	Meadow
Novo Selo	Rim of Kupres field	N 44^0 04' E 17^0 10'	1214.6	Pasture
Kupres	Kupres field	N 44^0 01' E 17^0 52'	1200.9	Meadow, lea
Maglajani	Lijevče field - mine	N 44^0 58' E 17^0 20'	108.2	In the filed next to the road

Collected populations of perennial ryegrass were previously sown in isolation at the experimental field in order to obtain sufficient quantities of seed for further research. In the fall of 2006 the seeding of space plant nursery (70 x 70 cm) was done. Each population and the cultivar was represented by 40 plants. During 2007 and 2008. the following parameters of seed yield and seed yield components of perennial ryegrass were monitored: number of generative tillers per plant, flowering time (number of days from April the 1st to the beginning of flowering), spike length, number of spikelets per spike, seed yield per spike and per plant and 1000 seed weight.

Obtained results were processed by factorial analysis of variance (ANOVA), a significant difference between mean values was determined by LSD test. Expression of ecological effects was presented by separating the total variance to genetic variance (σ^2_g), variance of interaction (population x year) (σ^2_g/σ^2_y) and phenotypic variance (σ^2_f). Also, heritability in the broad sense was calculated by the formula: $h^2=\sigma^2_g / \sigma^2_f$. Establishing relationships between the traits of perennial ryegrass populations was performed by calculating the coefficient of phenotypic (r_f). and genetic (r_g) correlation.

Also hierarchical cluster analysis of populations and varieties was performed on the basis of all observed traits and on data row by Ward method with Euclidean distances.

Environmental conditions

Soil Conditions - The trial was conducted at the experimental field of Agricultural Institute of Republic of Srpska in Banja Luka, on valley-brown soil on alluvial substrate of the river Vrbas. On the basis of chemical analysis of the arable layer (0-30 cm depth), the soil is slightly alkaline (pH 7.15 in H_2O) with low humus content (2.9%). Content of phosphorus (P_2O_5) is very good (28.4 mg/100 g), while the presence of potassium in the soil is good (20.2 mg/100 g of K_2O).

Precipitation and temperature - less rainfall and higher temperatures characterized weather conditions in the years of performing experiments, and which affected the researching results.

Table 2. Precipitation and mean monthly temperature for the growing period 2007-2008 and multi-year average (1961-2010)

Month	Rainfalls (l/m^2)			Temperatures (^{0}C)		
	2007	2008	1961-2010	2007	2008	1961-2010
IV	4.5	102.9	89.0	13.6	12.6	11.1
V	95.0	70.9	94.7	18.3	17.6	16.1
VI	81.0	79.6	114.4	22.7	21.5	19.5
VII	38.2	85.2	92.0	24.0	22.4	21.2
VIII	60.9	24.3	85.9	22.5	21.9	20.7
IX	154.5	106.7	95.3	14.8	15.6	16.1
Total	434.1	538.9	571.3			
Average				19.3	18.6	17.5

Total precipitation during the vegetation period (IV-IX) for long-term average (1961-2010), was 571.3 l/m^2 and was higher compared to the total precipitation for the same period in the study years (Table 2). In the same table can be seen that the average air temperature during the study were higher than the long-term average. Agro-climatic factors affected the plant growth and seed yield, which is in accordance with ZEBARINI and THOMAS (2003), IKANOVIC *et al.*, 2011, 2013, POPOVIC *et al.*, 2011, 2012, 2013, ZORIC *et al.*, 2012).

RESULTS AND DISCUSSION

Average values of two-year study of seed yield and seed yield components of six autochthonous populations and two varieties of perennial ryegrass are shown in table 3. Analysis of the results of generative tillers per plant indicates that there is a significant difference among studied populations and cultivars of perennial ryegrass. The highest number of generative tillers was found in the cultivar *Maja* (193.8), the lowest number was found in population *Dragočaj* (78.9), and statistical differences were highly significant. The number of generative tillers was also high in populations *Kupres* (186.7) and *Kosjerovo* (180.4). In paper SOKOLOVIĆ et al. (2011) the average number of generative tillers for the investigated collection consisted of 10 autochthonous populations of perennial ryegrass originating from Serbia was 376.2. The cause of the much smaller average number of genitive tillers in this studied population (127.5) lies in the unfavorable weather conditions that reduced plant growth (Table 2).

Table 3. Mean values of seed yield components and seed yield of autochthonous populations and cultivars of perennial ryegrass in the period 2007-2008.

Population	Number of generative tillers per plant	Time of flowering	Spike length	Number of spikelets per spike	Seed yield per spike	Seed yield per plant	Weight of 1000 seeds
Dragočaj	78.9	53.0	24.85	21.31	0.07	5.21	2.30
Kosjerovo	180.4	47.0	22.68	25.06	0.08	14.93	2.08
Laminci	119.4	43.0	20.94	22.13	0.05	5.41	2.00
Novo Selo	113.8	43.5	22.94	23.38	0.11	11.79	2.03
Kupres	186.7	45.0	22.86	24.13	0.09	15.40	1.98
Maglajani	85.8	46.0	22.38	24.69	0.10	8.11	1.94
$\overline{X}$ *of populations*	127.5	46.3	22.78	23.45	0.08	10.14	2.06
variety Maja	193.8	48.0	26.04	26.14	0.08	14.15	2.10
variety Calibra	92.4	56.5	25.36	23.70	0.05	4.41	2.60
LSD 0.05	44.26	0.99	2.94	2.91	0.03	2.72	0.19
0.01	58.47	1.31	3.88	3.84	0.04	3.59	0.26

The average time of flowering of investigated autochthonous populations of perennial ryegrass was 46.3 days from April the 1st, so the differences with cultivars *Calibra* and *Maja* were statistically significant. The longest period to beginning of flowering was determined in cultivar *Calibra* (56.5 days) and the shortest in population *Laminci* (43 days). By studying 50 different genotypes of perennial ryegrass MCGRATH *et* al. (2010) found that time of flowering ranged from 47.07 (ecotypes mainly from Ireland) to 52.89 as recorded for cultivars included in the study. MARTINIELLO (1998) points out that the early flowering in perennial ryegrass is important characteristics for seed yield improvement due to longer grain filling period.

The average spike length in the studied populations was 22.78 cm and was significantly lower regard to variety *Maja.* Maximum spike length in the examined populations was identified in the population *Dragočaj* (24.85 cm). For number of spikelets per spike, there are statistically significant differences between some populations and cultivar *Maja*. The high number of spikelets per spike was found in populations *Kosjerovo* (25.06), *Maglajani* (24.69) and *Kupres*

(24.13). ELGERSMA (1990) points out that the average length of a spike obtained for the three varieties of perennial ryegrass grown in different soil types in different years ranged from 21.4 to 28.3 cm. The average length of a spike of ten autochthonous populations originating in Serbia in two years investigation ranged from 22.2 to 25.7 cm (SOKOLOVIĆ *et* al., 2011).

The average seed yield per spike of examined populations was in level with variety *Maja*, and 37.5% higher than variety *Calibra.* The yield of seed per spike distinguished populations *Novo Selo* (0.11 g/class) and Maglajani (0.10 g/class). Seed yield per spike determined by studying the progeny obtained by crossing the 184 varieties of Italian and Danish varieties *Veyo* and *Falster* was 0.05 g (STUDER et al., 2008).

In the studied populations of perennial ryegrass determined average seed yield per plant was 10.14 g, and ranged from 5.21 g (*Dragočaj* population) to 15.40 g (*Kupres* population). Population *Kosjerovo* (14.93) also had high seed yield per plant. Best yielding populations had a higher yield than variety Maja which showed the best all seed yield components.

Weight of 1000 seeds was highest in tetraploid varieties *Calibra* and the smallest in population *Maglajani*. In the examined autochthonous populations of perennial ryegrass highest 1000 seeds weight was determined in the population *Dragočaj* (2.30 g), and the differences compared to the variety Maja were statistically significant. SOKOLOVIĆ *et* al. (2007) state that the 1000 seeds weight in the most of perennial ryegrass studied populations varied from 1.27 g to 2.42 g.

Table 4. ANOVA and parameters of the variability of observed traits of perennial ryegrass populations and cultivars

Parameters	Time of flowering	Number of generative shoots	Spike lenght	Number of spikelets per spike	Seed yield per spike	Seed yield per plant	Weight of 1000 seeds
MS genotype	556.39^{**}	36914.71^{**}	47.83^{**}	39.04^{**}	0.0036^{**}	338.13^{**}	0.39^{**}
MS year	2140.01^{**}	3570.13^{ns}	400.45^{**}	105.12^{**}	0.0001^{ns}	2.35^{ns}	0.79^{**}
MS interaction	8.12^{**}	2554.95^{ns}	27.94^{**}	15.05^{ns}	0.0004^{ns}	2.20^{ns}	0.04^{ns}
Genetic variance σ^2_g	22.85	2147.49	1.24	1.50	0.0004	21.00	0.044
Phenotypic variance σ^2_f	23.18	2307.17	2.99	2.44	0.0005	21.13	0.049
Heritability h2 (%)	98.54	93.08	41.58	61.44	89.84	99.35	89.47

Based on the results of analysis of variance it is clear that the influence of genotype on all the components of seed yield was highly significant (Table 4). Also is noticeable that different year had a significant effect on some traits, such as time of flowering, spike length, number of spikelets per spike and 1000 seed weight. The existence of a highly significant interaction (genotype x year) was found for time of flowering and spike length.

Proportion of genetic to phenotypic variance for time of flowering, the number of generative tillers, seed yield per spike and plant and 1000 seed weight indicate that the variability of these traits in this collection of genotypes, largely derived from plant genotype.

For spike length and number of spikelets per spike ratio of genetic and phenotypic variance was significantly different, indicating the significant influence of ecological factors on variability of these traits in the population collection.

Heritability for the number of generative tillers, time of flowering, seed yield per spike, seed yield per plant and 1000 seed weight is high, which means that these traits largely depend on genetic factors. The high heritability values determined for these traits, talk about the great proportion of genetic to total phenotypic variability of the traits and suitability for breeding. In our investigation broad sense heritability was lowest for number of spikelets (61.44%) and the length of spike (41.58%). Based on the results shown in Table 4. can be concluded that investigated traits are quite variable, so significant variation in some individual genotypes can be expected depending on environmental factors and the year of investigation.

Table 5. Genetic and phenotypic correlation coefficients of investigated traits of populations and cultivars of perennial ryegrass

Traits	Time of flowering	Number of generative tillers	Spike length	Number of spikelets per spike	Seed yield per spike	Seed yield per plant	Weight of 1000 seeds
Time of flowering		-0.39	0.98**	-0.15	-0.44	-0.47	0.97**
Number of generative tillers	-0.35		0.07	0.82*	0.21	0.91**	-0.37
Spike length	0.77*	0.05		0.22	-0.09	0.01	0.98**
Number of spikelets per spike	-0.09	0.66	0.24		0.53	0.89**	-0.27
Seed yield per spike	-0.43	0.13	-0.10	0.37		0.61	-0.71
Seed yield per plant	-0.46	0.88**	-0.01	0.69	0.58		-0.59
Weight of 1000 seeds	0.93**	-0.38	0.66	-0.24	-0.55	-0.54	

Phenotypic coefficients of correlations (below diagonal); Genetic coefficients of correlations (above diagonal)

* p > 0.95

** p > 0.99

For successful breeding on any plant species is necessary to know the genetic background, the structure of genetic and phenotypic variance, heritability, and the correlation of functionally related traits. Also, of particular importance are genetic correlations, suggest possible directions of change in different traits of plant material under the impact of selected and applied methods of breeding. Calculated values of the coefficients of phenotypic and genetic correlations of seed yield components of perennial ryegrass are shown in Table 5.

From the Table 5. with correlation coefficients it can be seen that the genetic correlations (above diagonal) are somewhat higher than the phenotypic (below diagonal). As for the correlation between the seed yield components of perennial ryegrass population, there were highly significant positive genetic correlation between time of flowering and spike length (0.98) and time of flowering and 1000 seed weight (0.97). At the same time, highly significant genetic and phenotypic correlations between the number of generative tillers and seed yield per plant (0.91 and 0.88 respectively) were obtained. This practically means that the seed yield per plant is mainly determined by the number of generative tillers. Also, a highly significant positive genetic correlation between spike length and 1000 seed weight was detected. Significant positive genetic correlation was found between the number of generative tillers and number of spikelets per spike (0.82). Calculated coefficients of phenotypic correlation were highly significant between time of flowering and 1000 seeds weight (0.93) and significant between time of flowering and spike length (0.77).

Assessment of genetic and phenotypic correlations between observed traits of perennial ryegrass, shows to the complexity of the inheritance of seed yield components and seed yield and large impact of external environment factors and their interaction (genotype *x* age).

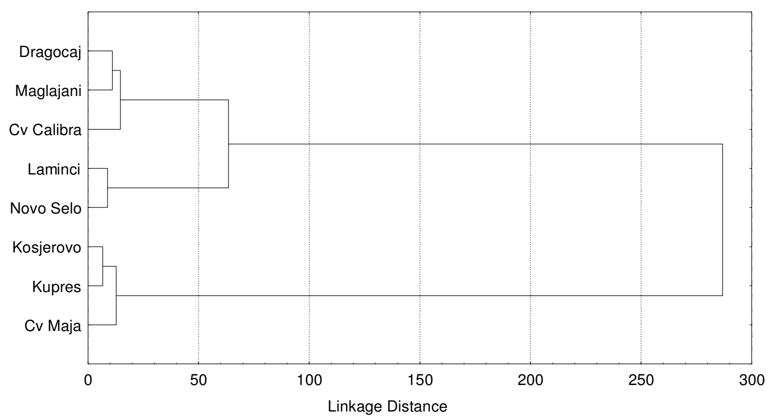

Figure 1. Cluster diagram of the investigated perennial ryegrass populations and cultivars

On the graphic formed as a result of hierarchical cluster analysis (Figure 1) two groups of genotypes can be observed. The first group was composed of two subgroups. In the first subgroup are genotypes with lowest number of generative tillers and seed yield per plant. The second subgroup is consisted of genotypes that are characterized by medial number of generative tillers. Also, these genotypes have earlier maturity than the rest of the collection. The second group is composed of genotypes that have the highest number of generative tillers and seed yield per plant. The resulting clustering of populations in the cluster diagram is not correlated with their geographical origin (Table 1).

CONCLUSION

In two-year study of the genetic variability of seed yield and seed yield components of six autochthonous populations and two cultivars of perennial ryegrass high genetic variability and very significant differences among the studied populations and varieties were observed.

In cultivar *Maja* was found most generative stems, the largest average size of spike and the highest number of spikelets per spike. The longest period before flowering had a variety *Calibra* and shortest population was *Laminci*. Highest seed yield per spike was detected in population *Novo Selo*, while the highest seed yield per plant was achieved within a population *Kupres*. Weight of 1000 seed of studied genotypes ranged from 1.94 g (*Maglajani* population) to 2.60 g (variety *Calibra*).

The influence of genotype on all traits was highly significant. Proportion of genetic to phenotypic variance, especially for time of flowering, number of generative tillers, seed yield per spike and plant and 1000 seed weight, indicating that the variability of these traits is largely derived from plant genotype and consequently the heritability of these traits was high.

In this collection of population it is determined presence of a highly significant positive genetic correlation between time of flowering and spike length, time of flowering and 1000 seed weight, number of generative tillers and seed yield per plant and spike length and 1000 seed weight.

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GENETIČKA VARIJABILNOST PRINOSA I KOMPONENTI PRINOSA SJEMENA AUTOHTONIH POPULACIJA *Lolium perenne* L.

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Izvod

Cilj ovog rada je da se ispitaju genetička varijabilnost, heritabilnost i korelacije komponenti prinosa i prinos sjemena potomstava autohtonih populacija i sorti engleskog ljulja. Istraživanja su obavljena na oglednom polju i u laboratorijama Poljoprivrednog instituta Republike Srpske, tokom 2007. i 2008. godine. Proučavano je 6 autohtonih populacija engleskog ljulja sakupljenih u Bosni i Hercegovini i dvije sorte *Maja* i *Calibra* kao standardi. Analizirane su sledeće osobine: broj generativnih stabljika po biljci, broj dana do početka cvjetanja, dužina klasa, broj klasića po klasu, prinos sjemena po klasu i biljci i masa 1.000 sjemena.

Analiza rezultata broja generativnih stabljika po biljci ukazuje da postoji visoko signifikantna razlika kod ispitivanih populacija i sorti engleskog ljulja. Najduži period do početka cvjetanja imala je sorta *Calibra*, a najkraći populacija Laminci. Prosječna dužina klasa kod ispitivanih populacija iznosila je 22,7 cm i bila je značajno manja u odnosu na sortu *Maja*. U broju klasića po jednom klasu postoje visoko signifikantne razlike između pojedinih populacija engleskog ljulja i sorte *Maja*. Prosječan prinos sjemena po klasu ispitivanih populacija bio je u nivou sorte *Maja*, a u odnosu na sortu *Calibra* viši za 37,5%. Kod ispitivanih populacija engleskog ljulja prinos sjemena bio je od 5,21 g (populacija Dragočaj) do 15,40 g (populacija Kupres). Masa 1.000 sjemena bila je najveća kod sorte *Calibra*.

Tokom ispitivanja utvrđeno je postojanje visoko značajne pozitivne genetičke korelacije između početka cvjetanja i dužine klasa, početka cvjetanja i mase 1.000 sjemena, broja generativnih izdanaka i prinosa sjemena po biljci i dužine klasa i mase 1.000 sjemena.

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