

**EVALUATION OF MORPHOLOGICAL TRAITS, DRY MATTER YIELD
AND QUALITY OF *Lolium perenne* L. AUTOCHTHONOUS POPULATIONS
FROM SERBIA THROUGH MULTIVARIATE ANALYSIS**

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Due to specific climatic conditions, perennial ryegrass breeding in
Serbia is focused on resistance or tolerance to abiotic stress factors,
especially to drought and high temperatures. These traits should be
associated with high dry matter yield and quality. Therefore, most
frequently used initial material is autochthonous populations and ecotypes
adapted to local agro-ecological conditions, but knowledge about their
variability of important traits for breeding is missing. Pre-selection
evaluation of ten populations of perennial ryegrass originating from Serbia

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is presented in this paper. Twenty five traits were investigated during the two-year period and processed using analysis of variance and multivariate statistical methods (cluster and principal components analysis). The goal was to determine diversity and genetic distances of investigated populations by phenotyping and to define traits considerably affecting the variability and discrimination of populations.

On cluster diagram two groups of population are observed, but geographic origin of populations (lowland, hilly, mountainous habitat) was not influence to clustering of collection. Factor analysis has clarified that first seven principal components (PC) described almost 95%. The traits which show high correlation coefficients with first principal component were plant height in first cut, leaf length and width, DM of generative tillers, spike and spikelet length and 1000 seed weight, and with second principal component time of heading, terminal internode length, DM of vegetative tillers, spikelet number and FSU.

It can be concluded that variability between populations was high and that differences of population were mainly affected by most important traits for breeding, such as components of dry matter production and some seed yield components.

Key words: perennial ryegrass, population, phenotyping, variability, principal components

INTRODUCTION

Breeding success basically depends on genetic variability of starting breeding genotypes and populations collection, or germplasm origin and breeding history. The investigation of the start breeding material represents the first basic step in the selection process, even germplasm originates from previous breeding circles or is completely new. To introduce exotic or wild germplasm of *L. perenne* in breeding programmes it is necessary to screen a wide range of variability in order to find some desirable adaptation traits. Knowledge of genetic structure and variability inside of wide and heterogeneous genotype collection and also genotypes geographic origin (GHESQUIERE *et al.* 2003; KATOVA *et al.* 2006) are priceless in further decisions which genotypes should be included in process of selection. If genetic variability is not sufficient for certain breeding model and criteria, it is crucial to increase variability with new germplasm introduction, from ecotypes and autochthonous populations (ROMANI *et al.* 2002; THOROGOOD and SKOT 2003) or from distant exotic origin (JAFARI *et al.* 2003).

Perennial ryegrass is native in Serbian flora (SOKOLOVIĆ *et al.* 2003), what enables constant broadening of genetic basis for breeding with new material acclimatised on local agro-ecological conditions. This richness of various genotypes in Serbia is the prerequisite for genetic improvement of commercial cultivars. Perennial ryegrass as forage crop has been in breeding process for a very long time, especially in Western Europe. Very often the initial breeding material are ecotypes (CAGAŠ *et al.* 2006; SOKOLOVIĆ *et al.* 2006) and multivariate methods are essential in

the beginning of breeding, especially factor analysis (GHARIANI *et al.* 2006) and cluster analysis (MUYLLE *et al.* 2003). These methods enable simultaneous processing of huge number of populations and data, with defining of interrelationship among populations and significance of traits impact on total variability (CASLER 1995; ROMANI *et al.* 2002). Therefore the aim of analyses in this article was to identify within and among ecotypes diversity, describe complex trait correlation structure in collection with less number of principal components and to define significant traits for population discrimination.

MATERIALS AND METHODS

The investigated autochthonous perennial ryegrass populations were collected in central Serbia representing all contrasting localities (SOKOLOVIĆ *et al.* 2003; Figure 1).

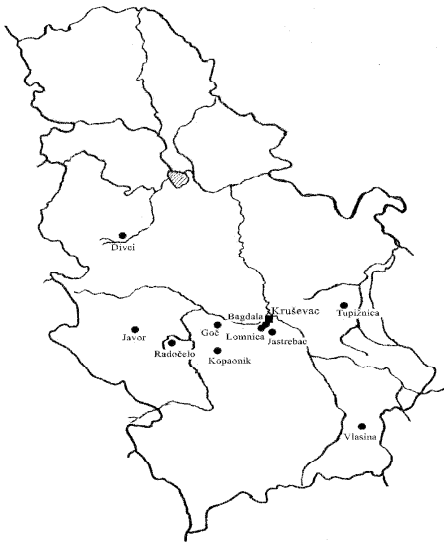


Figure 1. Geographic origin of *Lolium perenne* autochthonous populations

Three populations were collected from lowland meadows (up to 400 m asl.) and two populations from hilly meadows (between 400 and 700 m asl.), respectively (Table 1). Most of the populations were collected from mountain localities (above 700 m asl.). Population names were given after locality names.

Spaced plant trial (60x60cm) was established in autumn 2002. in completely randomized block design. Seed was sown in pots and transfer to field when plants formed three or four tillers. Population were represented with 60 plants planted in four replications (15 plants per replication).

Table 1. *Perennial ryegrass (Lolium perenne L.)* populations origin

No	Popul. name /Locality	Abbrev	Nearest place	Altitude (m)	Geographic		Habitat (locality) description
					Longitude	Latitude	
1	Jastrebac	JAS	Ravnište	683	21° 23'	43° 28'	Meadow, forest edge
2	Kopaonik	KOP	Ploča	1105	20° 52'	43° 29'	Meadow
3	Divci	DIV	Valjevo	185	20° 01'	44° 19'	Meadow, roadside
4	Lomnica	LOM	Lomnica	218	21° 20'	43° 30'	River bank
5	Goč	GOČ	Stanišinci	670	20° 54'	43° 25'	Meadow
6	Bagdala	BGD	Kruševac	195	21° 21'	43° 43'	Meadow, roadside
7	Vlasina	VLS	Vrla	857	22° 17'	42° 41'	Meadow, lake shore
8	Tupižnica	TUP	Lasovo	820	22° 15'	43° 44'	Meadow
9	Javor	JAV	Kušići	812	20° 05'	43° 30'	Meadow
10	Radočelo	RDČ	Rudno	1100	20° 25'	43° 25'	Meadow

Phenotyping

In 2003. and 2004. time of heading [HT] (number of days after 1st April) was scored when at least 3 shoots appeared on plant. Morphological traits (plant height [PH1] (cm), length [LL] and width [LW] of first leaf (cm), length of terminal internode [IL] (cm) and vegetative tillers number [VTN]) were measured in first cut and plant height in second cut [PH2] (cm). Components of forage and seed yield (spike length [SL](cm), number of spikelets per spike [SN], spikelets length [SLL](cm), generative tillers number [GTN], 1000 seed weight [SW] (g), floret site utilization [FSU], seed yield per plant [SY] (g), turf diameter [TD] (cm), dry matter of vegetative [DMV] and generative tillers [DMG] (g) and dry matter yield in the second cut [DMS] (g) were determined, as well. First defoliation has been made immediately after tillering and the second one month after first. Traits LL, LW, IL, SL, SS, SLL were measured on three representative tillers per plant. Turf diameter was measured in base of turf. FSU (%) represent ratio of number of flowers on inflorescences before pollination and obtained seed after pollination. Data for all traits were averaged for 2003. and 2004.

In the second year (2004) chemical composition of dry matter in first cut (ash [ASH], crude protein content [CPC], crude cellulose content [CCC], phosphorous [P], potassium [K], calcium [Ca] and magnesium [Mg] (gkg^{-1})) were also determined. All chemical analyses were carried out using standard laboratory methods (ASH, CPC and CCC by Weende system, P by spectrophotometry and K, Ca and Mg by AAS).

Statistical analyses

All two-years averaged data were analysed by ANOVA and significance of differences were determined by LSD test. Hierarchical cluster analysis was performed on row data by Ward method with Euclidean distances. Also, K-mean method was done to confirm cluster analysis results and to show what traits were significant for population grouping in clusters.

Factor analysis was carried out on populations two-years averages based on principal components (PC) extracting method. Dimension of factor area was determined based on principal components with eigenvalue above 1. After rotation by a variance maximizing method (Varimax normalised) converged in 25 iterations, importance of some traits was analysed based on their correlations coefficients with principal components higher than 0,7. All analyses were done with Statistica 8.0, Stat Soft inc. and SPSS 10.0 (RAYNALD 1996).

RESULTS

Results for examined traits indicate that expected high variability actually exists within (CV) and between autochthonous populations showing high significant differences among populations (LSD) (Table 2). The least intra population variability is obtained for time of heading and components of dry matter chemical composition (under 10%), with exception of Mg content. Other traits have shown higher intra population average genetic variability, particularly DMY components and seed yield (above 50%).

Hierarchical cluster analysis of traits two-years average values was performed and dendrogram was formed (Figure 2). Two groups can be noticed on diagram and they are consisted of four and six populations, respectively. Most closely related populations are Goč and Tupižnica and Bagdala and Radočelo, respectively. Population Jastrebac is most distinguished from the rest of collection.

Based on the fact that cluster analysis showed two groups of populations, K-means clustering method was used to confirm population distribution. Identical results were obtained. The first group consisted of populations Jastrebac, Bagdala, Radočelo and Javor and the second included Kopaonik, Goč, Tupižnica, Lomnica, Diveci and Vlasina. Analysis within and between population groups variability detected what traits discriminate populations within this cluster classification. High significant effect ($P < 0.01$) on that clustering had the following traits: leaf length, number of generative tillers, DM of generative tillers, 1000 seed weight and crude cellulose content. Leaf width, spike length and ash had also significant effect ($P < 0.05$). Other traits had no significant effect on population differentiation.

Factor analysis has clarified that first seven principal components (PC) described almost 95% of variability which determine dimension of factor area on 7 (Table 3).

First principal component has defined a large amount of variability among populations (27.79 %). Traits that showed significant correlation coefficients with first principal component were: plant height in first cut, leaf length and width, DM of generative tillers, spike and spikelet length and 1000 seed weight (Table 4).

Table 2. Average trait values for populations (2003 and 2004 averaged) with Lsd test and CV

Traits	JAS	KOP	DIV	LOM	GOČ	BGD	VLS	TUP	AV	RDC	Lsd 005;		
											Average	CV (%)	
HT	46.3	43.2	48.6	47.5	46.5	45.8	45.0	47.1	43.3	49.5	46.9	2.2; 2.9	7.73
PH1	82.9	84.9	82.7	78.8	82.1	77.3	84.7	83.5	87.3	80.6	82.7	4.7; 6.2	11.55
PH2	26.5	17.5	24.7	19.0	21.4	18.0	20.8	20.4	21.9	14.0	23.3	2.7; 3.5	25.98
LL	17.1	16.3	16.9	16.2	18.5	16.7	20.0	18.3	18.0	16.1	17.8	1.3; 1.7	14.69
LW	0.40	0.50	0.49	0.46	0.51	0.46	0.54	0.44	0.50	0.48	0.49	0.04; 0.05	16.93
IL	18.6	24.8	18.2	19.9	22.7	23.0	24.1	22.7	27.2	21.0	22.2	1.9; 2.5	17.93
SD	49.0	49.1	47.9	46.0	47.8	49.5	52.5	51.1	51.4	44.0	48.9	3.7; 4.9	15.79
GTN	419.4	393.5	369.5	354.6	400.5	396.8	398.5	358.0	424.7	371.0	376.2	46.6; 61.3	31.01
VTN	181.4	106.9	125.7	146.2	95.0	103.9	114.9	100.2	108.5	116.0	115.6	25.8; 33.9	53.80
DMG	161.0	138.3	158.0	132.2	163.3	134.2	164.8	134.8	155.5	134.5	149.8	23.5; 30.9	36.65
DMV	30.1	15.8	21.3	21.7	17.3	18.7	17.4	18.7	17.7	19.1	19.86	4.9; 5.5	50.98
DMS	30.1	15.6	40.4	31.7	22.0	27.6	30.9	23.8	32.2	27.4	28.7	7.3; 9.5	58.04
SL	22.9	22.2	24.3	23.7	24.1	23.1	25.7	24.6	23.8	23.1	23.9	1.6; 2.1	11.96
SLL	1.42	1.47	1.30	1.46	1.46	1.29	1.54	1.31	1.43	1.27	1.41	0.1; 0.2	15.13
SN	26.5	24.8	27.5	26.1	26.5	25.5	27.0	26.4	22.7	26.6	25.9	1.4; 1.8	9.48
SY	6.22	5.12	4.06	2.98	4.93	3.95	3.12	3.11	5.78	4.46	4.34	1.3; 1.7	68.53
SW	1.56	1.49	1.36	1.27	1.41	1.33	1.47	1.36	1.62	1.35	1.51	0.1; 0.8	14.50
FSU	0.67	0.75	0.69	0.72	0.73	0.71	0.58	0.66	0.88	0.58	0.7	0.06; 0.08	18.06
ASH	102	91	97	99	92	97	96	98	99	96	97.4	4.2; 5.7	3.39
CPC	198	182	177	202	164	193	181	175	188	197	174.9	11.5; 15.4	5.52
CCC	266	301	292	284	280	273	303	293	288	279	282.7	13.9; 18.6	3.47
P	3.6	3.3	3.2	3.3	3.0	3.0	2.7	2.4	2.8	3.1	3.09	0.1; 0.2	10.06
K	20.1	19.2	19.2	17.7	16.7	18.2	16.7	17.5	17.8	17.0	17.9	1.8; 2.4	7.66
Ca	4.8	5.6	4.9	5.1	4.4	4.8	4.3	4.6	5.4	5.3	4.94	0.8; 1.0	10.86
Mg	3.5	2.4	2.8	3.1	3.6	3.5	4.1	3.6	3.7	3.6	3.35	0.6; 0.8	15.18

Table 3. Principal components analysis and total variance explained

Component	Extraction sums of squared loadings		
	Eigenvalue	Total variance explained (%)	Cumulative variance (%)
1	6.95	27.79	27.79
2	6.08	24.34	52.13
3	4.63	18.53	70.66
4	2.03	8.13	78.79
5	1.60	6.39	85.18
6	1.43	5.70	90.88
7	1.01	4.02	94.90

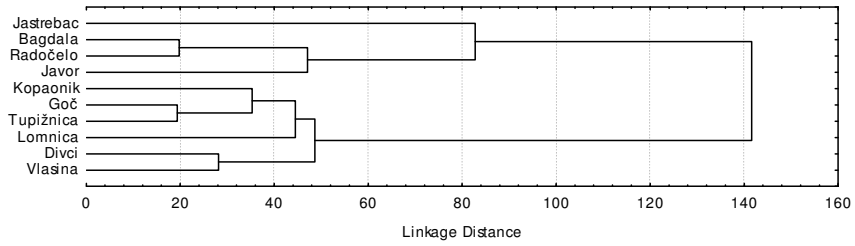


Figure 2. Grouping of populations based on all examined traits

Table 4. Rotated principal components matrix

Traits	Components		
	1	2	3
HT	0.014	-0.975	-0.022
PH1	0.770	0.126	0.024
PH2	0.630	-0.286	0.077
LL	0.837	-0.492	-0.178
LW	0.833	0.100	0.240
IL	0.120	0.923	0.134
SD	0.630	0.216	-0.403
GTN	-0.535	0.186	0.478
VTN	-0.566	-0.467	0.392
DMG	0.783	-0.543	0.190
DMV	0.180	-0.901	0.183
DMS	0.174	-0.494	0.093
SL	0.724	-0.555	-0.355
SLL	0.819	0.068	0.052
SN	0.019	-0.773	-0.417
SW	0.915	-0.249	0.226
FSU	0.083	0.723	0.333
SY	0.112	0.268	0.736
ASH	0.487	-0.611	0.255
CPC	-0.053	-0.402	0.625
CCC	-0.339	0.614	-0.441
P	0.231	-0.389	0.800
K	-0.249	0.059	0.599
Ca	0.010	0.331	0.753
Mg	-0.136	0.037	-0.548

Based on correlation with second principal component, which explains 24.34 % of total variability, time of heading, terminal internode length, DM of vegetative tillers, spikelet number and FSU had secondary significance. Internode length and FSU showed negative values and opposite direction. Fact that first three principal components describe more than 70% of collection variability can reduce showed correlation coefficients in table 4 to only three columns. All statistically significant coefficients are marked. The projection of populations on the plan defined by first three principal components is presented on figures 3.

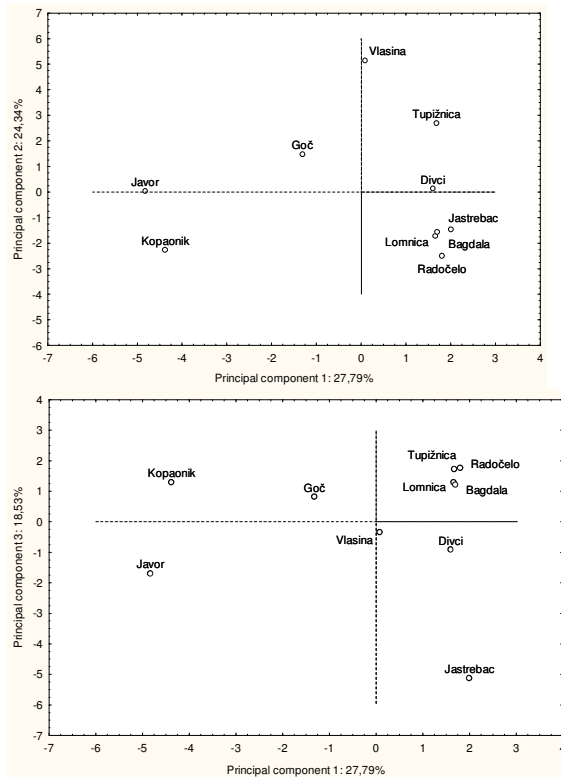


Figure 3. Biplots of 10 populations based on first three principal components

The first two PC of examined traits explain 52.13% of the total variability. Populations are scattered where 4 of them are slightly grouped. Those principal components categorize populations giving higher significance to production traits, time of tillering and for some traits of seed yield components (Table 4).

DISCUSSION

High genetic inter and intra population variability is starting point of successful grass forage breeding. There are a huge number of genotypes with level of production traits around average values, but very little have trait values that can preserve improved DM production of new cultivar. Also, different maturity of starting genotypes allows breeders to produce cultivars with different utilization time. All these depend on the starting breeding collection genetic variability for most important traits (THOROGOOD and SKOT 2003). In collection of ten perennial ryegrass Serbian populations the lowest level of variability was found for DM chemical composition, as was expected (SOKOLOVIĆ 2000). Nevertheless, CV for Mg content suggest that plant material is promising for the selection of genotypes for prevention of hypomagnesaemia, although Mg and other mineral concentration tends to be associated with forage yield and with animal voluntary intake of forage. Genetic variability for all important yield components and morphological traits and heritability for these traits from previous research show that breeding for improved perennial ryegrass cultivars is possible using reduced phenotypic recurrent selection. This is a common and effective breeding method for perennial ryegrass (WILKINS *et al.* 2003; SOKOLOVIĆ 2006).

Therefore, using multivariate methods for investigation of such variable material was the best choice to reduce number of factors and traits thus effecting populations variability and distribution.

Also, these methods mark some populations and genotypes with extreme or desired traits and emphasize certain traits which are main cause for population differentiation.

On the graphic formed as a result of hierarchical cluster analysis (Figure 2.) two groups of population can be observed. Cluster A consisted of four populations, subgroup with three populations and population Jastrebac. Populations in cluster A differed from the rest of the populations regard to higher tillers number, DM and seed yield per plant and better DM quality. Also, six populations in cluster B, within two subgroups, showed medium values for examined traits. It is clearly visible from this analysis that localities (altitude) where populations originated from had no impact on the regularity of traits expression and populations clustering. Geographic distribution of populations habitat on lowland, hilly, mountainous was not even similar with hierarchical clustering of collection. K-means clustering provided complete confirmation of population grouping from hierarchical clustering. Also, this analysis highlighted the most important traits for that grouping. Among them there are some very important for cultivar breeding. The traits of leafiness (leaf length and width), CCC and ash are important as components of DM quality. Also the number and DM of generative tillers had significant effect on total DMY and with spike length and 1000 seed weight on total seed yield.

The factor analysis showed that 70.66% variability among populations is described by diversity of 15 traits. Most important traits were plant height in first cut, leaf length and width, DM of generative tillers, spike and spikelet length and 1000 seed weight. These traits defined almost the third part of variability among

populations (27.79%). The first two PC of examined traits explain 52.13% of the total variability. The projection of populations defined by first and second principal components presented on figure 3 shows scattered populations where four of them are slightly grouped. Three of them are grouped on both figures 3 and 4, which means that populations Bagdala, Radočelo and Lomnica are most phenotypically similar according 70.66% of collection variability. Most phenotypically distinguished and unique populations are Javor and Kopaonik.

Factor analyses on different collections have indicated that every collection of genotypes is unique. Some investigations show that 83.8% of variability among Italian ryegrass genotypes is described by three principal components (JAFARI *et al.* 2003). The most important traits for that variability were plant height, DMY and persistency, and secondly phenological traits. In collection of 54 perennial ryegrass accessions most of variability was described with four principal components (BALFOURIER and CHARMET 1994). DMY and phenological traits were most significant. It is evident that agronomical most important traits of yield and maturity always have great variability in collection and therefore discriminate genotypes in collection, which was the case in our collection of 10 populations, as well. It was determined that variability and distinctness of populations originated from variability of most important traits for biomass and seed yield breeding. Finally, there are many genotypes with desirable traits which are suitable for breeding in projected model within all perennial ryegrass populations.

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**PROUČAVANJE MORFOLOŠKIH OSOBINA, PRINOSA I KVALITETA
SUVE MATERIJE AUTOHTONIH POPULACIJA *Lolium perenne*
POREKLOM IZ SRBIJE MULTIVARIJACIONIM ANALIZAMA**

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Usled specifičnih klimatskih uslova, oplemenjivanje engleskog ljulja u Srbiji je fokusirano na toleranciju abiotičkih faktora stresa, posebno na sušu i visoke temperature. Stoga se kao početni materijal u oplemenjivanju najčešće upotrebljavaju autohtone populacije i ecotipovi prilagođeni lokalnim agro-ekološkim uslovima. Međutim saznanja o varijabilnosti najvažnijih osobina kod autohtonih populacija su vrlo često oskudna. U ovom radu su predstavljena proučavanja varijabilnosti osobina deset populacija engleskog ljulja poreklom iz Srbije. Dvadeset pet osobina je ispitivano tokom dve godine, a podaci su obrađeni analizom varijanse i multivarijacionim statističkim metodama (PCA i klaster analiza). Cilj je bio da se utvrdi raznovrsnost i genetička udaljenosti ispitivanih populacija fenotajpingom i da se definišu osobine koje značajno utiču na varijabilnost i grupisanje populacija. Klaster analizom utvrđene su dve grupe populacija, ali geografsko poreklo populacija (ravničarska, brdska i planinska staništa) nije uticalo na pomenuto grupisanje. Faktorska analiza je pokazala da prvih sedam glavnih komponenti (PC) opisuje 95% varijabilnosti. Osobine koje pokazuju visok koeficijent korelacije sa prvom glavnom komponentom su visina biljaka u prvom otkosu, dužina i širina lista, suva metrija generativnih izdanaka, dušina klasa i klasića i težina 1000 semena. Utvrđena je visoka varijabilnost između populacija koja uglavnom potiču od oplemenjivački najvažnijih osobine, kao što su komponente prinosa suve materije i neke komponente prinosa semena.

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