GENETIC RELATEDNESS OF SOYBEAN GENOTYPES BASED ON AGROMORPHOLOGICAL TRAITS AND RAPD MARKERS

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Modern agriculture, breeding procedures, as well as competition among breeding institutions contribute to further reduction of already narrowed diversity of soybean commercial varieties. The objective of the study was to characterize eighteen soybean cultivars from three different breeding programs for agro-morphological traits and to reveal genetic diversity using molecular markers. Morphological description was performed with 13 qualitative and 9 quantitative traits. The genetic relationships were estimated using 21 RAPD markers. PIC was calculated for RAPD data, while the diversity of qualitative traits was described by Shannon genetic diversity index. Cluster analysis based on qualitative morphological characters showed clear separation of genotypes on the basis of their plant growth type. PC analysis performed for quantitative traits divided genotypes according to their maturity group. Grouping pattern based on molecular marker data was in agreement with pedigree of cultivars. A great similarity was found, primarily between the varieties under the same institution, and then among all examined varieties. Comparison of three methods in the assessment of diversity indicated that morphological markers might provide useful information in breeding process and allow classification by pedigree to some extent, but RAPD markers were found to be superior in assessing differences among genetically very similar genotypes.
Key words: agronomic traits, diversity, RAPD markers, soybean

INTRODUCTION

Soybean is the most important grain legume in the world because of its high oil and protein concentration. Commercially cultivated varieties developed in breeding programs all over the world have very narrow genetic base, since soybean is largely self-pollinated species and

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relatively small number of ancestors contributes to the genetic background of commercial cultivars. The practice of using crosses between elite lines minimizes genetic diversity and reduces possibility for yield advancement. Soybean populations derived from bi-parental crosses of genetically distant parents are more likely to have high genetic variance for yield than populations derived from crosses of parents that are more related (KISHA *et al*., 1997; CORNELIOUS and SNELLER, 2002; BAROSSO *et al.,* 2003; SUDARIC *et al.*, 2008). These studies indicate that sufficient genetic variation for yield can be obtained by crossing some high-yielding parents but that limited genetic diversity between parents renders many such crosses useless.

Different types of markers were used for assessing genetic variability of soybean genotypes–agronomic, morphological, biochemical traits and molecular marker polymorphisms (LI and NELSON, 2001; GIANCOLA *et al*., 2002; CHOWDHURY *et. al*., 2002; UDE *et al*., 2003; DONG *et al.,* 2004; BONATO *et al*., 2006; YAMANAKA *et al.,* 2007; MALIK *et al*., 2009; GOYAL *et al.,* 2012). All mentioned marker groups have limitations, but applied together they can provide reliable information about examined germplasm (SUDARIC *et al.* 2011).

Morphological characters, both quantitative and qualitative, have long been used to identify species, genera, to evaluate systematic relationships, and to discriminate between varieties (SMYKAL *et al.,* 2008). In breeding practice and seed production, the role of morphological descriptor is very important, since the distinguishing between varieties can be done quickly and precisely. Qualitative traits are usually controlled by a few genes, thus easily observable and suitable for cultivar differentiation and identification. On the other hand, quantitative traits have more limitations in cultivar description, since they are affected by environmental effects, developmental stage of the plant and the generation of selfing of breeding material.

In plants with a narrow genetic base, such as soybean, morphological markers may not be sufficient for detection of differences between varieties. In such cases, molecular markers can provide additional information about diversity of the existing germplasm (PRIOLLI *et al*., 2002), since they are highly polymorphic and not affected by environmental conditions. With the beginning and advancement of the molecular markers era significant progress in germplasm evaluation of different species has been made. RAPD markers can be successfully applied for genetic diversity evaluation because of the large data sets they can produce. This type of DNA markers has been extensively used in genetic diversity determination of soybean genotypes (LI and NELSON, 2001, NIKOLIC *et al*., 2004; CHEN and NELSON, 2005; DRINIC *et al.,* 2008).

It is evident that soybean cultivars grown in the eastern and south eastern parts of Pannonian Plain (Croatia and Serbia) have been originated from closely related genotypes, due to the practice of selection within the material existing in local germplasm collections (adapted to similar environmental conditions) and exchange between them. Eighteen soybean varieties were selected to represent diversity of cultivars developed in three breeding institutions in Pannonian part of Serbia and Croatia. In order to (i) determine the level of genetic and phenotypic diversity, (ii) analyze genetic relationships among cultivars and (iii) estimate the potential of each type of characterization for distinguishing between cultivars, molecular evaluation and agro-morphological description was done.

MATERIALS AND METHODS

Plant material

Experimental material consisted of 18 soybean cultivars from different breeding institutions - Maize Research Institute "Zemun Polje", Serbia (MRIZP), Institute of Field and

Vegetable Crops, Novi Sad, Serbia (IFVCNS) and Agricultural Institute Osijek, Croatia (AIOS) were chosen for agro-morphological and molecular characterization (Table 1). All analyzed genotypes are characterized by high genetic potential for seed yield, resistance to lodging and seed shattering, satisfactory field resistance to major diseases, and adapted to the growing conditions in our region.

Table 1. Soybean cultivars used for agro-morphological and molecular characterization

Genotype		Breeeding company	Pedigree	Maturity group	Origin
1	Laura	MRIZP	Kunitz/Novka	I	Serbia
2	Lana	MRIZP	Kunitz/Kador	II	Serbia
3	Olga	MRIZP	OS101/ZPS208	II	Serbia
4	Lidija	MRIZP	(Sibley/A1937)/Kunitz	II	Serbia
5	Nena	MRIZP	OS101/Elf	II	Serbia
6	ZPS015	MRIZP	NBSG1 popul. (USA)	0	Serbia
7	Balkan	IFVCNS	(Evans/Four)/S1346	I	Serbia
8	Kolubara	IFVCNS	unknown	0	Serbia
9	Afrodita	IFVCNS	S1346/Hodgson	0	Serbia
10	Ravnica	IFVCNS	S1346/Hodgson	I	Serbia
11	Vojvodjanka	IFVCNS	S1346/Hodgson	II	Serbia
12	Backa	IFVCNS	unknown	0	Serbia
13	Korana	AIOS	1 / 2	00	Croatia
14	Lucija	AIOS	(3 / 4)/4	00-0	Croatia
15	Julijana	AIOS	5 / 6 *5=a/b *6=c/d	0	Croatia
16	Vita	AIOS	7 / 8 *8=a/ e	0	Croatia
17	Ika	AIOS	(5/9)/10 *9=c/ f	0-I	Croatia
18	OS 101	AIOS	((5/9)/10)/11	I	Croatia

Field experiment

The field trials were carried out at the experimental fields of Maize Research Institute Zemun Polje, during the years 2011 and 2012, in randomized complete block design with three replicates. Each genotype was planted in plot containing 4 rows, 5 m long, with the row spacing of 0,5 m. The conventional agronomic practice was applied.

Morphological characterization was performed using 13 qualitative and 9 quantitative characters. The qualitative traits were: plant growth type, plant habit type, leaf blistering, size of lateral leaflet, shape of lateral leaflet, intensity of green color of the leaf, flower color, intensity of pod color, seed shape, seed coat color, seed coat luster, hilum color and pubescence color. Qualitative traits were described according to UPOV descriptor (UPOV, 1998). At growth stage R8, the samples of 30 plants per genotype were collected from the two central rows and scored for quantitative characters: plant height (cm), number of nodes per plant, pod number per plant, seed number per plant, seed yield per plant (g) and 1000 seed weight (g). Number of days to maturity was counted from the emergence to the date when 95% pods matured. Seed samples were analyzed for protein and oil content (expressed as a percentage on a dry matter basis) with a near infra-red reflectance spectroscopy (NIRS) using Infratec 1241 Grain analyzer.

Molecular characterization (RAPD amplification)

DNA was extracted from soybean seeds according to KAMIYA and KIGUCHI (2003) protocol. Thirty-three RAPD primers (Genosys Biotechnologies, USA and Operon Technologies, USA) were tested in this study, of which 21 gave clear and reproducible bands. List of selected primers and their sequences is given in Table 2. PCR amplification was done following the protocol of WILLIAMS *et a*l. (1990) with modifications in a total volume of 25 μl. Reaction contained 1x reaction buffer, 2.5 mM MgCl$_2$, 0.2 μM primers, 100 μM dNTPs, 2U of Taq polymerase and 20 ng of genomic DNA.

Thermocycling was performed in Biometra TProfessional Standard Thermocycler according to the following program: 5 minutes initial denaturation step at 94°C, 1 minute denaturation step at 94°C followed by 1 minute annealing cycles at 35°C, 2 minutes extension cycles at 72°C and a final extension step at 72°C for 7 minutes.

The amplification products were separated on 1.5% agarose gels prepared in 1xTBE buffer. After electrophoresis, gels were stained with ethidium bromide and photographed with BDA live system, Biometra.

Table 2. Primers used for RAPD analysis

PRIMERS	COMPANY	Seqence (5'-3')
GEN 1-70-3	GENOSYS	ACGGTGCCTG
GEN 1-70-9	GENOSYS	GGACTCCACG
GEN 4-70-5	GENOSYS	CATGTCCGCC
GEN 4-70-6	GENOSYS	GCACGTGAGG
GEN 4-70-9	GENOSYS	CCGGGGTTAC
GEN 4-70-10	GENOSYS	CGCAGACCTC
GEN 1-80-7	GENOSYS	GCACGCCGGA
GEN 1-80-10	GENOSYS	CGCCCTGGTC
GEN 2-80-4	GENOSYS	GCAGCTCCGG
OPB1	OPERON	GTTTGCTCC
OPB2	OPERON	TGATCCCTGG
OPB3	OPERON	CATCCCCCTG
OPB4	OPERON	GGACTGGAGT
OPB7	OPERON	GGTGACGCAG
OPB11	OPERON	GTAGACCCGT
OPB12	OPERON	CCTTGACGCA
OPB14	OPERON	TCCGCTCTGG
OPB15	OPERON	GGAGGGTGTT
OPB17	OPERON	AGGGAACGAG
OPB19	OPERON	ACCCCCGAAG
OPR10	OPERON	CCATTCCCCA

Statistical methods

The qualitative traits were transformed into binary data considering the presence or absence of each character state. Shannon genetic diversity index (TANG, 1997) was calculated to quantify diversity of qualitative traits according to formula:

$$H = -\sum_{n=1}^{s} - P_i \ln (P_i)$$

where *S* is the number of classes of each qualitative character, *i* is the *i*th class of a character, and P_i is the proportion of *i*th class in a character.

For the quantitative traits, dissimilarity estimates were calculated from standardized data matrix using Euclidean distance (ED). Normalization was applied to rescale ED to range from 0 to 1. To study the inter-relationships between the varieties, a principal components analysis was performed for quantitative traits, using software SPSS 15.0 for Windows Evaluation Version.

In the case of RAPD data, results were visually scored and presence/absence of specific DNA band for each primer was transformed in binary code (0, 1). The polymorphism information content (PIC) for molecular marker data was determined as:

$$PIC = 1 - \sum f_i^2$$

where f_i^2 is the frequency of *i*th allele. PIC provides information about discriminatory power of a locus taking into account the number of alleles that are expressed and also the relative frequencies of these alleles (LYNCH and WALSH, 1998).

Genetic similarity (GS) between cultivars was estimated with simple matching coefficient (SOKAL and MICHENER, 1958) for both RAPD and qualitative data. Cluster analysis was done by NTSYS-pc version 2.1 software (ROHLF, 2000), using UPGMA (Unweighted pair group method with arithmetic mean) method.

Comparison of dissimilarity matrices was done by application of Mantel's Z-statistics (SOKAL and ROHLF, 1995). Prior to this, similarity coefficients were converted to genetic distances as suggested by REIF *et al.* (2005).

RESULTS

Morphological description

Qualitative data

The highest similarity (0,888) was determined for following pairs of genotypes: Afrodita - Ravnica, Afrodita - Vojvodjanka, Ravnica – Vojvodjanka and Kolubara-Backa. First three pairs are closely genetically related since they have been developed from the same cross. The lowest similarity was found between genotypes Lana and Lucija (0,278). Average pairwise genetic similarity was 0,652.

The cluster analysis for the qualitative traits using UPGMA method classified genotypes into two groups. First group (I) was divided in two clusters (A and B). Cluster A consisted of varieties Laura, Nena and Julijana, identical in growth type, leaf blistering, leaf size, leaf shape, leaf color, seed shape and seed coat luster. Cluster B encompassed two subclusters (B$_1$ and B$_2$). Subcluster B$_1$ included genotypes of the same growth type, leaf blistering, leaf color, seed coat color, seed coat luster and hilum color (Lana, Lidija, OS 101 and Vita). Subcluster B$_2$ was further divided in two subclusters (b$_1$ and b$_2$). Subcluster b$_1$ encompassed cultivars Olga and Korana identical in the growth type, plant habit type, leaf size, leaf shape, leaf color, flower color, pod color, seed coat color, seed coat luster and pubescence. Subcluster b$_2$ comprised of 1 cultivar from AIOS (Ika) and 6 cultivars developed in IFVCNS: Balkan, Afrodita, Ravnica, Vojvodjanka,

Kolubara and Backa, having the same growth type, plant habit type, leaf size, seed shape, seed coat color, seed coat luster and pubescence. Group II encompasses two genotypes ZPS 015 and Lucija identical in plant habit type, leaf shape, leaf color, flower color, seed shape and seed coat luster (Figure 1).
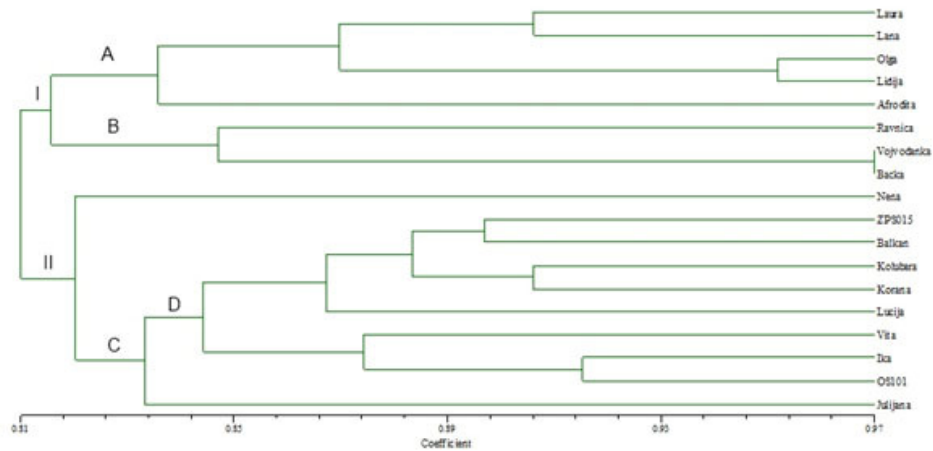

Figure 1. Dendrogram of 18 soybean genotypes based on 13 qualitative traits using Sokal and Michener coefficient of similarity

Table 3. The Shannon genetic diversity index (H) of qualitative traits in 18 soybean genotypes

Traits	Plant growth type	Plant Habit type	Leaf blistering	Size of leaflet	Leaflet shape	Leaf color	Flower color	Pod color	Seed shape	Seed coat color	Seed coat luster	Hilum color	Pubesc. color
H	0.348	0.668	1.040	0.591	0.687	0.868	0.529	1.089	0.451	0.683	0.636	1.725	0.636

The genetic similarity of 18 soybean cultivars was also analyzed using the Shannon diversity index, as a measure of phenotypic diversity of each trait (Table 3). Hilum color showed the highest variation among all qualitative traits, followed by pod color and leaf blistering. Plant growth type showed the lowest variation, since 88,9% of cultivars were of indeterminate type. Average Shannon genetic diversity index (H') was 0,766.

Quantitative data

The highest ED (1.00) was determined between cultivars Vojvodjanka and Vita, while genotypes Lana and Lidija were observed as the genotypes with the lowest ED (0.13). The overall pairwise genetic distance was 0.48.

Principal components analysis showed that different quantitative traits contributed differently to the total variation as described by eigenvalues as well as their weight and loading in different PC-s (Tab. 4). The data revealed that 3 major principal components with eigenvalues > 1 contributed 92,129% of total variation among 18 cultivars, accounting for 47,726%, 31,371% and 13,032% of variation, respectively.

Table 4: Matrix of eigenvalues and vectors of PCA for 9 quantitative traits of soybean, assessed in field trials

	Principal components		
	PC1	PC2	PC3
Eigenvalues			
Variance	4.295	2.823	1.173
% total contribution	47.726	31.371	13.032
% accumulated	47.726	79.097	92.129
Eigenvectors			
Protein content	-0.225	0.353	0.856
Oil content	0.058	-0.039	-0.970
Number of days to mat.	0.109	0.949	0.042
Plant height	0.053	0.945	0.141
Number of nodes/ plant	0.448	0.801	0.248
Number of pods/plant	0.959	0.171	0.034
Seed number/plant	0.975	0.101	-0.132
Seed yield/plant	0.975	0.062	-0.056
1000 seed weight	-0.821	-0.207	0.322

The first PC was highly positively correlated with the number of pods per plant, seed number per plant, seed yield per plant and negatively correlated to 1000 seed weight. For the second PC, traits with high loading were: number of days to maturity, plant height and number of nodes per plant. The third PC was strongly negatively associated with oil, and positively with protein content.

Position of genotypes at the PC1 axis reflects the performance of varieties that refers to yield components (number of pods per plant, seed number per plant, 1000 seed weight) and seed yield per plant (Figure 3). Cultivars with negative PC1 values had lower level of these parameters and *vice versa*. Cultivar Balkan was the most extreme, expressing the greatest values for 1000 seed weight and lowest values for number of pods per plant, number of seeds per plant and seed yield per plant. Three varieties had the extreme values for 3 traits: Vojvodjanka (the greatest seed yield per plant and number of seeds per plant, and the least 1000 seed weight), Lana (the greatest number of days to maturity, plant height and number of nodes per plant) and Lucija (the lowest values of protein content, plant height and number of nodes per plant) while extremities in only two characters were observed in Korana (the lowest number of days to maturity, and the greatest number of pods per plant) and Nena (maximum protein and minimum oil content). Short season varieties yielded less than late season cultivars. Meanwhile, cultivars Lana and Backa showed the

same values for seed yield, despite different growing season length, while Korana (short season cultivar) and Vojvodjanka (late season cultivar) had yielded similarly.
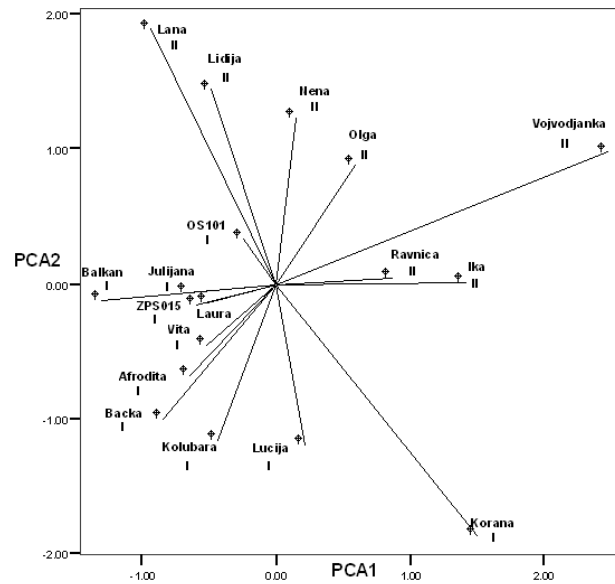

Figure 2: Scatter diagram of 18 soybean cultivars from 9 quantitative agro-morphological traits

With exception of cultivar OS 101, PC analysis divided soybean genotypes in two groups (I and II), according to their growing season length (Fig. 3). Genotypes with positive values for the second PC axis belong to medium to late maturing cultivars group (II) with growing season of about 135 days, while varieties with negative values for second PC axis have shorter vegetation length. Cultivars with the highest and the lowest PC2 score were Lana and Korana, with 146 and 112 days to maturity, respectively.

RAPD analysis

Among 33 random primers used in this study, 21 showed clear DNA profile of sufficient intensity and were submitted for evaluation of genetic similarity between soybean genotypes (Table 5). Amplification products for 8 primers (38.09% of 21 informative primers) were monomorphic - identical for all analyzed genotypes. The number of bands produced by each primer was from 1 (OPB3, GEN 1-80-7, GEN 2-80-4) to 18 recorded for primer GEN 4-70-6 (Figure 3) with average 5.1 bands per primer.

A total number of fragments amplified were 107, of which 46 (43 %) were polymorphic. PIC values for all polymorphic primers ranged from 0.027 to 0.7359 with mean value of 0.3887. RAPD fragments from 13 polymorphic RAPD primers with PIC>0.30 were considered as informative (Table 5).
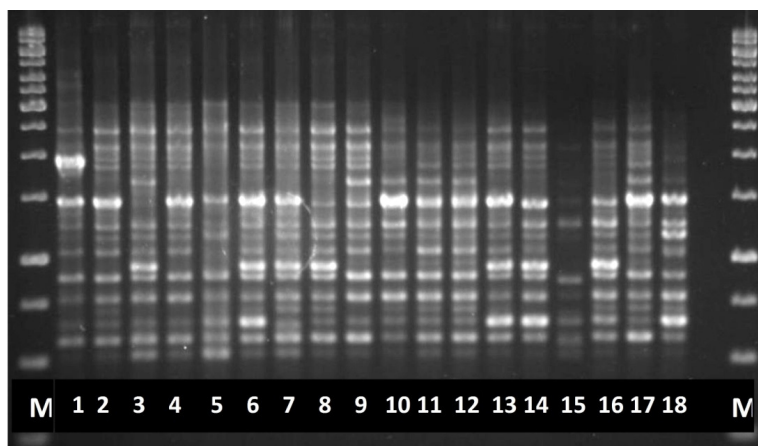
Figure 3. RAPD Primer GEN 4-70-6 banding pattern for 18 soybean genotypes (M –DNA marker, 1kb, lanes 1-18 soybean genotypes as listed in table 1).

Table 5. Number of bands per polymorphic primer, percentage of polymorphic bands and polymorphic information content (PIC) values

Primer	Total bands	Percent of polymorphic bands	PIC
GEN 4-70-6	18	61.11	0.392
GEN 4-70-9	10	60.00	0.486
GEN 4-70-10	7	28.57	0.108
GEN 1-80-10	11	27.27	0.164
OPB1	4	25.00	0.027
OPB2	7	28.57	0.545
OPB7	3	33.33	0.377
OPB11	3	66.66	0.336
OPB12	8	87.50	0.652
OPB14	7	71.43	0.652
OPB15	3	33.33	0.142
OPB19	6	50.00	0.437
OPR10	4	75.00	0.736

The lowest GS based on RAPD data was found between variety Laura and Ika (GS=0.74). Vojvodjanka and Backa were the closest pair of 18 soybean cultivars (GS=0.97). Average pairwise GS was 0.83.

UPGMA clustering method based on RAPD data similarity matrices distributed varieties in two major clusters (I and II). Cluster I included subclusters A and B and cluster II was consisted of genotype Nena loosely attached to cluster C. Subcluster A contained cultivars from MRIZP (Laura, Lana, Olga, Lidija) and one from IFVCNS (Afrodita). Three cultivars from IFVCNS (Ravnica, Vojvodjanka and Backa) were grouped together in subcluster B. Subcluster C was

consisted of genotype Julijana loosely attached to subcluster D which grouped cultivars from all three institutions in two subclusters. One of the subclusters contains genotypes from MRIZP and IFVCNS and the other three cultivars from AIOS (Figure 4).
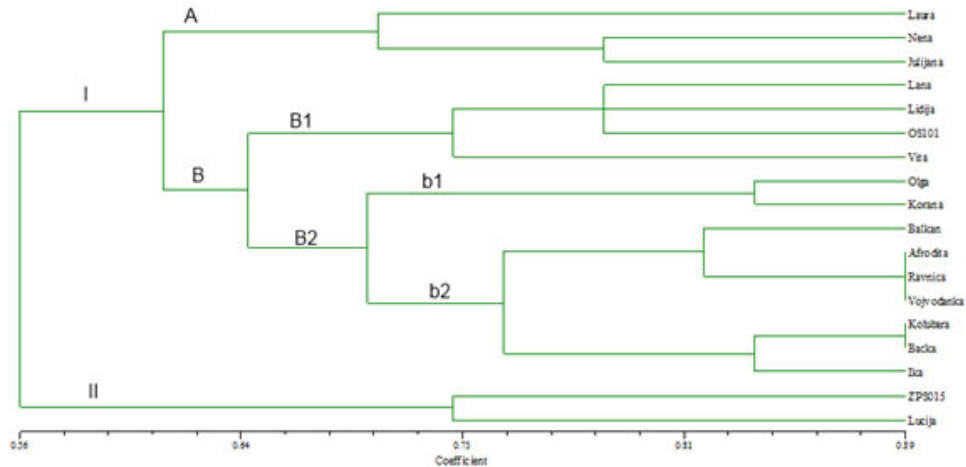

Figure 4. Dendrogram of 18 soybean cultivars based on RAPD data using Sokal and Michener coefficient of similarity

Comparison of similarity matrices by application of Mantel test resulted in rather low correlations between different marker data. Correlation coefficients between RAPD and quantitative distance matrices and RAPD and qualitative distance matrices were 0.01074 and 0.05651, respectively, indicating a complete absence of relationship between molecular and morphological distances.

DISCUSSION

Considering the pedigree data, studied set of 18 soybean genotypes was consisted mostly of genetically similar cultivars. Variety OS 101 was a common parent in two varieties (Olga and Nena) and closely related to cultivar Ika, 3 varieties (Laura, Lana and Lidija) were half-sib lines, while 3 varieties (Afrodita, Ravnica, Vojvodjanka) originated from the same cross. Results suggest that 3 characterization techniques described genetic diversity of examined cultivars in different ways.

Qualitative data
Accuracy of morphological data greatly depends on skills of observer, particularly for traits that are less observable and measurable, and therefore, diversity estimated by morphological data might be limited representative (CUPIC *et al.,* 2009). On the other hand, protocol for variety protection as well as different steps of breeding practice inevitably requires morphological

description. The problems of subjectivity with visual estimates of morphological characters can be minimized if observers strictly follow the instructions of descriptors and use recommended reference materials (check cultivars, drawings, color charts,) and methods (part of the plant, phenological conditions, plant growth stage, sampling methods).

Grouping of genotypes according to their qualitative morphological characters showed clear separation on the basis of their plant growth type to indeterminate (cluster I) and semi-determinate type (cluster II). Dendrogram generated from qualitative traits was partially congruent to the known pedigrees of genotypes, considering all cultivars from IFVCNS were placed in subcluster b_2, while cultivars ZPS 015 and Lucija, whose pedigree data revealed no relatedness to other cultivars, formed separate cluster (II) which appeared to be the most distinct from all the others. Still, when we compare pedigree data to the clustering pattern, several related varieties were spread.

Average Shannon index (0,766) indicated medium diversity among cultivars. Similar results are reported by MALIK *et al*., (2011), while WANG et al., (2006) and DONG *et al.,* (2004) found higher values ranging from 1.27 to 1.37, respectively.

In this present study, 13 qualitative traits used for characterization were sufficient to discriminate between 18 varieties. All cultivars were found to differ from each other in one or more individual characters and no duplicates were detected. Although a lot of studies reported morphological marker's limitations and weaknesses, our results suggest they may be very useful in cultivar differentiation and identification. Reliability of these findings lies in the choice of the morphological traits examined; they are all qualitatively inherited, controlled by single mendelian genes, no additive effect is present and they comply statistical assumption for variable independency.

Quantitative data

Agronomic, biochemical and phenological traits are among the first phenotypic markers used in germplasm management. They have a number of limitations including low heritability, late expression and vulnerability to environmental influences (SMITH and SMITH, 1992). However, the results of principal component analysis of 9 quantitative traits used in this study indicated this type of markers well suited to estimate differences between 18 soybean cultivars. This analysis confirmed a satisfactory degree of separation within varieties, dividing them in two groups.

PCA is a powerful method that removes interrelationships among the components and decreases the data set, reducing a larger number of correlated variables to a smaller number of artificial variables that are independent. Nine observed quantitative traits created 3 independent variables: PC1 axis (traits which refers to seed yield), PC2 axis (traits connected with vegetation length) and PC3 axis (seed quality parameters). Numerous studies reported various contributions of different traits to proportion of variation among cultivars (ZAFAR *et al*., 2008; SMYKAL *et al.,* 2008; CUPIC *et al*., 2009; OJO *et al*., 2012). Generally, the characters contributed positively to first principal component axis (number of pods per plant, seed number and seed yield per plant and 1000 seed weight) should be given considerable importance for the selection of material for the breeding program in the future.

RAPD data

Forty-six polymorphic bands (43%) were identified with 21 RAPD primers in this study. Similar results were shown by BARANEK *et al*., (2002), who detected 122 highly reproducible

fragments with 22 RAPD primers, and 55 of them were polymorphic (46%). As compared to the results of other authors, polymorphism found in our study had reached slightly higher values. GIANCOLA et al., (2002), CHOWDHURY et al., (2002) and BAROSSO et al., (2003) reported 31,4%, 31,0% and 18,7% of polymorphic RAPD loci, respectively. Polymorphism detected in this study was moderate; taking into account the materials analyzed were all from breeding programs that share the same germplasm, thus having a relatively narrow genetic base.

Polymorphism information content represents the gene diversity for specific locus. Mean value of PIC for markers used in this study was 0.3887. Diversity scores reported in other studies (GIANCOLA et al., 2002; CHOWDHURY et al., 2002; BROWN-GUEDIRA et al., 2000; LI and NELSON, 2001) were in strong agreement with this result.

High genetic similarity values obtained in present study confirmed the results of previous researches on RAPD (HELMS et al., 1997, CORREA et al., 1999), SSR (TANTASAWAT et al., 2011), RFLP (BONATO et al., 2006) and AFLP marker diversity in soybean (ZHU et al., 1999; MAUGHAN et al., 1996). The lower level of RAPD similarity indices were determined in studies of LI and NELSON (2001), GIANCOLA et al., (2002) and BAROSSO et al., (2003), while BROWN-GUEDIRA et al., (2000) and CHOWDHURY et al., (2002) reported medium similarity. Nevertheless, variability detected in present study assuredly indicated high similarity among soybean genotypes, confirming narrow genetic base and lack of diversity reported in numerous studies (GIZLICE et al., 1994; NARVEL et al., 2000).

Information about the genealogy of soybean cultivars was essential for the interpretation of the dendrogram based on RAPD data. Eighteen soybean genotypes were assigned into groups, which showed strong association with their pedigree data. Three of four soybean cultivars from MRIZP that were grouped in subcluster A have one common parent. Subcluster B was consisted of cultivars from IFVCNS and information about their origin indicated that they have been closely related, too. In subcluster D genotypes were grouped in two smaller subclusters. One of those subclusters comprised of three Croatian cultivars and two of them have the same components in their pedigree. Additionally, all genotypes from AIOS were allocated to subcluster C. Cluster analysis showed 21 RAPD markers were able to distinguish between 18 soybean varieties. Clustering pattern showed strong congruence between grouping of genotypes and their affiliation to respective breeding program. These results were in line with the findings of other authors (DIWAN and CREGAN, 1997; PRIOLLI et al., 2002; CUI et al., 2001; NIKOLIC, 2005; RISTOVA et al., 2009) and largely expected, since each breeding program has its own selection targets and exploits specific breeding stocks, mostly consisted of elite material. Consequently, reselection of elite commercial genotypes leads to reduced diversity within cultivars developed in the same program.

Mantel test showed low, non-significant correlation between molecular and morphological data. These findings are in agreement with the conclusions of other authors who studied diversity in soybean (GIANCOLA et al., 2002) as well as in other plant species (SMYKAL et al., 2008, TOMASSINI et al, 2003, CUPIC et al., 2009). A lack of correlation between molecular and morphological qualitative data could be explained by the fact that varieties that show high phenotypic similarity are not necessarily genetically similar as different gene pools can be manipulated to produce similar varieties. Close matching of molecular and morphological relationships indicates a very restricted commercial gene pool, where varieties shared the same genetic resources and have the same breeding objects (ROLDAN-RUIZ et al., 2001). Correlation between RAPD and quantitative morphological data was very low, too. The reason for this lies in the nature of molecular markers used in this study. RAPD markers are randomly distributed across

the genome and may not have been linked to loci for those quantitative traits that were evaluated for phenotypic characterization (HELMS *et al*., 1997; CERNA *et al*.; 1997, BAROSSO *et al*., 2003). The use of other marker systems (SSR) may increase the accordance of molecular and morphological data (GIANCOLA *et al*., 2009; TAVAUD-PIRRA *et al*., 2009; LI *et al.*, 2010). Furthermore, low correlation between molecular and quantitative data is attributed to the facts variation of agronomic traits does not always reflect real genetic variation because of genotype x environment interaction and the largely unknown genetic control of polygenic morphological and agronomic traits (SMYTH and SMYTH, 1992). Correlation between phenotypic distances and marker distances necessarily decreases with the number of loci involved in the variation of the traits of interest (BURSTIN and CHARCOSSET, 1997). Relationship between molecular and phenotypic based distances is noted for quantitative rather than qualitative traits (LI *et al.,* 2010). If there is a linkage disequilibrium, in the case when inbred lines are related by pedigree, a strong relationship is expected between quantitative and marker distances (BURSTIN and CHARCOSSET, 1997).

The highest average distance between 18 cultivars was found for qualitative data (GD= 0,585), followed by quantitative (GD=0,481) and RAPD data (GD=0,419). The values of morphological distances were slightly higher than those of molecular distances. Different studies presented similar results (CHOWDHURY *et al*., 2002; CUPIC *et al*., 2009) while GIANCOLA *et al.,* (2002) reported molecular diversity estimated with three different marker systems to be larger than morphological. Potential use of SSR markers in future would probably give a high degree of identity and differentiate among phenotypically similar genotypes.

Relative positions of the soybean cultivars in dendrograms based on RAPD and qualitative data were rather different. Genotypes grouping in dendrogram based on RAPD data showed better congruence with pedigree of soybean cultivars, as compared to grouping pattern based on morphological data. On the other hand, discrimination achieved by morphological characterization was more precise, as it separated genotypes on higher genetic distance level, and with respect to their maturity group and plant growth type. Nevertheless, RAPD markers generated different genetic profiles for the genotypes that appeared to be morphologically highly similar, emphasizing their ability to distinguish between closely related cultivars.

However, a medium level of genetic similarity and close relationships among analyzed elite materials from three former Yugoslavian breeding programs indicated that genetic base of soybean collections in region is considerably narrow. The similar findings are reported by TAVAUD-PIRRA *et al.,* 2009, who analyzed diversity of soybean cultivars maintained in INRA collection, as well as by RISTOVA *et al*., 2010, who studied diversity among genotypes from breeding programs in Southeastern Europe. Results of our study emphasize the need for new germplasm introgression of and molecular markers application.

Different studies in soybean (WANG *et al.,* 2000; TAVAUD-PIRRA *et al.,* 2009; GIANCOLA *et al*., 2002) and other plant species (ROLDAN-RUIZ *et al*., 2001; LI *et al*., 2010) advised that more data types should be used in order to accomplish the better representation of genotype collections. Genetic relationships determined with both molecular and agro-morphological data can reveal sources of desirable characteristics in closely related individuals, providing different but complementary information.

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GENETIČKA POVEZANOST GENOTIPOVA SOJE NA OSNOVU AGRO-MORFOLOŠKIH OSOBINA I RAPD MARKERA

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Izvod

Savremena poljoprivreda, oplemenjivačke procedure kao i kompeticija među selekcionerskim ustanovama doprinose dodatnom smanjenju već uskog genetičkog diverziteta sorti soje. Cilj istraživanja bio je da se izvrši karakterizacija osamnaest sorti soje iz tri različita oplemenjivačka programa na osnovu agromorfoloških osobina i utvrdi njihov genetička raznovrsnost primenom molekularnih markera. Agro-morfološki opis izvršen je pomoću 13 kvalitativnih i 9 kvantitativnih osobina. Genetička povezanost ocenjena je na osnovu 21 RAPD markera. PIC vrednosti su izračunate za RAPD podatke, dok je diverzitet kvalitativnih osobina izražen Shannon-ovim indeksom diverziteta. Klaster analiza na osnovu kvalitativnih morfoloških osobina pokazala je jasnu podelu genotipova prema tipu rasta. PC analiza kvantitativnih osobina grupisala je genotipove na osnovu dužine vegetacije. Grupisanje na osnovu molekularnih podataka bilo je u saglasnosti sa podacima o poreklu genotipova. Velika sličnost je utvrđena prvenstveno između genotipova stvorenih u okviru jedne selekcionerske ustanove, a zatim i između svih proučavanih genotipova. Poređenje tri metoda za procenu diverziteta pokazalo je da morfološki markeri mogu biti korisno sredstvo u procesu oplemenjivanja i donekle omogućiti klasifikaciju po poreklu, ali su RAPD markeri superiorniji u otkrivanju razlika između genetički veoma sličnih genotipova.