

GENETIC ASSESSMENT OF MAIZE LANDRACES FROM FORMER YUGOSLAVIA

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Ignjatović Micić D., D. Risić, V. Babić, V. Anđelković, K. Marković and J. Vančetović (2013): *Genetic assessment of maize landraces from former Yugoslavia*. Genetika, Vol 45, No. 2, 405-417.

A collection of 2217 landraces from former Yugoslavia region is maintained at Maize Research Institute Zemun Polje gene bank. All local varieties from the former Yugoslavia are classified into 18 groups. These agro-ecological groups encompass early introduced flint types grown on small and isolated areas, later introduced dent types that spread on wide areas of crop production and types created through hybridization between these two kernel types. The objective of this research was to study population structure, genetic diversity and relationships of nine flint and nine dent accessions belonging to different agro-ecological groups using phenotypic and simple sequence repeat (SSR) markers. The 18 analyzed landraces displayed great variation for most analyzed traits, but flint landraces were more diverse. Ten SSR probes revealed total of 56 and 62 alleles in flint and dent landraces, respectively. Eight specific alleles (i.e. alleles found only in one landrace or only within flint, i.e. dent landraces) were detected with five probes. One specific allele was found in flint and seven alleles in dent landraces. These differences in allele structure point to different origins and possibly different purposes of flint and dent genotypes. Both phenotypic and SSR analyses could distinguish most flint and dent landraces, but not agro-ecological groups. The results revealed a significant genetic heterogeneity indicating that the analyzed landraces could be valuable sources of genetic variability.

Key words: genetic diversity, landraces, maize, phenotype, SSR

INTRODUCTION

Maize is the most diverse crop species containing enormous variation in morphological traits and extensive polymorphism in DNA sequences (MATSUOKA *et al.*, 2002). Two randomly chosen modern maize lines have on the average one single-nucleotide polymorphism every 100bp (TENAILLON *et al.*, 2001). However, only 5% of maize variability is in commercial use

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(CARENA *et al.*, 2009). Even though this germplasm enables production of high yielding hybrids its narrow genetic base is adverse for maize adaptation to different biotic and abiotic stresses and in this context inadequate to meet the contemporary and future major challenges - population growth and climate changes.

The solution to this problem lies in the utilization of maize diversity preserved within maize genetic resources, which represent the raw material that farmers and plant breeders can use to improve the quality and productivity of crops. The significance of proper use of maize diversity in breeding programmes was recognised as far back as the 1960s when many national collections were formed. The collection from western Balkan (former Yugoslavia region) was created between 1962 and 1964. Classification into 18 agro-ecological groups/races (PAVLICIC and TRIFUNOVIC, 1968) was done using the natural classification method of ANDERSON and CUTLER (1942). A re-classification of the landraces, conducted by a concurrent analysis of the widest range of morpho-biological traits of all populations in one year, based on the CIMMYT/IBPGR descriptor, confirmed the validity of the original classification performed in 1968 (RADOVIĆ *et al.*, 2000). All local varieties from the former Yugoslavia were classified into 16 main and two derived groups. These agro-ecological groups encompass early introduced flint types grown on small and isolated areas, later introduced dent types that spread on wide areas of crop production and types created through hybridization between flint and dent types. Today, Maize Research Institute Zemun Polje maintains a collection of 2217 landraces.

The classification methods based on phenotypic traits were enhanced with the use of DNA markers in the last 15-odd years. Due to the rapid developments in the field of molecular genetics, a variety of different techniques have emerged to analyze genetic variation (SPOONER *et al.*, 2005). DNA provides many advantages that make it especially attractive in studies of diversity and relationships, such as independence from environmental and pleiotropic effects, a potentially unlimited number of available markers and selectively neutral nature of many molecular markers (SPOONER *et al.*, 2005). These advantages do not imply that other more traditional data used to characterize biodiversity are not valuable. On the contrary, morphological, ecological and other 'traditional' data will continue to provide practical and often critical information needed to characterize genetic resources.

Due to their high informativeness, reproducibility and ease of application Simple Sequence Repeats (SSR) are the most frequently used PCR based markers in maize diversity studies (DUBREUIL *et al.*, 2006; SHARMA *et al.*, 2010; CÖMERTPAY *et al.*, 2012). New marker technologies based on DNA sequencing (e.g. SNP – single nucleotide polymorphism) have been developed that could also be used for estimating genetic diversity, but they are still to be adjusted for studies regarding relatedness (YANG *et al.*, 2011).

The objective of this research was to study population structure, genetic diversity and relationships of flint and dent accessions belonging to different agro-ecological groups of landraces collected on former Yugoslavia territories. Both phenotypic and molecular (SSR) approaches in diversity assessment were conducted. Also, the concurrence of the results obtained with these two methods was estimated.

MATERIALS AND METHODS

Genetic material

The eighteen analyzed accessions from Maize Research Institute gene bank belong to three flint and three dent agro-ecological groups. They were Montenegrin flints, Macedonian

flints, Mediterranean flints, Dent type of USA Corn Belt dents, Dent type of Southern areas of USA and Serbian dents. Each of the group was presented with three landraces in this work. The flint landraces analyzed in this study are supposed to originate from the earliest introduced genotypes from Caribbean Islands, Mexican plateau and Andean slopes, while the dent landraces are the last introduced genotypes originating from New England and Canada. The list of the analyzed populations with geographical data is given in Table 1.

Table 1. Agro-ecological groups, landrace abbreviated name, latitude, longitude, altitude and country of collection of the analyzed maize landraces

Agro-ecological group	Group number	Landrace abbreviated name*	Latitude	Longitude	Altitude	Country of collection
Montenegrin flints	1	FI1	42.12	19.06	400	Montenegro
		FI2	42.35	21.24	747	Serbia
		FI3	42.51	29.46	912	Montenegro
Macedonian flints	4	FIV1	-	-	-	Montenegro
		FIV2	41.00	21.20	244	Macedonia
		FIV3	44.27	20.04	100	Serbia
Mediterranean flints	7	FVII1	43.08	17.44	41	Bosnia
		FVII2	-	-	-	Slovenia
		FVII3	44.53	15.4	459	Croatia
Dent type of USA Corn Belt dents	13	DXIII1	44.46	20.28	190	Serbia
		DXIII2	-	-	-	-
		DXIII3	44.23	20.4	212	Serbia
Dent type of Southern areas of USA	15	DXV1	45.17	17.11	200	Croatia
		DXV2	45.25	13.58	153	Croatia
		DXV3	45.02	13.53	230	Croatia
Serbian dents	16	DXVI1	45.5	16.17	100	Croatia
		DXVI2	43.37	21.42	214	Serbia
		DXVI3	43.13	21.59	350	Serbia

- data not available

* The abbreviations for each landrace consists of a letter indicating flint (F) or dent (D) affiliation, number of the agro-ecological group (given in Roman numerals) and number of the landrace from the particular agro-ecological group (1, 2 and 3).

Morphological analysis

All populations were sown in Zemun Polje in 2008, at two different sowing densities – 44640 and 64935 plants per ha. The experimental design was RCB (Random Complete Block design) with two replicas, four rows per replica and 20 plants per row. For morphological trait

measurements two medium rows per population were used. For each plot 16 morphological traits (Table 2) taken from 20 competitive plants (40 plants per population) and from two kernels per ear were measured.

Analysis of variance was performed in order to test the significance of variation between populations. This analysis enabled estimation of genotypic and environmental variances. Principal Component Analysis (PCA) was performed on the phenotypic correlation matrix of the adjusted means of the populations for the 16 descriptors. The matrix of distances between populations was calculated upon the standardized principal components with eigenvalue higher than one. All the statistical analyses were performed using program package SPSS 15.0 (<http://spss-for-windows-evaluation-version.software.informer.com/>).

Molecular analysis

SSR analysis was carried out using a DNA-pooled sampling strategy (bulk analysis). Each population was presented with 30 plants. Pooled-samples of each population contained 0.1g of lyophilized leaf tissue from which DNA was isolated by the CTAB method. PCR amplification was performed with 50 SSR primer pairs, but only ten were chosen for statistical analysis. The amplification reaction was carried out in 25 µl reaction volume containing DreamTaq™ Green PCR Master Mix (2X), 0.5 µM primers and 50 ng of DNA. The amplification profiles were: an initial denaturation at 95°C/5 min, followed by 15 cycles each of denaturation at 95°C/30 s, annealing at 63.5°C/1 min (-0.5°C/cycle) and extension at 72°C/1 min; another 22 cycles of 95°C/30 s, 56°C/1 min and 72°C/1 min were performed.

Genetic similarities were calculated on binary data (presence versus absence of alleles) using Jaccard's coefficient (JACCARD, 1908). Cluster analysis was performed with unweighted pairgroup method (UPGMA) and relationships between landraces were visualized as dendrogram. The co-phenetic coefficients values were computed and the significance of the co-phenetic correlation observed was tested using the Mantel matrix correspondence test (MANTEL, 1967). Statistical analysis was done by NTSYSp2.1 program package.

In order to determine allele frequencies of detected specific alleles (i.e. alleles found only in one landrace or only within flint, i.e. dent landraces) the bands were scored as pixel total percentage of individual bands within the sample, using UN-SCAN-IT gel 6.1 program package.

RESULTS

Phenotypic analysis

The 18 analyzed landraces displayed great variation for most analyzed traits, but flint landraces were more diverse. Range and average values of the measured morphological traits and anthesis-silking interval (ASI) of the flint and dent landraces are given in Table 2.

Relationships between the traits were estimated using principal component analysis. The first three principal components accounted for 80.86% of the total variation. In the first PC (57.10%) plant height, ear height, leaf number, husk leaf length, number of tassel primary branches, ear row number, number of kernels per row, ear diameter, kernel length and ASI were the most important traits. In the second PC (16.53%) predominant traits were tassel length, central spike length, branched part/tassel and kernel width. The third PC (7.23%) described variation in ear length and kernel thickness.

Table 2. Range and the average values of the measured morphological traits and anthesis-silking interval (ASI) of the analyzed flint and dent landraces

Traits	Flint landraces					Dent landraces			
	Range	FI	FIV	FVII	<i>F av.</i>	DXIII	DXV	DXVI	<i>D av.</i>
Plant height (cm)	<i>min</i>	151.	168.	126.7	186.7	208.3	212.1	203.6	222
	<i>max</i>	9	3	230.1		254.9	231.9	228.3	
Ear height (cm)	<i>min</i>	40.8	58.9	37.3	68.4	75.3	84.9	94.4	92.3
	<i>max</i>	73.5	92.8	92.4		111.6	98.3	96.6	
Leaf number	<i>min</i>	8.1	8.4	7.5	9.9	10.9	12.1	12	12.2
	<i>max</i>	10.9	11.6	12		13.5	13.1	12.7	
Husk leaf length (mm)	<i>min</i>	53.8	62.1	54.5	68.6	71.5	77	73.7	78.7
	<i>max</i>	67.4	77.3	85.8		81.6	84.8	80.6	
Tassel length (mm)	<i>min</i>	36.3	43	37.4	43.2	42.4	43.3	39.3	45
	<i>max</i>	46.5	49.2	47.5		47.8	50.2	46.9	
Central spike/tassel length	<i>min</i>	18.4	22.6	20.2	22.5	22	21.2	21.9	23.9
	<i>max</i>	25	24.8	27		25.4	25.7	27.3	
Number of tassel primary branches	<i>min</i>	6.9	12.2	8	11.5	10.5	16.4	12.4	15.3
	<i>max</i>	13.1	15.2	16.6		17.6	18.6	15.3	
Branched part/tassel length	<i>min</i>	6.8	11	6.7	10.6	8.4	7.6	8.9	9.7
	<i>max</i>	10	13.6	13.8		9.1	13.3	9.8	
Ear length (cm)	<i>min</i>	8.8	13.2	12.4	13.6	11.9	12.5	13.3	13.8
	<i>max</i>	13.6	15.3	15.9		15.5	13.9	15	
Ear row number	<i>min</i>	9.3	10.2	12.7	12	12.8	13.3	12.7	14.3
	<i>max</i>	11.9	13.9	15		18	16	14.2	
Kernel/row number	<i>min</i>	17.5	27.3	22.3	26.4	24.8	27.3	27.5	29.7
	<i>max</i>	29.2	30.4	32.5		31.9	30.4	31.7	
Ear diameter (mm)	<i>min</i>	3.2	3.1	3.4	3.7	3.9	4.5	4.4	4.4
	<i>max</i>	3.5	4.2	4.3		4.4	47	4.6	
Kernel length (mm)	<i>min</i>	0.7	0.8	0.7	0.8	0.9	1.1	0.9	1.1
	<i>max</i>	0.9	0.9	0.9		1.1	1.3	1.2	
Kernel width (mm)	<i>min</i>	0.7	0.8	0.8	0.8	0.7	0.7	0.9	0.8
	<i>max</i>	0.9	0.9	0.9		0.9	1	0.9	
Kernel thickness (mm)	<i>min</i>	0.4	0.4	0.4	0.44	0.4	0.4	0.4	0.4
	<i>max</i>	0.5	0.5	0.5		0.4	0.4	0.4	
ASI (days)	<i>min</i>	1.9	0.5	1.1	2.4	3.6	5.4	4	5.5
	<i>max</i>	2.9	3.5	6.5		6.5	8	6.4	

The analyzed landraces were plotted in the area defined by the first two PC (Figure 1). Flint and dent landraces were distinctly separated – majority of flint landraces (except FIV3 and FVII1) had negative PC1 value, while all dent landraces (except DXIII3) had positive PC1 value.

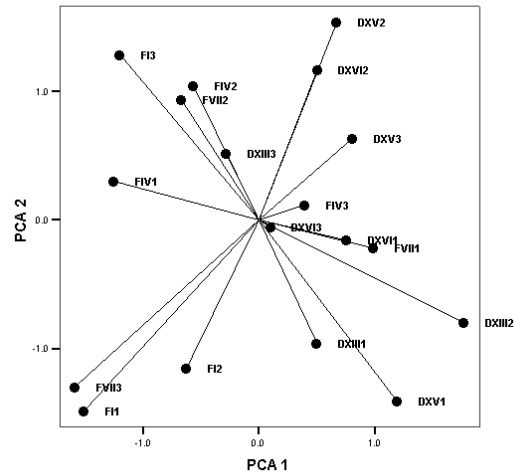


Figure 1. Distribution of the 18 maize landraces on the first two principal components PC1 and PC2 of the PCA performed for phenotypic data.

SSR analysis

Most of the 50 SSR primers used for PCR amplification could not be applied for landraces' analysis due to absence of amplification product (2 primers), poor amplification (15 primers) and complex pattern (28 primers). Ten informative primers with good amplifications and clear band patterns were chosen for statistical analysis (Table 3).

Total number of alleles revealed for all analyzed landraces was 63. The number of alleles varied from 3 (umc1418) to 9 (umc1474 and umc1492), with the average value of 6.3. For flint landraces alone total number of alleles was 56 (average value of 5.6), while for dent landraces it was 62 (average value of 6.2).

Eight specific alleles (i.e. alleles present only within flint, i.e. dent landraces) were detected with five probes – one allele in flint and seven alleles in dent landraces (Table 3). Only phi087 probe revealed specific alleles in both flint and dent landraces, while the other four probes (umc1109, umc1393, umc1274 and umc1492) revealed them only in dent landraces. Three different specific alleles were detected with phi087, two with umc1393 and one each with the remaining three SSR probes.

Table 3. List of the ten informative primers, including their chromosome position (bin), repeat motif and number of specific alleles detected within the analyzed landraces

	Probe	Bin	Repeat motif	Number of specific alleles	
				Flint	Dent
1	umc1282	1.01	(AT)6	0	0
2	umc2047	1.09	(GACT)4	0	0
3	umc1418	4.08	(GGAAG)4	0	0
4	umc1109	4.10	(ACG)4	0	1
5	umc1274	5.03	(TGC)5	0	1
6	phi087	5.06	(ACC)	1	2
7	umc1393	7.02	(GTC)4	0	2
8	umc1324	7.03	(AGC)5	0	0
9	umc1492	9.04	(GCT)4	0	1
10	umc1827	10.04	(GAC)6	0	0
				1	7

The genetic structure of the analyzed landraces is given in Table 4. The lowest (21) and the highest (41) number of alleles were found in landraces belonging to the Macedonian flints (FIV1 and FIV2, respectively). Only FIV3 among flint landraces carried a specific allele, revealed by phi087 and with frequency of 0.17. On the other hand, specific alleles were detected in all dent landraces except DXV2 and DXVII1. The highest number of specific alleles (eight) was detected within DXIII agro-ecological group. Specific allele frequencies were in the range from 0.01 to 0.49, with nine alleles possibly being rare alleles (frequency < 0.05).

Genetic similarity values calculated on Jaccard coefficient were in the range from 0.30 (FIV1 and DXVI2) to 0.81 (FI1 and FI3). The most similar landraces within an agro-ecological group were Montenegrin flints (FI, average GS=0.65) and the most diverse were Macedonian flints (FIV, average GS=0.47). Comparison between agro-ecological groups revealed that the most similar were Montenegrin and Mediterranean flints (FI and FVII, average GS=0.59) and the most distant Macedonian flints and Dent type of USA Corn Belt dents, i.e. Serbian dents (FIV and DXIII, i.e. DXVI, average GS=0.42). Altogether, no significant differences could be found between GS of landraces within and between agro-ecological groups.

Cluster analysis based on Jaccard genetic similarities separated most flint and dent landraces into different clusters, with several exceptions (Figure 2). Dendrogram can be divided into four clusters and two attached genotypes (DVI3 and FIV1). The first cluster consists of six flint landraces and the second cluster of five dent and one flint (FVII1) landrace. The third and the fourth clusters group two landraces each – FIV3 and DXV2 and DXIII1 and DXIII3, respectively. The co-phenetic correlation coefficient (which indicates the extent to which the clustering of genotypes depicted in the trees accurately represents the estimates of genetic distances between populations obtained with a particular marker system) was 0.76, indicating a good concurrence (fit).

Table 4. Total number of alleles, mean number of alleles per locus and population, and specific alleles for each of the analyzed landraces

Agro-ecological group	Landrace	Total number of alleles	Mean number of alleles per locus and population	Specific alleles		
				Probe	Number	Frequency
Montenegrin flints	FI1	37	3.7	-	-	-
	FI2	28	2.8	-	-	-
	FI3	33	3.3	-	-	-
Macedonian flints	FIV1	21	2.1	-	-	-
	FIV2	41	4.1	-	-	-
	FIV3	30	3	phi087* ¹	1	0.17
Mediterranean flints	FVII1	34	3.4	-	-	-
	FVII2	38	3.8	-	-	-
	FVII3	33	3.3	-	-	-
Dent type of USA Corn Belt dents	DXIII1	40	4.4	phi087* ^{2,3}	2	0.03, 0.02
				umc1393* ¹	1	0.05
				umc1492	1	0.03
	DXIII2	31	3.1	umc1393* ¹	1	0.11
				umc1393* ²	1	0.04
	DXIII3	39	3.9	umc1274	1	0.49
				umc1492	1	0.01
Dent type of Southern areas of USA	DXV1	28	2.8	umc1109	1	0.23
				umc1393* ²	1	0.04
	DXV2	29	2.9	-	-	-
Serbian dents	DXV3	40	4	umc1492	1	0.01
	DXVI1	33	3.3	-	-	-
				umc1109	1	0.23
	DXVI2	37	3.7	umc1393* ¹	1	0.06
				umc1393* ¹	1	0.08
DXVI3	27	2.7	umc1492	1	0.03	

*^{1,2,3} – indicates three different alleles detected with phi087

*^{1,2} – indicates two different alleles detected with umc1393

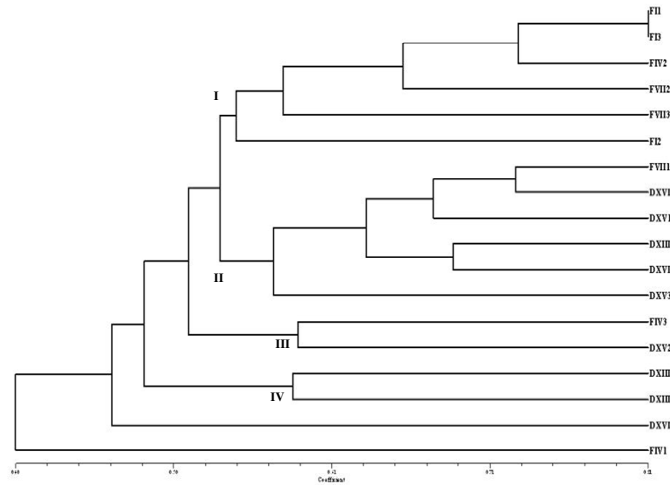


Figure 2. Dendrogram of 18 maize landraces (bulked population samples) constructed using UPGMA cluster analysis of *Jaccard* similarity values obtained by SSR binary analysis.

DISCUSSION

The paths of introduction and the origin of maize types from the regions of former Yugoslavia are very diverse. They originate from North American, South American and Mexican types of maize and their introduction lasted from the 16th to the 20th century. During the stated period introduced genotypes were either maintained unchanged (due to geographic and climatic similarity) or specific types were derived by hybridization and selection.

Montenegrin, Macedonian and Mediterranean flints are considered as the oldest types grown in this region, originating from South America. On the other hand, dents were the most recently introduced types. They were the first varieties used for the development of hybrids. Both Dents type of southern areas of USA and Dents type of USA Corn Belt dents, analyzed in this study, affected other groups of dents. Serbian Dents have traits that are intermediate between these two dent agro-ecological groups. It was assumed that they originated from Dents type of southern areas of USA or Mexican dents that had been crossed with flints existing in Serbia at that time (PAVLIČIĆ *et al.*, 1976).

In the present study, we have analyzed genetic diversity in a sample of 18 former Yugoslavian landraces by using both phenotypic traits and molecular markers. The results showed large genetic heterogeneity. Similar results were obtained for a set of different flint landraces analyzed with SSR and AFLP markers (IGNJATOVIĆ-MIČIĆ *et al.*, 2007; IGNJATOVIĆ-MIČIĆ *et al.*, 2008). The genetic diversity revealed for both morphological and molecular traits indicates that the landraces were highly adapted to specific environmental conditions and purposes through a long period of selection and local adaptation. In the last ten years different

research programs aimed at the identification of superior genotypes among genebank accessions, regarding stress tolerance (drought and herbicides), better nutritional quality (protein, starch, oil, phosphorus) and specific traits (cytoplasmic male sterility – CMS) are being carried out at the MRI (VANČETOVIĆ *et al.*, 2010; MLADENOVIĆ DRINIĆ *et al.*, 2011).

Phenotypic analysis showed great variation for most analyzed traits. However, flint landraces were more diverse compared to dent landraces. Also, ASI was much shorter in flint landraces (except FVIII). It was previously shown that the diversity of flint types is greater than diversity of dent types, due to their earlier and predominant introduction into this region. The greatest variability in Europe was found among flints grown in Italy, followed by flints grown in the former Yugoslavia (BRANDOLINI, 1968).

PCA analysis of morphological traits could separate most flint and dent landraces, but differentiation according to agro-ecological groups was not achieved. It could be the consequence of a small number of the analyzed landraces per agro-ecological group and/or the fact that morphological parameters are not the most reliable indicators of genetic relationships, especially when heterogeneous open-pollinated populations are considered. All the analyses were performed on the average values, although there was a significant variation within populations for most of the traits. Highly heritable traits can be easily modified by several cycles of selection and that morphological analysis does not seem fully appropriate for the classification of maize populations according to their genetic origin (REBOURG *et al.*, 2001).

SSR analysis of the flint and dent landraces' genetic structure was performed with a set of 10 probes. The average number of alleles per locus is in agreement with the allele number found in REIF *et al.* (2005), LABATE *et al.* (2003), CÔMERTPAY *et al.* (2012), but lower than found in DUBREUIL *et al.* (2006), MATSOUKA *et al.* (2002) and SHARMA *et al.* (2010). Different numbers of alleles detected in different studies can be attributed several factors. The type of SSR loci used can affect the results - tri-nucleotide or higher repeat motifs (although reducing the stuttering and giving more clear band patterns) are less polymorphic than di-nucleotide motifs and reveal lower number of alleles (VIGOUROUX *et al.*, 2002). Additionally, bulk approach might discard some alleles with frequencies < 0.02 (REIF *et al.*, 2005). Also, the number of genotypes analyzed affect the number of detected alleles - the more genotypes analyzed, larger the possibility of finding different alleles.

In the present study specific alleles (i.e. alleles present only within flint, i.e. dent landraces) were detected. The presence of alleles found in a single population may be attributed to possible selections of specific alleles in certain accessions associated with their morphology and area(s) of adaptation (SHARMA *et al.*, 2010). A set of seven alleles was found within dent landraces (one allele present in two to four accessions) and these differences indicate different origins and possibly different purposes of flint and dent genotypes.

Cluster analysis did not group the populations precisely with expectations based upon their origin, i.e., agro-ecological groups. Montenegrin landraces were the most similar as they grouped into one cluster, and Macedonian most diverse as they were scattered throughout the dendrogram. FIV3 and FVIII grouped with dent populations both in cluster and PCA analyses. This could be the consequence of hybridization between the flint landraces with dent genotypes - after the introduction of dent landraces, flint landraces were frequently crossed with them for deriving better maize performances. However, some discrepancies between phenotypic and SSR analysis (e.g. DXIII3 or FIV1 grouping with flint genotypes in PCA, but having different positions in the cluster analysis) warn that one approach for accession classification can be

insufficient and misleading - different information (morphological, ecological, geographic, genotypic, etc.) are needed to characterize genetic resources for efficient conservation and utilization.

CONCLUSION

Both morphological and SSR analyses distinguished flint and dent landraces, but not agro-ecological groups. The presence of specific alleles found within a single population may be a consequence of selection of the alleles associated with the population morphology and area(s) of adaptation. Differences in specific alleles content between flint and dent landraces point to their different origins and possibly different purposes. The results revealed a large genetic heterogeneity indicating that the analyzed landraces could be valuable sources of genetic variability. Both phenotypic and genetic studies are necessary for achieving the most accurate assessment of genetic diversity.

ACKNOWLEDGEMENT

This research was supported by the Ministry of education and science of Republic of Serbia through the project TR31028 *Exploitation of maize diversity to improve grain quality and drought tolerance*

Received August 03th, 2012

Accepted April 06th, 2013

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**FENOTIPSKA I SSR ANALIZA LOKALNIH POPULACIJA TVRDUNACA I ZUBANA
IZ REGIONA JUGOSLAVIJE**

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Izvod

Kolekcija od 2217 lokalnih populacija sa teritorija Jugoslavije se održava u banci gena Instituta za kukuruz Zemun Polje. Populacije su klasifikovane u 18 agro-ekoloških grupa, koje obuhvataju rano introdikovane tipove tvrdunaca, kasnije inrodukovane tipove zubana i tipove nastale njihovom hibridizacijom. Cilj ovog rada je bio da se utvrdi struktura i genetička divergentnost, pomoću fenotipskih i SSR markera, devet populacija tvrdunaca i devet populacija zubana koji pripadaju različitim agro-ekološkim grupama. Analizirane populacije su pokazale visok stepen varijacija za većinu analiziranih osobina, mada su populacije tvrdunaca bile raznovrsnije. Ukupno 56 alela je detektovano u populacijama tvrdunaca, odnosno 64 u populacijama zubana, pomoću deset SSR markera. Osam specifičnih alela (alela detektovanih samo među tvrduncima, odnosno zubanima) je identifikovano pomoću pet proba – jedan alel među tvrduncima i sedam među zubanima. Ove razlike u alelnoj strukturi ukazuju na različito poreklo i različite namene genotipova tvrdunaca i zubana. Fenotipska i SSR analiza su mogle da razdvoje većinu tvrdunaca od zubana, ali ne i agro-ekološke grupe. Rezultati su pokazali značajnu genetičku heterogenost analiziranih populacija, koja bi mogla biti dragocen izvor genetičke varijabilnosti.

Primljeno 03. VIII 2012.
Odobreno 06. IV. 2013.