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DIVERGENCE AMONG MAIZE GENOTYPES WITH DIFFERENT KERNEL TYPES ACCORDING TO SSR MARKER ANALYSIS

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Panels of diverse materials have proven to be very useful in evaluation of the organization of genetic diversity available for breeding at phenotypic and genotypic levels. In this study, a panel consisting of several groups of maize inbreds was tested using microstelite markers. The aim of this study was to test genetic diversity and define population structure of twenty-four genotypes differing in kernel type (dent, flint, popcorn, sweet maize) and kernel color (white, yellow, orange) with 21 SSRs. Genetic diversity parameters such as number of alleles, gene diversity, observed heterozygosity, PIC (*Polymorphism Information Content*) and number of unique alleles were determined. In addition, genetic distances according to Rogers distance were calculated. The values for all parameters were high, reflecting high genetic divergence of analyzed germplasm. Distance matrix based on UPGMA cluster analysis showed moderate congruence to available pedigree/origin or kernel type/color data. Genetic structure of tested genotypes was defined using Bayesian model-based clustering, without a considerable difference in comparison to cluster data analysis. The determined diversity parameters along with the results concerning genetic structure analysis provided a valuable information for improved selection efficiency.

Keywords: inbred line, kernel type/color, microsatellites, population structure, *Zea mays* L.

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INTRODUCTION

In Serbia, maize is the main crop and it is grown on more than one million hectares annualy (https://gain.fas.usda.gov/Belgrade_Serbia_4-10-2018.pdf). Statistical data show that Serbia holds the fifth position in Europe in maize production for years. These data indicate the importance of production of new improved maize hybrid varieties.

Besides high yield as the most important goal, production of hybrids with better nutritional properties and higher tolerance to biotic and abiotic stress is also of great importance. According to kernel texture, there are seven major types, including dent, flint, flour, sweet, pop, waxy and pod corn. Their major differences are based on the quality, quantity and pattern of endosperm composition. Endosperm composition may be changed by a single gene difference, as in the case of floury (*fl*) versus flint (*Fl*), sugary (*su*) versus starchy (*Su*), waxy (*wx*) versus non-waxy (*Wx*), and other single recessive gene modifiers that have been used in breeding specialty maize types (GWIRTS and GARCIA-CASAL, 2014).

Dents are maize varieties with kernels that have high starch content, whereas flints have kernels with a hard outer layer. The genetic divergence of these two major germplasm pools exploited in maize breeding can be explained by their historic geographical separation (i.e. origin) and adaptation to different environments (UNTERSEER *et al*., 2016). Dent inbred lines contribute with high productivity to hybrids and are slightly more efficient for processing and provide a higher digestibility index to livestock (SEVERINI *et al*., 2011). On the other hand, flint inbreds contribute with early vigor and good cold tolerance (REVILLA *et al*., 2014), having some extra natural insect resistance compared to dents (WIT *et al*., 2011).

Except for maize with standard kernel type (dents, flints and their intermediate types, i.e. semi-dent and semi-flint forms), development of speciality maize genotypes (e.g. sweet maize and popcorn) is also important. In sweet maize, i.e. standard sugary (*su*) maize, the sugary gene prevents or retards the normal conversion of sugar into starch during endosperm development. By accumulation of a water-soluble "phytoglycogen" polysaccharide, the dry, sugary kernels are wrinkled and glassy, thus adding a texture quality factor in addition to sweetness. At least 13 endosperm mutants, in combination with sugary, have been studied for improving sweet maize. Except for sugary, the genes used in breeding act differently to produce the taste and texture deemed desirable for sweet maize (PAJIĆ, *et al*., 2007). The popcorn type falls into the category of small-kernelled flint types, characterized by a very hard and corneous endosperm, having a very small portion of soft starch. The yellow and white are the primary groups of popcorn varieties. Yellow popcorn is the most common used for commercial applications, with kernels rounded in shape (pearl-like). White popcorn is characterized by a small white grain that looks similar to the grain of rice. Its pericarp is thinner, resembling some primitive semi-popcorns, such as the Argentine popcorns. On the other hand, some of more recently developed popcorns have thick pericarps (SILVA *et al*., 2009).

Kernel color depends upon pigments synthesized from one of two metabolic pathways – carotenoid (yellow and orange pigments) or anthocyanin pathway (red and purple pigments). The lack of pigments produced from either pathway results in white kernel color. Numerous genes are required for the synthesis of each pigment, including structural genes (coding for enzymes) and regulatory genes (called transcription activators and coding for proteins that control the transcription of structural genes). The presence of dominant *Pr1*, *C1* and *R1* genes results in purple or red aleurone. Their recessive versions block the anthocyanin formation. Dominant *Y1* gene is responsible for yellow or orange color of endosperm, while the homozygous recessive

version of (*y1*) gives a white starchy endosperm. Yellow flint and yellow dent corn has recessive *c1c1c1* and *Y1Y1Y1* genes (MOROHASHI *et al*., 2012).

Maize Research Insitute „Zemun Polje" gene bank contains maize genotypes with enormous genetic variability which could be potential source of beneficial alleles for various traits of interest. These alleles could be successffuly used in new breeding programs for making improved maize hybrid varieties (MESEKA *et al*., 2015). Moreover, for a hybrid with good performances, an adequate combination of parental components is also necessary (LIU *et al*., 2016). Thus, thorough examination of genetic diversity stands as the indispensable step at the beginning of a new breeding program process.

Molecular markers as a tool for characterization, detection of differences among maize accessions, their classification and genetic structure determination are present more than three decades and efforts for finding the best solution and the most adequate way of using this tool resulted in many generations and variations of them (GAUTHIER *et al*., 2002; HARTINGS *et al*., 2008; WARBURTON *et al*., 2011; ABDALLA *et al*., 2014; ROY and KIM, 2016). Simple Sequence Repeat (SSR) markers proved to be ones of the most frequently used in maize genotyping for a very long period of time – since their discovery in 1980s till nowadays. The importance and wide application of SSR markers in plant genotyping were reviewed recently (CARNEIRO VIEIRA *et al.*, 2016). The authors stated that over the period from 2010 to 2015 930 articles applying this technique in the genetic analysis of cultivated plants were published according to Web of Science (Web of Science™ Core Collection). This report proves the relevance of SSRs in plant genotyping in spite of the fact that SNPs (*Single Nucleotide Polymorphism*) are stated as improved marker technique with many advantages relative to other types of molecular markers.

This study is a evaluation of genetic diversity in a small set of maize genotypes with different kernel types and colours from Maize Research Institute „Zemun Polje". Genetic diversity was determined according to the polymorphism of 21 SSR markers, with the aim to perceive their potential for future breeding programs.

MATERIAL AND METHODS

Twenty-four genotypes from Maize Research Institute gene bank with different kernel type (dent, flint, sweet corn, popcorn) and kernel color (white, yellow and orange) were selected for molecular characterization using 21 highly informative SSR markers. Genotypes were selected according to their potential value for future breeding programs (considered as potential sources of beneficial alleles) or they have been proven to have good agronomical characteristics. Origin/pedigree and kernel type/color information of the teseted genotypes is shown in Table 1.

For each group of kernel type/colour, leaf samples were prepared for DNA isolation by taking a tissue from five individual plants for each tested line. Genomic DNA extraction was performed applying modified DOROKHOV and KLOCKE (1997) protocol. Fifty SSR markers were tested for genetic diversity assessment of the chosen genotypes. Finally, 21 markers gave clear and reproducible bands and were analysed. Primer sequences are available at the MaizeGDBwebsite (https://www.maizegdb.org/data_center/ssr) and their characteristics are given in Table 2. Also, we tried to include as little as possible markers with dinucleotide repeats (28.6%), considering the fact that they produce stuttering bands, which is not the case with the markers with other types of repeats as stated in many previous publications (IGNJATOVIC-MICIC *et al*., 2015; ACI *et al.*, 2018). Total volume of each PCR reaction was 25 µl and final concetrations of components were as follows: 1 x buffer, 0.5 µM primers, 0.8 mM dNTPs each,

1 U of Taq Polymerase (Thermo Scientific), 2 µl of 25 µg/µl genomic DNA and sterile water. PCR amplificaton was done using following program: 5 min intial denaturation step at 94°C, followed by 35 cycles of 94°C for 30s, 1 min of annealing at temerature specific for each primer, 2 min of extension at 72°C and final exension for 10 min at the same temperature.

Table 1. Origin/pedigree and kernel type/color of the tested inbreds

	Line number	Origin/pedigree	Kernel color/type
1	L1	-	Orange dent
2	L2	-	Orange flint
3	L3	-	Orange flint
4	L4	BSSS	Orange dent
5	L5	-	Orange flint
6	L6	-	Orange semi-flint
7	L7	IowaxLancaster	White dent
8	L8	BSSS	White dent
9	L9	-	White dent
10	L10	-	Sweet corn
11	L11	-	Sweet corn
12	L12	-	Sweet corn
13	L13	-	Sweet corn
14	L14	-	Sweet corn
15	L15	-	Popcorn
16	L16	-	Popcorn
17	L17	-	Popcorn
18	L18	BSSS	Yellow dent
19	L19	Lancaster	Yellow dent
20	L20	Lancaster	Yellow dent
21	L21	Lancaster×BSSS	Yellow dent
22	L22	BSSS	Yellow dent
23	L23	Lancaster	Yellow dent
24	L24	BSSS×Iowa	Yellow dent

Table 2. List of SSR primers used for genetic diversity determination and their characteristics

	Name	Chr/bin	Repeat type	Description
1.	umc1282	1.00	di-nucleotide	glyceraldehyde-3-phosphate dehydrogenase B1
2.	umc2083	1.06	tri-nucleotide	homeobox-transcription factor 84
3.	umc1535	2.05	di-nucleotide	hsbp1 - herbicide safener binding protein1
4.	umc2002	3.04	-	probed site
5.	bnlg1350	3.08	di-nucleotide	probed site
6.	umc2373	5.03	tri-nucleotide	probed site
7.	umc1784	5.03	tri-nucleotide	probed site
8.	umc1153	5.09	tri-nucleotide	inactive leucine-rich repeat receptor-like
9.	phi075	6.00	di-nucleotide	chloroplast ferredoxin 1
10.	umc1887	6.03	tri-nucleotide	L-type lectin-domain containing receptor kinase
11.	umc2375	6.06	tri-nucleotide	probed site
12.	umc1324	7.03	tri-nucleotide	NC domain-containing protein-related
13.	umc1412	7.04	tri-nucleotide	fused leaves1
14.	umc1799	7.04	di-nucleotide	probed site
15.	umc2355	8.03	tri-nucleotide	UDP-glucuronic acid decarboxylase 4
16.	umc1172	8.04	tri-nucleotide	pyruvate decarboxylase1
17.	bnlg1782	8.05	di-nucleotide	probed site
18.	phi080	8.08	penta-nucleotide	glutathione-S-transferase1
19.	umc2393	9.00	tri-nucleotide	transcription factor PRE3
20.	umc1492	9.04	tri-nucleotide	putative SAP DNA-binding domain family protein
21.	umc1506	10.05	tetra-nucleotide	serine/threonine-protein kinase GRIK1

Polyacrylamide electrophoresis was done for PCR products separation (8% gels, 1 hour at 60 mA, BioRAD tetra cell) and results were documented by photographing the gels under UV light using Biometra BioDocAnalyze Live gel documentation system. Size of the bands was estimated according to 20 bp ladder (Thermo Scientific).

Allele number, gene diversity (i.e. expected heterozygosity), observed heterozygosity, and PIC (*Polymorphism Information Content*) per marker was calculated using PowerMarker V3.25. Cluster analysis was done with MEGA 7 using matrices of genetic distances calculated in PowerMarker V3.25 applying Rogers' coefficient and UPGMA method.

Genetic structure of the analyzed maize genotype groups was determined with the software STRUCTURE (PRITCHARD *et al.*, 2000) using admixture model based clustering method. The number of subgroups (K) was set from 2 to 5 with five runs per each subgroup. A burn-in period was set to 50,000 with 100,000 MCMC (Markov Chain Monte Carlo) replications after burn-in. Visualization and evaluation of the best *K*-value for the STRUCTURE runs was

performed using CLUMPAK (KOPELMAN *et al*., 2015). Model choice criterion to detect the most probable value of K was both the LnP(D) value for each given K and ΔK.

RESULTS AND DISCUSSION

Summary of genetic diversity parameters calculated in PowerMarker are presented in Table 3. Total number of alleles was 139 with an average of 6.62 per marker. Number of alleles per primer was in a range from four (umc1887, umc1172, phi080) to 14 (umc2002). The average number of alleles was higher in comparison to other studies, taking into account the number of tested genotypes and markers. A possible reason for these results could be the fact that the tested material consisted of very divergent maize groups. OYEKUNLE *et al.* (2015) reported somewhat smaller total number of alleles (130) but for 42 early-maturing maize lines analyzed using 23 SSR markers. Also, the average number of alleles of 5.7 was slightly smaller comparing to our results. MUTHUSAMY *et al.* (2015) detected lower average number of alleles (3.88) for larger number of inbreds (48) and SSR markers (58), but much higher total number of alleles (225). For the purposes of effective hybrid breeding, almost the same number of maize inbred lines and SSR markers were analyzed (WENDE *et al.*, 2013). The authors reported that in 20 medium to late maturing inbreds using 20 microsatellites, a total of 108 alleles were detected with an average of 5.4. In our work, major allele frequency was lower than 0.5 for most of the SSRs, exceptions were only umc1172 and phi080 with 0.54 both. These results indicate diverse nature of loci and also wide genetic diversity in tested genotypes (MUTHUSAMY *et al.*, 2015). Gene diversity (i.e. expected heterozygosity) ranged from 0.62 for umc1172 and phi080 to 0.89 for umc2002. This parameter showed high values with an average of 0.77 and generally was greater than in the other studies, most probably because a very diverse set of inbreds was chosen for this analysis. A portion of dinucleotide repeat SSRs used was relatively small comparing to microsatellites with other types of repeats, and did not affect expected heterozygosity in this case. Observed heterozygosity was low, and equal to zero for most of the SSRs (average 0.083). Only three markers showed a certain level of heterozygosity: umc1172 (observed heterozygosity of 0.17), umc1535 and umc2355 (0.29), respectively, while only umc2002 showed heterozygosity equal to 1. A number of different factors could cause the presence of heterozygosity in inbreds, and one of the most probable in this case is the residual heterozygosity as explained in NEPOLEAN *et al*. (2013). PIC values ranged from 0.57 to 0.89, with average value of 0.73. A marker is considered highly informative if its PIC is greater than 0.5, and the whole set of the tested markers could be considered as useful in characterization of maize genotypes. All so far commented studies reported lower average PIC values. QU *et al.* (2013) compared results for PIC in 12 different studies on genetic diversity in maize inbred lines using microsatellites and showed that value of this parameter varied from 0.47 to 0.69 with an average of 0.607, which is lower but close to the results of our research. Differences among all discussed parameters in various studies are probably caused by the choice of different SSR markers. Also, the genotypes tested in our study belong to genetically diverse groups, thus high values of diversity parameters are not surprising.

Number of unique alleles was 32 (23%). Eight of them were found among genotypes with orange kernel color, while five were unique for white maize. Four, five and 10 of this type of alleles were detected in sweet, popcorn and yellow maize genotypes, respectively. The highest ratio between the number of genotypes per group and the number of unique alleles detected was 1.66 for popcorn maize. The importance of this type of alleles lies in the fact that they could be

used in identification of genome regions specific for a certain type of maize - sweet corn, popcorn (SENIOR *et al*., 1998). The percentage of rare alleles (alleles with a frequency lower than 5%) was 16.5%. Approximately half of all detected alleles had intermediate frequency. Within the whole set of genotypes evaluated, 84.2% of all detected alleles occurred at a frequency of 0.25 or less, and only 1.4% alleles had a frequency greater than 0.50. High allelic diversity could be expected due to high marker polymorphism and several kernel classes tested (SENIOR *et al*., 1998).

Table 3. Names of marker loci, major allele frequencies, allele numbers, gene diversities, expected heterozygosities and polymorphism information contents (PIC) of 21 SSRs studied

SSR marker	Major allele frequency	Allele number	Gene diversity	Observed heterozygosity	PIC
umc1282	0.25	9	0.84	0.00	0.82
umc2083	0.38	5	0.74	0.00	0.69
umc1535	0.33	6	0.76	0.29	0.72
umc2002	0.21	14	0.89	1.00	0.89
bnlg1350	0.33	7	0.76	0.00	0.72
umc2373	0.38	8	0.78	0.00	0.76
umc1784	0.29	6	0.80	0.00	0.77
umc1153	0.38	6	0.75	0.00	0.72
phi075	0.21	9	0.84	0.00	0.83
umc1887	0.33	4	0.71	0.00	0.65
umc2375	0.33	7	0.77	0.00	0.74
umc1324	0.38	5	0.74	0.00	0.69
umc1412	0.33	5	0.75	0.00	0.70
umc1799	0.29	7	0.82	0.00	0.80
umc2355	0.33	6	0.75	0.29	0.71
umc1172	0.54	4	0.62	0.17	0.57
bnlg1782	0.21	9	0.86	0.00	0.85
phi080	0.54	4	0.62	0.00	0.57
umc2393	0.42	5	0.74	0.00	0.70
umc1492	0.42	5	0.70	0.00	0.65
umc1506	0.29	8	0.82	0.00	0.80
Mean	0.34	6.62	0.77	0.083	0.73

Genetic distance calculated according to Rogers' coefficient was in a range from 0.38 to 0.99. The average genetic distance was high - 0.77, which is not surprising considering the fact that analyzed lines were taken from genetically distant groups. The lowest genetic distance was determined between two lines with yellow kernel color (L19 and L20) and this in accordance with their origin. Both lines belong to Lancaster Sure Crop group. There were only two more pairs of inbreds with genetic distance lower than 0.50. One pair are two popcorn lines (L15 and L17), and the other two are a line with orange (L4) and a line with white (L8) kernel color. It might be that the last two lines have common origin, in spite of the fact that the color of their kernels is different. A confirmation of concurrence between relatedness of inbreds by pedigree

and genetic distance calculated according to SSR marker data was shown in previous studies (OYEKUNLE *et al.*, 2015).

Two methods were implemented for revealing genetic structure of inbreds – cluster analysis by UPGMA method (MEGA 7) and Baesyan clustering (STRUCTURE software). The results of cluster analysis are presented in Figure 1. Cluster I included two subclusters. One of them, Ia, consisted of only three popcorn inbreds (L15, L16 and L17). The second one, Ib was divided in two smaller groups. One group comprised closely related Lancaster Sure Crop inbreds (L19 and L20), while the other comprised two lines with white (L7 and L9) and two with yellow kernel color (L21 and L23). Inbreds L7, L21 and L23 have Lancaster Sure Crop background and it is not surprising that they clustered together with L19 and L20. Thus, it might be concluded that popcorn genotypes also comprise Lancaster gemplasm. Incorporation of dent into popcorn germplasm, in order to improve traits as yield, resistance to desease, stalk and root lodging, was a frequent practice in maize breeding (BABU *et al*., 2006). The second cluster (II), included greater part of analysed genotypes, subdivided into two subclusters (IIa and IIb). Subcluster IIa comprised four genotypes with orange (L2, L3, L4, L5) and one with white (L8) kernel color. The closest were the lines with different kernel color (L4 and L8), but both of them belong to BSSS heterotic group and have dent endosperm type. The rest of the genotypes in this sub-cluster are of unknown origin, but all of them are flints. Clear classification according to kernel type/color was not present in sub-cluster IIb, and three out of five gerplasm groups tested were present. This part of dendrogram was divided into several groups. One of them was heterogenous, comprising sweet maize line (L13) and two inbreds of dent endosperm type with orange (L1) and yellow (L22) kernel color. Genotype L22 was of BSSS background and it is not impossible that the other two lines comprise the same germplasm since their pedigree is unknown. In the second group, dent inbreds L18 and L24 clustered together, which is expected considering the fact that both lines have BSSS pedigree. Four sweet maize genotypes (L10, L11, L12, L14) and one orange kernel semi-flint inbred (L6) constituted the last group. The only genotype with unknown origin in cluster II is inbred L6 and all the other are either sweet maize lines or genotypes with BSSS background. It is possible that sweet maize lines contain BSSS germplasm, since field maize has been extensively used for sweet maize improvement (BUTRÓN *et al*., 2008).

Model based analysis of population structure subdivided inbreds into five groups (Figure 2). In spite of some discrepancies, grouping of genotypes using this method was similar to classification according to cluster analysis. Results are shown in Figure 2. The estimated log probability of the data (LnP(D)) increased sharply at K=5. The ad hoc statistic ΔK showed the highest likelihood values at K=4. The results of different K values were compared with available data – pedigree/origin, kernel type/color, and grouping of genotypes at K=5 seemed to be the best. One group included popcorn genotypes together with two white and one yellow genotype. The next group consisted of two Lancaster Sure Crop lines with common origin. All discussed lines grouped together in cluster analysis. STRUCTURE gave somewhat better classification of these inbreds according to their origin, since two Lancaster lines are the closest in the whole set according to pedigree data. On the other hand, popcorn lines are genetically distant from genotypes with standard kernel type, thus more detailed analysis (e.g. greater number of markers) is needed for revealing relationships among chosen material. The grouping of inbreds in the rest of the three groups using STRUCTURE software, was similar to cluster II, although some lines assigned to some groups by structure analysis were placed in different groups using

cluster analysis. The third group in STRUCTURE analysis comprised four sweet maize genotypes and two yellow type endosperm genotypes with BSSS background (L24 and L21). The fourth group consisted of genotypes with different characteristics – one sweet (L13), one inbred line with orange (L1) and two with yellow kernel color (L18, L22). The last group contained four genotypes with orange (L2, L3, L4, L5) and one with white endosperm color (L8).

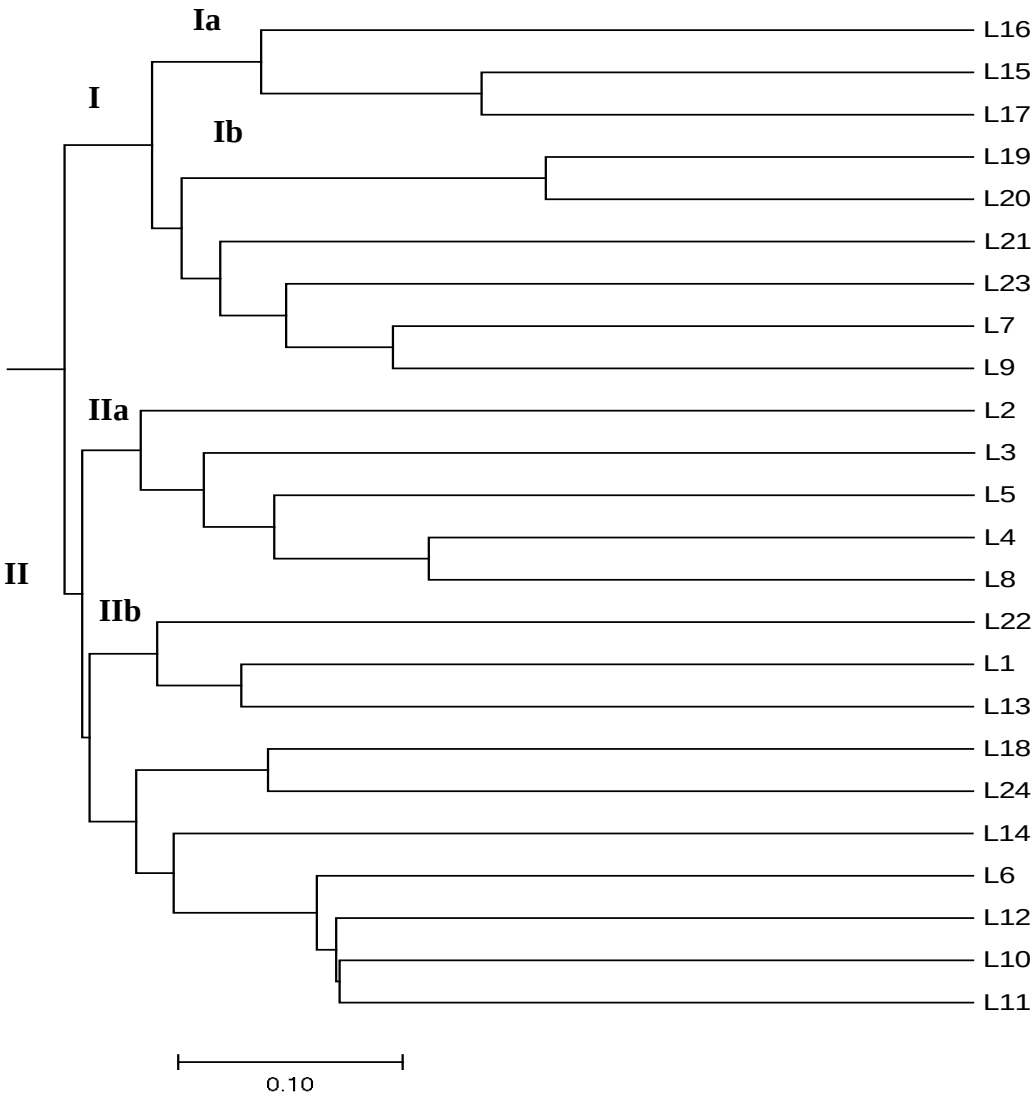

Figure 1. Classification of 24 maize inbreds revealed by UPGMA cluster analysis based on Rogers' distance

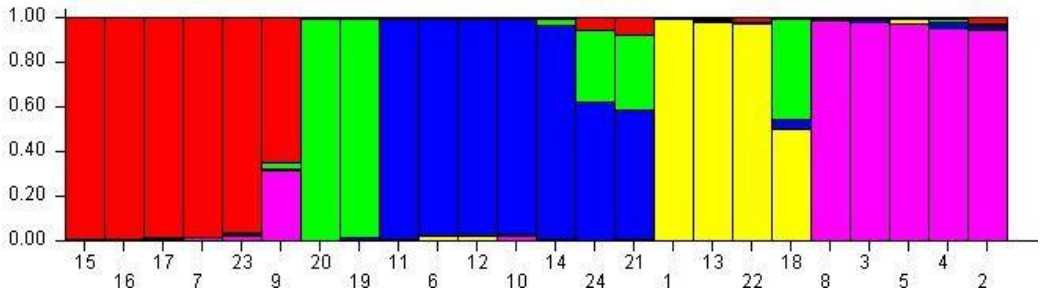

Figure 2. Population structure of 24 maize inbreds based on 21 SSR markers. Each vertical bar marked with belonging number (numbers as per Table 1) represents one inbred

It could be concluded that grouping of genotypes was not completely in agreement with kernel type/color. Also, absence of complete data for origin and pedigree affected potentially a better explanation of the presented classification. Similar results, partial agreement with mentioned data, were reported in many studies on genetic diversity of different maize breeding material using diverse molecular marker techniques (ADETIMIRIN, *et al.*, 2008; NIKOLIĆ *et al.*, 2016; SSERUMAGA *et al.*, 2019). XIA *et al.* (2004) reported the absence of separation between white and yellow maize germplasm according to SSR molecular data, which is in line with our study. However, the separate evaluation of these materials is imposed because of practiacal breeding considerations (XIA *et al.*, 2004). On the other hand, VAZ PATTO *et al.* (2004) discussed a possible role of kernel type and kernel color in classification and management of maize germplasm and their results suggest possible relationship between these traits and genetic variation *per se*, indicating a different origin. Sweet maize and popcorn genotypes grouped together with standard maize genotypes for both methods applied for genetic structure revealing. LIU *et al*. (2003) excluded these maize kernel type groups from model-based approach analysis because of small number of genotypes and great genetic divergence in comparison to genotypes of standard maize. But, as discussed earlier, inclusion of field maize germplasm in breeding of speciality maize is not uncommon practice and presented classification is not surprising. Also, such classification of genotypes could be influenced by size homoplasy of microsatellite markers (ŠIMIĆ *et al.*, 2009).

More intensive study with greater number of genotypes plus more SSR markers will be conducted. Also, field data and grain quality traits data will be combined with molecular marker information for some association studies.

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GENETIČKI DIVERZITET GENOTIPOVA KUKURUZA RAZLIČITOG TIPA ZRNA UTVRĐEN NA OSNOVU SSR MARKERA

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Izvod

Panel od 24 samooplodne linije kukuruza je analiziran primenom mikrosatelita. Genetički diverzitet i struktura populacije za dvadeset četiri genotipa različitih po tipu (zuban, tvrdunac, kukuruz šećerac, kokičar) i boji (narandžasta, žuta, bela zrna), definisani su primenom 21 SSR markera. Pored parametara genetičkog diverziteta kao što su broj alela, očekivana heterozigotnost, uočena heterozigotnost, PIC i broj jedinstvenih alela, izračunata je i genetička distanca između ispitivanih linija primenom Rodžerovog koeficijenta. Izračunate vrednosti za sve parametre su bile visoke što ukazuje na visok stepen diverziteta između analiziranih genotipova. Na osnovu matrica distanci urađena je klaster analiza koja je pokazala delimično poklapanje sa podacima o poreklu i tipu/boji zrna. Genetička struktura testiranih genotipova je definisana korišćenjem *Bayesian model-based* metode i nisu detektovane velike razlike u poređenju sa klasifikacijom na osnovu klaster analize. Definisani parametri genetičkog diverziteta i strukture populacije se mogu smatrati relevantnim za pružanje korisnih informacija u cilju povećanja efikasnosti selekcije.

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