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MOLECULAR CHARACTERISATION OF MAIZE HYBRIDS

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Abstract

Despite the huge diversity of maize germplasm, modern maize breeding programme and agricultural practices decrease the diversity of modern hybrids. Genetic characterization of maize hybrids allows knowledge of the genetic relationship among them, thus preventing the risk of increasing uniformity. Because of their high reproducibility, informativeness and easey application of microsatelites are the most frequently used molecular markers in maize genetic diversity studies. The aim of our work was to evaluate genetic diversity of maize hybrids by SSR markers and compare results with their pedigre information. Sixteen polymorphic SSR (*Simple Sequence Repeats*) markers were used to characterize 14 maize (*Zea mays* L.) hybrids belonging to different breeding programs and FAO groups (from 300 to 800). A total of 53 alleles were found, ranging from two to four alleles. Genetic similarities were calculated in NTSYSpc2 program package using Jaccard's coefficient based on binary data (presence or absence of alleles). The highest value of genetic similarity was 0.80 between H1 and H2, while the lowest value (0.26) was found between H12 and H13. Cluster analysis was done by unweighted pair group method (UPGMA) on the basis of genetic similarity matrix. Dendrogram analysis grouped maize hybrids in one cluster (most of the analyzed genotypes), one smaller cluster and one branch. The results revealed genetic heterogeneity between analyzed maize hybrids.

Keywords: *maize hybrids, genetic similarity, SSR markers, Zea mays L.*

Introduction

Maize, wheat and rice are the most important cereal crops grown in the world. Maize is used mainly for food, feed, as feedstock for food processing and in chemical industry. Due to huge genetic and phenotypic variability maize is adapted to different agroecological environments, edaphic and climatic conditions.

Maize has an important role in the field of scientific research such as the application of molecular genetic techniques and identifying genes and their functions. Maize is biological model system for genetics, evolution and domestication of cereals of the highest public interest (Wei et al., 2007). The main characteristic of maize is based on its variability in morphological traits and the high polymorphism of the DNA sequence (Matsuoka et al., 2002). During the breeding programs, maize hybrids have narrowed the genetic basis, leding to a significant reduction in diversity, so maize in commercial use contribute around 5% of the available germplasm (Carena et al., 2009).

In order to prevent genetic erosion, i.e. loss of individual genes and their combinations, as well as further narrowing of maize diversity, it is necessary to characterize existing elite lines, modern varieties and hybrids. Many methodologies are used for the assessment of genetic diversity in maize such as pedigree data, morphological traits and molecular markers. Compared with morphological variation, molecular polymorphism is generally considered to be independent of the environment and they are able to detect differences on DNA level on different individuals. (Ghebru et al., 2002; O'Neill et al., 2003).

Molecular diversity analyses can be performed using various kinds of methods including fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms

(AFLPs), randomly amplified polymorphic DNA markers (RAPD), simple sequence repeats (SSRs) or single nucleotide polymorphisms (SNPs). SSR markers have been the marker of choice for assessing maize genetic diversity due to the high level of polymorphism, multi-allelic nature, random distribution throughout the genome and cost effectivness (Barcaccia et al., 2006; Mason, 2015). In plant genomes they show an extensive variation in different individuals and genotypes (Cömertpay et al., 2012). Single nucleotide polymorphism (SNP) are new marker technologies that could also be used for estimating genetic diversity, but they are still to be adjusted for studies of genetic relatedness in maize (Yang et al.,2011).
In the present work SSR fingerprinting of 14 maize hybrids was done for molecular identification and assessment of genetic diversity, as well as to compare their clasification with their pedigre information.

Materials and Methods

A set of fortheen maize hybrids from the Maize Research Institute „Zemun Polje" that belong to different breeding programs were analyzed with molecular markers to evaluate genetic diversity. These 14 hybrids are covering different FAO groups, in the range from 300 to 800 (Table 1).

Table 1. List of the analyzed maize hybrids and their FAO groups

Hybrid	FAO group	Hybrid	FAO group
H1	300	H8	500
H2	300	H9	600
H3	600	H10	700
H4	300	H11	800
H5	500	H12	600
H6	500	H13	400
H7	500	H14	400

Genomic DNA was isolated from kernel using the CTAB (cetyl trimethylammonium bromide) procedure according to Doyle and Doyle (1987). Simple sequence repeat (SSR) characterization was done with 18 markers from the maize germplasm bank (www.maizegdb.org) (Table 1). Polymerase chain reaction (PCR) was carried out in 25µL reaction volume containing: 50ng of DNA sample, 1xBuffer, 0.8mM dNTP, 0.5µM of each primer pair and 1U Taq polymerase. The PCRs were performed using the following touch-down program: an initial denaturation at 95ºC/5min. by 15 cycles each of denaturation at 95ºC/30 s, annealing at 63.5/1min (-0.5ºC/cycle) and extension at 72 ºC /1min; and another 22 cycles of 95 ºC /30 s, 56ºC/1min and 72ºC/1min. Final elongation was at 72°C for 4min. Using vertical electrophoresis (Mini Protean Tetra-Cell BioRad) the amplified PCR fragments were separated on 8% polyacrylamide gel, with 20 bp ladder as a marker. Gels were photographed under UV light on BioDocAnalyse Biometra after staining with 0.5µg/µL ethidium bromide. Data were assembled into a binary matrix after SSR profiles were scored as presence or absence (1/0) of fragments in each sample. Genetic similarities (GS) between maize hybrids were calculated in accordance to Jaccard (1908): GSij = a/(a+b+c); where *a* is number of fragment shared by both individuals; *b* is number of fragments present in *i* but not in *j*; *c* is number of fragments present in *j* but not in *i.* For marker data analyses statistical NTSYSpc2 program package (Rohlf FJ, 2000) was applied.

Results and Discussion

The loss of maize diversity is considered one of today's most serious problem in maize production. Genetic biodiversity of maize is crucial for future breeding programmes in continuing advances in yield, grain quality improvement, disease and pest resistance.

In order to determine genetic diversity between 14 maize hybrids, molecular characterization was done using 18 SSR markers (Table 2). Two SSRs were not included in data analyses since they were monomorphic. Total of 53 alleles among the analysed 14 maize hybrids were identified. The number of alleles obtained with different primers varied from two (nc133) to four (bnlg1083, phi083, umc1448, umc1109, bnlg2235, umc1492 and umc1152) with the average value of 3.31 per locus. Similar number of alleles 3.33 was found in work with 38 Iranian maize hybrids (Shiri et al., 2015). Bantte and Prasanna (2003) reported slightly lower number of alleles (3.25) with 36 SSR loci. Some other works have shown considerably higher number of alleles. Nikkhoy and Shiri (2017) obtained 4.2 alleles per locus with 20 SSRmarkers on maize hybrids, while Lu and Bernardo (2001) found 4.9 alleles with 83 SSR loci, same as Warburton et al. (2002) with 85 SSR loci.

Table 2. List of 16 informative primers, with their chromosome position, repeat motif, number of alleles and allele range within analyzed maize hybrids

	Probe	Bin	Repeat motif	Number of alleles
1.	umc1282	1.00	(AT)6	3
2.	phi109275	1.03	AGCT	2
3.	bnlg1083	1.02	AG(29)	4
4.	umc1122	1.06	(CGT)7	3
5.	umc2047	1.09	(GACT)4	3
6.	phi083	2.04	AGCT	4
7.	umc1448	2.04	(GCT)5	4
8.	nc133	2.05	GTGTC	2
9.	umc1109	4.10	(ACG)4	4
10.	umc1153	5.09	(TCA)4	3
11.	phi452693	6.04	AGCC	3
12.	bnlg2235	8.02	AG(23)	4
13.	phi080	8.08	AGGAG	3
14.	umc1492	9.04	(GCT)4	4
15.	umc1152	10.01	(ATAG)6	4
16.	bnlg1526	10.04	AG(15)	3

Based on presense or absense of alleles in each sample coefficient of similarity was calculated by Jaccard. The genetic similarities were in range from 0.26 for H12 and H13 to 0.80 for two pairs of hybrids H1 and H2. Hybrids H12 nad H13 belong to specific types of maize, popcorne and sweet corn hybrids, respectively. On the other hand, single crosses hybrids H1 and H2 showed the higest similarity, because they have one same parental component and the other one are highly related inbred lines.

The cluster analysis using UPGMA method, based on Jaccard similarities distributed genotypes into two clusters (A and B) and one branch (c) showing good separation of hybrids and agrement with their pedigre data. Hybrids with very similar parental lines were grouped together within dendrogram. Cluster A contained most of the analyzed genotypes (11), dividing into subclusters A1 and A2. Subclaster A1 grouped hybrids with same genetic basis; hybrids H5 and H7 have one same parental component, and H4 and H6 are three way crosses and they have one parental line in common. This subclaster contained all hybrids from 300

FAO group, two from 500 FAO group and one from 600 FAO group. In subcluster A2 were grouped hybrids from 500 to 800 FAO groups, which have also same genetic basis. H9 and H10 have very similar parental lines, from te same source. Sweet corn hybrids (H13 and H14) formed cluster B, while branch c was formed of popcorn hybrid H12.

The average similarity of 0.54 among 14 maize hybrids showed satisfying variability between hybrids. The lower variability was reported in Bauer et al. (2005) with RAPD markers on maize hybrids, where Jaccard's coefficient of similarity ranged from 0.69 to 0.93. Also, Chen et al. (2008) reported higer genetic similarities with average value of 0.77 among 186 maize hybrids in China.

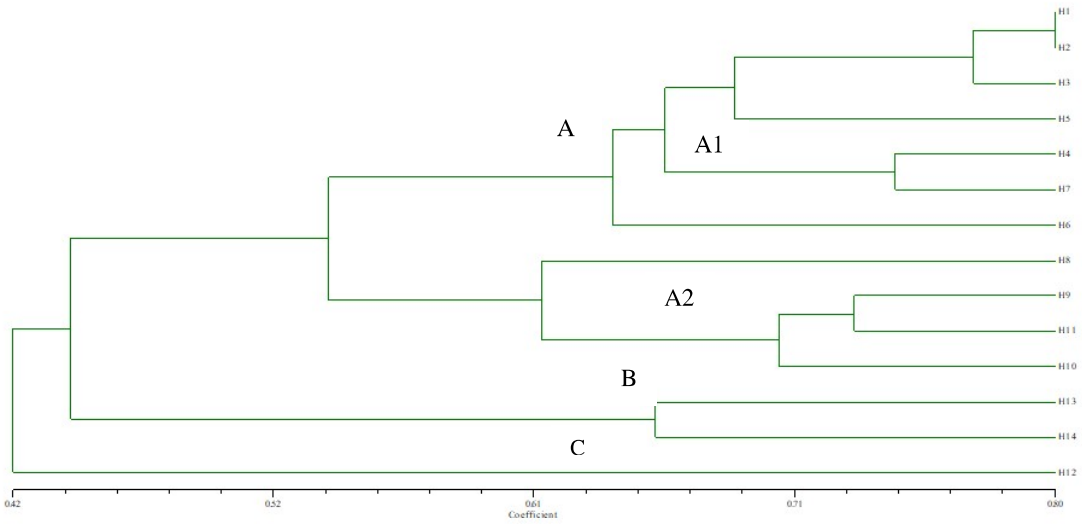

Figure 1. Dendrogram of 14 maize hybrids constructed using UPGMA cluster analysis of genetic similarity values (Jaccard, 1908) obtained from SSR data.

In his study SSR analysis determined variability among maize hybrids, as well as their grouping by genetic background. The dendrogram constructed from UPGMA method distinguishing hybrids to clusters according to their parental lines.

Conclusions

SSR markers is powerful tool for genetic characterization of maize hybrids and their clasification comparing with pedigree data. In this work 16 polymorphic SSR markers classified 14 maize hybrids into different groups in accordance to their pedigree data. Molecular marker could be successful and significant in evaluation of maize diversity assigned the most of the maize hybrids to their genetic background.

References

Bantte, K. & Prasanna, B. (2003): Simple sequence repeat polymorphism in Quality Protein Maize (QPM) lines. Euphytica: Volume 129, Issue 3, pp 337–344.

Barcaccia G, Meneghetti S, Albertini E, Triest L, Lucchin M (2003): Linkage mapping in tetraploid willows: segregation of molecular markers and estimation of linkage phases support an allotetraploid structure for Salix alba × Salix fragilis interspecific hybrids. Heredity 90:169–180

Bauer I, S. Mladenovic Drinic, M. Filipovic and K. Konstantinov (2006): Genetic characterization of early maturing maize hybrids (*Zea mays* L.) obtained by protein and RAPD markers. Genetika, vol.37, no.3., pp.235-243.

CarenaM .J., G. Bergman, N. Riveland, E. Eriksmoen, and M. Halvorson (2009): Breeding maize for higher yield and quality under drought stress. Maydica 54:287–296.

Chen F, K. Yang, T. Rong G. Pan (2008): Analysis of genetic diversity of maize hybrids in the regional tests in Sichuan and Southwest China. Frontiers of Agriculture in China. 2:162

Cömertpay G., F.S. Baloch, B. Kilian, A.C. Ülger, H. Özkan (2012): Diversity assessment of Turkish maize landraces based on fluorescent labelled SSR markers. Plant Molecular Biology Reporter 30:261-274

Doyle J.J. and J.L. Doyle (1987): A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19:11-15.

Ghebru B, Schmidt RJ, Bennetzen JL. (2002): Genetic diversity of Eritrean sorghum landraces assessed with simple sequence repeat (SSR) markers. Theor Appl Genet. 105:229–236. doi: 10.1007/s00122-002-0929-x

Jaccard P. (1908): Nouvelles recherches sur la distribution florale. Bulletin Société Vaudoise des Sci Naturelles 44(2): 223-270.

Lu H., Bernardo R. (2001): Molecular diversity among current and historical maize inbreds. Theor Appl Genet 103:613–617

Mason AS (2015): SSR Genotyping. In: Batley J (ed) Plant Genotyping.Springer, New York, NY, pp 77-89.

Matsuoka Y., Vigouroux Y., Goodman M. M., Sanchez G. J., Buckler E., Doebley J. (2002): A single domestication for maize shown by multilocus microsatellite genotyping. Proc. Natl. Acad. Sci. U.S.A. 99, 6080–608410.1073/pnas.052125199

Farideh Nikkhoy and Mohammadreza Shiri (2017): Genetic Diversity Analysis of Maize Hybrids Through Morphological Traits and Simple Sequence Repeat Markers. J Plant Mol Breed 5(1): 49 – 60

O'Neill, R., Snowdon, R. J. and Kohler, W.(2003): Population Genetics Aspects of Biodiversity. Progress Bot., 64: 115-137

Rohlf, F.J. (2000): NTSYS-Pc: Numerical Taxonomy and Multivariate Analysis System. Version 2.1., Exeter Publishing Led., New York.

Shiri, M. R., Choukan, R., and Aliyev, R. T. (2014): Study of genetic diversity among maize hybrids using SSR markers and morphological traits under two different irrigation conditions. Crop Breeding Journal 4 (1): 65-72.

Warburton, M.L., Xianchun, X., Crossa, J., Franco, J., Melchinger, A.E., Frisch, M., Bohn, M. and Hoisington, D. (2002): Genetic characterization of CIMMYT inbred maize lines and open pollinated populations using large scale fingerprinting methods. Crop Sci., 42(6): 1832-1840.

Wei F, Coe E, Nelson W, Bharti A.K, Engler F, Butler E, Kim H, Goicoechea J.L, Chen M, Lee S, Fuks G, Sanchez-Villeda H, Schroeder S, Fang Z, McMullen M, Davis G, Bowers JE, Paterson A.H, Schaeffer M, Gardiner J, Cone K, Messing J, Soderlund C, Wing R.A. (2007): Physical and genetic structure of the maize genome reflects its complex evolutionary history. PLoS Genet. 3 e123

Yang X, Y, Xu, T, Shah, H, Li, Z, Han, J, Li, J, Yan (2011): Comparison of SSRs and SNPs in assessment of genetic relatedness in maize. Genetica 139:1045–1054.