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ESTIMATION OF GENETIC DIVERSITY AMONG MAIZE INBRED LINES

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Abstract

One of the most effective method in maize (*Zea mays* L) selection of adapted material is to create synthetic populations from inbred lines of known origin and superior properties. The methods used for inter- and intra-population synthetic improvement are some of the recurrent selection techniques. Success of recurrent selection depends on the choice of parent components and the method that will be used to obtain new inbred lines. The aim of this paper was to apply molecular markers for estimation of genetic variability of inbred lines, as potential parent components of synthetic populations. Molecular characterization of 26 inbred lines was done with 18 polymorphic SSR (*Simple Sequence Repeat*) markers. The total number of obtained alleles was 54, and ranged from two alleles for primers: phi033, phi036, phi087 and umc1013 to five alleles for primer umc1040. Genetic similarity values were calculated using Dice coefficient in the NTSYSpc2 program package. The highest similarity value (0.96) was calculated between inbred lines L22 and L24, while the lowest value (0.26) was between inbred lines L7 and L21. Cluster analysis divided the inbred lines into three groups mostly in accordance with their origin. The variability detected using SSR markers could be useful in selecting best parental combinations in creating synthetic populations.

Keywords: *inbred lines, synthetic population, SSR markers.*

Introduction

Maize is open-pollinated crop species with huge genetic diversity, containing wide variation in morphological traits and large-scale polymorphism on DNA level (Matsuoka *et al*., 2002). Long-term selection as well as development of modern maize hybrids have led to narrowing of the genetic variability of maize populations, i.e. to reducing the pre-conditions for the expression of heterosis. On the other hand, practical application of heterosis has been eliminated significance of the variety production and led to the genetic uniformity of the commercial material. Maize breeders are under constant pressure to create new commercial inbred lines, so they increasingly use F2 populations, reverse crossings and synthetic populations of narrow genetic bases in their programs, which has led to a reduction in the use of local populations as a carrier of high genetic variability (Ho *et al.,* 2005). Maize breeders recognized the importance of the genetic variability of the starting material for the expression of heterosis in the F1 generation. Molecular studies have confirmed that genetic divergence is positively correlated with heterosis (Ajmoone Marsan *et al*., 1998). For the successful program of creating synthetic populations as the source of new inbred lines besides choosing the selection method, the choice of parental components is very important. In order to study the genetic variability of the breeding material, divergence and belonging to heterotic groups, breeders can use data on the origin of the lines, test methods or genetic markers. Recently, in short-term selection programs, F2 generations and narrow base synthetic populations of narrow genetic bases are mainly used as a source of new lines. In contrast, long-term programs use synthetic populations and composites. It is very important to choose the right selection method of working with synthetic populations and parental components for their formation.

The use of molecular markers in diversity estimation and genetic structure is of great help when starting a selection program, creating synthetic populations as a working material and choosing the parents for that purpose. Due to the rapid developments in the field of molecular genetics, various types of techniques are used to estimate genetic diversity (Spooner *et al*., 2005, Semagn *et al.,* 2012, Dao *et al.,* 2014). *Simple sequence repeats* (SSRs) markers provides many advantages that make it especially applicable in studies of diversity and relationships, such as independence from environmental and pleiotropic effects, co-dominant inheritance, high reproducibility, locus specificity and their random distribution across the genome (Morgante *et al*., 2002; Barcaccia *et al*., 2006). The advantages of DNA markers in diversity estimation do not diminish the significance of phenotypic traits, on the contrary, morphological data provide practical information in characterisation of breeding material. In the past decade due to their high informativeness and reproducibility SSR markers become quite useful and frequently used PCR based markers in maize diversity studies (Dubreuil *et al*., 2006; Sharma *et al*., 2010). The objectives of this study were assessment of genetic diversity and relationships of inbred lines, as potential parent components of synthetic populations.

Materials and Methods

A set of 26 maize inbred lines from Maize Research Institute "Zemun Polje", which belongs to diferent heterotic groups and with different genetic background, was analyzed using molecular markers. Genomic DNA for each sample was isolated from seed bulk using the CTAB (cetyl trimethylammonium bromide) procedure according to Doyle and Doyle (1987). Molecular characterization was done with 18 polymorphic SSR markers (Table 1) from the maize germplasm bank (www.maizegdb.org). Polymerase chain reaction (PCR) was carried out in 25μL reaction volume containing: 50 ng of DNA sample, 1xBuffer, 0.8 mM dNTP, 0.5 μM of each primer pair and 1U *Taq* polymerase. The PCRs were performed in Thermocycler Biometra TProfessional Standard 96 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 95ºC/30 s, 56ºC/1min and 72ºC/1min. Final elongation was at 72°C for 4 min. The amplified fragments were separated by vertical electrophoresis (Mini Protean Tetra-Cell BioRad) on 8% polyacrylamide gel, with 100bp ladder as a marker. After staining with 0.5 μg/μL ethidium bromide gels were photographed under UV light on BioDocAnalyse Biometra. SSR profiles were scored as presence/absence of fragments in each sample and the data were assembled into a binary matrix. Genetic similarities between inbred lines were calculated by Dice (1945) and Unweighted Pair Group Method with Arithmetic mean (UPGMA) method was applied for cluster analysis. Statistical NTSYSpc2 program package (Rohlf FJ, 2000) was applied for marker data analyses.

Results and Discussion

Variability of initial genetic material is an important precondition for breeding. One way to increase the genetic variability of the breeding material is to create synthetic populations with a broad genetic base and to further apply the appropriate selection method. To determine the genetic similarity between 26 inbred lines, 18 SSR markers were used. Out of the 22 SSRs, four were not included in data analyses due to absence of amplification product (two primers) and poor amplification (two primers). Total of 54 alleles among the analysed 26 lines were identified. The number of alleles richness varied from two (phi033, phi036, phi087 and umc1013) to five (umc1040) with the average value of 3.15 per locus (Table 1). That is similar to previous study where Bantte and Prasanna (2003) reported 3.25 alleles using 36 SSR loci. Some other investigations have shown considerably higher number of alleles. Lu

and Bernardo (2001) obtained 4.9 alleles with 83 SSR loci, same as Warburton *et al*. (2002) with 85 SSR loci.

Table 1. List of 18 informative primers, with their chromosome position, repeat motif, number of alleles and allele range within analyzed inbred lines

	Probe	Bin	Repeat motif	Number of alleles	Allele range (bp)
1.	umc1013	1.08	(GA)9	2	120-160
2.	umc2047	1.09	(GACT)4	4	120-300
3.	umc1265	2.02	(TCAC)4	3	100-120
4.	umc2129	2.07	(CGC)5	3	90-110
5.	phi036	3.04	AG	2	60-80
6.	umc1350	3.08	(AG)13	3	140-160
7.	umc1418	4.08	(GGAAG)4	3	145-280
8.	umc1109	4.10	(ACG)4	4	100-120
9.	bnlg557	5.03	-	3	100-120
10.	phi087	5.06	(ACC)	2	150-180
11.	phi075	6.00	CT	3	200-220
12.	umc1006	6.02	(GA)19	3	100-120
13.	umc1015	7.03	(GA)45	3	90-110
14.	umc1782	7.04	(GAC)4	3	120-140
15.	bnlg2235	8.02	AG(23)	3	160-200
16.	phi033	9.01	AAG	2	220-240
17.	umc1040	9.01	CT11	5	80-200
18.	umc1492	9.04	(GCT)4	3	130-160

Genetic similarity (GS) values calculated by Dice (1945) between the pairs of all 26 inbred lines ranged from 0.26 (L7 and L21) to 0.96 (L22 and L24), with an average 0.55. The high value of genetic similarity between L22 and L24 can be explained by the same base population derivation. The largest part (53.5%) of obtained GS values was between 0.41 and 0.6 (Figure 1). The estimated average genetic similarity shows relatively high level of diversity among the analysed genotypes.

This study showed 9.8% of pairwise comparisions with GS values of GS greater than 0.7, making them a valuable source in creating synthetic populations. Beside genetic similarity of potential perental lines, their affiliation to heterotic groups should be considered.

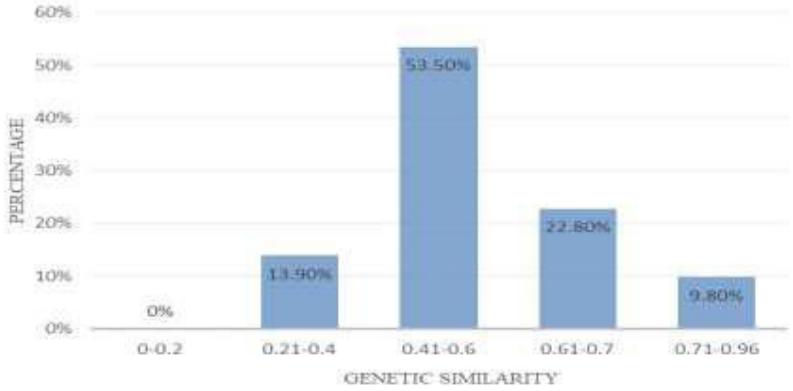

Figure 1. Dispersion og pairwise Dice's genetic similarity values of 26 maize inbred lines obtained from SSR data

The cluster analysis based on Dice′s genetic similarities distributed 26 inbred lines into three clusters (Figure 2) mostly in accordance with their origin. Cluster „A" was consisted of only two inbred lines (L1 and L19) with diferent background, quality protein maize (QPM) line with unknown genetic origin and Lancaster Sure Crop (LSC). QPM lines had unknown genetic orgin, so another goal was to classified them into appropriate heterotic group.
Out of the 26 inbred lines in total, twelve lines were grouped in cluster „B". The related lines belong to QPM lines (L2, L3, L4, L5 and L6), BSSS background mixed with other germplasm (L7, L9, L10, L11 and L25) and mixed local genetic basis and BSSS background (L13 and L18).

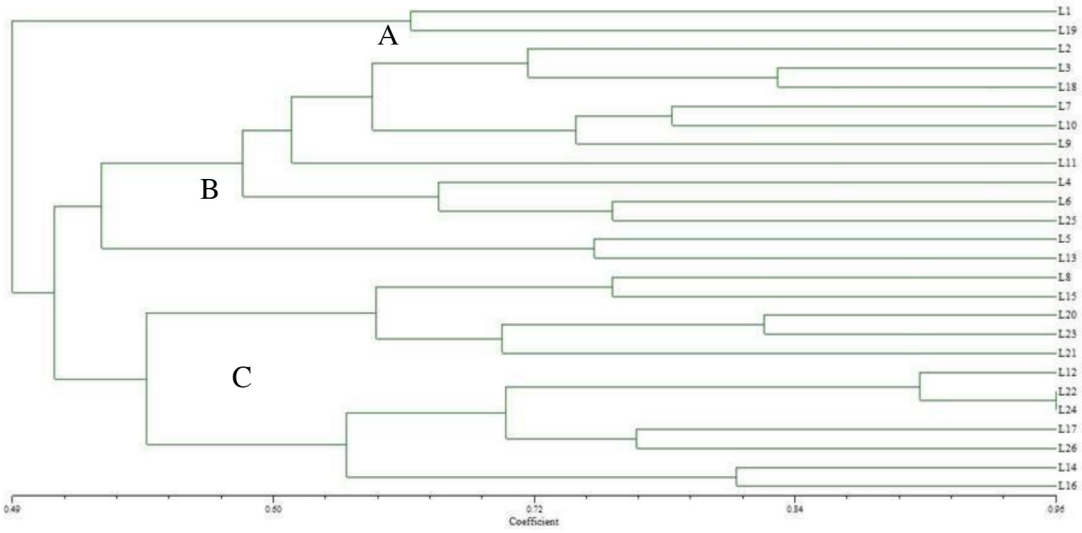

Figure 2. Dendrogram of 26 maize inbred lines constructed using UPGMA cluster analysis of genetic similarity values (Dice, 1945) obtained from SSR data

Remaining 12 genotypes formed cluster „C", which includes eight lines that belong to LSC germplasm (L12, L17, L20, L21, L22, L23, L24 and L26), three lines to BSSS (L8, L14 and L15) and one to local genetic basis (L16).
Using a small number of markers, it may be difficult to clearly evaluate the heterotic grouping due to the absence of some key loci for the differentiation of heterotic groups, indicating the use of larger number of markers.

Conclusions

Molecular marker analysis assigned the most of the inbred lines to their genetic background, substantiate them significant in evaluation of maize diversity. The variability detected using SSR markers could be useful in selecting best parental combinations in creating synthetic populations and new elite lines through breeding programs.

References

Ajmone Marsan, P., Castiglioni, P., Fusari, F., Kuiper, M., Motto, M. (1998): Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers. Theor Appl Genet 96: 219-227.

Bantte, K. and Prasanna, B.M. (2003): Simple sequence repeat polymorphism in Quality Protein Maize (QPM) lines. Euphytica, 129(3): 337-344.

Barcaccia, G., Pallottini, L., Parrini, P., Lucchin, M. (2006): A genetic linkage map of a flint maize (*Zea mays* var. *indurata* L.) Italian landraces using one way pseudo-test cross strategy and multilocus PCR based markers. Maydica, vol. 51, pp. 469-480.

Dao, A., Sanou, J., Mitchell, S.E., Gracen, V., Danquah, E.Y. (2014): Genetic diversity among INERA maize inbred lines with single nucleotide polymorphism (SNP) markers and their relationship with CIMMYT, IITA, and temperate lines. BMC Genetics. 2014;15:127. doi:10.1186/s12863-014-0127-2.

Dice, L.R. (1945): Measures of the amount of ecologic association between species. Ecology, 26: 297-302.

Dubreuil, P., Warburton, M., Chastanet, M., Hoisington, D., Charcosset, A. (2006): More on the introduction of temperate maize into Europe: large-scale bulk SSR genotyping and new historical elements. Maydica 51: 281-291.

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

Ho, J.C., Kresovich, S., Lamkey, K.R. (2005): Extent and Distribution of Genetic Variation in U.S. Maize: Historically Important Lines and their Open-Pollinated Dent and Flint Progenitors. Crop Sci. 45: 1891-1900.

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

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. PNAS 99: 6080-6084.

Morgante, M., Hanafey, M., Powell, W. (2002): Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. Nature Genetics, vol. 3, pp. 194-200.

Rohlf, F.J. (2000): NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 2.0 Exeter Software, Setaket, N.Y.

Sharma, L., Prasanna B.M., Ramesh B. (2010): Analysis of phenotypic and microsatellite-based diversity of maize landraces in India, especially from the North East Himalayan region. Genetica 138: 619-631.

Semagn, K., Magorokosho, C., Bindiganavile, S.V., Makumbi, D., Beyene, Y., Mugo, S., Prasanna, B.M., Warburton, M.L. (2012): Molecular characterization of diverse CIMMYT maize inbred lines from eastern and southern Africa using single nucleotide polymorphic markers. BMC Genomics, 13(1): 113. doi: 10.1186/1471-2164-13-113.

Spooner, D., Van Treuren, R., De Vicen, M.C. (2005): Molecular markers for genebank management. IPGRI Technical Bulletin No. 10, International Plant Genetic Resources Institute, Rome, Italy.

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.