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Research Article

Molecular characterisation of Maize [*Zea mays* (L.)] germplasm accessions

A. Subramanian* and N. Subbaraman

Centre for Plant Breeding and Genetics,
Tamil Nadu Agricultural University,
Coimbatore – 641 003,India.
*Email : subbi25@yahoo.com

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Abstract :
Genetic distances within crop species are measures of average genetic divergence between populations and it provides an index for parental selection. This study was undertaken to identify diverse inbreds from a group of 38 maize inbreds using 27 Random Amplified Polymorphic DNA (RAPD) primers. The data obtained was subjected to genetic diversity analysis by Sequential Agglomerative Hierarchical Non-overlapping (SAHN) clustering using Dice's coefficient and Unweighted Paired Group Method with Arithmetic Average (UPGMA). Genotypes were broadly classified into seven clusters. Similarity coefficient at molecular level was highest between UMI-852 and UMI-752. Based on the study, 11 inbreds were selected for use in heterosis breeding.

Key words : Maize, Diversity, Germplasm, RAPD

Introduction

Maize [*Zea mays* (L.)] is the third most important food crop next to Rice and Wheat. About 66 per cent of the maize produced in the world is used as feed, 17 per cent as food and as industrial product and the remaining is used as seed . It is used primarily as a food for human in third world countries whereas about 80 per cent of crop produced is fed to livestock in developed countries (CIMMYT, 2000). It has several industrial uses and is one of those crops whose advantageous features were gauged and exploited from time immemorial. Crop improvement in maize has passed through several phases. Selection as a method of crop breeding probably dates back to the beginning of domestication (Mukherjee, 1997). Other breeding methods for maize improvement are mass selection , ear to row selection , varietal hybridisation and development of hybrid maize. Among these methods, varietal hybridisation and development of hybrid maize, which takes advantage of the allogamous nature of the crop, has gained much importance. However, success of any projected experiment in this direction hinges on availability of genetic variability in base population. To understand usable variability, grouping or classification of genetic stocks based on suitable scale is quite imperative. The present study was undertaken to assess the genetic diversity at molecular level among 38 maize inbred lines.

Material and Methods

The study was carried out with 38 maize inbred lines (Table 1) maintained in Maize Breeding Unit, Department of Millets, and Centre for Plant Breeding and Genetics (CPBG), Tamil Nadu Agricultural University (TNAU). DNA was extracted by following the method described by Mc Couch *et al*. (1998) from fresh leaves of etiolated maize seedlings, germinated in roll towels. DNA samples were quantified in a fluorometer (DyNA-Quant-200, Hoeffer-Pharmacia) and the concentration adjusted to 10 ng/ µl. RAPD reaction was done in 200 µl thin walled Tarsons PCR tubes in a PCR–300 (Perkin Elmer) thermal cycler programmed with the following program: initial denaturation step for 2 minutes at 92°C, followed by 40 cycles of 1 minute at 92°C , 1 minute at 34°C and 2 min at 72° C and a extended run at 72°C for 10 minutes. The reaction mixtures were made up to 20 µl with 10 mM Tris HCl (pH-9.0), 50 mM KCl, 1.5 mm $MgCl_2$, 0.001 per cent gelatin, dATP, dCTP, dTTP and dGTP – 0.1 mM each , 1.0 pg of primer, 20 –30 ng of genomic DNA and 0.5 unit of *Taq* DNA polymerase (Bangalore Genei PVT, Ltd., Bangalore). A total of 27 arbitrary decamer oligonucleotide DNA primers (Table 2) from Operon technologies Inc., Alameda, CA, USA were employed for amplification. Amplified products were subjected to electrophoresis in 1.4 per cent agarose gel in 1X TBE buffer at 120V for 3.5 hours

using Hoefer super submarine electrophoresis unit (Pharmacia biotech). The electronic image of ethidium bromide stained gel was captured using Kodak digital science DC-120 zoom digital camera (Eastman Kodak co, Rochester, NY) and the gel was documented using electrophoresis documentation and analysis system (EDAS-120) 1D image analysis software (Scientific imaging systems, Eastman Kodak company, N.Y).

Clear and unambiguous bands were scored as the score 1 indicating their presence and 0 indicating their absence. The data matrix of binary codes thus obtained was subjected to genetic diversity analysis by Sequential Agglomerative Hierarchical Non-overlapping (SAHN) clustering using Dice's coefficient and Unweighted Paired Group Method with Arithmetic Average (UPGMA). The entire analysis was performed using NTSYS pc version 2.02 (Rohlf, 1998) software.

Results and discussion

Genetic distances within crop species are measures of average genetic divergence between populations. It helps to avoid redundancies in germplasm banks and provides an index for parental selection (Souza and Sorrels, 1989; Tsegaye *et al*., 1996). Choice of parents for developing base population is crucial in breeding of cultivars because, it largely predetermines the outcome of subsequent selection steps and affects the optimum allocation of resources in breeding programmes.

The genetic diversity of plants has been assessed more efficiently after the introduction of methods that reveal polymorphism directly at the biochemical and DNA levels. Markers based on isoenzymes (Lankey *et al*., 1997) and RFLP (Lee *et al*., 1986; Bernardo, 1994) were the first molecular markers used in maize breeding programs. More recently, markers based on polymerase chain reaction (PCR), such as RAPD have been used in analysis of genetic distance in many plant species by Irvin *et al*. (1998); Colombo *et al*., (2000) and several other workers. Comparison among the different types of markers has contributed to the selection of the most appropriate technique related to desired objectives. RAPD markers are commonly used because they are quick and simple to obtain, enabling genetic diversity analysis in several types of plant materials, such as natural populations, populations in breeding programs and germplasm collections (Ferreira and Grattapaglia, 1996). RAPD markers were superior than RFLP when compared to simplicity and cost involved (Dos Santos *et al*., 1994).

In the present experiment, 38 genotypes listed in Table 1 were subjected to RAPD analysis with 27 random primers. The bands produced by the primers ranged from 3 to 11. A total of 124 bands were produced with an average of 4.7 bands per primer. Among these bands, 101 bands were polymorphic (81.45 per cent). Both strong and weak bands were produced in the RAPD reactions. Since, weak bands result from low homology between the primer and the pairing site on the DNA strand (Thormann *et al*., 1994); they were disregarded for scoring to increase the precision. A binary matrix was generated based on the presence or absence of markers. The data matrix was converted to Dice's (1945) similarity matrix. A dendrogram was generated by SAHN clustering with UPGMA method (Fig. 1). At a truncation limit of 0.83, the genotypes could be broadly classified into seven clusters. The genotype UMI 433 was found to be a solitary member of one of the clusters and this indicated that it could have evolved from divergent genealogy. Similarity coefficient at molecular level was highest between UMI-852 and UMI-752 followed by UMI-720 and UMI-757. Most of the genotypes had high similarities with the exception of some pairs, which displayed divergence. The most plausible explanation for the comparatively low genetic distances between the inbreds is that they might probably have descended from a common ancestral population.

Bruel *et al.,* (2006) observed positive correlations between genetic divergences, detected by RAPD, and the averages determined in dialellic crossings, concerning the characteristics plant height, ear corn height, production, and seed weight. This corroborates with the hypothesis that genetic divergence in lines is directly related to hybrid performance, emphasizing the efficiency of RAPD markers in the prediction of hybrid behaviour. Leal *et al.,* (2010) reported that RAPD markers were efficient for determining genetic diversity among maize lines, dividing them into different heterotic groups, and therefore, it was useful in the selection of superior lines for crossings, thus reducing the number of crossings for evaluation in the field. Based on the diversity observed in the present study, 11 parents *viz.*, UMI-438, UMI-470, UMI-497, UMI-532, UMI-556, UMI-577, UMI-615, UMI-679, UMI-757, UMI-852 and UMI-946 were selected for hybridisation and further analysis of the heterotic pattern.

References

Bernardo R (1994) Prediction of single-cross performance using RFLPs and information from related hybrids. *Crop Science* **34**:20-25.

Bruel DC, Carpentieri-Pípolo V, Gerage AC, Fonseca Júnior NS, et al. (2006). Genetic distance estimated by RAPD markers and its relationship with hybrid performance in maize. *Pesq. Agropec. Bras.* **41**: 1491-1498.

CIMMYT, 2000. World maize facts and trends report. pp. 45-57.

Colombo C, Second G and Charrier A (2000) Diversity within American cassava germplasm based on

RAPD markers. *Genetics and Molecular Biology* **23**:189-199.

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

Dos Santos JB, Nienhuis J, Skroch P, Tivang J and Slocum MK (1994) Comparison of RAPD and RFLP genetic markers in determining genetic similarity among *Brassica oleracea* L.genotypes. *Theor. Appl. Genet.* **87**:909-915.

Ferreira ME and Grattapaglia D (1996) Introdução ao uso de marcadores moleculares em análise genética. 2nd ed. EMBRAPA-CENARGEN, Brasília, pp 121-130.

Irvin SV, Kaufusi P, Banks K, de la Penha R and Cho JJ (1998) Molecular characterization of taro (*Colocasia esculenta*) using RAPD markers. *Euphytica* **99**:183-189.

Lankey KR, Hallauer AR and Kahler AL (1997) Allelic difference at enzyme loci and hybrids performance in maize. *Journal of Heredity* .**78**:231-234.

Leal. A.A., Mangolin. C.A., Do Amaral Júnior. A.T, Gonçalves, L.S.A, Scapim C.A., Mott. A.S., Eloi. I.B.O., Cordovés. V and M.F.P. Da Silva.2010. Efficiency of RAPD versus SSR markers for determining genetic diversity among popcorn lines. *Genetics and Molecular Research*. **9** (1): 9-18

Lee, T.C., G.J. Shieh, C.L. Ho and J.R. Juang. 1986. Analysis of diallel sets of flint maize inbreds for combining ability and heterosis. *J. Agric. Res. (China),* **35**: 145-164

McCouch, S.R., G. Kochert, G., Z.H. Yu, Wang, Z.Y., G.S. Khush, W.R. Coffman and S.D. Tanksley. 1998. Molecular mapping of rice chromosomes. *Theor. Appl. Genet.*, **76**: 815-829

Mukherjee, B.K. 1997. Breeding procedures for cross pollinated crops: Maize. In: Plant Breeding (ed.) V.L. Chopra, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, pp. 199-212.

Rohlf, F.J. 1998. Numerical taxonomy and multivariate analysis system, version 2.02 i. 100. North Country Road, Setanket, New York.

Souza, E. and M.E. Sorrells. 1989. Pedigree analysis of North American oat cultivars released from 1951 to 1985. *Crop Sci*., **29**: 595-601.

Thormann CE, Ferreira ME, Camargo LEA, Tivang JG and Osborn TC (1994) Comparison of RFLP and RAPD markers for estimating genetic relationships within and among cruciferous species. *Theor. Appl. Genet.***88**:973-980.

Tsegaye, S., T. Tesemma and G. Belay. 1996. Relationships among tetraploid wheat (*Triticum turgidum* L.) land race populations revealed by isozyme markes and agronomic traits. *Theor. Appl. Genet*., **93**: 600-605.

Table 1. List of maize genotypes used in diversity analysis and their parentage

Accession No.	Parentage	Source
UMI – 433	UMI-40 x UMI –101	Coimbatore
UMI – 438	EH – 450879	DMR,Delhi
UMI – 456	ALR –4	Coimbatore
UMI – 458	ALR –6	Coimbatore
UMI – 465	KLD –7	DMR, Delhi
UMI – 470	K1	Kovilpatti
UMI – 479	EM – 456979	DMR, Delhi
UMI – 480	EH – 459379	DMR, Delhi
UMI – 487	Dho –79	Bihar
UMI – 492	Not known	Bihar
UMI – 497	Not known	Bihar
UMI – 510	T-433 / 980 K	Kanpur
UMI – 524	(Sarhad x Suwan –1) x Suwan –1	Kanpur
UMI – 532	UMI –79	Coimbatore
UMI – 536	Hawaii Sugar	Hawaii
UMI – 540	UMI – 14 x UMI –12	Coimbatore
UMI – 550	UMI – 115 x UMI –3	Coimbatore
UMI – 551	UMI – 126 x UMI – 80	Coimbatore
UMI – 556	UMI – 140 x UMI –126	Coimbatore
UMI – 561	UMI –269 x UMI – 146	Coimbatore
UMI – 577	M-13	Coimbatore
UMI – 615	(Sakthi x CM – 202) x C. Rattan x CM .111	Coimbatore
UMI – 677	UMI – 165 x UMI –150	Coimbatore
UMI – 679	Not known	Kanpur
UMI – 694	Not known	Kanpur
UMI – 696	Not known	Kanpur
UMI – 720	7292 /2 (W)	DMR, Delhi
UMI – 752	EH – 4003	DMR, Delhi
UMI – 757	UMC –5	Coimbatore
UMI – 761	Deccan 103	Hyderabad
UMI – 763	Not known	Bihar
UMI – 773	Euchan No. 5	South Korea
UMI – 803	Bs 11 (FR) C6	DMR, Delhi
UMI – 842	8824 ME x 2451	DMR, Delhi
UMI – 852	RICA 8926 Mex x 2474	DMR, Delhi
UMI – 886	Not known	DMR, Delhi
UMI – 926	Not known	DMR, Delhi
UMI – 946	Hyd 92 R / 1040	Hyderabad

Table 2. List of RAPD primers used for characterization of maize genotypes

S.No.	Primer code	Sequence 5' to 3'	S.No.	Primer code	Sequence 5' to 3'
1	OPAK –02	CCATCGGAGG	15	OPAM –10	CAGACCGACC
2	OPAK – 04	AGGGTCGGTC	16	OPAM – 11	AGATGCGCGG
3	OPAK – 05	GATGGCAGTC	17	OPAM –13	CACGGCACAA
4	OPAK – 07	CTTGGGGGAC	18	OPAM –16	TGGCGGTTTG
5	OPAK – 08	CCGAAGGGTG	19	OPAB – 01	CCGTCGGTAG
6	OPAK– 09	AGGTCGGCGT	20	OPAB – 03	TGGCGCACAC
7	OPAK–16	CTGCGTGCTC	21	OPAB – 04	GGCACGCGTT
8	OPAK – 17	CAGCGGTCAC	22	OPAB – 09	GGGCGACTAC
9	OPAK – 19	TCGCAGCGAG	23	OPAB –13	CCTACCGTGG
10	OPAK – 20	TGATGGCGTC	24	OPAB –18	CTGGCGTGTC
11	OPAM – 03	CTTCCCTGTG	25	OPAL – 01	TGTGACGAGG
12	OPAM –04	GAGGGACCTC	26	OPAW – 02	TCGCAGGTTC
13	OPAM –05	GGGCTATGCC	27	OPAW – 05	CTGCTTCGAG
14	OPAM –07	AACCGCGGCA			

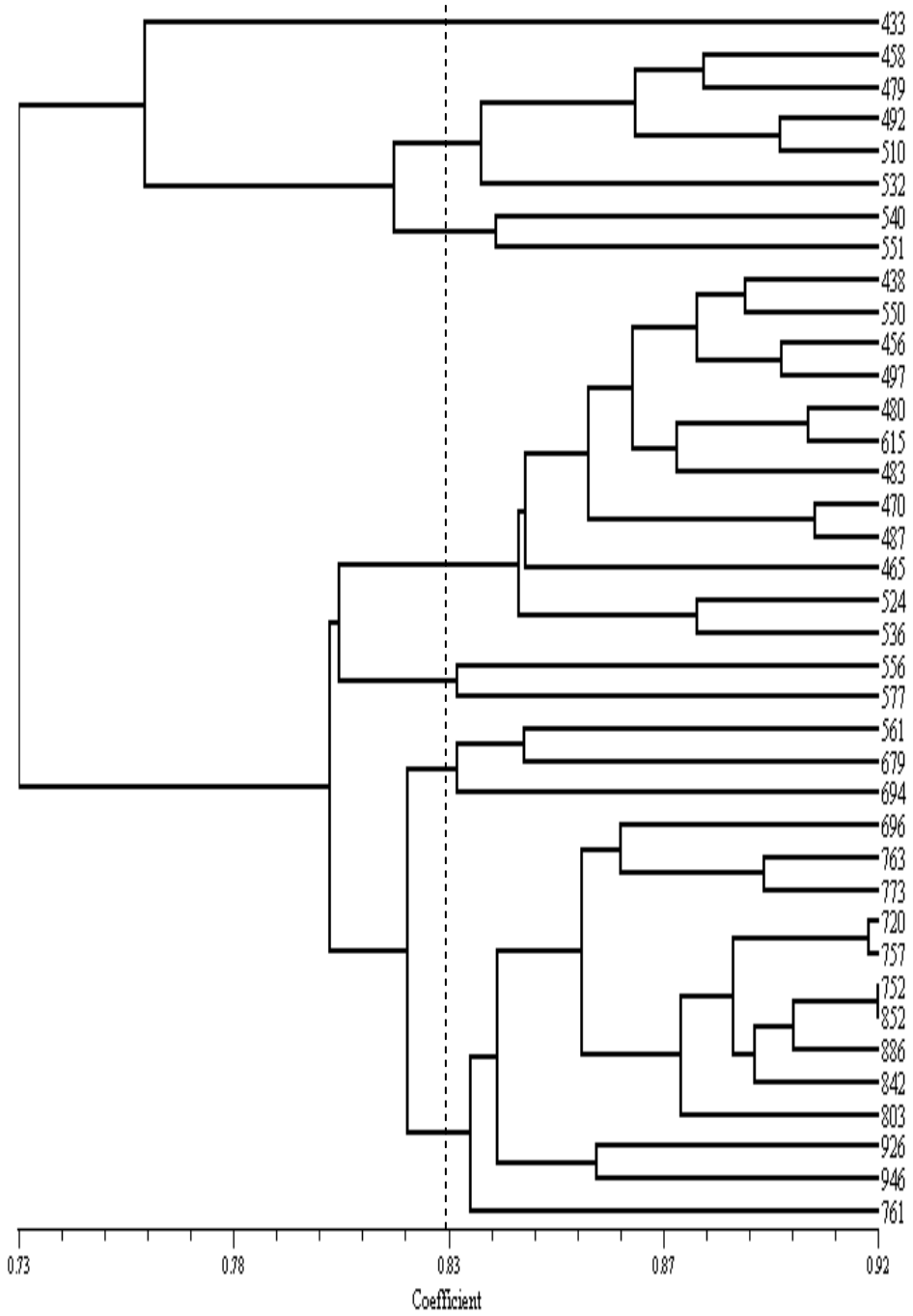

Figure 1. Clustering of maize genotypes based on RAPD markers