Plant Archives Volume 20 No. 1, 2020 pp.708-712 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ASSESSMENT OF MOLECULAR DIVERSITY IN MAIZE INBREDS DERIVED FROM DIFFERENT BASE POPULATIONS.

B. Bharathi Veeramani[1] And M. Prakash[2]*

[1]Foliage Crop Solutions Private Limited, R&D Farm, Attur-636 102 (Tamilnadu) India.
[2*]Depart. of Genetics and Plant Breeding, Faculty of Agri., Annamalai University, Annamalainagar-608002 (TN)

Abstract

Maize is one of the most widely grown crops in the world and exceeds wheat and rice in production. Because of its open-pollinated nature, there is tremendous genetic diversity in the maize genome. The main objective of this experiment was to assess the genetic diversity of 144 maize inbreds using Mahalanobis D^2 analysis and molecular diversity among 10 selected inbreds through screening of RAPD markers. In the present investigation, 144 maize inbreds derived from four different base populations *i.e.*, advanced generations of single-cross hybrids, three-way cross hybrids, hybrid mixtures and composites, were tested for genetic diversity using Mahalanobis D^2 analysis based on 14 morphological characters. Ten selected parents were subjected to molecular diversity analysis through screening of RAPD markers. The grouping of inbreds into 10 clusters revealed that the inbreds derived from composites were more divergent followed by hybrid mixture base population. The clustering pattern observed in RAPD analysis was similar to the pattern in morphological diversity observed through Mahalanobis D^2 analysis and it also confirmed the diverse nature of the composite and the hybrid mixture base population. The results of the present study showed that composites, followed by the hybrid mixture are the best base populations for deriving genetically diverse inbreds in maize.

Key words: Inbred, Maize, Molecular diversity, Yield.

Introduction

Maize (*Zea mays* L.; 2n = 2x = 20) is one of the most important cereal crops in the world, providing food, feed and bioenergy (Linehan *et al*., 2013, Ranum *et al*., 2014), it is cultivated for its nutritive and economical importance. In India, maize contributes with a productivity of 2.5 mt hectare^{-1}, which is much less than global productivity. Despite its lower productivity, there is an increasing demand for its uses including poultry feed, pharmaceuticals, cosmetics, corn oil, protein and al-coholic beverages etc.

After realizing the advantages of single-cross hybrids in maize improvement, the thrust in present breeding has been in this direction. With this orientation towards breeding of single cross hybrids, it has become imperative to use diverse source populations for deriving inbreds that are not only divergent but whose crosses are heterotic and productive. The superiority of inbreds directly depends on the presence of desirable genes and gene complexes in the base population. Besides single cross hybrids, elite line synthetics/composites, F_2 populations, backcross populations, pools and experimental varieties are also used as source materials (Kaul *et al*., 2011). Maize breeders currently exploit genetically narrow base populations as a resource by deriving the recombinant lines from F_2 populations of commercial single cross hybrids (Chandel *et al*., 2014). Though widely followed by maize breeders, studies of the genetic divergence and usefulness of inbreds derived from such narrow-base populations are very limited.

Singh *et al*., (2005) estimated genetic divergence in 23 genotypes of maize using D^2 analysis and concluded that genotypes included in the diverse clusters can be used as promising parents for hybridization in order to obtain better segregants and thus high heterotic response in maize. Of several techniques available for assessing genetic diversity using molecular techniques, the most common molecular markers used to assess genetic diversity in maize include restriction fragment length polymorphism (RFLP), random am-plified polymorphic (RAPD), microsatellite or simple sequence repeats (SSRs), amplified fragment length polymorphism (AFLP)

***Author for correspondence :** E-mail: geeth_prakash@yahoo.co.in.

Table 1: Details of 144 maize inbreds used for diversity analysis.

S. No	Source population	No. of inbreds	Inbred accession numbers
1.	Advanced generations of Single-cross hybrids.		
	Chola	7	FI-1, FI-2, FI-3, FI-4, FI-5, FI-6, FI-7
	Ashoka	4	FI-29, FI-30, FI-31, FI-32
	SCH 55	20	FI-33, FI-34, FI-35, FI-36, FI-37, FI-38, FI-39, FI-40, FI-41, FI-42, FI-43, FI-44, FI-45, FI-46, FI-47, FI-48, FI-49, FI-50, FI-51, FI-52
2.	Advanced generations of Three-way cross hybrids.		
	C555	7	FI-25, FI-26, FI-27, FI-28
	TWH 001	17	FI-8, FI-9, FI-10, FI-11, FI-12, FI-13, FI-14, FI-15, FI-16, FI-17, FI-18, FI-19, FI-20, FI-21, FI-22, FI- 23, FI-24
3	Hybrid mixtures	62	FI-53, FI-54, FI-55, FI-56, FI-57, FI-58, FI-59, FI-60, FI-61, FI-62, FI-63, FI-64, FI-65, FI-66, FI-67, FI-68, FI-69, FI-70, FI-71, FI-72, FI-73, FI-74, FI-75, FI-76, FI-77, FI-78, FI-79, FI-80, FI-81, FI-82, FI-83, FI-84, FI-85, FI-86, FI-87, FI-88, FI-89, FI-90, FI-91, FI-92, FI-93, FI-94, FI-95, FI-96, FI-97, FI-98, FI-99, FI-100, FI-101, FI-102, FI-103, FI-104, FI-105, FI-106, FI-107, FI-108, FI-109, FI-110, FI-111, FI-112, FI-113, FI-114
4	Composite - CMP001	30	FI-115, FI-116, FI-117, FI-118, FI-119, FI-120, FI-121, FI-122, FI-123, FI-124, FI-125, FI-126, FI-127, FI-128, FI-129, FI-130, FI-131, FI-132, FI-133, FI-134, FI-135, FI-136, FI-137, FI-138, FI-139, FI-140, FI-141, FI-142, FI-143, FI-144

and single nucleotide polymor-phism (SNP) (Molin *et al*., 2013). Among the different markers, RAPD and SSR markers are of main interest since they are hyper variable and present throughout the genome (Anandan *et al*., 2017). Randomly Amplified Polymorphic DNAs (RAPD) have been widely utilized by several researchers (Pham *et al*., 2010). The advantages of RAPD include ease and rapidity of analysis, the use of a general set of universal random primers for DNA amplification and their requirement for minimal substrate DNA. RAPD can efficiently generate both randomly dispersed markers as well as markers linked to specific genes (Bauer *et al*., 2005). Studies on genetic diversity analysis among maize lines based on morphological and molecular data was reported by Thirunavukkarasu *et al*., (2013). In the current study, we assessed the genetic diversity of 144 maize inbreds using Mahalanobis D^2 analysis and molecular diversity among 10 selected inbreds through RAPD analysis.

Material and Methods

The material for the study was comprised of 144 inbred lines originated from different base populations of unknown pedigree *i.e.*, advanced generations of single-cross hybrids, three-way cross hybrids, hybrid mixtures and composites, their accession number are given in table 1. The experiment was laid out in a 12 × 12 simple lattice design with three replications at the R&D Farm, Foliage Crop Solutions, Private Limited, Attur, Tamil Nadu, India in two seasons and the mean value for the traits was recorded. Data were recorded on 14 characters *i.e.*, days to 50% tasseling, days to 50% silking, anthesis-silking interval, plant height (cm), ear height (cm), tassel length (cm), number of tassel branches, number of kernel rows, number of kernels / row, number of kernels / ear, days to maturity, hundred-seed weight (g), shelling percentage and grain yield / plant (g). The statistical analysis was carried out using mean values of ten plants over three replications for each character.

Ten inbreds were studied for molecular diversity through Random Amplified Polymorphic DNA (RAPD) analysis (Table 2). The 10 inbreds were grown in pots under protected cultivation to raise healthy seedlings. Twelve-day old young leaves from the seedlings were utilized for DNA extraction, done as per the modified CTAB proce-dure of Irfan *et al*., (2013). Twenty decamer primers of the OPA series (Operon Technologies, USA) were used to amplify two randomly chosen accessions included in the study. Out of these, 15 primers which

Table 2: Inbred lines selected for RAPD analysis.

S. No	Inbred no	Source population	GCA for grain yield
Females			
1	FI-7	Advanced generation of single-cross hybrids	0.25
2	FI-24	Advanced generation of three-way cross hybrids	0.45
3	FI-54	Hybrid Mixture	2.06
4	FI-101	Hybrid Mixture	-0.38
5	FI-114	Hybrid Mixture	0.71
6	FI-127	Composite	-0.09
7	FI-141	Composite	0.32
8	FI-144	Composite	-0.02
Males			
1	FI-109	Hybrid Mixture	0.56
2	FI-142	Composite	0.85

Table 3: Selected RAPD primers used in PCR amplification.

S. No	Oligo Name	DNA sequence (5'-3')
1	A-01	CAGGCCCTTC
2	A-02	TGCCGAGCTG
3	A-04	AATCGGGCTG
4	A-05	AGGGGTCTTG
5	A-06	GGTCCCTGAC
6	A-07	GAAACGGGTG
7	A-08	GTGACGTAGG
8	A-09	GGGTAACGCC
9	A-11	CAATCGCCGT
10	A-12	TCGGCGATAG
11	A-13	CAGCACCCAG
12	A-16	AGCCAGCGAA
13	A-17	GACCGCTTGT
14	A-18	AGGTGACCGT
15	A-20	GTTGCGATCC

gave satisfactory amplification and band resolution were chosen for further study, the list of selected primers is presented in table 3.

PCR amplification was carried out using Master Thermal Cycler 5331 - Eppendorf model 2.30, 31-09, Germany. PCR conditions were as follows:

1. Initial denaturation at 95°C for 5 minutes.

2. Forty cycles of denaturation at 94°C for one minute, primer annealing at 36°C for 1 minute and primer extension at 72°C for 2 minutes.

3. Final 8 minute extension at 72°C for 10 minutes.

After completion of PCR amplification, 1.0 µl of 6x loading dye was added to each PCR tube. A 0.8% Agarose gel in 1X TBE buffer with 6µl Ethidium Bromide per 100ml of gel volume was prepared. Electrophoresis was carried out at 90V for 1.5 hours, followed by 70V for 2 hours till the Bromophenol Blue trevelled less than two third the length of the gel. The resolved amplification products were visualized under UV light on UV-Trans illuminator. After completion of PCR amplification, electrophoresis was carried out and resolved amplification products were visualized under UV light on UV-Trans illuminator. The amplification products were scored across the lanes comparing their respective molecular weights. The presence of band was scored as "1" and the absence of a band as "0". Jaccard's similarity metric, J, was used to calculate similarity between pairs of accessions, utilizing the formula:

$$J = \frac{a}{(n\text{-}d)}$$

where, n = Total sample size.

The genetic associations between accessions were studied by calculating the Jaccard's similarity coefficient of pair-wise comparisons based on the proportion of shared bands produced by the primers. The similarity matrix was subjected to cluster analysis by unweighted pair group method for arithmetic mean (UPGMA) and a dendrogram was generated. The

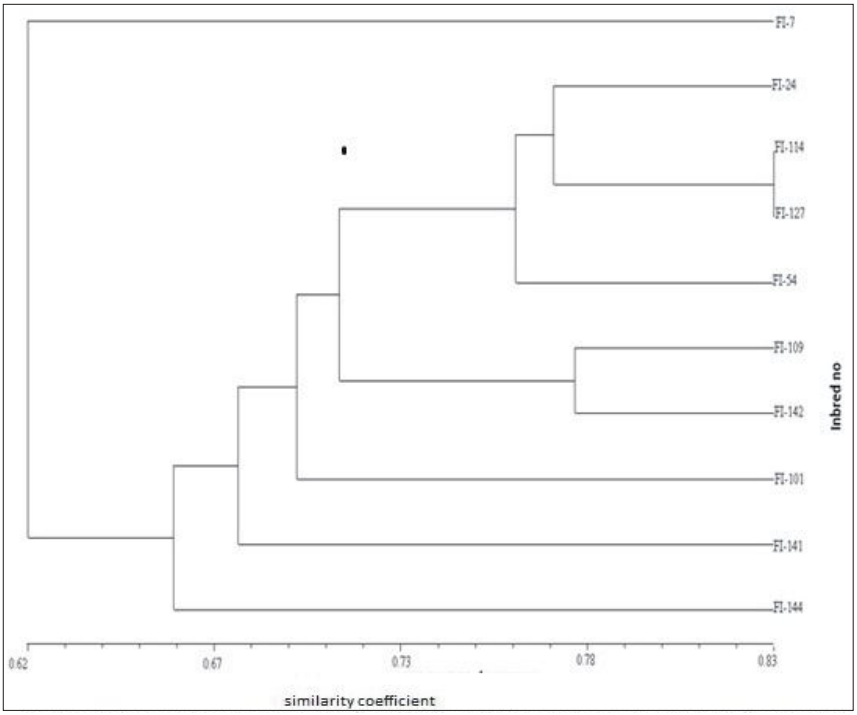


Fig. 1: UPGMA dendrogram showing the genetic relationship among 10 accessions of maize obtained from RAPD data.

Table 4: Jaccard's similarity coefficient between ten maize inbreds selected.

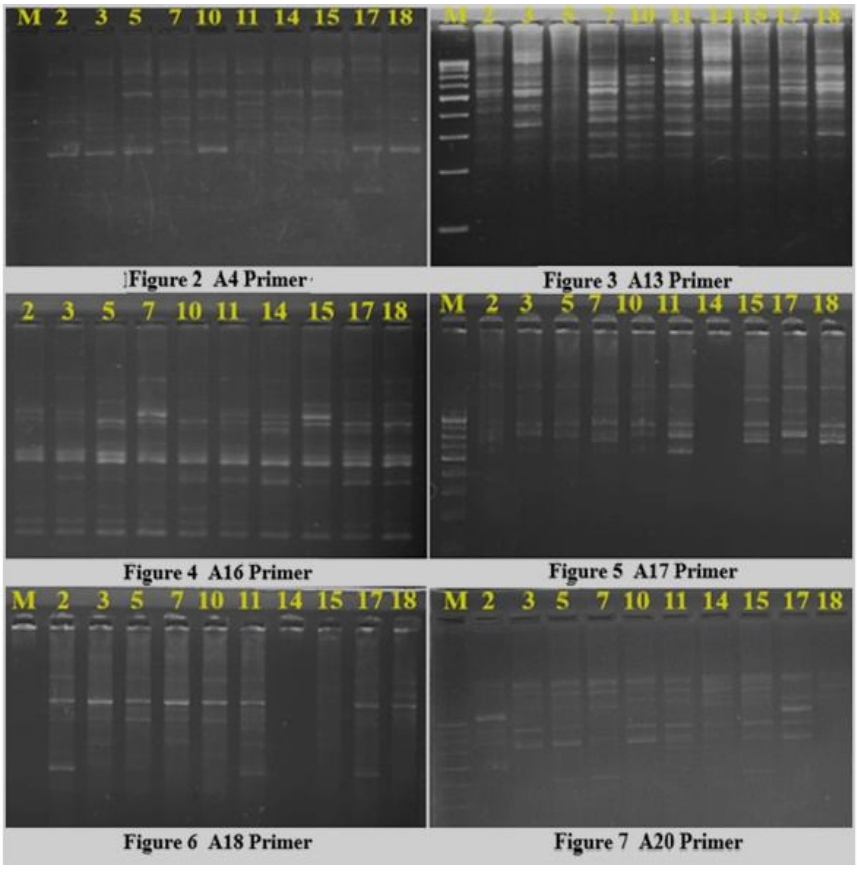
Inbreds	FI-7	FI-24	FI-54	FI-101	FI-114	FI-127	FI-141	FI-144	FI-109	FI-142
FI-7	1.00									
FI-24	0.71	1.00								
FI-54	0.69	0.76	1.00							
FI-101	0.65	0.67	0.68	1.00						
FI-114	0.67	0.78	0.79	0.72	1.00					
FI-127	0.60	0.76	0.73	0.73	0.83	1.00				
FI-141	0.53	0.69	0.62	0.63	0.71	0.74	1.00			
FI-144	0.54	0.62	0.68	0.60	0.70	0.71	0.64	1.00		
FI-109	0.57	0.68	0.67	0.68	0.74	0.72	0.67	0.63	1.00	
FI-142	0.61	0.68	0.67	0.70	0.76	0.75	0.69	0.69	0.78	1.00

computations were performed using the program NTSYS-PC version 1.8.

Results and Discussion

Assessment of genetic diversity among the germplasm within and between species has gained several importance in crop improvement program. Hence, estimates of genetic diversity can be utilised to identify cultivars, screen parents for hybridization, to map qualitative and quantitative traits and also in marker-assisted selection. DNA markers such as RAPD, RFLP, ISSR, AFLP and SSR have been extensively used to elucidate the genetic relationship and molecular diversity among different crop species (Singh *et al*., 2014; Sheikh *et al*., 2015; Ahlawat *et al*., 2016; Leonte *et al*., 2016 and Mohammad *et al*., 2017).

In the present study, Jaccard's similarity coefficient varied from 0.53 to 0.83 (Table 4), indicating the presence of substantial genetic diversity among the chosen inbred lines. The lowest similarity coefficients observed between inbred line FI-7 with the inbred lines FI-141, FI-144 and FI-109 indicate that FI-7 is genetically diverse from other three inbreds. Inbred line FI-7 was derived from single-cross hybrid, FI-109 was derived from hybrid mixture and FI-141 and FI-144 originated from composite base population. The lowest similarity coefficient of 0.83 was between inbred lines FI-114 and FI-127, both coming from composite base population. These observations confirm that inbreds derived from different base populations show high genetic distance and also that the genetic distance between inbreds derived from same base population is low.



Using the Unweighted Pair Group Method with Arithmetic mean (UPGMA), the 10 accessions were grouped into six different clusters (Fig. 1). The four inbreds derived from composite base population (FI-127, FI-141, FI-142 and FI-144) were grouped into four different clusters, indicating the relatively diverse nature of these inbreds. Four inbreds derived from hybrid mixture base population (FI-54, FI-101, FI-109 and FI-114) were grouped into three different clusters. The inbred line FI-24 derived from three-way cross hybrid was grouped into cluster II which also had inbreds from hybrid mixture and composite base population. The inbred derived from single-cross hybrid FI-7 formed a separate mono-cluster.

In the present study, ten inbred lines were analysed for their diversity using RAPD marker. The pattern in which these 10 inbreds from the four different base populations were grouped in molecular diversity analysis was more or less similar to the grouping pattern of morphological diversity analysis using Mahalanobis D^2 analysis. This shows that both approaches are effective for assessing genetic divergence among inbred maize lines. Beyene *et al.*, (2005) found significant correlation between diversity among 15 morphological traits and molecular genetic analysis using AFLP and SSR markers for Ethiopian maize. Information about the genetic diversity and re-lationships among diverse genetic resources is valuable in crop improvement programmes and for strategic conservation of genetic resources (Abera *et al*., 2012; Kage *et al.*, 2013; Wu *et al*., 2014). In our context, the results of the present study regarding molecular diversity using RAPD marker showed that composite, followed by hybrid mixture, are the better base populations for deriving genetically diverse inbreds in Indian maize and the present finding will be useful for designing selective breeding and hybridization strategies (Fig. 2).

Acknowledgement

The support provided by M/S Foliage Crop Solutions Private Limited, Chennai, India to carry out the research work is greatfully acknowledged.

References

Abera, W., H. Shimelis, J. Derera, W. Mosisa, J. Danson and M.D. laing (2012). Genetic interrelationships among medium to late maturing tropical maize in-bred lines using selected SSR markers. *Euphytica.*, **10:** 1-9.

Ahlawat S.P., R.V. Kumar, R. Ranjan, S.K. Pandey, D.C. Joshi and S.K. Dhyani (2016). Morphological and molecular level of genetic diversity among Pongamia (*Pongamia pinnata* (L.) Pierre) accessions. *Indian Journal of Biotechnology*, **15:** 85-94.

Anandan R., T. Deenathayalan, M. Prakash, B. Sunilkumar and G. Sathiyanarayanan (2017). Assessment of genetic diversity among Sesame (*Sesamum indicum* L.) germplasm as revealed by RAPD and SSR markers. *Indian Journal of Biochemistry & Biophysics.*, **55:** 143-150.

Bauer, I., M.S. Drinic and M.F. Konstanti (2005). Genetic characterization of early maturing maize hybrids (*Zea mays* L.) obtained by protein and RAPD markers. *Genetika.*, **37(3):** 235-243.

Beyene, Y., A.M. Botha and A.M. Alexander (2005). A comparative study of molecular and morphological methods of describing genetic relationships in traditional Ethiopian highland maize. *Afr. J. Biotechnol*., **4(7):** 586-595.

Chandel, U., B.S. Mankotia and K.S. Thakur (2014). Assessment of recombinant lines of maize hybrids for inbred development. *Bangl. J. Bot.*, **43(3):** 363-366.

Irfan, M., Z.T. Ting, W. Yang, Z. Chunyu, M. Qing, Z. Lijun and L. Feng (2013). Modification of CTAB protocol for maize genomic DNA extraction. *Res. J. Biotech.*, **81:** 41-45.

Kaul, J., R.S. Dass, R. Saikumar, P. Bansal, O. Prakash, U. Nara and B. Ahmad (2011). Single cross hybrid breeding technology in maize. Directorate of Maize Research, Technical Bulletin No: 2011.

Kage, U., D.M.L. Malakannavar and P. Ganagashet-ty (2013). Genetic diversity studies in newly derived inbred lines of maize (*Zea mays* L.). *Mol. Plant Breed*., **4:** 77-83.

Leonte, C. and M.C. Arsene (2016). Assessment of genetic diversity of some *Brassica napus* L. cultivars revealed by molecular markers and phenotypic evaluation. *Indian Journal of Biotechnology*, **15:** 568-575.

Linehan, V., S. Thorpe, C. Gunning-Trant, E. Heyhoe, K. Harle, M. Hormis and K. Harris-Adams (2013). Global food production and prices to 2050: Scenario analysis under policy assumptions, Australian Bureau of Agricultural and Resource Economics and Sciences Con-ference paper 13.6, Canberra.

Mohammad N., S. Mahesh, Y.K. Jain and S.A. Ansari (2017). Effect of discrete (individual) and mixed (bulk) genomic DNA on genetic diversity estimates and population structure in Teak (*Tectona grandis* L. f.). *Indian Journal of Experimental Biology.*, **55:** 44-48.

Molin, D., C.J. Coelho, D.S. Máximo, F.S. Ferreira, J.R. Gardin-go and R.R. Matiello (2013). Genetic diversity in the germplasm of tropical maize landraces deter-mined using molecular markers. *Genet. Mol. Res.*, **12:** 99-114.

Pham, T.D., M. Geleta, T.M. Bui, T.C. Bui, A. Merker and A.S. Carlsson (2010). Comparative analysis of genetic diversity of sesame (*Sesamum indicum* L.) from Vietnam and Cambodia using agro-morphological and molecular markers. *Hereditas.*, **148:** 28.

Ranum, P., J. P. Pena-Rosas and M.N. Garcia-Casal (2014). Global maize production, utilization and consumption. *Ann. NY Acad. Sci.*, **1312:** 105-112.

Sheikh, W., S. Acharya, J.B. Patel, S.R. Kalaskar, A.S. Shinde and K.A. Patel (2015). Genetic fingerprinting of A and R lines of pigeonpea (*Cajanus cajan* (L.) Millsp.) using RAPD and SSR markers. *Indian J. of Biotechnology.*, **14:** 328-333.

Singh, A., H.K. Dikshit, N. Jain, D. Singh and R.N. Yadav (2014). Efficiency of SSR, ISSR and RAPD markers in molecular characterization of mungbean and other *Vigna* species. *Indian Journal of Biotechnology.*, **13:** 81-88.

Singh, P., S. Dass, V.K. Dwivedi, Y. Kumar and O. Sangwan (2005). Genetic divergence studies in maize (*Zea mays* L.). *Annals of Agri. Bio. Research.*, **10(1):** 43-56.

Thirunavukkarasu, N., F. Hossain, K. Shiriga, S. Mittal, K. Arora, A. Rathore, S. Mohan, T. Shah, R. Sharma and P.M. Namratha (2013). Unraveling the genetic architecture of subtropical maize (*Zea mays* L.) lines to assess their utility in breeding programs. *B.M.C. Genomics.*, **14:** 877.

Wu, K., M. Yang, H. Liu, Y. Tao, J. Mei and Y. Zhao (2014). Genetic analysis and molecular characterization of Chinese sesame (*Sesamum indicum* L.) cultivars using insertion-deletion (indel) and simple sequence repeat (SSR) markers. *B.M.C. Genomics.*, **35:** 1-15.