

Original Research Article

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## Diversity Analysis of Maize Inbred Lines

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### ABSTRACT

#### Keywords

Morphological diversity, Genetic diversity, SSR markers, Cluster, Mahalanobis distance

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A set of 10 lines and three testers of maize were grown under optimal and waterlogging conditions, to study the diversity among them. 13 morphological traits were studied and D<sup>2</sup> analysis is done. The genotypes were grouped into four clusters by Tocher's method based on Mahalanobis Euclidean distance. Genetic diversity based on SSR markers using 25 markers was also studied. 15 SSR markers with PIC value ranging from 0.13 to 0.32 exhibited polymorphism and the genotypes were grouped into two clusters based on Dice similarity coefficients. The molecular study, did not support the morphological diversity, which can be attributed to the insufficient SSR markers used in the study. However, the trueness of the hybrids can be assessed using the SSR markers. From the diversity study, diverse parents from different cluster can be selected for hybridization programme.

### Introduction

Maize (*Zea mays* L.) is one of the most important food crops that represents as the staple food of many developed and developing countries. Besides using as food grain, maize is also used as animal feed, fodder and in industries for various purposes, chiefly for starch. It can also be processed and

value-added to various processed products. It is greatly preferred by the farmers for its versatility and good yield potential. Therefore maize is considered as a potential crop for crop improvement works. Being predominantly cultivated as a *kharif* crop in India, maize faces drought and waterlogging stresses during *kharif*. Waterlogging stress affects the reproductive stage of the crop and

results in yield loss. However, tolerance to waterlogging is found to be present in the crop and improvement of maize for waterlogging tolerance could be a good scope. Selection of diverse parents is essential for hybridization programme. The objective of the present study is to reveal the diversity in the inbred lines and testers based on morphological traits and simple sequence repeat markers.

## Materials and Methods

The details of the inbred lines and testers used in the investigation are presented in Table 1 and 2 respectively.

### Morphological diversity analysis

The 13 genotypes were evaluated sown in *kharif*, 2019 in randomized block design with two replications under optimal and waterlogging environments. The waterlogging treatment is given to the waterlogging environment by maintaining stagnant water for seven days, first at knee-high stage and second at tasseling stage. The morphological traits including pre-harvest traits namely, days to 50 per cent tasseling (day), days to 50 per cent silking (day), anthesis – silking interval (day), days to 75 per cent brown husk (day), plant height (cm) and ear height (cm) and post-harvest traits cob length (cm), cob girth (cm), number of rows per cob, number of grains per row, test weight (g), shelling percentage (%) and grain yield at 15% moisture ( $\text{Kg ha}^{-1}$ ) were recorded for both environments.

The genetic divergence between genotypes was estimated using Mahalanobis's  $D^2$  statistic (1936). The genetic distance between any two populations was computed using the formula

$$D^2_p = d^1 S^{-1} d$$

Where,

$D^2_p$  = Square of distance considering 'p' variables

d = Vector observed differences of the mean values of all the characters ( $x_i 1 - x_i 2$ )

$S^{-1}$  = inverse of variance and covariance matrix

The lines and testers are clustered into different groups following Tocher's method (Rao, 1952). The intra and inter-cluster distance were calculated as described by Singh and Chaudhary (1977).

### Genomic DNA isolation

Genomic DNA was extracted from fresh leaf tissue of each genotype using modified Dellaporta method of DNA extraction as described by Dellaporta *et al.*, (1983). For this, Two hundred milligrams of leaf samples were cut into small bits with the help of sterile scissors and grinded with 500  $\mu\text{l}$  of pre-heated extraction buffer in a pestle and mortar and taken into eppendorf (1.5 ml) tube. The tube is incubated at 65°C in water bath for 45 minutes with occasional mixing. Then the tubes were removed from the water bath and 200  $\mu\text{l}$  of 5 M potassium acetate was added and mixed by inversion for 15 minutes. It was then kept in -20 °C for 10 minutes and then centrifuge at 10,000 rpm for 10 minutes at room temperature. Then the supernatant was transferred to a fresh Eppendorf tube. Equal volume of chilled isopropanol was added to it and mixed gently by inversion and then kept in -20°C deep freezer for 15 minutes. Then it was centrifuged at 10,000 rpm for 10 minutes at room temperature. Supernatant was discarded and DNA pellet was washed twice with 70% ethanol (200  $\mu\text{l}$ ) and then final wash with 100% ethanol. After each washing, tubes were centrifuged at 3000 rpm for 5 minutes at room temperature. Then the alcohol was decanted and DNA pellet was air dried, after which it was dissolved in 100  $\mu\text{l}$

of 10mM Tris and Stored at -20°C. The quality of DNA was checked by agarose gel electrophoresis. After quantification, the DNA was diluted to a concentration of 40 ng/μl for SSR analysis.

### **PCR amplification through SSR primers**

The Diluted DNA samples were subjected to PCR amplification, using the selected SSR primers in automated thermal cycler. 10μL reaction volume contained 2 μL (100 ng) DNA, 1μL 10 X PCR buffer [MgCl<sub>2</sub>], 0.2μL 0.2mM dNTPs, 0.2μL of 0.2 μM forward primer, 0.2μL of 0.2 μM reverse primer, 0.5 μL *Taq* DNA Polymerase enzyme (1U/μL) and 5.9μL autoclaved molecular biology grade water. Reaction condition was programmed as initial hold at 94°C for 4 min followed by 25 cycles of denaturation at 94°C for 30s, annealing of primer at 55°C for 40s and extension at 72°C for 40s. Further extension was allowed at 72°C for 5 min. On completion of reaction 2 μL gel loading buffer was added.

### **Scoring and data analysis**

The clear and distinct bands amplified by SSR primers were scored visually for their presence (1) or absence (0) of the corresponding band among the 13 maize genotypes. The polymorphic information content (PIC) values of each primer were calculated using Power Marker 3.5.

The binary data scored was used to construct a dendrogram. Similarity matrix was generated using the SIMQUAL programme of NTSYS-pc software, version 2.02 (Rohlf, 1998). The similarity coefficients were used for cluster analysis and dendrogram was constructed by the Unweighted Pair-Group method (UPGMA) (Sneath and Sokal, 1973).

## **Results and Discussion**

### **Morphological diversity**

On the basis of recorded observations, mean and range for the traits were presented in Table 3. The analysis of variance revealed that the genotypes are significantly different for all the traits studied under both environments. The environment was found to be significant for all the traits except days to 50 per cent tasseling and days to 50 per cent silking. From the D<sup>2</sup> analysis, the genotypes were grouped into four clusters. The dendrogram obtained on the basis of morphological diversity is presented in fig. 1. Cluster I consists of seven genotypes namely L<sub>5</sub>, T<sub>1</sub>, L<sub>2</sub>, L<sub>7</sub>, L<sub>1</sub>, L<sub>9</sub> and L<sub>8</sub>, cluster II consists of four genotypes namely, L<sub>3</sub>, L<sub>4</sub>, L<sub>6</sub> and L<sub>10</sub>, while cluster III and cluster IV consists of one genotype each, T<sub>2</sub> and T<sub>3</sub> respectively. The maximum inter-cluster distance was observed between cluster III and cluster IV (90.50), while the minimum inter-cluster distance was observed between cluster I and cluster II (33.94).

### **Molecular diversity**

The diversity observed based on morphological traits could not be exact, due to environmental interaction. The use of DNA markers can overcome the short back. 15 SSR markers out of 25 showed polymorphism in the 13 genotypes. From the molecular analysis of the 13 genotypes using 15 SSR markers, 43 alleles were observed. The number of alleles per locus ranged from 2 (umc1245, umc2199, bnlgl1017, bnlgl2046 and bnlgl1367) to 4 (bnlgl1556, phi109275 and bnlgl1401). The gel documentation of 13 genotypes using 15 SSR markers is presented in fig. 3. The details of the 15 SSR markers used in the molecular study is presented in Table 4.

**Table.1** Details of lines

S.No.	Code	Pedigree	Source
1	L <sub>1</sub>	(CML451-B*4//CML451-BBB/ZEWBc1F2-216-2-2-B-2-B*4-1-B-1-BB//CML451-B*4//CML451-BBB/CML444-1-BB)/(CML451-B*4//CML451-BBB/LaPostaSeqC7-F86-3-1-2-1-B*6//CML451-B*4//CML451-BBB/DTPWC9-F24-2-3-1-3-2-1-2-B*4)-B-3-2-BBB1-B	CIMMYT, Hyderabad
2	L <sub>2</sub>	((CML451-B*4//CML451-BBB/LaPostaSeqC7-F18-3-2-2-3-B*7//CML451-B*4//CML451-BBB/DRB-F2-60-1-1-1-BBB-3-B)-BB/(LPSC7-F96-1-2-1-1-BBB**/OFP39)-6-1-1-1-2BB)-B-11-BB-B2-B	CIMMYT, Hyderabad
3	L <sub>3</sub>	(POP501C5#9/GEMS-0053)-B-9-2-1-1-BB	CIMMYT, Hyderabad
4	L <sub>4</sub>	(HSBC1F1-12)DH10-B-#-BBB	CIMMYT, Hyderabad
5	L <sub>5</sub>	(CLQRCYQ44-B*8/[(CTS013082/P3011F2-5-3-1-3-B*4/Nei402011)-BB/G17C3H883-1-BB-3-3-B)-BBB-1-B-B	CIMMYT, Hyderabad
6	L <sub>6</sub>	(POP-501C5#2/GEMS-0009)-B-15-3-1-1-BBB	CIMMYT, Hyderabad
7	L <sub>7</sub>	(AMDROUT1(DT-Tester)C1F1-36-B*5/(CML451/LH82//CML451)-B-8-1-1-1-B)-B-3-B-B1-B	CIMMYT, Hyderabad
8	L <sub>8</sub>	((CML466/CML165-B//CML466)-BB-9-B*4/(CML465/CML165-B//CML465)-BB-36-B*5)-B-10-B-B1-B	CIMMYT, Hyderabad
9	L <sub>9</sub>	(AMDROUT2C2-17-B*4/POP502C5#4/GEMN-0159)-B-19-3-1-1-B)-B-3-2-B1-B	CIMMYT, Hyderabad
10	L <sub>10</sub>	((CA34505xCA00302)-B-2-1-B-1-B(T)/ZEWBC1F2-216-2-2-B-2-B*4-1-B-1-BB)-B-B1-B-2-BB1-B	CIMMYT, Hyderabad

**Table.2** Details of testers

S.No	Code	Name	Source
1	T <sub>1</sub>	CLO2450	CIMMYT, Hyderabad
2	T <sub>2</sub>	CM501	IIMR, Ludhiana
3	T <sub>3</sub>	BML-7	ANGRAU, Hyderabad

**Table.3** Characters with mean and range

S.No	Character	Mean	Range
1	Days to 50 per cent tasseling (day)	62	56 – 67
2	Days to 50 per cent silking (day)	69	62 - 74
3	Anthesis – silking interval (day)	7	5 – 9
4	Days to 75 per cent brown husk (day)	91	86 - 95
5	Plant height (cm)	109.42	78.50 – 151.75
6	Ear height (cm)	43.90	27.25 – 58.00
7	Cob length (cm)	11.70	7.30 – 14.29
8	Cob girth (cm)	3.00	2.46 – 3.60
9	No. of rows per cob	13	11 – 15
10	No. of grains per row	23	17 – 29
11	Test weight (g)	213.53	164.50 – 280.50
12	Shelling percentage (%)	64.17	54.50 – 74.67
13	Grain yield at 15% moisture (Kg/ha)	2380.05	1940.75 – 2850.81

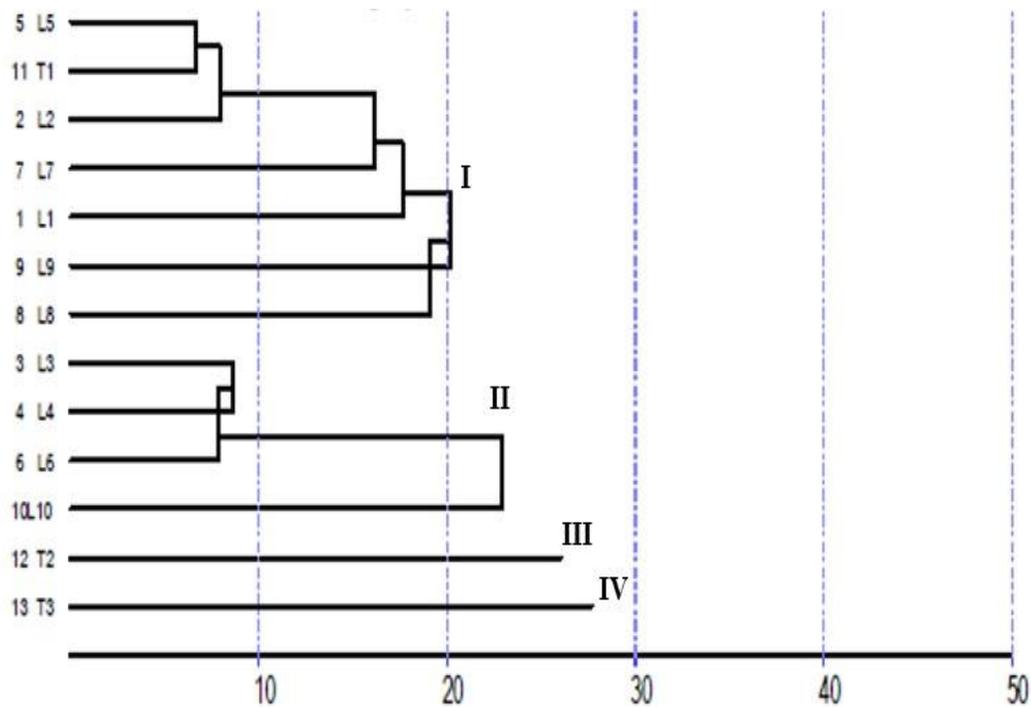
**Table.4** Details of SSR markers used in the study

S. No.	Marker name	Forward primer	Reverse primer	Chromosome	Number of allele	PIC value
1	bnlg1064	CTGGTCCGAGATGATGGC	TCCATTTCTGCATCTGCAAC	2	3	0.29
2	umc2205	ATGGTGAGCGAGTGAAAGAGAGAT	CATGATCATTTGGCGATGGTAAT	2	3	0.30
3	bnlg1556	ACCGACCTAAGCTATGGGCT	CCGGTTATAAACACAGCCGT	1	4	0.28
4	phi109275	CGGTTCATGCTAGCTCTGC	GTTGTGGCTGTGGTGGTG	1	4	0.28
5	umc1245	TGGTTATGTGCATGATTTTTCTG	CATGCGTCTGATCTTCAGAATGTT	1	2	0.30
6	umc1519	CTCGAGACTCTGGTTCAATCCAAT	CATGCACGTACTIONCCCTGATTTTT	9	3	0.29
7	umc1911	CCCGGAAGCTGTTTTCTTTTAAT	CGATCTGGAACGTAAGGATCATCT	10	3	0.30
8	umc2199	ACCGAGTTAAGATTACATCACGCC	TGTTTTCCCCTAATAAAGCAAATGAA	8	2	0.13
9	bnlg1525	AGGAATTGCGAGTCTTCCAA	CAACCCCCAAAATGAACAAA	9	3	0.27
10	umc2038	ACAGAAACCAATGCATGTGATGAG	TGCATGGTTGCTTCAGCAGT	4	3	0.32
11	bnlg1017	ATTGGAAGGATCTGCGTGAC	CAGCTGGTGGACTGCATCTA	2	2	0.31
12	bnlg2046	TTGGTGAAACGGTGAAATGA	CTGGTGAGCTTCACCCTCTC	8	2	0.13
13	umc1657	ATGGATGAATATGATCCCACGG	GATCCGCACGTAGCTTTTCG	9	3	0.29
14	bnlg1401	CACTCGGTTTTTGCTTAGCC	GTGTCGTCGAGTGCATGC	9	4	0.27
15	bnlg1367	CGACGGCGTACAGAGAGAG	GGTCGCCACCCACCT	7	2	0.13

**Table.5** Dice similarity coefficient matrix

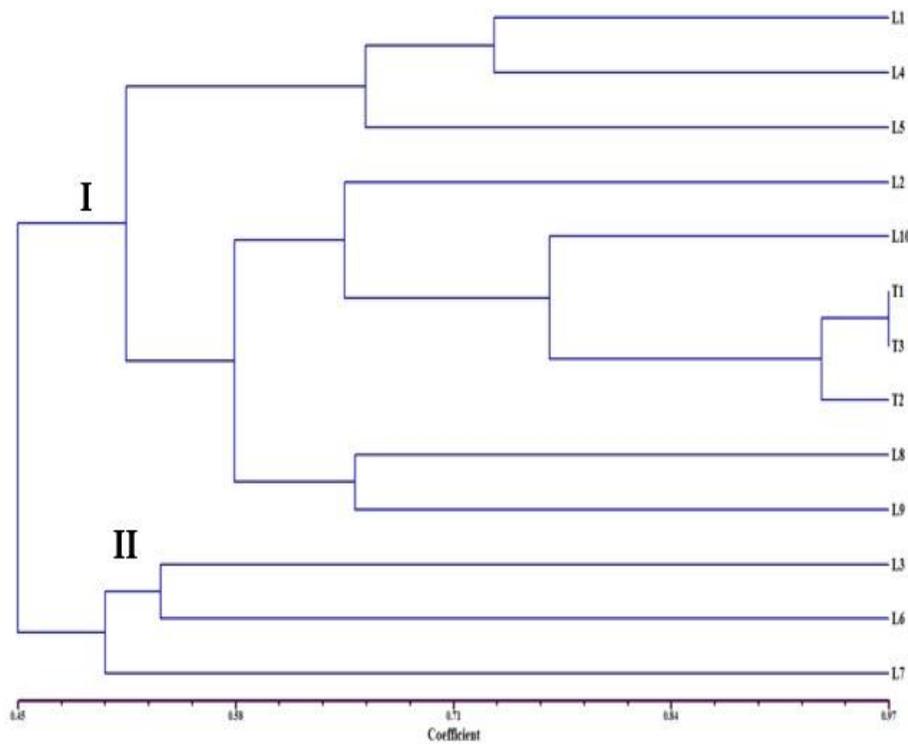
	L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>	L <sub>4</sub>	L <sub>5</sub>	L <sub>6</sub>	L <sub>7</sub>	L <sub>8</sub>	L <sub>9</sub>	L <sub>10</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
L <sub>1</sub>	1.00												
L <sub>2</sub>	0.56	1.00											
L <sub>3</sub>	0.47	0.25	1.00										
L <sub>4</sub>	0.73	0.56	0.47	1.00									
L <sub>5</sub>	0.69	0.59	0.38	0.63	1.00								
L <sub>6</sub>	0.60	0.38	0.53	0.53	0.44	1.00							
L <sub>7</sub>	0.53	0.31	0.47	0.60	0.38	0.53	1.00						
L <sub>8</sub>	0.32	0.56	0.32	0.43	0.41	0.32	0.32	1.00					
L <sub>9</sub>	0.48	0.51	0.36	0.61	0.57	0.36	0.48	0.65	1.00				
L <sub>10</sub>	0.69	0.59	0.57	0.57	0.59	0.51	0.57	0.48	0.47	1.00			
T <sub>1</sub>	0.44	0.65	0.44	0.56	0.41	0.44	0.56	0.62	0.63	0.76	1.00		
T <sub>2</sub>	0.47	0.67	0.47	0.53	0.50	0.47	0.53	0.63	0.65	0.82	0.94	1.00	
T <sub>3</sub>	0.45	0.67	0.39	0.58	0.42	0.45	0.52	0.63	0.59	0.72	0.97	0.91	1.00

**Fig.1** Dendrogram based on morphological diversity



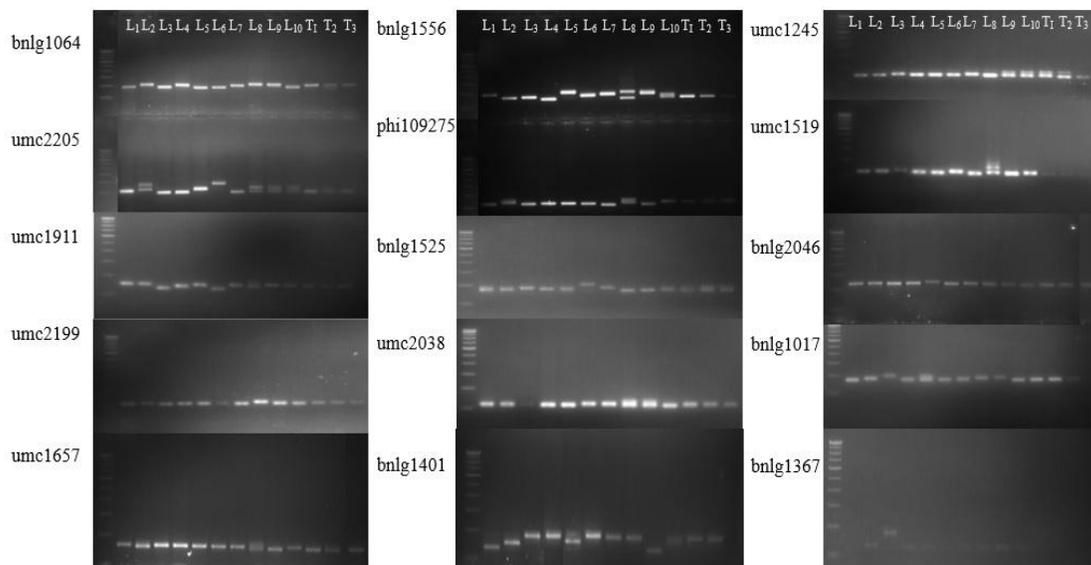
The roman numerals indicate the cluster number

**Fig.2** Dendrogram based on molecular diversity



The roman numerals indicate the cluster number.

**Fig.3** Ethidium bromide stained 2% agarose gel revealing polymorphism in 13 genotypes by various SSR markers



The PIC value for the 15 SSR markers ranges between 0.13 (umc2199, bnlg2046 and bnlg1367) to 0.32 (umc2038). Highest PIC value was an indication that SSR markers are efficient tools for polymorphism analysis (Brown *et al.*, 1996).

### Cluster analysis

Dice similarity coefficient matrix for the 13 genotypes is presented in Table 5. The minimum similarity was observed between L<sub>2</sub> and L<sub>3</sub> (0.25), while maximum similarity was observed between T<sub>1</sub> and T<sub>3</sub> (0.97). Based on the Dice similarity coefficient for the 13 genotypes, two clusters were detected. The cluster I was found to have ten genotypes namely, L<sub>1</sub>, L<sub>2</sub>, L<sub>4</sub>, L<sub>5</sub>, L<sub>8</sub>, L<sub>9</sub>, L<sub>10</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> and the cluster II has three genotypes L<sub>3</sub>, L<sub>6</sub> and L<sub>7</sub>. The dendrogram showing clusters based on molecular analysis is presented in fig. 2

It is concluded, from the diversity analysis based on morphological data, four clusters were obtained in the 13 genotypes. The molecular diversity, however contradicts this by revealing two clusters in the 13 genotypes. The results suggest that a better marker system with more number of markers should be considered, due to the genome size and cross pollinated nature of maize. Nevertheless, the SSR markers can be applied in the assessment of the purity of the lines and trueness of the hybrids, which can be developed. The genotypes that are diverse and present in different clusters can be used in hybridization programme.

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