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Full Length Research Paper

Genetic diversity of maize genotypes on the basis of morpho-physiological and simple sequence repeat (SSR) markers

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In this investigation, an attempt was made to assess the genetic diversity among 91 maize (*Zea mays* L.) genotypes using morpho-physiological and molecular markers. Variability was observed for six morpho-physiological traits namely, SPAD chlorophyll meter reading, canopy temperature, plant height, yield per plant, fodder yield and plant biomass as well as with simple sequence repeat (SSR) markers. All the amplification products with 40 SSRs were in the range of 58 to 410 bp. A total of 124 alleles were generated and the number of alleles scored for 40 SSR loci ranged from 2 to 5 with a mean of 3.1 alleles per locus. Polymorphism information content ranged from 0.054 to 0.82 with a mean of 0.55 suggesting that all the selected genotypes possessed high level of polymorphism. The study indicates that five genotypes, RJR-247, RJR-159, NSJ-179, RJR-55 and Z101-15 were most diverse, so it is suggested that they may be used as genetic resources for maize improvement programme in future quantitative trait loci (QTL) mapping for different agronomic traits and for developing new varieties with adaptation to a broad range of environments.

Key words: Maize, SSR markers, genetic diversity, dendrogram.

INTRODUCTION

Maize (*Zea mays* L.) is an important cereal and fodder crop which occupy a pivotal role in the world economy (White and Johnson, 2003). Diversity among maize germplasm is important for identifying parental lines for successful breeding programme, and hybrid development (Kostova et al., 2006; Losa et al., 2011). Since there is a rapid increase in climate change, so there is need to develop high yielding genotypes which can tolerate various environmental stress conditions, like drought, increased salinity in soil, cold and heat stresses. On these backgrounds plant breeders need to look deeply for sources of genotypes, which can be effectively used as parents to develop new variety with high yield and good agronomic traits with adaptation to a broad range of environments.

Several studies have been carried out on genetic diversity study in maize. Morpho-physiological markers were used to study genetic diversity in different maize landraces (Beyene et al., 2005; Comertpay et al., 2012). Among the different kind of molecular markers, simple sequence repeats (SSRs) are one of the most promising

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Abbreviations: SSR, Simple sequence repeat; **PIC,** polymorphism information content; **CTAB,** cetyl tri-methyl ammonium bromide; **UPGMA,** unweighted pair-group method with arithmetic mean.

molecular marker for genotypic studies due to their high levels of polymorphism (Senior et al., 1998), co-dominant inheritance, high polymorphism information content, high reproducibility, locus specificity, extensive genome coverage and high allelic diversity (Powell et al*.,* 1996; Mohan et al., 1997). SSR markers have become quite useful in various aspects of molecular genetic studies in the past decade including assessment of genetic diversity in maize (Nguyen et al., 2012; Babu et al., 2012), marker assisted selection and genetic studies such as construction of linkage maps and QTL mapping (Prasanna et al., 2009a) or evolution studies (Xia et al., 2004; Prasanna et al., 2009b, 2010).

In the present study an effort was made to identify diverse genotypes for genetic enhancement of drought tolerance. Both morpho-physiological and genotypic variations among 91 maize genotypes were carried out using six phenotypic traits and SSR markers related to drought tolerance. Hence, the diverse genotypes selected based on its study could be used for the identification of QTLs for drought tolerance.

MATERIALS AND METHODS

Plant materials and their field evaluation

Ninety-one maize genotypes received from three different sources *viz*., Directorate of Maize Research, New Delhi, India, regional station of National Bureau of Plant Genetic Resources, Hyderabad, and regional centre of CIMMYT, Hyderabad were used in the present study (Table.1). Seeds were sown in augmented block design with a row to row spacing of 60 cm and plant to plant spacing of 25 cm. Experiment was carried out at Central Research Institute for Dryland Agriculture, Hyderabad, during rainy season of 2011. The recommended fertilizer dose and cultural practices along with plant protection measures were taken to raise the crop.

Morpho-physiological study

Data was recorded from three plants of each genotype on the basis of six traits namely, SPAD chlorophyll meter reading, canopy temperature (°C), plant height (cm), yield per plant (g), fodder yield (g) and plant biomass (g). Light absorbance at specific wavelengths helps to estimate the amount of chlorophyll in leaf. There were three replications for each measurement. Leaf chlorophyll estimation and canopy temperature was carried out in three leaf stage using SPAD-502, Minolta, Tokyo, Japan and by IR-Thermometer, Fluke/568 respectively. Plant height was measured from the soil surface to the tip of the central axis. Yield, fodder yield and after drying plant biomass per plants were recorded. Pair wise similarities were calculated using Euclidean distances from the mean values. These values were used to construct sequential agglomerative hierarchical nested (SAHN) clustering.

Genotyping

Young leaves were collected from three to four weeks old plants of each line and genomic DNA was extracted following CTAB protocol (Doyle and Doyle, 1990) with minor modification. Previously reported 48 total SSR markers related to different agronomic traits (under drought, yield controlling traits) were selected based on repeat units and bin location to provide uniform coverage of entire maize genome. Details of SSR primers of 10 maize chromosomes were extracted using website *www.maizegdb.org/.* PCR amplification was carried out with 25 µl reaction mixture containing 50 ng template DNA, 2.5 µl of 10 X reaction buffer (10 mM Tris HCl, pH 8.3 and 50 mM KCl), 2.5 mM $MgCl_2$, 0.5 U Taq DNA polymerase, 200 µM dNTPs and 0.4 µM primer. Amplification was performed in Applied Biosystem Thermal Cycler programmed as one cycle of initial denaturation at 94°C for 5 min, 40 cycles each of denaturation at 94°C for 30 s, annealing step was performed for 1 min, 30 s at an optimum temperature for each primer, about 0.8°C to 1.2°C above its Tm, and primer extension was done at 72°C for 1 min, 30 s and final elongation at 72°C for 10 min. Each SSR marker was examined by carrying out 2 to 3 independent PCR reaction and gel analysis to ensure that the amplification obtained with the primers is reproducible and consistent. Amplified PCR products were resolved through electrophoresis at 80 volts for 1 h and 30 min using 1 X, TAE buffer in 3.5% agarose (Agarose SFR^{TM}) gel containing 5 µl (1 mg/ml) ethidium bromide, then photographed under ultraviolet light with VILBER LOURMAT gel documentation system.

The SSR gel images and marker data were processed using Biovision Software. The bands were sized then binary coded by 1 or 0 for their presence or absence respectively in the selected 91 genotypes for each SSR primer pairs and it was used for calculation of similarity matrix based on Jaccard coefficients (Jaccard, 1908). Cluster analysis was based on similarity matrices obtained with the unweighted pair-group method using the arithmetic mean (UPGMA) to generate the dendrogram. All the data analysis were carried out using the software package NTSYS-pc2.0 (Rohlf, 1998). The mean PIC values for each SSR were estimated by determining the frequency of alleles per locus using the following formula:

PIC= 1- $\Sigma\ x_i^2$; where x_i is the relative frequency of the i^{th} allele of the SSR loci; markers were classified as informative when PIC was ≥ 0.5 (Sharma et al., 2009).

RESULTS AND DISCUSSION

Analysis of genotypes based on morpho-physiological traits

The performances of 91 maize genotypes were studied with respect to six morpho-physiological traits. SPAD chlorophyll meter reading was lowest (40.80) in genotype NSJ-179, while it was highest (65.50) in R2HKI-46. Lowest canopy temperature (16.05°C) was recorded in Z93-154 while the highest (28.33) was recorded in genotype R1HKI-659-3. Minimum (25 cm) plant height was recorded in genotype Z101-57 while maximum (273 cm) was in RJR-159. Yield per plant was recorded minimum (0.10 g) in genotype R1HKI-161 and the maximum (2.20 g) in NSJ-221. Fodder yield was minimum in genotype Z59-9 and maximum in R1HKI-164D4. Plant biomass was maximum (3.90 g) in genotype R1HKI-164D4 (Table 2).

Cluster analysis of genotypes based on morpho-physiological traits

The taxonomic distance matrix of six morpho-physiological traits for 91 maize genotypes was constructed. Euclidean distances varied widely (data

Table 1. Description of the 91 maize genotypes used for genetic diversity on the basis of morpho-physiological and SSR markers.

S/N	Genotype	Source	S/N	Genotype	Source
1	R1HKI-161	DMR, New Delhi	47	Z162-10	CIMMYT, Hyderabad
2	R2HKI-161	DMR, New Delhi	48	Z162-12	CIMMYT, Hyderabad
3	R1HKI-164D4	DMR, New Delhi	49	NSJ-99	NBPGR, Regional Station, Hyderabad
4	R2HKI-164D4	DMR, New Delhi	50	RSR-025	NBPGR, Regional Station, Hyderabad
5	R1HKI-1035-10	DMR, New Delhi	51	NSJ-221	NBPGR, Regional Station, Hyderabad
6	R2HKI-1035-10	DMR, New Delhi	52	NSJ-366	NBPGR, Regional Station, Hyderabad
7	R1HKI-3-4-8-6ER	DMR, New Delhi	53	NSJ-245	NBPGR, Regional Station, Hyderabad
8	R2HKI-3-4-8-6ER	DMR, New Delhi	54	NSJ-211	NBPGR, Regional Station, Hyderabad
9	R1HKI-766(0)	DMR, New Delhi	55	RJR-163	NBPGR, Regional Station, Hyderabad
10	R2HKI-766(0)	DMR, New Delhi	56	NSJ-285	NBPGR, Regional Station, Hyderabad
11	R1HKI-1040-4	DMR, New Delhi	57	RJR-198	NBPGR, Regional Station, Hyderabad
12	R2HKI-1040-4	DMR, New Delhi	58	RJR-208	NBPGR, Regional Station, Hyderabad
13	R1HKI-46	DMR, New Delhi	59	RJR-247	NBPGR, Regional Station, Hyderabad
14	R2HKI-46	DMR, New Delhi	60	PSR-13247	NBPGR, Regional Station, Hyderabad
15	R1HKI-325-17AN	DMR, New Delhi	61	RJR-375	NBPGR, Regional Station, Hyderabad
16	R2HKI-325-17AN	DMR, New Delhi	62	PSRJ-13122	NBPGR, Regional Station, Hyderabad
17	R1HKI-659-3	DMR, New Delhi	63	PSRJ-13041	NBPGR, Regional Station, Hyderabad
18	R2HKI-659-3	DMR, New Delhi	64	PSRJ-13007	NBPGR, Regional Station, Hyderabad
19	R1HKI-L-287	DMR, New Delhi	65	SNJ-2011-70	NBPGR, Regional Station, Hyderabad
20	R2HKI-L-287	DMR, New Delhi	66	SNJ-2011-03	NBPGR, Regional Station, Hyderabad
21	R1LM6	DMR, New Delhi	67	SNJ-2011-26	NBPGR, Regional Station, Hyderabad
22	R2LM6	DMR, New Delhi	68	SNJ-2011-15	NBPGR, Regional Station, Hyderabad
23	R1HKI-164-7-4	DMR, New Delhi	69	SNJ-2011-37	NBPGR, Regional Station, Hyderabad
24	Z60-87	CIMMYT, Hyderabad	70	RJR-132	NBPGR, Regional Station, Hyderabad
25	Z40-19	CIMMYT, Hyderabad	71	RJR-075	NBPGR, Regional Station, Hyderabad
26	Z61-34	CIMMYT, Hyderabad	72	PSRJ-13154	NBPGR, Regional Station, Hyderabad
27	Z59-9	CIMMYT, Hyderabad	73	PSRJ-13038	NBPGR, Regional Station, Hyderabad
28	Z101-57	CIMMYT, Hyderabad	74	RJR-049	NBPGR, Regional Station, Hyderabad
29	Z101-61	CIMMYT, Hyderabad	75	RJR-55	NBPGR, Regional Station, Hyderabad
30	Z59-11	CIMMYT, Hyderabad	76	RJR-037	NBPGR, Regional Station, Hyderabad
31	Z101-68	CIMMYT, Hyderabad	77	NSJ-315	NBPGR, Regional Station, Hyderabad
32	Z32-12	CIMMYT, Hyderabad	78	RJR-138	NBPGR, Regional Station, Hyderabad
33	Z93-194	CIMMYT, Hyderabad	79	RJR-159	NBPGR, Regional Station, Hyderabad
34	Z49-7	CIMMYT, Hyderabad	80	PSR-13255	NBPGR, Regional Station, Hyderabad
35	Z93-154	CIMMYT, Hyderabad	81	RJR-270	NBPGR, Regional Station, Hyderabad
36	Z101-15	CIMMYT, Hyderabad	82	RJR-328	NBPGR, Regional Station, Hyderabad
37	Z59-17	CIMMYT, Hyderabad	83	RJR-363	NBPGR, Regional Station, Hyderabad
38	Z59-41	CIMMYT, Hyderabad	84	RJR-385	NBPGR, Regional Station, Hyderabad
39	Z60-72	CIMMYT, Hyderabad	85	PSRJ-13099	NBPGR, Regional Station, Hyderabad
40	Z32-87	CIMMYT, Hyderabad	86	PSRJ-13059	NBPGR, Regional Station, Hyderabad
41	Z93-170	CIMMYT, Hyderabad	87	NSJ-179	NBPGR, Regional Station, Hyderabad
42	Z40-183	CIMMYT, Hyderabad	88	NSJ-189	NBPGR, Regional Station, Hyderabad
43	Z32-62	CIMMYT, Hyderabad	89	NSJ-155	NBPGR, Regional Station, Hyderabad
44	Z49-65	CIMMYT, Hyderabad	90	PSRJ-13086	NBPGR, Regional Station, Hyderabad
45	Z96-5	CIMMYT, Hyderabad	91	RJR-068	NBPGR, Regional Station, Hyderabad
46	Z162-9	CIMMYT, Hyderabad			

not shown) showed a scattered distribution of 91 genotypes in the dendrogram (Figure 1). All the genotypes were grouped into two major clusters, one larger cluster (Cluster I) with 73 genotypes and two smaller one with 18 (Cluster II). Further, cluster I was subdivided into three sub clusters. Due to wider level of diversity based on six morpho-physiological traits, 18 genotypes were clearly separated and grouped in a distinct cluster. Clusters suggest wide variability among all selected genotypes.

Analysis of genotypes based on SSR markers

Out of the 48 SSR markers validated, 40 were

Table 2. Mean of the six morpho-physiological traits of 91 maize genotypes.

Name of genotype	SPAD	Canopy (°C)	Pl Ht (cm)	Yield (g)	Fodder yield (g)	Biomass (g)
R1HKI-161	46.60	26.93	80	0.10	0.56	0.66
R2HKI-161	47.63	24.47	79	0.85	0.45	1.30
R1HKI-164D4	51.50	26.90	85	0.85	3.05	3.90
R2HKI-164D4	58.43	26.93	85	0.95	1.77	2.72
R1HKI-1035-10	57.13	26.40	67	0.90	0.75	1.65
R2HKI-1035-10	54.33	26.00	68	0.90	2.10	3.00
R1HKI-3-4-8-6ER	54.27	27.40	67	0.75	1.19	1.94
R2HKI-3-4-8-6ER	58.83	28.07	67	0.80	1.16	1.96
R1HKI-766(0)	49.87	28.13	67	0.75	0.95	1.70
R2HKI-766(0)	49.93	26.93	66	0.75	0.89	1.64
R1HKI-1040-4	48.43	25.27	61	0.69	0.98	1.67
R2HKI-1040-4	62.63	25.17	61	0.69	1.50	2.19
R1HKI-46	55.60	26.00	64	0.70	0.65	1.35
R2HKI-46	65.50	27.00	63	0.92	1.15	2.07
R1HKI-325-17AN	58.50	27.00	64	0.90	1.17	2.07
R2HKI-325-17AN	54.40	27.00	64	0.80	0.84	1.64
R1HKI-659-3	57.57	28.33	65	0.75	1.47	2.22
R2HKI-659-3	52.17	25.00	65	0.75	2.50	3.25
R1HKI-L-287	58.63	24.50	62	0.69	1.80	2.49
R1HKI-L-287	57.17	24.17	62	0.75	1.42	2.17
R1LM6	55.73	24.00	50	0.90	1.50	2.40
R1LM6	59.73	24.00	52	0.80	1.46	2.26
R1HKI-164-7-4	59.27	24.67	37	0.90	1.75	2.65
Z60-87	56.90	24.68	48	0.75	0.45	1.20
Z40-19	52.90	24.00	56	0.85	0.72	1.57
Z61-34	51.60	24.02	36	0.85	0.86	1.71
Z59-9	56.57	24.22	35	0.50	0.43	0.93
Z101-57	55.00	24.17	25	0.90	2.90	3.80
Z101-61	55.00	24.67	40	0.90	0.56	1.46
Z59-11	54.60	24.17	43	0.92	0.44	1.36
Z101-68	48.57	24.33	50	0.90	1.50	2.40
Z32-12	50.77	24.00	35	1.10	0.56	1.66
Z93-194	53.63	24.27	45	0.80	1.20	2.00
Z49-7	56.67	25.00	39	0.92	0.65	1.57
Z93-154	57.20	16.05	30	0.90	0.44	1.34
Z101-15	52.53	24.07	75	0.50	2.07	2.57
Z59-17	54.13	24.13	55	0.90	0.65	1.55
Z59-41	53.13	24.20	48	0.70	0.95	1.65
Z60-72	63.90	24.07	60	0.92	1.45	2.37
Z32-87	60.57	23.90	59	0.90	2.50	3.40
Z93-170	54.33	24.07	85	0.35	0.81	1.16
Z40-183	47.40	24.00	60	0.55	2.39	2.94
Z32-62	48.47	24.30	69	0.91	0.78	1.69
Z49-65	55.10	24.27	70	0.50	0.95	1.45
Z96-5	52.07	24.07	65	0.90	0.61	1.51
Z162-9	52.40	24.00	70	0.90	1.49	2.39
Z162-10	51.83	24.13	75	0.90	0.86	1.76
Z162-12	42.60	23.97	72	0.35	1.33	1.68
NSJ-99	52.77	24.43	57	0.90	0.77	1.67
RSR-025	53.23	24.40	100	0.90	0.58	1.48
NSJ-221	48.73	24.23	70	2.20	1.20	3.40

Table 2. Continued.

NSJ-366	53.17	24.20	100	0.91	1.26	2.17
NSJ-245	52.97	24.50	115	0.50	0.93	1.43
NSJ-211	52.00	25.00	118	0.90	0.65	1.55
RJR-163	52.20	25.33	120	0.78	0.93	1.71
NSJ-285	50.50	25.00	120	1.50	0.82	2.32
RJR-198	55.10	25.60	115	0.90	1.30	2.20
RJR-208	46.67	25.07	100	0.92	1.82	2.74
RJR-247	57.03	25.00	130	0.90	0.50	1.40
PSR-13247	47.63	25.07	120	1.10	0.65	1.75
RJR-375	53.90	25.17	91	0.80	0.90	1.70
PSRJ-13122	54.47	25.00	103	0.92	0.60	1.52
PSRJ-13041	52.73	24.93	80	0.90	1.83	2.73
PSRJ-13007	53.87	24.40	102	0.90	0.75	1.65
SNJ-2011-70	56.47	24.70	104	0.90	1.43	2.33
SNJ-2011-03	56.43	24.30	110	0.78	0.83	1.61
SNJ-2011-26	53.57	25.13	120	1.15	0.85	2.00
SNJ-2011-15	55.30	25.00	120	1.25	1.24	2.49
SNJ-2011-37	53.07	25.53	100	0.90	1.31	2.21
RJR-132	55.00	25.70	121	1.10	0.81	1.91
RJR-075	51.30	25.80	110	0.80	0.44	1.24
PSRJ-13154	59.10	26.83	100	0.92	0.87	1.79
PSRJ-13038	54.87	26.80	100	1.00	1.10	2.10
RJR-049	52.60	24.43	216	1.25	0.80	2.05
RJR-55	50.57	25.00	215	0.78	0.75	1.53
RJR-037	54.37	24.00	238	1.50	0.50	2.00
NSJ-315	47.87	23.80	237	0.90	0.86	1.76
RJR-138	62.70	23.80	209	1.89	0.77	2.66
RJR-159	46.30	23.13	273	0.90	1.50	2.40
PSR-13255	42.63	22.60	259	0.75	0.65	1.40
RJR-270	48.87	22.60	215	0.88	0.99	1.87
RJR-328	56.13	22.13	246	0.70	0.53	1.23
RJR-363	48.43	23.00	250	0.92	0.98	1.90
RJR-385	51.70	22.00	257	0.72	0.52	1.24
PSRJ-13099	49.03	22.33	208	1.50	0.66	2.16
PSRJ-13059	60.77	22.67	193	1.00	0.97	1.97
NSJ-179	40.80	22.57	191	2.10	1.56	3.66
NSJ-189	47.03	23.00	210	0.99	1.00	1.99
NSJ-155	48.07	23.17	240	1.23	0.58	1.81
PSRJ-13086	54.47	24.27	194	1.88	0.75	2.63
RJR-068	44.97	24.33	206	1.50	0.85	2.35

polymorphic, 2 were monomorphic and 6 were unable to amplify the genomic DNA of maize. All the polymorphic primer pairs produced distinct reproducible amplification which was used to examine the degree of genetic variation among 91 maize genotypes (Table 3). The SSR profile with markers bnlg1614 is represented in Figure 2. The lowest size of PCR fragment (58 bp) was revealed by locus umc1056 and the highest size (410 bp) was revealed by locus bnlg 2248, respectively. A total of 124 alleles were generated with 40 SSR markers and the number of allele scored for SSR loci ranged from 2 to 5 with mean of 3.1 alleles per locus.

In this investigation, the mean number of alleles (3.1) was considerably lower than those reported previously (Legesse et al., 2007; Comertpay et al., 2012) and the highest mean alleles 21.7 per loci was reported by Liu et al. (2003). However, our finding (3.1 alleles per locus) is closely related to the finding reported by Bantte et al. (2003) who reported mean of 3.25 alleles per locus with 36 SSR loci. The present study supports the previous

Table 3. Characteristics of 40 SSR marker loci; repeat type, bin no., no. of allele and PIC value

S/N	Markers	Repeat type	Bin no.*	No. of alleles	Product size	PIC value
1	bnlg1179	AG(16)	1.01	3	158-255	0.39
2	bnlg1014	AG(14)	1.01	2	156-170	0.70
3	umc2189	(CAG)4	1.10	2	140-171	0.82
4	umc1542	(AG)10	2.02	2	130-155	0.67
5	phi053	ATAC	3.05	2	172-208	0.68
6	bnlg1136	AG(14)	6.07	3	192-236	0.34
7	umc1016	(CT)25	7.02	5	80-160	0.44
8	bnlg1346	AG(24)	5.07	4	107-220	0.44
9	bnlg1564	AG(24)	1.07	3	114-166	0.61
10	bnlg1035	AG(13)	3.05	2	95-112	0.57
11	bnlg2328	AG(33)	2.05	4	170-245	0.48
12	bnlg2190	AG(31)	10.06	4	148-249	0.64
13	bnlg1225	AG(14)	2.06	3	121-167	0.61
14	phi022	GTGC	9.03	2	143-169	0.74
15	bnlg1537	AG(16)	2.03	5	185-269	0.49
16	nc012	CT	6.05	3	118-152	0.61
17	bnlg1327	CT(25)	2.02	4	190-305	0.54
18	bnlg1812	AG(22)	8.05	3	173-229	0.53
19	bnlg1241	AG(21)	4.01	2	149-180	0.49
20	bnlg1063	AG(42)	3.06	3	117-293	0.35
21	phi081	GAT-TAC	6.05	2	175-187	0.054
22	bnlg1655	AG(21)	10.03	3	105-148	0.28
23	bnlg1614	AG(15)	1.02	3	175-250	0.79
24	bnlg1083	AG(29)	1.02	4	84-170	0.61
25	bnlg1209	AG(12)	9.04	3	92-265	0.45
26	bnlg1082	AG(11)	9.02	3	189-280	0.16
27	umc1143	AAAAT	6.00	3	170-259	0.69
28	bnlg439	NA	1.03	2	155-229	0.71
29	bnlg1297	AG(32)	2.02	4	153-248	0.63
30	umc1042	GA17	2.07	3	81-108	0.71
31	umc1922	(ATA)6	2.05	3	113-256	0.54
32	bnlg1866	AG(11)	1.03	4	91-136	0.41
33	umc1056	(AGCA)4	5.03	4	58-165	0.61
34	dup13	-	1.08	4	129-168	0.49
35	umc1069	(GGAGA)6	8.08	3	127-156	0.76
36	umc1962	-	10.03	5	131-185	0.48
37	bnlg1028	AG(12)	10.06	2	147-160	0.79
38	umc1344	(GTTC)5	10.07	2	96-114	0.47
39	bnlg2248	AG(30)	2.03	4	195-410	0.66
40	umc2252	(TCC)4	2.05	2	86-130	0.74
				Mean 3.1		Mean 0.55

* Location of allele in the chromosome of maize genome.

reports, that SSR marker can be used for estimation of genetic diversity in maize improvement (Xia et al., 2004; Shah et al., 2009).

The used SSR marker provided high PIC value with mean of 0.55 ranging from 0.054 to 0.82 (Table 3). The lowest PIC value (0.054) was recorded with markers phi 081 on bin locus 6.05 and the highest (0.82) was recorded with markers umc 2189 on bin locus 1.10. Three loci showed ≥0.39 and 17 loci showed ≥ 0.69 and 20 loci showed ≥ 0.99 PIC value respectively. The SSR markers containing trinucleotide $(CAG)_4$ repeat had the highest PIC value and hexanucleotide (GATTAC) repeat had the lowest PIC value. Here, the mean PIC value was in the range as reported by Senior et al. (1998) and

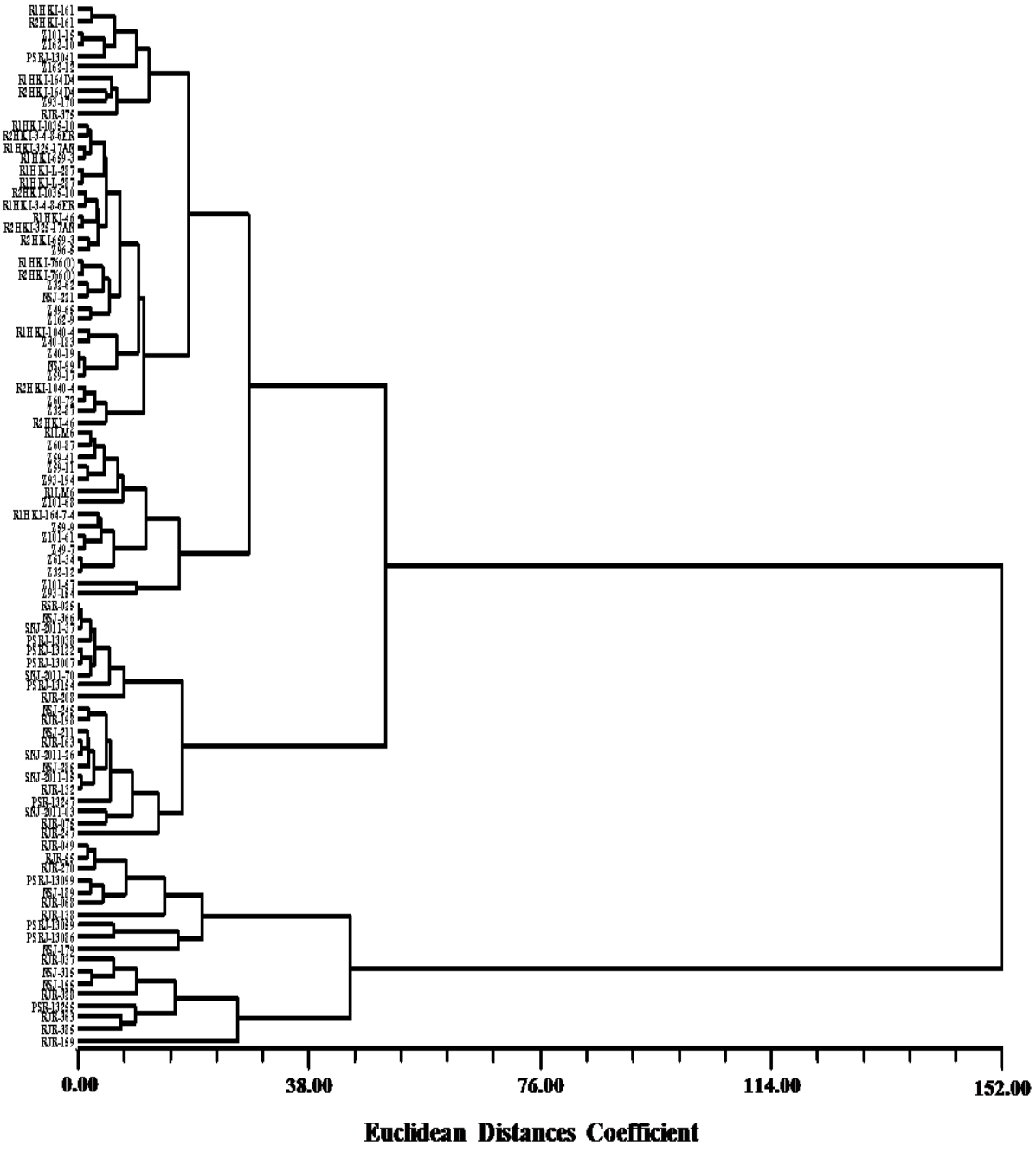

Figure 1. Association of the 91 maize genotypes as revealed by UPGMA cluster analysis obtained using six different agronomic traits**.**

Heckenberger et al. (2002); and this value was similar to those reported by Legesse et al. (2007) in their genetic diversity study of African maize inbred lines. It was lower than those reported previously (Beyene et al., 2005; Sharma et al., 2010). However, higher mean PIC value was reported by Smith et al. (1997); this difference may be associated with the use of acrylamide gels for detection in their study.

Cluster analysis of the genotypes based on SSR marker

From the data generated by 40 SSR markers, the similar classes were also fused together from the most similarity to the least similarity levels and plotted as dendrogram (Figure 3). Genetic similarity measured through analysis of data on the 40 SSR markers from the 91 maize

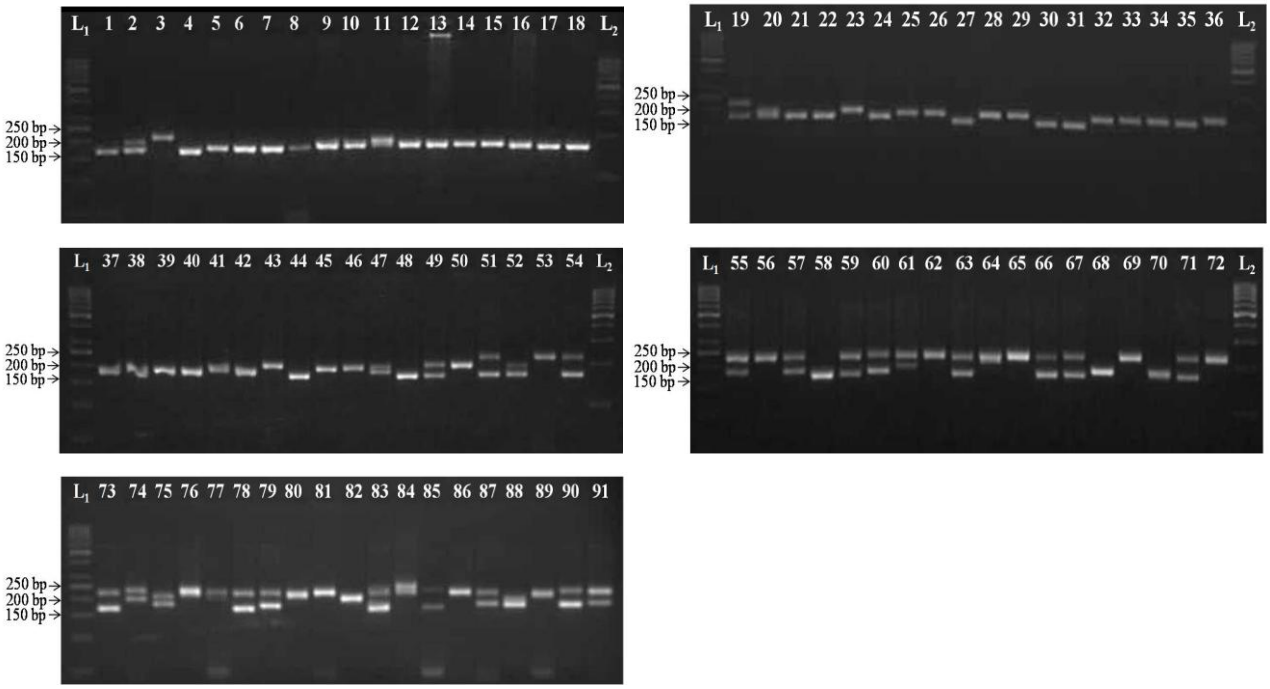

Figure 2. PCR amplification profile of 91 maize genotypes with SSR marker bnlg1614 (Bin 1.02). L_1= Ladder 50 bp, L_2 = ladder 100 bp, 1 to 23 = source DMR, New Delhi; 24 to 48 = source CIMMYT, Hyderabad; 49 to 91 = source NBPGR, Regional Station, Hyderabad (details of sample number 1-91 is presented in Table 1).

genotypes revealed varying degree of genetic diversity ranging from 0.25 to 0.85 in Jaccard coefficient. The highest (0.85) similarity coefficient was observed between genotypes R1HKI-659-3 and R2HKI-659-3 and the second highest (0.83) was observed between genotypes R1HKI-1035-10 and R2HKI-1035-10 which confirms that these genotypes were closely related .

The dendrogram separated the 91 genotypes into two major clusters, one small cluster I with 5 genotypes and a large cluster II with 86 genotypes (Figure 3). The large cluster II was further subdivided into two sub clusters; sub cluster IIA containing 34 genotypes in the first cluster and 52 genotypes in the sub cluster IIB, at a similarity coefficient level of 0.27. The sub cluster IIA of the 34 genotypes was further subdivided into two groups; the smaller group with 4 genotypes and the larger group with 30 genotypes at 0.28 coefficient level. The second sub cluster IIB of 52 genotypes were further subdivided into two groups; the smaller group with 2 genotypes only and one larger group with 50 genotypes. Again, these 50 genotypes were re-subdivided into two groups with 31 genotypes in one group and 19 genotypes in the second group at 0.30 coefficient levels. Minimum genetic distance between R1HKI-1305-10 and R2HKI-1035-10 and R1HKI-659-3 and R2HKI-659-3 was a good indication confirming the efficiency of SSR markers to distinguish closely related inbred lines (Smith et al., 1997). In our investigation, the highest genetic distance among 91 maize genotypes was 0.85 which indicates high level of polymorphism among the selected genotypes.

Genotypes R1HKI-1035-10 and R2HKI-1035-10, R1HKI-659-3 and R2HKI-659-3, and genotypes R1LM6 and R2LM6 were closest to each other as evident from the dendrogram generated by SSR marker as well as in the dendrogram generated by phenotypic traits. This may be due to narrow diversity among them. Similarly, many of the genotypes grouped in single cluster, were expected because they may have originated from same source. Genotypes Z59-41, Z40-183, Z40-19, Z61-34, R1HKI-164-7-4, Z93-170 and Z101-15 were divergent as they fall in side of the dendrogram (Figure 3). Genotypes RSR-025 and NSJ-366 came out to be the most diverse based on quantitative traits as well as SSR marker and clearly separated from the rest of the genotypes. This grouping in most instants revealed evidence of associations related to their pedigree records. This is in agreement with earlier findings of Reif et al. (2003) and Legesse et al. (2007) who demonstrated the correspondence of SSR marker distance with pedigree information in maize. Cross pollination with highly variable genotypes and multiple origins with different sources are all considered to be factors contributing to the extreme genetic diversity among the selected genotypes.

Conclusion

Ninety-one selected maize genotypes were characterized and classified using SSR marker system indicating the

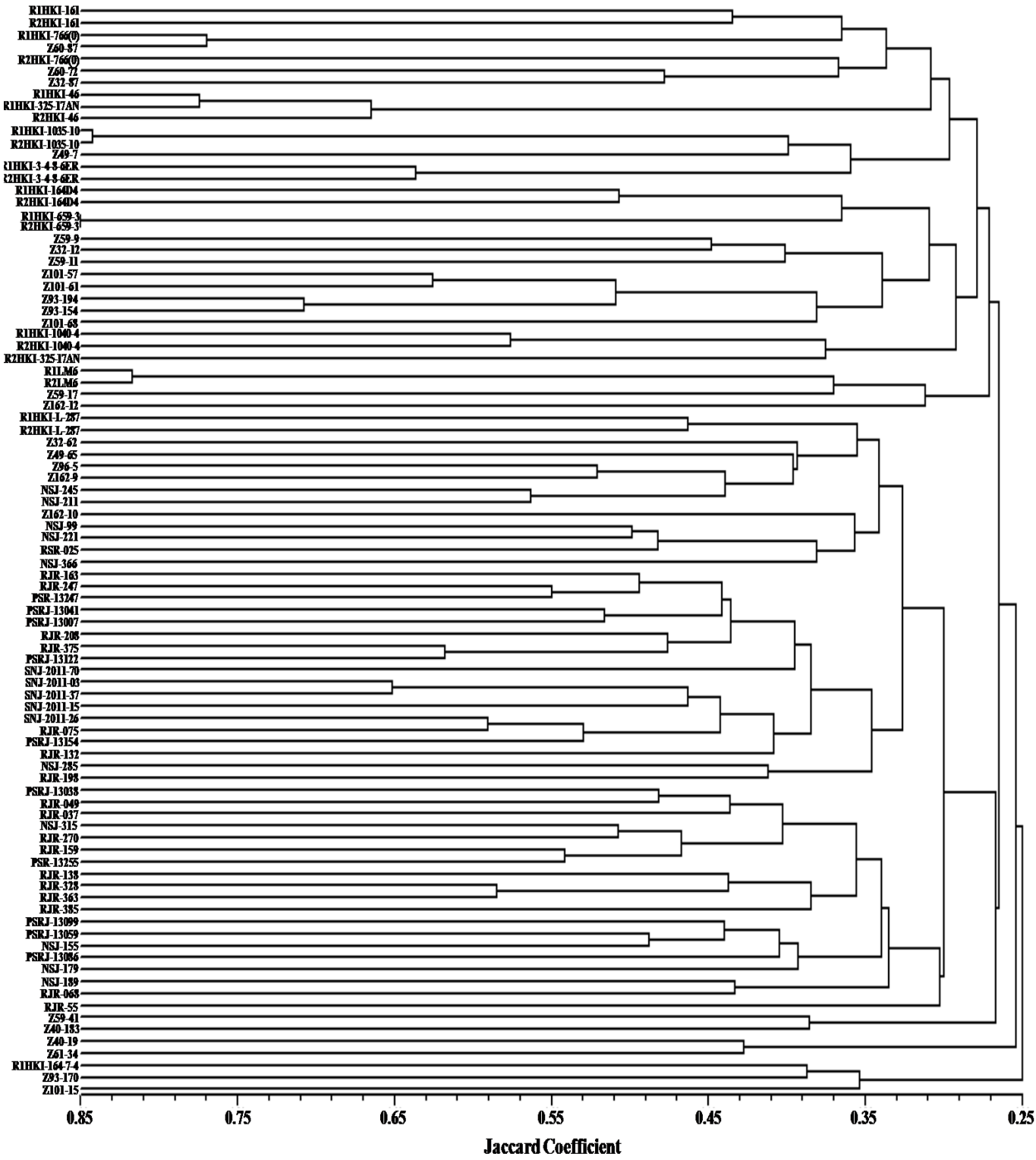

Figure 3. Association of the 91 maize genotypes as revealed by UPGMA.

robustness of SSR markers for diversity analysis and inbred grouping. A degree of genetic distance ranged between 0.25 to 0.85 with mean PIC value 0.55 indicate that these genotypes are genetically diverse and possess high level of polymorphism. DNA polymorphism provided a detailed differentiation which may be utilized for

verifying the authenticity of genotypes, selecting the best genotypes for breeding, for better estimation of heterosis and verifying the pedigree. High genetic diversity detected among the 91 maize genotypes by morpho-physiological and SSR marker suggests the opportunity to exploit the most diverse genotypes for future QTL mapping and maize improvement programme. Genetically distinct genotypes have been identified that could be potentially important sources of germplasm for drought tolerance maize improvement programme. The result could assist plant breeders in selecting diverse sources of germplasm for the maximum heterosis and making new cultivars with adaptation to a broad range of environments.

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