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Research Article

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Genetic Diversity of Maize (*Zea mays* L.) Genotypes Assessed by SSR Markers under Temperate Conditions

Asima Gazal[1*], Dar ZA[1], Lone AA[1], Shikari AB[2] and Habib M[1]

Abstract

A set of twenty four maize lines comprising fifteen drought promising inbred lines, four drought susceptible inbred lines and five drought tolerant checks were analyzed for molecular characterization. These inbred lines belong to AICRP (All India Co-ordinated Research Programme) Maize Srinagar Centre and the checks were selected from CIMMYT (International Maize and Wheat Improvement Centre) material.. Molecular characterization was done using a set of 45 SSR markers having genome wide coverage. The marker data was analyzed using Power Marker Software (Version 3.25). The markers detected a total of 271 alleles with an average of 8.46 alleles per locus in twenty four maize lines. The average polymorphism information content (PIC) ranged from 0.56 and 0.89 with a mean of 0.78. The level of heterozygosity in the inbred panel was significantly low. The mean value of heterozygosity was 0.05 implying that most of the loci attained homozygosity. Dendrogram derived from UPGMA cluster analysis showed presence of two major clusters, one of which had many subgroups. Phenotyping of these inbreds was done by using morphological, maturity, physiological, yield and yield attributing traits. Genotyping data complemented by phenotyping data would be used to identify a number of pair-wise combinations for the development of mapping populations for drought tolerance related traits and potential heterotic pairs for the development of drought tolerant hybrids.

Keywords

Zea mays; Simple sequence repeats; Diversity; Inbreds; Drought tolerance

Introduction

Maize is an important crop under temperate conditions next to rice and is gaining more popularity due its multiple uses. It is grown mainly in Kharif season and under rainfed conditions facing erratic precipitation which leads to yield losses. Due to global warming the most important abiotic stress affecting maize crop production and productivity worldwide is moisture stress which in turn is becoming one of the topmost priorities in maize research programmes [1]. Assessment of genetic diversity in every crop species is important to understand variability and the pattern of genetic and evolutionary relationship between crop germplasm which further guides to sample genetic resources in a more systematic fashion for conservation and plant improvement [2]. In maize germplasm genetic relationships were morphologically characterized since 1970's [3]. But as morphological characters are more influenced by environment and have low heritability, mostly having stage specific expression, are less in number and less polymorphic [4] therefore, DNA markers are preferred in genetic characterization and diversity studies [5]. Among many marker systems, simple sequence repeats (SSRs) are considered as the markers of choice because of their abundance in the eukaryotic and prokaryotic genome, reproducibility, high variability, and uniform distribution in the genome. SSRs are PCR-based, codominant in inheritance and multiallelic marker system [6]. Several studies have extensively used molecular markers to assess the genetic relationship of genotypes in maize [7-17]. In maize many mapped SSR markers are available which can be utilized for molecular characterization. The objective of this study was to perform molecular characterization of a set of elite maize inbred lines using SSR markers. The purpose of this study was to identify inbred lines to develop a trait specific mapping populations for drought tolerance and later on to develop drought tolerant hybrids which would be able to cope up with the climate change conditions prevailing in the valley especially erratic rainfall pattern mainly during the critical crop stage (flowering stage) of the maize crop which results in appreciable yield losses.

*Corresponding author: Asima Gazal, Dryland (Karewa) Agriculture Research Station, Budgam, India, Jammu and Kashmir, India, E-mail: asimagazal@gmail.com

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Materials and Methods

Plant material – The experimental material comprised of a set of twenty four maize lines comprising fifteen drought promising inbred lines, four drought susceptible inbred lines and five drought tolerant checks. These inbred lines belong to AICRP (All India Co-ordinated Research Programme) Maize Srinagar Centre and the checks were selected from CIMMYT (International Maize and Wheat Improvement Centre) Mexico, AAU, Anand and MPUAT, Udaipur (Table 1).

DNA isolation

The extraction of plant DNA was carried out by CTAB (Cetyl-Tri Methyl Ammonium Bromide) method as described by Murray and Thompson (1980) with minor modifications from a pool sample of leaves from 15 seedlings. Fresh leaf samples (1000 mg) ground to fine powder in liquid nitrogen, were transferred to 50 ml centrifuge tubes containing 10 ml pre warmed CTAB extraction buffer (1M Tris-Cl, 0.5M EDTA, 2% CTAB, pH 8.0). Sample tubes were incubated in water bath at 65°C for 1 h with occasional swirling. Equal volume of chloroform: isoamyl alcohol (24:1 v/v) was added to the tubes and mixed thoroughly followed by centrifugation at 10,000 rpm for 10 min at 20°C. Aqueous phase was pipetted out and pre-chilled iso-propanol @ 0.6 volume of the content in tube was added followed by gentle inversion until fibrous mass was visible. Tubes were shifted at 4°C for overnight. Tubes were then centrifuged at 5000 rpm for 10 minutes at 4°C to obtain a precipitate. The supernatant was drained by gently inverting the tubes. The tubes were left inverted with lids open on blotting paper to drain the residual iso-propanol. After a while, the DNA pellet was washed twice with 70% ethanol, and kept overnight at room temperature for drying the pellet. 100-200 µl of TE buffer (pH 8.0) was added to dissolve the pellet. After 6-8 h RNase (10 mg/ml) was added to the DNA voil @ of 2 µl/100 ul of crude DNA. Mixture was incubated in a water-bath for 1 hour at 37°C with intermittent mixing.

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Table 1: List of inbred lines used in the investigation.

S. No.	Line	Institution
1.	KDM-361A	SKUAST-K, Srinagar
2.	KDM-932A	SKUAST-K, Srinagar
3.	KDM-961	SKUAST-K, Srinagar
4.	KDM-717	SKUAST-K, Srinagar
5.	KDM-463	SKUAST-K, Srinagar
6.	KDM-912A	SKUAST-K, Srinagar
7.	KDM-343A	SKUAST-K, Srinagar
8.	KDM-1051	SKUAST-K, Srinagar
9.	KDM-402	SKUAST-K, Srinagar
10.	KDM-918A	SKUAST-K, Srinagar
11.	KDM-1156	SKUAST-K, Srinagar
12.	KDM-1236	SKUAST-K, Srinagar
13.	KDM-372	SKUAST-K, Srinagar
14.	CM-129	IIMR, New Delhi
15.	KDM-331	SKUAST-K, Srinagar
16.	KDM-969	SKUAST-K, Srinagar
17.	KDM-741	SKUAST-K, Srinagar
18.	KDM-344	SKUAST-K, Srinagar
19.	KDM-375	SKUAST-K, Srinagar
20.	GM-6	AAU, Anand
21.	MahiDhawal	MPUAT, Udaipur
22.	H17	SKUAST-K, Srinagar
23.	CML-440	CIMMYT, Mexico
24.	CML-470	CIMMYT, Mexico

Primers and PCR amplification

A set of forty SSR primers (four for each chromosome), distributed uniformly on both arms of all the 10 chromosomes was used in the study (Table 2). The marker sequences were retrieved from www.maizegdb.org. After the initial screen, eight SSR markers which did not amplified were rejected from the experiment. Rest thirty-two SSR markers amplified and generated good and reproducible products for all the lines.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) was performed in a thermal cycler (Eppendorf, Hamburg, Germany) with thermal regimes given in Table 3. PCR reaction mix contained 25 ng of DNA, 10X PCR Hi Buffer A (100 mM Tris-HCl, pH 9.1, 500 mM KCl, 25 mM $MgCl_2$), 10 mM dNTP solution set (MolBio HIMEDIA), 5 pmol each of forward and reverse primer and 3 U/ µl of Taq DNA polymerase (MolBio HIMEDIA) in a reaction volume of 10 µl (Table 4).

Resolution of amplified PCR products

Gel electrophoresis is a widely used technique for the analysis of nucleic acids and proteins. Here we used the technique for size separation of amplified DNA. A concentration of 3.5% (w/v) agarose gel was prepared by dissolving 7 g of weighed agarose powder in 200 ml of 1XTAE [196 ml double distilled water + 4 ml of 50x TAE buffer (242.2g Tris base: Mwt. 121.14; 100 ml of 0.5M EDTA: PH 8.0; 57.1ml Glacial acetic acid: Molecular weight: 61.83 g; make volume to 1000 ml using de-ionized Milli-Q water)] in a conical flask. The suspension was heated in microwave oven for 6 minutes at 600 watt till clear solution was obtained. The solution was allowed to cool down and to this was added 8 µl (0.05 µl/ml of 1X TAE) of Ethidium Bromide stock solution (10 mg/ml of double distilled water). After gentle shaking, the gel was poured onto gel casting tray. After 15-20 minutes the gel was immersed inside the gel tank filled with 1X TAE (PH 8.0). To each PCR product of 10 µl was added 1 µL 6 loading dye (0.25% bromophenol blue; 0.25% xylene cyanol FF; 40% sucrose). With the help of 10 µl pipettes samples were loaded in individual wells. In parallel, was also loaded 50 bp size reference ladder (MolBio HIMEDIA). The power pack was adjusted at 5 Volts/cm of run and the total duration of electrophoresis varied from 1.5 to 2.5 hours. After optimum run of samples the gel slabs were visualized under UV trans-illuminator and documented in gel documentation system (Bio-Rad Laboratories Inc., USA). This was followed by scoring of bands with the help of 50 bp DNA size standard (MolBio HIMEDIA).

SSR data analysis

Scoring of the SSR alleles was performed manually in terms of positions of the bands relative to the ladder sequentially from the smallest to the largest-sized bands. Diffused bands or bands revealing ambiguity in scoring were considered as missing data and designated as '999' in comparison with the base pair reading in the data matrix. Genotypes showing two allelic bands with equal intensity were considered as heterozygous for the locus. Polymorphism information content (PIC) values for each of the primer was estimated using formula given by Goodman and Bird [18] i.e., PIC=1- , where Pij is the frequency of jth allele in ith primer and summation extends over 'n' patterns. Power Marker version 3.25[19] was also used to calculate the average number of alleles, gene diversity, and polymorphic information content (PIC) values. Based on the electrophoretic banding pattern of 32 SSR markers, pair wise genetic distance amongst genotypes were estimated and a dendrogram was generated using UPGMA clustering. Phylogenetic reconstruction was based on the neighbor joining method was conducted using computer software programme Dissimilarity Analysis and Representation for Windows (DARwin) 5.0 [20].

Results and Discussion

The summary statistics of marker related parameters are presented in Table 5. The 32 SSR markers produced as many as 271 alleles with an average of 8.43 alleles per locus in the 24 genotype panel. The average number of alleles per loci (6 to 13) obtained in the present study was higher considering the number of genotypes examined in this study. The results were in accordance with the previous report [15]. The heterozygosity level in the inbred panel was significantly low. The mean value of heterozygosity was 0.06 revealing that most of the loci attained homozygosity. However, for the loci umc-2372 (Figure 1) the heterozygosity was 0.50. The study indicated instances where the SSR primers for the inbreds displayed clear deviation from the unexpected pattern where inbreds are assumed to be highly homozygous and therefore are expected to reveal only a single amplification product (allele) per locus, at least for a large majority of the loci analyzed for the locus umc-2372. The presence of heterozygosity in the inbred lines might be due to a number of probable causes including residual heterozygosity, pollen or seed contamination, mutation at specific SSR loci, or amplification of similar sequences in different genomic regions due to duplication [21,22]. Since maize is a highly cross-pollinated crop, pollen or seed stock contamination during maintenance could be the most plausible explanation could be the residual heterozygosity which is not uncommon in maize. By virtue of its cross pollinated nature, maize maintains 5 to 10 per cent heterozygosity which is called residual heterozygosity. As a result of this, inbreds tend to segregate for a few loci/characters despite repeated cycles of selfing over many generations. Mutations at specific SSR loci, and

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Table 2: SSR primers selected for the study.

S.No.	SSR		Primer Sequence (5'-3')	Bin location	Annealing Temperature (˚C)
1	Umc2383	F R	CATAGACGTGCCCCTTGTCATC CTCGCAACTGCGCTTCTAGATACT	1.02-1.03	57.7 58.6
2	Umc1664	F R	AATTGTTTACTGCGCTGAAACTCC CCTCTTTGCCTGTACCGTGTATTC	1.06	57.5 56.3
3	Umc1147	F R	GAGAAACCATCGACCCTTCCTAAC TTCCTATGGTACAGTTCTCCCTCG	1.07	57.1 57.5
4	Umc2100	F R	AAAGGCATTATGCTCACGTTGATT TGACGTGCAAACAACCTTCATTAC	1.12	56.2 55.6
5	Umc2245	F R	GCCCTGTTATTGGAACAGTTTACG CGTCGTCTTCGACATGTACTTCAC	2.01	56.3 57.4
6	Umc1696	F R	CTAGGGTTTAACCAACGGGGAG TAAGGAGAGGGTCGATGAACACAT	2.1	57.3 57.4
7	Umc1823	F R	AAAGCCTTACTGTTATTAGGCTAGGCA AGAAAACCAGCCCCAGATGTTC	2.03	57.8 57.6
8	Umc1026	F R	TCGTCGTCTCCAATCATACGTG GCTACACGATACCATGGCGTTT	2.04	56.8 54.4
9	Umc2372	F R	ACCCCTTGCGTTCTCTTCTGTT CACCAGGCGTAGTGAGACAGC	2.06	57.6 58.3
10	Umc2144	F R	CCAGCCCCTATCTATTTGCTTGT GAATACTATATCACGGTCGGTCGG	2.08	57.0 56.4
11	Umc1594	F R	GCCAGGGGAGAAATAAAATAAAGC CACTGCAGGCCACACATACATA	3.09-3.1	54.4 57.3
12	Umc2071	F R	ACTGATGGTGTTCTTGGGTGTTTT ATACACGCAGTTACCCGAAGGTT	3.01	57.2 58.4
13	Umc2369	F R	TTCGTCTGATGAAAGGTTCAGAGG GATCCTCATCAAGACCAGCAGAGT	3.02-3.03	56.6 58.2
14	Umc1644	F R	CCATAAACTGTTCCTTTGGCACAC CTTTCACGTGTTAAGGGAGACACC	3.06	56.7 57.6
15	Bnlg1890	F R	ACCGGAACAGACGAGCTCTA GTCCTGCAAAGCAACCTAGC	4.11	57.6 56.6
16	Bnlg1621	F R	CTCTTCGATCTTTAAGAGAGAGAGAG ACACGAGGCACTGGTACTAACG	4.06	53.9 58.8
17	Umc1478	F R	GAAGCTTCTCCTCTCGCGTCTC CAGTCCCAGACCCTAGCTCAGTC	5.01	59.3 60.5
18	Umc1800	F R	TTATGGGTGCTGGTGATGTGTATC GAAAAGCAATCGCTTCTGAGAAAA	5.05	56.8 54.4
19	Umc1766	F R	ACAAGAAGGAATCGAGAGCAAATG CTTCGGGATGGAGTCGTAGTTC	5.01	55.5 56.9
20	Bnlg1306	F R	CACCTTGAAAGCATCCTCGT CAAAAACAAATGGCAGCTGA	5.07	55.2 52.2
21	Umc1918	F R	CACAAGAACATTATGACGACCGAG AAGCAGGAGACATCGTTTAAGTCG	6.03	55.7 57.0
22	Umc1762	F R	CTTACTCCAGGCACTCCATACCAT ATCCAGGTGAATGGTGTTTACGAT	6.06	58.1 56.4
23	Umc1063	F R	AGGCCACTGAGCAGGTGAAG GTGATGGTAGAGGAGTCCTTGGTG	6.07	59.7 58.6
24	Umc1018	F R	GAACGGATATTGGAACCTGTGC GTGCACGGTGTCGTACTTGAAC	6.01	56.1 58.4
25	Phi452693	F R	CAAGTGCTCCGAGATCTTCCA CGCGAACATATTCAGAAGTTTG	6.04	56.7 52.4
26	Umc1424	F R	CCGGCTGCAGGGGTAGTAGTAG ATGGTCAGGGGCTACGAGGAG	6.06	61.0 60.6
27	Phi129	F R	GTCGCCATACAAGCAGAAGTCCA TCCAGGATGGGTGTCTCATAAAACTC	6.05	59.3 58.2
28	Umc1002	F R	AGCTAGCTATACACCGCCAGG TCAGTTTGGAACAGGGAAAAGTA	6	58.6 54.2
29	Phi051	F R	GGCGAAAGCGAACGACAACAATCTT CGACATCGTCAGATTATATTGCAGACCA	7.05	60.3 58.3
30	Umc1036	F R	CTGCTGCTCAAGGAGATGGAGA GACACACATGCACGAGCAGACT	7.02	58.7 59.7
31	Umc1708	F R	GATATGTCGAGCTTCGCTGGAG CGCACACTAAAGCATCCTTAACCT	7.04	57.4 57.3
32	Bnlg1056	F R	ATCGTTGTTGGGTACACGGT ACGGGTAGTGGTGAAGATGC	8.08	56.7 57.1

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33	Umc1141	F R	AGAGGAGAAAGAGACAGACAGGCA CAGGAACTGAATGAAAGCAACTCA	8.06	59.2 55.6
34	Umc1415	F R	GTGAGATATATCCCCGCCTTCC AGACTTCCTGAAGCTCGGTCCTA	8.04	56.8 59.1
35	Umc1786	F R	ACCGTGACTTCCTCCTCATAACTG CATTTTTCGCATTTAGGAAATCCA	8.01	58.1 52.5
36	Phi067	F R	CTGCAAAGGTAAGCACTAGGATGCT CATCATTGATCCGGGTGTCGCTTT	9.01	59.0 59.7
37	Phi061	F R	GACGTAAGCCTAGCTCTGCCAT AAACAAGAACGGCGGTGCTGATTC	9.03	58.9 60.4
38	Umc1077	F R	CAGCCACAGTGAGGCACATC CAGAGACTCTCCATTATCCCTCCA	10.04	58.6 57.2
39	Mmc0501	F R	TGCTGAACACTCTAAGCAATAC ATTACTCTACTCGCTGCCTG	10.02	52.8 54.0
40	Bmc1655	F R	ATTAAAATCTTGCTGATGGCG TTCTGTTCCCGCCTGTACTT	10.03	51.6 56.2

Table 3: PCR thermal regimes and conditions.

Step	Reaction	Temperature	Time	Cycle
I	Initial denaturation	94°C	4	1
II	Denaturation	94°C	1	
	Annealing	55°C	1	35
	Extension	72°C	2	
III	Final extension	72°C	7	1

Table 4: The PCR reaction mixture.

Reagent	Stock conc.	Aliquot	Final conc.
DNA	25 ng/µl	1 µl	25 ng
PCR buffer	10x	1 µl	1x
dNTP mix	2mM	1 µl	0.2mM
Forward Primer	5 pM	0.5 µl	
Reverse Primer	5 pM	0.5 µl	
Taq DNA polymerase:	3 U/µl	0.2 µl	0.6 U
Milli-Q water	-	5.8 µl	-
Total		10 µl	-

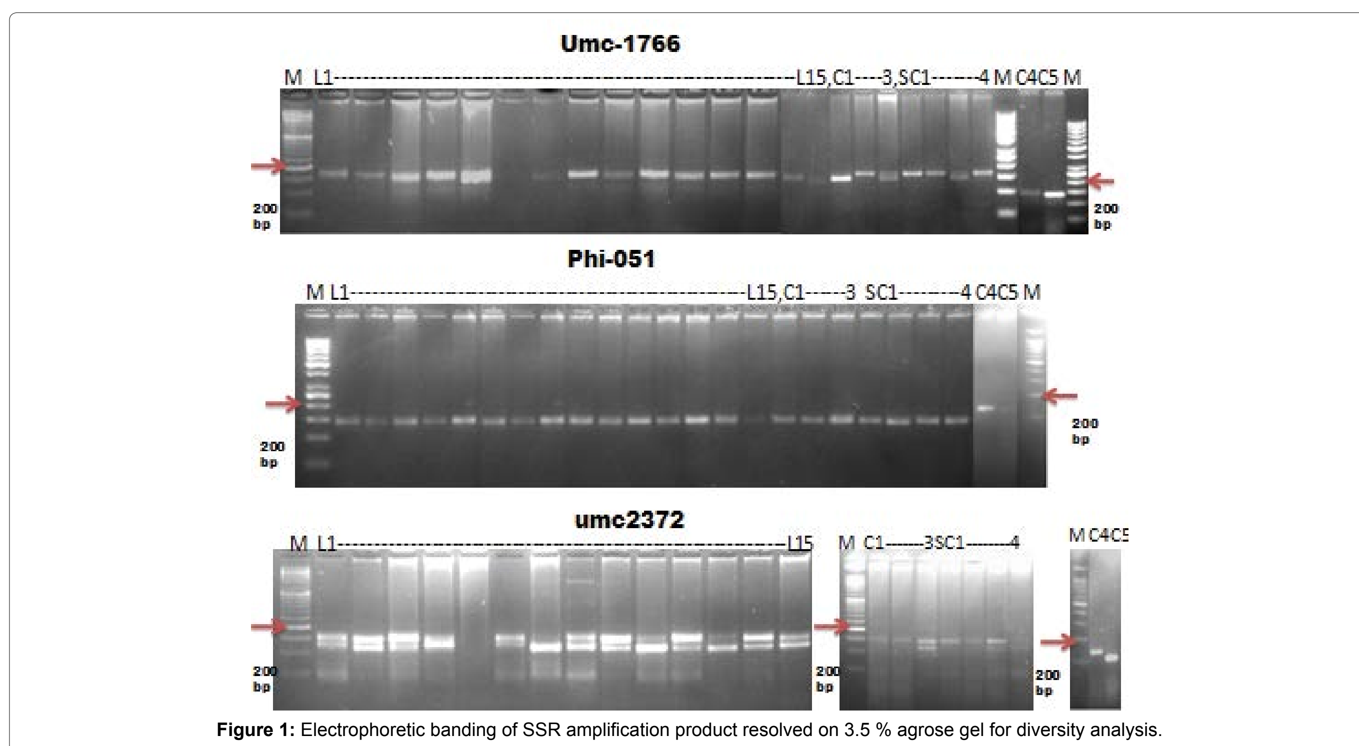

Figure 1: Electrophoretic banding of SSR amplification product resolved on 3.5 % agrose gel for diversity analysis.

Table 5: Allele frequency, alleles5 per locus, gene diversity, heterozygosity and PIC values obtained using SSR locus.

S.No.	Locus	Alleles per locus	Gene Diversity	Heterozygosity	PIC value	Major allelic frequency	Minor allelic frequency
1	Umc2383	7	0.854	0.000	0.837	0.21	0.05
2	Umc1664	10	0.878	0.125	0.779	0.32	0.05
3	Umc1147	8	0.823	0.000	0.835	0.29	0.04
4	Umc1823	10	0.853	0.167	0.802	0.29	0.04
5	Umc1026	7	0.833	0.000	0.820	0.30	0.09
6	Umc2372	10	0.825	0.500	0.563	0.43	0.04
7	Umc2144	12	0.861	0.333	0.735	0.39	0.06
8	Umc1594	9	0.855	0.042	0.843	0.22	0.06
9	Bnlg1621	6	0.798	0.083	0.742	0.31	0.06
10	Umc1478	7	0.778	0.000	0.744	0.36	0.05
11	Umc1766	7	0.799	0.000	0.799	0.33	0.04
12	Bnlg1306	12	0.891	0.083	0.875	0.16	0.05
13	Umc1918	9	0.837	0.250	0.662	0.37	0.05
14	Umc1762	8	0.740	0.042	0.740	0.43	0.07
15	Umc1063	8	0.857	0.042	0.824	0.23	0.05
16	Umc1018	10	0.854	0.000	0.854	0.21	0.04
17	Phi452693	9	0.859	0.042	0.837	0.26	0.04
18	Umc1424	7	0.868	0.000	0.851	0.18	0.09
19	Phi129	7	0.823	0.000	0.796	0.28	0.06
20	Umc1002	7	0.795	0.000	0.761	0.35	0.06
21	Phi051	7	0.701	0.000	0.701	0.42	0.04
22	Umc1036	8	0.766	0.125	0.738	0.33	0.04
23	Umc1708	11	0.889	0.000	0.889	0.17	0.04
24	Bnlg1056	7	0.677	0.000	0.708	0.50	0.08
25	Umc1141	6	0.799	0.000	0.757	0.38	0.05
26	Umc1415	6	0.809	0.000	0.771	0.33	0.05
27	Umc1786	11	0.844	0.000	0.898	0.13	0.06
28	Phi067	6	0.778	0.000	0.744	0.41	0.05
29	Phi061	7	0.819	0.000	0.793	0.32	0.05
30	Umc1077	11	0.855	0.042	0.875	0.18	0.06
31	Mmc0501	13	0.840	0.000	0.891	0.25	0.06
32	Bmc1655	8	0.817	0.042	0.773	0.38	0.05
	Total	271					
	Mean	8.46	0.821	0.060	0.788	0.30	0.05
	Range	6-13	0.891 - 0.677	0.000 – 0.500	0.563 – 0.898	0.13 - 0.43	0.04 - 0.09

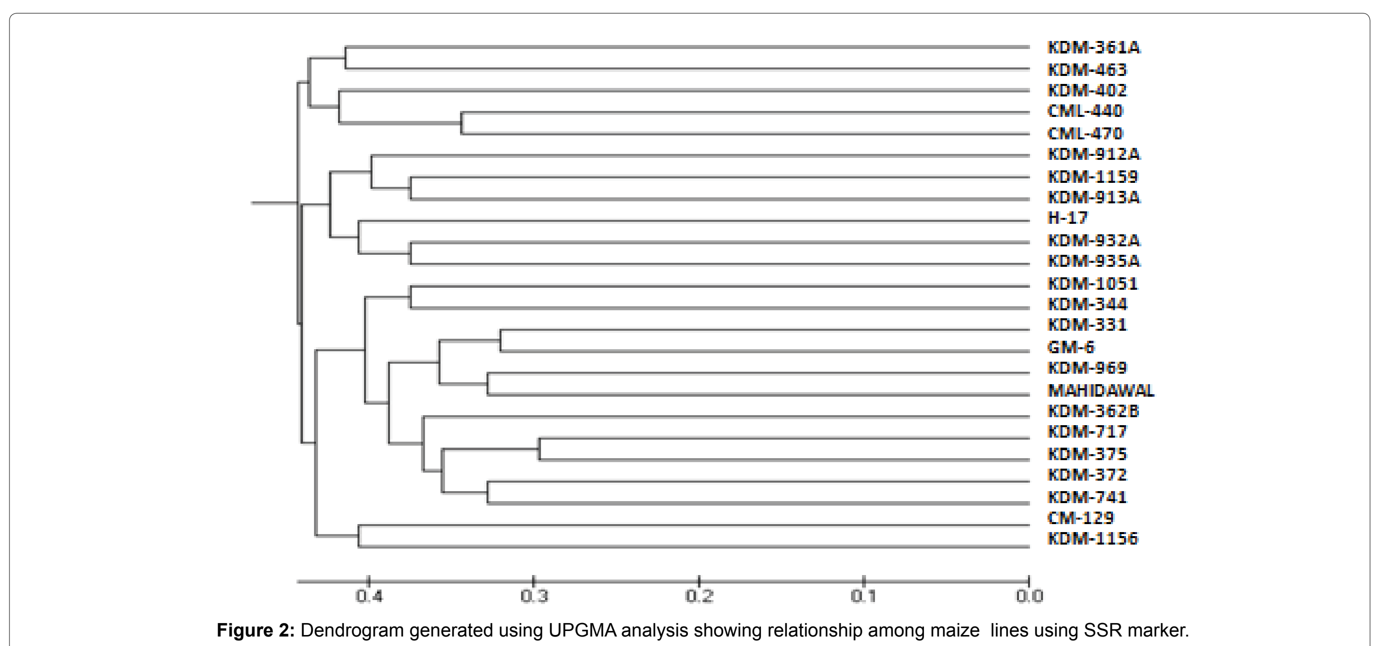

Figure 2: Dendrogram generated using UPGMA analysis showing relationship among maize lines using SSR marker.

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amplification of similar sequences in different genomic regions due to duplications possibly explains the occurrence of 'double - bands' [21] when analyzed with locus umc-2372. However, the low level of heterozygosity in the inbred lines revealed that they have been maintained properly and the reported heterozygosity was inherent.

PIC values are influenced by a number of factors *viz*., nature of germplasm, number of SSR loci as well as the inbred lines analyzed, SSR loci assayed, in terms of the nature and type of repeats and methodology employed for allele detection (PAGE versus agrose). The mean PIC value was 0.788 with locus umc-2372 having the lowest PIC value (0.563) whereas the locus umc1786 had the highest PIC value (0.898). Closely related lines would express lower PIC, whereas genetically diverse lines exhibit higher PIC values. Apart from being a different marker system which can detect polymorphisms, the ability to resolve the alleles also plays a crucial role in detecting the number of alleles. The mean PIC value was comparable with the previous findings [15,23], because of the nature of markers were chosen for polymorphism. PIC and alleles per locus indicated that selected primers were highly polymorphic and the degree of diversity among the lines was high and PIC was sufficient to group the population into different clusters. The results were comparable with the findings in Mishra et al. and Wasala et al. [24-26].

Genetic diversity is defined as the probability that two randomly chosen alleles from the population are different among the maize genotypes [19]. The maximum amount of gene diversity was exhibited by the marker Bnlg1306 (0.891) whereas, the minimum gene diversity of (0.677) was recorded for Bnlg1056. The mean value of the gene diversity was 0.821. Since the genetic diversity and PIC values recorded in these studies were within those recorded for genetic diversity studies in maize, these SSR markers are considered useful for genetic purity analysis of maize varieties. Major allelic frequency ranged from 0.13 - 0.43 with a mean of 0.30 whereas the minor allelic frequency ranged from 0.04 to 0.09 with a mean of 0.05 respectively.

The lines got grouped into two major clusters (Figure 2). One group had five lines viz., KDM-361A, KDM-463, KDM-402, CML-440 and CML-470. Another group was further separated into two clusters one having six lines viz., KDM-912A, KDM-1159, KDM-913A, H-17, KDM-932A and KDM-935A and second group had thirteen lines with further classification viz., KDM-1051, KDM-344, KDM-331, GM-6, KDM-969, Mahidhawal, KDM-362B, KDM-717, KDM-375, KDM-372, KDM-741, CM-129 and KDM-1156. The weighted Neighbor Joining (NJ) method (Figure 3) grouped the 24 lines into three major groups (a, b and c). The group A had thirteen lines and it was further divided into two subgroups. The second group (B) had five lines and the third group (C) had six lines. Two lines bred at CIMMYT for drought tolerance were grouped together with elite lines in group A. Group B had lines susceptible to drought stress. This study opened up a lot of possibility of development of hybrids tolerance to drought stress by exploiting the diversity pattern. The SSR-based clustering and the relationship among parental lines can be further used for development of new hybrids. Genetic diversity studies in maize to determine the genetic distance (GD) using various operational taxonomic units (OTUs) are a useful practice. From the genetic diversity analysis results of present study, maize inbreds lacking their pedigree data could be identified based on their GD to make hybridization between them to result in the development of a good hybrid.

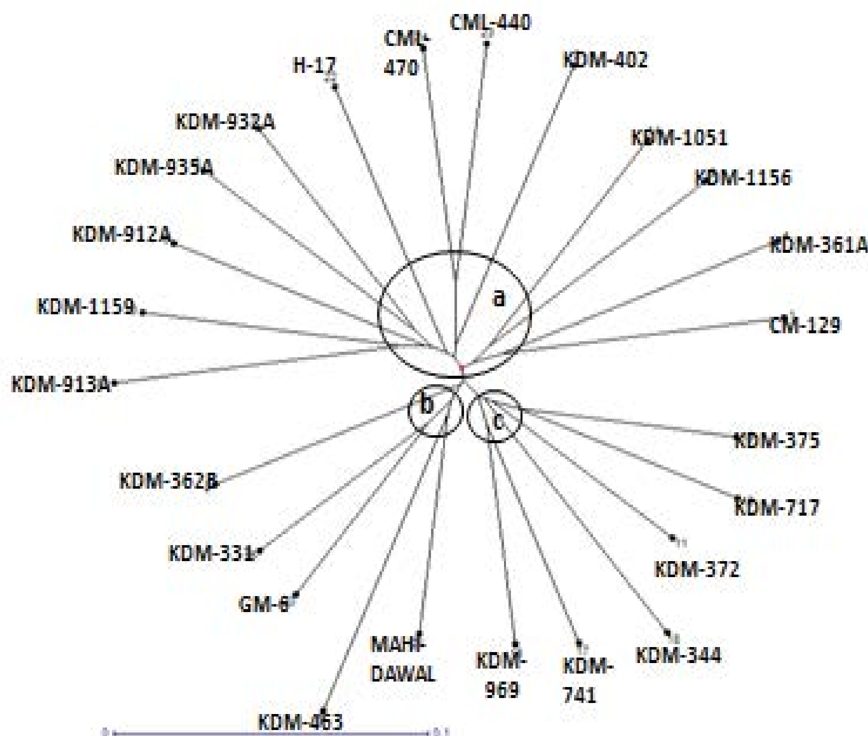

Figure 3: Neighbor joining NJ tree (Radial) generated using DARwin showing relationship among maize inbred lines using SSR marker.

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Author Affiliations

Top

[1]Dryland (Karewa) Agriculture Research Station, Budgam, India

[2]Division of Genetics and Plant breeding, SKUAST-K, Shalimar, India

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