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DNA FINGERPRINTING OF PAKISTANI MAIZE HYBRIDS AND PARENTAL LINES USING SIMPLE SEQUENCE REPEAT MARKERS

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Abstract

Maize is important cereal crop which is used a major source of dietary ingredients. Due to implementation of Plant Breeders Rights, plant variety protection is absolute necessity. Plant variety protection using morphological markers is not a reliable source due to heavy influence of environmental factors. However, DNA fingerprinting using molecular markers is reliable technique as these are unaffected by environment. Present study was carried out for DNA fingerprinting of 08 maize genotypes comprising of 03 hybrids and 05 parental lines using 209 Simple Sequence Repeat markers. Sixteen SSR markers were not amplified, 19 were found monomorphic and 174 were polymorphic. A sum of 1015 alleles was amplified and 783 were found polymorphic. Maximum number of alleles (21) was recorded for umc1676, maximum number of polymorphic alleles (16) were recorded for p-Phi008. Polymorphic Information Content values ranged from 0.0 (umc1179) to 0.94 (umc1676) with an average 0.67. Jaccard's similarity coefficient was used to construct a dendrogram based on unweighted pair group method with arithmetic mean and SAHN clustering. Maize hybrids and parental lines were classified to 03 clusters i.e. Group I, II and III on 96% similarity coefficient. DNA fingerprints were also developed for identification of maize hybrids as well as parental lines which will be useful for variety protection in future.

Key words: Clsuter analysis, Polymerase Chain reaction, Polymorphic information content, Variety identification.

Introduction

Maize (*Zea mays* L.) is an important cereal crop after wheat and rice which is used as human food, feed for animals, means for income and source of employment (Anon., 2010). It contains rich source of nutrients such as starch, vitamin A and B, proteins, oil, fiber and sugar. In Pakistan, maize is third major crop which accounts 4.8% of crop cultivated area and approximately 3.5% of agricultural output. Maize production has increased largely due to use of new and upgraded technologies. According to Plant Breeders' Rights Rules, 2018 of Pakistan, the varieties are differentiated on the basis of Distinctness, Uniformity and Stability (DUS) and examination of morphological characteristics. However, characterization merely on morphological basis is not sufficient as these characters may be offered because of environmental conditions, poor sampling and unknown genetic control (Ye-Yun *et al.,* 2005).

There is need of a robust and reliable technique to characterize, differentiate, purify and study the genetic variability among the cultivars in order to improve production and help in breeding programs. Biochemical analysis *i.e.* reversed-phase high performance liquid chromatography and electrophoresis of storage proteins in seed can also be used for the identification of hybrid (Asif *et al.,* 2006, Salgado *et al.,* 2006). DNA fingerprinting approaches using DNA markers are considered most effective genomic tools having high resolving power for hybrid/elite genotype identification (Perry 2004, Salgado *et al.,* 2006; Sadia *et al.,* 2018).

DNA fingerprinting is an effective method to help breeders in placement of breeding lines to accurate heterotic group, enabling to distinguish the hybrids at different stages of plant development and allowing comparison of the plants with control and its parental lines (Warburton *et al.,* 2002). Further, technique is not affected by environmental interaction and spatial-temporal expression. It employs the use of polymerase chain reactions (PCR) and is based on several types of markers. To understand, assess and elucidate the genetic diversity, variability and inter-relatedness among varieties, inter and intra species at molecular level, molecular markers have been proved to be the powerful tools. Various markers have been used to characterize the species; these include RAPD, AFLP, SSR, STS, SCAR, RAMP, ISSR, RFLP and SNPs (Pejic *et al.,* 1998; Warburton *et al.,* 2002; Reddy *et al.,* 2009, Iqbal *et al.,* 2019). Nowadays, microsatellites, also known as SSR markers, are widely accepted to be the best choice for characterization of varieties at molecular level, genome analysis and gene mapping in different species of crops such as barely (Liu *et al.,* 1996), maize (Legesse *et al.,* 2007) , sorghum (Agrama *et al.,* 2003), wheat (Salem *et al.,* 2015), rice (Rabbani *et al.,* 2010; Shah *et al.,* 2015; Singh *et al.,* 2016) datepalm (Zhao *et al.,* 2012), pearl millet (Chandra-Shekara *et al.,* 2007) and cotton (Bourgou *et al.,* 2017).

SSR markers are comprised of 2–5 nucleotides tandemly repetitive sequence that is randomly interspersed in eukaryotic genome. They are co-dominantly inherited, hyper-variable, highly polymorphic, highly reproducible, easily scorable, abundant and multi-allelic, reliable and exhibit cross specie transferability within and between species and populations (Rakoczy-Trojanowska *et al.,* 2004). In this study, 209 SSR markers have been utilized to generate genotypic data providing unique allelic profile in order to discriminate 8 maize cultivars. We have evaluated the degree of polymorphism of the markers using polymorphism information content (PIC) (Smith *et al*., 1997). Moreover, a standardize-able reference based on

DNA finger prints establishing unique genotypic identity was established. This study will be useful for maize variety/hybrids protection in future. Further this study has provided useful information about effectiveness of SSR markers for study of genetic diversity and DNA fingerprinting.

Materials and Methods

The current research was carried out at Biotechnology Laboratory, Agricultural Biotechnology Research Institute (ABRI) Ayub Agricultural Research Institute, Faisalabad, Pakistan, during 2018 to 2019. The germplas comprised of eight maize genotypes, of which 03 were commercial hybrids and 05 were parental inbred lines. The detail of hybrids and their parental lines is given in Table 1.

Table 1. Detail of plant material and its origin.

Origin	Hybrids	Male parent	Female parent
Maize & Millets Research Institute, Yusaf wala, Sahiwal	YH-1898	Y-27	Y-22
Maize Research Station, Faisalabad	FH-949	F-165	F-271
	FH-1046	F-165	F-308

Table 2. Chromosome wise detail of 209 SSR markers.

Chromosome No.	p-nc	p-Phi	Umc	Bnlg	Total
1	-	02	15	02	19
2	-	01	08	-	09
3	-	03	13	-	16
4	02	11	25	01	39
5	01	06	13	01	21
6	02	06	17	01	26
7	-	02	13	-	15
8	-	02	06	-	08
9	-	10	07	-	17
10	-	05	34	-	39
Total	**5**	**48**	**151**	**5**	**209**

Seeds of each variety were sown in pots in green house at 28°C following the standard agriculture practices. Each genotype was planted in 5 different pots wherein, each pot contained 2 seeds per genotype. After germination and seedling development till 3-4 leaves, 05 seedlings for each genotype were harvested and stored at -40°C until DNA was extracted. The genomic DNA was isolated using modified Cetyl Trimethyl Ammonium Bromide (CTAB) method of Salgado *et al*., (2006). DNA was quantified using Nanodrop spectrophotometer (ND 2000, Thermo Scientific, USA). DNA was considered pure when A_{260}/A_{280} ratio ranged between 1.80 and 2.0. The quality of extracted DNA was also assessed by loading DNA 20 ng/ µl on 0.8% (w/v) agarose gel stained with ethidium bromide. All the DNA extracts were stored at -40°C.

To obtain a well representative sampling of maize genome, a total of 209 evenly distributed SSR markers across the genome were selected on the basis of bin locations and repeating units and synthesized according to the sequence information retrieved from Maize Genome Database (http//:www.maizegdb.org/SSR.php) (Table 2). The selected markers belonged to four different series, p-nc, p-Phi, Umc and Bnlg. Each series contained 5, 47, 151 and 5 markers, respectively.

Entire genome of maize was covered such that maximum number of selected SSR markers was 39 from chromosome number 04 and 10 m whereas; minimum number of SSR markers was 9 that were screened from chromosome 02 (Table 2).

PCR was assembled using standard procedure. The reaction mixture (50 µL) comprised of 30 ng/µl genomic DNA, 25µl of 2X PCR Master Mix (DreamTaq Green PCR Master Mix of Thermofisher Scientific Catalogue No. K 1081), 0.6 µM of each forward and reverse primers and nuclease-free water. Negative controls without DNA were also included. The PCR reaction was carried out in Qantarus Thermal cycler under the following parameters: initial denaturation at 94°C for 5 min; followed by 35 cycles of 94°C for 1 min, annealing for 1 minute at variable temperatures according to the primers (Table 3), polymerization at 72°C for 1 min, finally, an elongation at 72°C for 7 min.The PCR Products were subjected to 6% Polyacrylamide (19:1 acrylamide: bis-acrylamide) Gel Electrophoresis (PAGE) for migration at 16 W, 300 V and 3000 mA using 0.5X TAE Buffer in vertical gel electrophoresis system. The amplified product was detected by silver nitrate staining. The gels were soaked in fixative (10% ethanol, 10% acetic acid) for 15 minutes followed by 08 minutes treatment on shaker with staining solution (1.75 mg/ml silver nitrate) and visualized by placing the gels in developer (12 g NaOH pellets and 08 ml 37% of formaldehyde solution dissolved in 800 ml d_3H_2O) until the bands were clearly observed. A 50 base pair ladder was used as a molecular weight marker and sizes of alleles was evaluated visually. The products of different sizes obtained from same primer were considered as different alleles. The gels were photo-documented under ultraviolet light on Gel Documentation System (Syngene).

The amplified fragments produced by the genotypes (hybrid and parental lines) were entered in binary form (0 and 1). Scoring of alleles was done by considering each band as an allele. Binary data was used for estimation of allelic diversity to find the total number of alleles per SSR, Polymorphic alleles per SSR and Polymorphic Information content (Smith *et al.,* 1997). Moreover, genetic similarity relationship was also analyzed using the NTSYSpc program version 2.0 (Rohlf, 1998). Jaccard similarity coefficients following unweighted pair group method with arithmetic mean (UPGMA) and SAHN clustering (Sneath and Sokal, 1973) were used to construct dendrogram. DNA bands that were unique in each hybrid/parental lines were recorded as DNA Fingerprints for identification of hybrids/Parental lines.

Table 3. Primers name, sequence and polymorphic status of SSR primers used in study.

Sr. No.	Marker Name	Forward Primer Forward Primer Forward Primer	Reverse Primer	PIC	Polymor. Alleles	No of Alleles	Ta	Chro. Loc
1.	p-nc004	TGCGAAGAAGCAGTAGCAAA	TGGAGGTAGAAGACGCACG	0.88	12	12	52	04
2.	p-nc005	CCTCTACTCGCCAGTCGC	TTTGGTCAGATTTGAGCACG	0.86	10	10	52	04
3.	p-nc007	ACTGTTCCACCAAACCAAGC	CTCCATGGAGAAGACGCG	0.80	0	5	52	05
4.	p-nc010	TGAGCTGACGACGAGCAG	CATTATCTGTTCGGCCCG	0.91	12	14	52	06
5.	p-nc012	TAATTTAAACACCACACCACCG	ACACACGCCAAAGAAAAACC	0.55	1	3	52	06
6.	p-phi001	TGACGGACGTGGATCGCTTCAC	AGCAGGCAGCAGGTCAGCAGCG	0.87	5	9	56	1
7.	p-phi006	AGGCGGCGTGCTGAACACCT	CGCTTCATCTCCCGTGACAATG	0.92	10	13	56	4
8.	p-phi008	CGGCTACGGAGGCGGTG	GATGGGCCCACACATCAGTC	0.92	16	18	56	5
9.	p-phi015	GCAACGTACCGTACCTTTCCGA	ACGCTGCATTCAATTACCGGGAAG	0.82	7	7	56	8
10.	p-phi016	TTCCATCATTGATCCGGGTGTCG	AAGGAGCAACATCCCATCCAGGAA	0.67	0	3	56	9
11.	p-phi017	CGTTGGCGACCAGGGTGCGTTGGAT	TGCAACAGCCATTCGATCATCAAAC	0.77	5	6	56	9
12.	p-phi019	TCCGCCTTTGTACCAATACAAGCCA	ATCCATCTTCAGGTAGCAGGGGT	0.79	7	8	58	4
13.	p-phi021	TTCCATTCTCGTGTTCTTGGAGTGGTCCA	CTTGATCACCTTTCCTGCTGTCGCCA	0.71	5	5	58	4
14.	p-phi022	TGCGCACCAGCGACTGACC	GCGGGCGACGCTTCCAAAC	0.66	4	5	58	9
15.	p-phi024	ACTGTTCCACCAAACCAAGCCGAGA	AGTAGGGGTTGGGGATCTCCTCC	0.50	2	2	58	5
16.	p-phi025	GCAACATCCTGGAGAGCCACTACAAGG	ACAGCCTGTTTTCCTGGACAGTGAACTC	0.68	2	4	58	6
17.	p-phi026	TAATTCCTCGCTCCCGGATTCAGC	GTGCATGAGGGAGCAGCAGGTAGTG	0.72	4	4	58	4
18.	p-phi027	CACAGCACGTTGCGGATTTCTCT	GCGTACGTACGACGAAGACAC	0.78	5	5	58	9
19.	p-phi028	TCTCGCTGTCCTTCGATTAGTACGG	AATGCAGGCGATGGTTCTCCGGCCT	0.77	2	5	58	9
20.	p-phi029	TTGTCTTTCTTCCTCCACAAGCAGCGAA	ATTTCCAGTTGCCACCGACGAAGAACTT	0.72	1	4	58	3
21.	p-phi031	GCAACAGGTTACATGAGCTGACGA	CCAGCGTGCTGTTCCAGTAGTT	0.89	10	12	56	6
22.	p-phi032	CTCCAGCAAGTGATGCGTGAC	GACACCCGGATCAATGATGGAAC	0.50	2	2	56	9
23.	p-phi033	ATCGAAATGCAGGCGATGGTTCTC	ATCGAGATGTTCTACGCCCTGAAGT	0.70	5	6	56	9
24.	p-phi044	TTATTGGTCCCTCTCCCGTCCCAGA	AGCATACCCCAATGGTCAACAGGGA	0.75	1	4	56	9
25.	p-phi048	GCAAACCTTGCATGAACCCGATTGT	CAAGCGTCCAGCTCGATGATTTC	0.50	0	2	56	5
26.	p-phi049	GATTGCGATAACATTGCGGCAAGTTGT	CTTCTGTTCCGCCATCCAGTATGTT	0.79	3	5	56	3
27.	p-phi050	TAACATGCCAGACACATACGGACAG	ATGGCTCTAGCGAAGCGTAGAG	0.50	0	2	56	10
28.	p-phi052	CAGAATGGGACGACAAGGTCATC	GGGACACTTCTAGCAGGATCTGTTT	0.75	0	4	56	10
29.	p-phi058	AGGTGCTGGACACAGACTTCAAC	ACTGAGATCCAGGCTCCTCTTC	0.66	3	4	56	5
30.	p-phi059	AAGCTAATTAAGGCCGGTCATCCC	TCCGTGTACTCGGCGGACTC	0.50	2	2	56	10
31.	p-phi061	GACGTAAGCCTAGCTCTGCCAT	AAACAAGAACGGCGGTGCTGATTC	0.71	4	5	56	9
32.	p-phi062	CCAACCCGCTAGGCTACTTCAA	ATGCCATGCGTTCGCTCTGTATC	0.66	3	3	56	10
33.	p-phi065	AGGGACAAATACGTGGAGACACAG	CGATCTGCACAAAGTGGAGTAGTC	0.64	3	4	56	9
34.	p-phi070	GCTGAGCGATCAGTTCATCCAG	CCATGGCAGGGTCTCTCAAG	0.49	1	2	56	6
35.	p-phi071	GGAGTTCATCAGCTACCCCATCT	TTCTGCTTGTTGATCTGCACCCAC	0.40	1	2	56	10

Table 3. (Cont'd.).

Sr. No.	Marker Name	Forward Primer Forward Primer Forward Primer	Reverse Primer	PIC	Polymor. Alleles	No of Alleles	Ta	Chro. Loc
36.	p-phi072	ACCGTGCATGATTAATTTCTCCAGCCTT	GACAGCGCGCAAATGGATTGAACT	0.81	6	7	56	4
37.	p-phi073	GTGCGAGAGGCTTGACCAA	AAGGGTTGAGGGCGAGGAA	0.92	6	13	54	3
38.	p-phi074	CCCAATTGCAACAACAATCCTTGGCA	GTGGCTCAGTGATGGCAGAAACT	0.80	4	6	56	4
39.	p-phi075	GGAGGAGCTCACCGGCGCATAA	AAAGGTTACTGGACAAATATGCGTAACTCA	0.79	5	5	56	6
40.	p-phi076	TTCTTCCGCGGCTTCAATTTGACC	GCATCAGGACCCGCAGAGTC	0.61	4	4	56	4
41.	p-phi078	CAGCACCAGACTACATGACGTGTAA	GGGCCGCGAGTGATGTGAGT	0.84	7	8	56	6
42.	p-phi079	TGGTGCTCGTTGCCAAATCTACGA	GCAGTGGTGGTTTCGAACAGACAA	0.86	8	8	56	4
43.	p-phi080	CACCCGATGCAACTTGCGTAGA	TCGTCACGTTCCACGACATCAC	0.50	2	3	56	8
44.	p-phi081	AAGGAACTGGTGAGAGGGTCCTT	AGCCCGATGCTCGCCATCTC	0.76	5	5	56	6
45.	p-phi082	CACAGCACAGGCAGTTCG	CGCGGCAAAAGATCTTGAACACCT	0.76	5	5	54	7
46.	p-phi083	CAAACATCAGCCAGAGACAAGGAC	ATTCATCGACGCGTCACAGTCTACT	0.00	0	1	56	2
47.	p-phi084	AGAAGGAATCCGATCCATCCAAGC	CACCCGTACTTGAGGAAAACCC	0.80	0	5	56	10
48.	p-phi085	AGCAGAACGGCAAGGGCTACT	TTTGGCACACCACGACGA	0.90	14	14	54	5
49.	p-phi092	GTGGGGGAGCCTACTACAGG	GACGAGGCCATCATCACGGT	0.83	6	7	54	4
50.	p-phi093	AGTGCGTCAGCTTCATCGCCTACAAG	AGGCCATGCATGCTTGCAACAATGGATACA	0.66	2	4	56	4
51.	p-phi095	CCGATCGGCTTTATCACTGTTTAGC	ATGCACCATTCTAGCACTATAGCAACACT	0.84	4	8	56	1
52.	p-phi096	TCCACCATTTGACACTTAGGCA	GCGTAGGACGACCGTTGAA	0.83	8	8	54	4
53.	p-Phi113	GCTCCAGGTCGGAGATGTGA	CACAACACATCCAGTGACCAGAGT	0.22	2	2	56	5
54.	p-Phi114	CCGAGACCGTCAAGACCATCAA	AGCTCCAAACGATTCTGAACTCGC	0.82	4	7	56	7
55.	bnlg118	CTTCCAGCCGCAACCCTC	CCAACAACGCGGACGTGA	0.91	13	14	54	5
56.	bnlg490	GCCCTAGCTTGCTAATTAACTAACA	ACTGTAAGGGCAGTGGACCTATA	0.99	2	2	54	4
57.	bnlg1124	TCTTCATCTCTCTATCAAACTGACA	TGGCACATCCACAAGAACAT	0.59	3	3	52	1
58.	bnlg1136	TAACCGGATGAGCATCTTCC	CATCAGCTTCAACGAGTTCG	0.76	5	5	54	6
59.	bnlg1429	CTCCTCGCAAGGATCTTCAC	AGCACCGTTTCTCGTGAGAT	0.71	4	4	54	1
60.	umc1002	AGCTAGCTATACACCGCCAGG	TCAGTTTGGAACAGGGAAAAGTA	0.85	8	8	54	6
61.	umc1003	AATAGATTGAATAAGACGTTGCCC	TGTTCCAATGCTTTTGTACCTCTA	0.93	4	4	54	2
62.	umc1006	AATCGCTTACTTGTAACCCACTTG	AGTTTCCGAGCTGCTTTCTCT	0.00	0	1	54	6
63.	umc1008	TCTAGCTTGTGGTGGTGGTTGA	ACATGAGCACAAAGACTGACGC	0.74	4	5	54	4
64.	umc1009	AGCAGCTCTGGTGATGGAAGAA	ATCCTAACAGGCGCATACCAGA	0.50	2	2	54	1
65.	umc1010	TCCATGTATGTGTGTGTGACGTG	AAACCAAACAGCCAAAAGGACA	0.66	3	3	54	3
66.	umc1014	GAAAGTCGATCGAGAGACCCTG	CCCTCTCTTCACCCCTTCCTT	0.85	7	10	56	6
67.	umc1016	GTGATACCGGGTAATCTGGTGC	GATGATGGGTGATCATCGGTTC	0.81	5	6	56	7
68.	umc1017	GAAGAGGTAAGGACGACGACGA	GCACCTGCAGTGAACGTCAGTA	0.86	8	9	56	4
69.	umc1018	GAACGGATATTGGAACCTGTGC	GTGCACGGTGTCGTACTTGAAC	0.83	4	7	56	6
70.	umc1019	CCAGCCATGTCTTCTCGTTCTT	AAACAAAGCACCATCAATTCGG	0.79	7	7	54	5

Table 3. (Cont'd.).

Sr. No.	Marker Name	Forward Primer Forward Primer Forward Primer	Reverse Primer	PIC	Polymor. Alleles	No of Alleles	Ta	Chro. Loc
71.	umc1020	CCTGGAGAGCCACTACAAGGAA	TCAGCCTGAGCTCACATCATCT	0.81	7	7	56	6
72.	umc1022	AACAAGTTTTGTTTGACAAGCCG	ATGATCACCCCGTCAGCG	0.79	3	7	56	4
73.	umc1023	CTTGTGCCACCACATGCAGTA	CAGTTTGGAACAGGGAAAAGTACG	0.67	3	3	56	6
74.	umc1035	CTGGCATGATCACGCTATGTATG	TAACATCAGCAGGTTTGCTCATTC	0.84	6	7	56	1
75.	umc1045	GCTCGTCCATGAGCAGCATC	AAGCTGAAGATGCGGAGGTTG	0.80	6	7	56	10
76.	umc1048	TGCGCTGTTTCCTACTCAGACTAA	AAGACAAGTCCAGTGACGAAGAGC	0.00	0	1	56	5
77.	umc1050	CGATACACATCCATCTTCAGGTAGC	GCCTTTGTACCAATACAAGCCAAG	0.45	3	3	56	4
78.	umc1051	CTGATCTGACTAAGGCCATCAAAC	AATGATCGAAATGCCATTATTTGT	0.88	10	10	54	4
79.	umc1053	CTTGTATCATCAGCTAGGGCATGT	TCAACTTATGTCAACTGCATGCTT	0.82	7	7	54	10
80.	umc1054	CCGTCTTCTTCAGGGTGTTCC	GTGGAGTTAGTAGGGTCGTTGCAC	0.77	3	5	56	10
81.	umc1056	CGGATCGCTTTTTACCGTCTATAA	AGCAAGAGTAGCGTTCCATTTCAG	0.61	3	3	56	5
82.	umc1057	GCCACGCTCAACTACGACAAC	GAACCCCTCCACGTAGCTCAG	0.49	1	2	56	3
83.	umc1058	AGCAAGCAGTTCGAAACAAGGAT	GACACCAGCACCACTTGAACG	0.69	4	4	56	4
84.	umc1061	AGCAGGAGTACCCATGAAAGTCC	TATCACAGCACGAAGCGATAGATG	0.24	2	2	54	10
85.	umc1062	GGGATACATGTTCATCATCAGCAG	GTGTTGAGCGGATTCGGATACTAC	0.64	2	3	54	3
86.	umc1063	AGGCCACTGAGCAGGTGAAG	GTGATGGTAGAGGAGTCCTTGGTG	0.83	7	9	56	6
87.	umc1065	ACAAGGCCATCATGAAGAGCAGTA	CACGGTCTGGCACACTAACCTTAT		Not Amplified		56	2
88.	umc1067	ACTTGTACTACGCAGGACAGTTCG	AGCCTCTGTCTGGATGACTGAAC	0.79	4	6	56	4
89.	umc1068	AGTCGTTTTCAAAGGCTGCTGATA	TGAGTCACCTCATTTCTTCTGGTTC	0	0	1	54	7
90.	umc1069	AGAGAATCCCCAAGCAAACAAAC	CTTCATCGGAGCCATGGTGT	0.63	2	3	54	8
91.	umc1082	CCGACCATGCATAAGGTCTAGG	GCCTGCATAGAGAGGTGGTATGAT	0.82	4	6	56	1
92.	umc1088	TCATCCTCCTAGCTCCTCTACTCG	AAAACAGTCAGCAGAACCCACTTT	0.91	8	12	56	4
93.	umc1094	GCTACTCTCGTGGACTGGTGGT	TGAAGGCTTAGTGGTGATCCGT	0.80	0	5	55	9
94.	umc1095	ATCCCTCGTTGACGATACTAGCTG	CAGATGCATGCACCCATTAGAGT	0.84	4	7	55	7
95.	umc1103	AATTTTCAGGGTGTTACGTGGATG	TGGAGAGAGCTCAACTGGTAGCTT	0.55	2	3	55	7
96.	umc1113	ATCATGCGTCATCACTCTCAGAAC	GCTGGAGCTAGCTGTAGTGTAGCA	0.70	3	4	55	10
97.	umc1115	TGGAAGGGGATATCAGGATTTAGA	TGTGATGACCATGAATGTAAGCTG		Not Amplified		55	10
98.	umc1124	GAAAGGAATCTTTCAGCTCACACC	ACCTGGCGAGCAGTAGCAGTAG	0.83	7	7	56	1
99.	umc1140	GCCTCTACCACCTCGTCCATC	CGAGCAAAGAGAGGGAGAGAGA		Not Amplified		56	3
100.	umc1143	CGTGGTGGGATGCTATCCTTT	GACACTAGCAATGTTCAAAACCCC	0.74	3	5	56	6
101.	umc1144	ATGGCCCACTCATCATATCTCTGT	TGTGTTGATTAGCAGCGGATAAAA	0.80	5	6	52	1
102.	umc1152	CCGAAGATAACCAAACAATAATAGTAGG	ACTGTACGCCTCCCCTTCTC	0.69	4	4	56	10
103.	umc1158	CGACGAATCGAGAAAAGATATTTGA	AATGCAACTGCTTCAGCTCCTACT	0.50	0	2	56	3
104.	umc1161	GCTCGCTGTTGGTAGCAAGTTTTA	GGTACCGCTACTGCTTGTTACTGC	0.32	1	2	56	8
105.	umc1164	AAATAAACGCTCCAAAGAAAGCAA	GCACGTGTGTGTGTGTTGTTTTTA	0.75	0	4	52	4

Table 3. (Cont'd.).

Sr. No.	Marker Name	Forward Primer Forward Primer Forward Primer	Reverse Primer	PIC	Polymor. Alleles	No of Alleles	Ta	Chro. Loc
106.	umc1165	GTCGATTGATTTCCCGATGTTAAA	TATCTTCAGACCCAAACATCGTCC	0.88	11	12	54	2
107.	umc1167	CCTGCATGCATTAGGTATACGAAG	GTTTCTTCCAAGTTTTTGGCTTGA	0.21	1	3	52	3
108.	umc1169	CAGGCTAGAATAACATCCCGAAGA	TAGCCAACAGTCCAACATTTTTCA	0.88	10	11	54	1
109.	umc1171	ACGTACTACAGATAATGGGCGACG	CGCCGTACCCATGAGTATAATGTAA	0.70	2	4	54	5
110.	umc1172	ATGAAGCAGAGGCAGTCTTTCTTG	CTCCTCCATCCAACACTGAACC	0.72	4	4	54	8
111.	umc1173	ATCCGCCAAAAAGGGGAAAA	TAGAAGTAGCACACGCGCCG	0.88	3	9	52	4
112.	umc1178	CTGTCGTAAGAGCGCCAACAG	GTCTGAACGATGAACAGTACACGC	0.50	2	2	54	6
113.	umc1179	AGCTCCCCATCTTAATCCGTAAA	ATTGAGCTCGGCGTAGAAAAA	0.00	0	1	52	10
114.	umc1183	GCATGTACACAACACAACCTTTCA	ATGTCATTTTTGGCTTCTCGAAAT	0.78	4	6	52	3
115.	umc1186	TCAAGAACATAATAGGAGGCCCAC	AGCCAGCTTGATCTTTAGCATTTG	0.72	4	5	54	6
116.	umc1187	ATGTGCATGCTCTTGTTCCTATCA	AGAGCTACCACCCCTCTTATCCC	0.00	0	1	55	6
117.	umc1196	CGTGCTACTACTGCTACAAAGCGA	AGTCGTTCGTGTCTTCCGAAACT	0.77	5	5	55	10
118.	umc1197	GGTGTAATTTAGGGAGTGTTTGTTCG	CCGCATAGATGTGCTTTCTAGGAG		Not Amplified		55	4
119.	umc1202	CACCATGACCACCAGCTTCAC	AAGAAGAAGAAGATGGCGACACTG	0.76	4	5	55	8
120.	umc1203	ATGGCTGGAGAACCTAGTGTGTTG	GCTTGTCTCCTCCAGCAGAAGTT	0.85	7	8	55	3
121.	umc1207	GGTGAAGGAGAAGGCGGAGTAT	CACTGGATCACCACACCAACAT		Not Amplified		55	2
122.	umc1209	CCAGCTAGTCGTCGTAGCCAAG	GTCGTCGACCACTACGTCCAC	0.69	1	4	55	3
123.	umc1213	GTACGTCCACCCCGTGCTCT	CACGCTCGATCACTGAAGCAT	0.72	5	5	55	7
124.	umc1215	CAACCAAATTCAGGGGTTACCT	CCCTCGCCTAATTCACTTACTTTT		Not Amplified		55	6
125.	umc1216	TTGGTTGTTGGCTCCATATTCA	GTTATATGCCCGTGCATTGCTA	0.80	5	5	55	7
126.	umc1219	AGTCGTCCAAGGGAGGCAAT	ACGCCTTTCTGGGTTTGCTT		Not Amplified		55	3
127.	umc1232	GGAATTACCACAACAAACTAAACTTGG	AGGCTCTAGCTACCTGGCTACGTT	0.86	8	11	55	4
128.	umc1239	ATCAACACACCTTTCGATTTCTGG	CGGTGATTAGTCGATGAAGAGTGA	0.77	2	5	55	10
129.	umc1241	TGAAGCAAGTCACTGGTAAGAGCA	TGACACACCCATACTTCCAACAAG	0.63	2	4	55	7
130.	umc1246	TCGAGTTTGCTTCTCTCCAGTTTC	TGCAGCATATGGCTCTTTATTCAA	0.67	1	3	55	10
131.	umc1249	GACCAGCAGCACTAGAGGACATTT	CTTCTGTTACTTTGGCAGCGGTT	0.74	2	4	55	10
132.	umc1255	GGACTACATCACGCCGGAGAT	TTTGGGAGAACAATCGGTTCTGTA	0.83	6	6	55	4
133.	umc1266	CACAGGTAAAAGTAAACGCACACG	CTCGTCATTTTCAACGTCCTCTTT	0.67	0	3	55	3
134.	umc1269	TATATTAGAGGCACCTCCCTCCGT	AGCTGCTTCAGCGACTTTGG	0.00	0	1	55	1
135.	umc1272	CTCTGACAGACCTGCAGATAGGGT	ATCGAGGGGCTAATCAGCAAG	0.67	4	5	55	10
136.	umc1276	CTACCTTGTTCCTAGGGCCGTCTA	ACGCAATTATTACTGCCACACGTC	0.00	0	1	55	4
137.	umc1280	AACAGCCAGTTTTGGGCTGTATAA	AAAATCCATGGCTTCTTTCTTTCC	0.73	4	5	55	10
138.	umc1287	AGAAGGAGGCCCACTACGAGAG	ATGGGATGATCAGTCGTTTCAGTC	0.00	1	1	55	8
139.	umc1291	ACTGCTCCAGGGTGAACTGAAC	CAAGTCGTGATCATGCGTAGGTAG	0.22	2	2	56	10
140.	umc1293	GTATCCGTTTCTCATGCAACACAC	GATCTCGATCTGCTTCATCATCTG	0.80	6	6	55	10

Table 3. (Cont'd.).

Sr. No.	Marker Name	Forward Primer Forward Primer Forward Primer	Reverse Primer	PIC	Polymor. Alleles	No of Alleles	Ta	Chro. Loc
141.	umc1298	AGGACAAGAAAAAGAAGAAGCACG	AGCTGAACAAAATAAACGGAACGA		Not Amplified		55	1
142.	umc1299	CGCTACAAACAAGTGGCGTTTAAT	CTTGGGTTCTTCTCTCCTATGGGT	0.63	3	3	55	4
143.	umc1312	AAAGTTACTGCTGCCAAAGCTGTC	AGATCGAGCGGTGGATATGGT	0.47	1	2	55	10
144.	umc1318	ACTTCGTCTAGTGTCCCTCCGTT	TGCCAGATTAAAAGCAACACAAGA	0.86	9	9	55	10
145.	umc1319	TGAGAGCCACCTTCTTGAGCTACT	TTCCTTGAAGGCGAAGGTAGGTAT	0.62	3	3	55	10
146.	umc1328	TACAAGGAGGAGGCCGCTGT	ATCCAGTCTCCGGACTTCCAAC	0.73	3	5	55	4
147.	umc1330	AGCAAAGAAGCCAAAGAGCAACT	GTCACCACCGTCTGCTGGTA	0.32	1	2	55	10
148.	umc1336	GTACAAATGATAAGCAAGGGGCAG	CTCTGTTTTGGAAGAAGCTTTTGG		Not Amplified		55	10
149.	umc1337	TGGATCTTTTATTTATGTTTTTATTCGG	CTGCCTGTAACGAATATGAATGC	0.75	4	4	55	10
150.	umc1341	GTCTACCAGGACGTTTACCTGTGG	CCTCAATCCTTTGTGGACAAACAC	0.44	1	2	55	10
151.	umc1343	CTTCCTGGCACGTAACATTACCAT	ATCGGTCTTCCAACCAGATCATTA	0.81	3	6	55	6
152.	umc1344	GCGCTCTGACTTAATTAGAGGAGTTG	GGCAGCAGATCTATGTCCAAGAAG	0.00	0	1	55	8
153.	umc1358	AGAACCTCCCGCTTGACGAC	ACCTCAACCTCGACCTCTGCAT	0.81	7	7	55	10
154.	umc1359	GCAGAGCCAGAATTCGACCTT	CATCGTCATCATTCGAGCAGAG	0.64	3	3	55	1
155.	umc1380	CTGCTGATGTCTGGAAGAACCCT	AGCATCATGCCAGCAGGTTTT	0.68	2	4	55	7.
156.	umc1402	TACACGCAGCTCTGGGTTTTG	GTGATCCGGGTAGAGGAATGTG	0.75	4	4	55	10
157.	umc1423	TAGTATGGTCCATTGATGCTGGC	GAGCAGGCGGAGGATACTAGC		Not Amplified		55	5
158.	umc1453	AATACCAAGCTGCACTCAGAAACC	CGTCAAATCCAGCCTAAGCATC	0.79	1	5	55	10
159.	umc1466	CGAATAGTGGTCTCGCGTCTATCT	GATCCACTAGGGTTTCGGGGT		Not Amplified		55	4
160.	umc1482	GAACAAAGAATCACAACACGATGC	CAGGTTCTGAGGAAAGCAAGGTT	0.86	10	10	55	5
161.	umc1491	GATTTGAGGCCATAGTGCTCCTTA	TAATAATCCCAAACCACCAAAAGG	0.73	4	4	55	5
162.	umc1496	GATTACAACCCACCGGAGTTACAG	GCTCTTCCTAGGTGCAGACAAAGA	0.60	3	3	55	5
163.	umc1498	AACGTCCATTCGCTTGTCTACATC	GATGGTGTCCATATCCATATCCGT	0.80	3	7	55	6
164.	umc1505	TTACACAGAAGCCCATTTGAAGGT	GGATGGTTGTTGGTGGTGTAGAAT	0.60	3	3	55	9
165.	umc1506	ATAAAGGTTGGCAAAACGTAGCCT	AAAAGAAACATGTTCAGTCGAGCG	0.76	5	5	55	10
166.	umc1507	GATTCAAACCAAACACTTTTCCCA	CGAACCTTGCTGTGTGTTTATCAG	0.60	4	4	55	10
167.	umc1509	CTTTCTGCAGATTCACCGTTTCTT	TTGGTTCTTTTGACCATAGACAAGC	0.54	3	3	55	4
168.	umc1511	CAGACAGATCCATCCAGCACATAC	GTTTGTAGGCTTCGTTTTCCTTCA	0.50	1	2	55	4
169.	umc1515	AGAGAGGCTGCTTCAATAAGTTGC	TTAGTAGTTTCGGTGTCCGTTTCC	0.79	5	6	55	1
170.	umc1535	CAAGGCACCCACACACATACATA	GGCAGAGAGATGAAAAAGAATGGA	0.71	4	5	55	2
171.	umc1537	CATGAATCACACTTGGATGTGGTC	AGAAGCTGTCCTCGTTCAAGCTC	0.74	2	4	55	5
172.	umc1539	GAGCAGCACACGAGGACCAG	GAGTCCAGGCAGCACGCTAGT	0.85	10	11	55	3
173.	umc1542	TAAAGCTATGATGGCACTTGCAGA	CATATTTGCCTTTGCCCTTTTGTA	0.73	5	5	55	2.
174.	umc1545	GAAAACTGCATCAACAACAAGCTG	ATTGGTTGGTTCTTGCTTCCATTA	0.66	5	5	55	7
175.	umc1546	GTCACAGCAAAGTCATCCTCCTCT	CTGGTCTTGGCCTTGGACTTCT	0.90	12	15	55	7

Table 3. (Cont'd.).

Sr. No.	Marker Name	Forward Primer Forward Primer Forward Primer	Reverse Primer	PIC	Polymor. Alleles	No of Alleles	Ta	Chro. Loc
176.	umc1550	GTGCCTCCAACGCCTAGTTTTT	CGGGGTAATTGGGTACATAACCTC	0.00	0	1	55	4
177.	umc1564	AAGAAGAAAGAGAAGAAGCACGGG	GGACAGCTCGTATTATAACCTGCG	0.82	5	8	55	5
178.	umc1566	ATCTCGTCTACCTAACCCACCCTC	CAGGTGAAGAATCTGGTGAGGTC	0.76	7	8	55	1.
179.	umc1568	AAGTCCAGCCAAGTTCATCAAAGA	ACTGTAACTAAACTGGGTGTGCCC	0.72	5	5	55	1
180.	umc1570	GTCGTAGAGGTGGTGCTGCTG	CAGGAGATGATGAGCGGGAG	0.84	7	7	55	9
181.	umc1571	CACCGAGGAGCACGACAGTATTAT	GCACTTCATAACCTCTCTGCAGGT	0.80	5	5	55	9
182.	umc1572	AATCCTTCTCTGCGTCCTTTCTCT	CAAGGTGTCCTTGGTGTGTATCAG	0.69	4	4	55	6
183.	umc1573	ACGACGTCGGTACTTGCTGG	GTCCCTCCTCCTGCACACAC	0.78	5	5	55	4
184.	umc1574	GTCATGCAAGTATCCGCTGTCTT	TTTCATGTGCTTGCAGAGTTTGAC		Not Amplified		55	4
185.	umc1575	GAGTCGAGACTGCCGTCCTTC	GCCTAGACGTCATGGACAACG	0.62	4	5	55	5
186.	umc1576	CTCGTCATCCTTTCTGCAGTGTAT	TGTACAAAATTACAAGGTGGCAGC	0.55	2	3	55	10
187.	umc1577	AAGAACTCCTTCAAGCTGCCG	TTTCCCTTCTTGGCAGGAGC	0.67	3	4	55	7
188.	umc1582	GTGCGTGTGAGAGTGATATCGAG	AGATTACGTAGCCACGCTTATTCG	0.64	4	5	55	10
189.	umc1585	AAGGGAAAGAATAATCCAACCGTC	CGGCCTATGTAACAATCCCTAGC	0.85	8	9	55	7
190.	umc1588	GGATGAAGCAAACCAAGCACATAC	TGACAACAGCTATGTGTCTGCTCC	0.75	4	5	55	9
191.	umc1589	CAGGTGGTAGTTGAGCTTGGTCAC	CAGGATCACTTTGCGATACATCCT	0.75	1	4	55	10
192.	umc1594	CACTGCAGGCCACACATACATA	GCCAGGGGAGAAATAAAATAAAGC		Not Amplified		55	3
193.	umc1595	CGCTTGAAATGGAAAGGTAGAAAG	GCTGCTGGTCTACAACCTCTTGTT		Not Amplified		55	6
194.	umc1610	CGTCTCCTCCATCTCTACTCGTTC	CTTGAGATCTGTGGCGTCGTC	0.79	5	5	55	4
195.	umc1614	TCACTTGCATGAGCAACTTCAGTA	GAGCTACTCAGCCAAGACGAAAAG	0.84	7	7	55	6
196.	umc1622	CCTCGGATTTTCCAAAACATTTCT	CGCTACAAATCCTACTGGTGCTTT	0.24	2	2	55	2
197.	umc1623	GAGACCAGCAGGTAGTTCTTGGAA	TCCAGCAGAACACCAACCTATTAGA	0.59	3	3	55	4
198.	umc1624	GAGACCAGATTCTTGGAACGGTAA	GAGAGGTCGTCGTCGCTACTG	0.32	2	2	55	5
199.	umc1630	CAGACCTTCGAGGGCAAGAACT	AGTTTTGGCTTCTTCTCCCAAGTC		Not Amplified		55	1
200.	umc1635	GCTGAGCAGATCTTTCCTTGTTTC	AAGGAGCAGAACTCGGAGACG	0.79	6	6	55	2
201.	umc1636	CATATCAGTCGTTCGTCCAGCTAA	GTACTGGTACAGGTCGTCGCTCTT	0.88	10	10	55	9
202.	umc1640	ACTACACGGTGTGAGATGTGATCG	GTCGTCGCAAGAACAACAAGG	0.73	4	4	55	10
203.	umc1648	CTGCAGTACGTGAGCCTGTACG	GCTTGAGCTGTGAGGAAGTTTTG	0.79	5	5	55	10
204.	umc1676	AGTCGTACGATGACGGAGGC	GCACCACCGACTGATCAAGA	0.94	12	21	55	1
205.	umc1682	AGCAAGCAAGCAAGTCACTGAGTA	GAGCTAGCCGGAGATAGAGAGGAG	0.73	3	5	55	4
206.	umc1688	AGCAGTAGCCGCAAGCAGAG	ATCTGGAGCTGCGTGCTGTC	0.72	5	5	55	9
207.	umc1689	GAGGCGGAGGAGGAACACAG	GAACGAGTAGGGCAGCGTCAG	0.35	2	2	55	1
208.	umc1692	AGAGACGAACTGAAGCCTGAAGTG	GATGTCCACGTCCTGGTAGAAGTT	0.70	5	7	55	5
209.	umc1694	ATCATTCTGCAGGTCACGAGAAG	AGAGACGAAAACCGACCATTCAT		Not Amplified		55	7

Results and Discussion

Plant variety/cultivar identification is important aspects in agricultural system (Shinwari *et al.,* 2013; Jan *et al.,* 2017; Jan *et al.,* 2019). The cultivated varieties are almost alike which require an effective method for their identification. The large number of varieties/hybrids among crop plants has made it difficult to identify and characterize varieties merely on morphological characters only because they are non-stable and are affected by the environmental and climatic conditions (Asif *et al.,* 2006; Kostova *et al.,* 2006). Molecular biology methods, especially DNA fingerprinting techniques, have promising applications in identification of plant genotypes including varieties and cultivars (Shinwari *et al.,* 2018). Nearly 1000 SSR markers are available in maize under public domain facilitating their utilization for diverse purposes in genetics and plant breeding and are also used as an important tool for purity identification of maize hybrid (Wu *et al*., 2010). Nikhou *et al*., (2013) studied polymorphism among maize varieties by application of SSR markers and found distinctiveness for varieties that served as an identity to diagnose maize varieties.

SSR markers analysis: In present study 03 hybrids along with 05 parental lines were used to study polymorphism and develop DNA Fingerprints for varietal identification. A sum total of 209 SSR evenly distributed across maize genome were used for genetic Fingerprinting of locally bred maize hybrids and inbred lines. 16 SSR markers i.e. umc1065, umc1115, umc1140, umc1197, umc1207, umc1215, umc1219, umc1298, umc1336, umc1423, umc1466, umc1574, umc1594, umc1595, umc1630 and umc1694 were not amplified. Nineteen SSR markers i.e. p-nc007, p-Phi016, p-Phi048, p-Phi050, p-Phi052, p-Phi083, p-Phi084, umc1006, umc1048, umc1094, umc158, umc1164, umc1179, umc1187, umc1266, umc1269, umc1276, umc1344 and umc1550, were found monomorphic. Whereas remaining 174 SSR markers were polymorphic (Table 3). These monomorphic markers were not considered for DNA fingerprinting as these amplified a uniform banding pattern and could not differentiate genotypes (Lukman *et al.* 2008).

The 183 markers amplified 1015 alleles among which 783 were polymorphic whereas 232 alleles were monomorphic. Some representative gels showing some polymorphic SSR markers i.e. umc1018, umc1020, umc1022, p-Phi09642, p-Phi113, p-Phi114, umc1491, umc1496, umc1498, umc1144 and umc1167 showing diversity among 08 maize genotypes and one monomorphic SSR umc1164 are given in Fig 1. Maximum number of alleles (21) were recorded for umc1676 followed by 18 alleles observed for p-Phi008 whereas maximum number of polymorphic alleles 16 were recorded for p-Phi008 followed by 14 alleles observed in p-Phi085. Whereas minimum number of polymorphic alleles (01 allele) was observed in p-nc012, p-Phi029, p-Phi044, p-Phi070, p-Phi071, p-Phi085, umc1057, umc1161, umc1167, umc1209, umc1246, umc1287, umc1312, umc1330, umc1341, umc1453, umc1511 and umc1589. On an average 5.25 alleles per locuswas observed in this study (Table 4) was higher than earlier reports of 3.25, 3.85, 4.9 except 5.3 alleles using 36, 27, 85 and 80 polymorphic SSR loci, respectively (Warburton *et al*., 2002, Bantte & Prasanna, 2003, Patto *et al.,* 2004, Legesse *et al.,* 2007). The average number of alleles amplified per SSR locus are influenced by type of SSR loci and repeat types, genetic diversity among genotypes and methodology adopted for detection of polymorphic markers (Gupta & Singh, 2010).

Polymorphic Information Content is (PIC) is used to measure the effectiveness of a genetic markers for linkage studies. PIC value of 209 SSR markers was calculated using Smith *et al*., (1997) method. PIC values ranged from 0.0 (for umc1179, umc1187, umc1269, umc1287, umc1344 and umc1550) to 0.94 for umc1676 with an average 0.67. 124 SSR marker showed PIC value greater than average 0.67 (Table 3). These results indicated that genotypes used in this study are highly polymorphic. Shiri (2011) obtained similar results in maize using 40 SSR markers observing PIC values 0.23 to 0.79. Pandit *et al*., (2016) observed PIC values ranging from 0.00 to 0.87 with an average of 0.65 using 18 SSR markers in maize.

Cluster analysis and dendrogram: Dendrogram was constructed from similarity/dissimilarity coefficient (Table 5) using UPGMA algorithm which showed variable genetic similarity 0.69 to 0.96 among maize genotypes (Table 5). Genotypes were classified into 03 groups (Fig 1). Group I comprised of two genotypes YH-1898 (hybrid) and Y-22 (female inbred line) from Maize and Millets Research Institute, Yusaf wala Sahiwal sharing 77% genetic similarity. Whereas group II was subdivided to IIa and IIb each comprising of two genotypes. IIa comprised of two genotypes *i.e.* Y-27 and F-165 which were both male inbred lines, former from Maize and Millets Research Institute, Yusaf wala Sahiwal and later from Maize Research Station Faisalabad sharing 78% genetic similarity. Both these male inbred lines may possibly share the common origin. IIc comprised of two genotypes FH-949 and FH-1046 both were hybrids from Maize Research Station Faisalabad and were highly similar with almost 96% similarity (Fig. 1). Group III comprised of two genotypes *i.e.* F-271 and F-308 both were female inbred lines from Maize Research sharing 72% genetic similarity (Table 5). Most distantly related genotypes were YH-1989 and F-308 sharing 61% genetic similarity (Fig. 2).

Genetic Similarity coefficient among genotypes from Maize and Millets Research Institute, Yusafwala Sahiwal ranged 0.748 to 0.777 with an average 0.749 whereas genotypes from Maize Research Station, Faisalabad showed a genetic similarity coefficient 0.592 to 0.936 giving an average 0.883 (Table 6). These results suggested that genotypes from Maize Research Station except hybrids (FH-949 & FH-1046) have high genetic distance as compared to genotypes from Maize and Millets Research Institute, Yusaf wala Sahiwal. Kumari *et al*., (2018) also studied eight maize genotypes using 22 SSR markers which were also clustered to 03 groups and reported genetic similarity coefficient varying from 0.21 to 0.64. Kanagarasu *et al*., (2013) studied genetic diversity in 27 maize inbred lines using 10 SSR markers and clustered genotypes into five major heterotic groups at 0.62 similarity coefficient. Kumar *et al.* (2016) studied genetic diversity in 13 maize genotypes using 22 SSR markers which were grouped to five clusters.

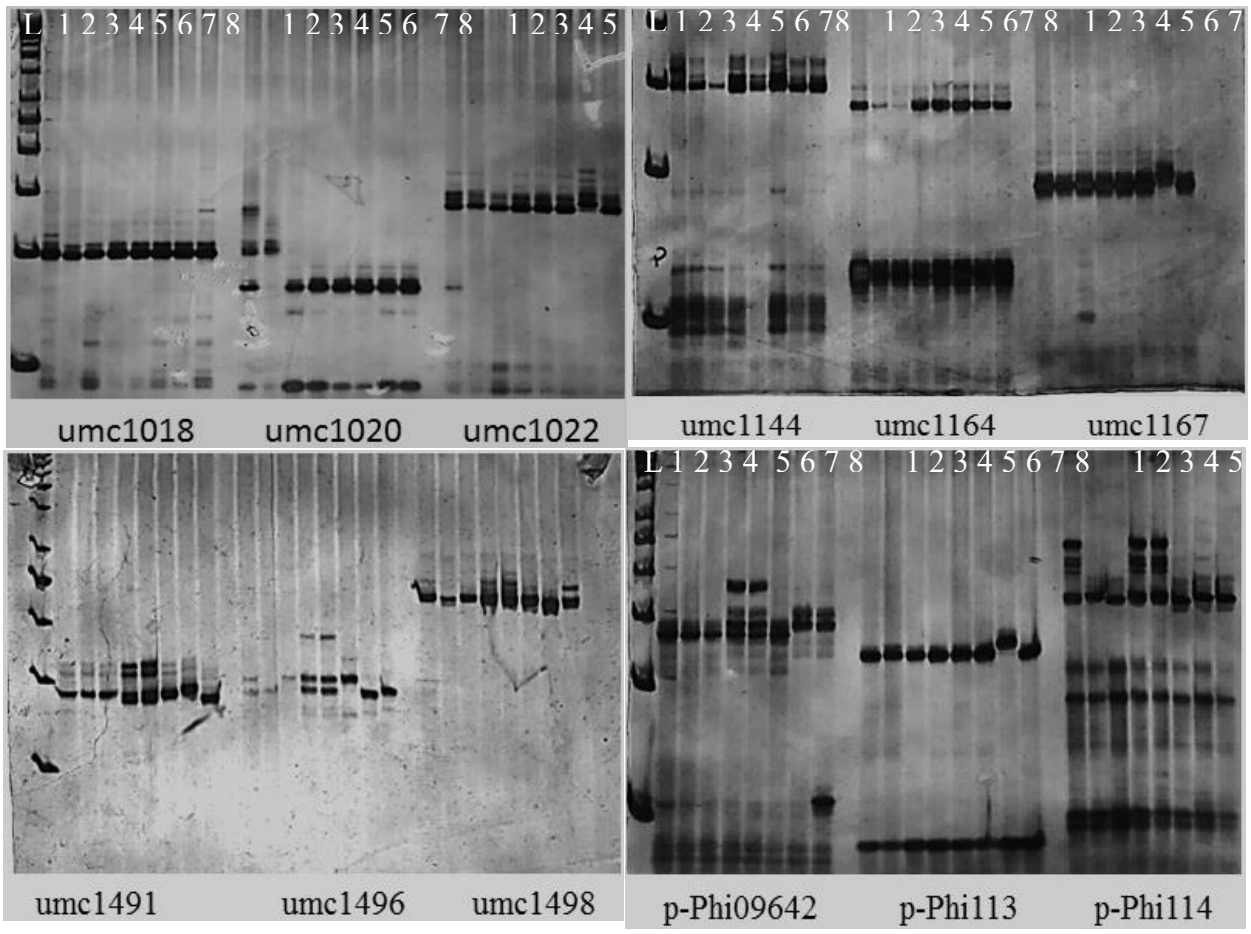

Fig 1. Different amplified alleles with polymorphic (umc1018, umc1020, umc1022, umc1144 and umc1167 umc1491, umc1496, umc1498 p-Phi09642, p-Phi113, p-Phi114) and monomorphic (umc1164) SSR markers for DNA fingerprinting of Maize. L, 50 bp ladder; 1, YH-1898; 2, Y-22; 3, Y-27; 4, FH-949; 5, FH-1046; 6, F-165; 7, F-271; 8, F-308.

Table 4. Summary of allelic diversity parameters for SSR marker used for DNA fingerprinting of commercial maize hybrids and their parental lines.

Allelic diversity parameters	Values
Total SSR Markers used	209
Total SSR Marker amplified	193
Total SSR Marker no amplified	16
Total Polymorphic SSR Markers	174
Total Monomorphic SSR markers	19
Proportion of amplified SSR marker	96.5%
Total number of amplified alleles	1015
Total polymorphic alleles	783
Proportion of Polymorphic Alleles	77.2%
Total Monomorphic alleles	232
Alleles Per Locus	5.25
Polymorphic Alleles Per Locus	4.05
Maximum Alleles Per SSR	21
Maximum Polymorphic Alleles Per SSR	16

DNA fingerprinting: DNA Fingerprints are used for identification of varieties/genotypes for variety protection. 209 SSR markers were used for development of DNA fingerprinting profile of 08 maize genotypes comprising of 03 hybrids and 05 inbred lines (Parents). Forty one unique DNA regions using 32 SSR markers were identified which could be used as an identification mark or DNA fingerprint for YH-1898. The size of DNA fingerprints varied from 85 bp for SSR marker umc1020 to 400 bp for SSR marker umc1676. Y-22 which is female parent (inbred line) was identified with the help of umc1067 (152 bp) and umc1682 (150 bp). Similarly male parent (Inbred Line) Y-27 was identified with the help of 03 SSR markers i.e. p-Phi019 (200, 500, 520 bp), p-Phi026 (80 bp) and umc1564 (80, 90 bp) (Table 7).

Table 5. Genetic similarity matrix of eight maize genotypes using Amplified 193 Simple Sequence Repeat Loci.

Accessions	YH-1898	Y-22	Y-27	FH-949	FH-1046	F-165	F-271
Y-22	0.77						
Y-27	0.75	0.78					
FH-949	0.66	0.61	0.69				
FH-1046	0.65	0.59	0.67	0.94			
F-165	0.68	0.68	0.78	0.72	0.73		
F-271	0.61	0.66	0.67	0.59	0.58	0.71	
F-308	0.61	0.67	0.67	0.70	0.69	0.68	0.72

Table 6. Range and mean genetic similarity between maize genotypes from different source.

Genotype source	Similarity coefficients	
	Range	Mean
Maize and Millets Research Institute, Yusafwala Sahiwal	0.748-0.777	0.749
Maize Research Station, Faisalabad	0.592-0.936	0.883

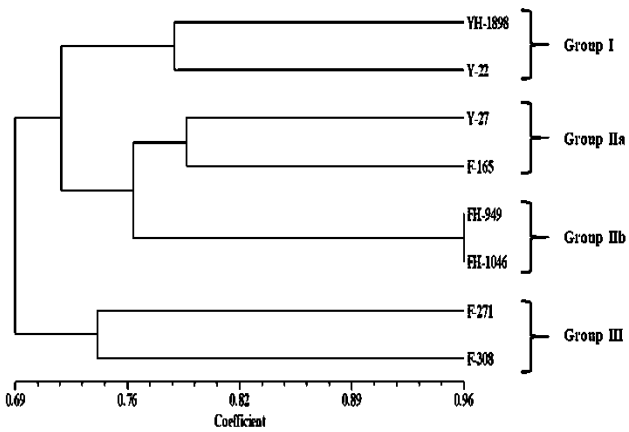

Fig 2. Dendrogram of 08 maize genotypes constructed based on Jaccords similarity coefficients following Unweighted Pair Group Method of Arithmetic Means (UPGMA) and SAHN clustering.

DNA fingerprints were also developed for genotypes from Maize Research Station Faisalabad. Maize hybrid FH-949 was identified with the help of 02 SSR markers *i.e.* umc1209 (175 bp) and umc1232 (200 bp) whereas maize hybrid FH-1046 was identified with the help of 01 SSR marker umc1063 (190 bp). Male parent F-165 which is common parent for both hybrids was identified with 11 SSR markers giving 20 DNA fingerprints. Maximum number of DNA fingerprints for F-165 was recorded with p-Phi085 (180, 195, 290, 330, 600 and 800 bp). F-271 which is female parent for FH-949 was identified with help of 48 DNA fingerprints using 34 SSR markers. Similarly F-308 which is female parent for FH-1046 was uniquely identified with help of 20 DNA fingerprints using 12 SSR markers. Maximum DNA Fingerprints for F-308 was observed with umc1692 (185, 200, 250, 300, 400 bp) (Table 7).

Jhansi *et al*., (2015) also developed DNA fingerprints of 05 maize commercial hybrids and their parental line using 100 SSR markers. Sharma *et al*., (2014) also developed DNA fingerprints for 07 commercial maize hybrids using 19 SSR markers. They reported that SSR markers *i.e.* umc087, umc1088, umc1389, umc2281, p-Phi022, p-Phi112 and p-Phi114 could be used for identification of KH9374, KH 404, KH 2005, KH 9452, POLO, KH 789 and KH 717 hybrids respectively.

Table 7. Identification of Maize inbred lines and hybrids through banding pattern with SSR markers and their respective base pair.

Genotypes	DNA Fingerprints (Markers along with allele sizes)
YH-1898	p-nc004 (250 bp), p-Phi017 (125 bp), p-Phi076 (190, 205 bp), umc1017 (160 bp), umc1018 (110 bp), umc1020 (85 bp), umc1019 (135 bp), umc1103 (115 bp), umc1124 (200 bp), umc1143 (245 bp), umc1165 (95 bp), umc1169 (240, 250 bp), p-Phi033 (260, 270 bp), umc1144 (160 bp), umc1196 (205 bp), umc1203 (225 bp), umc1213 (190 bp), umc1241 (225 bp), umc1280 (320 bp), umc1293 (200 bp), umc1318 (200 bp), umc1358 (75 bp), umc1507 (225, 260 bp), umc1545 (150 bp), umc1564 (170 bp), umc1566 (140 bp), umc1575 (225, 240 bp), umc1622 (80 bp), umc1635 (190 bp), umc1676 (400 bp), umc1688 (90, 125 bp) and p-Phi021 (100 bp)
Y-22	umc1682 (150 bp) and umc1067 (152 bp)
Y-27	umc1564 (80, 90 bp), p-Phi019 (200, 500, 520 bp) and p-Phi026 (80 bp)
FH-949	umc1209 (175 bp) and umc1232 (200 bp)
FH-1046	umc1063 (190 bp)
F-165	p-Phi008 (150 bp), p-Phi085 (180, 195, 290, 330, 600, 800 bp), p-Phi092 (150 bp), umc1183 (175 bp), umc1213 (160 bp), umc1232 (160, 210 bp), umc1482 (165 bp), umc1539 (150, 165, 190, 225 bp), umc1542 (200 bp), umc1566 (180 bp) and umc1582 (130 bp)
F-271	bnlg1429 (185 bp), bnlg118 (98, 160, 260 bp), bnlg1124 (315 bp), p-nc005 (225 bp), p-nc010 (90 bp), p-nc012 (115 bp), p-Phi008 (320, 420 bp), p-Phi015 (90 bp), p-Phi072 (148, 155 bp), p-Phi079 (185 bp), p-Phi095 (150, 190 bp), p-Phi085 (250 bp), p-Phi096 (130, 150, 162 bp), p-Phi113 (125 bp), umc1016 (160 bp), umc1022 (145, 155 bp), umc1045 (180 bp), umc1050 (105, 120 bp), umc1058 (115 bp), umc1061 (105 bp), umc1167 (100 bp), umc1202 (148 bp), umc1272 (100 bp), umc1291 (110 bp), umc1318 (150 bp), umc1498 (230 bp), umc1506 (165 bp), umc1546 (145, 150, 160 bp), umc1566 (175, 225, 350 bp), umc1568 (130 bp), umc1571 (105 bp), umc1576 (105 bp), umc1585 (135, 145 bp) and p-Phi065 (190 bp)
F-308	p-Phi033 (235, 270 bp), umc1014 (160, 170, 180 bp), umc1165 (250 bp), umc1318 (60 bp), umc1509 (193 bp), umc1535 (190 bp), umc1582 (140, 160 bp), umc1636 (60 bp), umc1692 (185, 200, 250, 300, 400 bp), p-Phi019 (650 bp), p-Phi022 (250 bp) and umc1063 (275 bp)

Conclusion

DNA fingerprints were developed for 02 maize hybrids (FH-949 and FH-1046) and 03 inbred lines (F-165, F-271 and F-308) from Maize Research Station, Faisalabad and 01 maize hybrid (YH-1898) and 02 maize inbred lines (Y-22 and Y-27) from Maize and Millets Research Institute, Yusafwala Sahiwal. These DNA fingerprints will be helpful in variety protection and registration process. Further it was observed that genotypes belonging to Maize and Millets Research Institute, Yusafwala Sahiwal are genetically more similar whereas genotypes from Maize Research Station Faisalabad were dissimilar. Further PIC values for 209 SSR markers reported in this study will assist maize breeders who are working on the study of maize genetic diversity.

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