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MORPHOLOGICAL CHARACTERIZATION AND SSR BASED DNA FINGERPRINTING OF ELITE COMMERCIAL MANGO CULTIVARS

Muhammad Abubakkar Azmat[1,*], Asif Ali Khan[2], Iqrar Ahmad Khan[1], Ishtiaq Ahmad Rajwana[3], Hafiza Masooma Naseer Cheema[2] and Ahmad Sattar Khan[4]

[1]Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad- Pakistan; [2]Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad- Pakistan; [3]University College of Agriculture, Bahauddin Zakariya University Multan-Pakistan; [4]Institute of Horticultural Sciences, University of Agriculture, Faisalabad- Pakistan.
[*]Corresponding author's e-mail: knnike@yahoo.com

Thirteen premium quality commercial mango cultivars grown in Pakistan were assayed with seven SSR primer series *viz.*, mMiCIR, MiSHRS, MIAC, MITGIT, LMMA, UBC and MiIIHR. Among the 120 primers used, 101 produced bands and revealed a narrow genetic base ranging from 0.62-0.8 with maximum similarity among the cvs. 'Anwar Ratole' and 'Sensation' while the maximum divergence was between cvs. 'Sindhri' and 'Sensation'. Chaunsa was genetically more similar (74.2%) and 'Kala Chaunsa' was more dissimilar (31.2%) respectively when compared with all the other cultivars. Of 101 SSR primers, we have identified 30 (29.7%) highly informative primers (PIC value ≥0.6), which could be useful in the molecular characterization of mango germplasm. The primer series MIAC, UBC and MiIIHR had the maximum percentage of highly informative primers in descending order. The UPGMA based Euclidian dendrogram constructed with similarity indices placed all the cultivars according to regions of their origin and magnitude of diversity among them. The morphological characterization of the commercial cultivars was also done using 11 quantitative traits; however, none of the traits except fruit weight was informative for the assessment of genetic diversity. SSR based molecular characterization appeared to be reliable, reproducible and cost effective.

Keywords: *Ceratocystis manginecans,* genetic variability, MQWD, polymorphism, multivariate analysis.

INTRODUCTION

Mango (*Mangifera indica* L.) belonging to family *Anacardiaceae* is a very delicious and nutritious tropical/subtropical fruit. Indo-Pak subcontinent-Myanmar region is the consensus centre of origin for mango (Yamanaka *et al.,* 2006; Jha *et al.,* 2010). Buddhist monks have played key role in the domestication of mango, 400-500 BC in the eastern regions including Pakistan, Bangladesh, Sri Lanka, Northeastern India and Myanmar (Singh, 1976; Rajwana *et al.,* 2011). Pakistan is the home of premium quality delicious mangoes. Due to its matchless taste, fine aroma and excellent texture, the demand for Pakistani mangoes has been increasing in international market. Despite the increase in the demand, mango production in Pakistan has tumbled down due to insect-pest infestation and different physiological disorders. Especially Mango Quick Wilt Disease (MQWD) caused by *Ceratocystis manginecans* is widespread in the commercial fruit producing areas of the country, which is a serious threat to the production of quality mangoes. Most of the commercial mango cultivars grown in Pakistan have been developed by seedling selection from the natural populations of 'Samar Bahisht Chaunsa' (commonly called as Chaunsa), (Rajwana *et al.,* 2008), which is more prone to MQWD. Narrow genetic base of commercial mango cultivars and growing threat of MQWD make the situation more threatening. Moreover, raising mango orchards is a long term investment and the inability to precisely identify desired cultivar at nursery stage may result in the loss of huge amount of time and investment

Genetic resistance is the only long-term strategy to overcome any disease but breeding in mangoes is a long term affair (Lavi *et al.,* 1989; Lavi *et al.,* 1993). The breeder's work could be accelerated by proper identification of resistant germplasm along with its ecological adaptations (Mukherjee *et al.,* 1968). The availability of workable genetic diversity is a pre-requisite for the success of any breeding program (Lavi *et al.,* 1993; Ravishankar *et al.,* 2000; Azmat and Khan, 2010; Azmat *et al.,* 2011). Usually, morphological traits are used for diversity assessments and characterization of mango genotypes, though easy but are not a reliable. The inferences derived on the basis of morphological assessment can be misleading as they are prone to change in response to environmental fluctuations (Sankar *et al.,* 2011). The use of molecular markers (RAPD, SSR, ISSR and AFLP) is the most appropriate, cost effective and efficient alternative of morphological assessment for the estimation of genetic diversity (Duneman, 1994; Ravishankar *et al.,* 2004;

Yamanaka *et al.,* 2006; Azmat and Khan, 2010). SSRs are the most appropriate, cost effective and preferred DNA markers being used for the estimation of genetic diversity, identification of different agronomic traits and disease resistance genes in different field and horticultural crops (Michelmore *et al.,* 1991; Schnell *et al.,* 2006; Azmat and Khan, 2010).

Realizing the importance of genetic diversity in the scenario of MQWD progression, 13 elite commercial mango cultivars were assayed with SSR markers. The knowledge sought has been used for preliminary recommendation on MQWD tolerant cultivars for commercial cultivation, especially in MQWD hit regions. The identification of highly informative SSR markers from a huge number of SSRs was also one of the objectives of this study.

MATERIALS AND METHOD

Plant material and DNA isolation: The young and tender leaves of 13 elite commercial mango cultivars were collected from Mango Germplasm Unit, Khanewal and Mango Research Station, Shujahabad, Pakistan (Table 1). The leaf samples were washed with distilled water, dried, packed in zipper bags and stored at -80°C until utilized. DNA was extracted from the leaves using modified CTAB method (Azmat *et al.,* 2012). After purification, genomic DNA was quantified spectrophotometrically and the integrity of DNA was assessed by agarose gel electrophoresis. Three dilutions (15, 20 and 25 ng μL^{-1}) of DNA were prepared in d_3H_2O to optimize the DNA concentration for amplification.

***Morphological characterization*:** Data for 11 quantitative fruit traits were recorded for three consecutive years, 2010-2012 (Table 1), following IPGRI's (International Plant Genetic Resources Institute) mango descriptor (Rajwana *et al.,* 2011). The data recorded over three years were pooled for further analysis. Means of all observations were calculated for all the quantitative traits of each cv. and subjected to principal component analysis (PCA). The principal components with Eigen-values >1.0 were selected as proposed by Jeffers (1967). Statistical analyses were carried out using SPSS 12, MVSP 3.1 and Microsoft Excel (QI Macros).

***SSR analysis*:** A set of 120 SSR/ISSR primers were used for fingerprinting of 13 commercial mango cultivars (Table 2). PCR reaction mixture (25 µL) contained 30 ng μL^{-1} template DNA; 10X *Taq* buffer (pH 8.3); 50 mM $MgCl_2$; 10 mM dNTPs; 1 U of *Taq* DNA polymerase (MBI, Fermentas, Vilnius, Lithuania); 10 µM each of forward and reverse

Table 1. Morphological characterization of commercial mango cultivars on the basis of eleven quantitative traits.

Cultivars	Origin	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Pulp content (%)	PTSS* (Brix)	Stone length (cm)	Stone width (cm)	Stone thickness (cm)	Stone weight (g)	Seed weight (g)	Fruit storage life (days)
Sindhri	Tharparkar-Pakistan	407.2	15.02	23.1	0.882	17.26	12.72	3.48	2.42	47.988	31.564	5
Langra	Sindh-Pakistan	349.8	10.82	31.54	0.91	21.16	9.88	7.88	2.06	33.3	22.78	5
Malda	Sindh-Pakistan	173	8.7	18.56	0.85	23.94	7.16	2.36	1.34	29.25	23.904	4
Dusehri	Northern India	199.9	10.32	19.18	0.86	20.1	9.02	3.16	1.92	28.046	20.986	5
Anwar Ratole	Northern India	179	8.52	19.68	0.855	26.36	7.04	3.66	1.92	26.03	17.842	6
Sensation	Florida (Exotic)	300	10.1	22.14	0.859	18.63	8.1	5.01	2.5	27.45	20.67	6
Chaunsa	Rahim- -yar Khan	369.3	12.68	23.68	0.885	25.42	10.34	4.02	2.2	42.354	28.954	5
New Sindhri	Tharparkar-Pakistan	450.4	18.16	22.82	0.917	17.84	15.6	2.82	1.36	37.252	23.95	6
Faiz Kareem	Multan-Pakistan	326.6	9.84	25.48	0.932	25.2	7.28	4.38	1.68	22.248	11.906	5
Fajri	Northern India	399.8	13.18	24.6	0.896	20.42	10.74	4.42	2.26	41.77	25.226	5
Kala Chaunsa	Multan-Pakistan	280	11.46	20.64	0.872	21.32	9.24	3.5	2.04	34.272	23.618	3
Late Ratole No. 12	Multan-Pakistan	193.1	9.36	19.9	0.883	20.18	7.72	3.26	2.18	22.652	16.164	6
Sufaid Chaunsa	Multan-Pakistan	540.2	13.38	27.32	0.898	22.64	10.36	4.46	2.2	55.272	25.288	10
Minimum		172.99	8.52	18.56	0.85	17.26	7.04	2.36	1.34	22.25	11.90	3
Maximum		540.17	18.16	31.54	0.932	26.36	15.6	7.88	2.42	55.27	31.56	10
Average		320.35	11.66	22.97	0.88	21.57	9.63	4.03	2.01	34.45	22.53	5.62
CV		0.37	0.24	0.17	0.03	0.13	0.26	0.35	0.18	0.29	0.24	0.33

*Pulp total soluble solids (°Brix)

Table 2. Primer sequences, annealing temperature, Number of alleles and PIC values for 101 SSR loci found in thirteen mango cultivars.

Sr. No.	Primer name	Sequence (5'-3')	Annealing temperature (°C)	No. of alleles	PIC value
Duval *et al.* (2005)					
1	mMiCIR001	F:TGAGTTGTTGTCCTGCT R: GGTGCTTGTTTCTCGT	51	1	0
2	mMiCIR002	F:AAACAAAGAATGGAGCA R:TGGACTGAATGTGGATAG	51	2	0.31
3	mMiCIR003	F:GATGAAACCAAAGAAGTCA R:CCAATAAGAACTCCAACC	51	1	0
4	mMiCIR004	F:CTTGAAAGAGATTGAGATTG R:AGAAGGCAGAAGGTTTAG	51	1	0
5	**mMiCIR005¥**	**F:GCCCTTGCATAAGTTG R:TAAGTGATGCTGCTGGT**	51	**3**	**0.62**
6	mMiCIR006	F:TCTAAGGAGTTCTAAAATGC R:CTCAAGTCCAACATACAATAC	51	2	0.50
7	**mMiCIR008**	**F:GACCCAACAAATCCAA R:ACTGTGCAAACCAAAAG**	51	**3**	**0.67**
8	**mMiCIR009**	**F:AAAGATAAGATTGGGAAGAG R:CGTAAGAAGAGCAAAGGT**	51	**3**	**0.64**
9	mMiCIR010	F:TAGGGATATAGCTGGAGG R:ACGCAGTAGAACCTGTG	51	1	0
10	mMiCIR011	F:CAGCCTTATGTGTTGAAG R:AAACTAAACAAGCTGAACC	51	2	0.50
11	mMiCIR012	F:CTTCATTTCTCCACTTTTG R:ATGAAATACTGGCTGGTT	51	1	0
12	mMiCIR013	F:GCGTAAAGCTGTTGACTA R: TCATCTCCCTCAGAACA	48	3	0.59
13	mMiCIR014	F:GAGGAACATAAAGATGGTG R:GACAAGATAAACAACTGGAA	51	2	0.50
14	**mMiCIR016**	**F:TAGCTGTTTTGGCCTT R:ATGTGGTTTGTTGCTTC**	**51**	**3**	**0.62**
15	mMiCIR018	F:CCTCAATCTCACTCAACA R:ACCCCACAATCAAACTAC	51	2	0.50
16	mMiCIR020	F: GACTTGCAGTTTCCTTTT R: TCAAGAACCCCATTTG	53	1	0
17	mMiCIR021	F: CCATTCTCCATCCAAA R: TGCATAGCAGAAAGAAGA	51	2	0.50
18	mMiCIR022	F: TGTCTACCATCAAGTTCG R: GCTGTTGTTGCTTTACTG	51	2	0.50
19	mMiCIR024	F: ATTTTGATTCCCGTTCT R: ATTCGATCATGGTTTTG	51	2	0.50
20	mMiCIR025	F: ATCCCCAGTAGCTTTGT R: TGAGAGTTGGCAGTGTT	51	2	0.27
21	**mMiCIR027**	**F: ACGGTTTGAAGGTTTTAC R:ATCCAAGTTTCCTACTCCT**	**51**	**3**	**0.64**
22	mMiCIR029	F: GCGTGTCAATCTAGTGG R: GCTTTGGTAAAAGGATAAG	51	1	0
23	**mMiCIR030**	**F: GCTCTTTCCTTGACCTT R: TCAAAATCGTGTCATTTC**	**51**	**3**	**0.62**
24	mMiCIR033	F:GTATAAATCGCGTGCAT R:AGTTTCCCTCCTTGTATCT	48	2	0.50

25	mMiCIR034	F: TCGGTCATTTACACCTCT R:TTATTGAGCTTCTTTGTGTT	51	2	0.50
26	mMiCIR036	F:ACCACGAAAAGACAACTC R:TCATCTTTGTTAAATAGGTTAAT	51	2	0.50
Schnell *et al.* (2005)					
27	MiSHRS-1	F: TAACAGCTTTGCTTGCCTCC R: TCCGCCGATAAACATCAGAC	46	2	0.50
28	MiSHRS-4	F: CCACGAATATCAACTGCTGCC R: TCTGACACTGCTCTTCCACC	52	2	0.50
29	MiSHRS-18	F: AAACGAGGAAACAGAGCAC R: CAAGTACCTGCTGCAACTAG	42	2	0.50
30	MiSHRS-29	F: CAACTTGGCAACATAGAC R: ATACAGGAATCCAGCTTC	48	2	0.42
31	MiSHRS-32	F: TTGATGCAACTTTCTGCC R: ATGTGATTGTTAGAATGAACTT	48	1	0
32	**MiSHRS-33**	**F:CGAGGAAGAGGAAGATTATGAC R: CGAATACCATCCAGCAAAATAC**	**54**	**4**	**0.71**
33	MiSHRS-34	F: TGTGAAATGGAAGGTTGAG R: ACAGCAATCGTTGCATTC	48	1	0
34	**MiSHRS-36**	**F: GTTTTCATTCTCAAAATGTGTG R: CTTTCATGTTCATAGATGCAA**	**51**	**3**	**0.62**
35	**MiSHRS-37**	**F: CTCGCATTTCTCGCAGTC R: TCCCTCCATTTAACCCTCC**	**51**	**3**	**0.61**
36	MiSHRS-39†	F: GAACGAGAAATCGGGAAC R: GCAGCCATTGAATACAGAG	51	1	0
Kittipat (2007)					
37	**MIAC-2**	**F: GCTTTATCCACATCAATATCC R: TCCTACAATAACTTGCC**	**53**	**4**	**0.73**
38	**MIAC-3**	**F: TAAGCTAAAAAGGTTATAG R: CCATAGGTGAATGTAGAGAG**	**53**	**3**	**0.64**
39	MIAC-4	F: CGTCATCCTTTACAGCGAACT R: CATCTTTGATCATCCGAAAC	55	2	0.50
40	**MIAC-5**	**F: AATTATCCTATCCCTCGTATC R: AGAAACATGATGTGAACC**	**53**	**6**	**0.77**
41	**MIAC-6**	**F: CGCTCTGTGAGAATCAAATGGT R:GGACTCTTATTAGCCAATGGGATG**	**53**	**9**	**0.65**
42	**MIAC-11**	**F: GTGCGAGGAGATATCTGT R: CTGGTTCTTCATTGTTGAGATG**	**53**	**6**	**0.68**
43	**MITGI75**	**F:TGCGTCTTGTGTGTGTGTGT R:GGAATGCTGTGTGTGTGTG**	**54**	**8**	**0.74**
44	MITG436-2	F:GGTCAGCTGTGTGTGTGTG R:CAATTCAATGCTTTGGATGCT	53	4	0.44
45	MITGg62	F:TGTTCGATTTGCAAACTTTTT R:GGCCTAATGTGTGTGTGTG	52	3	0.59
46	**MICA23I-1**	**F:TGGAAGGACCATGCTTGAAT R:GGTCACACACACACACACA**	**55**	**8**	**0.78**
47	MICA23I.2	F:CGGCACACACACACACA R:AAGGTCATTGGGTTCATTCG	54	2	0.46
48	MICA235	F:TGTCACACACACACACACA R:AATGGAAGGACCATGCTTGA	53	5	0.57
49	MIGA I79	F:CCTGAGAGAGAGAGAGAGA R:GAGAGAGAGAGAGAGGTGG	53	2	0.50
50	**MIGA2O3**	**F: TGAAGGATAGGTGTGGTG R:CATGAGAGAGAGAGAGAGA**	**52**	**9**	**0.76**

51	MIGA224	F:CACGAGAGAGAGAGAGAGA R:GGGTCTCAGAGGGAGGATTT	55	2	0.50
52	MIGA253	F:CATGAGAGAGAGAGAGAGAGA R:AAAGGAAAGGCAGGGAAATG	53	1	0
53	MIGA326	F: GACAGACAAAGCCAGCAGAA R:CCCGAGAGACAGAGAGAGAGA	55	2	0.19
54	MIAC25I-I	F: CCTTGGGTTCATTCGCTAAA R: GGACGCCACACACACACAC	55	3	0.59
55	**MIAC25I-2**	**F: TGGCGCTACACACACACAC R: CACACACACACACACACACG**	**55**	**4**	**0.71**
56	MIAC326	F: TGGTATTCAAGCATGGTCCTC R: TGGCATCACACACACACAC	53	1	0
57	MITCI38	F: TCTCCCTTCATCGATTGTCC R: GGAGCGTCTCTCTCTCTCCA	55	2	0.35
Viruel *et al.* (2005)					
58	LMMA1	F: ATGGAGACTAGAATGTACAGAG R: ATTAAATCTCGTCCACAAGT	49	1	0
59	LMMA2a	F: AAATAAGATGAAGCAACTAAAG R: TTAGTGATTTTGTATGTTCTTG	49	1	0
60	LMMA3	F: AAAAACCTTACATAAGTGAATC R: CAGTTAACCTGTTACCTTTTT	49	2	0.42
61	LMMA4	F: AGATTTAAAGCTCAAGAAAAA R: AAAGACTAATGTGTTTCCTTC	49	2	0.27
62	LMMA6a	F: ATATCTCAGGCTTCGAATGA R: TATTAATTTTCACAGACTATGTTC	49	2	0.50
63	LMMA7	F: ATTTAACTCTTCAACTTTCAAC R: AGATTTAGTTTTGATTATGGAG	49	2	0.50
64	**LMMA8**	**F: CATGGAGTTGTGATACCTAC R: CAGAGTTAGCCATATAGAGTG**	**49**	**4**	**0.71**
65	LMMA9	F: TTGCAACTGATAACAAATATAG R: TTCACATGACAGATATACACTT	49	1	0
66	LMMA10	F: TTCTTTAGACTAAGAGCACATT R: AGTTACAGATCTTCTCCAATT	49	3	0.59
67	LMMA11b	F: ATTATTTACCCTACAGAGTGC R: GTATTATCGGTAATGTCTTCAT	49	3	0.59
68	LMMA12	F: AAAGATAGCATTTAATTAAGGA R: GTAAGTATCGCTGTTTGTTATT	49	1	0
69	LMMA14	F: ATTATCCCTATAATGCCCTAT R: CTCGGTTAACCTTTGACTAC	49	1	0
70	LMMA15	F: AACTACTGTGGCTGACATAT R: CTGATTAACATAATGACCATCT	49	1	0
71	LMMA16	F: ATAGATTCATATCTTCTTGCAT R: TATAAATTATCATCTTCACTGC	49	1	0
University of British Columbia (UBC, Vancouver-USA)					
72	**UBC 809**	**AGA GAG AGA GAG AGA GG**	**57**	**5**	**0.77**
73	**UBC 841**	**GAG AGA GAG AGA GAG AYC**	**57**	**11**	**0.68**
74	UBC 868	GAA GAA GAA GAA GAA GAA	57	5	0.20
75	UBC 810	GAG AGA GAG AGA GAG AT	57	2	0.50
76	UBC 811	GAG AGA GAG AGA GAG AC	57	2	0.54
77	***UBC 812***	***GAG AGA GAG AGA GAG AA***	**57**	***4***	***0.79***
78	UBC 813	CTC TCT CTC TCT CTC TT	57	1	0
79	UBC 815	CTC TCT CTC TCT CTC TG	57	2	0.50
80	UBC 834	AGA GAG AGA GAG AGA GYT	57	2	0.50
81	UBC 836	AGA GAG AGA GAG AGA GYA	57	2	0.50

82	**UBC 845**	**CTC TCT CTC TCT CTC TRG**	**57**	**3**	**0.67**
83	**UBC 852**	**TCT CTC TCT CTC TCT CRA**	**57**	**6**	**0.74**
Ravishankar *et al.* (2011)					
84	MiIIHR01a	F: GGATGCACAACAACAAGCAC R: TCAGCAAGCAATCCCTTCTT	55	2	0.50
85	**MiIIHR02c**	**F: CCCCAACATTTCATAAACACA R: CCTCCTTACATGCCTCCTTG**	**55**	**3**	**0.69**
86	MiIIHR03a	F: GTCGATGCCTGGAATGAAGT R: AAGCATCGAACAGCTCCAAT	55	3	0.59
87	**MiIIHR04c**	**F: CGTTTTTGACCCTCTTGAGC R: CCGCATACTTCCCTTCACAT**	**55**	**5**	**0.71**
88	MiIIHR05c	F: CTCTCCCTCACTTGCTCCAC R: AGACCACCGACAACGAAAAC	55	1	0
89	**MiIIHR06**	**F: CGCCGAGCCTATAACCTCTA R: ATCATGCCCTAAACGACGAC**	**55**	**5**	**0.68**
90	**MiIIHR07a**	**F: GCCACTCAGCTAAATAGCCTCT R: TGCAGTCGGTAAAGTGATGG**	**55**	**3**	**0.62**
91	MiIIHR08	F: TGCTCTCTACTGCCCCGTAT R: GTCACACCAATCGGGAATCT	55	1	0
92	MiIIHR10c	F: CGATTCAAGACGGAAAGGAA R: TTCAAGCACAGACGACCAAC	55	1	0
93	MiIIHR11a	F: CAGTGAAACCACCAGGTCAA R: TGGCCAGCTGATACCTTCTT	55	3	0.54
94	MiIIHR12a	F: GCCCCATCAATACGATTGTC R: ATTTCCCACCATTGTCGTTG	55	3	0
95	MiIIHR14	F: CCGAAACAACTCTTCCTCCA R: TGCTCTCTGGCCTCTTCTTC	55	3	0
96	MiIIHR15	F: CTAACCATTCGGCATCCTCT R:TCTGTGATAGAATGGCAAAAGAA	55	1	0
97	MiIIHR16a	F: TTTCACTTGGTTCTGGATTGC R: ATTTCCCACCATTGTCGTTG	55	1	0
98	MiIIHR17	F: GCTTGCTTCCAACTGAGACC R: GCAAAATGCTCGGAGAAGAC	55	2	0.50
99	MiIIHR18	F: TCTGACGTCACCTCCTTTCA R: ATACTCGTGCCTCGTCCTGT	55	1	0
100	MiIIHR19a	F: TGATATTTTCAGGGCCCAAG R: AAATGGCACAAGTGGGAAAG	55	2	0
101	**MiIIHR20a**	**F: CCTAACGCGCAAGAAACATA R: ACCCACCTTCCCAATCTTTT**	**55**	**3**	**0.64**
Minimum				1	0
Maximum				11	0.79
Average				2.69	0.40
Standard deviation				1.92	0.28

¥Primers in bold text are highly informative.

primers. The amplifications were carried out in thermal cycler (Bio-Rad C-1000) using a program configured for an initial denaturation step of 5 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 46°C to 57°C and 1 min at 72°C (Table 2). The program ended with one final extension at 72°C for 10 min. The amplified products were resolved using on 2.5% agarose gel containing Ethidium bromide in electrophoresis chamber.

***Data analysis*:** The electrophoresed gels were examined under ultra violet Transilluminator and photographed using UVP® Gel Documentation System. All the amplification products were scored as present (1) or absent (0) for each of the 13 cultivars with all primers. The ambiguous bands that could not be clearly distinguished were not scored. The data generated from the detection of polymorphic fragments were analyzed through *Popgene32* software (Ver. 1.44) (Yeh *et al.*, 2000). The polymorphism information content (PIC) was

calculated by following the equation developed by Botstein *et al*. (1980).

RESULTS

***Morphological characterization*:** Analysis of the data recorded on different quantitative traits indicated variability among the commercial mango cultivars (Table 1). Wide range and high coefficients of variability (0.24%-0.37%) were observed for seven quantitative traits (fruit weight, fruit length, stone length, stone width, stone weight, fruit storage life and seed weight) in all the cultivars (Table 1). Considering all the cultivars, a low coefficient of variability was observed for fruit diameter (0.17%), pulp content (0.03%), pulp total soluble solids (TSS) (0.13%) and stone thickness (0.18%) (Table 1).

Multivariate analysis of the cultivars revealed that the first two PCs (PC1 and PC2) had Eigen-values >1 and cumulatively accounted for 99.79% of total quantitative variation (Table 3). The first PC axes accounted for 99.44% of total multivariate variation and the second accounted for only 0.35%. The variation of only one trait (fruit weight) was associated with PC1 while PC2 was associated only with stone weight and seed weight, respectively (Table 3). The variation for the remaining eight quantitative traits did not contribute significantly to any of the principal components.

Table 3. Percentage of explained and cumulative variances and eigenvectors on the first two principal components for eleven quantitative characters in commercial mango cultivars.

Parameter	PC1	PC2
Eigen-values	14310.7	50.91
Explained proportion of variance (%)	99.43	0.354
Cumulative proportion of variation(%)	99.43	99.79
Variable	***Eigen-vectors***	
Fruit weight (g)	**0.997** [a]	-0.070
Fruit length (cm)	0.019	-0.001
Fruit diameter (cm)	0.022	-0.002
Pulp Content	0.000	0.000
Pulp total soluble solids (°Brix)	-0.008	0.001
Stone length (cm)	0.015	-0.001
Stone width (cm)	0.004	0.000
Stone thickness (cm	0.000	0.000
Stone weight (g)	0.072	**0.781**
Seed weight (g)	0.022	**0.621**
Fruit storage life (Days)	0.006	0.000

[a]Bold values are greater than the arithmetic mean of the highest and lowest absolute values of eigenvectors within the column.

***Molecular characterization*:** One hundred one of 120 SSR/ISSR primers yielded good score-able amplifications. Considering all the primers and mango cvs a total of 271 alleles were amplified, of which 217 (80.07%) were polymorphic (Table 2). An average of 128 alleles per cultivar was amplified in the commercial mango cvs. ranging from 116-141 alleles. The maximum number of alleles was amplified in 'Langra' while the minimum number of bands was produced in 'Faiz Kareem'. The genetic similarity indices ranged from 0.62-0.8 with an average similarity index of 0.713, indicating a relatively narrow genetic base. With 101 SSR primers, the maximum similarity (80.5%) was observed between the cvs. 'Anwar Ratole' and 'Sensation' while the maximum divergence (37.9%) was observed between 'Sindhri' and 'Sensation' (Table 4; Fig. 1). The cv. Chaunsa was most similar (74.2%) compared to the other cultivars followed by 'Sensation', 'Malda' and 'Fajri' having the genetic similarity index of 0.73 (Table 4). The cv. 'Kala Chaunsa' was the most diverse (68.8%) among all the other commercial cultivars (Table 4). The number of alleles amplified per primer varied from 1 to 11 with an average of 2.68 alleles per primer. The maximum numbers of alleles (11) were amplified by the primer UBC 841 followed by MIGA 2O3 and MIAC-6 with nine alleles each (Table 2).

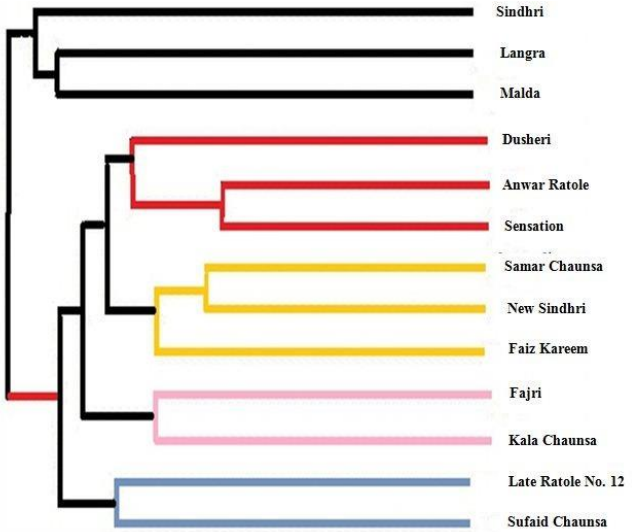

Figure 1. UPGMA based Euclidian dendrogram developed using similarity coefficient data after SSR analysis.

The polymorphism information content calculated for 101 SSR/ISSR primers ranged from 0 to 0.79 with an average PIC value of 0.4 (Table 2). Among the 101 primers, 34 (33.7%) were ranked as 'uninformative' with PIC value ≤ 0.30 while only 30 (29.7%) primers were 'highly informative' with PIC value ≥ 0.60. There were 37 (36.6%) SSR primers with PIC value 0.31-0.59, making the largest group of primers named as 'moderately informative' (Table 5). The primer UBC 812 had maximum PIC value (0.79) followed by MICA 231-1 (0.78), UBC 809 (0.77), MIAC-5 (0.77) and MIGA 203 (0.76), respectively (Table 5). The PIC value based

Table 4. Similarity matrix of 13 mango cultivars by using 101 SSR primers.

Cultivar	Dusheri	Late Ratole No. 12	Sufaid Chaunsa	Fajri	Anwar Ratole	Langra	Sensation	Kala Chaunsa	Malda	Samar Chaunsa	New Sindhri	Faiz Kareem	Average
Sindhri	0.7125	0.6958	0.7187	0.6462	0.6318	0.7027	***0.6207***	0.6782	0.6973	0.7251	0.7261	0.7184	0.717
Dusheri		0.7250	0.7392	0.6895	0.7755	0.6652	0.7273	0.7140	0.6515	0.7282	0.7559	0.7124	0.724
Late Ratole No. 12			0.7489	0.7151	0.7590	0.7068	0.7193	0.7483	0.6848	0.7200	0.7118	0.7401	0.705
Sufaid Chaunsa				0.6874	0.6934	0.6874	0.6725	0.7037	0.6736	0.7257	0.6908	0.6914	0.704
Fajri					0.7049	0.6794	0.7088	0.7645	0.6513	0.7430	0.7113	0.7044	0.729
Anwar Ratole						0.7049	**0.8054**	0.7299	0.6493	0.7354	0.7273	0.7560	0.700
Langra							0.6681	0.7259	0.7126	0.7265	0.7113	0.6534	0.712
Sensation								0.7171	0.6706	0.7225	0.7771	0.7065	0.730
Kala Chaunsa									0.7365	0.7433	0.7535	0.7126	0.688
Malda										0.7210	0.7393	0.6559	0.730
Samar Chaunsa											0.7965	0.7434	0.742
New Sindhri												0.7908	0.721
											Average	0.7150	------

Table 5. Informativeness of different SSR primer series among thirteen commercial mango cultivars.

informativeness of the seven SSR primer series *viz.*, mMiCIR, MiSHRS, MIAC, MITGIT, LMMA, UBC and MiIIHR was also assessed separately. It was found that the primer series MIAC (Kittipat, 2007) was highly informative with 83.3% primers having the PIC value ≥ 0.60 followed by UBC (University of British Columbia, Vancouver-Canada), MiIIHR (Ravishankar *et al.,* 2011), MiSHRS (Schnell *et al.,* 2005) and MITGIT (Kittipat, 2007) series with 41.6%, 39%, 30% and 26.6% primers, respectively, having PIC value ≥ 0.60 (Table 5). The primer series mMiCIR (Duval *et al.,* 2005) and LMMA (Viruel *et al.,* 2005) had the minimum number of highly informative (PIC value ≥ 0.60) primers *i.e.* 19.2% and 7.14% primers respectively (Table 5). Moreover a significant positive correlation (0.66) was found among the number of alleles amplified by a particular primer and its PIC value.

A UPGMA and Euclidian distance based dendrogram was generated using Nei's similarity coefficients with the help of *PopGene32* software (version 1.44) to estimate the genetic distances and relatedness among the cultivars. The dendrogram segregated all the commercial mango cvs. into four clusters (Fig. 1). The cluster 'A' was comprised of three cvs. *i.e.* 'Sindhri', 'Langra' and 'Malda' and among them, 'Langra' and 'Malda' were more similar (71.2%) while the Sindhri cultivar also showed close resemblance to these two (Fig. 1). In the cluster 'B' there was six cultivars *viz.*, 'Dusheri', 'Anwar Ratole', 'Sensation', 'Chaunsa', 'New Sindhri,' and 'Faiz Kareem' making two sub-clusters. In the first sub-cluster 'B_1', 'Anwar Ratole' and 'Sensation' showed maximum similarity (80.5%) while 'Dusheri' was also similar to other cultivars within sub-cluster. The sub-cluster 'B_2' also had three cvs. of which 'Chaunsa' and 'New Sindhri' were most similar (79.6%) to each other followed by 'Faiz Kareem' within the group (Fig. 1). The third cluster 'C' had only two cvs. *viz.*, 'Fajri' and 'Kala Chaunsa' having 76.4% similarity among them. Similarly cluster 'D' also had two genetically similar (74.8%) cvs. namely 'Late Ratole No. 12' and 'Sufaid Chaunsa' (Fig. 1).

Considering all the morphological traits and SSR/ISSR based dendrogram it was found that each cluster had some unique characteristics and it was also found that the cultivars in cluster 'B' and 'D' had comparatively good performance with respect to commercially important traits. The cluster 'D' had cultivars with high fruit weight (366.7 g) and maximum fruit storage life (8 days), in addition the cultivars of this cluster have late maturing time while the cultivars in cluster 'B' had more pulp TSS (23); less stone weight (31.2 g) and seed weight (20.7 g), respectively (Table 1).

DISCUSSION

The availability of wide range of diverse germplasm is a key strength for success, sustainability and improvement of a domesticated plant species. The genetic variability also provides shield against different biotic and abiotic stresses especially against different disease epidemics. For seven of the 11 quantitative morphological traits (fruit weight, fruit length, stone length, stone width, stone weight, fruit storage life and seed weight), wide ranges were observed but the multivariate analysis has indicated that only one trait (fruit weight) contributed >99% to the total variability and the rest of the traits cumulatively contributed <1% to total variation among the 13 commercial mango cultivars (Table 3). This shows that morphological characterization alone with a limited number of parameters may lead to erroneous conclusions about the extent of genetic variability among a set of cultivars. The assessments of genetic diversity on the basis of molecular markers have shown their significance in different crop species. Beyond any debate of efficiency, ease and cost effectiveness, DNA based marker systems, are the best and reliable option for the assessment of genetic variability among all the species of plant and animal kingdoms (Eiadthong *et al.,* 2000; Schnell *et al.,* 2006; Azmat and Khan, 2010; Ravishankar *et al.,* 2011).SSR markers are co-dominant in nature, which put them on advantage over the dominant markers (RAPD, RFLP and AFLP). Thus, SSR markers can differentiate homozygous genotypes/cultivars from heterozygous for a particular locus. Though the isozymes markers are also co-dominant but these markers are prone to changes due to environmental fluctuations (Pascua *et al.,* 1996; Eiadthong *et al.,* 1998). Due to the co-dominant nature of SSR markers and being highly specific and highly variable, SSR markers are highly suitable for diversity studies in related populations and/or cultivars.

The genetic variability revealed by 101 SSR/ISSR primer combinations among 13 commercial mango cultivars has indicated very low genetic back ground which puts a caution for mango production in general and specifically in the perspective of MQWD in the mango growing areas of Pakistan (Fig. 1). Our findings suggest that the cultivar Chaunsa had maximum similarity with all other commercial cultivars, which is a popular cultivar in Pakistan and is the major victim of *Ceratocystis manginecans* (Rajwana *et al.,* 2008). Among the 13 mango cultivars, Kala Chaunsa had shown maximum genetic divergence with other commercial cultivars followed by Anwar Ratole and both have good fruit quality and yield traits as well (Table 1). Pakistan lies at the western edge of the natural range of monoembryonic mango domestication and considered as its centre of diversity (Rajwana *et al.,* 2008). Although mango quick wilt disease is now widespread in the commercial fruit producing areas of the country, it has not yet become established further north, in Jammu and Kashmir, where native mango flourishes since centuries. However, this ancient and valuable genetic resource is under serious threat due to unprecedented domestication and lack of ownership by the growers. Since native *Mangifera* germplasm is understudied, needs to be evaluated and preserved, not only for its intrinsic worth, but also for the potential presence of valuable resistance against *C. manginecans* Native germplasm having resistance against *C. manginecans* could be preserved and exploited as Scion and/or root stock to widen our varietal base for export and indigenous markets of the future.

As a result of this study we were able to identify highly informative (PIC≥ 0.60) SSR/ISSR primers, determined on the basis of their corresponding PIC values, which would facilitate molecular characterization and preservation of mango cultivars and germplasm (Table 2). It is also noteworthy that by using SSR/ISSR primers the UPGMA based dendrogram placed mango cultivars according to their regions of origin, establishing some link between the geographical distribution of the cultivars and their corresponding response to different morphological and pathological traits.

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