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Genetic Purity Analysis in Maize under Temperate Conditions

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A B S T R A C T

In the present study SSR markers were shown to be polymorphic between parents which would help in establishing the genetic purity of hybrids in commercial seed lots. Also, few SSRs were found to discriminate among the set of hybrids which will ease the seed identity of competing hybrids in seed chain. Low probability of identity based on marker information suggests that a set of markers identified in present study can be efficiently used in establishing distinctness, identity and purity of hybrids in open markets after their release.

Introduction

Maize (*Zea mays* L.) is the second most important cereal crop in the world after wheat (Asif *et al.*,2006) and is produced on nearly 100 million hectares in developing countries with almost 70% of the total maize production in the developing world coming from low to lower middle income countries. The demand for maize is projected to get doubled by the year 2050 and the crop may attain highest production globally by 2025 (Rosergent *et al.*, 2009). In India the crop occupies an area of 8.55 million hectares and the production is 21.76 million tons with an average yield of 2.48 tones ha-[1](Anonymous, 2013). In Jammu and Kashmir State, maize is grown as rainfed crop in marginal hilly terrains of Kashmir Valley and occupies an area of 0.1 million hectares with production and productivity of 0.15 million tones and 1.2 tones per hectare (Anonymous, 2012). Although, number of varieties has been released for valley basin and high altitude areas of J&K, however, few hybrids/ composites have become popular in maize growing regions and have successfully been grown by farmers with the aim of achieving high production. The maize hybrids are known for their improved yield performance, genetic uniformity, resistance to pests and diseases and are being preferred by the farmers. Thus, genetic purity of seed becomes much more important since seed is the initial lever for bolstering production potentiality and means of creating surplus in commercial markets (Srivastava and Jaffee, 1993). Hybrid corn has helped to achieve a quantum jump in yield. Before dispensing seeds to the farmer its purity testing is essential to ensure its trueness. It is estimated

that for every percent impurity in hybrid seed the yield reduction can go upto100 kg ha^{-1} (Li Liu *et al.,* 2000). In maize it is even higher as the reduction in the yield goes to 135 kg ha^{-1}(Mao *et al.,* 1996). Moreover, the contamination reduces the genetic and physiological quality of seeds that consequently decreases the crop productivity (Kalinka *et al.,* 2006). Since detailed morphological descriptors of the plants and seeds have been used for identification but this method is ambiguous and time consuming. The methods like grow out test normally demand considerable time and labour. Hybrid purity testing is best achieved by use of DNA markers. Molecular markers which could clearly distinguish the hybrids from its parental lines have been identified and developed in major agricultural and horticulture crops (Pallavi *et al.,* 2010). SSR markers have proven to be preferred and robust molecular markers for purity identification, due to their high efficiency, reproducibility and simplicity (Wu *et al.,* 2010). Genetic purity of commercial seed lots is of immense importance in realization of full yield potential of varieties per unit area. Therefore, present study was broadly aimed to identify the DNA based markers for hybrid purity testing.

Materials and Methods

The plant materials comprised of six maize (*Zea mays* L.) inbreds (KDM-361A,KDM-343A,KDM-332A,KDM-914A, KDM-916A, KDM-362A,CM-128) and five single cross hybrids namely H1 (KDM-361A X KDM-343A), H2 (KDM-332A X CM-128),H3 (KDM-914A X KDM-362A),H4 (KDM-916A X CM-128),H5 (KDM-332A X KDM-916A). A set of inbreds and hybrids was laid in Replicated Block Design with three replications. The observations were recorded for plant height, ear height, tassel length, ears per plant, days to tassel emergence, days to silk emergence, days to maturity, ear length, ear girth, number of rows per ear, number of grains per row, number of grains per ear, grain yield per plant. Analysis of variance was performed following Singh and Chowdhury (1985). Besides, total of thirty one morphological traits were studied as per Maize descriptor (IIMR, New Delhi).DNA extraction from young leaves was carried out by CTAB (Cetyl- Tri Methyl Ammonium Bromide) method as described by Murray and Thompson (1980).PCR assay was performed using 45 SSR markers retrieved from www.maizegdb.org (Table 1). PCR reaction mix contained ~25 ng of DNA, 10x PCR buffer (10 mMTris, pH 8.4, 50 mMKCl, 1.8 mM $MgCl_2$), 2 mM dNTPs (MBI, Fermentas, Lithuania, USA), 5 pmol each of forward and reverse primer and 1 U of Taq DNA polymerase (Bangalore GeneiPvt.Ltd., Bangaluru, India) in a reaction volume of 10 µl. Polymerase chain reaction (PCR) was performed in a thermal cycler (Eppendorf, Hamburg, Germany) with following thermal regimes: After initial denaturation for 4 min at94°C, each cycle comprised 1 min denaturation at 94°C, 1 min annealing at 55°C, and 2 min extension at 72°C with a final extension for 5 min at 72°C at the end of 35 cycles. The PCR products were mixed with bromo-phenol blue gel loading dye and were analyzed by electrophoresis on3.5% (w/v) agarose gel. The gels were stained in 0.5 mg/ml ethidium bromide and were documented using gel documentation system (Bio-Rad Laboratories Inc., USA). This was followed by scoring of bands with the help of 50 bp DNA size standard (Fermentas, Lithuania, USA).

Results and Discussion

Evaluation for grain yield and contributing traits

The hybrids and inbreds were evaluated for various agronomic and yield related traits (Table 2). The mean values for plant height

were recorded between 100 and 215 cm for inbreds CM-128 and KDM-916A, respectively. Among hybrids the plant height ranged from 166 cm for H_3 to 240 cm H1.

Hybrid H_1 was followed by hybrid H_4, H_2 and H_5.The inbreds recorded an average plant height of 125.29 cm against 205.70 cm for hybrids. The range of variability for days to tassel emergence across a set of inbreds and hybrids was recorded between 59 for KDM-343A to 67 KDM-914A DAS. Average of 61.9 and 60.7 DAS were recorded for inbreds and hybrids, respectively. The mean values for days of silk emergence ranged from 61.5 and 67.0 days within inbreds and from 63.5 to 64.5 within hybrids. The early silk emergence was recorded for CM-128 with 61.50 DAS while all the hybrids recorded around 64.0 days to emergence of silk with low CV (1.40%). Days to maturity ranged from 126.5 to 136 days among inbreds with inbred KDM-361A recording 126.5 days and KDM-916A recording 136.0 days. Hybrid H_2recorded early maturity with 127 DAS while as Hybrid H3 with 137 days recorded maximum days to maturity. Maximum ear length was recorded for inbred KDM-332A with 10.10 cm while the minimum ear length was recorded for CM-128 with 8.50 cm. Ear length ranged from 14.8 cm to 18.5cm for H2 and H1, respectively. Among the inbreds, the range of variability for ears per plant was recorded from 1.0 cm to 1.70 cm with the mean of 1.29 cm. KDM-332A and KDM-914A recorded highest number of ears per plant with 1.7 ears each followed by KDM-343A and KDM-362A both having 1.20 ears per plant. Hybrids H1 was found to be prolific with 2.10 ears per plant. Inbreds recorded the mean kernel rows per ear of 9.86 versus hybrids with average value of 14.51. Among inbreds, highest test weight of 18 gm was recorded for KDM-914 A. Test-weight ranged from 26.5 to 29.5 gm among hybrids with the mean of the 28.40 gm. The mean grain yield per hectare ranged from 19.71 to 25.60 q/ha for CM-128 and KDM-362A, respectively. Grain yield in hybrids ranged from 54.16 to 78.84 q/ha (H1) with a mean of 65.08 q/ha.

Characterization of single cross hybrids for different morphological attributes

The five single cross hybrids were evaluated for 31 descriptive traits following (DMR, DUS descriptor). It was found that only nine out of 31 traits studied were polymorphic among hybrids and rest of the traits were completely uniform and monomorphic across all the five hybrids. Out of these nine polymorphic traits, only 'Ear: anthocyanin colouration of glumes of cob' had three different classes, while rest eight were grouped in two contrasting phenotypes. The eight polymorphic traits included: Leaf: Altitude of blade, Tassel: Angle between main axis and lateral branches, Tassel: Altitude of lateral branches, Tassel: Length of main axis, Plant ear placement, Leaf: Width of blade, Ear: Length and Ear: color of top of grain. As many as eight out of 31 traits differed between H3 and H4 whereas; H1 and H4 differed with respect to only three traits (Table 3 and Fig. 1).

Amplification profile of SSR markers across parents and hybrids

The marker Umc-2383 amplified 135 bp allele in parents KDM-361A and KDM 343A and 150bp allele in rest of five parents (Fig. 2). Allele size of 135 bp allele was scored in hybrids H_1 and H_2 while 150bp allele was scored in rest of three hybrids.Umc-2100 amplified 100 bp allele in parents KDM 361A and KDM 343A and 125 bp allele in rest of five parents. Fragment size of 100 bp was scored in a single hybrid H_1 while other four hybrids record 125 bp allele. The marker Umc-2245 amplified 125 bp in two parents and amplified 100 bp allele in other five.

Table.1 The list of SSR markers used for identification of polymorphic markers between parents and multi-locus profiling of hybrids

S No	Marker	Forward primer sequence	Reverse primer sequence	Bin location
1	Umc2383	CATAGACGTGCCCCTTGTCATC	CTCGCAACTGCGCTTCTAGATACT	1.02-1.03
2	Umc1664	AATTGTTTACTGCGCTGAAACTCC	CCTCTTTGCCTGTACCGTGTATTC	1.06
3	Umc1147	GAGAAACCATCGACCCTTCCTAAC	TTCCTATGGTACAGTTCTCCCTCG	1.07
4	Umc2100	AAAGGCATTATGCTCACGTTGATT	TGACGTGCAAACAACCTTCATTAC	1.12
5	Umc2245	GCCCTGTTATTGGAACAGTTTACG	CGTCGTCTTCGACATGTACTTCAC	2.01
6	Umc1696	CTAGGGTTTAACCAACGGGGAG	TAAGGAGAGGGTCGATGAACACAT	2.1
7	Umc1823	AAAGCCTTACTGTTATTAGGCTAGGCA	AGAAAACCAGCCCCAGATGTTC	2.03
8	Umc1026	TCGTCGTCTCCAATCATACGTG	GCTACACGATACCATGGCGTTT	2.04
9	Umc2372	ACCCCTTGCGTTCTCTTCTGTT	CACCAGGCGTAGTGAGACAGC	2.06
10	Umc2144	CCAGCCCCTATCTATTTGCTTGT	GAATACTATATCACGGTCGGTCGG	2.08
11	Umc1594	GCCAGGGGAGAAATAAAATAAAGC	CACTGCAGGCCACACATACATA	3.09-3.1
12	Umc2071	ACTGATGGTGTTCTTGGGTGTTTT	ATACACGCAGTTACCCGAAGGTT	3.01
13	Umc2369	TTCGTCTGATGAAAGGTTCAGAGG	GATCCTCATCAAGACCAGCAGAGT	3.02-3.03
14	Umc1644	CCATAAACTGTTCCTTTGGCACAC	CTTTCACGTGTTAAGGGAGACACC	3.06
15	Bnlg1890	ACCGGAACAGACGAGCTCTA	GTCCTGCAAAGCAACCTAGC	4.11
16	Bnlg1621	CTCTTCGATCTTTAAGAGAGAGAGAG	ACACGAGGCACTGGTACTAACG	4.06
17	Umc1478	GAAGCTTCTCCTCTCGCGTCTC	CAGTCCCAGACCCTAGCTCAGTC	5.01
18	Umc1800	TTATGGGTGCTGGTGATGTGTATC	GAAAAGCAATCGCTTCTGAGAAAA	5.05
19	Umc2136	CCAGATGCGGAAGTAGACGG	GATTCGGAGGTGATCTGACCTGT	5.08
20	Umc1766	ACAAGAAGGAATCGAGAGCAAATG	CTTCGGGATGGAGTCGTAGTTC	5.01
21	Bnlg1306	CACCTTGAAAGCATCCTCGT	CAAAAACAAATGGCAGCTGA	5.07
22	Umc1918	CACAAGAACATTATGACGACCGAG	AAGCAGGAGACATCGTTTAAGTCG	6.03

23	Umc1762	CTTACTCCAGGCACTCCATACCAT	ATCCAGGTGAATGGTGTTTACGAT	6.06
24	Umc1063	AGGCCACTGAGCAGGTGAAG	GTGATGGTAGAGGAGTCCTTGGTG	6.07
25	Umc1018	GAACGGATATTGGAACCTGTGC	GTGCACGGTGTCGTACTTGAAC	6.01
26	Phi452693	CAAGTGCTCCGAGATCTTCCA	CGCGAACATATTCAGAAGTTTG	6.04
27	Umc1424	CCGGCTGCAGGGGTAGTAGTAG	ATGGTCAGGGGCTACGAGGAG	6.06
28	Phi129	GTCGCCATACAAGCAGAAGTCCA	TCCAGGATGGGTGTCTCATAAAACTC	6.05
29	Umc1002	AGCTAGCTATACACCGCCAGG	TCAGTTTGGAACAGGGAAAAGTA	6
30	Phi051	GGCGAAAGCGAACGACAACAATCTT	CGACATCGTCAGATTATATTGCAGACCA	7.05
31	Umc1036	CTGCTGCTCAAGGAGATGGAGA	GACACACATGCACGAGCAGACT	7.02
32	Umc1708	GATATGTCGAGCTTCGCTGGAG	CGCACACTAAAGCATCCTTAACCT	7.04
33	Umc2392	CAGAGACCTCGACTTCGACCAC	CTTCTGCTTCTGCTCGACCTTCT	7.01
34	Bnlg1056	ATCGTTGTTGGGTACACGGT	ACGGGTAGTGGTGAAGATGC	8.08
35	Umc1141	AGAGGAGAAAGAGACAGACAGGCA	CAGGAACTGAATGAAAGCAACTCA	8.06
36	Umc1415	GTGAGATATATCCCCGCCTTCC	AGACTTCCTGAAGCTCGGTCCTA	8.04
37	Umc1786	ACCGTGACTTCCTCCTCATAACTG	CATTTTTCGCATTTAGGAAATCCA	8.01
38	Phi067	CTGCAAAGGTAAGCACTAGGATGCT	CATCATTGATCCGGGTGTCGCTTT	9.01
39	Phi061	GACGTAAGCCTAGCTCTGCCAT	AAACAAGAACGGCGGTGCTGATTC	9.03
40	Umc1310	AACTCCGAGATCTACGACAACAGC	GAGGAAGAGTTGGCCAGGATG	9.06
41	Umc1675	GTTCTTCCTCTTCCCCATCAGTCT	ATAGCTGCGCGTAAAGCAACC	9.07-08
42	Umc1640	ACTACACGGTGTGAGATGTGATCG	GTCGTCGCAAGAACAACAAGG	10.07
43	Umc1077	CAGCCACAGTGAGGCACATC	CAGAGACTCTCCATTATCCCTCCA	10.04
44	Mmc0501	TGCTGAACACTCTAAGCAATAC	ATTACTCTACTCGCTGCCTG	10.02
45	Bmc1655	ATTAAAATCTTGCTGATGGCG	TTCTGTTCCCGCCTGTACTT	10.03

Table.2 Evaluation of parental inbreds and hybrids for yield and yield contributing traits

Entry	PH	DT	DS	DM	EL	EP	KE	KR	ED	SW	GY
KDM-361A	102.3	60.3	63.5	126.1	8.8	1.1	9.2	15.4	3.1	14.5	20.1
KDM-343A	108.5	59.4	63.3	130.4	9.7	1.2	11.5	12.5	3.5	15	20.6
KDM-332A	102.7	63.3	64.6	133.4	10	1.7	9.2	16.5	2.8	17.2	25.2
KDM-914A	128.6	67.3	67.7	131.2	9.4	1.7	11.5	12.9	3.1	18.8	25.5
KDM-362A	120.4	64.6	65	131.8	9.7	1.2	10.1	16	2.3	16.1	25.6
KDM-916A	215.3	60.6	63.5	136.3	9.6	1.1	11.8	12.5	2.8	14.4	19.9
CM-128	100.3	59.4	61.1	135.3	8.5	1.0	8.8	15.4	2.3	16.3	19.7
H_1(KDM-361A X KDM-343A)	240.3	59.5	63.2	131.4	18.5	2.1	16.3	40.8	5.5	29.5	78.8
H_2 (KDM-332A X CM-128)	206.3	59.3	63.7	127.1	14.8	1.5	13.5	34	3.8	29.4	54.1
H_3 (KDM-914A X KDM-362A)	166.4	60.4	64.2	137.7	17.3	1.9	15.0	40.5	4.8	29.4	70.4
H_4 (KDM-916A X CM-128)	227.5	61.8	63.1	129.1	15.6	2.0	13.2	40.9	5.2	26.5	57.4
H_5 (KDM-332A X KDM-916A)	188.0	63.4	64.2	132.3	17.4	1.7	14.4	40.7	4.0	27.7	64.4
Mean	158.9	61.6	63.9	131.8	12.4	1.5	12.0	24.8	3.6	21.2	40.1
Sd	54.3	2.5	1.5	3.5	3.9	0.4	2.5	13.0	1.1	6.6	22.9
CV (%)	34.2	4.1	2.4	2.7	31.4	25.4	20.5	52.4	30.2	30.9	57.0
Min	100.3	59.3	61.1	126.1	8.5	1.0	8.8	12.5	2.3	14.4	19.7
Max	240.3	67.3	67.7	137.7	18.5	2.1	16.3	40.9	5.5	29.5	78.8
Mean Inbreds	215.3	67.3	67.7	136.3	10	1.7	11.8	16.5	3.5	18.8	25.6
Mean Hybrids	240.3	63.4	64.2	137.7	18.5	2.1	16.3	40.9	5.5	29.5	78.8

PH: Plant height (cm); DT: Days to tassel emergence; DS: Days to silk emergence; DM: Days to maturity; EL: Ear length (cm); EP: Ears per plant; KE: Kernel rows per ear; KR: Kernels per row; ED: Ear diameter (cm); SW: 100-seed weight (g); GY: Grain yield (q/ha)

Table.3 Characterization of single cross hybrids for different morphological attributes

S No.	Character	KDM-361A X KDM-343A	KDM-332A X CM-128	KDM-914A X KDM-362A	KDM-916A X CM-128	KDM-332A X KDM-916A
1	Leaf angle between blade and stem (on leaf just above upper ear)	Small	Small	Small	Small	Small
2	Leaf : Altitude of blade (on leaf just above upper ear)	Straight	Straight	Drooping	Straight	Drooping
3	Stem : Anthocyanin colouration of brace roots	Absent	Absent	Absent	Absent	Absent
4	Tassel : Time of anthesis	Early	Early	Early	Early	Early
5	Tassel : Anthocuanin colouration of base of glume	Absent	Absent	Absent	Absent	Absent
6	Tassel : Anthocyanin colouration of glumes excluding base	Absent	Absent	Absent	Absent	Absent
7	Tassel : Anthocyanin colouration of anthers (in middle third of main axis on fresh anthers)	Absent	Absent	Absent	Absent	Absent
8	Tassel : Density of spikelets (in middle third of main axis)	Sparse	Sparse	Sparse	Sparse	Sparse
9	Tassel : Angle between main axis and lateral branches (in lower third of tassel)	Wide	Narrow	Narrow	Wide	Narrow
10	Tassel : Altitude of lateral branches (in lower third of tassel)	Curved	Straight	Straight	Curved	Straight
11	Ear : Time of silk emergence	Early	Early	Early	Early	Early
12	Ear : Anthocyanin colouration of silks (on day of emergence)	Absent	Absent	Absent	Absent	Absent
13	Leaf Anthocyanin colouration of sheath (below the ear)	Absent	Absent	Absent	Absent	Absent
14	Tassel : Length of main axis above lowest side branch)	Medium	Medium	Medium	Long	Long
15	Plant ear placement	Medium	Low	Medium	Low	Low
16	Leaf : Width of blade (leaf of upper ear)	Broad	Medium	Broad	Broad	Medium
17	Ear : Length without husk	Long	Long	Long	Medium	Long
18	Ear. Diameter without husk (in middle)	Large	Large	Large	Large	Large
19	Ear : Shape	Conical	Conical	Conical	Conical	Conical
20	Ear : Number of rows of grains	Many	Many	Medium	Many	Many
22	Ear : Type of grain (in middle third of ear)	Flint	Flint	Flint	Flint	Flint
23	Ear: color of top of grain	Yellow	Yellow with cap	Yellow with cap	Yellow	Yellow
24	Ear : anthocyanin colouration of glumes of cob	White	Dark purple	Light purple	White	Light purple
25	Kernel : row arrangement (middle of ear)	Straight	Straight	Straight	Straight	Straight
26	Kernel : poppiness	Absent	Absent	Absent	Absent	Absent
27	Kernel : sweetness	Absent	Absent	Absent	Absent	Absent
28	Kernel : waxiness	Absent	Absent	Absent	Absent	Absent
29	Kernel : apaqueness	Absent	Absent	Absent	Absent	Absent
30	Kernel : Shape	Toothed	Toothed	Toothed	Toothed	Toothed
31	Kernel : 1000 kernel	Medium	Medium	Medium	Medium	Medium

Table.4 Simple sequence repeat marker based profile of five single cross hybrids

S No	Marker Name	KDM-361A X KDM-343A	KDM-332A X CM-128	KDM-914A X KDM-362A	KDM-916A X CM-128	KDM-332A X KDM-916A
1	**Umc-2383**	**135**	**150**	**150**	**135**	**150**
2	**Umc-1664**	**140/120**	**140/120**	**140/120**	**140/120**	**140/120**
3	**Umc-1147**	**100/85**	**120/85**	**85**	**120/85**	**85**
4	Umc-2100	100	125	125	125	125
5	**Umc-2245**	**100**	**100**	**125**	**100**	**100**
6	Umc-1696	NA	150	150	150	150
7	Umc-1823	100	100	100	100	100
8	Umc-1026	150	150	150	150	150
9	**Umc-2372**	**150/120**	**140/120**	**150/120**	**140/120**	**150/120**
11	**Umc-1594**	**140/100**	**140**	**140**	**140**	**140**
12	Umc-2071	150	150	150	150	150
13	Umc-2369	300	300	300	300	300
16	Bnlg-1621	200	200	200	200	200
17	Umc-1478	150	150	150	150	150
18	Umc- 1800	150	150	150	150	150
19	Umc-2136	150	150	150	150	150
20	Umc-1766	165	165	165	165	165
21	Bnlg-1306	175	175	175	175	175
24	Umc-1063	150	150	150	150	150
26	Umc-1036	175	175	175	175	175
27	Phi-129	100	100	100	100	100
28	Phi-051	140	140	140	140	140
30	Umc-2392	90	90	90	90	90
31	Umc-1141	100	100	100	100	100
33	Phi-067	200	200	200	200	200
37	Umc-1675	150	150	150	150	150
38	Umc-1640	125	125	125	125	125
41	Phi-061	80/60	60/80	80/60	80/60	80/60
42	**Umc-1002**	**160/140**	**140**	**150/170**	**140**	**140**
43	**Umc-1424**	**140**	**140/110**	**140/110**	**140**	**140**
44	**Phi-452693**	**125**	**125**	**125**	**125**	**105**

Bold figured markers are highly polymorphic in discriminating the hybrids

Table.5 The Probability of identity between hybrids based on amplification SSR barcode using ten SSR markers

	H_1	H_2	H_3	H_4	H_5
H_1		**0.16**	**0.10**	**0.15**	**0.13**
H_2			**0.15**	**0.21**	**0.18**
H_3				**0.13**	**0.14**
H_4					**0.16**
H_5					

Fig.1 Close view of single cross hybrid

Fig.2 Allelic diversity across five single cross hybrids

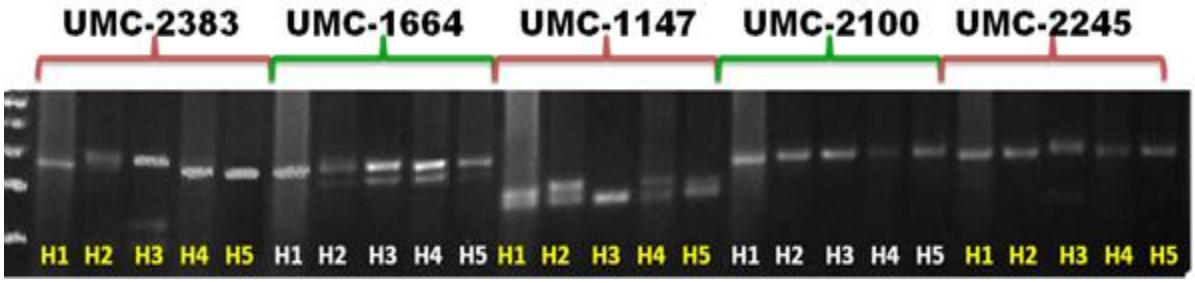
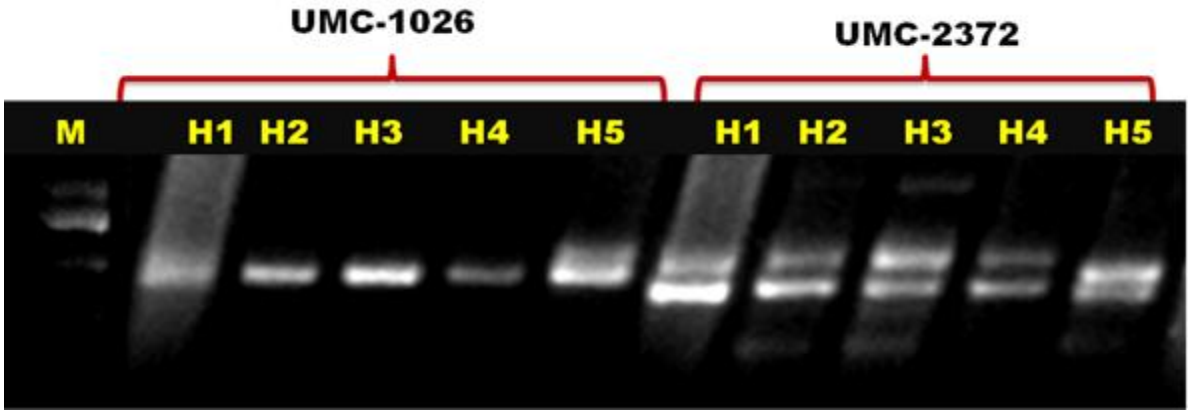


Fig.3 Amplification profile of single cross hybrids against SSR marker UMC-1026 and UMC-2372



A marker UMC-2372 was polymorphic across the five hybrids.
Designation of single cross hybrids: H_1: KDM-361A X KDM-343A; H_2: KDM-332A X CM-128; H_3: KDM-914A X KDM-362A; H_4: KDM-916A X CM-128; H_5: KDM-332A X KDM-916A; M: 50 bp DNA Ladder (MBI, Fermentas, Luthuania)

Allele size of 125 bp was scored in a single hybrid H_3 while as rest of 4 hybrids showed 100bp allele.Umc-1696 amplified 150 bp allele in most of the parents and hybrids. Marker Umc 2372 amplified 150 bp allele in parents KDM 361A and KDM 914 A, 140 bp allele in parent CML 128 and in rest four parents 120 bp allele was scored.

In hybrids this marker was polymorphic across all the five hybrids with allele size of 150/120, 140/120,150/120, 140/120, 150/120 bp in hybrids H_1, H_2, H_3, H_4 and H_5 respectively (Fig. 3). The marker Umc-1708 showed polymorphism between the parents of hybrid H_1 with 80 bp and 60 bp alleles whereas; hybrids H_2 and H_5 amplified 80 bp and 60 bp alleles, respectively. Marker phi 061 was polymorphic across all hybrids with 80 bp (KDM342A, KDM914A, KDM 916A and CM-128) and 60 bp (KDM361A, KDM 332A and KDM 362A) sized alleles (Table 4).

The markers revealed lack of polymorphism across all parents and hybrids with fragment size of 90 bp for the marker Umc-2392; 100 bp for Phi-129, Umc-1141, Bulg-1056, Umc-1823; 140 bp for Phi-051; 195 bp for Umc-1036; 150 bp for Umc-1063, Umc-1478, Umc-1675, Umc-1800,Umc-1026 and Umc-2136; 165 bp for Umc-1766; 175 bp for Umc-

1306; 200 bp for Phi-067, Umc-1415, Bnlg 1621; 300 bp with respect to markers Umc-2071 and Umc-2369.

Fingerprinting of single cross hybrids

Multi-locus profiling of five single cross hybrids was carried out using 45 genome wide SSR markers. Ten most polymorphic markers were identified based on their PIC values and included the markers, Umc-283, Umc-1664, Umc-1147, Umc-2245, Umc-2372, Umc-1594, Phi -061, Umc-1002, Umc-1424 and Phi -452693. These ten markers were used to generate DNA profile for five single cross hybrids. SSR Marker Umc-2383 amplified 135 bp allele in hybrids H_1, H_4 and 150 bp allele in H_2, H_3, and H_5. Marker Umc-1664 was found to be polymorphic across all the five hybrids amplifying a fragment size of 140 and 120 bp in each of the five hybrids. Umc-1147 showed polymorphism between constitutive parents of hybrids H_1, H_2 and H_4 and were monomorphic in H_3 and H_5. H_1 with respect to this marker had an allele size of 100 and 85 bp. The marker amplified 120 bp and 85 bp in H2. The fragment size of 120 and 85 bp was scored in hybrid H_4. The marker Umc-2245 amplied 100 bp fragment in H_1, H_2, H_4 and H_5. Umc-2375 was again polymorphic between corresponding parents of all the single cross hybrids. Umc-1594 showed fragment size of 140 bp and 100 bp in H1 and unique allele of 140 bp in rest four hybrids.

Ph-061 amplified 2 bands each in hybrids H_1, H_4 and H_5 with a size of 80 and 60 bp. Hybrid H_1 and H_3 were characterized having two alleles each against marker Umc-1002. Umc-1424 amplified two fragments each in H_2 and H_3. Phi 452693 amplified 125 bp across all the five hybrids. Taken together each hybrid revealed distinct allele profile across a set of ten markers. Hybrids H_1, and H_5 recorded 16 and 13 alleles against 10 SSR markers, whileH_2, H_3, H_4 amplified 14 alleles each. Based on allelic profile generated for at 10 SSR loci, the hybrids showed maximum genetic divergence of 90% between H_1 and H_3. The probability of identity was highest (0.21) between hybrids H_2 and H_4 and lowest (0.10) between H_1 and H_3 (Table 5).

During hybrid seed production, greater emphasis is directed towards managing the process to ensure maximum kernel set and high levels of genetic purity (Fonseca *et al.*, 2003). It is necessary to set up a fast, economical and effective system for testing the purity in order to prevent impure seeds from entering the market (Dou *et al.*, 2102).Normally, seed certification for purity and variety distinctness is based on morphological evaluation of seeds and growing plants. For an experimenter Grow out Test is a standard technique to evaluate purity of hybrid seed by comparing seeds and plants in the same stage in identical environmental conditions. These evaluation methods often involve field inspection, which are rigorous, resource and time intensive, prone to error and have very little precision. However, now it has been possible to evaluate the large number of plants at DNA or protein level in a much reproducible manner with greater precision. Among these are use of molecular markers (Perry, 2004; Ali *et al.*, 2008) particularly SSRs (Tautz, 1989) which have proved to be the preferred molecular marker systems for purity identification in some crops (due to their co-dominant nature, high efficiency and simplicity (Wu *et al.*, 2010).

In the present study, the SSR markers Umc-2383, Umc-2100, Umc-2245, Umc-1696, Umc-1708 were able to discriminate between parents of one or the other hybrid. The markers Umc-1664, Umc-2372, Phi-061revealed polymorphism across parents of all the five hybrids and belonged to chromosomes 1, 2 and 9, respectively.

Apart from the fact that genetic diversity of maize germplasm is important for planning breeding programmes and conservation of maize germplasm, etc, it is also important that farmers have an opportunity to choose among hybrids the one that will be a choice for highest yields and suitable for environmental stress conditions (Troyer *et al.,* 1983). In Kashmir province, several different hybrids are being pumped by private sector besides few known public bred hybrids those have occupied considerable area. Most of times it becomes difficult for a grower or even seed certification agencies to identify a given hybrid in market merely on the basis of morphological markers or descriptive traits. The hybrids with similar seed characteristics like color, indentation, size and pattern may not easily discern the identity when offered to grower. A set of polymorphic markers in present study was thus used to finger print hybrids. Finally a sub-set of 10 ploymorphic markers were used in order to discriminate five single cross hybrids H_1, H_2, H_3, H_4 and H_5. Collectively, twelve different alleles (60, 80, 85, 100, 110, 120 125, 135, 140, 150, 160 and 170 bp) were amplified with the size range of 60 bp to 170 bp. Hybrid H_1, H_2, H_3, H_4 and H_5 amplified 16, 14, 14, 14 and 13 alleles, respectively. Based on SSR profile using 10 markers, the hybrids showed distinct molecular profile amongst themselves. Low probability of identity was recorded between H_1 and H_3 (0.10). Probability that H_2 and H_4 shared alleles at 10 markers loci was only 21% that was the highest among all the paired combinations. The SSR profile standardized here holds a promise and could be used as a key to establish genetic identity of hybrids sampled form open market or farmers' custody, with extreme precision and greater reliability.

Protection of Plant varieties and Farmers Right authority insists on characterization and registration of extant, farmers and new varieties as a part of national and botanical asset. Pinnisch *et al.,* (2012) advocated characterizing inbred lines which serve as the seed parents to commercially grown maize hybrids. Therefore, a set of inbreds was characterized using 31 morphological traits following Maize descriptor (DMR, New Delhi). Only nine out of 31 traits studied were polymorphic among hybrids among ear characters only traits 'ear length' and 'grain color' were found polymorphic. As many as eight out of 31 traits differed between H_3 and H_4 whereas, H_1 and H_4 differed with respect to only three traits, therefore, revealed high similarity. The marker analysis based on SSRs revealed 4-5 fold more genetic divergence among hybrids when compared to that based on morphological traits. In fact only 10 SSR markers revealed 80-90% divergence among hybrids that was much higher than what was found based on phenotypic trait variation among the five hybrids. SSRs have been known and several occasions advocated to be markers of choice for estimation traits diversity among hybrids and also for establishing identify and genetic purity of hybrids in crop like maize (Natalya *et al.,* 2002; Wang *et al.,* 2011).

References

Ali, M.A., *et al.,* 2008. Hybrid authentication in upland cotton through RAPD analysis. Aus. J. of Crop Sci., 2(3), 141-149.

Anonymous, 2012. Digest of statistics., Directorate of Economics & Statistics Planning and Development Department, Govt. of J&K, Srinagar pp. 94-100.

Anonymous, 2013. Project Director Review, All India Coordinated Annual Maize Workshop, 6-8, April, ANGRAU, Hyderabad.

Asif, M., Rahman M and Zafar Y 2006. Genotyping analysis of six maize (*Zea mays* L.) hybrids using DNA

fingerprinting technology. *Pak. J. Bot*., 38(5): 1425-1430.

Dou, X., Yan M, Xu Y, Hussain K, Liu Y, Lin F. 2012. Identification and purity testing of maize hybrids with one parent in common by ultrathin-layer isoelectric focusing of seed salt-soluble proteins. Turk J Agric For 36: 267-273.

Fonseca, A.E., *et al.*, 2003. Tassel morphology as an indicator of potential pollen production in maize. Crop Management. Online doi, 10.1094/CM-2003-0804-01-RS.

Kalinka Carla Padovani de Carvalho Salgado, *et al.*, 2006. Genetic purity certificate in seeds of hybrid maize using molecular markers. Rev. Bras. Sementes, 28(1), Pelotas.

Liu-Jie, Liu-Gongshe, Li-Dianrong, Ri-Dongmei. Wang Hao and Wang-Daojie, 2000. Identification of genetic purity of seed of hybrid rape with RAPD.*Chinese J. Appl. Envorn. Biol.*, 6(6): 526-529.

Mao, C. X., Vermani, S. S., and Kumar, I. 1996. Technological innovations to lower the cost of hybrid rice seed production.Proc, of 3rd Intsymp. On hyb. Rice. pp: 111-123.

Natalya, S., McMullen MD, Schultz L and Edward H. C., 2002. Development and mapping of SSR markers for maize. Plant Molecular Biology 48: 463-481

Pallavi, H. M., Maithreyee. M. N. and Gowda R 2010. Application of molecular markers in hybrid purity testing. Summer school on advances in quality seed production, processing and marketing. pp: 220-223.

Perry, D.J., 2004. Identification of Canadian durum wheat varieties using a single PCR. Theor. App. Gen., 109(1), 55-61.

Pinnisch, R., Mowers R, Trumpy H, Walejko R, Bush D 2012. Evaluation of maize (*Zea mays* L) inbred lines for yield component traits and kernel morphology. Maydica 57:1-5.

Singh, R.K., and Chaudhary, B. D. 1985. Biometrical Methods in Quantitative Genetic Analysis, Kalyani Publishers, New-Delhi. Revd Ed. pp: 39-68.

Srivastava, J., And Jaffee, S. 1993. Best practices for moving seed technology. The World Bank, Washington DC, Technical Paper No 213,

Troyer, A.F., Openshaw S.J. and Knittle K.H. 1983. Measurement of genetic diversity among commercial corn hybrids. Method. In: Agronomy abstracts. ASA, Madison, WI, p: 83.

Wang, F., Tian, H, Zhao J, Yi H, Wang L, Song W. 2011. Development and characterization of a core set of SSR markers for fingerprinting analysis of Chinese maize varieties. *Maydica* 56: 1-11.

Wu, M., *et al.*, 2010. Rapid and reliable purity identification of F1 hybrids of Maize (*Zea mays* L.) using SSR markers. *Plant Breeding*, 4(3), 381-384.

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