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Parentage validation for certain hybrids of mango (*Mangifera indica* L)

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Abstract
The mango is highly cross-pollinated and heterozygous. It has delicate flowers and the fruit set is only about 0.01% ([8]Iyer, 1991). In the absence of morphological markers, it is extremely difficult to make out whether the resultant hybrid progenies are from the parents, which were utilized for crossing or not. The morphological observations recorded shows that the colour of the variety 'Sensation' and Janardhan Pasand as male parent imparts young leaf colour (reddish brown) to the hybrid. The hybrid Arunika, also had a colour similar to its male parent 'Vanraj'. These results help in developing pre-selection indices for progeny selection. The molecular characterization of thirty-eight hybrids carried out with eight SSR (Simple Sequence Repeat), revealed paternal allele inheritance for the hybrids Arka Udaya, Konkan Ruchi, Manjeera and AU-Rumani to the extent of 50, 50, 54.54 and 50%, respectively. The five hybrids *viz.*, Arka Anmol, Arka Udaya, Konkan Ruchi, Manjeera, AU-Rumani were confirmed for their parentage by the allele transmission from parents to offspring. The validation of hybrids using total phenolics and flavonoids indicated both paternal and maternal inheritance.

Keywords: Triclosan, TCS, determination, detection, sensor Mango, Heterozygosity, hybrids, parent, Pre-selection, SSR marker, validation

Introduction

The mango (*Mangifera indica* L) regarded as one of the choicest fruits of the world, belongs to the family Anacardiaceae. Its origin is traced back to 4000 years (De Candolle, 1884) [5]. In India more than thousand varieties are under cultivation with large diversity (Mukherjee, 1953) [10]. Mango being highly cross pollinated, most of the cultivated types represent land races that originated as seedling selections and have since been maintained through vegetative propagation (Mukherjee *et al*., 1968 and Ravishankar *et al*., 2004) [11, 13].

Despite the drawbacks, ailing mango breeding like, high heterozygosity, and single seed per fruit, breeding can be successful because of a number of positive attributes *viz.*, wide range of available genetic variation and the ease with which a selected hybrid can be vegetatively propagated (Iyer and Schnell, 2009) [9]. The presence of delicate flowers, complex floral biology, poor fruit set and absence of pre-selection indices have made validation a necessity for determining the parentage of a hybrid. Analysis of hybrids and their parents is essential to know the contribution of each parent to their progenies, which will help in further analysis of hybridization programs (Vasanthaiah, 2009) [21]. Mango cultivars are often identified by the morphological traits like leaf and fruit characteristics (Campbell, 1992 and He *et al*., 2007) [3, 6]. But, morphological markers have certain limitations as they vary with the environmental conditions (Tanksley *et al*., 1989) [19]. Of all the markers 'Simple Sequence Repeats (SSR)' show great potential for mango improvement and can be performed for variety identification and validation of parentage (Anshuman *et al*., 2012) [1], estimation of genetic variation in existing populations and characterisation of rootstocks (Brettell *et al*., 2002) [2].

Materials and Methods

The mango hybrids along with their parents were utilized for validation. Leaf samples for analysis were taken from Field Gene Bank (FGB) maintained at IIHR, Bengaluru. Leaf morphometric characters like colour and shape was noted from 5-10 days old leaves collected from three plants. This was recorded for the hybrids Arka Aruna, Arka Puneet, Arka Anmol, Arka Neelkiran, Ratna, Sindhu, Amrapali, Mallika, Kodur Mango Hybrid-1, Swarnajehangir, Neeleshan, Au-Rumani, Manjeera, Ambika, Arunika, Pusa Shreshth, Pusa Pratibha and Pusa Arunima along with their parents, using 'Bioversity International Descriptors' for mango.

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Phenols and flavonoids in the leaves were estimated for hybrids and parents using the protocol of Shivashankara *et al.* (2012) [18].

Molecular characterization for thirty-eight hybrids and their parents was carried out using eight SSR markers *viz.*, MiIIHR17, MiIIHR23, MiIIHR26, MiIIHR30, MiIIHR31, MiIIHR34 and MiIIHR36 developed by Ravishankar *et al.* (2011) [14]. The genomic DNA was isolated using CTAB (cetyl trimethylammonium bromide) method by taking leaf samples. The PCR reactions were carried out in 10µl reaction mixture. The amplified PCR products were then separated in 1.5% Agarose gel and viewed under UV light gel documentation system (UVi PRO, UK). The SSR profiling was carried out according to Ravishankar *et al.* (2015) [15]. The sizes of the PCR products were obtained by automated ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA). Parentage validation was done based on the allele transmission from parents to offspring at each SSR locus.

The mango hybrids and their parents used for biochemical and molecular analysis are portrayed in Table 1.

Results and Discussion
Morphological characterization
Leaf colour (reddish brown) in the hybrids Pusa Pratibha, Pusa Shreshth and Pusa Arunima derived from the parentage Amrapali x Sensation, was similar to the paternal parent Sensation, whereas, shape differed from their parents. Leaf colour (reddish brown) in the hybrid Ratna was found to be inherited from its paternal parent Alphonso. Hybrid KMH-1 and Arka Anmol differed from their parents with respect to leaf colour and shape, Manjeera had leaf colour (reddish brown) similar to its paternal parent Neelum. Leaf colour (reddish brown) and leaf shape (lanceolate) in Arka Neelkiran was similar to its paternal and maternal parents Neelum and Alphonso, respectively. Au-Rumani had its leaf colour (reddish brown) and shape (elliptic) similar to its paternal parent Mulgoa. Leaf shape (lanceolate) was observed to be similar to that of its maternal parent Alphonso in the hybrid Arka Puneet. Swarnajehangir had its leaf shape (lanceolate) similar to its maternal parent Suvarnarekha and leaf colour (light green with brownish tinge) similar to paternal parent Jehangir. Arka Aruna had a leaf shape (oblong) similar to its maternal parent Banganapalli, showing the maternal inheritance of this character.

The observation recorded in various hybrids on the colour of the young leaf showed that the variety Sensation as male parent imparts similar colour to the hybrids (reddish brown). This is also seen when Janardhan Pasand is used as one of the parents. This is evident from previous studies of Iyer and Subramanyam (1987) [7] that Janardhan Pasand when crossed with a green coloured cultivars variation of color was seen in progenies, indicating colour is governed by a number of loci. In the hybrids wherein Dashehari is one of the parents, the hybrid progenies tend to get only light green young leaves, which is evident in the hybrids Mallika and Amrapali. One hybrid Arunika, which has Vanraj as one of the parents also had coloured young leaf. This also shows that with the evaluation of increased number of progenies in a particular cross these characters can be very effectively used as pre-selection indices in mango breeding. It was observed that progenies of red coloured varieties exhibited gradation of red colour (Sharma *et al*, 1987) [16], indicating that colour could be transmissible to hybrids. In the case of highly heterozygous crop like mango, large number of progenies can only give the

indication of the combining ability of the parent for a particular character.

Biochemical characterization
Total phenolic content in the leaf extract of hybrids and parents showed a difference in content (2977.60 mg/100 g FW (fresh weight) in Ratna to 7627.43 mg/100 g FW) in Amrapali whereas Arka Anmol recorded maximum total flavonoids (443.17 mg/100 g FW) (Table 2). In case of total phenolics, 18 hybrids were observed to have phenolic content intermediate between their parents. In 12 hybrids phenolic content exceeded both maternal and paternal parent. The phenolic content was observed to be lower than the parents in the hybrids KMH-1 and Ratna in which Alphonso is the paternal parent. However, as maternal parent, the total phenolic content exceeded in progenies. Total phenols are being used as biochemical idex for screening mango progenies (Sharma *et al*, 2000) [17]. In total flavonoids, 10 hybrids were observed to have flavonoid content intermediate between their parents. In 17 hybrids, the flavonoid content exceeded both maternal and paternal parent. The flavonoid content in the hybrids Neeleshan, Neelphonso, Neeleshwari, and Neeleshan Gujarat in which Neelum is the maternal parent was observed to be lower than the parents. Some of the workers have attempted chemical profiling in recent times in pickling mango varieties viz., Appemidi (Vasugi *et al.*, 2012) [22] and other mango cultivars for fruit volatiles (Pandit *et al*, 2009) [12], to study their genetic diversity. In some studies leaf volatiles are being used for the primary selection of progenies (Campbell and Zill, 2006) [4].

Perennial crop breeding is time consuming and the prediction of progeny performance is extremely difficult due to the fact that they are highly heterozygous (Iyer and Schnell, 2009) [9]. Pre-selection indices in crop breeding play an important role. Biochemical markers can help in the primary screening of the progenies.

Molecular characterization
A total of 81 alleles were obtained from 8 SSR loci for 62 genotypes including hybrids and parents. The number of alleles per locus ranged from 5 (MiIIHR31) to 14 (MiIIHR34), with an average of 10.13. Maximum number of heterozygote alleles (21 out of 55 individuals typed) for genotypes was observed in MiIIHR34 loci and minimum heterozygote alleles of 1 was seen in MiIIHR18 loci and MiIIHR26 for the 60 and 42 individuals typed. Of the 8 markers used 3 (MiIIHR17, MiIIHR30, MiIIHR36) were found to be homozygous for all the individuals typed (Table 3).

Percentage of allele transmission from maternal and paternal parent to their offspring are presented in Table 4. Out of thirty eight hybrids five hybrids were confirmed for their parentage. Hybrid Arka Udaya (Amrapali x Arka Anmol) showed 50% paternal allele inheritance, whereas, hybrid Manjeera had paternal allele inheritance to the extent of 54.54% and AU-Rumani had paternal allele inheritance to the extent of 50%. The hybrid Konkan Ruchi showed paternal allele inheritance to the extent of 50%.

For the hybrids, Arka Udaya and Arka Anmol, one out of eight SSR loci showed polymorphism between their parental cultivars *viz.*, Amrapali and Arka Anmol, Alphonso and Janardhan Pasand, respectively. In the hybrid Arka Udaya, the 156 bp and the 169 bp alleles at the MiIIHR18 SSR were considered to be derived from Amrapali and Arka Anmol, respectively. Whereas, in Arka Anmol, the 231 bp and the

238 bp alleles at the MiIIHR34 were considered to be derived from Janardhan Pasand and Alphonso, respectively. The variation might be due to the variation in the repeat motif number of an allele at that locus which is occurred due to replication slippage or recombination. There has been considerable amount of deviation in the expected allele size of the hybrids in comparison to that of their respective parental lines ranging from 1bp to 8bp. additionally, some alleles haven't been called and picked up by the genetic analyzer, though expected amplicon was observed on the agarose gel.

Considering the allele transmission from parents to offspring total of five hybrids *viz.,* Arka Anmol, Arka Udaya, Konkan Ruchi, Manjeera, AU-Rumani were confirmed for their parentage. Nullifying 2bp (This *et al*, 2004) [20] difference of allele length between parents and hybrids resulted in the identification of true hybridity of Sonpari at MiIIHR30 with respect to allele 200 and 198 inheriting from their parents. In this study dialect in the simple mendelian inheritance was seen. The reasons for absence of simple mendelian inheritance in case of SSR allele might be due to recombination, slippage or error in sizing.

In conclusion, mango, which is highly heterozygous the progeny parentage is not confirmative. As in most cases the progenies do not resemble the parents due to the heterozygous nature. Hence, there is a need to validate them. In this case morphological (leaf colour) and biochemical (phenolics and flavonoids) traits can be used as pre-selection indices in fruit breeding. As an alternative to morphological markers, molecular markers are used in validating hybrids. In this experiment the molecular characterization of thirty-eight hybrids carried out with eight SSR (Simple Sequence Repeat), revealed paternal allele inheritance for the hybrids Arka Udaya, Konkan Ruchi, Manjeera and AU-Rumani to the extent of 50, 50, 54.54 and 50%, respectively.

Table 1: List of mango hybrids and their parents utilized for biochemical and molecular analysis

Sl. No.	Hybrids	Parents	Stations/ Institutions
1	Arka Aruna	Banganapalli × Alphonso	IIHR, Bengaluru
2	Arka Puneet	Alphonso × Banganapalli	
3	Arka Anmol	Alphonso × Janardhan Pasand	
4	Arka Neelkiran	Alphonso × Neelum	
5	Arka Udaya	Amrapali × Arka Anmol	
6	Ratna	Neelum × Alphonso	KKV, Maharashtra
7	Sindhu	Ratna × Alphonso	
8	Konkan Raja	Bangalora × Himayuddin	
9	Konkan Ruchi	Neelum × Alphonso	
10	Amrapali	Dashehari × Neelum	IARI, New Delhi
11	Mallika	Neelum × Dashehari	
12	Pusa Shreshth	Amrapali × Sensation	
13	Pusa Pratibha	Amrapali × Sensation	
14	Pusa Arunima	Amrapali × Sensation	
15	Pusa Lalima	Dashehari × Sensation	
16	Pusa Pitamber	Amrapali × Lal Sundari	
17	Kodur Mango Hybrid-1	Cherukurasam × Khader	YSRHU, AP
18	Swarnajehangir	Suvarnarekha × Jehangir	
19	Neelgoa	Neelum × Mulgoa	
20	Neeleshan	Neelum × Baneshan	
21	Au-Rumani	Rumani × Mulgoa	
22	Manjeera	Rumani × Neelum	
23	Ambika	Amrapali × Janardhan Pasand	CISH, Lucknow
24	Arunika	Amrapali × Vanraj	
25	H-87	Kalapadi × Allampur Baneshan	KAU, Kerala
26	H-85	Kalapadi × Allampur Baneshan	
27	H-151	Kalapadi × Neelum	
28	H-56	Bennet Alphonso × Himayuddin	
29	PKM-1	Chinna Suvarnarekha × Neelum	TNAU, Tamil Nadu
30	PKM-2	Neelum × Mulgoa	
31	Neeleshan Gujarat	Neelum × Baneshan	GAU, Paria
32	Neelphonso	Neelum × Alphonso	
33	Neeleshwari	Neelum × Dashehari	
34	Sonpari	Alphonso × Baneshan	
35	Al Fazli	Alphonso × Fazli	BAU, Sabour
36	Prabhashankar	Bombai × Kalapadi	
37	Mahmood Bahar	Bombai × Kalapadi	
38	Sabri	Gulabkhas × Bombai	

Table 2: Flavonoids and Phenolics of the genotypes

Genotypes	Total flavonoids @ 510 nm	Total phenolics @ 700 nm	Genotypes	Total flavonoids @ 510 nm	Total phenolics @ 700 nm	Genotypes	Total flavonoids @ 510 nm	Total phenolics @ 700 nm
	mg/100g fresh wt.			mg/100g fresh wt.			mg/100g fresh wt.	
KMH-1	202.53 ± 0.98	3230.86 ± 32.46	H-87	441.38 ± 2.76	5597.67 ± 258.97	Rumani	198.79 ± 6.75	5193.58 ± 103.63
Arka Udaya	380.99 ± 2.23	4607.00 ± 72.57	Neelphonso	154.55 ± 6.57	4247.60 ± 58.08	Suvarnarekha	131.23 ± 20.73	3646.12 ± 102.14
Arka Anmol	443.17 ± 20.66	6065.08 ± 298.87	Neeleshwari	201.93 ± 5.70	4595.83 ± 34.48	Banganpalli	250.51 ± 3.51	4119.11 ± 17.76
Manjeera	195.95 ± 0.93	4100.49 ± 101.38	Sonpari	264.85 ± 7.38	4249.46 ± 84.12	Bangalora	147.97 ± 9.26	4191.74 ± 78.10
Arka Neelkiran	305.81 ± 10.54	3793.23 ± 84.04	Neeleshan Gujarat	173.38 ± 3.06	3744.82 ± 62.23	Chinna Suvarnarekha	202.08 ± 5.39	3644.26 ± 44.49
AU-Rumani	229.88 ± 1.66	3990.62 ± 33.87	Ambika	283.84 ± 7.00	4640.52 ± 75.11	Bennet Alphonso	287.57 ± 5.65	4755.97 ± 64.61
Arka Puneet	307.15 ± 3.13	4214.08 ± 122.23	Arunika	342.58 ± 4.10	5452.42 ± 203.91	Gulabkhas	207.01 ± 8.89	4215.95 ± 127.16
Swarnajehangir	209.70 ± 6.39	4072.56 ± 80.56	Pusa Pratibha	228.68 ± 1.95	4815.56 ± 1121.14	Janardhan Pasand	276.06 ± 4.56	4800.67 ± 45.87
Arka Aruna	204.47 ± 8.59	3804.41 ± 48.80	Pusa Shreshth	290.41 ± 5.58	5465.46 ± 51.74	Neelum	220.76 ± 2.10	3858.41 ± 14.54
Neeleshan	218.37 ± 11.83	4858.39 ± 96.88	Pusa Lalima	203.87 ± 10.02	5767.13 ± 13.03	Mulgoa	225.25 ± 6.20	3966.42 ± 45.15
Ratna	197.00 ± 5.14	2977.60 ± 84.60	Pusa Pitamber	265.15 ± 4.56	6571.59 ± 391.32	Jehangir	193.26 ± 1.81	4957.09 ± 16.55
Sindhu	283.09 ± 5.14	3519.50 ± 83.85	Pusa Arunima	204.32 ± 5.11	4161.94 ± 119.42	Himayuddin	245.72 ± 4.68	6906.78 ± 115.45
Konkan Ruchi	249.91 ± 5.28	3584.67 ± 108.53	Amrapali	316.12 ± 10.67	7627.43 ± 256.76	Allampur Baneshan	229.28 ± 3.37	4713.14 ± 104.82
Konkan Raja	191.17 ± 6.24	3951.52 ±20.98	Mallika	271.88 ± 4.60	5552.98 ± 181.99	Dashehari	292.95 ± 6.75	4093.04 ± 92.15
Neelgoa	202.08 ± 10.30	3754.13 ± 116.64	Prabhashankar	255.74 ± 12.28	4169.39 ± 71.56	Vanraj	250.95 ± 8.76	3592.12 ± 78.80
PKM-2	256.48 ± 4.53	3189.89 ± 11.62	Mahmood Bahar	292.66 ± 8.46	4767.15 ± 79.15	Sensation	236.31 ± 7.97	3564.19 ± 47.51
PKM-1	174.13 ± 11.10	3180.58 ± 54.09	Sabri	211.64 ± 3.62	3666.61 ± 97.04	Kalapadi	200.43 ± 10.36	3543.70 ± 93.99
H-85	239.89 ± 9.41	5526.91 ± 110.81	Al Fazli	281.15 ± 16.92	5228.96 ± 89.61	Bombai	207.01 ± 6.25	6275.50 ± 152.42
H-151	236.31 ± 5.93	4029.73 ± 76.75	Cherukurasam	202.08 ± 5.39	5452.42 ± 79.26	Fazli	172.78 ± 4.78	3664.74 ± 132.35
H-56	285.63 ± 11.44	5143.30 ± 183.48	Alphonso	201.63 ± 7.10	3523.22 ± 48.41	Lal Sundari	100.74 ± 3.28	4089.32 ± 90.82
Total flavonoids			Total phenols					
F-test	*		F-test	*				
S.Em. ±	7.79		S.Em. ±	117.16				
CD at 5%	21.83		CD at 5%	328.02				

*Significant at 5%

Table 3: Details of 8 SSR markers used in parentage analysis (Source: Ravishankar et al., 2011) [14]

Locus	Repeat motif	H$_O$	H$_e$	PIC	F(Null)
MiIIHR17	(GT)13GAGT(GA)10	0.050	0.510	0.470	+0.8258
MiIIHR18	(GT)12	0.000	0.782	0.744	+1.0000
MiIIHR 23	(GA)17 GG(GA)6	0.017	0.728	0.693	+0.9541
MiIIHR 26	(GA)14 GGA(GAA)2	0.000	0.757	0.718	+1.0000
MiIIHR 30	(CT)13	0.044	0.762	0.713	+0.8910
MiIIHR 31	(GAC)6	0.024	0.885	0.862	+0.9469
MiIIHR 34	(GGT)9 (GAT)5	0.389	0.876	0.855	+0.3847
MiIIHR 36	(TC)17	0.000	0.845	0.818	+1.0000

H$_O$ – Observed heterozygosity H$_e$ – Expected heterozygosity PIC – Polymorphic Information Content
F(Null) – Frequency of null allele

Table 4: Percentage of allele transmission from maternal and paternal parent to their offspring

Sl. No.	Hybrids list	Maternal inherited allele (%)	Paternal inherited allele (%)
1	Arka Udaya (Amrapali x Arka Anmol)	16.66	50
2	Arka Puneet (Alphonso x Banganapalli)	37.5	25
3	Arka Anmol (Alphonso x Janardhan Pasand)	20	10
4	Arka Aruna (Banganapalli x Alphonso)	11.11	0
5	Arka Neelkiran (Alphonso x Neelum)	25	25
6	Amrapali (Dashehari x Neelum)	25	12.5
7	Mallika (Neelum x Dashehari)	12.5	0
8	Pusa Arunima (Amrapali x Sensation)	40	0
9	Pusa Pratibha (Amrapali x Sensation)	12.5	37.5
10	Pusa Shresht (Amrapali x Sensation)	44.44	11.11
11	Pusa Lalima (Dashehari x Sensation)	0	37.5
12	Pusa Pitamber (Amrapali x Lal Sundari)	12.5	0
13	Ambika (Amrapali x Janardhan Pasand)	55.55	11.11
14	Arunika (Amrapali x Vanraj)	62.5	0
15	Sindhu (Ratna x Alphonso)	50	2
16	Ratna (Neelum x Alphonso)	28.57	42.85
17	Konkan Raja (Bangalora x Himayuddin)	0	28.57
18	Konkan Ruchi (Neelum x Alphonso)	37.5	50
19	Manjeera (Rumani x Neelum)	0	54.54
20	AU-Rumani (Rumani x Mulgoa)	0	50
21	KMH-1 (Cherukurasam x Khader)	25	25
22	Swarnajehangir (Suvarnarekha x Jehangir)	22.22	11.11
23	Neelgoa (Neelum x Mulgoa)	12.5	12.5
24	Neeleshan (Neelum x Baneshan)	14.28	42.85
25	PKM-1 (Chinna Suvarnarekha x Neelum)	14.28	42.85
26	PKM 2 (Neelum x Mulgoa)	0	14.28
27	Prabhashankar (Bombai x Kalapadi)	0	0
28	Sabri (Gulabkhas x Bombai)	25	0
29	Al Fazli (Alphonso x Fazli)	12.5	25
30	Mahmood Bahar (Bombai x Kalapadi)	50	16.66
31	H-85 (Kalapadi x Allampur Baneshan)	25	12.5
32	H-151 (Kalapadi x Neelum)	14.28	28.57
33	H-56 (Bennet Alphhonso x Himayuddin)	28.57	0
34	H-87 (Kalapadi x Allampur Baneshan)	0	0
35	Sonpari (Alphonso x Baneshan)	0	2
36	Neeleshan Gujarat (Neelum x Baneshan)	0	14.28
37	Neeleshwari (Neelum x Dashehari)	0	12.5
38	Neelphonso (Neelum x Alphonso)	37.5	0

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