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Development of polyembryonic x monoembryonic hybrid progenies and their hybridity confirmation in mango through SSR markers

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Abstract
In crosses involving polyembryonic mango varieties as a female parent the identification of resultant hybrid progenies is challenging. Therefore to confirm the hybridity, SSR markers were tried. The present study was carried out using the polyembryonic genotype, Vellaikolamban as a female parent and seven monoembryonic cultivars (Alphonso, Dashehari, Totapuri, Banganapalli, Neelum, Rumani and Amrapali) as male parents. The Vellaikolumban x Alphonso combination recorded the highest fruit set percentage, minimum days taken for germination, highest germination percentage, maximum plant height and highest rate of leaf emergence. However, seedling girth was found to be highest in Vellaikolumban x Banganapalli cross combination. The maximum fruit retention percentage was recorded in Vellaikolumban x Totapuri followed by Vellaikolumban x Alphonso and minimum in Vellaikolumban x Neelum. While, F_0 fruits of 'Vellaikolumban x Rumani was taken maximum number of days for germination and recorded lowest germination percentage. Out of 16 SSR primers used, two markers namely MiIIHR 18 (for Vellaikolamban x Alphonso and Vellaikolamban x Totapuri cross combinations) and MiIIHR 30 (Vellaikolamban x Totapuri and Vellaikolumban x Banganapalli) gave distinct parent specific alleles.

Keywords: Mango, SSR markers, hybridity, Vellaikolumban, MiIIHR

1. Introduction
Mango (*Mangifera indica* L.) is a member of Anacardiaceae family and is believed to be originated in the Indo-Burma region. It is imperative to note that, selection by man from seedlings of unknown parentage has played the most significant role in the development of new mango cultivars. Mostly the mango breeding programs have relied mainly on classical breeding practices, which are based on controlled crossings and selecting the superior individuals. Some varieties mango have nucellar embryony, which poses a substantial obstacle to cross-breeding research since it results in multiple asexual embryos. This feature reduces the possibility of finding out the true hybrid seedlings (Schnell and Knight, 1992) [20]. In mango, the nucellar embryos are developed from the nucellar tissue that surrounding the embryo sac, and the seedlings descended from these embryos are genetically similar to the mother plant (Aron *et al.*, 1998) [2]. However, the zygotic embryo is the goal of breeding programmes for the selection of superior genotypes and desirable traits. The zygotic embryo is derived from fertilisation by self-pollination or by cross-pollination. (Rocha *et al.*, 2014) [18].
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) [24]. Mango cultivars are often identified by the morphological traits like leaf and fruit characteristics (Campbell, 1992 and He *et al.*, 2007) [3]. But, morphological markers have certain limitations as they vary with the environmental conditions (Tanksley *et al.*, 1989) [23]. However, molecular markers are useful in addressing these problems as they are robust and not influenced by the environment. Of all the markers, 'Simple Sequence Repeats (SSR)' show greater potential for mango improvement and can be performed for variety identification and validation of parentage (Singh *et al.*, 2012) [1]. In the present study, 16 SSR primers were used for the confirmation of hybridity the crosses of polyembryony x monoembryony.

2. Material and Methods

The present study was carried out by using the polyembryonic genotype, Vellaikolamban as a female parent and seven monoembryonic cultivars (Alphonso, Dashehari, Totapuri, Banganapalli, Neelum, Rumani and Amrapali) as male parents at ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta, Bengaluru-560089, Karnataka. Hand pollination was attempted using the technique described by Mukherjee *et al.* (1961) [9] for the crossing. The hybrids obtained from these crosses were sown in polybags having coco-peat. After 45 days of germination, the hybrids from each kernel were separated and transplanted in polybags containing a mixture of soil, sand and FYM in 1:1:1 ratio v/v. The initial observations on number of fruit-set was recorded and fruit-set percentage was calculated by dividing the number of fruit-set by total number of flowers crossed and multiplied by 100. Fruit retention percentage was recorded at lemon size stage and calculated by dividing the number of fruits retained by the total number of fruits retained at lemon size stage. The morphological observations on germination *viz*. number of days taken for germination of first seedling and germination % (Number of seedlings germinated divided by total number of seeds sown) were recorded. Their growth parameters were recorded at an interval of 30 days up to 180 days. Seedling height was measured using a meter scale and expressed in cm, seedling girth (mm) was measured using 'Vernier calipers, and the rate of leaf emergence was counted manually and colour of emerging leaf was determined using RHS colour chart.

For the hybrid confirmation, total genomic DNA was isolated from the newly sprouted leaves of the parents and F_1 progenies by using modified CTAB method (Ravishankar *et al.,* 2000) [16]. Two grams of leaf tissue were ground to a fine powder with liquid nitrogen. 50 mg PVPP was added and mixed. The content was transferred into a centrifuge tube containing 10 ml CTAB buffer preheated to 60 ºC and shaken gently. The tubes were incubated for one hour at 60 ºC, with intermittent shaking every 10 minutes, and later cooled to room temperature. 10 ml of $CHCl_3$: Isoamyl alcohol (24:1) was added and mixed gently by inverting tubes to form an emulsion. The tubes were centrifuged at 10000 rpm for 15 minutes. If cloudy 6 ml of $CHCl_3$: Isoamyl alcohol can be added and this step can be repeated. The clear aqueous phase was transferred to fresh centrifuge tubes. 2.5 ml of 5 M NaCl was added and mixed. 10 ml cold ethanol was added; gently mixed and refrigerated overnight at -20 ºC. The tubes were centrifuged at 10000 rpm for 10 minutes at room temperature. The supernatant was poured off; pellets were washed with 200 µl of 70 percent ice-cold ethanol and centrifuged as above for two minutes. The washing was repeated twice or more. The supernatant was drained out; ethanol was removed and then the DNA pellet was air dried DNA by leaving the tubes uncovered at 37 ºC for 20-30 minutes or vacuum drying at room temperature. The pellets were re-suspended in 50 µl TE buffer. 1 µl RNAse (10 mg /ml) was added to the dissolved DNA. The tubes were incubated at 37 ºC for 30 minutes and stored at -20 ºC. A total of 16 SSR markers were employed in this study (Tables 1; Ravishankar *et al*., 2011; 2015) [16-17]. The PCR amplification conditions were as follows: initial denaturation at 94 ºC for 1 min, followed by 35 cycles of denaturation, annealing and polymerisation steps (94 ºC for 30 s, 55 ºC for 30 s and 72 ºC for 1 min, respectively). A final extension of polymerisation was done at 72 ºC for 5 min. PCR amplification was performed in a 20-µL reaction volume containing 3 µL of 20ng DNA, 2 µL Taq Buffer 10x (Tris pH with 15 mm MgCl2), dNTPs 1 µL (10 mM), 1 µL of forward primer M13 tail (5 pM), 1 µL of reverse primer (5 pM), 1 µL of fluorescent probes FAM, VIC, PET and NED (5 pM) M13 primers, 0.2 µL of *Taq* polymerase (3 u per µL), and nuclease free water (10.8 µL). All the PCR reactions were performed using a Bioer Life Pro Thermal cycler (Bioer Technology, China). The amplified products were labelled with FAM, VIC, PET or NED were multiplexed pooled before separation on an automatic 96-capillary automated DNA sequencer (ABI 3730 DNA Analyzer, Applied Biosystems, USA). The data obtained were further analyzed using the Peak Scanner software (Applied Biosystems, USA) to determine exact fragment size of the PCR product. The products were analyzed based on the intensity of fluorescence in expected product size range. The per cent paternal and maternal alleles were classified based on inheritance.

3. Result and Discussion

In Mango, the fruit set and fruit retention are poor in monoembryony (ME) x monoembryony (ME) combinations. Since we did ME x ME hybridization, the fruit set and fruit retention parameters were recorded as an additional information. The result (Table 2) revealed that the polyembryony (PE) x ME, the fruit set and fruit retention were found to be more as compared to ME xME. The results revealed that, the highest fruit set percentage (9.8) was recorded in the Vellaikolumban x Alphonso combination whereas, the least percentage in Vellaikolumban x Amrapali (0.98). The maximum fruit retention percentage (86.66) was recorded in Vellaikolumban x Totapuri followed by Vellaikolumban x Alphonso (82.97) and a minimum in Vellaikolumban x Neelum (11.11). Such inter-varietal hybridization was first described by Mukherjee *et al.* (1961) [9] with a success rate of 1.45%. However, Pinto and Byrne (1993) [13] have suggested an improvement to the existing technique and increased the fruit set to 6 % which promotes the enlargement of the hybrid population. Interestingly, Srivastav *et al.,* (2014) [22] have observed that, self-pollination in Amrapali resulted in rapid decline in fruit retention than open- and cross-pollination (Amrapali × Sensation) during 20 days after pollination (DAP). Out of 1,133 self-pollinated flowers, only three have set fruits (0.26%) after 25 days of pollination. In contrast, cross-pollination with Sensation (637 flowers) resulted in 32 fruits (5.02%) after 25 days of pollination. However, with regard to the average fruit weight, no significance difference was observed. The F_0 seeds from Vellaikolumban x Alphonso cross combination took minimum days (15.3) for germination and also the highest germination percentage (83.33). On the other hand, the F_o seeds of Vellaikolumban x Rumani combination took maximum number of days (19.9) for germination and recorded lowest germination percentage (28.57). The gradation of colours in emerging leaves were observed which were mostly the light green to grey orange and yellow group (Table 2) each and every cross combination had the similar emerging leaf colour, so its not possible to identify zygotic seedlings. The similar range on germination aspects after removal of hard seed coat was in agreement with Murlidhara *et al.,* (2016). The cultivars with less stone weight exhibited slow germination due to less endosperm content in the stone, which might have supplied less nutrient and food material for germination (Rao and Reddy, 2006) [15].

Data presented in table 3 on plant height, rate of leaf emergence and seedling girth respectively of different cross combination after transplanting revealed that, highest plant height was noted in Vellaikolumban x Dashehari (22.8cm) combination followed by Vellaikolumban x Rumani (21.8cm) in 30 DAT. However, at 180 DAT, tallest plants were observed in Vellaikolumban x Alphonso (46.8cm) followed by Vellaikolumban x Dashehari (45.3cm) combination, whereas, the shortest plants were seen in Vellaikolumban x Neelum (27.8cm) combination. The highest rate of leaf emergence was found in Vellaikolumban x Alphonso (6.3) at 30 DAT and Vellaikolumban x Amrapali (18.8) followed by Vellaikolumban x Banganapalli (18.2) at 180 DAT, whereas the lowest rate of leaf emergence was noticed in 30 (3.2) and 180 (11.6) DAT in Vellaikolumban x Dashehari combination. The seedling girth was highest in Vellaikolumban x Banganapalli at 30 (3.9mm) and 180 (7.0mm) DAT and lowest in Vellaikolumban x Amrapali (2.3mm) at 30 and 180 DAT. The results reported by earlier workers on polyembryonic genotypes by Abirami *et al*, (2011) [1] observed maximum seedling height, girth and number of leaves in Nekkare and concluded that Starch, Peach and Kurukkan were the less vigorous genotypes. Khobragade *et al.* (1999) [7] noted Peach as dwarf genotype. Based on these parameters we are unable to distinguish the true hybrid seedlings.

The data in table 4 and 5 of Vellaikolamban x Alphonso cross combination revealed that P1a (single seedling) was true hybrid seedling whereas all the other F_1 progenies were showing 50 percent maternal allele and 50 percent unknown allele except P1a, which was similar to the mother plant. The allelic data of four primers (MiIIHR 18, 30, 99, 11588) indicated polymorphism between female and male parent but only one (MiIIHR 18) detected a true hybrid (P1a). In the Vellaikolumban x Dashehari cross combination, all seedlings revealed 50 percent maternal allele and 50 percent unknown allele also inherited from the maternal parent. The total 16 SSR primer were used out of those eight primers was found polymorphic in nature. For Vellaikolamban x Totapuri cross combination, the last emerged seedling i.e. P4b and P5c was detected as true hybrids respectively in the two stones. The remaining seedlings showed 50 percent maternal allele, 15-50 percent unknown allele from the maternal allele and some novel allele. The seven markers were polymorphic and in those two primers (MiIIHR 18 & MiIIHR 30) detected the true F_1 hybrid (P4b and P5c). Data on Vellaikolumban x Banganapalli indicated seedling P7a as the true hybrid seedling whereas the remaining were nucellar in origin being similar to mother plant. Out of four markers MiIIHR 30 detected the hybrid. The percent data of Vellaikolumban x Neelum and Vellaikolumban x Rumani crosses revealed that all the progenies were nucellar seedlings with 25 percent novel allele in both cross combinations. The Vellaikolumban x Amrapali cross combination showed that stone had 50 percent maternal allele, 15 percent paternal allele and 35 percent novel allele composition while stone two first 2 seedlings had 50 percent maternal allele and 50 percent unknown allele inherited from the maternal parent and last emerged seedling showing 50 percent maternal allele, 15 percent paternal allele and 35 percent novel allele. Out 16 SSR primers used, two markers such as MiIIHR 18 (for Vellaikolamban x Alphonso and Vellaikolamban x Totapuri cross combinations) and MiIIHR 30 (Vellaikolamban x Totapuri and Vellaikolumban x Banganapalli) could be useful for hybridity confirmation purpose in a particular cross combination because these markers are varietal specific. However no clear cut references regarding the sequential emergence of the zygotic embryo could be derived as the results varied among the markers and parents involved.

The F_1 progenies were showing stronger affinity towards maternal parents similar result were observed by Singh *et al.*, (2012) [21] also he reported that high genetic variability found in F_1 population. Nesara *et al.*, (2018) [12] used eight SSR markers from that they concluded 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. Polyembryony is characterized by the development of more than one embryo in a single seed, and one may be zygotic and the others, nucellar or all may be nucellar (Degani *et al.*, 1993) [1]. Fatimah *et al.*, (2016) [5] revealed the SSR primers could not distinguish sharply between groups into zygotic or nucellar. Ledesma *et al.*, (2017) [8] used the MiIIHR primers for study and noticed that some of the seedlings may not be hybrids because of self-compatibility and/or polyembryony. Sankaran *et al.*, (2020) [19] reported that out of 11 SSR markers, three primers showed high polymorphism and confirmed the hybridity based on the allelic range variation among the four parental mangoes. A total of 16 SSR primers were used across all combinations of which polymorphism was observed with few primers with specific cross combination. Hence it can be concluded that the primers are genotype specific and cross combination specific.

Table 1: SSR primers used in the study

S. No.	Locus	Primers (5′–3′) details
1.	MiIIHR 23	F: TCTGACCCAACAAAGAACCA R: TCCTCCTCGTCCTCATCATC
2.	MiIIHR 17	F: GCTTGCTTCCAACTGAGACC R: GCAAAATGCTCGGAGAAGAC
3.	MiIIHR 18	F: TCTGACGTCACCTCCTTTCA R: ATACTCGTGCCTCGTCCTGT
4.	MiIIHR 30	F: AGCTATCGCCACAGCAAATC R: GTCTTCTTCTGGCTGCCAAC
5.	MiIIHR 31	F: TTCTGTTAGTGGCGGTGTTG R: CACCTCCTCCTCCTCCTCTT
6.	MiIIHR 26	F: GCGAAAGAGGAGAGTGCAAG R: TCTATAAGTGCCCCCTCACG
7.	MiIIHR 34	F: CTGAGTTTGGCAAGGGAGAG R: TTGATCCTTCACCACCATCA
8.	MiIIHR 36	F: TCTATAAGTGCCCCCTCACG R: ACTGCCACCGTGGAAAGTAG
9.	MiKVR 80	F-GTAAAACGACGGCCAGTTACAGGCTGCCCAGAAAGAT R-GTTTCTTGCGCATGCTGGGATTAGTAT
10.	MiKVR 71	F-GTAAAACGACGGCCAGTAAAAGTGCCACAGAAAACATGTAA R-GTTTCTTATGCCTCAACCTGTTATGCC
11.	MiIIHR 99	F-GTAAAACGACGGCCAGTCTTCATCGAATCCAAGGCAT R-GTTTCTTCTTCCATGGCACGAGTAGGT
12.	MiMRD 88	F-GTAAAACGACGGCCAGTAAAATGGACGCCACAAAGTG R-GTTTCTTGTTTCGGATTTCTCATGGGA
13	MiKVR 81	F-GTAAAACGACGGCCAGTGTGTTCAGAATACCGGCCAT R-GTTTCTTACCCCTGCATGATTTTGACT
14	MiIIHR 333	F-GTAAAACGACGGCCAGTAAACCATTGTGGATGTGGGT R-GTTTCTTTCCCAGTCTGGAAAAAGAAAAA
15	MiIIHR	F-GTAAACGACGGCCAGTGAGAAATGGTTCCAGCAA R-GTTCTTCACGCGAAGTAAACCAAA

	11588	
16	MiIIH 16400	F-GTAAAACGACGGCCAGTAAATCCCGTACCTTCATCCC R-GTTTCTTTGCCAGAACTGCTCTCTTCA

Table 2: Artificial hybridization, days to germination, Germination (%) and colour of emerging leaf in different cross combinations in mango

Cross Combinations	Fruit set (%)	Fruit retention (%)	Fruit weight (g)	Days to germination	Germination %	Colour of emerging leaf
Vellaikolumban x Alphonso	9.8	82.97	253.97	15.3	83.33	Greyed orange Grp 177 A
Vellaikolumban x Dashehari	6.8	68	246.17	16.2	68.00	Greyed Yellow Grp 160 A
Vellaikolumban x Totapuri	8.3	86.66	272.81	16.3	78.26	Greyed orange Grp 166 A
Vellaikolumban x Banganapalli	7.2	42.68	239.72	16.2	65.00	Greyed Yellow Grp 160 A
Vellaikolumban x Neelum	9.2	11.11	214.87	16.8	60.00	Greyed Orange Grp 170 A
Vellaikolumban x Rumani	8.03	32.96	232.14	19.9	28.57	Greyed Yellow Grp 160 A
Vellaikolumban x Amrapali	0.98	50.00	288.30	17.5	50.00	Greyed orange Grp 165 A

Table 3: Growth parameters of different cross combinations recorded after transplanting

Cross combination	Plant Height (cm)		Rate of leaf emergence		Plant Girth (mm)	
	30 Days	180 Days	30 Days	180 Days	30 Days	180 Days
Vellaikolumban x Alphonso	20.5	46.8	6.3	17.1	3.7	6.5
Vellaikolumban x Dashehari	22.8	45.3	3.2	11.6	3.4	6.2
Vellaikolumban x Totapuri	20.4	41.3	5.4	13.6	3.5	6.5
Vellaikolumban x Banganapalli	17.4	33.1	5.7	18.2	3.9	7.0
Vellaikolumban x Neelum	15.3	27.8	4.9	16.8	2.8	5.6
Vellaikolumban x Rumani	21.8	36.5	4.8	15.8	3.7	5.9
Vellaikolumban x Amrapali	16.1	36.4	5.4	18.8	2.3	6.2
C.D.	5.2	5.6	1.5	3.8	0.5	0.7
SE(m)	1.6	1.8	0.4	1.2	0.1	0.2
SE(d)	2.3	2.5	0.6	1.7	0.2	0.3
C.V. (%)	15.1	8.1	16.2	13.2	9.4	6.7

Table 4: Fragment analysis and allele inheritance of the seven cross combinations

Cross Combinations	Stone Number	Hybrids	% maternal allele	% paternal allele	% unknown allele
Vellaikolumban x Alphonso	1	P1a -Seedling 1	50	25	25***
	2	P2a -Seedling 1	50	0	50*
	3	P3a -Seedling 1	50	0	50*
	4	P4a- Seedling 1	50	0	50*
		P4b- Seedling 2	50	0	50*
	5	P5a- Seedling 1	50	0	50*
		P5b- Seedling 2	50	0	50*
	6	P6a -Seedling 1	50	0	50*
		P6b -Seedling 2	50	0	50*
		P6c -Seedling 3	50	0	50*
	7	P7a -Seedling 1	50	0	50*
		P7b -Seedling 2	50	0	50*
		P7c -Seedling 3	50	0	50*
		P7d -Seedling 4	50	0	50*
	8	P8a -Seedling 1	50	0	50*
		P8b -Seedling 2	50	0	50*
		P8c -Seedling 3	50	0	50*
		P8d -Seedling 4	50	0	50*
		P8e -Seedling 5	50	0	50*
Vellaikolumban x Dashehari	1	P1a	50	0	25:25**
	2	P2a	50	0	50*
	3	P3a	50	0	50*
		P3b	50	0	50*
	4	P4a	50	0	50*
		P4b	50	0	50*
	5	P5a	50	0	50*
		P5b	50	0	50*
	6	P6a	50	0	50*
		P6b	50	0	50*
		P6c	50	0	50*
	7	P7a	50	0	50*
		P7b	50	0	50*
		P7c	50	0	50*
	8	P8a	50	0	50*
		P8b	50	0	50*

		P8c	50	0	50*
	9	P9a	50	0	50*
		P9b	50	0	50*
		P9c	50	10	40*
		P9d	50	0	50
Vellaikolumban x Totapuri	1	P1a	50	0	50*
	2	P2a	50	0	50*
	3	P3a	50	0	50*
		P3b	50	0	30:20**
	4	P4a	50	0	50*
		P4b	50	20	30***
	5	P5a	50	0	50
		P5b	50	05	15:35**
		P5c	50	20	30*
	6	P6a	50	0	50*
		P6b	50	0	50*
		P6c	50	0	50*
	7	P7a	50	0	25:25**
		P7b	50	0	50*
		P7c	50	0	20:30**
Vellaikolumban x Banganpalli	1	P1a	50	0	25:25**
	2	P2a	50	0	50*
	3	P3a	50	0	25:25**
	4	P4a	50	0	50*
	5	P5a	50	0	50*
		P5b	50	0	50*
	6	P6a	50	0	25:25**
		P6b	50	0	50*
	7	P7a	37.5	12.5	50***
		P7b	50	0	50*
	8	P8a	50	0	50*
		P8b	50	0	50*
		P8c	50	0	50*
Vellaikolumban x Neelum	1	P1a	50	0	25:25**
	2	P2a	50	0	25:25**
		P2b	50	0	25:25**
Vellaikolumban x Rumani	1	P1a	50	0	25:25**
	2	P2a	50	0	25:25**
	3	P3a	50	0	25:25**
	4	P4a	50	0	25:25**
	5	P5a	50	0	25:25**
		P5b	50	0	25:25**
Vellaikolumban x Amrapali	1	P1a	50	15	35***
		P1b	50	15	35***
		P1c	50	15	35***
	2	P2a	50	0	50*
		P2b	50	0	50*
		P2c	50	15	35***

*** Complete Novel allele (%)
**Maternal allele: Novel allele (%)
*Maternal allele (%)

Table 5: Allelic data of the seven cross combinations

Vellaikolumban x Alphonso

Primer id	Vel	Alphonso	P1a	P2a	P3a	P4a	P4b	P5a	P5b	P6a	P6b	P6c	P7a	P7b	P7c	P7d	P8a	P8b	P8c	P8d	P8e
MiIIHR 18	162/166	166/169	162/169	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166
MiIIHR 30	198/201	166/170	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201
MiIIHR 99	206/207	166/171	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207
MiIIHR 11588	297/298	166/172	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298

Vellaikolumban x Dashehari

Primer id	Vel	Dash	P1a	P2a	P3a	P3b	P4a	P4b	P5a	P5b	P6a	P6b	P6c	P7a	P7b	P7c	P8a	P8b	P8c	P9a	P9b	P9c	P9d
MiIIHR 23	128/129	99/100	128/129	128/129	128/129	128/129	128/129	128/129	128/129	128/129	128/129	128/129	128/129	128/129	128/129	128/129	128/129	128/129	128/129	128/129	128/129	128/129	128/129
MiIIHR 17	105/106	156/156	141/143	105/106	105/106	105/106	105/106	105/106	105/106	105/106	105/106	105/106	105/106	105/106	105/106	105/106	105/106	105/106	105/106	105/106	105/106	105/106	105/106
MiIIHR 18	162/166	154/156	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	154/156	162/166
MiIIHR 30	198/201	200/202	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201
MiIIHR 26	136/138	147	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138
MiMRD 88	206/209	243	206/209	206/209	206/209	206/209	206/209	206/209	206/209	206/209	206/209	206/209	206/209	206/209	206/209	206/209	206/209	206/209	206/209	206/209	206/209	206/209	206/209
MiIIHR 11588	297/298	208	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298
MiIIHR 16400	291/295	210	291/295	291/295	291/295	291/295	291/295	291/295	291/295	291/295	291/295	291/295	291/295	291/295	291/295	291/295	291/295	291/295	291/295	291/295	291/295	291/295	291/295

Vellaikolumban x Totapuri

Primer id	Vel	Totapuri	P1a	P2a	P3a	P3b	P4a	P4b	P5a	P5b	P5c	P6a	P6b	P6c	P7a	P7b	P7c
MiIIHR 23	128/129	120/126	128/129	128/129	128/129	99/100	128/129	99/100	128/129	128/129	128/129	99/100	128/129	99/100	128/129	99/100	128/129
MiIIHR 18	162/166	167/169	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	166/169	162/166	162/166	197/203	197/203	162/166
MiIIHR 30	198/201	193/195	198/201	198/201	198/201	198/201	198/201	193/198	198/201	198/201	198/201	200/202	198/201	198/201	198/201	202/204	198/201
MiIIHR 26	136/138	145/147	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138
MiIIHR 36	222/223	228/231	222/223	222/223	222/223	222/223	222/223	222/223	222/223	222/223	222/223	222/223	222/223	222/223	222/223	222/223	222/223
MiIIHR 99	206/207	159/160	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207	206/207
MiIIHR 11588	297/298	207/208	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298	297/298

Vellaikolumban x Banganpalli

Primer id	Vel	Bang	P1a	P2a	P3a	P4a	P5a	P5b	P6a	P6b	P7a	P7b	P8a	P8b	P8c
MiIIHR 23	128/129	107/109	99/100	128/129	99/100	128/129	128/129	128/129	99/100	128/129	99/100	128/129	128/129	128/129	128/129
MiIIHR 18	162/166	166/169	162/166	162/166	162/166	162/166	162/166	162/166	162/166	162/166	197/203	162/166	162/166	162/166	162/166
MiIIHR 30	198/201	197/199	198/201	198/201	198/201	198/201	198/201	198/201	198/201	198/201	195/198	198/201	198/201	198/201	198/201
MiIIHR 26	136/138	157/159	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138	136/138

Vellaikolumban x Neelum

Primer id	Vel	Neelum	P1a	P2a	P2b
MiIIHR 30	198/201	194/198	198/201	198/201	198/201
MiIIHR 26	136/138	160/163	136/138	136/138	136/138
MiIIHR 36	221/223	243/247	221/223	221/223	221/223

Vellaikolumban x Rumani

Primer id	Vel	Rumani	P1a	P2a	P3a	P4a	P5a	P5b
MiIIHR 23	128/129	100/109	128/129	128/129	128/129	128/129	128/129	128/129
MiIIHR 18	162/166	160/162	162/166	162/166	162/166	162/166	162/166	162/166
MiIIHR 30	198/201	196/197	198/201	198/201	198/201	198/201	198/202	198/201

Vellaikolumban x Amrapalli

Primer id	Vel	Amrapalli	P1a	P1b	P1c	P2a	P2b	P2c
MiIIHR 18	162/166	154/156	162/166	162/166	162/166	162/166	162/166	162/166
MiIIHR 30	198/201	194/201	190/193	198/201	198/201	198/201	198/201	198/201
MiMRD 88	206/209	243	206/209	243/245	243/245	206/209	206/209	243/245

4. Conclusion

The mango breeders are challenged when polyembryonic genotype are used as female parent, due to difficult to identify the hybrids among multiple seedlings. 16 SSR markers used for hybrid confirmation in this study, ten showed the polymorphism of which two markers namely MiIIHR 18 (for Vellaikolamban x Alphonso and Vellaikolamban x Totapuri cross combinations), and MiIIHR 30 (Vellaikolamban x Totapuri and Vellaikolumban x Banganapalli) could be useful for hybridity confirmation in particular cross combination being varietal specific.

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