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Development of interspecific hybrid progenies of mango and their characterization

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ABSTRACT

A total 26 interspecific mango hybrids population were obtained from the crosses involving Alphonso, Amrapali and NR34 as a female parents and *Mangifera odorata* Griff, as male parent. Morphological traits revealed that all the interspecific hybrids as well as parent trees were found to be erect and also spreading habit with circular, semicircular and oblong crown shape. The results of cytological studies showed that interspecific hybrids were also possessed the same somatic chromosome number 2n = 40 as that of mango. Most of the interspecific hybrids showed the intermediate to dense foliage density. 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.

Key words: *Mangifera indica*, Amrapali, Alphonso, SSR markers, hybridity.

INTRODUCTION

Mango (*Mangifera indica* L.) belongs to the family Anacardiaceae is one of the most important commercially grown fruit crops of the country. The wide hybridization involving interspecific and intergeneric crosses, would introgress the new traits to the off springs. Wild relative species of the cultivated crops are the potential sources of new genes, which provide both biotic and abiotic stress tolerance (Bowley and Taylor, 2).

In mango, the introgression of gene/genes are possible because all the *Mangifera* species have the same chromosome number (2n = 40). Therefore, they can inter cross easily (Mukherjee, 6). There are a very few reports on interspecific hybridization in mango, however, none of the hybrids has been released so far for commercial cultivation. Bhujanga Rao *et al*. (1) reported about 32 interspecific hybrids of *Mangifera odorata* and *Mangifera zeylanica*. *Mangifera odorata* has got an unique aroma and taste, and these traits can be transferred on to other varieties of mango. In this present study, an attempt was made to cross *Mangifera odorata* with the commercial cultivars such as Amrapali, Alphonso and NR 34 to improve the aroma and quality of fruits. The chromosome count, morphological and molecular characterization of the interspecific hybrids has been done to confirm the hybridity of progenies.

MATERIALS AND METHODS

The present study was carried during 2012-2017 by using three important mango cultivars, namely Amrapali, Alphonso and NR34 local as female parents and *Mangifera odorata* as a male parent at the Division of Fruits, ICAR-IIHR, Bengaluru, Karnataka. The selection of mango cultivars was made on the basis of their importance in mango breeding programme. Hand pollination technique was employed as described by Mukherjee *et al*. (5) for crossing. The hybrids obtained from these crosses were sown in polybags having soil and sand mixture as growth media. A total of interspecific hybrid progenies derived from the above crosses were used along with their parents for study.

Two to three years old mango hybrid seedlings were used for morphological characterization as per the IPGRI descriptors (IPGRI, 3). The traits such as tree growth habit, foliage density, crown shape and leaf characters such as leaf shape, leaf length, leaf texture, leaf margin variation as well as leaf length and width were recorded.

Actively growing shoot tip with small leaflets of 2-3 mm in length were excised and pretreated with 0.003M 8 hydroxyquinone for 2 h at 14-16°C. Then it was rinsed in distilled water and fixed in Carnoy's-II fixative *viz.,* 6:3:1 of absolute alcohol: glacial acetic acid: chloroform and stored for 24 hours. Later those were transferred to 70% alcohol after 24 h of fixation for long term storage (about 2-4 months). The stored shoot tips then rinsed in distilled water and hydrolyzed in a water bath with 1N HCL at 60°C for 5 min or in 5N HCL at room temperature for 30 min. The hydrolyzed shoot tips were transferred to Schiff's reagent (Lillie, 4) also known as Feulgen

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stain after rinsing in distilled water and stored in dark for 11/2 to 2 h. Schiff's reagent stains the actively dividing meristematic tissue to deep magenta colour. The stained tips were squashed with drop 1% aceto-carmine or orcein. The slides were sealed with wax and observed under microscope in the same day or the next day. The slides were scanned using Olympus BX-51 research microscope for the well spread metaphase chromosomes under 100x oil immersion objective (Rekha *et al*., 10).

Total genomic DNA was isolated from the newly sprouted leaves modified CTAB method (Ravishankar *et al*., 9). Eleven mango SSR markers were used in the study. PCR was carried out using in 15 µl reaction volume each reaction containing 1.5 µl of reaction buffer A (pH 9.0, 10 mM Tris with 15 mM $MgCl_2$, 50 mMKCl and 0.01 % gelatin), 1.5µl of 25 Mm $MgCl_2$, 1.0 µl of 10 mM dNTPs, 1.5 µl (5 pmol) of fluorescently labelled (FAM, VIC, NED and PET) forward primer, 1.5µl of reverse primer (5 pmol), 0.5µl (3 U/µl) of Taq DNA polymerase, 3µl of template DNA and 4.3µl of nuclease-free water. The amplification program included an initial step of 2 min at 94°C, followed by 35 cycles of denaturation at 94°Cfor 30 sec, annealing at 53°C for 30 sec, and elongation at 72°C for 1 min. A final extension was performed at 72°C for 5 min. The amplified products were confirmed by 1.5 % agarose gel electrophoresis, and the PCR products were sent for fragment analysis. Using ABI automated sequence at MS Eurfin facility, Begaluru.

The fragment size results were extracted by using Peak scanner software. The products are analysed based on the intensity of fluorescence in expected product size range. The per cent paternal and maternal alleles were classified based on inheritance.

RESULTS AND DISCUSSION

The interspecific hybrids had shown a wide variation for morphological characters (Fig. 1 & 2). Significant differences were noticed for the leaf characters such length, width and petiole length (Table 1). Leaf length varied from 18.26 cm to 36.3 cm within the interspecific progenies. Among the hybrid progenies, R_7P_4 plant recorded maximum leaf length (36.3cm) and lowest was in female parent Amrapali (18.26 cm). Leaf width ranged from 3.96 to 10.48 cm where as the petiole length ranged from 2.0 to 3.8 cm. Variations in leaf characteristics are reported to be due to genetic divergence of mango cultivars (Srivastava, *et al*. 14). The leaf shape was oblong in all the hybrids of Alphonso and *Mangifera odorata* except in Amrapali, R_7P_4 and R_7P_{10}. Shape of leaf blade was observed to be acute in all the interspecific hybrids devoid of R_7P_3 as also in one of the parent i.e. *Mangifera odorata.* The leaf colour of the hybrids and parents varies from green to dark green.

Root tips are the common material used to examine the chromosome number, for which plant has to be uprooted for collecting root tips. Moreover the roots carry soil particles and sometimes it is difficult to get healthy root-tips due to pest and disease infection. The axillary leaf buds can be obtained at any stage of plant growth without disturbing the plant. Hence, it becomes an ideal material for chromosome studies. Karyotypic study exhibited that the chromosomes were very small

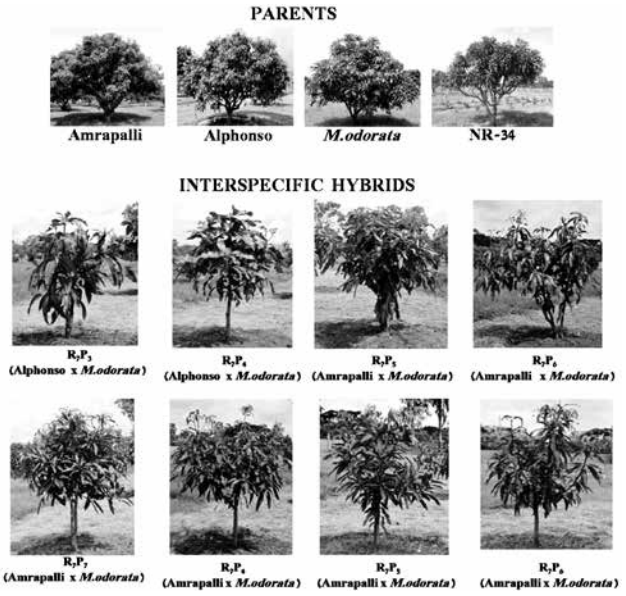

Fig. 1. Interspecific hybrid varieties tress

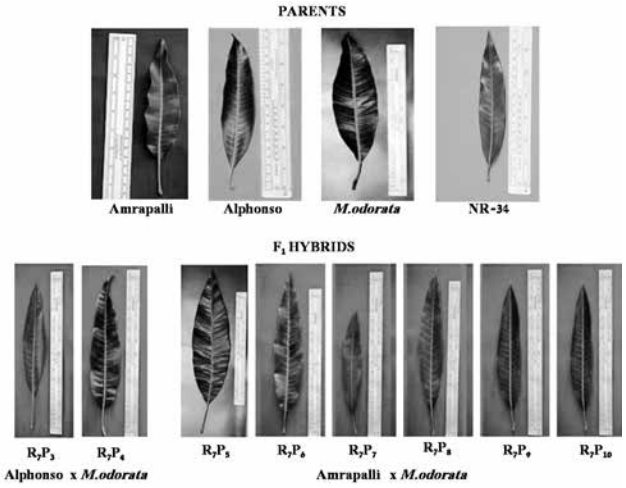

Fig. 2. Leaf structure of interspecific hybrid varieties

Table 1. Leaf characters of interspecific mango hybrids.

Sl. No.	Interspecific mango hybrids	Leaf blade length (cm)	Leaf blade width (cm)	Petiole length (cm)	Leaf blade: shape of base	Leaf blade :shape of apex	Leaf texture	Leaf shape	Leaf margin	Colour of fully developed leaf
1	Alphonso	18.7	4.5	3.2	Acute	Acute	Coriaceous	Oblong	Entire	Dark green
2	Amrapali	18.2	3.9	2.1	Acute	Acute	Coriaceous	Lanceolate	Entire	Dark green
3	Nr.34	12.3	4.7	2.6	Acute	Acute	Coriaceous	Elliptic	Entire	Light green
4	Mangifera odorata	18.5	5.1	3.1	Obtuse	Acute	Chartaceous	Oblong	Entire	Dark green
5	R_7P_3 (Alphonso × *Mangifera odorata*)	27.1	8.14	4.1	Obtuse	Acute	Chartaceous	Oblong	Wavy	Green
6	R_7P_4 (Alphonso × *Mangifera odorata*)	36.3	10.4	9.7	Acute	Acuminate	Coriaceous	Lanceolate	Wavy	Dark green
7	R_7P_5 (Amrapali × *Mangifera odorata*)	19.7	10.4	9.7	Acute	Acuminate	Coriaceous	Elliptic	Entire	Green
8	R_7P_6 (Amrapali × *Mangifera odorata*)	26.7	6.5	3.8	Acute	Acute	Coriaceous	Oblong	Wavy	Dark green
9	R_7P_7 (Amrapali × *Mangifera odorata*)	20.5	5.1	3.6	Acute	Acute	Coriaceous	Oblong	Entire	Green
10	R_7P_8 (Amrapali × *Mangifera odorata*)	27.1	6.4	3.5	Acute	Acute	Chartaceous	Oblong	Wavy	Green
11	R_7P_9 (Amrapali × *Mangifera odorata*)	21.7	4.3	2.6	Acute	Acute	Chartaceous	Oblong	Entire	Green
12	R_7P_{11} (Amrapali × *Mangifera odorata*)	22.6	4.7	3.0	Acute	Acute	Coriaceous	Lanceolate	Entire	Green
13	R_1P_4 (Amrapali × *Mangifera odorata*)	18.57	4.30	3.00	Acute	Attenuate	Coriaceous	Elliptic	Entire	Green
14	R_1P_5 (Amrapali × *Mangifera odorata*)	12.17	3.80	2.00	Obtuse	Acute	Chartaceous	Ovate	Entire	Green
15	R_1P_6 (Amrapali × *Mangifera odorata*)	14.67	4.23	2.17	Obtuse	Acute	Chartaceous	Ovate	Wavy	Green
16	R_1P_8 (Amrapali × *Mangifera odorata*)	11.33	3.20	1.80	Acute	Acute	Coriaceous	Ovate	Wavy	Green
17	R_1P_9 (Nr-34 × *Mangifera odorata*)	12.83	3.20	1.77	Acute	Acute	Chartaceous	Elliptic	Wavy	Green
18	R_1P_{10} (Nr-34 × *Mangifera odorata*)	10.67	3.27	5.53	Obtuse	Acute	Chartaceous	Elliptic	Wavy	Green
19	R_1P_{12} (Nr-34 × *Mangifera odorata*)	23.17	5.23	4.33	Acute	Attenuate	Coriaceous	Elliptic	Wavy	Green
20	R_1P_{13} (Nr-34 × *Mangifera odorata*)	17.17	3.30	1.93	Acute	Attenuate	Coriaceous	Elliptic	Wavy	Green
21	R_1P_{57} (Amrapali × *Mangifera odorata*)	20.43	5.83	4.70	Obtuse	Attenuate	Chartaceous	Ovate	Wavy	Green
22	R_1P_{62} (Amrapali × *Mangifera odorata*)	20.80	5.17	2.73	Acute	Attenuate	Chartaceous	Ovate	Wavy	Green
23	R_1P_{63} (Amrapali × *Mangifera odorata*)	23.27	5.77	2.50	Acute	Attenuate	Coriaceous	Elliptic	Wavy	Green
24	R_1P_{64} (Amrapali × *Mangifera odorata*)	21.97	4.60	3.83	Acute	Attenuate	Chartaceous	Ovate	Wavy	Green
25	R_1P_{71} (Amrapali × *Mangifera odorata*)	30.97	7.60	4.77	Acute	Attenuate	Coriaceous	Elliptic	Wavy	Green
26	R_1P_{72} (Amrapali × *Mangifera odorata*)	24.90	6.10	4.90	Acute	Acute	Coriaceous	Ovate	Wavy	Green
27	R_1P_{73} (Amrapali × *Mangifera odorata*)	15.60	4.00	2.83	Acute	Attenuate	Chartaceous	Elliptic	Wavy	Green
28	R_1P_{74} (Amrapali × *Mangifera odorata*)	13.67	3.60	1.60	Acute	Acute	Chartaceous	Ovate	Wavy	Green
29	R_1P_{75} (Amrapali × *Mangifera odorata*)	16.03	4.33	2.47	Acute	Attenuate	Chartaceous	Ovate	Wavy	Green
30	R_1P_{76} (Amrapali × *Mangifera odorata*)	18.63	6.53	2.17	Acute	Attenuate	Chartaceous	Elliptic	Wavy	Green
C.D		0.986	0.265	0.260	-	-	-	-	-	-
C.V. (0.5)		3.026	3.187	4.813	-	-	-	-	-	-
SE(M)		0.347	0.093	0.092	-	-	-	-	-	-

and sticky, which needed extra tapping to get well spread chromosomes. The somatic chromosomes were 40 (2n=40) in all interspecific hybrids and both types of hybrid seedlings *viz*., normal leaf and broad leaf (Fig. 3).

In spite of variations in leaf morphology, no variation in chromosome number could be observed in any of the hybrids that might be due to heterozygous nature of the crop. Usually hybrids are found to exhibit a difference in ploidy number when compared (Samuel and Bavappa, 11) to parents. Cytological characters such as chromosome number are said to be useful in modern plant taxonomy for differentiating species like *Piper*. However, use of cytology for differentiating *Mangifera* sp. was not found be of any significance as all of them had the same ploidy level. Similar studies have been conducted in *Cinnamomum* to find the difference in chromosome number and ploidy among different species but failed to find any difference (Sritharan *et al.,* 13). Interspecific crossing studies among five *Paspalum* species were also found to result in the production of sexual diploid hybrids along with apomictic tetraploid cytotypes. All hybrids had 2n = 20 chromosomes, and the degree of meiotic pairing was high in most of them (Quarin and Norrmann, 8). However, detailed studies on karyo-morphology, microsporogenesis*, etc.* could prove useful in differentiating hybrids.

According to the banding patterns obtained from 11 SSR loci for 26 interpescific hybrid population, the SSR markesr such as MiBNG_a619- VIC, MIIHR 36, MiKVR_l976 FAM and MiIIHR31 showed more polymorphism based on allelic size. Confirmation of hybrid based on the banding pattern and variation in allelic size and range (Fig. 4 & Table 2) has been done.

Interspecific hybrid R7P5 (Amrapali × *Mangifera odorata*), R1P62 (Amrapali × *Mangifera odorata*), R1P70 (Amarapali × *Mangifera odorata*), R1P71 (Amrapali × *Mangifera odorata*), R1P72 (Amrapali × *Mangifera odorata*), R1P73 (Amrapali × *Mangifera odorata*), R1P74 (Amrapali × *Mangifera odorata*), R1P75 (Amrapali × *Mangifera odorata*) and R1P76

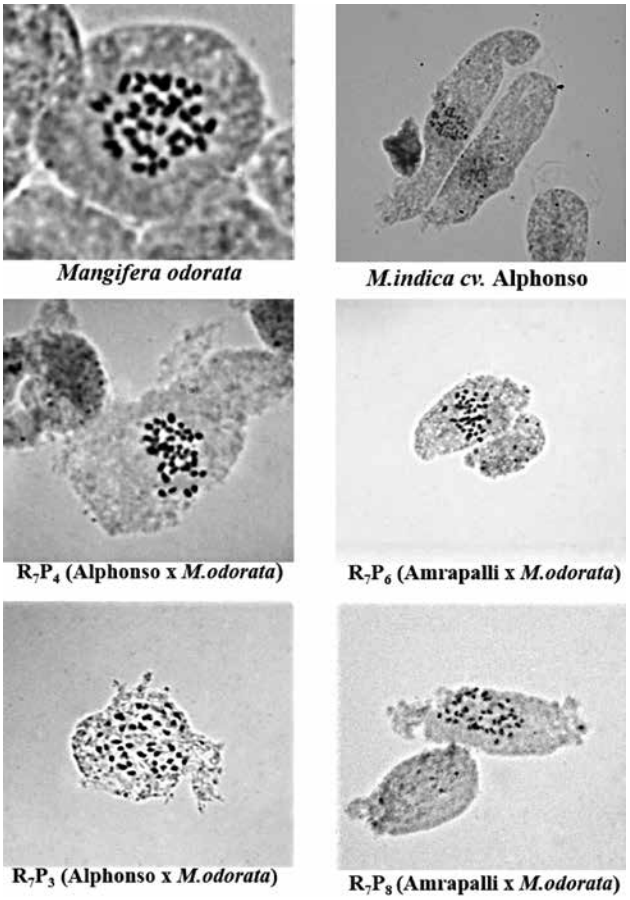

Fig. 3. Cytological studies of mango.

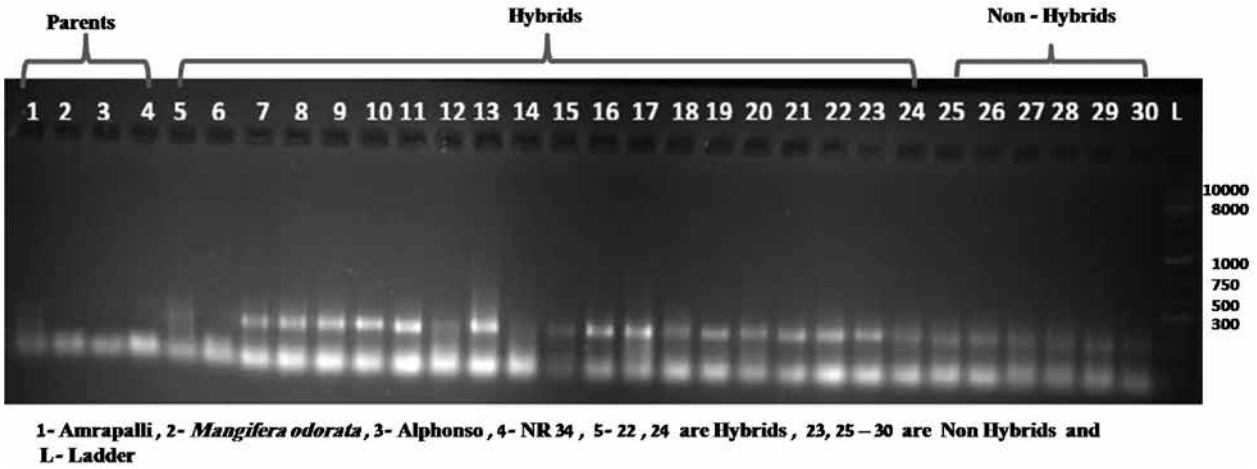

Fig. 4. MIIHR 36 primer images of the amplified PCR products of interspecific hybrids.

Table 2. Characteristics of the 11 SSR markers.

S. No.	Locus	Primer (5´- 3´)	Product size (bp)
1	MiIIHR17	F: GCTTGCTTCCAACTGAGACC R: GCAAAATGCTCGGAGAAGAC	230- 269
2	MiIIHR18	F: TCTGACGTCACCTCCTTTCA R: ATACTCGTGCCTCGTCCTGT	148- 193
3	MiIIHR23	F: TCTGACCCAACAAAGAACCA R: TCCTCCTCGTCCTCATCATC	107- 156
4	MiIIHR26	F: GCGAAAGAGGAGAGTGCAAG R: TCTATAAGTGCCCCCTCACG	190- 213
5	MiIIHR30	F: AGCTATCGCCACAGCAAATC R: GTCTTCTTCTGGCTGCCAAC	127- 171
6	MiIIHR31	F: TTCTGTTAGTGGCGGTGTTG R: CACCTCCTCCTCCTCCTCTT	207- 260
7	MiIIHR36	F: TCTATAAGTGCCCCCTCACG R: ACTGCCACCGTGGAAAGTAG	210-250
8	MiBNG_c268 FAM	F: TATCGCCTACCTTTGAGGGA R: TTTTGTTTGTGGGTGCACAT	160- 220
9	MiKVR_I230VIC	F: GCACAACCATGCACTTAACC R: CAACCTAGGATGAACAAGGAGAA	178- 211
10	MiKVR_I976 FAM	F: CATTTGTTTGACACTAAAGAGCG R: ATCAAGGAACCCAGATGCAG	208- 276
11	MiBNG_a619- VIC	F: GCAAGGAAGCTGATTCTCCA R: TACCACTTTGTCCAAAGCCC	142- 186

(Amrapali × *Mangifera odorata*) showed 50% of maternal and paternal allele cross combinations (Table 3). Similar results were reported in mango hybrids using SSR markers (Nayak, 7). The Fig. 4 Reveals that the lane number 7 to 13 and 16 to 30 are believed to be hybrids and they are matching with the morphological characters. The results of the present study is in the line of the work conducted by Singh *et al*. (12) who have studied genetic diversity in closely related mango hybrids using SSR markers and concluded that hybrids had a stronger affinity towards maternal parent Amrapali.

Interspecific hybrid R7P5 (Amrapali × *Mangifera odorata*), R1P62 (Amarapali × *Mangifera odorata*), R1P70 (Amrapali × *Mangifera odorata*), R1P71 (Amrapali × *Mangifera odorata*), R1P72 (Amrapali × *Mangifera odorata*), R1P73 (Amrapali × *Mangifera odorata*), R1P74 (Amrapali × *Mangifera odorata*), R1P75 (Amrapali × *Mangifera odorata*) and R1P76 (Amrapali × *Mangifera odorata*) showed 50% of maternal and paternal allele cross combinations and they are matching with the morphological characters. SSR markers such as MiBNG_a619- VIC, MIIHR 36, MiKVR_I976 FAM and MiIIHR31 could be useful for confirming the hybridity.

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Table 3. Fragment analysis and allele inheritance.

Sl. No.	Genotypes	Paternity (%)	Maternity (%)	New Allele (%)
1	Amrapali			
2	Mangifera odorata			
3	R7P7(Amrapali × *Mangifera odorata)*	80	20	0
4	R7P6(Amrapali × *Mangifera odorata)*	71.42	14.29	14.29
5	R1P60(Amrapali × *Mangifera odorata)*	71.42	14.29	14.29
6	R1P61(Amrapali × *Mangifera odorata)*	62.50	25	12.50
7	R1P57(Amrapali × *Mangifera odorata)*	55.56	22.22	22.22
8	R7P5(Amrapali × *Mangifera odorata)*	50	50	0
9	R1P62(Amrapali × *Mangifera odorata)*	50	33.33	16.67
10	R1P63(Amrapali × *Mangifera odorata)*	37.50	37.50	25
11	R1P70(Amrapali × *Mangifera odorata)*	37.50	50	12.5
12	R1P71(Amrapali × *Mangifera odorata)*	50	33.33	16.67
13	R1P72(Amrapali × *Mangifera odorata)*	25	50	25
14	R1P73(Amrapali × *Mangifera odorata)*	40	40	20
15	R1P74(Amrapali × *Mangifera odorata)*	50	33.33	16.67
16	R1P75(Amrapali × *Mangifera odorata)*	50	50	0
17	R1P76(Amrapali × *Mangifera odorata)*	33.33	50	16.67
18	R1P64(Amrapali × *Mangifera odorata)*	66.67	33.33	0
19	R1P6(Amrapali × *Mangifera odorata)*	57.14	42.86	0
20	R1P8(Amrapali × *Mangifera odorata)*	37.5	50	12.5
21	R1P4(Amrapali × *Mangifera odorata)*	28.57	14.29	57.14
22	R1P5(Amrapali × *Mangifera odorata)*	50	16.67	33.33
23	Alphonso			
24	R7P3 (Alphonso × *Mangifera odorata)*	77.78	0	22.22
25	R7P4 (Alphonso × *Mangifera odorata)*	71.42	0	28.58
26	NR34			
27	R1P11(NR34 × *Mangifera odorata)*	20	0	80
28	R1P9 (NR34 × *Mangifera odorata)*	20	0	80
29	R1P13 (NR34 × *Mangifera odorata)*	16.67	0	83.33
30	R1P12 (NR34 × *Mangifera odorata)*	40	0	60

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