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Intraspecific hybridization of low chill peach cultivars for superior fruit quality and their hybridity confirmation by SSR markers

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ABSTRACT

The present investigation was carried out to develop of low chill peach hybrids for superior fruit quality during the year 2016 and 2017. Crosses were made between low chill peach cultivars, taking Flordaglo and Florda Crest as a female parent and Florda Prince, Florda Grand, Florda Crest and Yumyoeng as male parents. Among the hybrids, the maximum fruit set (59.28%) was observed in H_3, while the minimum fruit set (38.56%) was recorded in H_6. Among the parents crossed, the maximum fruit set (82.32%) and fruit retention (53.23%) was observed in Florda Prince while the minimum fruit set (56.42%) and fruit retention (33.56%) was noticed in Yumyoeng. In the low chill hybrids developed, fruit retention percent was recorded the maximum (74.50%) and the minimum (41.27%) in H_1 and H_4. Crosses of Florda Crest as female noticed the maximum fruit drop percentage with H_5 having highest (74.61%) fruit drop percentage while hybrid H_1 observed the minimum (25.84%) fruit drop percentage. Among the parents, Yumyoeng showed maximum (53.79%) while Florda Crest showed minimum (43.94%) fruit drop percentage. Seeds of hybrid H_6 took the maximum days for stratification (77 days) while H_2 took the minimum days for stratification (69 days). The minimum (10.30 days) and the maximum (15.58 days) number of days taken for seed germination was noticed in H_3 and H_5, respectively. After sowing the seeds in the protrays, H_1 observed the maximum seed germination percentage (90.41%) while minimum (43.38%) percent of seed germination was recorded in H_6. A significant variation was recorded between the traits *viz.*, seedling height, intermodal length and rosetting percentage among different peach hybrids. Among the 22 SSR markers, only six markers (MA015a, MA020a, MA023a, CPPCT-022, CPPCT-030 and UDP96-005) were found to be able to test the hybridity of F1 seedling.

Keywords: *Prunus persica*, morphological characterization, SSR markers, hybridity confirmation.

INTRODUCTION

Peach [*Prunus persica* (L.) Batsch] originated in China is well adapted to temperate and sub-tropical regions. In 2016, China was the leading producer of peach i.e., about 58% share of the total world production followed by Italy, Spain and USA (Anonymous, 2). Total world production and area under peaches and nectarines is 2, 49, 75649 MT and 16, 39,925 ha, respectively (FAO, 7). In India, peach occupies an area of 18.10 (000 ha) and production is 107.19 (000 MT) (NHB, 11). Peach is a diploid plant having haploid chromosome number n = 8. The genome size is comparatively small i.e., 5.9×10^8 bp in diploid nucleus, with a haploid size of 265 Mb which is approximately twice the size of the *Arabidopsis* genome (Verde *et al*., 16). It is considered as one of the important fruits in the world and ranked third after apple and pear in the Rosaceae family.

Peach industry in India had been dependent on introduction of varieties bred in USA and Europe. In the entire world, most of the peach breeding program is now being undertaken by private companies which do not share their germplasm due to commercial interests. Also the consumer preferences are changing day by day. Improving the fruit colour, shape, size, texture, flesh color, freedom from loose fibre and non-browning of the flesh are some of the important goals of cultivar development in peach. Improvement of all these traits in combination develops a variety which is having fruits of superior quality. Under sub-tropical climatic conditions, low chilling requirement is also the main characteristic for the economic cultivation of peaches.

Based on the characteristics of fruit firmness and textural changes during ripening, peach cultivars can be classified into three groups: melting (M), non-melting (NM), and stony hard (SH) flesh types. Most peach and nectarines varieties have melting flesh which is good to eat and have good flavour but, lacks post-harvest storage life. Non-Melting flesh types are suitable for processing but, they lack tree ripe flavour hence, not liked for fresh consumption. Stony hard (SH) peaches have firm flesh with tree ripe flavour and longer storage life found in cultivars such as 'Jingsu' from China, 'Yinggetao' from Taiwan, 'Hakuto' from Japan and 'Yumyeong' from Korea.

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In order to understand the genetic background and the breeding value of the available germplasm, systemic study related to characterization and evaluation of germplasm is of great importance for current and future breeding and genetic improvement of the crop. Morphological characters include both qualitative and quantitative characteristics, which are the strongest determinants of the agronomic value and systematic classification of fruit crops. Compared with other means, morphological evaluations are direct, inexpensive and easy, leading to identification of varieties and its wild forms is essential for sustainable fruit production. But in the last few years, use of microsatellites markers (SSR) is becoming the appropriate method for molecular characterization and genetic diversity studies in peaches (Alvarez *et al.,* 1). Microsatellite or simple sequence repeats (SSRs) are abundant and ubiquitous along the eukaryotic genome, having high level of polymorphism, co-dominant inheritance, high discrimination power and easiness of detection. A number of SSR markers for *Prunus persica* and other species of the same genus are available for different purpose (Aranzana *et al.,* 3) and are used for molecular characterization of peaches.

Thus, to meet the above said goals, the present investigation was done to develop hybrids by crossing low chill peach cultivars and then morphologically characterized. Later the hybridity was confirmed with the use of SSR markers.

MATERIALS AND METHODS

The present investigation was carried out during the year 2015-16 and 2016-17 at Fruit Research Farm, Department of Fruit Science, Punjab Agricultural University, Ludhiana. Crosses were made among different low chill cultivars viz. H_1 (Flordaglo × Florda Prince), H_2 (Flordaglo × Florda Grand), H_3 (Flordaglo × Florda Crest), H_4 (Flordaglo × Yumyoeng), H_5 (Florda Crest × Florda Prince) and H_6 (Florda Crest × Florda Grand).

Branches with unopened flower buds at popcorn stage were selected and emasculated by removing sepals, petals and stamens to prevent self-pollination. All the previously fully opened flowers were removed. Pollination was done at the same time of emasculation either with the fresh pollen (cultivars in which flowering period coincided) or with stored pollen (cultivars in which flowering periods did not coincide. Among the above said crosses, Flordaglo × Florda Prince and Flordaglo × Florda Grand were crossed in 2016 with fresh pollen while Flordaglo × Florda Crest, Flordaglo × Yumyoeng, Florda Crest × Florda Prince and Florda Crest × Florda Grand were crossed in 2017 with stored pollen. The stigmas were pollinated with the help of camel hair brush. Bagging or protection of pollinated flowers is not done because emasculated flowers do not attract pollinators (Monet and Bassi, 9).

Data on fruit set, fruit retention and fruit drop percentage was recorded in the hybrids as well as in the parents. After full maturation of fruits, these are harvested separately and seeds were extracted from them. Seeds were kept in stratification media containing cocopeat, vermiculite and perlite in the ratio 2:1:1. Stratification media was moistened with 1 per cent Carbendazim to avoid any fungal infestation. Seeds were properly labeled and kept at 4±2°C till more than 75% of seeds show radicle emergence. Data regarding days taken for stratification was recorded when the seeds kept for stratification starts radicle emergence. After radicle emergence, seeds were sown in portrays and kept in growth chamber for germination of seeds. After germination, portrays were transferred to polyhouse for further growth of the seedlings. Data on days taken for germination was recorded at this stage. The seedlings were transferred to polybags for their proper growth and development. Data regarding germination percentage, seedling height, internodal length and leaf rosetting percentage was recorded.

Hybridity testing of these hybrids were tested by using 22 SSR primer pairs. Protocol of DNA extraction and purification was as follows:

Young leaves were selected for the extraction of DNA following the procedure of Paterson *et al.,* (12). Young emerging leaves were plucked from parents and hybrids, placed in glassine bags and stored in ice. Leaves were ground in liquid nitrogen to fine powder with the help of mortar and pestle. The powdered sample was transferred immediately to a 2 ml microfuge tubes. The CTAB extraction buffer and β-Mercaptoethanol was added to each sample. After addition of buffer, the tubes were incubated at 65 °C for 45 minutes in a water bath and were mixed occasionally. Saturated phenol: chloroform: isoamyl alcohol (25:24:1) was added after incubation and tubes were churned, till it made a dark green emulsion. The tubes were then placed on a rotary shaker for 30 minutes and then centrifuged at 10,000 rpm for 10 minutes at room temperature for separation of phases. After centrifugation, the upper aqueous phase was transferred to a clean sterile 1.5 ml microfuge tubes and chilled isopropyl alcohol was added to them. The tubes were inverted gently several times. A white cotton thread like precipitate of DNA was formed and good quality of DNA floated atop. Tubes were then kept in refrigerator for 20 minutes at -4 °C. After that it was again centrifuged for 10 minutes at 12,000 rpm. The supernatant were discarded as the

pelleted DNA is attached to the bottom of the tubes. Washing of the pelleted DNA was done with 70 per cent ethanol for five minutes to remove any residual salts followed by re-centrifugation. The tubes were then put upside down on a blotting paper to remove leftover ethanol and was completely air dried at room temperature for one to two hours. The pellets were then dissolved in 100-200 µl volume of 1X TE buffer (Tris EDTA buffer- 10mM Tris HCl, 1mM EDTA, pH 8.0) and were left overnight at room temperature for dissolving of DNA pellets. The DNA samples were stored at 4 °C until used.

A set of 22 SSR markers (Table 1) were initially tested for DNA amplification. Out of these, 6 primers produced the polymorphic bands and were selected for further analysis. Amplification of DNA was carried out in 10 µl reactions containing 50 ng of template DNA, 0.2 mM of total dNTPs, 0.5 µM primer, 1.0 µl of 10X PCR buffer, 1.5 mM of magnesium chloride and 1 unit of *Taq* polymerase. Following cycle of processes was involved in the amplification process: initial denaturation step for 4 minutes at 94 °C, followed by 35 cycles at 94 °C for 1 minute (denaturation), 52- 60 °C for 1 minute (annealing) 35 cycles and 72 °C for 1 minute (elongation) 35 cycles, followed by a final extension step at 72 °C for 7 minutes and 1 cycle. 2.5 percent of superfine resolution agarose gel was prepared in 0.5X TBE buffer and 0.5 µg/ml aqueous solution of ethidium bromide for resolving of the PCR products. About 10 µl of reaction products (2 µl of loading buffer 6X) were loaded and visualized under UV light.

The experiment was laid out as completely randomized design (CRD) with three replications. The data was analysed using SAS v9.0.0 software and separation of means was done using Least Significant Difference (Fisher's LSD) test at ≤ 0.05 level of significance.

RESULTS AND DISCUSSION

Among the parents used, the maximum fruit set (82.32 %) and fruit retention (53.23 %) percentage was recorded in Florda Prince, while the minimum fruit set (56.42 %) and fruit retention (33.56 %) was observed in Yumyoeng. The minimum (43.94 %) per cent of fruit drop was observed in Florda Crest, while the maximum (53.79 %) fruit drop percentage was seen in Yumyoeng (Table 2). Comparing the hybrids with parents, relatively less fruit set was noted among them but they showed more fruit retention

Table 1: SSRs primers pairs used for the hybridity testing of low chill peach hybrids.

Locus name	Forward sequence (5'-3')	Reverse Sequence (3'-5')	Repeat motif	Size (bp)	Chromosome No.
CPPCT027	AATTTTCTCTTTCATTTCTCATATC	CCTCCTCGTCTTCTCTGTGC	$(CT)_{30}$	114	G1
EPPCU1902	TGTTTTTCCAGTTCTCCTTTTTG	ACTGTGACTGCGAGTGGTTG			
MA0056a	GTCTTGTCTCCATTAGTCCC	GAACTTGATGGATTGGTTTG			G1
M15a	GAGGGTCCTTAGCTCTCTCT	ATGAGAAACGACTGGAAAAG	$(CT)_{16}$	135	G1
BPPCT020	CGTGGATGGTCAAGATGC	ATTGACGTGGACTTACAGGTG	$(AG)_{14}GG(AG)_7AT(AG)_8$	121	G1
BPPCT016	GATTGAGAGATTGGGCTGC	GAGGATTCTCATGATTTGTGC	$(AG)_{14}$	96	G1
MA015a	TGAGTTCGATGGAGCCTCCT	GGTTACTCCCCCCATTGTCA	$(AG)_{21}$	177	G3
BPPCT017	TTAAGAGTTTGTGATGGGAACC	AAGCATAATTTAGCATAACCAAGC	$(GA)_{28}$	174	G5
BPPCT038	TATATTGTTGGCTTCTTGCATG	TGAAAGTGAAACAATGGAAGC	$(GA)_{25}$	135	G5
BPPCT009	ATTCGGGTCGAACTCCCT	ACGAGCACTAGAGTAACCCTCTC	$(CT)_{14}$	171	G4
MA020a	CTTGCCCATTTATGTACTGA	TATATCGCATAATCACGGTC	$(AG)_{23}$	180	G7
MA023a	AGAAGCAAAGCTAACAGCC	GATGACTCATTGACGCAAGA	$(AG)_{24}$	192	G8
UDP98-024	CCTTGATGCATAATCAAACAGC	GGACACACTGGCATGTGAAG	$(GT)_{19}TC(TG)_7$	105	G4
CPPCT-022	CAATTAGCTAGAGAGAATTATTG	GACAAGAAGCAAGTAGTTTG	$(CT)_{28}CAA(CT)_{20}$	250	G7
CPPCT-030	TGAATATTGTTCCTCAATTC	CTCTAGGCAAGAGATGAGA	$(CT)_{30}$	186	G6
BPPCT 030	AATTGTACTTGCCAATGCTATGA	CTGCCTTCTGCTCACACC	$(AG)_{25}$	175	G2
MA036a	ACAGAAGAGAGAAGGGGAA	CCACCATGCTACAGACAACT	$(AG)_{26}$	241	
MA049a	CCTTTTGGCAAGATTGAGAG	CGGTTGTTTAATTATGTACG	$(GA)_{19}$	278	
MA069a	GGAAATGAACACATCTCGTCAGTAA	AACAGCCAAAAGGAGACAACC	$(GA)_{28}$	127	G2
UDP96-008	TGCTGAGGTTCAGGTGAGTG	TGCTGAGGTTCAGGTGAGTG	$(CA)_{23}$	165	G3
UDP96-005	GTAACGCTCGCTACCACAAA	CACCCAGCTCATACACCTCA	$(AC)_{16}TG(CT)_2CA(CT)_{11}$	155	G1
UDP96-003	TTGCTCAAAAGTGTCGTTGC	ACACGTAGTGCAACACTGGC	$(CT)_{11}(CA)_{28}$	143	G4

Table 2: Fruit set, fruit retention and fruit drop in low chill peach cultivars.

Cultivars	Fruit set (%)		
	2016	2017	Mean
Flordaglo	82.16[a]	80.71[a]	81.44[a]
Florda Grand	83.61[a]	80.92[a]	82.27[a]
Florda Prince	83.04[a]	81.60[a]	82.32[a]
Florda Crest	82.77[a]	81.07[a]	81.92[a]
Yumyoeng	59.35[b]	53.49[b]	56.42[b]
$LSD_{0.05}$	2.72	2.44	1.49
	Fruit retention (%)		
Flordaglo	52.69[ab]	50.14[a]	51.42[b]
Florda Grand	51.74[b]	47.11[b]	49.43[c]
Florda Prince	54.66[a]	51.80[a]	53.23[a]
Florda Crest	53.78[ab]	49.92[ab]	51.86[b]
Yumyoeng	35.07[c]	32.04[c]	33.56[d]
$LSD_{0.05}$	2.16	2.99	1.27
	Fruit Drop (%)		
Flordaglo	47.92[b]	50.46[b]	49.19[b]
Florda Grand	46.18[bc]	49.27[b]	47.23[b]
Florda Prince	46.93[bc]	43.90[c]	45.42[c]
Florda Crest	45.32[c]	42.56[c]	43.94[c]
Yumyoeng	51.99[a]	55.56[a]	53.79[a]
$LSD_{0.05}$	2.42	2.36	1.65

*Values with the same letters are not significantly different according to Fisher's LSD test at 5% level

Table 3: Fruit set, fruit retention and fruit drop in crosses made between low chill peach hybrids.

Parents	Fruit set (%)
Flordaglo × Florda Prince	59.08[a]
Flordaglo × Florda Grand	48.71[b]
Flordaglo × Florda Crest	59.28[a]
Flordaglo × Yumyoeng	44.96[bc]
Florda Crest × Florda Prince	40.10[c]
Florda Crest × Florda Grand	38.56[c]
$LSD_{0.05}$	6.41
	Fruit retention (%)
Flordaglo × Florda Prince	74.50[a]
Flordaglo × Florda Grand	66.51[c]
Flordaglo × Florda Crest	70.78[b]
Flordaglo × Yumyoeng	41.27[e]
Florda Crest × Florda Prince	46.38[d]
Florda Crest × Florda Grand	41.38[e]
$LSD_{0.05}$	1.92
	Fruit Drop (%)
Flordaglo × Florda Prince	25.84[e]
Flordaglo × Florda Grand	30.26[d]
Flordaglo × Florda Crest	34.95[c]
Flordaglo × Yumyoeng	62.96[b]
Florda Crest × Florda Prince	75.61[a]
Florda Crest × Florda Grand	75.07[a]
$LSD_{0.05}$	2.47

*Values with the same letters are not significantly different according to Fisher's LSD test at 5% level

percentage. Hybrid H_3 resulted in the maximum fruit set (59.28 %), while H_6 observed the minimum fruit set (38.56 %) (Table 3). The maximum (74.50 %) and the minimum (41.27 %) fruit retention was calculated in H_1 and H_4, respectively. Hybrid H_1 recorded the minimum (25.84 %) and H_5 observed the maximum (75.61 %) fruit drop percentage. It can clearly be observed from the table that when Flordaglo was used as female parent, maximum fruit set, fruit retention and minimum fruit drop percentage was noted. The climatic condition during blooming time, pollen production, pollen quality, presence of pollinators, nutrition of tree, diseases, seed abortions or the need of chilling and heat accumulation of cultivar are one of the reasons for fluctuating fruit set, fruit retention and fruit drop. Additionally, the degeneration and abortion of the ovule may occur due to environmental stress such as rainfall, humidity, temperature, etc. leading to low fruit set and retention percentage (Nava *et al.,* 10). Eroglu *et al.* (6) observed fruit set to be 78.27% and 73.10% for two years, respectively.

Stratification of seeds is needed to break the dormancy of the embryo. A strong effect of embryo genotype on the chilling requirement of the seeds was observed by Bruckner *et al.* (4). Days taken for stratification, germination and germination percentage of the low chill peach hybrids are depicted in Table 4. Maximum days taken for stratification (77.00 days) were observed in H_6, while minimum (69.00 days) was recorded in H_2. The difference in stratification period might be due to the need of different chilling duration in different genotypes (Bruckner *et al.,* 4).

When the seeds show radicle emergence, data regarding days taken for seed germination was recorded. It was observed that hybrid H_3 took the minimum (10.30 days) days for germination. In contrast, the maximum (15.58 days) days taken for germination were by H_5. In comparison to Florda Crest, seeds obtained from Flordaglo crosses took

Table 4: Days taken for stratification, germination and germination percentage of low chill peach hybrids.

Parents	Days taken for stratification	Days taken for germination	Germination (%)
Flordaglo × Florda Prince	70.00^{cd}	10.73^{e}	90.41^{a}
Flordaglo × Florda Grand	69.00^{d}	12.42^{d}	88.94^{a}
Flordaglo × Florda Crest	72.00^{bcd}	10.30^{e}	84.18^{b}
Flordaglo × Yumyoeng	73.67^{abc}	13.58^{c}	82.94^{b}
Florda Crest × Florda Prince	76.00^{ab}	15.58^{a}	57.78^{c}
Florda Crest × Florda Grand	77.00^{a}	14.59^{b}	48.38^{d}
$LSD_{0.05}$	4.24	0.81	2.69

*Values with the same letters are not significantly different according to Fisher's LSD test at 5% level.

lesser time for germination because of longer fruit development period, matured seeds and accumulation of more dry matter in the seeds.

Seed germination percentage was calculated after one month of seed germination. Maximum germination percentage (90.41 %) was observed in H_1, while H_6 resulted in the minimum (48.38 %) germination percentage. Flordaglo as a female parent has resulted in the maximum seed germination percentage as compared to Florda Crest. This may be due to the proper development of embryo in Flordaglo as FDP in this is more than Florda Crest. Less seed germination in Florda Crest crosses may be due to immature embryos because of shorter FDP and less dry matter content in the seeds. Genotypes with fruit development period of more than 105 days and seed stratification without endocarp has resulted in decreased length of days taken for germination and increased germination rate (Tukey and Carlson, 14).

A quantitative study of the vegetative characters has also been done after four months of germination of seeds. Significant variations have been observed among the hybrids (Table 5). Maximum (34.05 cm and 2.40 cm) and minimum (21.33 cm and 1.87 cm) seedling height and intermodal length were recorded in H_1 and H_3 respectively. This difference in seedling height and intermodal length might be due to different genotypes used for crossing. Minimum (11.37%) and maximum (18.57%) rosetting percentage have been observed in H_1 and H_2. It may be sometimes due to seed stratification without endocarp and also dependent on variety (Topp *et al*., 13). Byrne *et al*., (5) reported that seeds of genotypes with less than 110 days FDP can produce a high rate of rosetted seedlings and this rate drops rapidly when the length of FDP increases.

Hybrid seedlings resulted from crossing between diverse parents are very difficult to identify when either of the parents have a convenient dominant character. Different methods have been developed by various scientists to identify these characters. These methods mainly include morphologic, chromatographic and isoenzymatic procedures for the recognition of hybrids but none of these methods provide perfect confirmation to identify hybrid seedlings (Tusa *et al.,* 15). To overcome these problems, new methods are being used that is use of molecular markers which give frequent results in the exclusion of true hybrid seedlings from segregating populations.

In the present experiment, 22 SSR markers were used for the hybridity testing but only six (MA015a, MA020a, MA023a, CPPCT-022, CPPCT-030 and UDP96-005) of them have been found to be

Table 5: Seedling height, internodal length and rosetting percentage of low chill peach hybrids.

Parents	Seedling height (cm)	Internodal length (cm)	Rosetting (%)
Flordaglo × Florda Prince	34.05^{a}	2.40^{a}	11.37^{c}
Flordaglo × Florda Grand	29.69^{abc}	2.33^{a}	18.57^{a}
Flordaglo × Florda Crest	21.33^{d}	1.87^{b}	14.33^{b}
Flordaglo × Yumyoeng	25.24^{cd}	2.05^{b}	13.37^{b}
Florda Crest × Florda Prince	28.78^{bc}	2.03^{b}	13.49^{b}
Florda Crest × Florda Grand	30.18^{ab}	1.92^{b}	12.53^{bc}
$LSD_{0.05}$	4.64	0.22	1.91

*Values with the same letters are not significantly different according to Fisher's LSD test at 5% level.

polymorphic and are able to confirm the hybridity of the F1 seedlings. The list of markers confirming the hybridity is presented in Table 6 and Fig. 1. These polymorphic SSR primers pair with the genomic DNA from both the parents. The respective hybrids run on 2.5% agarose gel and confirm the hybridity having two amplicons in the hybrid whereas parents had alternate amplicon. The results confirm the authenticity of the peach crosses made and their further use for future breeding programs. Further studies needs to be carried out to decipher the interplay of factors during the fruit set and fruit drop to enable harnessing of these factors to prolong fruit retention in the hybrids.

Hybridity of the hybrid lines have been tested by different types of molecular markers such as

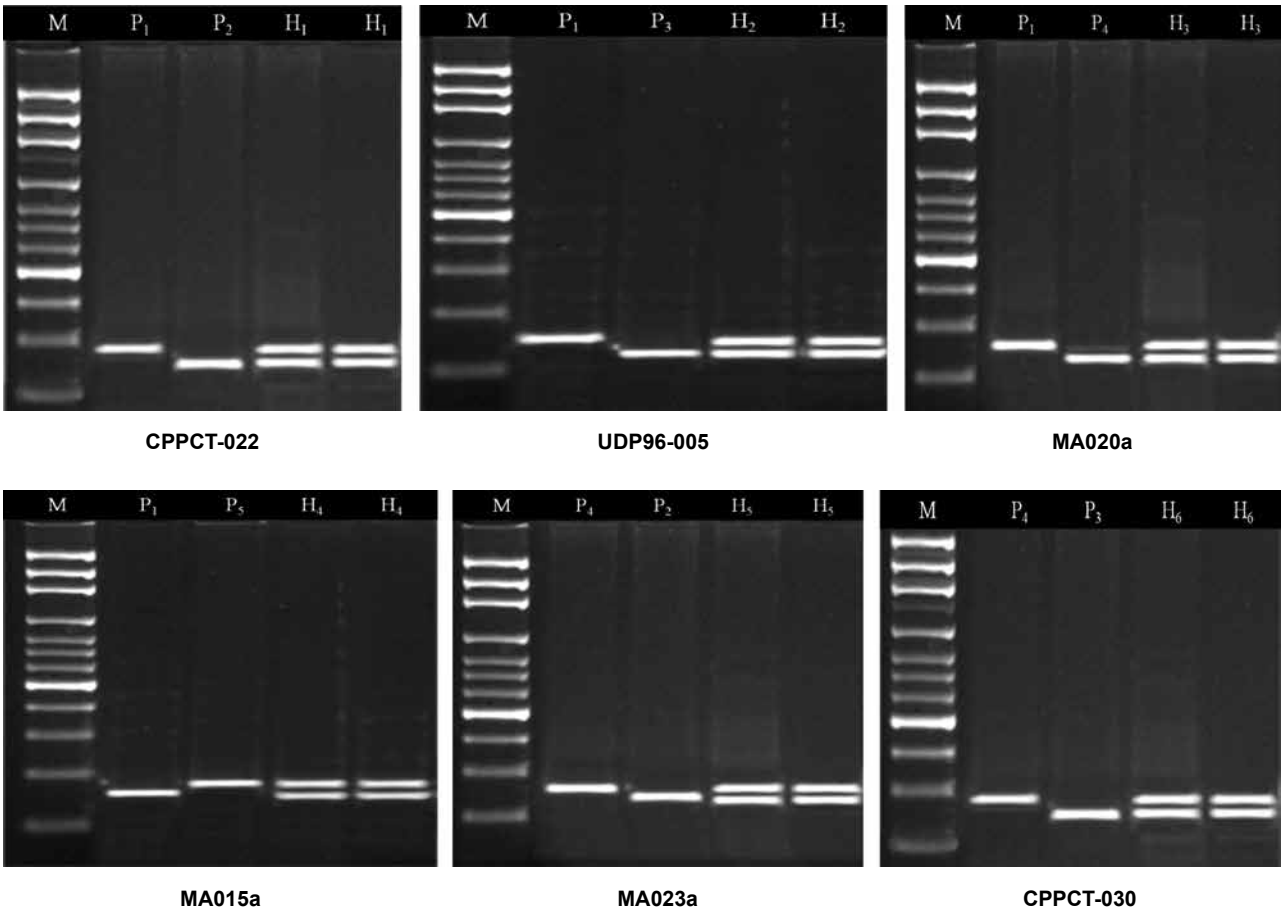

Figure 1: Hybridity confirmation by SSR markers.

M- 1000 bp (DNA ladder); P_1 – Flordaglo; P_2- Florda Prince; P_3- Florda Grand; P_4- Florda Crest; P_5- Yumyoeng; H_1- Flordaglo × Florda Prince; H_2- Flordaglo × Florda Grand; H_3- Flordaglo × Florda Crest; H_4- Flordaglo × Yumyoeng; H_5- Florda Crest × Florda Prince; H_6- Florda Crest × Florda Grand

Table 6: Hybridity confirmation by SSR polymorphic markers.

Hybrid number	Pedigree	Result	Marker (s)
H_1	Flordaglo × Florda Prince	Hybridity confirmed	CPPCT- 030, CPPCT- 022, UDP96-005
H_2	Flordaglo × Florda Grand	Hybridity confirmed	UDP96-005, CPPCT- 022
H_3	Flordaglo × Florda Crest	Hybridity confirmed	CPPCT- 030, MA020a
H_4	Flordaglo × Yumyoeng	Hybridity confirmed	CPPCT- 030, MA015a
H_5	Florda Crest × Florda Prince	Hybridity confirmed	CPPCT- 030, MA023a, UDP96-005
H_6	Florda Crest × Florda Grand	Hybridity confirmed	CPPCT- 030, MA023a, MA015a

Randomly Amplified Polymorphic DNA (RAPD) by different scientists to distinguish nucellar seedlings from zygotic seedlings. Use of molecular markers is a very useful aid helping the breeders to give immediate results. SSRs provide more reliable method for parentage analysis because of its co-dominant inheritance and large number of alleles per locus. Hybrid status between Zhonghuashoutao peach (*Prunus persica*) × Xinshiji apricot (*P armeniaca*) and Zhonghuashoutao peach × President plum (*P. saliciana*) was checked using SSR markers by Liu *et al.* (8). In the combination of peach and apricot, five specific bands from female and two from the male have been inherited in the hybrids. In the cross between peach and plum, hybrids possessed several specific bands from both parents, thus confirming the hybridity.

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