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Morphological characterization and hybridity confirmation of low chill peach (*Prunus persica*) hybrids using SSR markers

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ABSTRACT

A hybridization programme was conducted during 2015 and 2016 with the main objective to widen the varietal range of early ripening cultivars. Crosses were made between low chill peach [*Prunus persica* (L.) Batsch] cultivars, taking Shan-i-Punjab and Tropic Sweet as a female and crossed with Florda Prince, Flordaglo and Prabhat and crosses of Shan-i-Punjab x Florda Prince showed maximum fruit set (72.63%), whereas minimum fruit set was recorded in Tropic Sweet × Flordaglo (18.87%). Although in Tropic Sweet crosses, fruit set was lower but they showed significantly higher percentage of fruit retention and lower percentage of fruit drop than the crosses made with Shan-i-Punjab cultivar. After ripening seeds were extracted from the fruits and were kept at low temperature for stratification until the seeds showed radicle emergence. Crosses made between Tropic Sweet × Flordaglo took maximum days for stratification (88.33). After sowing in the field maximum percentage of seed germination 90.43% was recorded in Tropic Sweet × Florda Prince seeds and maximum seedling height in Tropic Sweet × Flordaglo (36.03 cm). Very less variations were recorded among different crosses for petiole length and intermodal length and more rosetting was observed in Tropic Sweet hybrids. Among 22 SSR markers only six markers (MA015a, MA020a, MA023a, CPPCT-022, CPPCT-030 and UDP96-005) were able to test the hybridity of F1 seedling.

Key words: Hybridity testing, Morphological characterization, Peach hybrids, SSR markers

Peach [*Prunus persica* (L.) Batsch] is native to China and is well adapted to temperate and sub-tropical regions. It is a diploid plant has a comparatively small genome 5.9 × 108 bp or 0.61pg in diploid nucleus, with a haploid size of 300 Mb (Baird *et al.* 1994) which is approximately twice the size of the *Arabidopsis* genome (Arumuganathan and Earle 1991). It is a popular fruit and is considered as one of the important fruit in the world and is a highly genetically characterized fruit tree and now considered as a model species for the Rosaceae family (Monet and Bassi 2008, Arus *et al.* 2012). In India, its cultivation is confined to the warm temperate and sub-tropical parts of Jammu and Kashmir, Himachal Pradesh, Punjab, Haryana, Uttarakhand, parts of Uttar Pradesh, Tamil Nadu and North Eastern states. China is the leading producer of peach with about 54% share of the total world production and is followed by Italy, Spain and USA. Area under peach is increasing rapidly in the sub-tropics of northern India due to the availability of suitable cultivars and higher returns on a unit area bases. Peach fruit species has the largest number of commercial cultivars, which represents a diverse international germplasm. With the advancement of breeding efforts, low chilling peach cultivars have been developed and their cultivation stretches from temperate to sub-tropical world.

As the consumer standards are increasing rapidly, breeding of high quality peach and nectarines cultivars has become a major concern. Currently many peach breeding programs are pursuing with the objective to improve the fruit quality and productivity within locally adapted germplasm (Monet and Bassi 2008, Byrne *et al.* 2012). Initially the peach breeding goals were only to improve external fruit quality, post harvest life, disease and pest resistance and greater range of fruit maturities and types (Byrne 2005) but now, improved fruit eating quality including nutritional composition, has also been targeted and such varieties can only developed through breeding programmes. The important tree and fruit quality parameters may not be independent of each other (Cantin *et al*. 2010, Abidi *et al*. 2011) and might be anticipated owing to their complex genetic and physiological control. Genetic control of traits affecting plant growth and architecture, yield, blooming and harvesting time are usually quantitative (Dirlewanger *et al*. 1999). Fruit size is reported to be a polygenic trait with a low to moderate heritability (Souza *et al*. 1998) and so largely affected by environmental conditions, plant nutrition, and cultural practices. In this genus (*Prunus*) a great advances have been achieved during last 100 years

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using the traditional methods of genetic improvement such as crossing, selection, evaluation of superior lines and *in vitro* propagation of new cultivars (Hancock *et al*. 2008, Okie and Hancock 2008, Iezzoni 2008). These traditional methods has led to the development and commercialization of highly productive, good quality and resistant cultivars to biotic and abiotic conditions. So the current breeding programme was carried out with the main objective is to widen the varietal range of early ripening cultivars characterized by low chilling requirement.

MATERIALS AND METHODS

The present study was conducted at Fruit Research Farm and Tissue Culture Laboratory, Department of Fruit Science, Punjab Agricultural University, Ludhiana during 2015 and 2016. The crosses were made between low chill peach cultivars, viz. Shan-i-Punjab (♀) × Florda Prince (♂), Shan-i-Punjab (♀) × Flordaglo (♂), Shan-i-Punjab (♀) × Prabhat (♂), Tropic Sweet (♀) × Florda Prince (♂), Tropic Sweet (♀) × Flordaglo (♂) and Tropic Sweet (♀) × Prabhat (♂). Branches with unopened blossom at popcorn stage were selected and were emasculated to prevent self-pollination. All the opened flowers and undeveloped buds were removed. Emasculated flowers were pollinated on the same day either with fresh pollen (cultivars in which flowering period coincided) or with stored pollen (cultivars in which flowering periods did not coincided). The pollens were applied to stigmas with camel hair brush and pollinated flowers were not bagged or protected because emasculated flowers do not attract pollinators (Monet and Bassi 2008). Data on fruit set, fruit retention and fruit drop was recorded. When fruits ripened they were harvested separately and seeds were excised from the fruits in the laboratory. Seeds were kept in media containing cocopeat, vermiculite and perlite (2:1:1) and was moistened with Bavistin to avoid the fungal diseases. Seeds were kept at 4±2°C temperature till the maximum seeds show radicle emergence. After that they were sown in pot trays in the field kept in growth chamber initially but when the seeds showed germination they were transferred in polyhouse. Data regarding days taken for stratification was recorded when the seeds kept for stratification start radicle emergence. After germination in field, seedlings were evaluated for total seed germination percentage, days taken for seed germination, seedling height, intermodal length, petiole length, leaf area and leaf rosetting percentage. Rosetting is the curling of leaves and shortening of internodes of shoots and branches, producing a bunchy growth habit. Hybridity of these hybrid seedlings were tested by using 22 SSR primer pairs. Samples were taken from the seedlings grown in the polyhouse. Procedure of DNA extraction and purification is as:

DNA extraction: DNA was extracted from young leaves according to Paterson *et al*. (1993). Young emerging leaves from parents and hybrid seedlings were harvested, placed in glassine bags and stored in ice. Leaves were grounded to fine powder after adding liquid nitrogen and transferred immediately to a 2 ml microfuge tubes. The CTAB extraction buffer and β- Mercaptoethanol was added to each sample. After adding buffer the tubes were incubated at 65°C for 45 minutes in a water bath and were mixed occasionally while maintaining at 60 °C. Saturated phenol: chloroform: isoamyl alcohol (25:24:1) was added after incubation and tubes were swirled, till it made a dark green emulsion. The tubes were placed on a rotary shaker for 30 min and than centrifuged at 10 000 rpm for 10 minutes at room temperature for separate the phases. After centrifugation, the upper aqueous phase was transferred to a clean sterile 1.5 ml microfuge tubes. Chilled isopropyl alcohol was added and the tubes were inverted gently several times. White cotton like precipitate of DNA was formed and good quality DNA floated atop. Tubes were kept in refrigerator for 20 min at -4 °C and than centrifuged for 10 min at 12 000 rpm and than supernatant were discarded. The pelleted DNA was washed with 70% ethanol for five minutes so as to remove any residual salts followed by re-centrifugation. The leftover ethanol was completely dried up by turning down microfuge tubes on a blotting paper and allowed to air dry at room temperature for one to two hours. The pellets were than dissolved in 100-200 µl volume of 1X TE (Tris EDTA buffer-10mM Tris HCl, 1mM EDTA, pH 8.0) and were left for few hours at room temperature to allow DNA to dissolve. The DNA samples were stored at 4 °C until used.

DNA amplification: For DNA amplification 22 SSR markers (Table 1) were initially tested and finally six primers that produced polymorphic bands were selected for further analyses. DNA amplification was carried out in 10 µl reactions containing 50 ng of template DNA, 0.2 mM 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. Amplifications were performed under the following cycle program: initial denaturation step for 4 min at 94°C, followed by 35 cycles at 94°C for 1 minute (denaturation), 52 – 60°C for 1 min (annealing) 35 cycles and 72°C for 1 min (elongation) 35 cycles, followed by a final extension step at 72°C for 7 min and 1 cycle. PCR products were resolved on 2.5 per cent superfine resolution agarose gel which was prepared in 0.5X TBE buffer and 0.5 µg/ml aqueous solution of ethidium bromide. About 10 µl of reaction products (with 2 µl of loading buffer 6X) were loaded and visualized under UV light.

The experiment was laid out as randomized block design (RBD) with three replications. The data were analysed using SAS v9.0.0 software and means were compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The data pertaining to fruit set, fruit retention and fruit drop of crosses is presented in Table 2. Among the crosses made between low chill peach cultivars Shan-i-Punjab × Florda Prince showed maximum mean fruit set (72.63%) but non significant differences in fruit set was recorded

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)	Chro-mosome 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)14GG(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)19TC (TG)7	105	G4
CPPCT-022	CAATTAGCTAGAGAGAATTATTG	GACAAGAAGCAAGTAGTTTG	(CT)28CAA (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)16TG(CT)2CA(CT)11	155	G1
UDP96-003	TTGCTCAAAAGTGTCGTTGC	ACACGTAGTGCAACACTGGC	(CT)11(CA)28	143	G4

in crosses made between Shan-i-Punjab × Florda Prince, Shan-i-Punjab × Flordaglo and Shan-i-Punjab × Prabhat. Minimum mean fruit set was recorded in Tropic Sweet × Flordaglo (18.87 %). In this hybridization programme it was observed that those crosses where Shan-i-Punjab was taken as a female showed higher fruit set, whereas the crosses made with Tropic Sweet as a female exhibited less than 25 % fruit set during both the year. The reason of low fruit set might be due to the temperature fluctuations and thus Tropic Sweet plants failed to undergo dormancy and showed staggered flowering. Hesse (1975) found that initial fruit set in peach crosses can vary from 10% to 90% and this information is consistent with our data showing less fruit set in Tropic Sweet crosses compared to Shan-i-Punjab crosses. Eroglu *et al.* (2016) reported 78.27% and 73.10% fruit set for two years in different peach crosses.

Although in Tropic Sweet crosses, fruit set was lower but they showed significantly higher percentage of fruit retention and lower percentage of fruit drop than the crosses made with Shan-i-Punjab cultivar (Table 2). The highest fruit retention was recorded in Tropic Sweet × Florda Prince crosses (74.12 %) followed by Tropic Sweet × Prabhat (70.41 %) and minimum in Shan-i-Punjab × Flordaglo (22.99 %). As far as fruit drop is concerned, it was maximum in Shan-i-Punjab × Flordaglo (76.99 %) and Shan-i-Punjab × Florda Prince (75.80) followed by Shan-i-Punjab × Prabhat (69.05) whereas minimum fruit drop was recorded in Tropic Sweet × Florda Prince (25.84 %). Among Tropic Sweet crosses maximum fruit drop was observed in Tropic Sweet × Flordaglo (34.08 %) followed by Tropic Sweet × Prabhat (29.57 %) and minimum in Tropic Sweet × Florda Prince (25.84 %).

Data given in Table 3 is pooled data of the year 2015 and 2016 which represent the days taken for stratification, days taken for germination and germination percentage of hybrid seeds. Crosses made between Tropic Sweet × Flordaglo took maximum days for stratification (88.33), whereas those crosses where Shan-i-Punjab was taken as a female took slightly lesser time for stratification and minimum days taken for stratification was recorded in Shan-i-Punjab × Florda

Table 2 Fruit set, fruit retention and fruit drop in crosses made between low chill peach cultivars

Parents	2015	2016	Mean
		Fruit set	
Shan-i-Punjab × Florda Prince	76.31	68.95	72.63a*
Shan-i-Punjab × Flordaglo	75.16	66.16	70.66a
Shan-i-Punjab × Prabhat	76.20	67.39	71.79a
Tropic Sweet × Florda Prince	24.78	21.82	23.30b
Tropic Sweet × Flordaglo	20.06	17.68	18.87d
Tropic Sweet × Prabhat	22.36	20.12	21.24c
LSD (P = 0.05)	0.55	0.37	1.98
		Fruit retention	
Shan-i-Punjab × Florda Prince	26.9	21.45	24.17e
Shan-i-Punjab × Flordaglo	25.83	20.15	22.99e
Shan-i-Punjab × Prabhat	37.49	24.28	30.88d
Tropic Sweet × Florda Prince	75.11	73.14	74.12a
Tropic Sweet × Flordaglo	67.73	64.05	65.89c
Tropic Sweet × Prabhat	70.96	69.87	70.41b
LSD (P = 0.05)	0.81	0.88	2.65
		Fruit drop	
Shan-i-Punjab × Florda Prince	73.07	78.54	75.80a
Shan-i-Punjab × Flordaglo	74.17	79.81	76.99a
Shan-i-Punjab × Prabhat	62.40	75.71	69.05b
Tropic Sweet × Florda Prince	24.82	26.86	25.84e
Tropic Sweet × Flordaglo	32.00	36.17	34.08c
Tropic Sweet × Prabhat	29.03	30.12	29.57d
LSD (P = 0.05)	0.44	0.96	2.64

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

Table 3 Days taken for stratification, days taken for germination and germination percentage of hybrid seeds

Parents	Days taken for stratification	Days taken for germination	Seed germination percentage
Shan-i-Punjab × Florda Prince	76.00d	16.00b	57.73c*
Shan-i-Punjab × Flordaglo	86.00ab	20.50a	48.41d
Shan-i-Punjab × Prabhat	82.83c	20.73a	47.76d
Tropic Sweet x Florda Prince	85.66b	11.33d	90.43a
Tropic Sweet × Flordaglo	88.33a	15.25bc	88.94ab
Tropic Sweet × Prabhat	86.83ab	14.50c	85.11b
LSD CP = 0.05	2.35	1.07	4.95

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

Prince (76.00) and data of this parameter was recorded when the seeds kept for stratification showed 100 per cent radicle emergence. Seeds from all the crosses has taken more than 75 days for radicle emergence. Biggs (1966) demonstrated that seeds from different cultivars differed due to the duration of chilling needed for stratification. Bruckner *et al*. (2012) also found the strong effect of embryo genotype on the chilling requirement of the seeds. Stratification is used to break embryo dormancy and found that stratification treatment of 10 weeks increased the per cent germination over 3 weeks stratification (Mendez 2005). In present studies seeds without endocarp were kept for stratification at 4°C until the seeds showed radicle emergence and seeds of all crosses took 76 to 88 days for radicle emergence and this is in accordance with the results of Eroglu *et al,* (2016). They stratified the seeds of different peach crosses without endocarp at 4-5°C for 40 to 90 days and reported differential response of the crosses.

After the chilling requirement was fulfilled seeds were sown in field and the data of days taken for germination was taken after the seeds started germination in the field. Among all the crosses made, Tropic Sweet × Florda Prince seeds showed germination in minimum days (11.33 days) and Shan-i-Punjab × Prabhat and Shan-i-Punjab × Flordaglo took maximum mean days for seed germination (20.73 days and 20.50 days respectively). In comparison to Shan-i-Punjab, seeds obtained from Tropic Sweet crosses took lesser time for germination after sowing in the field because of longer fruit development period, matured seeds and more dry matter in the seeds of Tropic Sweet crosses. After 30 days of sowing total seed germination was observed and mean maximum percentage of seed germination 90.43% was recorded in Tropic Sweet × Florda Prince which was statistically at par with the seed germination of Tropic Sweet × Flordaglo (88.94 %) followed by Tropic Sweet × Prabhat which showed 85.11% germination, Shan-i-Punjab × Florda Prince seeds showed 57.73% germination whereas minimum seed germination was recorded in Shan-i-Punjab × Prabhat (47.76 %) and Shan-i-Punjab × Flordaglo (48.41%). In Tropic Sweet hybrid seeds, very quick germination was observed and more than 70% seeds germinated even after 10 days of sowing but hybrids made with Shan-i-Punjab showed very less percentage of seed germination. This may be due to the immature embryo of Shan-i-Punjab crosses because of short fruit development period (FDP, days from flowering to harvest) and embryo of these seeds have little reserve and unable to achieve maximum dry weight thus they are too weak to germinate, whereas Tropic Sweet cultivar took more time for ripening, thus embryo was matured and showed higher germination. Bacon and Byrne (1995) reported up to 85% seed germination from genotypes with fruit development period of more than 105 days and seeds stratified without endocarp has increased the germination rate and shorten the length of germination duration (Tukey and Carlson 1945).

Observations of the parameters such as seedling height, intermodal length, leaf area, petiole length and leaf rosetting

were recorded after six months growth of seedlings (Table 4). Maximum seedling height was observed in Tropic Sweet × Flordaglo (36.03 cm) which was non significant to the seedling height in Tropic Sweet × Florda Prince and Tropic Sweet × Prabhat (35.59cm and 35.87 cm). In the seedlings of Shan-i-Punjab × Flordaglo and Shan-i-Punjab × Prabhat mean minimum seedlings height (27.00 cm and 27.20 cm respectively) was observed. This difference in seedling height might be due to the different genotypes used for crossing. In internodal length very less difference was observed in all crosses and maximum was found in seedlings of Tropic Sweet × Flordaglo and Tropic Sweet × Prabhat which was recorded 1.60 cm in both crosses. Non-significant difference was observed in internodal length of Shan-i-Punjab × Florda Prince and Shan-i-Punjab × Flordaglo seedlings. Maximum leaf area was observed in Shan-i-Punjab × Florda Prince (20.16 cm^2) which was statistically at par with the leaf area of Tropic Sweet × Prabhat (19.79 cm^2) and minimum in Shan-i-Punjab × Flordaglo and Shan-i-Punjab × Prabhat (18.35 cm^2 and 18.40 cm^2). Ahmed Emad-Eldin *et al.* (2012) while working on some new F_1 hybrid seedlings also found variation in leaf area of different hybrids. Wang *et al*. (2006) reported that leaf characters such as leaf area, petiole length and petiole thickness are genetically inherited and varied from variety to variety. Very less variation was found among the hybrids for petiole length. Seedling of maximum crosses has recorded the petiole of same length, i.e 0.65cm. Seedlings of Tropic Sweet hybrids showed more rosetting as compared to the Shan-i-Punjab hybrids and mean maximum rosetting percentage was recorded in Tropic Sweet × Prabhat (37.25 %) which was non-significant to the rosetting percentage in Tropic Sweet × Florda Prince (36.85 %) and Tropic Sweet × Flordaglo (36.46 %). Sherman and Beckman (2003) indicated that in lot of stone fruits rosetting can be seen and can occur in those seeds which are stratified without endocarp and it is also variety dependent (Topp *et al*. 2008). In our results also Shan-i-Punjab crosses showed lesser rosetting as compared to the seedlings of Tropic Sweet crosses.

Table 4 Seedling height, intermodal length, leaf area, petiole length and leaf rosetting in hybrid seedlings

Parents	Seedling height	Internodal length	Leaf area	Petiole length	Leaf rosetting
Shan-i-Punjab × Florda Prince	30.34b	1.41c	20.16a	0.65a	15.08a*
Shan-i-Punjab × Flordaglo	27.00c	1.38c	18.35d	0.61ab	18.43a
Shan-i-Punjab × Prabhat	27.20c	1.32d	18.40d	0.56b	18.03a
Tropic Sweet × Florda Prince	35.59a	1.53b	18.78cd	0.65a	36.85b
Tropic Sweet × Flordaglo	36.03a	1.60a	19.21bc	0.65a	36.46b
Tropic Sweet × Prabhat	35.87a	1.60a	19.79ab	0.65a	37.25b
LSD CD (P=0.05)	0.76	0.03	0.60	0.06	5.18

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

Hybridity test of F_1 seedling using SSR markers

It is very difficult to identify hybrid seedlings resulting from the cross between diverse parents, when either parent has a convenient dominant character. Many scientists have developed different procedures, including morphologic, chromatographic and isoenzymatic methods to recognize different hybrids. However, none of these methods provide perfect confirmation to identify hybrid seedlings (Ruiz *et al*. 2000, Tusa *et al*. 2002), therefore, molecular markers are used to give frequent results in the exclusion of true hybrid seedlings from segregating populations. In this experiment 22 SSR markers were used for hybridity confirmation and only six markers (MA015a, MA020a, MA023a, CPPCT-022, CPPCT-030 and UDP96-005) were found to produce the polymorphic amplicon and these were able to test the hybridity of F_1 seedling. List of markers which has confirmed the hybridity of F_1 seedling is given in Table 5. These polymorphic SSR primer pairs with genomic DNA from both parents and their respective hybrids run on 2.5% agarose gel and confirm the hybridity having two amplicon in the hybrid, whereas parents had alternate amplicon. This confirms the authenticity of the peach crosses made and their further use for future breeding programme. Different types of molecular markers such as Randomly amplified polymorphic DNA (RAPD) were used by many workers to test the Hybridity of hybrid lines and to distinguish nucellar seedlings from zygotic seedlings (Rodriguez *et al*. 2005), whereas in citrus the use of simple sequence repeat (SSR) markers were described as an alternative method to distinguish sexual from nucellar seedlings (Ruiz *et al*. 2000). It is a very useful tool to help plant breeders in giving immediate results (Asin *et al*. 1998). Liu *et al*. (2007) also carried out SSR analysis for confirming the hybrid status of *Prunus persica* with *P. armeniaca* and *P.*

Table 5 Hybridity confirmation by SSR polymorphic markers

Hybrid number	Pedigree	Result	Marker(s)
H-1	Shan-i-Punjab × Florda Prince	Hybridity confirmed	CPPCT-030, CPPCT-022
H-2	Shan-i-Punjab × Flordaglo	Hybridity confirmed	CPPCT-030, CPPCT-022, MA020a
H-3	Shan-i-Punjab × Prabhat	Hybridity confirmed	CPPCT-030, MA023a, MA020a
H-4	Tropic Sweet × Florda Prince	Hybridity confirmed	CPPCT-030, MA015a, UDP96-005
H-5	Tropic Sweet × Flordaglo	Hybridity confirmed	CPPCT-030, MA020a
H-6	Tropic Sweet × Prabhat	Hybridity confirmed	CPPCT-030, MA015a

salicina. In the combination of Zhonghuashoutao peach × Xinshiji apricot, the hybrid inherited five specific bands from the female parents and two from the male parent. In the other combinations also Zhonghuashoutao peach with President plum or Morettini plum, the hybrids possessed several specific bands from both parents, thus confirming that they were indeed hybrids.

REFERENCES

Abidi W, Jiménez S, Moreno M A and Gogorcena Y. 2011. Evaluation of antioxidant compounds and total sugar content in a nectarine [*Prunus persica* (L.) Batsch] progeny. *Int. J. Mol. Sci.* **12**: 6919–35.

Ahmed Emad-Eldin A H, Shaltout A D, Elseoudy Alia A M and Khalil B M. 2012. Horticultural evaluation of some new F1 peach hybrid seedlings. *Aust. J. Basic. Appl. Sci*. **6**(3): 840–8.

Arumuganathan K and Earle E. 1991. Nuclear DNA content of some important plant species. *Plant. Mol. Biol. Reporter* **9**: 208–18.

Arus P, Verde I, Sosinski B, Zhebentyayev A T And Abbott A G. 2012. The peach genome. *Tree. Genet. Genomes* **8**: 531–47.

Asin M J, Mestre P F, Herrero R, Novarro L and Carbonell E A. 1998. Molecular markers: a continuously growing biotechnology area to help citrus improvement. *Fruits* **53**: 293–302.

Bacon T A and Byrne D H. 1995. Relationships of fruit development period, seed germination, seedling survival, and percent dry weight of ovule in peach. *Hort. Science* **30**: 833.

Baird W V, Estager A S and Wells J K. 1994. Estimating nuclear DNA content in peach and related diploid species using laser flow cytometry and DNA hybridization. *J. Amer. Soc. Hort. Science* **119**: 1312–6.

Biggs R H. 1966. Germination of 'okinawa1 peach seeds under the conditions of Florida. *Proc. Fla. State. Hortic. Soc* **74**: 370–3.

Bruckner C H, Osmar da J, Silva C, Cruz C D, Wagner Junior V and Moreno Sánchez M A. 2012. Effect of the embryo genotype on the chilling requirement for overcoming peach seed dormancy. *Acta. Hort* **13**: 145–7.

Byrne D H. 2005. Trends in stone fruit cultivar development. *Hort. Technol* **15**: 494–500.

Byrne D, Raseira M, Bassi D, Piagnani M, Gasic K, Reighard G, Moreno M and Pérez S. 2012. Peach. (*In*) *Fruit Breeding,* pp 505-69 Badenes M L and Byrne D H (Eds). Springer Verlag, New York.

Cantín C M, Gogorcena Y and Moreno M A. 2010. Phenotypic diversity and relationships of fruit quality traits in peach and nectarine [*Prunus persica* (L.) Batsch] breeding progenies. *Euphytica* **171**: 211–26.

Dirlewanger E, Moing A, Rothan C, Svanella L, Pronier V, Guye A, Plomion C and Monet R. 1999. Mapping QTLs controlling fruit quality in peach [*P. persica* (L.) Batsch]. *Theor. Appl. Genet*. **98**: 18–31.

Eroglu Z O , Misirli A and Kuden A B. 2016. The cross-breeding performances of some peach varieties. *Yyu. J. Agr. Sci* **26**: 89–97.

Hancock J F, Scorza R and Lobos G A. (2008) Peaches. (*In*) *Temperate Fruit Crop Breeding*, pp 265–98. Hancock J F (Ed). Springer, USA.

Hesse C O. 1975. Peaches. (*In) Advances in Fruit Breeding*, pp 285–305. Jannick J and Moore J N (Eds). Purdue University Press, West Lafayette, Indiana.

Iezzoni A F. 2008. Cheries. (*In*) *Temperate Fruit Crop Breeding*, pp 151–75. Hancock J F (Ed). Springer, USA.

Liu X, Reighard G, Swire-Clark G A and Baird W V. 2007. Peach rootstock identification by DNA-fingerprinting with microsatellite (SSR) markers. *J Amer Pomol Soc* **61**: 162–6.

Mendez E. 2005. Seed pregermination treatments and endocarp affects the percent germination and seedling height of *Prunus persica*. Department of Horticulture Science, Texas A&M University, Texas.

Monet R and Bassi D. 2008. Classical genetics and breeding. (*In*) *The Peach Botany, Production and Uses*, pp 61-84. Layne D R and Bassi D (Eds). CAB International, Wallingford (UK).

Okie W R and Hancock J F. 2008. Plums. (*In*) *Temperate Fruit Crop Breeding*, pp 337–57. Hancock J F (Ed). Springer, USA.

Paterson A H, Brubaker C L and Wendel J F 1993. A rapid method for extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP or PCR analysis. *Plant. Mol. Biol. Rep*. **11**: 122–7.

Rodriguez M, Monter A, Espinosa A, Castaneda C G and Velazquez A. 2005. Polyembryony and RAPD markers for identification of zygotic and nucellar seedlings in citrus. *Agrociencia* **39**: 371–83.

Ruiz C, Breto M P and Asins M J. 2000. A quick methodology to identify sexual seedlings in citrus breeding programs using SSR markers. *Euphytica* **112**: 89–94.

Sherman W B and Beckman T G. 2003. Climatic adaptation in fruit crops. *Acta. Hort* **622**: 411–28.

Souza V A B, Byrne D H and Taylor J F. 1998. Heritability, genetic and phenotypic correlations, and predicted selection response quantitative traits in peach. II. An analysis of several fruit traits. *J. Am. Soc. Hort.* Sci **123**: 604–11.

Topp B L, Sherman W B and Raseira C B. 2008. Low-chill cultivar development. (*In*) *The Peach: Botany, Production and Uses*, pp 106–138. Layne D R and Bassi D (Eds). CAB International.

Tukey H B and Carlson R F. 1945. Breaking the dormancy of peach seed by treatment with thiourea. *Plant. Physiol* **20**: 505–16.

Tusa N, Abbate L, Ferrante S, Lucretti S and Scarano M T. 2002. Identification of zygotic and nucellar seedlings in citrus interploid crosses by means of isozymes, flow cytometry and ISSR-PCR. *Cell. Mol. Biol. Lett* **7**: 703–8.

Wang L, Zhu G M and Fang W C. 2006. The evaluation criteria of some botanical quantitative charaters of peach genetic resources. *Agri. Sci* **5**: 905–10.