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Development and characterization of intraspecific hybrids of low chill peach [*Prunus persica* (L.) Batsch]

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Abstract

An experiment was conducted to broaden the range of early-ripening peach cultivars through hybridization. Low chill peach cultivars, specifically Shan-i-Punjab and Tropic Sweet, were selected as female parents and crossed with Florda Prince, Flordaglo, and Prabhat. Among the crosses, the highest fruit set (72.63%) was observed in Shan-i-Punjab x Florda Prince, while the lowest fruit set was recorded in Tropic Sweet × Flordaglo (18.87%). Despite the lower fruit set in Tropic Sweet crosses, they exhibited a significantly higher fruit retention percentage and a lower fruit drop rate than those involving the Shan-i-Punjab cultivar. Following ripening, seeds were extracted from the fruits and subjected to stratification at low temperatures until radicle emergence occurred. The stratification duration for the hybrid seedlings ranged from 76.00 days in Shan-i-Punjab × Florda Prince to 88.33 days in Tropic Sweet × Flordaglo. After sowing the seeds in the field, the highest seed germination percentage of 90.43 was recorded in Tropic Sweet × Florda Prince, which did not show a significant difference from Tropic Sweet × Flordaglo (88.94%), followed by Tropic Sweet × Prabhat (85.11%). Regarding seedling growth, Tropic Sweet × Flordaglo exhibited the maximum seedling height of 36.03 cm, while minimal variations were observed among different crosses regarding petiole length, leaf area, and internodal length.

Key words: Hybridization, morphological characterization, F_1 hybrids, peach [*Prunus persica* (L.) Batsch]

Introduction

Peach (*Prunus persica* L.) is a significant fruit crop belonging to the Rosaceae family, valued for its fresh and canned fruits. Originating in China, its cultivation dates back at least 4000 years. With successful breeding efforts, peach has adapted to various sub-tropical regions worldwide due to its wider climatic adaptability. It is a diploid plant with a relatively small genome of 5.9 × 108 bp or 0.61 pg in the diploid nucleus, with a haploid size of 300 Mb (Baird *et al.,* 1994). This genome size is approximately twice that of the Arabidopsis genome. Peach is globally recognized as an important fruit and is highly genetically characterized, making it a model species for the Rosaceae family (Monet and Bassi, 2008; Arus *et al.,* 2012).

In India, peach cultivation is limited to the warm temperate and sub-tropical regions of Jammu and Kashmir, Himachal Pradesh, Punjab, Haryana, Uttarakhand, parts of Uttar Pradesh, Tamil Nadu, and the North Eastern states. Peach occupies an area of 18,000 ha in India, producing 111,000 MT (NHB, 2021). China leads the world in peach production, accounting for approximately 58% of the total global production, followed by Italy, Spain, and the USA. The cultivation area for peach is expanding rapidly in the subtropical regions of northern India due to the availability of suitable cultivars and higher returns per unit area. Breeding efforts have led to the development of low-chilling peach cultivars, enabling their cultivation from temperate to sub-tropical regions worldwide.

Initially, peach breeding goals focused primarily on improving external fruit quality, post-harvest life, disease and pest resistance, and a greater range of fruit maturities and types (Byrne, 2005). However, as consumer standards have increased, there is now a growing emphasis on improving fruit eating quality, including nutritional composition. Numerous peach breeding programs are currently striving to enhance fruit quality and productivity within locally adapted germplasm (Monet and Bassi, 2008; Byrne *et al.,* 2012). Important tree and fruit quality parameters are often interconnected, as complex genetic and physiological factors govern them. Traits related to plant growth, architecture, yield, blooming, and harvesting time are typically influenced by multiple genes (Dirlewanger *et al.,* 1999). Fruit size, for example, is a polygenic trait with low to moderate heritability, influenced by environmental conditions, plant nutrition, and cultural practices (Souza *et al.,* 1998).

Significant advancements have been made in the genus Prunus over the past century, utilizing 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 breeding methods have resulted in the development and commercialization of highly productive, good quality, and resistant cultivars to both biotic and abiotic conditions. Therefore, the current breeding program aims to expand the range of early-ripening peach cultivars characterized by low chilling requirements, summer stratification, and controlled germination conditions to recover peach hybrid seedlings.

Materials and methods

The hybridization programme was started in 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 (♂) at Fruit

Research Farm, Department of Fruit Science, Punjab Agricultural University, Ludhiana. 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. The flower buds of the female parent were emasculated at balloon stage in the morning (9-11 am) and pollinated with camels hair brush during the day (11.30 am to 2.00 pm) on the same day. Emasculated flowers were pollinated either with fresh pollen (cultivars in which the flowering period coincided) or stored pollen (cultivars in which flowering periods did not coincide). The anthers from the male parents were collected at balloon stage and dehisced in a silica gel desiccator. The pollen was collected in 10 mL vials and stored at 5 °C till use. 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 until the maximum seeds showed radicle emergence. After that they were sown in pot trays and 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 started 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 and other leaf characters such as leaf area, shape, arrangements, margins and presence of nectaries. The experiment was laid out as randomized block design (RBD) with three replications. The data were analyzed using SAS v 9.0.0 software and means were compared using Duncan's Multiple Range Test (DMRT).

Table 1. The parentage of the cultivars used for hybridization

Cultivar	Parentage
Shan-i-Punjab	(Southland x Jewel) F_2 x June Gold
Florda Prince	Fla.2-7 x Fla. 13-72 (Maravilha)
Flordaglo	Sundowner nectarine x Maravilha peach
Prabhat	Sharbati x Flordasun
Tropic Sweet	Fla. 46-95 x Fla. A5-107 (Keygold nectarine)

Results and discussion

Maximum mean fruit set was observed in Shan-i-Punjab × Florda Prince (72.63 %), however the differences in fruit set was non significant in crosses Shan-i-Punjab × Florda Prince, Shan-i-Punjab × Flordaglo and Shan-i-Punjab × Prabhat (Fig.1). Minimum mean fruit set was recorded in Tropic Sweet × Flordaglo (18.87 %). It has been observed that those crosses where Shan-i-Punjab was taken as a female parent showed higher fruit set, whereas the crosses made with Tropic Sweet as a female exhibited less than 25 % fruit set during both the years. The low fruit set might be due to the temperature fluctuations; 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 (Fig. 1). 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 %).

Peach seeds require stratification (low chilling treatment) for

Fig. 1. Fruit set, fruit retention and fruit drop in crosses made between low chill peach cultivars

SP x F*P*=Shan-i-Punjab x Florda Prince, SP x FG=Shan-i-Punjab x Flordaglo, SP x *P*=Shan-i-Punjab x , Prabhat, TS x F*P*=Tropic Sweet x Florda Prince, TS x FG=Tropic Sweet x Flordaglo, TS x P =Tropic Sweet x Prabhat

germination. F_1 seeds of Tropic Sweet × Flordaglo took maximum days for stratification (88.33), whereas F_1 seeds obtained from 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 Prince (76.00) and data was recorded when the seeds kept for stratification showed 100 per cent radicle emergence (Fig. 2). Seeds from all the crosses has taken more than 75 days for radicle emergence (Fig 3). 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 obtained from 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

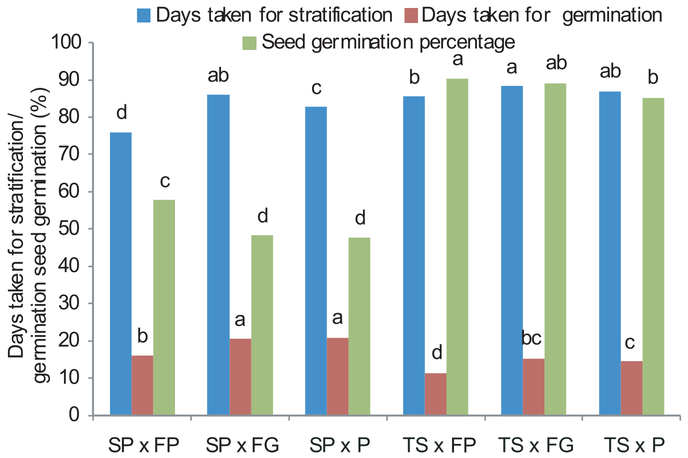

Fig. 2. Days taken for stratification, days taken for germination and germination percentage of hybrid seeds

SP x *FP*=Shan-i-Punjab x Florda Prince, SP x FG=Shan-i-Punjab x Flordaglo, SP x *P*=Shan-i-Punjab x , Prabhat, TS x *FP*=Tropic Sweet x Florda Prince, TS x FG=Tropic Sweet x Flordaglo, TS x P =Tropic Sweet x Prabhat

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 germinated in minimum days (11.33 days) and F_1 seeds of Shan-i-Punjab × Prabhat and Shan-i-Punjab × Flordaglo crosses took maximum mean days for 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. After 30 days of sowing total seed germination was observed and mean maximum seed germination 90.43% was recorded in F_1 seeds of Tropic Sweet × Florda Prince which was statistically at par with the seed germination of Tropic Sweet × Flordaglo (88.94 %) followed by Tropic Sweet × Prabhat (85.11%). F_1 seeds of Shan-i-Punjab × Florda Prince crosses 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 F_1 hybrids obtained from 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).

The seedling height, internodal length, leaf area, and petiole length of the hybrid seedlings were recorded after six months of growth (Table 2). The maximum seedling height of 36.03 cm was observed in the F_1 seeds of Tropic Sweet × Flordaglo, which did not show a significant difference from the seedling height of Tropic Sweet × Florda Prince and Tropic Sweet × Prabhat crosses (35.59 cm and 35.87 cm, respectively). The seedlings of Shan-i-Punjab × Flordaglo and Shan-i-Punjab × Prabhat crosses had the minimum mean seedling height of 27.00 cm and 27.20 cm, respectively. This variation in seedling height can be attributed to the different genotypes used in the crosses.

In terms of internodal length, the minimal difference was observed among the hybrid seedlings of all crosses, with the maximum observed in Tropic Sweet × Flordaglo and Tropic Sweet × Prabhat (1.60 cm). No significant difference was observed in the internodal length of Shan-i-Punjab × Florda Prince and Shan-i-Punjab × Flordaglo hybrid seedlings. The maximum leaf area was observed in Shan-i-Punjab × Florda Prince (20.16 cm²), which was statistically similar to the leaf area of Tropic Sweet × Prabhat (19.79 cm²), while the minimum leaf area was found in Shan-i-Punjab × Flordaglo and Shan-i-Punjab × Prabhat (18.35 cm² and 18.40 cm², respectively). Similar variations in leaf area were also reported by Ahmed Emad-Eldin *et al.* (2012) in their study on F_1 hybrid seedlings. Wang *et al.* (2006) mentioned that leaf characteristics, such as leaf area, petiole length, and petiole thickness, are genetically inherited and can vary among different varieties. There was very little variation observed among the hybrids for petiole length, with most crosses having a petiole length of 0.65 cm.

The leaf shape and leaf arrangement of all hybrids (Table 3) did not show any significant variation. All hybrids exhibited a consistent leaf shape, specifically lanceolate, and an alternate

Table 2. Seedling height, intermodal length, leaf area and petiole length of hybrid seedling

Parents	Seedling height	Internodal length	Leaf area	Petiole length
Shan-i-Punjab x Florda Prince	30.34b*	1.41c	20.16a*	0.65a*
Shan-i-Punjab x Flordaglo	27.00c	1.38c	18.35d	0.61ab
Shan-i-Punjab x Prabhat	27.20c	1.32d	18.40d	0.56b
Tropic Sweet x Florda Prince	35.59a	1.53b	18.78cd	0.65a
Tropic Sweet x Flordaglo	36.03a	1.60a	19.21bc	0.65a
Tropic Sweet x Prabhat	35.87a	1.60a	19.79ab	0.65a
LSD $_{0.05}$	0.76	0.03	0.60	0.06

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

Table 3. Leaf characters and presence of nectaries in hybrid seedlings

Parents/Crosses	Leaf shape	Leaf arrangement	Leaf margins	Nectaries
Shan-i-Punjab x Florda Prince	LAN*	ALT	SS	AB
Shan-i-Punjab x Flordaglo	LAN	ALT	SS	AB
Shan-i-Punjab x Prabhat	LAN	ALT	SS	AB
Tropic Sweet x Florda Prince	LAN	ALT	SDS	AB
Tropic Sweet x Flordaglo	LAN	ALT	SS	AB
Tropic Sweet x Prabhat	LAN	ALT	SS	AB

*LAN= Lanceolate, ALT= Alternate, AB= Absent, SS= Shallow Serrate, SDS=Shallow and Deep Serrate leaf arrangement. These results are consistent with the findings of Chalak *et al.* (2006), who reported a lanceolate leaf shape for all peach accessions. Similarly, no notable differences were observed in leaf margins among the hybrids. Shallow serrate leaf margins were observed in all hybrids, except for Tropic Sweet x Florda Prince, where both shallow and deep serrate leaf margins were observed. Furthermore, nectaries were absent in all hybrids.

The results of the present study indicate that using Shan-i-Punjab as the female parent in crosses results in higher fruit set and fruit drop rates, with relatively low fruit retention. In contrast, when Tropic Sweet is used as the female parent, crosses display significantly higher fruit retention and lower fruit drop percentages. Furthermore, Tropic Sweet crosses demonstrate a higher seed germination percentage of F1 seeds, likely due to their longer growth period compared to Shan-i-Punjab.

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