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Summer stratification and germination: A viable option for recovery of hybrid seedlings in low chill peach and nectarines

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ABSTRACT

***In-vitro* embryo rescue; and summer stratification and germination under controlled conditions was tested for recovering hybrid seedlings in crosses involving low chilling peach and nectarines. The embryos from all the cross combinations showed very high *in vitro* embryo germination (>85%) on basal MS medium, which can be effectively performed at hard mature or full ripe stage. The stratification period of the hybrid seed varied from 36.3 days in Shan-i-Punjab × Florda Prince to 44.7 days in Tropic Beauty × Florda Grand. At the end of four weeks of transfering the cultures to culture room, maximum plant height (33.7 mm) was recorded in Shan-i-Punjab × Tropic Beauty, which did not differ significantly from the cross Florda Crest × Tropic Beauty. The fruit development period of the seed parent varied from 78.3 days in Shan-i-Punjab × Tropic Beauty and Shan-i-Punjab × Florda Prince to 104.7 days in Tropic Beauty × Florda Grand. Stratification media of cocopeat + vermiculite + perlite (2:1:1) resulted in the highest seed germination but, the actual germination percentage varied with cross combinations. Highest germination (81.5%) was recorded in the cross FlordaGlo × Tropic Sweet, which did not differ significantly from Tropic Beauty × Florda Grand. It was followed by seed germination (68.0%) in Florda Grand × Tropic Beauty. Under high density nursery system, the hybrid seedlings of FlordaGlo × Tropic Sweet showed highest growth (160 cm) and branches (13). The germination of hybrid seeds was positively correlated (0.86) with fruit development period of the seed parent (FDP) and negatively correlated (-0.85) with chilling requirement of seed parent. The proportion of rosseted seedlings was negatively correlated with FDP (-0.61) and positively correlated (0.39) with chilling requirement of the seed parent. The cross combinations with higher FDP of seed parent resulted in higher seed germination of hybrid seed. Hence, controlled climate stratification and germination after harvest can be a viable option for recovery of hybrid seedlings and reducing the breeding cycle in crosses involving seed parents with higher FDP.**

Key words: Hybridization, *in vitro* embryo rescue, *Prunus.*

INTRODUCTION

In India, peach and nectarines are grown in Uttarakhand, Himachal Pradesh, Jammu and Kashmir, Punjab, Sikkim, Tamil Nadu and Mizoram over an area of 18,000 ha with an annual production of 1,07,000 tonnes (NHB, 9). The introduction of low chill, early maturing and better quality peach and nectarine cultivars from USA by Punjab Agricultural University, Ludhiana during 1968 to 2001 played a significant role in the spread of peach cultivation in subtropical regions of India (Singh *et al*., 11). The restriction on exchange of improved varieties due to patent laws and commercial interests of private players of the western countries is hampering the growth of peach industry in India. Local peach and nectarine breeding programme will help in creating a huge lot of locally adaptable genotypes for rigorous selection. Apart from low chilling requirement, better skin colour, flesh colour, firmness, consistency, sugar: acid ratio and storage life are the major breeding targets (Byrne *et al*., 3). The present challenge for peach industry is to increase the fruit consumption, which mainly relies on enhancing taste and nutritional quality (Desnoues *et al*., 5). Of late, higher sugar content is the primary objective of new peach breeding programmes in the western countries to revert the decreasing consumer demand for peach (Cirill *et al*., 4). Besides, low chilling and early ripening trait in peach is an important objective in peach fruit breeding programmes due to higher returns from these varieties (Anderson and Byrne, 1). Crosses involving early ripening parents can lead to higher proportion of progeny with early ripening trait. However, hybrid seeds from these parents germinated poorly due to their immature embryos or embryo abortion when fruits are ripe. Embryo culture technique has been used with great success with early ripening peach and nectarine (Byrne *et al*., 3). Peach embryos rescued at 75 days after hybridization resulted in maximum germination when stratified at 4ºC for 45 days (Sundouri *et al*., 12). Embryo rescue is a valuable tool for the development of early ripening cultivars, however, the use of traditional stratification-germination is preferable wherever possible (Bacon and Byrne, 2). Further,

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in subtropics, sometimes there is high mortality of embryo rescued seedlings as the hardening time coincides with the hot weather. Stratifying the hybrid seeds immediately after harvest during summers under controlled conditions can be viable option for higher recovery of hybrids and shortening the breeding cycle. Hence, the present studies were conducted to study the efficacy of embryo rescue; and summer stratification and germination under controlled conditions for recovery of peach and nectarine hybrid seedlings involving early maturity and low chill seed parents.

MATERIALS AND METHODS

Controlled pollinations were performed among different peach and nectarine varieties in the Fruit Research Farm of Department of Fruit Science, Punjab Agricultural University, Ludhiana during February, 2014. The treatments consisted of the crosses as described in Table 1 and 2. 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 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. The *in vitro* embryo rescue was used to rescue the seedlings and the results are presented in Table 1. In the embryo rescue experiments, the fruits were harvested at firm mature stage and ripe stage when the fruit just start softening from the distal end. The fruits were surfaced sterilized with mercuric chloride (0.1%) for 15 min. The fruit were then cracked open under a laminar flow under aseptic conditions with a double blade secateur (Kuker Art 72). The seed coat was peeled off and the embryo (8-10 mm) was placed on basal MS medium (Murashige and Skoog, 8) in test tubes with 20 ml medium. The test tubes containing cultured embryos were stratified at 3-4°C in dark conditions till >75% radicle emergence and the stratification period for each cross were recorded. After this the test tubes were transferred to a culture room with 25 ± 2°C temperature and 16 hour photoperiod for four weeks. The experiment was laid out as completely randomized design and the data regarding the embryo germination (%), plant height, root length, number of root hairs, and rosseting were recorded after 4 week. The experiment was laid as completely randomized design with three replications.

In view of the higher cost and *ex vitro* mortality; and poor recovery of hybrid seedlings with *in vitro* embryo rescue during the past years, summer stratification and germination under controlled conditions was also tested. In case of summer stratification and germination

Table 1. Germination, growth and stratification period of *in vitro* embryos rescued seedlings of low chill peach and nectarine genotypes.

Parentage	Embryo germination (%)		Plant height (mm)	Internodal length (mm)	Root length (mm)	Root hairs (No.)	Rosseting (%)	Stratification period (days)	Fruit developmental period (days)	Chilling hours of seed parent (CU)	Seedling growth period (days)
	Fruit at ripe stage	Fruit at hard mature stage									
Florda Grand × Tropic Beauty	91.67^{a} (9.57)	89.0^{a} (9.43)	24.7^{c}	6.2^{c}	25.0^{a}	20.3^{a}	7.7	40.7^{ab}	96.7^{b}	100	6.0
Shan-i-Punjab × Florda Prince	85.33^{c} (9.24)	87.3^{ab} (9.35)	29.0^{b}	8.8^{abc}	15.3^{c}	11.0^{c}	6.0	36.3^{b}	78.3^{e}	300	6.0
Shan-i-Punjab × Tropic Beauty	86.67^{bc} (9.31)	85.0^{bc} (9.22)	33.7^{a}	9.4^{ab}	27.0^{a}	20.3^{a}	6.4	40.3^{ab}	78.3^{e}	300	5.3
Florda Crest × Tropic Beauty	89.00^{ab} (9.43)	86.0^{b} (9.11)	32.3^{a}	9.9^{a}	19.7^{b}	18.7^{a}	7.3	40.3^{ab}	90.0^{c}	350	5.0
Tropic Beauty × Florda Grand	87.33^{bc} (9.35)	83.0^{c} (9.11)	24.3^{c}	6.8^{bc}	20.0^{b}	17.3^{ab}	7.3	44.7^{a}	104.7^{a}	150	5.3
Suncoast × Sun Rise	89.33^{ab} (9.45)	87.0^{ab} (9.33)	23.7^{c}	7.3^{abc}	18.0^{bc}	14.7^{b}	6.0	42.7^{a}	80.7^{d}	375	5.7
$LSD_{0.05}$	(0.17)	(0.13)	2.87	2.71	3.46	3.45	NS	5.48	1.95	-	NS

Means within a column followed by similar letter do not differ significantly (p≤0.05).Values in parenthesis are square root transformed values.

Table 2. Seed germination, seedling growth and rosseting in hybrid seedlings of peach and nectarine genotypes recovered by summer stratification.

Parentage	Germination (%)	Height (cm)	Branches (No.)	Rosseting (%)	Fruit developmental period	Chilling units of seed parent (cu)
Sun Coast × Punjab Nectarine	37.7^{c} (6.13)	130.0^{b}	8.7^{c}	5.6	95.7bc	375
Tropic Beauty × Florda Grand	80.3^{a} (8.96)	116.0^{b}	9.0^{b}	0.0	104.3a	150
Florda Grand × Tropic Beauty	68.0^{b} (8.23)	115.6^{b}	8.7^{b}	0.0	96.3c	100
Punjab Nectarine × Suncoast	43.1^{c} (6.58)	115.0^{b}	7.0^{b}	0.0	92.3	325
FlordaGlo × Tropic Sweet	81.5^{a} (9.02)	160.0^{a}	13.0^{a}	7.3	103.7a	150
$LSD_{0.05}$	(0.52)	16.41	2.37	NS	3.81	-

Means within a column followed by similar letter do not differ significantly (p≤0.05). Values in parenthesis are square root transformed values.

of seed under controlled conditions the fruits were collected at full maturity during second fortnight of May stored at 5°C up to two days till the seed is extracted. The fruit development period of the seed parent was also recorded from fruit set. The extracted seeds were washed properly and divided into lots of 30 seeds. The seeds were stratified in different media, *viz*. cocopeat + perlite (2:1), cocopeat + vermiculite (2:1), cocopeat + vermiculite + perlite (2:1:1) and sand. The stratification media were sprinkled with carbendazim (Bavistin®) suspension @ 1 g/l to the moist media. The seed lots were stored in the different stratification media at 4±2°C for stratification till 25-30% radicle emergence is seen in the seeds (10 weeks). After stratification the seeds were sown in solarized medium having soil, farm yard manure and cocopeat (2:1:1) in root trainers with 300 cc cells and maintained under a plant growth chamber with 25±2°C day and 21±2°C night temperature. The data regarding the germination percentage and rossetting percentage was recorded 45 days after sowing. The data regarding the hybrid seedling growth and branch number was recorded 11 months after transferring in the hybrid seedlings in field under high density nursery system (HDN) at 0.3 m × 1.0 m. The experiment was laid as completely randomized design with three replications whereas under HDN system the seedlings were planted following randomized block design. The data regarding the FDP was analysed as per randomized block design. The data was subjected to analysis using statistical software SAS (V 9.3 SAS Institute Inc, USA). When the interactions among treatments were significant ($p \leq 0.05$) mean separations were done by least significant difference (LSD).

RESULTS AND DISCUSSION

In general, higher *in vitro* embryo germination was recorded in embryos rescued from fruits at ripe stage as compared to hard mature maturity stage of fruit (Table 1). The advancement in the development stage might have led to greater embryo maturity, which might have led to better embryo germination with embryos harvested at ripe stage. However, culturing the embryos at full ripe stage resulted in very high microbial contamination as the fruit become fully soft and juicy. Developmental stage for successful embryo culture varies with variety and climatic conditions where the trees are grown. Hence, successful embryo culture at one location may not conform in another place (Scorza and Sherman, 10). Infanter and González (6) concluded that the embryo survival was higher in peach when harvested from the most advanced mature fruits. Further, irrespective of the stage of the embryo germination more than 80 per cent embryo germination was recorded in all the cross combinations. At the end of four weeks of transferring the cultures to culture room, tallest plants (33.7 mm) were recorded in Shan-i-Punjab × Tropic Beauty, which did not differ significantly from plant height in cross Florda Crest × Tropic Beauty. The highest internodal length (9.9 mm) was recorded in Florda Crest × Tropic Beauty, which was at par with Shan-i-Punjab × Tropic Beauty. Similarly, the highest root length (27.0 mm) and root hairs (20.3) was recorded in Shan-i-Punjab × Tropic Beauty. In all the cross combinations, the plant height more than 20 mm, was ideal for handling of the plant material. Rosseting was also observed in the hybrid seedlings (Fig. 2) and it ranged from 6.0 per cent in seedlings from Suncoast × Sun Rise and Shan-i-Punjab × Florda Prince to 7.7 per cent in open-pollinated Florda Grand × Tropic Beauty. Jeengool and Boonprakob (7) studied the effect of plant growth regulators on *in vitro* embryo germination and most embryos germinated well (>85%) with or without plant growth regulators, *viz*. benzyl adenine and gibberellic acid. The stratification period of the hybrid seed varied from 36.3 days in Shan-i-Punjab × Florda Prince to 44.7 days in Tropic

Beauty × Florda Grand. The fruit development period of the seed parent varied from 78.3 days in Shan-i-Punjab × Tropic Beauty and Shan-i-Punjab × Florda Prince to 104.7 days in Tropic Beauty × Florda Grand. The *ex vitro* survival of *in vitro* embryo rescued hybrid seedlings was around 20 per cent due to very high atmospheric temperatures during their transplanting in July. Even under plant growth chamber the high humidity resulted in root rot and high mortality.

In summer stratification and germination experiment under controlled conditions, the seed germination varied with the genotype combination (Table 2). Highest germination (81.5%) was recorded in Florda Glo × Tropic Sweet, which did not differ significantly from seed germination in Tropic Beauty × Florda Grand. It was followed by seed germination (68.0%) in Florda Grand × Tropic Beauty. The lowest germination (37.7%) was recorded in Sun Coast × Punjab Nectarine, which did not differ significantly from seed germination in Punjab Nectarine × Suncoast. Higher seed germination and seedling growth was recorded from crosses in which the seed parent had lower chilling requirement and higher FDP. The highly significant positive correlation (0.86) between FDP and germination per cent indicate that crosses involving seed parent with higher FDP resulted in higher seed germination. Further, seed germination also showed a negative correlation with chilling period. Seed viability was poor in early ripening peach genotypes and *in vitro* embryo culture is needed to ensure germination (Byrne *et al.*, 3). In the present studies, higher germination was obtained in the hybrid seed from crosses involving. The seedlings from Florda Glo × Tropic Sweet showed highest plant height (160.0 cm) and number of branches (13) after 11 months of transplanting under high density nursery system at 0.3 × 1.0 m (Table 2), whereas, the seedling height in all the other cross combinations did not differ significantly. The stratification media significantly affected the hybrid seed germination in different cross combinations. Irrespective of the parentage, stratification medium cocopeat + vermiculite + perlite (2:1:1) resulted in highest seed germination during stratification, while, minimum seed germination was recorded with sand (Fig. 1). Highest water holding capacity (Fig. 1), better aeration and other physical properties of the media might have affected the seed germination. The correlation studies showed high positive correlation (0.86) between hybrid seed germination and fruit development period of the seed parent, which was also statistically significant (Table 3). The hybrid seed germination and chilling requirement of seed parent were negatively correlated (-0.85). Fruit developmental period of the seed parent was also negatively correlated (-0.61) with the proportion of rosseted seedlings. Hence, seed stratification and germination under controlled conditions after fruit harvest during summers is a viable option for higher recovery of hybrid seedlings over embryo rescue in low chilling peach and nectarine genotypes with longer developmental period. This can save around 7-8 months of a breeding cycle in a year.

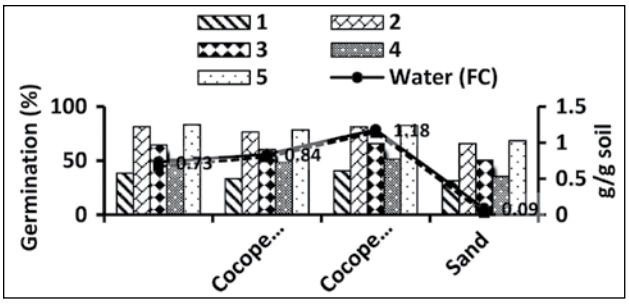

Fig. 1. Effect of stratification media on germination (%) of hybrid seeds and water holding capacity at field capacity (FC) and permanent wilting point (PWP). 1: Sun coast × Punjab Nectarine; 2: Tropic Beauty × Florda Grand; 3: Florda Grand × Tropic Beauty; 4: Punjab Nectarine × Suncoast; 5: FlordaGlo × Tropic Sweet.

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Table 3. Correlation between various variables.

Parameter	Fruit developmental period	Chilling unit of seed parent	Rosseting
Seed germination	0.86**	-0.85**	-0.19
Fruit developmental period	1.00	-0.69	-0.61**
Chilling of seed parent	-0.69	1.00	0.39*

*Significant P≤0.05; **Significant P≤0.01

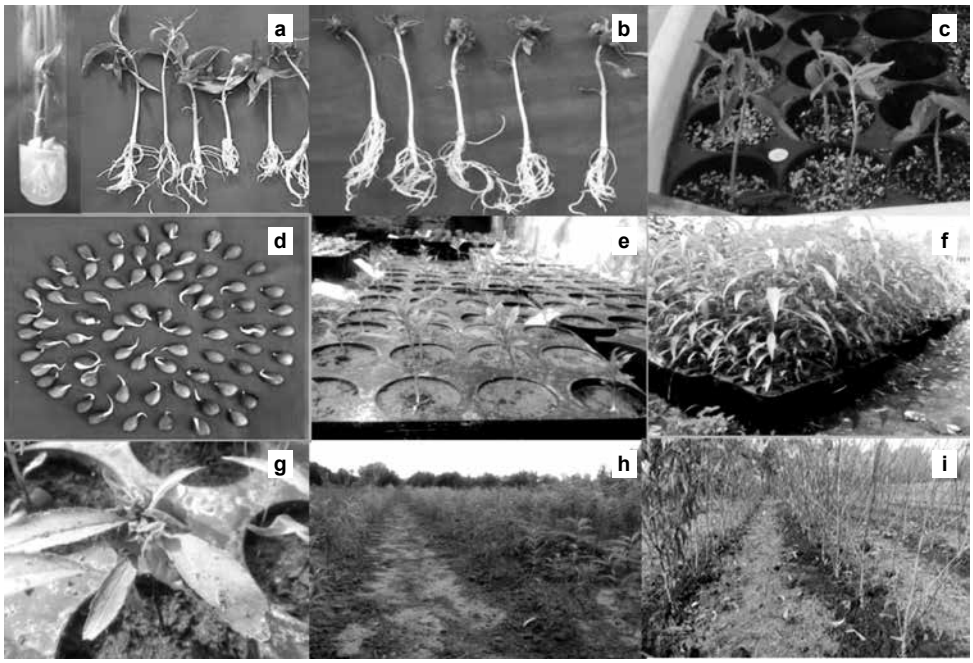

Fig. 2. Different stages in the development hybrid seedlings (A) *In vitro* embryo rescued seedlings, (B) *In vitro* rosseting, (C) Hardening *in vitro* seedlings, (D) Summer stratified seeds of Flordaglo × Tropic Sweet in cocopeat + vermiculite + pertile (2:1:1). (E, F) Seedlings of Flordaglo × Tropic Sweet at 30 and 90 DAS (G). *Ex vitro* rosseting. (H,I) HDN at planting (7-month-old seedlings) and after 17 months (1st winter).

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