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In-vitro germination of hybrid embryo rescued from low chill peaches as affected by stratification period and embryo age

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ABSTRACT

***In-vitro* embryo rescue is necessary to recover viable hybrid seeds from low chill peach hybrids, which is an important objective in many stone fruit breeding programmes. The effect of stratification on embryo germination in different parents (SP and FP, SP and P, SP and EG) and their hybrids (H-1 and H-2, H-3 and H-4, H-5 and H-6) indicated that parent show highly germination than their hybrids. The maximum embryo germination in all parents and their respective hybrids were observed when the embryos were stratified for 45 days at 4ºC. The highest embryo germination was recorded in parent Partap (78.00%) and hybrid H-3 (Shan-i-Punjab × Partap). The maximum germination (52.56%) was observed after 75 days of crossing, followed by 41.67% after 60 and least 12.45% after 45 days of crossing, while in hybrids, highest germination (40.75%) was recorded in H-2 and least in H-3 (29.5%). The study concluded that embryo rescued at 75 days after crossing gave maximum germination in rescued embryos when stratified at 4ºC for 45 days.**

Key words: Peach, embryo rescue, stratification, hybrids.

INTRODUCTION

One of the main goals of peach [*Prunus persica* (L.) Batsch.] breeding programme is widening the number of the early ripening and low chilling cultivars. In order to reach this objective crosses between early ripening cultivars were carried out. It is well known that seeds from early ripening cultivars do not germinate because of the immaturity of the zygotic embryo. For this reason, it is necessary to use *in vitro* embryo rescue technique. This technique has been applied since long time by carrying out the modification of already developed protocols of *in vitro* regeneration to match genotypes. Peach plantlets were regenerated by callus tissue derived from immature zygotic embryos (Hammerschlag *et al.*, 7), endosperm (Meng and Zhou, 12), cotyledon (Pooler and Scorza, 13) and leaf disc (Damiani and Palombi, 3). Of these, embryo culture is used in the sub-tropical peach breeding programme for germination of seed from parents with short fruit development periods (Hamill *et al.*, 6) and had been extensively used to rescue embryos from early maturing cultivars where the flesh matures before the seed and conventional germination methods provide low recoveries (Topp *et al.*, 18).

The early ripening trait in peach is genetically controlled and is an important objective in many stone fruit breeding programmes. Crosses between both early ripening parents could generate greater proportion of progeny with trait resulting in better chance of making new selections. However, hybrid seeds from these parents germinated poorly due to their immature embryos or embryo abortion when fruits are ripe (Tukey, 19). Therefore, an attempt was made to standardized the stratification period and age of embryo for developing hybrid cultivars through *in vitro* embryo rescue technique.

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MATERIALS AND METHODS

The present research was carried out at New Orchard and Tissue Culture Laboratory, Department of Fruit Science, Punjab Agricultural University, Ludhiana. The treatment consists of following crosses, *viz.*, Shan-i-Punjab (♀) × Flordaprince (♂), Shan-i-Punjab (♀) × Partap (♂), Shan-i-Punjab (♀) × Earligrande (♂), Flordaprince (♀) × Shan-i-Punjab (♂), Partap (♀) × Shan-i-Punjab (♂) and Earligrande (♀) × Shan-i-Punjab (♂). Pollination of the emasculated flower buds was done on the same day either with fresh pollen (in case of cultivars were flowering period coincided) or with stored pollen (in case of cultivars where flowering periods did not coincided). The pollens were applied to stigmas with camel hair brush and bagged. Each flower was labeled and bagged to avoid any contamination and bags were removed after fruit set. Fruits of crossed peach were collected after 45, 60 and 75 days after crossing (DAC). The excised embryos were cultured on the basal MS medium. After inoculating the embryo in culture medium, the culture vessels were kept at 5ºC in the dark chamber for 45, 60 and 75 days for stratification. Data were recorded for sprouting percentage before transferring to incubation room. The culture vessels

then transferred after receiving 45, 60 and 75 days of stratification to incubation room. The media were replaced before keeping for incubation. The culture vessels were incubated at 25 ± 2°C with 16 h of continuous fluorescent light (5,000 lux) followed by a dark period of 8 h. The incubation temperature and photoperiod were similar in all the experiments. The data were recorded on the embryo germination.

The treatments were distributed according to a complete randomized design (CRD) with three replicates. Data were analyzed for variance by using the SAS (V 9.3, SAS Institute Inc., USA) package. When interaction between treatments were significant (P ≤ 0.05), the effect of each treatment was determined separating the means by least significant difference (LSD).

RESULTS AND DISCUSSION

The effect of stratification on embryo sprouting in different parents (SP and FP, SP and P, SP and EG) and their respective hybrids (H-1 and H-2, H-3 and H-4, H-5 and H-6) indicate that the same trend was observed for embryo sprouting (Figs. 1, 2 & 3) but parents show higher sprouting percentage than their hybrids. The maximum embryo sprouting in all parents and their respective hybrids was observed when the embryos were stratified for 45 days at 4ºC. The embryo sprouting percentage was significantly reduced at lower as well as higher period of stratification in all parents and their respective hybrids. This indicates that all parents and hybrids show the response to different periods of cold stratification.

The highest embryo sprouting was recorded in Partap (78.00%) which was followed Shan-i-Punjab (77.00%), whereas least in Flordaprince (71.00%) and Earligrande (71.00%) with 45 days of stratification. In case of hybrid embryos, sprouting

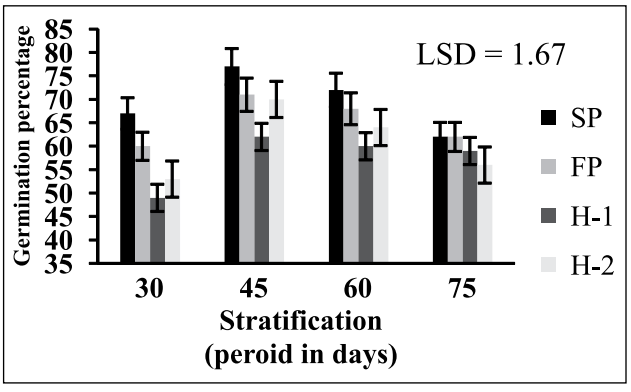

Fig. 1. Effect of stratification on the embryo germination in parents and their hybrids. Vertical bars represents ± S.E.

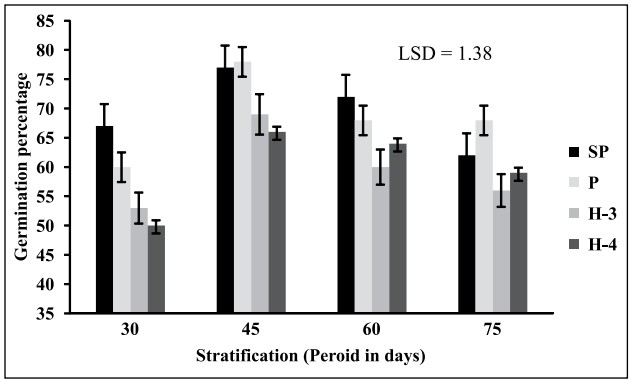

Fig. 2. Effect of stratification on the embryo germination in parents and their hybrids. Vertical bars represents ± SE.

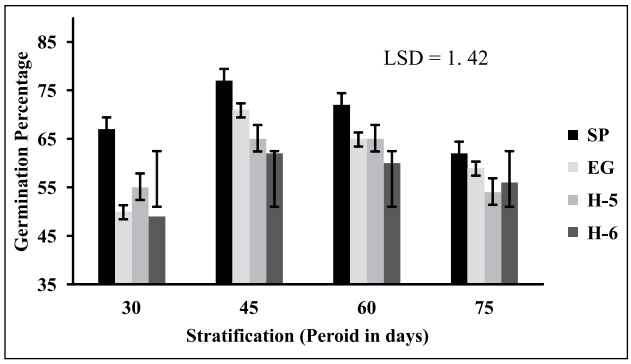

Fig. 3. Effect of stratification on the embryo germination in parents and their hybrids. Vertical bars represents ± SE.

was highest in H-3 (Shan-i-Punjab × Partap), followed by H-2 (Flordaprince × Shan-i-Punjab), H-3 (Shan-i-Punjab × Partap), H-4 (Partap × Shan-i-Punjab), (H-6 (Earligrande × Shan-i-Punjab), H-1 (Shan-i-Punjab × Flordaprince) and H-5 (Shan-i-Punjab × Earligrande) which recorded 70.00, 69.00, 66.00, 65.00, 62.00 and 62.00%, respectively with 45 days of stratification period at 4ºC. The least embryo sprouting was noticed for 30 days of stratification period in parent genotype Earligrande (50.00%), followed by Partap (60.00%), Flordaprince (61.00%) and Shan-i-Punjab (67.00%), while in hybrids it was 47.00, 49.00, 50.00, 53.00, 54.00 and 56.00%, in hybrids, H-6, H-1, H-4, H-2, H-3 and H-5 at 4ºC, respectively.

The stratification temperature played a critical role in germination of peach embryos. Higher number of germinated plants were recorded at 4ºC, although the effect of low temperature has been shown to be genotype-dependent. The mechanism of dormancy is present in the seeds of *Prunus* species (Garcıa-Gusano *et al.*, 5). The dormancy period

was required, even for germination of immature embryos of *Prunus domestica* and to avoid rosette-type plants (Kukharchyk and Kastrickaya, 9). The results observed in the study are in accordance with others on several *Prunus* species. Results different temperatures used for seed stratification, range from 0.5ºC (Emershad and Ramming, 4) to 5ºC (Anderson and Byrne, 1). Thus, stratification showed its consequence both for *in vitro* culture and conventional methods. The results are in line with findings of Sharma and Singh (15) who reported that the stratification had a significant influence on seed germination which was enhanced with increase in stratification periods from 15-75 days. Hence, it is concluded that seed germination increased at a faster rate from 45 to 60 days of stratification.

In the present study, chilling period of the embryo was genotype-specific and chilling depends on the presence of short and long term dormancy that affects the germination rate and seedling development. The short periods of chilling can improve germination by forcing to stratification (Lieda *et al.*, 10). Also, Sharma and Singh (16) found that the rate of increase in germination was more rapid at 10°C than at 7°C upto 75 days. Both free and bound ABA-like substances were very high in dormant seed tissues. However, during stratification at low temperature, *i.e.*, 10° and 7°C these declined either to zero or non-measurable quantity. Decline in ABA-like inhibitor substance was overcome by low temperature treatment only.

The data pertaining to germination and seedling growth of the excised embryos is shown in Fig. 4, whereas parents and those of their hybrids in Figs. 5 & 6. The maximum germination (52.56%) was observed after 75 days of crossing, followed by 41.67% after 60 and least 12.45% after 45 days of crossing. The embryos showed least germination after 45 days of crossing seemed them to be very immature. The result can be explained on the basis of the study conducted by Hesse and Kester (8) who developed a growth index designated as PF_1 (embryo length/seed length) to measure the comparative embryo development of the different peach cultivars and found that embryos with PF_1 of less than 0.7 were the least developed and could not be successfully germinate and cultured. However, germination and seedling growth occurred after 60 and 75 days of crossing irrespective of stratification period. In case of parents, maximum germination (51.37%) was recorded in the Shan-i-Punjab, followed by Partap (48.12%), Flordaprince (37.68%) and least in Earligrande (32.87%). While in hybrids, highest germination (40.75%) was recorded in H-2, followed by H-4 (35.75%), H-3 (37.5%) and least in H-3 (29.5%). The maximum germination and seedling growth was obtained when embryos extracted from firm unripe fruits and can be correlated with the increased embryo size and growth at 60 and 75 days after full bloom. Excising embryos at consecutive intervals throughout the season

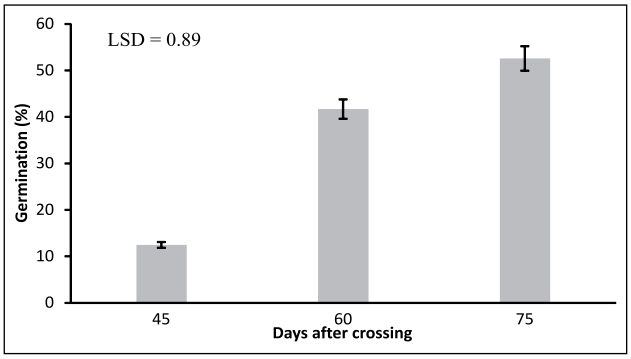

Fig. 4. Effect of days after crossing on germination of peach embryos. Vertical bars represents ± SE.

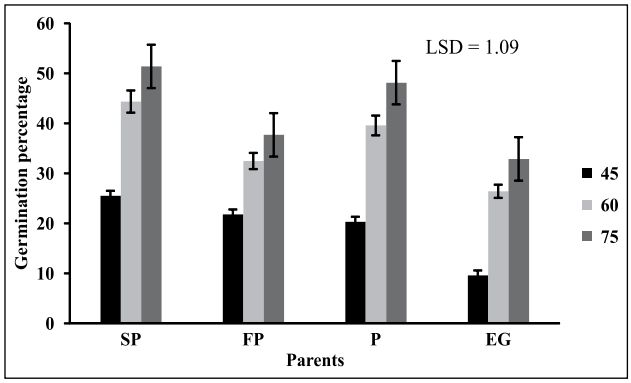

Fig. 5. Response of parents on the embryo germination after DAC. Vertical bars represents ±SE.

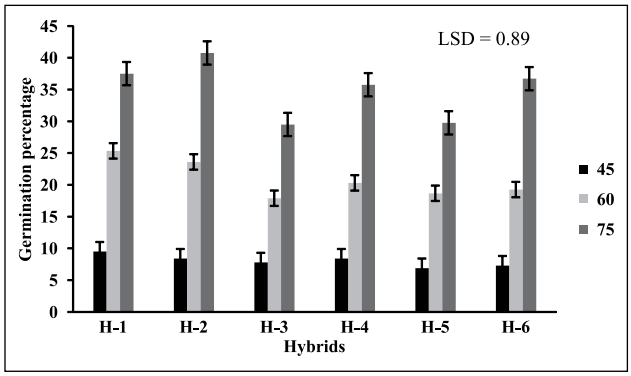

Fig. 6. Response of hybrids on the embryo germination after DAC. Vertical bars represents ± SE.

had shown the gradual acquisition of the ability to germinate in a single peach genotype.

The germination rate was strongly affected by embryo age and size. The main factor limiting the successful embryo cultures was the age of the embryo at the time of transfer onto the nutrient medium (Bridgen, 2). The embryo size depends on the embryo developmental stage and many efforts have been devoted in *Prunus* to find the optimum age at which germination will be successful (Liu *et al.*, 11; Sinclair and Byrne, 17). There is a critical size under which direct embryo germination is not possible, and thus the entire ovule culture should be given time to allow the small embryos to reach the minimum size needed for germination (Sharma *et al.*, 14), although this size differs among species and conditions. In the present study, an embryo size of 65% of the final seed size (5 mm in length) had high germination rate (55%). Below this size (30% of the final seed size) had a 40% rate of germination, and at even smaller sizes (2 mm or less), a 10% germination rate was achieved, thus allowing for germination of a relatively high percentage of seeds to occur. However, in other *Prunus* species, a similar decrease has been seen with correlation between germination and seed age and size. In *Prunus avium* hybrids, when embryo size was one-third of the seed size, the germination rate was only 40% (Kukharchyk and Kastrickaya, 9). Similarly, in *Prunus persica* hybrids, a critical size of 50% of seed size was stated to be required for germination (Liu *et al.*, 11).

From the present study, it was concluded that embryo rescued at 75 days after crossing gave the maximum germination in peach when stratified at 4ºC for 45 days.

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