Effect of developmental stage and medium on embryo culture of low chill peach hybrids

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The main objective of the present programme was to widen the varietal range of early ripening peach cultivars. Crosses were made between Shan-i-Punjab × Florda Prince, Shan-i-Punjab × Flordaglo and Shan-i-Punjab × Prabhat. The embryos of these crosses were rescued after 65, 75 and 85 days of crossing and cultured in MS basal medium supplemented with varying concentration of BAP (0 to 2 mg/l) and IBA (0 to 1 mg/l). After stratification at 4°C, the embryo cultured tubes were transferred to a growth chamber at 24 ± 2°C for germination. Seeds harvested at 85 days after crossing showed maximum embryo germination (75.26%). Among these crosses, hybrid-3 (Shan-i-Punjab × Prabhat) showed maximum germination (81.66%) in M2 medium (MS medium + BAP 0.25 mg/l + IBA 0.05 mg/l) when rescued after 85 days of pollination. Embryos harvested at fully matured stage (85 days after pollination) took minimum days to germinate.

Keywords: Embryo germination, growth stages, low chill peach hybrids, media.

Peach (*Prunus persica* L.) is an important fruit crop of the Rosaceae family and valued for its fresh and canned fruits. It is native to China where its culture dates back at least 4000 years. In India, it is grown in the mid-hill zone of the Himalayas extending from Jammu and Kashmir to North Eastern states at an altitude of 1000–2000 m above mean sea level (amsl). It has wide climatic adaptability and is now successfully cultivated in various sub-tropical regions of the world with the breeding efforts for low chill peach cultivars[1]. An early ripening peach variety has advantages of marketing window and good profit. The early ripening trait is genetically controlled and is an important breeding objective in many stone fruit breeding programmes[2]. Poor germination of seeds of early-maturing peach varieties is a major obstacle in the development of early ripening peaches because of immaturity of the zygotic embryo in hybrid seeds[3]. Thus, embryo culture technique is necessary to recover hybrid seeds. The first successful embryo culture of fruit trees on a synthetic medium was achieved by Tukey[4] in cherry. Since then, much work has been conducted to optimize embryo rescue technique in several crops. Blake[5] was the first to employ embryo culture in a peach breeding programme.

Embryo culture is an *in vitro* technique used to save the hybrid product of fertilization when they might otherwise degenerate. It involves isolating and growing an immature or mature zygotic embryo under sterile conditions on an aseptic nutrient medium. This technique is useful when there is poor embryo development or abortion. Embryo abortion occurs in early ripening genotypes of *Prunus* where the flesh matures before seed maturity. Emerging seeds of early maturing fruits have low germination percentage attributed to reduced embryo development. Embryo culture can also shorten the breeding cycle by overcoming dormancy in seeds. Embryo culture is important in breeding programmes where extreme early ripening is a major goal. Breeding programmes in fruit trees are usually based on the introgression of genes via inter-specific distant crosses. These hybridizations produce aborted seeds because their development is arrested at an early stage therefore, it is difficult for seeds to germinate with conventional methods. Low viability seeds also occur either when embryos come from early maturing fruits where seeds do not reach mature stage or when embryos come from stenospermic seedless fruits, such as in grape varieties. In all these cases embryo rescue has been successfully used to overcome the low viability of these seeds[6]. Successful embryo rescue depends on many factors such as genotype, developmental stage of embryo, basic composition of culture media, growth regulators and culture conditions. Angelo *et al.*[7] obtained 76% plants from interspecific hybrids of *Elaeis guineensis* × *E. oleifera* using embryo culture. Uma *et al.*[8] cultured zygotic embryos of banana at different maturity stages to determine the best maturity stage for embryo rescue, and found that fully matured embryos regenerated directly into plantlets without producing callus whereas, immature embryos required medium supplemented with plant growth regulators (PGRs) for successful regeneration. Eighty-eight per cent survival in hybrid banana was achieved. In mango hybrids more than 80% germination was obtained through embryo culture[9]. Embryos of some fruits require a cold treatment of 4°C to break dormancy. The growth of sweet cherry embryos was possible only when immature and mature embryos were treated at 4°C cold treatment for 40 days and 60 days respectively[10]. The optimum temperature for growth of embryos depends on plant species. Plant growth regulators play an important role in embryo culture and enhance the embryo growth[11,12]. Liu *et al.*[13] successfully cultured the embryos of peach (*Prunus persica*), apricot (*P. armeniaca*) and plum (*Prunus salicina*) in modified MS medium supplemented with 1.0 mg/l 6-BA and 1.0 mg/l IBA. Hence, it was hypothesized that the growth stages and medium will affect embryo germination of low chill peach hybrids. The present investigation was conducted to determine the best development stage for embryo rescue and to study

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Table 1. Response of different growth stages and MS medium supplemented with growth regulators on embryo germination percentage (%) of low chill Peach hybrids

Treatments	65 DAP			75 DAP			85 DAP			
MS + Growth regulators (mg/l)	Hybrid-1	Hybrid-2	Hybrid-3	Hybrid-1	Hybrid-2	Hybrid-3	Hybrid-1	Hybrid-2	Hybrid-3	Mean
M_0: Control	68.33	65.33	71.00	73.33	72.40	75.00	76.06	76.06	78.33	72.87[b]
M_1: BAP (0.125) + IBA (0.01)	70.00	65.66	71.66	73.40	73.00	76.66	76.00	74.10	80.66	73.46[ab]
M_2: BAP (0.25) + IBA (0.05)	71.66	68.73	73.33	75.00	74.50	78.33	78.66	76.00	81.66	75.31[a]
M_3: BAP (0.50) + IBA (0.1)	70.66	63.83	75.00	74.21	73.33	76.20	75.00	75.00	78.33	73.50[b]
M_4: BAP (1) + IBA (0.25)	66.23	62.00	66.00	70.16	71.00	73.50	74.26	73.33	78.00	70.49[c]
M_5: BAP (1.5) + IBA (0.50)	61.60	60.26	61.73	66.90	67.00	70.16	72.48	70.26	73.86	67.13[d]
M_6: BAP (2) + IBA (1)	60.66	60.00	61.06	66.83	66.66	71.66	69.00	70.00	73.46	66.59[d]
Days mean		66.62[c]			72.34[b]			75.26[a]		
Hybrids mean		Hybrid-1			71.02[a]		$LSD_{0.05}$: Trt – 1.66			
		Hybrid-2			69.45[c]		Crosses – 1.09			
		Hybrid-3			73.76[a]		Days – 1.09			
							Trt × crosses – 1.45			
							Trt × days – 1.45			
							Crosses × days – 1.88			
							Trt × crosses × days – 4.99			

Hybrid-1, Shan-i-Punjab × Florda Prince; Hybrid-2, Shan-i-Punjab × Flordaglo; Hybrid-3, Shan-i-Punjab × Prabhat.

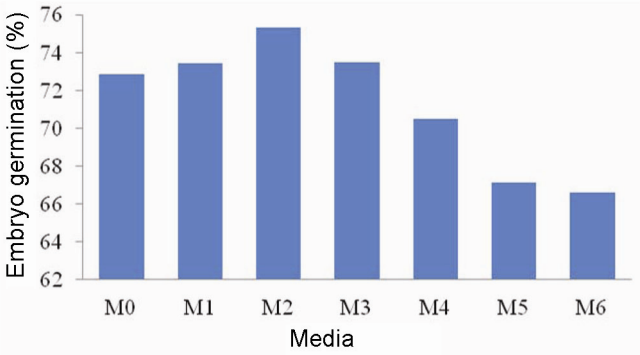

Figure 1. Effect of growth regulators on germination of low chill peach hybrid embryo.

the effect of growth regulator combinations on embryo culture of low chill peach hybrids.

The present studies were conducted at the Fruit Research Farm and Tissue Culture Laboratory, Department of Fruit Science, Punjab Agricultural University, Ludhiana. The crosses were made between low chill peach cultivars, viz. Shan-i-Punjab (♀) × Florda Prince (♂), Shan-i-Punjab (♀) × Flordaglo (♂) and Shan-i-Punjab (♀) × Prabhat (♂). Emasculated flower buds were pollinated on the same day with stored pollen grains because flowering of Shan-i-Punjab cultivar does not coincide with Florda Prince, Flordaglo and Prabhat. Fruits from these crosses were harvested at 65, 75 and 85 days after pollination. Seeds were extracted from fruits under laboratory conditions. Extracted seeds were surface-sterilized with 70% ethanol for 15–20 min and washed 2–3 times with sterile distilled water. Seeds were dissected with transversal cut to separate the seed coat. Embryo with half their cotyledons was excised under laminar air flow and cultured in test tubes containing culture media ($n = 30$). Culture medium used for this experiment was MS basal medium supplemented with different concentrations of BAP (0–2 mg/l) and IBA (0–1 mg/l). Seven media were prepared, i.e. M_0 (MS basal medium), M_1 (MS medium + BAP 0.125 mg/l + IBA 0.01 mg/l), M_2 (MS medium + BAP 0.25 mg/l + IBA 0.05 mg/l), M_3 (MS medium + BAP 0.50 mg/l + IBA 0.1 mg/l), M_4 (MS medium + BAP 1 mg/l + IBA 0.25 mg/l), M_5 (MS medium + BAP 1.5 mg/l + IBA 0.50 mg/l) and M_6 (MS medium + BAP 2 mg/l + IBA 1 mg/l). After inoculating the embryo in culture medium the cultured test tubes were kept at 4°C for stratification. An embryo was considered germinated after its radicle emerged out and grew into the medium ≥5 mm. After stratification the embryo cultured tubes were transferred to a growth chamber at 24 ± 2°C for germination. The data regarding embryo germination percentage was recorded 2 to 3 weeks after embryo culture in light. The experiment was laid out as factorial completely randomized design (CRD) with three stages of embryo growth and seven growing media. The data were analysed using SAS v9.0.0 software and means were compared using Duncan's Multiple Range Test (DMRT).

The data pertaining to the effect of different growth stages and media on embryo germination percentage is presented in Table 1 and Figures 1–5. The highest embryo germination percentage (81.66%) was recorded in hybrid seed form (Shan-i-Punjab × Prabhat) harvested at 85 days of pollination and cultured in M_2 medium (MS medium + BAP 0.25 mg/l + IBA 0.05 mg/l). The lowest embryo germination (60%) was found in 65-day-old

Table 2. Response of different growth stages and MS medium supplemented with growth regulators on days to germinate embryo of low chill peach hybrids

Treatments	65 DAP			75 DAP			85 DAP			
MS + Growth regulators (mg/l)	Hybrid-1	Hybrid-2	Hybrid-3	Hybrid-1	Hybrid-2	Hybrid-3	Hybrid-1	Hybrid-2	Hybrid-3	Mean
M_0: Control	45.00	47.83	47.66	42.00	43.33	43.33	41.00	41.66	42.33	43.79[a]
M_1: BAP (0.125) + IBA (0.01)	41.66	45.06	45.00	40.26	42.00	42.00	39.33	40.66	39.33	41.70[b]
M_2: BAP (0.25) + IBA (0.05)	40.00	43.40	43.60	38.66	39.33	40.00	38.00	39.33	38.00	40.03[c]
M_3: BAP (0.50) + IBA (0.1)	40.00	46.00	44.06	39.00	40.66	41.33	38.33	38.00	40.00	40.82[bc]
M_4: BAP (1) + IBA (0.25)	41.66	43.26	43.00	40.00	41.33	40.66	40.00	40.00	40.66	41.17[b]
M_5: BAP (1.5) + IBA (0.50)	43.33	44.86	43.16	39.33	40.00	39.33	39.00	39.33	41.33	41.07[c]
M_6: BAP (2) + IBA (1)	43.33	43.66	43.60	40.00	42.00	41.33	39.33	40.00	40.67	41.54[b]
Days mean		43.77[a]			40.75[b]			39.82[c]		
Hybrids mean		Hybrid-1			40.44[b]		$LSD_{0.05}$: Trt – 1.04			
		Hybrid-2			41.98[a]		Crosses – 0.68			
		Hybrid-3			41.92[a]		Days – 0.68			
							Trt × crosses – 1.80			
							Trt × days – 1.80			
							Crosses × days – 1.18			
							Trt × crosses × days – 3.12			

Hybrid-1, Shan-i-Punjab × Florda Prince; Hybrid-2, Shan-i-Punjab × Flordaglo; Hybrid-3, Shan-i-Punjab × Prabhat.

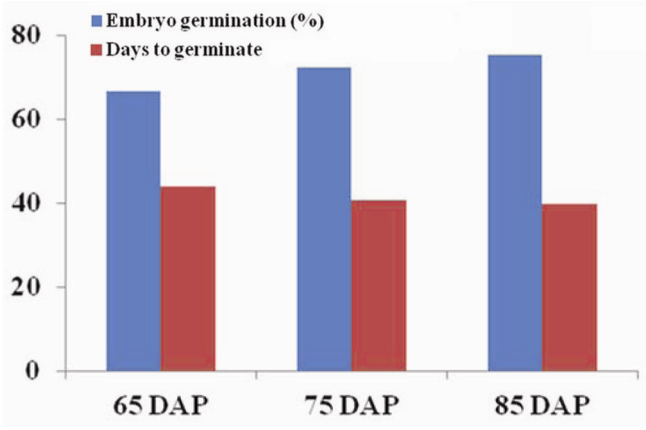

Figure 2. Effect of growth stage on germination and days to germinate in embryo of low chill peach hybrids.

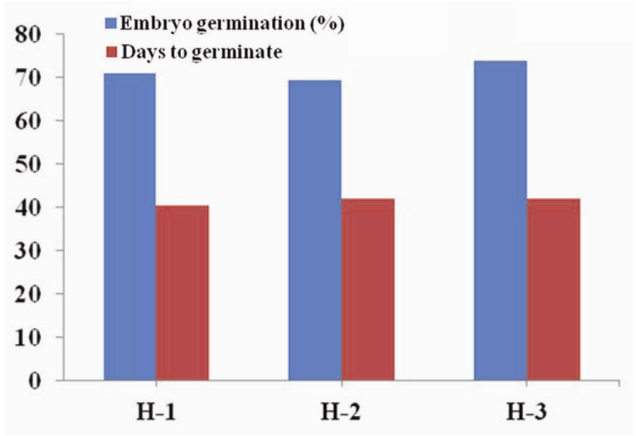

Figure 3. Effect of genotype on germination and days to germinate in embryo of low chill peach hybrids.

embryos from the hybrid-2 (Shan-i-Punjab × Flordaglo) in M_6 medium (MS medium + BAP 2 mg/l + IBA 1 mg/l). In M_0 (control), 72.87% of embryo germination was recorded. The increase in embryo age during culture resulted in an increase in embryo germination in all hybrids. The highest embryo germination was recorded in embryos rescued at 85 days after pollination and minimum in 65 days after pollination, which accords with the results of Bridgen[14] who concluded that germination percentage was strongly affected by embryo age. The main factor limiting the successful embryo cultures was the age of embryo at the time of transfer onto the nutrient medium. Infante and Gonzalez[15] concluded that the maximum percentage of embryo germination and embryo survival was recorded when embryos were harvested from the most advanced mature peach fruits, even when they were overripe and picked 25 days after commercial harvest.

Plant growth regulators play an important role in enhancing the embryo growth. Among all the media, maximum germination (75.31%) was recorded in M_2 medium (MS media + BAP 0.25 mg/l + IBA 0.05 mg/l) and significant differences were observed for different concentrations of BAP and IBA. Minimum germination (66.59%) was recorded in M_6 medium (MS medium + BAP 2 mg/l + IBA 1 mg/l). BAP and IBA induced the shoot, root and callus proliferation and embryo germination, but it was dependent on genotypes and hormone concentrations. When BAP and IBA were applied simultaneously, the per cent germination of immature embryos was more when compared to those when applied

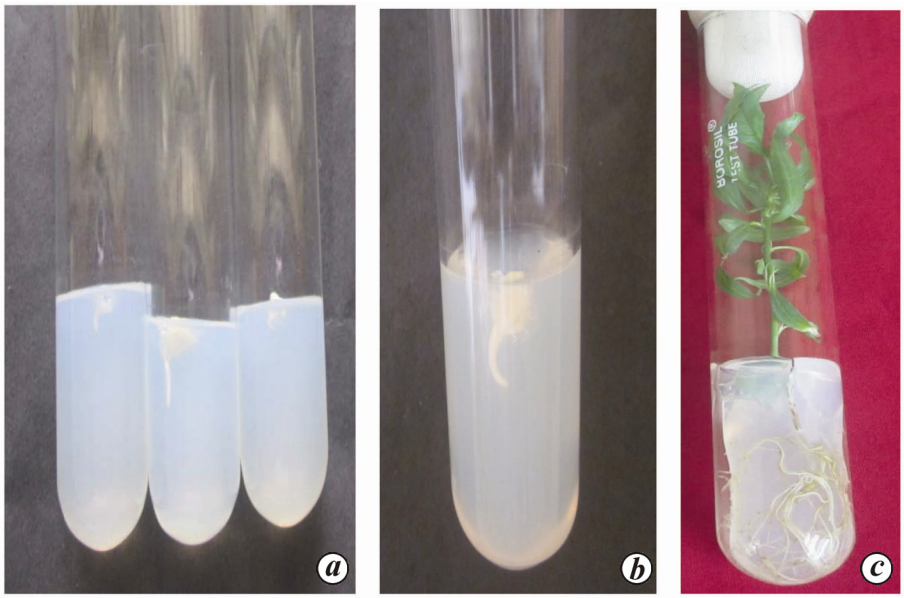

Figure 4. ***a***, ***b***, Radicle emergence in a peach embryo after 45 days of stratification at 4°C. ***c***, Germinated embryo 30 days after growth in a culture chamber showing both the developed shoot and root.

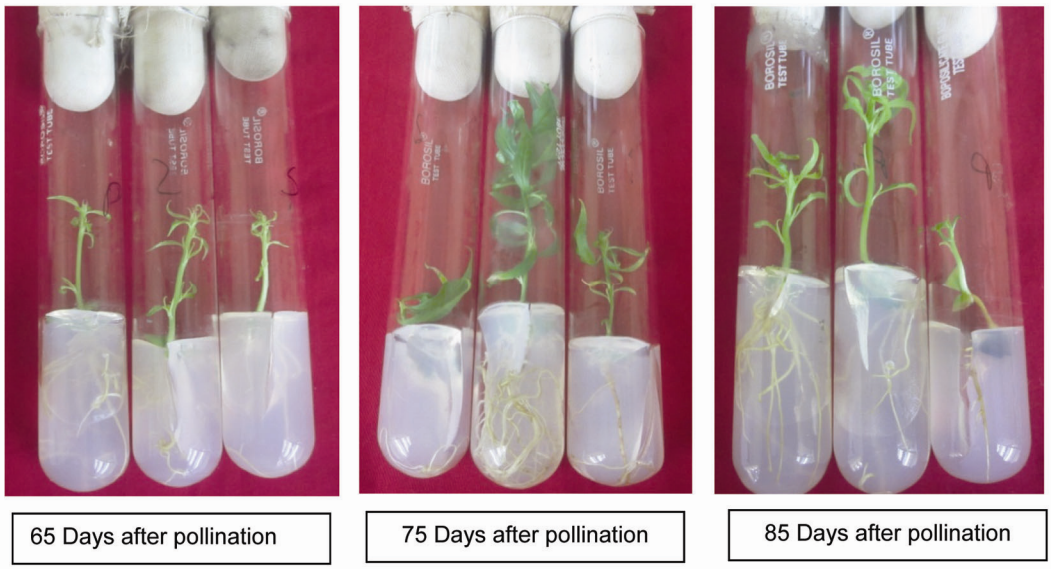

Figure 5. Embryo germination of low chill peach hybrids harvested at different growth stages.

separately[12]. Liu *et al.*[13] also studied different medium and concentrations of hormones for successful embryo culture and suggested that the abortion of embryo occurs due to improper growth regulators balance whereas excess growth regulators can often induce formation of callus[16]. In our experiment, lower concentration of growth regulators showed better germination. However, as the concentration of growth regulators was increased, the embryo germination percentage decreased and callus formation in embryo was recorded. When BAP and IBA were added at concentration of 0.25 and 0.05 mg/l, highest germination was observed and thereafter showed decrease in germination percentage. Gurel and Gulsen[17] also observed an extensive amount of callus when 1.0 mg/l IBA was used in the culture medium. In MS basal medium, without any growth regulators higher embryo germination was found when compared to those containing higher concentration of growth regulators.

The data on the number of days to germinate is shown in Table 2 and Figures 2 and 3. Significant differences were found for days to germinate with respect to the different growth stages and media. Fruits harvested at full maturity (85 days after pollination) took lesser time for embryo germination (39.82 days) when compared to

those harvested at earlier stages. Embryos harvested from 65-day-old fruits took maximum days to germinate (43.77 days). Days to germinate were reduced with increase in age and this reduction might be because of the reason that 85-day-old embryos had already grown on the plant itself and thus took less time to germinate. Similar findings were reported by Kumar and Arora[18]. They cultured hybrid peach embryos at three stages from the date of pollination and reported that, 9-week-old embryos took least number of days to grow than 7 and 8-week-old embryos. Growth regulators also played an important role in influencing the period of embryo germination. Embryos grown in M_0 medium (without growth regulators) needed longer duration to germinate (43.79 days) followed by M_1, M_4 and M_6 medium, whereas embryos grown in M_2 medium were the earliest to germinate. This medium was fortified with BAP (0.25 mg/l) + IBA (0.05 mg/l). Among the hybrids, Hybrid-1 (Shan-i-Punjab × Florda Prince) was fast growing when compared to Hybrid-2 (Shan-i-Punjab × Flordaglo) and Hybrid-3 (Shan-i-Punjab × Prabhat). This may be because of early maturing behaviour of the Florda Prince variety which was used as a male parent in Hybrid-1. In all three stages of embryo culture (65, 75 and 85 days aged embryos), Hybrid-1 (Shan-i-Punjab × Florda Prince) took minimum days to germinate.

Embryo rescued from fully matured embryos (85 days old) showed more embryo germination percentage and took lesser days to germinate than those harvested at early stage of growth. Among different medium used, MS medium supplemented with lower concentration of growth regulators (MS medium + BAP 0.25 mg/l + IBA 0.05 mg/l) showed higher embryo germination percentage and lesser days to germinate. MS basal medium also gave better results in terms of embryo germination compared to the MS medium fortified with higher level of growth regulators. There was significant effect of genotype on embryo germination and days to germinate with respect to different medium and growth stages.

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