Egypt. J. Plant Breed. 26(2):171– 186 (2022)

IN VITRO GROWTH AND DEVELOPMENT OF IMMATURE EMBRYOS IN FLORDA PRINCE PEACH CULTIVAR

A.S.M. Ismail* and E.H. El-Bassel*
Breeding Research Department for Fruit Trees, Ornamental and Woody Plants, Horticulture Research Institute, Agricultural Research Center, Egypt
*Corresponding Author: asm8019@yahoo.com & emadelbassel@yahoo.com

ABSTRACT

In early cultivars, fruit maturation precedes embryo maturation. Thus, this study was conducted during the 2020 and 2021 seasons to overcome an embryo abortion problem in early maturing Florda Prince peach cultivar through in vitro embryo rescue and culture technique. Then induce germination, growth and development of these rescued embryos up to complete seedlings and to determine the optimal time of successful excision of these embryos as well as the best media and supplements for embryo formation and germination under in vitro conditions. Open-pollinated immature fruits were collected at five developmental stages; 40, 50, 60, 70, and 80 days after full bloom (DAFB), and then the ovules were in vitro separated and cultured on three embryo formation media; MS1, Nitsch1, and WPM1, supplemented with 1.5 mgl^{-1} IAA and 1 mgl^{-1} GA_3. Ovule cultures subjected to cold pre-treatment were 1) stratified in the dark at 4°C and 2) without stratification in the dark at 25°C. For embryo germination, all formed embryos were excised and then transferred to MS2, Nitsch2, and WPM2 media supplemented with 0.5 mgl^{-1} BA and 3 mgl^{-1} GA_3. The WPM1 medium with stratification treatment at 60 DAFB, recorded a higher significant value of embryo formation (83.33%) in the first season and at 60 and 70 DAFB (80.00 and 81.82%) in the second season, respectively, than other media. In the first season, WPM2 medium, at 70 DAFB, recorded the highest value of embryo germination (62.09%) in the first season and at 60 and 70 DAFB (61.73 and 61.84%) in the second season, respectively. The addition of 3.0 mgl^{-1} IBA or NAA to half strength MS medium resulted in the highest percentage of rooting, the greatest average number of roots per shoot, and the longest root length. It can be recommended for breeding and improvement programs in Florda Prince peach that the optimal time for embryo excision is 60-70 days after pollination or full bloom by in vitro ovule culture on WPM1 medium with stratification at 4° C for 4 weeks, and then transferring excised embryos to WPM2 medium for germination.

Key words: ***Peach (Prunus persica L.), Embryo rescue, In vitro culture, Breeding***.

INTRODUCTION

Peach tree is one of the most important deciduous fruit trees grown in Egypt. Extension of the cultivated area nowadays is due to its high economic value, exporting potential, and the introduction of low-chilling peach cultivars such as "Florda Prince" (El-Baz *et al* 2007)**.** Peach cv. "Florda Prince" is an early ripening variety under local environmental conditions. It recorded superior yield, and exhibited high adaptation to the local environmental conditions (Shaltout 1987).

In early maturing peach genotypes such as Florda Prince, fruit maturation precedes embryo maturation, resulting in an immature, rudimentary, and abortive embryo. In the genus *Prunus*, the inability of the hybrid zygote to produce a viable embryo is due to the degeneration of the

endosperm, which is unable to provide proper nutrition to the developing embryo, especially in the early maturing peach cultivars (Ramming 1985**)**. This problem has thus hampered the evolution of superior early maturing genotypes through conventional breeding. Hence, the embryo culture technique proved to be the most effective alternate method, allowing us to exploit it. The first successful embryo culture of fruit trees on a synthetic medium was achieved by Tukey (1933) in cherry. Since then, much work has been conducted to optimize embryo rescue techniques in several crops. Blake (1939) was the first to employ embryo culture in peach breeding programs.

Embryo culture protocols in *Prunus* now permit the rescue of these immature embryos from early maturing stone fruit, thereby allowing them to be used as seed parents. Successful embryo rescue depends on many factors, such as genotype, embryo developmental stage, culture media, growth regulators, and culture conditions.

Plant growth regulators play an important role in embryo culture and enhance embryo growth (Jeengool and Boonprakob 2004). Embryos were successfully cultured in several media, including Gilmore and Monet media (George *et al* 1987), Brooks and Hough medium (Scorza and Sherman 1996) and Woody Plant medium (Emershad and Ramming 1994)**.**

The goals of this study were to: 1) overcome an embryo abortion problem in early maturing Florda Prince peach cv. using an *in vitro* embryo rescue and culture technique, then induce germination, growth, and development of these rescued embryos up to complete seedlings, 2) determine the optimal time of successful excision of these embryos as well as the best media and supplements for embryo formation and germination under *in vitro* conditions, and 3) increase the efficiency of breeding and genetic improvement programs in peaches later.

MATERIALS AND METHODS

Plant material:

This study was conducted at the plant tissue culture laboratory, breeding research department for fruit trees, ornamental and woody plants, Horticulture Research Institute, Agricultural Research Center, Egypt during the 2020 and 2021 seasons on seven-year-old trees of Florda Prince Peach

cv. **(***Prunus persica* L.), grown in a private orchard in Giza governorate, that were used as a source of plant material.

Embryo excised time:

The embryo excised time or fruit developmental stage refers to the number of days after full bloom (DAFB). The open-pollinated immature fruits were harvested at five developmental stages; 40, 50, 60, 70, and 80 DAFB (Fig. 1 a-e). The diameter, length, and weight of fruits were recorded at all developmental stages.

***In vitro* ovule culture and embryo rescue:**

Immature open-pollinated fruits of early-ripening 'Florda Prince' peach from each developmental stage were randomly harvested and were subjected to continuous tap water flow for about 20 minutes, surface sterilized in the laminar flow hood by immersion for 30 seconds in 70% (v/v) ethyl alcohol, and then submerged in sodium hypochlorite solution (about 2% active chlorine in water) for 20 minutes. Finally, it was underwent three rinses with sterile distilled water, lasting five minutes each. After sterilization, flesh and shell (endocarp) were removed by nutcracker to extract ovules.

All ovules were cultured for 4 weeks on three media based on each of the MS (Murashige and Skoog, 1962), Nitsch (Nitsch and Nitsch, 1969), and Woody Plant Medium (WPM) (Lloyd and McCown, 1980) media based on the basic salts and vitamins. These media were compared for growth and development of immature embryos. 30 gl^{-1} of sucrose, 1.5 mgl^{-1} of filter-sterilized indole-3-acetic acid (IAA), 1 mgl^{-1} of filter-sterilized gibberellic acid (GA_3), and 7 gl^{-1} of agar were added to the previous media; [MS1, Nitsch1 and WPM1]. Ovule cultures were subjected to cold pre-treatment; 1) stratification in the dark at 4°C and 2) without stratification in the dark at 25°C.

After 4 weeks of culture, all ovules were placed on a sterile Petri dish and dissected with a scalpel blade and forceps, and then the testa (ovule coat) was peeled off to release embryos. With the use of a histology needle and a binocular, all formed embryos at the micropyle end of the ovule were examined and excised. The percentage of embryo formation was recorded.

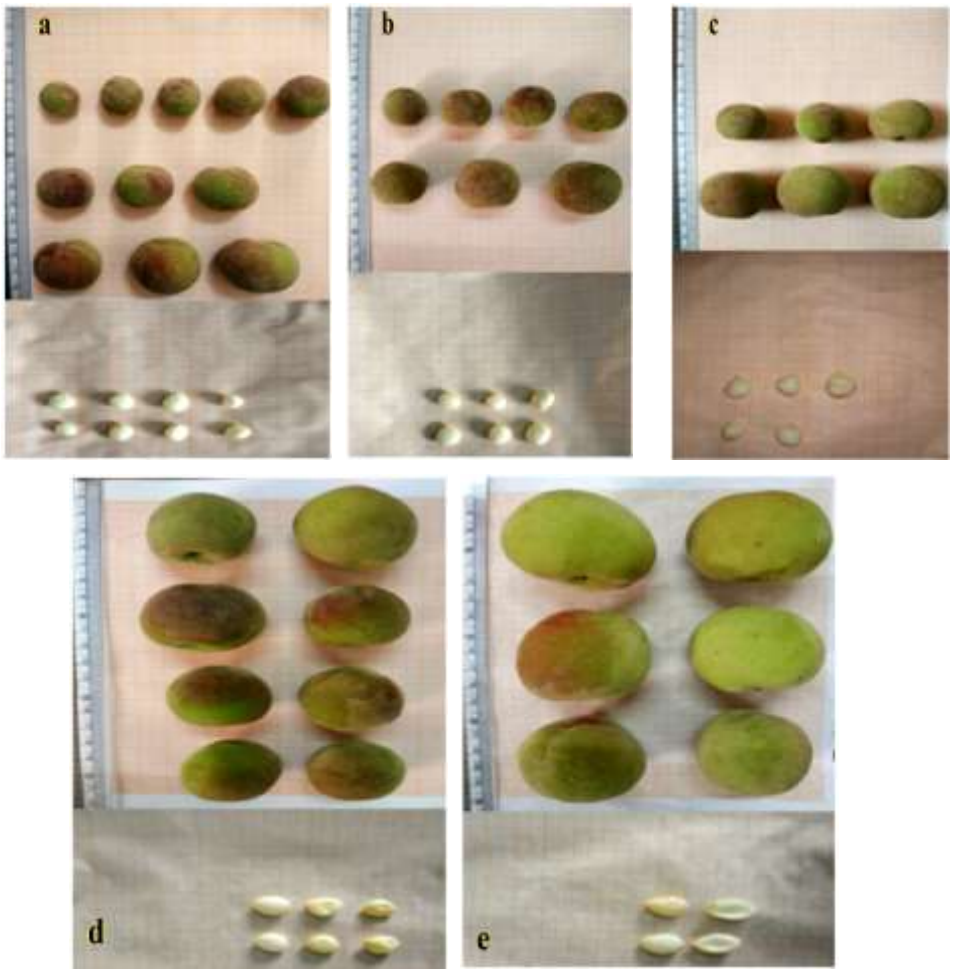

Fig. 1. a-e: Open-pollinated immature fruits and ovules of Florda Prince peach at different developmental stages.
a: 40, **b:** 50, **c:** 60, **d:** 70 and **e:** 80 DAFB.

The experiment included 30 treatments (3 media × 2 pre-treatment × 5 embryos excised time) as a factorial experiment. There were five replicates, each comprising one glass jar with 5 ovules.

***In vitro* embryo germination:**

For excised embryo germination, all embryos were transferred to the same previous media supplemented with 0.5 mgl^{-1} benzyl adenine (BA) and 3 mgl^{-1} filter-sterilized gibberellic acid (GA_3); [MS2, Nitsch2 and WPM2], for further 4 weeks. The experiment comprised 15 treatments (3 media × 5 embryos excised times). There were four replicates, each comprising one glass jar with four embryos. After 4 weeks of culture, the embryo germination percentage was recorded for each embryo excised time.

Seedling root multiplication and adaptation:

To multiply the roots of the seedlings obtained from rescued embryos, 20 ml of the basal salts and vitamins of MS medium at half strength supplemented with indole-3-butyric acid (IBA) or naphthalene acetic acid (NAA), each at concentrations of 0.0, 1.0, 2.0, and 3.0 mgl^{-1} were added. This experiment used a completely randomized design with 7 treatments and 10 replicates for each treatment (each replicate was one seedling). The average number of roots formed per shoot and average root length (cm) were recorded after 4 weeks of culture on the rooting media. The rooted seedlings were placed in plastic pots with peat moss and sand (1:1). The pots were covered with plastic bags and then placed in the glasshouse for adaptation. The seedlings were irrigated with a volume of MS medium every 3 days. Finally, the healthy, good seedlings were transferred to the orchard.

***In vitro* culture conditions:**

All the media were autoclaved for 20 minutes at 100 Kpa (15 P.S.I.) and 121°C after being adjusted to a pH of 5.7 0.1 with HCl or NaOH. Ovule cultures were incubated in a growth room at 27±1°C for 8 weeks in the dark, then the isolated embryos were placed under cool white fluorescent lamps (4 lamps per shelf) for the following subcultures to provide a light intensity of 4200-4400 lux at explant level (30 cm from the light).

All of the aforementioned investigations used Duncan's multiple range tests at 5% probability level to differentiate means (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

Effect of embryo excised time on the average of fruit diameter, length and weight

Embryo excised time (fruit developmental stage) had an effect on the average of fruit diameter, length, and weight as shown in Table 1. In both seasons, the fruits harvested 40 days after full bloom (DAFB) recorded the lowest average for diameter, length, and weight (27.14 mm, 30.36 mm, 9.44 g) and (26.96 mm, 31.33 mm, 10.83 g), respectively. Progressively, these values increased until they reached (42.74 mm, 47.80 mm, 42.72 g) and (44.36 mm, 48.65 mm, 44.67 g) at 80 DAFB, respectively. Generally, fruit diameter, length, and weight increased dramatically as the developmental stage of the fruit progressed.

Table 1. Effect of embryo excised time on the average of fruit diameter, length, and weight.

Embryo excised time (DAFB)	Season 2020			Season 2021		
	Fruit diameter (mm)	Fruit length (mm)	Fruit weight (g)	Fruit diameter (mm)	Fruit length (mm)	Fruit weight (g)
40	27.14 c	30.36 c	9.44 d	26.96 b	31.33 d	10.83 d
50	29.78 bc	33.31 c	13.05 d	28.48 b	33.25 cd	14.22 cd
60	34.63 a-c	38.73 bc	19.66 c	35.64 ab	39.52 bc	20.04 c
70	38.86 ab	43.46 ab	25.06 b	39.07 a	44.43 ab	26.56 b
80	42.74 a	47.80 a	42.72 a	44.36 a	48.65 a	44.67 a

Means followed by the same letter(s) in each column are not significantly different at (P < 0.05) using Duncan's multiple range test.

Other studies on early maturing peach genotypes 'Copiapo', 'May Glo', 'Early Majestic' and 'Venus' were carried out by Infante and González (2002). The fruit growth curves of the "Copiapo" and "Early

Majestic" genotypes were similar. Fresh weight accumulation and fruit diameter followed a simple sigmoid curve, with a slow growth rate phase until 49 DAFB, then a rapid growth rate until 105 DAFB, and finally a decrease in growth rate. The fresh weight and the fruit diameter of the 'May Glo' genotype slowly grew until 49 DAFB, and then increased in both variables until the end of the measurement period. A fruit slow growth rate phase II was seen in "Venus" at 77 and 96 DAFB, then the rapid growth rate resumed and continued until 130 DAFB, where it then again reduced (phase III), forming a double sigmoid curve. A double sigmoid curve was also seen in the fruit diameter, but phase III was less obvious than it was in the double sigmoid curve described by Scorza and Sherman (1996) for the 'Lowell' peach.

Effect of embryo excised time, medium type and pre-treatment on the percentage of embryo formation

It's clear that the form, growth, and development stage of immature embryos inside ovules were greatly affected by the sampling time, medium type, and pre-treatment as shown in **(**Table 2) (Fig. 2 a, b) . In the first season, the highest mean percentage of embryo formation was (68.89 and 68.47%) at 60 and 70 DAFB, respectively, while the lowest values were (47.08 and 51.35%) at 40 and 80 DAFB, respectively, with non-significant differences between them. In the second season, the highest mean percentage of embryo formation was (68.81%) at 70 DAFB, and the lowest value was (48.45%) at 40 DAFB. Concerning medium type, WPM1 medium recorded the highest mean percentage of embryo formation (66.73 and 65.98%), while Nitsch1 medium recorded the lowest values (51.09 and 52.57%) in both seasons, respectively. As regard to pre-treatment, stratification treatment gave the highest mean percentage of embryo formation (63.19 and 64.30%) as compared with no stratification treatment, which gave the lowest values (54.64 and 54.20%) in both seasons, respectively. The interaction between the three studied factors, WPM1 medium with stratification treatment at 60 DAFB, recorded a higher significant value (83.33%) in the first season and at 60 and 70 DAFB (80.00 and 81.82%) in the second season, respectively, than other media.

Table 2. Effect of embryo excised time, medium type and pre-treatment on the percentage of embryo formation.

	Season 2020						
Pre-treatment	**Stratification (B1)**			**Without Stratification (B2)**			**Means (A)**
Medium type / Embryo excised time (DAFB)	**MS1 (C1)**	**Nitsch1 (C2)**	**WPM1 (C3)**	**MS1 (C1)**	**Nitsch1 (C2)**	**WPM1 (C3)**	
40 (A1)	**50.00b-d**	**40.00cd**	**60.00a-d**	**41.67cd**	**37.50d**	**53.33a-d**	**47.08b**
50 (A2)	**66.67a-d**	**54.55a-d**	**70.00a-d**	**54.55a-d**	**45.45b-d**	**61.54a-d**	**58.79ab**
60 (A3)	**72.73a-c**	**63.64a-d**	**83.33a**	**64.29a-d**	**57.89a-d**	**71.43a-c**	**68.89a**
70 (A4)	**75.00ab**	**71.43a-c**	**76.92ab**	**63.64a-d**	**53.85a-d**	**70.00a-d**	**68.47a**
80 (A5)	**54.55a-d**	**45.45b-d**	**63.64a-d**	**46.15b-d**	**41.18cd**	**57.14a-d**	**51.35b**
Means (B)	**63.19a**			**54.64b**			
Means (C)	**58.93ab**		**51.09 b**		**66.73 a**		
Season 2021							
40 (A1)	**50.00ab**	**50.00ab**	**58.33ab**	**46.67ab**	**35.71b**	**50.00ab**	**48.45c**
50 (A2)	**69.23ab**	**54.55ab**	**72.73ab**	**53.33ab**	**47.06ab**	**57.14ab**	**59.01a-c**
60 (A3)	**75.00a**	**66.67ab**	**80.00a**	**61.11ab**	**54.55ab**	**68.75ab**	**67.68ab**
70 (A4)	**72.73ab**	**70.00ab**	**81.82a**	**60.00ab**	**55.56ab**	**72.73ab**	**68.81a**
80 (A5)	**58.33ab**	**46.15ab**	**60.00ab**	**46.67ab**	**45.45ab**	**58.33ab**	**52.49bc**
Means (B)	**64.37 a**			**54.20 b**			
Means (C)	**59.31 ab**		**52.57 b**		**65.98 a**		

Means followed by the same letter(s) in each column are not significantly different at (P < 0.05) using Duncan's multiple range test.

Other studies on early maturing peach genotypes, 'Copiapo', 'May Glo', 'Early Majestic', and 'Venus' were carried out by Infante and González (2002). In the 'Copiapo', 'May Glo', and 'Early Majestic' genotypes, embryos were visible at 80 DAFB. While in 'Venus', embryos

were visible at 68 DAFB. At 85 and 90 DAFB, all the embryos of previous genotypes were rescued, but the majority of the embryos failed to germinate. These embryos produced normal plants when harvested at 106, 118, and 125 DAFB. Srivastav *et al* (2004) excised and cultured ovules from two early-maturing peach cultivars, namely Saharanpur Prabhat and Flordasun. They found that 60-day old embryos showed better culture establishment, maturation, and germination than 70 and 80-day old embryos. On the other hand, Sundouri *et al* (2014) found that the maximum of Florda Prince embryo formation was observed when the ovules were stratified for 45 days at 4ºC.

Effect of embryo excised time and medium type on the percentage of embryo germination

The data pertaining to the effect of sampling time (DAFB) and medium type on the percentage of embryo germination is presented in (Table 3) (Fig. 2 c-e). The percentage of developed embryo germination derived from detached immature ovules increased gradually with increasing the developmental stage of the fruit, reaching 54.84 and 53.85% at 70 DAFB in the two seasons, respectively. Then these values started to decrease at 80 DAFB, which were 40.74 and 39.03% in the two seasons, respectively. WPM2 medium recorded the highest mean percentage of embryo germination (47.54 and 49.15%) followed by MS2 (40.33 and 39.76%) in the two seasons, respectively. On the other hand, Nitsch2 medium recorded the lowest mean percentage of embryo germination (34.05 and 33.78%) in the two seasons, respectively. The interaction between the two studied factors, WPM2 medium at 70 DAFB, recorded a higher significant value (62.09%) in the first season and at 60 and 70 DAFB (61.73 and 61.84%) in the second season, respectively, than other media.

Concerning embryo excised time, the previous results are in harmony with those obtained by Ahmed (2012) in Egypt, who stated that the best excised age of Florda Prince peach embryos ranged between 60-70 days after pollination, cultured on Nitsch medium supplemented with 0.2 mgl^{-1} BA and 2.0 mgl^{-1} GA_3 showed the highest germination percentage.

Table 3. Effect of embryo excised time and medium type on the percentage of embryo germination.

	Season 2020				Season 2021			
Medium type / Embryo excised time (DAFB)	MS2 (B1)	Nitsch2 (B2)	WPM2 (B3)	Means (A)	MS2 (B1)	Nitsch2 (B2)	WPM2 (B3)	Means (A)
40 (A1)	22.51de	17.68e	29.28b-e	23.16c	24.54b	18.20b	31.21ab	24.65b
50 (A2)	36.03a-e	27.88c-e	41.84a-e	35.25bc	38.96ab	28.38ab	44.00ab	37.11ab
60 (A3)	48.34a-d	41.62a-e	57.67ab	49.21ab	47.60ab	40.15ab	61.73a	49.82a
70 (A4)	54.61a-c	47.83a-d	62.09a	54.84a	50.44ab	49.28ab	61.84a	53.85a
80 (A5)	40.15a-e	35.24a-e	46.83a-d	40.74ab	37.26ab	32.89ab	46.95ab	39.03ab
MEANS (B)	40.33ab	34.05b	47.54a		39.76ab	33.78b	49.15a	

Means followed by the same letter(s) in each column are not significantly different at (P < 0.05) using Duncan's multiple range test.

Also, Sundouri *et al* (2014) in India, found that the maximum germination (52.56%) of Florda Prince peach embryos was observed after 75 days of crossing, followed by 41.67% after 60 and at least 12.45% after 45 days of crossing. Chaparro and Sherman (1994) reported that the germination percentage of embryos increased significantly at 60-67 days after full bloom. While, Srivastav *et al* (2004) concluded that embryos of "Saharanpur Prabhat" and Flordasun aged 60 days after open pollination showed better germination than 70 and 80-day old embryos. Regarding medium type, similar results were obtained by San *et al* (2014) who reported that the addition of different combinations of BA and GA_3 to the Murashige and Skoog (MS) medium significantly increased the germination ratios of embryos without cotyledons in peach, the best treatment (86.7% germination) was MS medium supplemented with 0.5 mgl^{-1} BA and 3.0 mgl^{-1} GA_3. While, Unek *et al* (2017) stated that the best germination rate of commercial peach cultivar 'Flored' (87.5%) occurred on MS medium followed by WPM medium (85%) supplemented with 1 mgl^{-1} BA and 0.1 mgl^{-1} GA_3. Jeengool and Boonprakob (2004) showed that woody plant

medium (WPM) with 0.1 mgl^{-1} GA_3 and either 0.5 or 1.0 mgl^{-1} BA yielded 100% germination of ‘EarliGrande’ peach embryos.

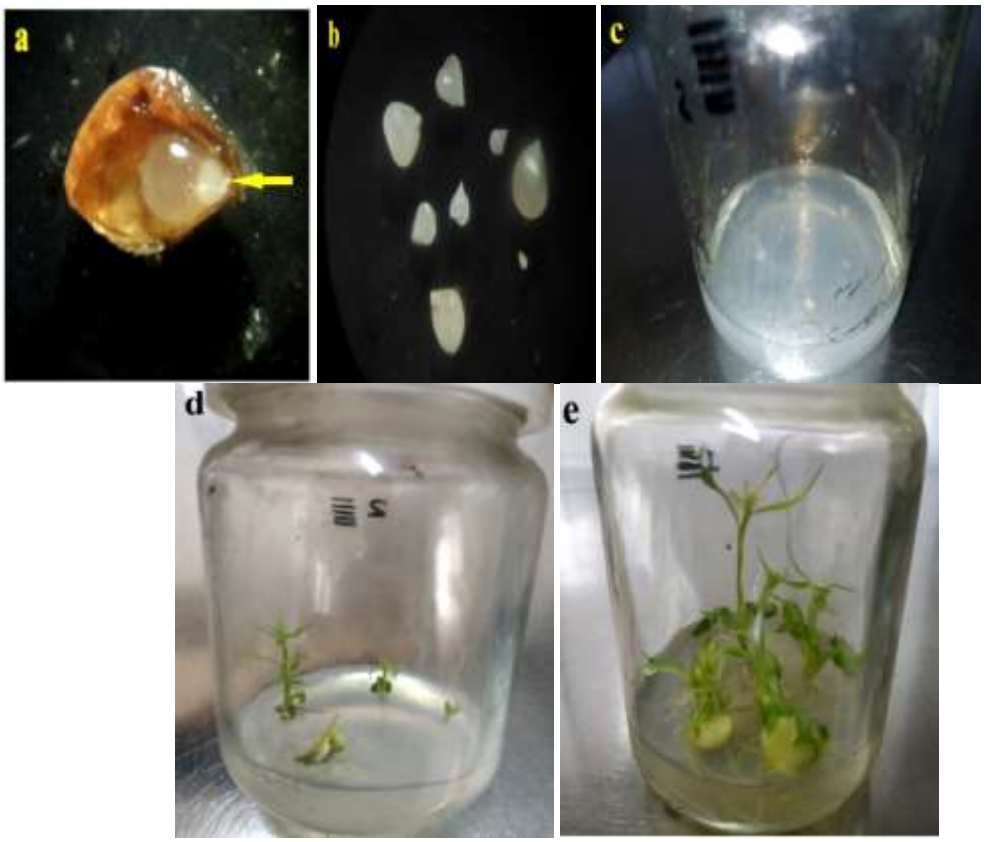

Fig. 2. a Embryo formation, set in the middle at the micropyle apex of the embryo sac (28× magnification).
b Excised embryos at different developmental stages (28× magnification).
c *In vitro* embryo culture.
d Initial embryo germination.
e Seedlings derived from excised embryo germination.

Effect of IBA and NAA concentrations on seedling root multiplication

Data in Table 4 showed the effect of IBA and NAA concentrations on the root multiplication of seedlings derived from embryo germination. In both seasons, the highest percentage of rooting (85.27 and 92.46%) was obtained on half-strength MS medium supplemented with 3.0 mgl^{-1} IBA, while the highest percentage of rooting (65.56 and 72.43%) was obtained at a concentration of 3.0 mgl^{-1} NAA. No rooting percentage was obtained on half-strength MS medium without auxin. The maximum average number of roots per shoot (6.26 and 5.38) was obtained when IBA was added to half-strength MS medium at a concentration of 3.0 mgl^{-1}. The addition of 3.0 mgl^{-1} IBA or NAA to half strength MS medium resulted in maximum average root lengths of 7.17 and 6.63 cm in the first season, and 8.10 and 7.56 cm in the second season, respectively. Greatly, root proliferation enhanced the acclimatization success of regenerated seedlings (Fig. 3).

Table 4. Effect of IBA and NAA concentrations on seedling root multiplication.

Treatments (mgl^{-1})		Season 2020			Season 2021		
		Rooting (%)	Average no. roots/shoot	Average root length (cm)	Rooting (%)	Average no. roots/shoot	Average root length (cm)
Control	0.0	0.00 e	0.00 e	0.00 e	0.00 e	0.00 e	0.00 e
IBA	1.0	66.53 bc	3.40 bc	4.77 cd	65.29 b-d	3.89 bc	4.36 d
″	2.0	72.49 ab	4.03 b	5.83 bc	78.34 ab	4.56 ab	6.15 bc
″	3.0	85.27 a	6.26 a	7.17 a	92.46 a	5.38 a	8.10 a
NAA	1.0	50.38 d	1.93 d	3.93 d	55.71 d	2.12 d	3.64 d
″	2.0	53.74 cd	2.77 c	5.50 bc	58.29 cd	3.23 cd	5.85 c
″	3.0	65.56 bc	3.70 b	6.63 ab	72.43 bc	4.10 b	7.56 ab

Means followed by the same letter(s) in each column are not significantly different at (P < 0.05) using Duncan's multiple range test.

These results are in harmony with those obtained by Abd Alhady (2018) in Egypt, who stated that 90% of the shoots of Nemaguard peach rootstock rooted on MS medium supplemented with 3.0 mgl^{-1} IBA with the maximum average root number/shoot (5.8). The maximum root length (8.5 and 7.2 cm) obtained when IBA or NAA were supplemented to half strength MS media at 3.0 mgl^{-1}. On the other hand, Liu *et al* (2007) found that the combination of 0.5 mgl^{-1} IAA and 0.2 mgl^{-1} NAA constituted the best combination for root induction with normal shape and many lateral roots for peach hybrids.

Fig. 3. Complete Florda Prince peach seedlings derived from embryo rescue.

REFERENCES

Abd Alhady, M.R.A. (2018). *In vitro* propagation for peach rootstock (Nemaguard). Egyptian Res. J. Desert. 68(1): 1-13.

Ahmed, E.A.H. (2012). Studies for improving characteristics of some peach cultivars grown in Egypt. Ph.D. Thesis, Fac. of Agric., Ain Shams Univ., Cairo, Egypt.

Blake, L.M. (1939). Some results of crosses of early ripening varieties of peaches. Proc. Am. Soc. Hortic. Sci., 37: 232–241.

Chaparro, J.X. and W.B. Sherman (1994). Culture date and germination procedure affects success of nectarine ovule and embryo culture. Fruit Varieties Journal. 48(3): 173-175.

El-Baz, E.T., A.A. Arafa and W.M. Awad (2007). Comparative study on growth and fruiting of some peach cultivars growing in two different locations. J. Agric. Sci., Mansura Univ., 52(8): 5863-5873.

Emershad, R.L. and D.W. Ramming (1994). Effect of media on embryo enlargement in earlyripening genotypes of *Prunus* growth *in vitro*. Plant Cell, Tissue and Organ Cult. 37: 55-59.

George, E.F., D.J.M. Puttock and H.J. George (1987). Plant Culture Media Volume 1: Formulation and Uses. Exegitics Limited, England. 567p.

Infanter, R. and J. González (2002). Early maturing peach embryo rescue and *in vitro* survival at different fruit growth stages. Acta Hortic., 592: 89-92.

Jeengool, N. and D. Boonprakob (**2004)**. Rescue of peach embryo in culture media with additional of 6-benzyladenine and gibberellic acid. *Nat. Sci.*, 38: 468–474.

Liu, W., X. Chen and G. Liu (2007). Interspecific hybridization of *Prunus persica* with *P. armeniaca* and *P. salicina* using embryo rescue. Plant Cell Tiss. Organ Cult., 88: 289–299.

Lloyd, G. and B.H. McCown (1980). Commercially-feasible micropropagation of mountain Laurel, Kalmia latifolia, by use of shoot-tip culture. Combined Proceedings-International Plant Propagator's Society, 30: 421-427.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Plant Physiology, 15: 473-497.

Nitsch, J.P. and C. Nitsch (1969). Haploid plants from pollen grains. Science, 163: 85-87.

Ramming, D.W. (1985). *In vitro* embryo culture of early maturing *Prunus*. Hort. Sci., 25: 393-398.

San, B., A.N. Yildirim and F. Yildirim (2014). An *In Vitro* germination technique for some stone fruit species: the embryo isolated from cotyledons successfully germinated without cold pre-treatment of seeds. HortScience. 49(3): 294–296.

Shaltout, A.D. (1987). 'Florda Prince' a promising peach cultivar recently introduced to Egypt. Bull. Fac. of Agric., Cairo Univ., 38: 381-391.

Snedecor, G.W. and W.G. Cochran (1994). Statistical Methods, 8th Ed. Iowa State University Press, Ames, Iowa, USA.

Scorza, R. and W. Sherman (1996). Peaches. Pages 325-440. In. Janick, J. and Moore, J.N. (eds.). Fruit Breeding. Volume I. Tree and Tropical Fruits. John Wiley & Sons, NY.

Srivastav, M., S.K. Singh, R.L. Arora and B. Krishna (2004). Embryo culture studies in subtropical peach (*Prunus Persica* Batsch.). Acta Hortic. 662, 297-301.

Sundouri, A.S., H. Singh, M.I.S. Gill, A. Thakur and A.K. Sangwan (2014). *In-vitro* germination of hybrid embryo rescued from low chill peaches as affected by stratification period and embryo age. Indian J. Hort. 71(2): 151-155.

Tukey, H. B. (1933). Growth of peach embryo in relation to growth of fruit and season of ripening. Proc. Am. Soc. Hortic. Sci., 30: 209–218.

Unek, C., E. Tanrıver and A.B. Kuden (2017). Embryo rescue for breeding of early peach cultivar 'Flored' and determination of Sharka resistant genotypes with marker-assisted selection. Acta Hortic. 1187, 285-290.

نمو وتطور الأجنة غير الناضجة في صنف الخوخ فلوردا برنس معمليا

أحمد سعيد محمد إسماعيل ، عماد حامد الباسل

قسم بحوث تربية الفاكهة و نباتات الزينة و الأشجار الخشبية

معهد بحوث البساتين – مركز البحوث الزراعية

أجريت هذه الدراسة بمعمل زراعة الأنسجة النباتية بقسم بحوث تربية الفاكهة و نباتات الزينة و الأشجار الخشبية بمعهد بحوث البساتين , مركز البحوث الزراعية خلال موسمي (٢٠٢٠ , ٢٠٢١) و ذلك للتغلب علي مشكلة إجهاض الجنين في صنف الخوخ فلوردا برنس مبكر النضج من خلال تكنيك إنقاذ وزراعة الجنين ثم دفع هذه الأجنة المنقذه للإنبات والنمو والتطور إلي شتلات كاملة, بالإضافة إلي تحديد الموعد الأمثل لفصل هذه الأجنة, كذلك البيئات والإضافات الأفضل لتكوين وإنبات الأجنة تحت الظروف المعملية. جمعت البويضات المفصولة من الثمار غير الناضجة من التلقيح المفتوح عند ٥ مراحل تطور (٤٠, ٥٠, ٦٠, ٧٠, ٨٠ يوم من التزهير الكامل), ثم زرعت معمليا علي ٣ بيئات لتكوين الجنين (MS1 , Nitsch1 , WPM1) مدعمه بتركيزات من ١,٥ ملليجم/لتر IAA, ١ ملليجم/لتر GA_3. خضعت مزارع البويضات إلي معاملة برودية (٤° م في الظلام) ومعاملة لابرودية (٢٥° م في الظلام). لإنبات الأجنة, تم فصل كل الأجنة المتكونة و نقلت إلي بيئات (MS2 , Nitsch2 , WPM2) مدعمه بتركيزات من ٠,٥ ملليجم/لتر BA , ٣ ملليجم/لتر GA_3. سجلت بيئة WPM1 مع معاملة البرودية المبدئية للأجنة المفصوله بعد ٦٠ يوم من التزهير الكامل وكانت أعلي قيمة معنوية لتكوين الجنين من البيئات الأخري كانت (٨٣,٣٣%) في الموسم الأول , (٨١,٨٢, ٨٠,٠٠%) بعد ٧٠, ٦٠ يوم من التزهير الكامل في الموسم الثاني علي التوالي. سجلت بيئة WPM2 للأجنة المفصوله بعد ٧٠ يوم من التزهير الكامل القيمة

الأعلي لإنبات الجنين (٦٢,٠٩%) في الموسم الأول, وبعد ٧٠, ٦٠ يوم من التزهير الكامل (٦١,٨٤, ٦١,٧٣%) في الموسم الثاني علي التوالي. وقد أدت إضافه IBA أو NAA بتركيز ٣ مللجم/لتر إلي بيئة MS نصف قوة إلي الحصول علي النسبة الأعلي من التجذير, ومتوسط الحد الأقصي من عدد الجذور/فرع , وطول الجذر. مما سبق يمكن التوصية لبرنامج تربية وتحسين الخوخ صنف فلوردا برنس بأن أنسب موعد لفصل الجنين بعد إجراء التلقيح هو (٦٠ – ٧٠ يوم من التلقيح أو الإزهار الكامل) عن طريق زراعة البويضات على بيئة WPM1 مع المعاملة البرودية على ٤° م لمدة ٤ أسابيع ثم نقل الأجنة المفصولة إلى بيئة WPM2 للإنبات, وبعد ذلك التجذير والوصول إلي نبات كامل.