Middle East Journal of Applied Sciences
Volume: 13 | Issue: 02| April – June | 2023

EISSN: 2706 -7947 ISSN: 2077- 4613
DOI: 10.36632/mejas/2023.13.2.17
Journal homepage: www.curresweb.com
Pages: 206-218

Micropropagation of Avocado cv. Fuerte by Embryo Culture Techniques

[1]Rohim F. M., A. Abd El-Hamid[2], Eman A. Abd El-Moniem[1] and T. F. El-Sharony[1]

[1]Horticultural Crops Technology, National Research Centre, Dokki, Giza, Egypt.
[2]Horticulture Dept., Faculty of Agriculture, Ain Shams University, Egypt.

Received: 20 Jan. 2023 **Accepted:** 30 Mar. 2023 **Published:** 20 April 2023

ABSTRACT

Embryo rescue is an *in vitro* technique that has been used to save the hybrid products of fertilization especially when they might be degenerated. A protocol for *in vitro* Zygotic embryo age micro propagation of Avocado (*Persea americana,* Mill) cv. Fuertes Was development during two successive seasons (2011 and 2012) in the tissue culture laboratory, Horticulture Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Khima, Egypt. The effect of sterilization with Clorox, embryo age from full bloom, medium type and sucrose concentration were investigated during establishment stage. The effect of medium types (MS&B5), Cytokinin type (Kinetin & BAP) and Subcultures number in multiplication stage were study. The effect of auxin type (IBA and NAA) at different concentration (0.5, 1.0,2.0 and 4.0 mg/L treatment in rooting stage. Different medium combinations (Sand: Prelate: Vermiculite) with different ratio (by volume) were tested during acclimatization stage. The obtained results showed that, sterilization the avocado embryos with Clorox at 30% gave the lowest Contamination and highest survival percentages. Culture of embryos after 180 and 210 days from Full bloom gave the highest germination percentage on MS&B5 media containing either 30 or 45 g/L Sucrose of avocado embryos. While, embryos cultured after 180 and 210 days from full bloom on MS medium Supplemented with 30 g/L sucrose are the most suitable for germination percentage and average shoot length. During multiplication stage, MS medium recorded the highest average shoot length as compared with B5 medium. In addition BAP at different concentrations surpassed Kinetin in on creasing number of proliferated shoots/explants. Also, Kinetin surpassed BAP in increased average shoot length. The second subculture gave number of proliferated shoots/explants. IBA at 2-0 mg/L to the culture medium increased *in vitro* rooting percentage, number of roots and root length of avocado Cv. Fuerte. Medium mixtures of sand: peat: vermiculite at (1: 2: 1 by volume) recorded the highest survival percentage during acclimatization stage. Sterilization the avocado embryos with Clorox at 30% gave the lowest Contamination and highest survival percentages. Culture of embryos after 180 and 210 days from Full bloom gave the highest germination percentage on MS&B5 media containing either 30 or 45 g/L Sucrose, respectively, without significant differences between them of avocado embryos to rescue of sexual zygote of avocado varieties.

Keywords: Avocado*, Persea americana* Mill, Fuerte, propagation, embryo culture, Sterilization

1. Introduction

The avocado (*Persea americana* Mill) originally to *Lauraceae* family that originated in Guatemala and Mexico but it's now grown throughout the tropical and subtropical countries.

Avocado fruit has a high value in its native regions, where it is considerable as an essential component of the daily diet due to its high oil content and well balanced supply of proteins, carbohydrates, minerals and vitamins. In addition, the avocado fruit has been gaining acceptance within North America and European consumers as a salad additive (Knight, 2002). Avocado oil is also used as a skin softener in some cosmetic products (Olaeta, 2003). This species is highly het-erozygous, with alone juvenile phase and a high rate of flower abscission and immature fruit drop (Litz *et al.,* 2005).

Corresponding Author: Rohim F.M., Horticultural Crops Technology, National Research Centre, Dokki, Giza, Egypt. Email: - aridrohim@gmail.com

Due to these problems, breeding programs have been relatively failed, and the most important cultivars have been obtained by open-pollinated tree selection (Litz *et al.,* 2007).
Embryo rescue is an *in vitro* technique that has been used to save the hybrid products of fertilization especially when they might be degenerated.

In vitro embryo age may allow premature embryos to be able to germinate along with change in plants (Burgos and Ledbetter, 1993). Embryos saving occurred to be created for many species and so are generally used in breeding programs. This technological know-how permit the actual restoration of an enhanced progeny, which includes progeny from interesting crosses that will usually lost (Sharma *et al.,* 1996).

The culture of immature embryos is used to rescue embryos that will abort or others that would not undergo the progressive sequence of ontogeny. This process is very difficult due to the tedious dissection necessary and the complex nutrient medium requirements. This type of culture could be easy and successfully depends strongly on the developmental stage of the embryo when it is isolated. The culture of mature embryos from ripened seeds is used to eliminate seed germination inhibitors or to shorten the breeding cycle.

The aim of the current investigation is to develop an *in vitro* culture protocol for recovery of immature avocado embryos by manipulating culture media and some other conditions. Along this manual, the effects of Clorox concentration, the age of the embryo on contamination and survival percentage, the effect of sucrose concentration and medium types on germination percentage and average shoot length of germinated embryos of avocado cv. Fuerte were studied. Also, cultivation of the plants resulting from embryos culture and study the effect of medium type, cytokinin and subcultures number in multiplication stage and auxin treatment in rooting stage.

2. Material and Methods

This investigation was carried out during 2011 and 2012 in the Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture; Ain shams University, Cairo, Egypt.

2.1. Culture media and incubation condition

Two types of media presented MS (Murashige and Skoog, 1962) and B5 (Gamborg *et al.,* 1968) at full strength were solidified with bacto agar at 7g/L. The pH was adjusted to 5.7 using NaOH and HCl. The media were autoclaved at 100 K. pa (15 P.S.I) and 121° C for 20 minutes, then the media left to cool and harden for 24 hours before being used.

2.2. Plant material and embryo culture

Avocado fruits (*Persea americana* Mill.) cv. Fuerte were collected from a private orchard located at Giza governorate. The fruits were collected in monthly intervals from full bloom to fruit maturity. Fruits of avocado were harvested at monthly intervals (30, 60, 90, 120, 150, 180 and 210 days) from full bloom. Fruits were surface sterilized by immersion in 25% Clorox solution, (5.25% (v/v) sodium hypochlorite solutions) containing Tween 20 (1 drop/100 ml) for 20 min, and rinsed three times in sterile distilled water at 10 min for each. Fruits were carefully cut lengthways under sterile conditions and zygotic embryos were separated from seeds with a small part of cotyledons. Avocado embryos were cultured in two types of medium MS (Murashige and Skoog, 1962) and B5 (Gamborg *et al.,* 1968) at full strength containing30 or 45 g/l sucrose. The pH of all media was adjusted to 5.7± 0.02 before autoclaving. Media were solidified using Bacto agar at 7g/l. twenty five ml of medium was dispensed into glass jars (350 ml) capped with plastic caps. The media were autoclaved at 121ºC and 100 KPa for 20 min. Avocado embryo cultures were incubated at 25± 2°C. Cultures were incubated in darkness during embryo germination stage then transferred to light intensity of about 2500 lux, the photoperiod was 16 h light and 8 h dark. Germination percentage and average shoot length (mm) were recorded after 6 weeks. Embryos were considered germinated when shoot elongation was >5 mm.

2.3. Effect of Clorox concentration and embryo age on contamination and survival percentage of avocado cv. Fuerte

Fruits collected after 30, 60, 90, 120, 180, 210 days after full bloom were surface sterilized by immersion in Clorox solution at (20, 25, and 30%) (Sodium hypochlorite solutions 5.25% available

chlorine containing Tween 20 (1drop/100 ml) for 25 min, and rinsed three times with sterile distilled water at 10 min for each. Fruits were carefully cut lengthways under sterile conditions and zygotic embryos were separated from seeds with a small part of cotyledons.

Contamination percentage, survival percentage were recorded after 4 weeks of avocado embryo cv. Fuerte. Each treatment consisted of three replicates and each replicate represented by five explants.

2.4. Effect of embryo age from full bloom, sucrose concentration and medium type on germination percentage and average shoot length of germinated embryos of avocado cv. Fuerte

Fruits of avocado cv. Fuerte were harvested at monthly intervals 30, 60, 90, 120, 150, 180 and 210 days from full bloom to fruit maturity. Zygotic embryos were cultured on two medium types (MS and B5) at full strength containing30 or 45 g/L sucrose. Germination percentage and shoot length (mm) were recorded after 6 weeks. Embryo were considered germinated when shoot elongation was >5 mm.

2.5. Effect of medium type, cytokinin and number of subcultures on number and length of proliferated shoots during multiplication stage

Stem node explants about 1-2 cm length produced from germinated embryos during the previous stage were cultured on multiplication medium (MS and B5) at full strength containing kinetin (kin) at 5, 10 and 20 mg/l or benzyl adenine (BA) at 1, 2 and 5 mg/l. All media were containing IBA at 0.2 mg /l. The proliferated shoots were subcultured into fresh medium after four weeks for six subcultures. Number of proliferated shoots/explant and average shoot length (mm) were recorded after four weeks.

2.6. Effect of auxin on number of roots/explant and average root length during rooting stage

Shoots about 3-5 cm of avocado cv. Fuerte produced after the 6^{th} subculture were transferred to half strength MS medium containing IBA or NAA at 0.5, 1, 2, and 4 mg /l. Rooting percentage, number of roots and average root length (mm) were recorded after six weeks on rooting medium.

2.7. Effect of medium combinations on survival percentage of avocado cv. Fuerte plantlets during acclimatization stage

Plantlets of avocado cv. Fuerte were rinsed carefully with sterile distilled water to remove adhering medium and transplanted into plastic pots (100 ml) containing a combinations of sand: peat: vermiculite with different ratio (by volume) at (1:1:1),(1:2:1),(1:1:2),(2:1:1),(2:2:1),(2:1:2),(1:2:2). Plantlets were incubated under greenhouse conditions. Survival percentages were recorded after six weeks from transplanting.

2.8. Experimental design and statistical analysis

All experiments were arranged in a completely randomized design. Each treatment contained three replicates with 5 explants for each replicate. Recorded data were subjected to Analysis of Variance (ANOVA). Duncan's multiple range test was employed for means comparisons according to Snedecor and Cochran (1982).

3. Results

3.1. Establishment stage

3.1.1. Surface sterilization

Table (1) shows the effect of clorox concentration, embryo age and their interaction on contamination and survival percentages of avocado embryos c.v. Fuerte. His clear that the lowest contamination (15.55%) and the highest survival % were exhibited with Clorox at 30%. on the other hand, using Clorox at 20% gave the highest contamination and the lowest survival percentage. However, the highest contamination % were recorded with embryos cultured 180 and 210 days after full bloom. Also, the lowest value was noticed with 180 days after full bloom. In addition, the highest survival % was obtained with embryos culture bo and 90 days after full bloom. The interaction between Clorox concentration and embryo age found that the lowest contamination % (13.34%) with Clorox at 30% and embryo cultured 120, 180 and 210 days after full bloom and embryos cultured 30,60 and 90 days after full bloom and disinfected with 25% Clorox. Meanwhile, the highest survival% (86-66%) were obtained with Clorox concentration at 30% and embryo age after 30, 60, 90, 120,180 and 210 days

from full bloom as compared with the other used treatment. Generally, the lowest contamination percent and the highest survival percentage were exhibited with Clorox concentration at 30% and embryo age 120,180 and 210 days after full bloom. These results are agreement with the finding of Shahin *et al.,* (2003) on mango, and Simon *et al.,* (2008) on avocado. They declared that Sodium by hypochlorite showed good results in decreased contamination percentage and highest survival percentage.

Table 1: Effect of clorox concentration, embryo age and their interaction on Contamination and survival percentages of avocado embryos cv. Fuerte.

Embryo age after full bloom (days)	Total contamination Clorox concentration %			Mean
	20	25	30	
30	46.67 a	20.0 c	13.34 d	**26.68 B**
60	33.33 b	20.0 c	13.34 d	**22.22 C**
90	26.67 bc	20.00 c	6.67 e	**17.78 D**
120	26.67 bc	33.33 b	20.0 c	**26.66 B**
180	40.00 a	33.33 b	20.0 c	**31.11 A**
210	46.67 a	26.67 bc	20.0 c	**31.11 A**
Mean	**36.67 A**	**25.55 B**	**15.55 C**	
Embryo age after full bloom (days)	**Survival % Clorox concentration %**			**Mean**
	20	**25**	**30**	
30	46.63 d	73.33 ab	86.66 a	**68.87 B**
60	60.0 b	73.33 ab	86.66 a	**73.33 A**
90	66.66 b	80.00 a	93.33 a	**79.99 A**
120	66.66 b	60.00 b	80.00 a	**68.88 B**
180	46.66 d	60.00 b	73.33 ab	**59.99 C**
210	46.66 d	66.66 b	80.00 a	**64.44 BC**
Mean	**55.54 C**	**68.88 B**	**83.33 A**	

Means in each column and rows with similar letter (s) are not significantly different at 0.05%.

3.1.2. Effect of embryo age, medium type and sucrose concentration.

Data presented in table (2-a) showed that, the highest of germination percentage (10.00) and average shoot length (52.0 and 49.3 mm) were recorded with embryos cultured after 180 and 210 days from full bloom followed by embryos culture after 120 and 150 days from full bloom as compared with the other embryo age after full bloom. However, Data in table (2-b) indicated that MS medium recorded higher significant average shoot length (30.6 mm) as compared with B5 medium. on the other hand, no significant different between both MS and B5 medium when germination percentage was concerned. Table (2-b) showed that embryos cultured after 180 and 210 days on MS & B5 medium recorded the highest germination percentage (100%) as compared with the other used treatments. In addition, embryos cultured after 210 days from full bloom on MS medium and embryo cultured 180&210 days from full bloom on BS medium gave the highest significant average shoot length.

Table 2: Effect of embryo age, medium type and sucrose concentration on germination percentage and average shoot length (mm) of avocado cv. Fuerte embryos.

Table 2-a: Effect of embryo age.

Embryo age after full bloom (days)	Germination %	Average shoot length (mm)
30	20.0 D	12.6 D
60	28.7 C	8.5 E
90	56.7 B	28.2 C
120	75.0 B	39.1 B
150	73.3 B	37.2 B
180	100.0 A	52.0 A
210	100.0 A	49.3 A

Means in each column with similar letters are not significantly different at 0.05%.

Table 2-b: Effect of medium type and embryo age date.

Medium type	Embryo age after full bloom(days)	Germination %	Average shoot length (mm)
MS	30	20.0 d	17.2 g
	60	24.0 c	7.0 i
	90	56.7 b	32. 8 d
	120	76.7 b	45.7 c
	150	76.7 b	44.6 c
	180	100.0 a	49.8 b
	210	100.0 a	51.3 ab
B5	30	20.0 d	8.0 hi
	60	23.3 d	10.2 h
	90	56.7 b	23.5 f
	120	73.3 b	32.5 de
	150	70.0 b	29.8 e
	180	100.0 a	54.2 a
	210	100.0 a	51.6 ab

Means in each column with similar letters are not significantly different at 0.05%

Data in table (2-c) indicated that embryos age after 180&210 days from full bloom on both MS&B5 medium supplemented with sucrose at 30 and 45 g/L gave the highest germination percentage (100%). Meanwhile, embryos after 180 and 210 from full bloom on MS medium supplemented with sucrose at 30 g/L gave the highest significant shoot length followed by embryos cultured after 180 days from full bloom on MS medium supplemented with 30 g/L sucrose. On the other hand, the lowest values were noticed with embryos cultured after 60 days from full bloom on MS medium with sucrose at 45 g/L and B5 medium with 30 g/L sucrose. In general, embryos age after 180 and 210 days from full bloom on MS medium Supplemented with 30 g/L sucrose are the most suitable for germination percentage and average shoot length of avocado.

Table 2-c: Effect of medium type, sucrose concentration and embryo age.

Medium type	Sucrose conc.	Germination %						
		Embryo age after full bloom(days)						
		30	60	90	120	150	180	210
MS	30 g/l	20.0e	28.0e	60.0cd	80.0b	80.0b	100.0a	100.0a
	45 g/l	20.0e	20.0e	53.3d	73.3bc	73.3bc	100.0a	100.0a
B5	30 g/l	20.0e	20.0e	53.3d	66.7c	66.7c	100.0a	100.0a
	45 g/l	20.0e	26.7e	60.0cd	80.0b	73.3bc	100.0a	100.0a
Medium type	**Sucrose conc.**	**Average shoot length (mm)**						
		Embryo age after full bloom(days)						
		30	60	90	120	150	180	210
MS	30 g/l	22.7jk	9.3n	37.0 f	50.3cd	54.0c	66.0a	60.4b
	45 g/l	11.6mn	4.7o	28.7hi	41.0e	35.2f	33.7fg	55.0c
B5	30 g/l	8.0no	3.9o	25.3ij	20.7k	30.0gh	48.3d	53.4cd
	45 g/l	8.0no	15.7l	21.7jk	44.3e	29.6 h	60.0b	51.3cd

Means in each column or row with similar letters are not significantly different at 0.05%.

3.2. Proliferation stage

3.2.1. Effect of medium types, cytokinin treatments and number of subcultures.

Data in table (3-a) showed that BAP at different concentrations gave higher number of proliferated shoots/explants (1.4,1.3 and 1.4) followed by kin at 20 mg/L than kin at 10 mg/L as compared with kin at 5 mg/L in a descending Order. On the other hand, kin at 5 and 20 mg/L gave the highest significant average shoot length (27.1&25.4 mm) without significant difference between then. Data in table (3-b) revealed that, the second subculture recorded the highest significant number of shoots (1.7) followed by the third subculture (1.2). While, the lowest value was noticed with the 4th subculture. However, the first subculture achieved the highest significant average shoot length (33.9 mm) followed by the second subculture (31.5 mm) Also, the lowest value of average shoot length (18.9&20.6 mm) was found with the 4th and 5th subcultures.

Table 3: Effect of cytokinin treatments and number of subcultures on number of proliferated shoots/explant and average shoot length (mm) of avocado cv. Fuerte during multiplication stage.

Table 3-a: Effect of cytokinin treatments.

Cytokinin treatment (mg/L)	Number of proliferated shoots/explant	Average shoot length (mm)
Kin 5.0	0.3 D	27.1 A
Kin 10.0	0.5 C	24.6 BC
Kin 20.0	0.9 B	25.4 AB
BA 1.0	1.4 A	21.4 D
BA 2.0	1.3 A	24.3 BC
BA 5.0	1.4 A	23.2 CD

Means in each column with similar letters are not significantly different at 0.05%.

Table 3-b: Effect of number of subcultures.

Number of subcultures	Number of proliferated shoots/explant	Average shoot length (mm)
1st Subculture	0.98 C	33.9 A
2nd Subculture	1.7 A	31.5 B
3rd Subculture	1.2 B	23.1 C
4th Subculture	0.6 E	18.0 E
5th Subculture	0.7 DE	18.9 DE
6th Subculture	0.8 CD	20.6 D

Means in each column with similar letters are not significantly different at 0.05%

The interactions between medium type, cytokinin treatments and number of subcultures, data in table (3-c) showed that the highest number of proliferated shoots/explant was achieved with B5 medium supplemented with BA at 1.0, 2.0 and 5.0 mg/L during the2nd subculture without significant differences among them. On the other hand, the lowest values were recorded with B5 medium supplemented with Kin at 5.0 and 10.0 mg/L during the 4th, 5th and 6th subculture and MS medium with Kin at 5.0 and 10.0 mg/L during the 4th and 5th subculture. MS medium supplemented with Kin at 5.0, 10.0 and 20.0 mg/L during the 1st and 2nd subcultures gave the highest average shoot length without significant differences among them. On the other hand, the least values were noticed with B5 medium supplemented with BA at 1.0 mg/L during the 5th and 6th subcultures.

Table 3-c: Effect of medium type, cytokinin treatments and number of subcultures.

Medium type	Cytokinin treatments (mg/L)	Number of proliferated shoots/explants					
		Number of subcultures					
		1st	2nd	3rd	4th	5th	6th
MS	Kin 5.0	0.6 ijk	0.6 ijk	0.6 ijk	0.2 k	0.2 k	0.4 jk
	Kin 10.0	0.8 hij	0.8 hij	0.6 ijk	0.2 k	0.2 k	0.6 ijk
	Kin 20.0	1.2 fgh	1.4 efg	0.8 hij	0.4 jk	0.8 hij	0.8 hij
	BA 1.0	2.0 cd	2.2 bc	1.6 def	1.4 efg	1.2 fgh	1.2 fgh
	BA 2.0	1.8 cde	1.4 efg	1.2 fgh	1.5 def	1.0 ghi	0.9 ghi
	BA 5.0	1.6 def	1.4 efg	1.2 fgh	1.2 fgh	1.4 efg	1.4 efg
B5	Kin 5.0	0.2 k	0.6 ijk	0.6 ijk	0.2 k	0.2 k	0.2 k
	Kin 10.0	0.6 ijk	1.4 efg	1.0 ghi	0.2 k	0.2 k	0.4 jk
	Kin 20.0	0.4 jk	2.0 cd	1.6 def	0.4 jk	0.6 ijk	0.6 ijk
	BA 1.0	0.8 hij	2.6 ab	1.8 cde	0.6 ijk	0.8 hij	1.4 efg
	BA 2.0	0.8 hij	2.6 ab	2.0 cd	0.8 hij	1.0 ghi	0.8 hij
	BA 5.0	1.0 ghi	3.0 a	1.8 cde	1.0 ghi	1.2 fgh	1.6 def
Medium type	**Cytokinin treatment (mg/L)**	**Average shoot length(mm)**					
		Number of subcultures					
		1st	2nd	3rd	4th	5th	6th
MS	Kin 5.0	49 a	47 a	27 e-i	16 nop	23 h-m	16.3 nop
	Kin 10.0	47 a	43 ab	18.7 k-o	19 k-o	21 i-o	18 l-p
	Kin 20.0	45 ab	45 ab	23 h-m	21 i-o	20 j-o	15.3 op
	BA 1.0	39 bc	32 def	20 j-o	22 h-n	17 m-p	16 nop
	BA 2.0	44 ab	35 cd	24 h-l	22 h-n	17 m-p	23 h-m
	BA 5.0	36 cd	32 def	31 d-g	16 nop	23.3h-m	20 j-o
B5	Kin 5.0	33.3cde	24 h-l	25 g-k	17 m-p	20 j-o	28 e-h
	Kin 10.0	25 g-k	24 h-l	23 h-m	15 op	17 m-p	24 h-l
	Kin 20.0	21 i-o	27 e-i	21 i-o	20 j-o	21 i-o	26 f-j
	BA 1.0	20 j-o	22 h-n	21 i-o	12 p	15 op	21 i-o
	BA 2.0	27 e-i	25 g-k	18 l-p	21 i-o	16 nop	19 k-o
	BA 5.0	21 i-o	22 h-n	25 g-k	15 op	17 m-p	20 j-o

Means in each column or row with similar letters are not significantly different at 0.05%.

3.3. Rooting stage

Table (4) showed the effect of auxin type and concentrations on rooting percentage, root number and root length of avocado cv. Fuerte microshoots. In is clear that supplementation of culture medium with 2.0 mg/L IBA caused a significant increase of rooting percentage. While, addition of either IBA at 2.0 and 4.0 mg/L or NAA at 2.0 mg/L gave the highest significant number of roots. However, supplementation of culture medium with 2.0 and 4.0 mg/L IBA and NAA recorded the highest significant root length. On the other hand, supplementation of culture medium with 0.5 mg/L NAA caused a significant decrease of rooting percentage and number of roots/micro shoots (Rohim *et al.,* 2013).

In addition, the lowest significant value of average root length was observed with IBA at 0.5 mg/L and NAA at 0.5 mg/L in micro shoots of avocado cv. Fuerte.

Table 4: Effect of different auxin treatments on rooting percentage, root number and root length (cm) of avocado cv. Fuerte microshoots during rooting stage.

Auxin treatments (mg/L)	Rooting %	Number of roots / microshoot	Average root length (cm)
IBA at 0.5	36 C	2.41 C	2.30 C
IBA at 1.0	40 B	2.76 BC	3.66 BC
IBA at 2.0	52 A	3.43 A	5.23 A
IBA at 4.0	48 B	3.21 AB	4.50 AB
NAA at 0.5	28 D	1.0 D	2.10 C
NAA at 1.0	36 C	2.33 C	3.73 BC
NAA at 2.0	44 B	3.02 AB	5.10 AB
NAA at 4.0	48 B	2.36 C	5.67 A

Means in each column with similar letters are not significantly different at 0.05%.

3.4. Acclimatization stage

As for the effect of medium mixtures on survival percentage of avocado cv. Fuerte, data illustrated in Table (5) in dictated that medium mixture continuing sand: peat : vermiculite (1:2:1 by volume)recorded the highest survival percentage (53.33%) followed by medium mixture continuing sand peat: vermiculite (2:2:1 by volume), and sand : peat : vermiculite (1:2:2 by volume) then medium mixture continuing sand : peat : vermiculite (2:1:1: by volume) as compared with both medium mixture continuing sand : peat: vermiculite (1:1:1 by volume) and sand: peat : vermiculite (1:1:2 by volume) in a descending order. Results clearly indicated that medium mixtures of sand: peat: vermiculite at 1:2:1 by volume recorded the highest survival percentage of avocado cv. Fuerte. These results are in accordance with those found by Bondouk *et al.* (1989) on banana, Abd El-Gawaad *et al.* (2006) on banana and Cortina (2008) on pear. They found that the combination treatment of different medium mixture continuing such as sand, peat, vermiculite, perlite and loan induced the highest survival percentage.

Table 5: Effect of medium mixtures on survival% of avocado cv. Fuerte plantlet during acclimatization stage.

Medium mixtures	Survival % of cv. Fuerte
Sand: Peat: Vermiculite 1 : 1 : 1	20.00 C
Sand: Peat: Vermiculite 1 : 2 : 1	53.33 A
Sand: Peat: Vermiculite 1 : 1 : 2	20.00 C
Sand: Peat: Vermiculite 2 : 1 : 1	26.67 BC
Sand: Peat: Vermiculite 2 : 2 : 1	53.33A
Sand: Peat: Vermiculite 2 : 1 : 2	40.00 B
Sand: Peat: Vermiculite 1 : 2 : 2	53.33 A

Means in each column with similar letters are not significantly different at 0.05%.

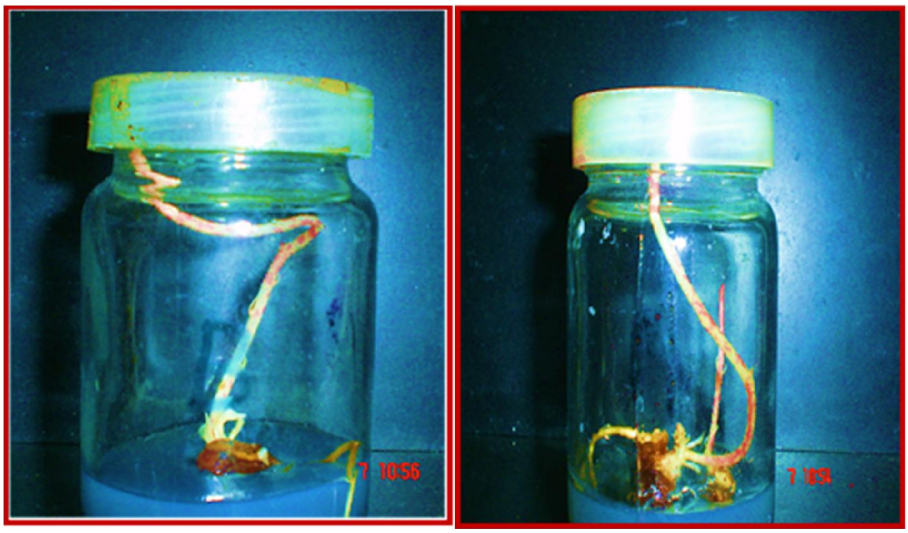

Plate 3: Effect of embryo age and medium type on germination % and average shoot length (mm) of avocado cvs Fuerte embryos.

a. Germination of avocado cv. Fuerte embryos cultured 210 days after full bloom on B5 medium.
b. Germination of avocado cv. Fuerte embryos cultured 210 days after full bloom on B5 medium.

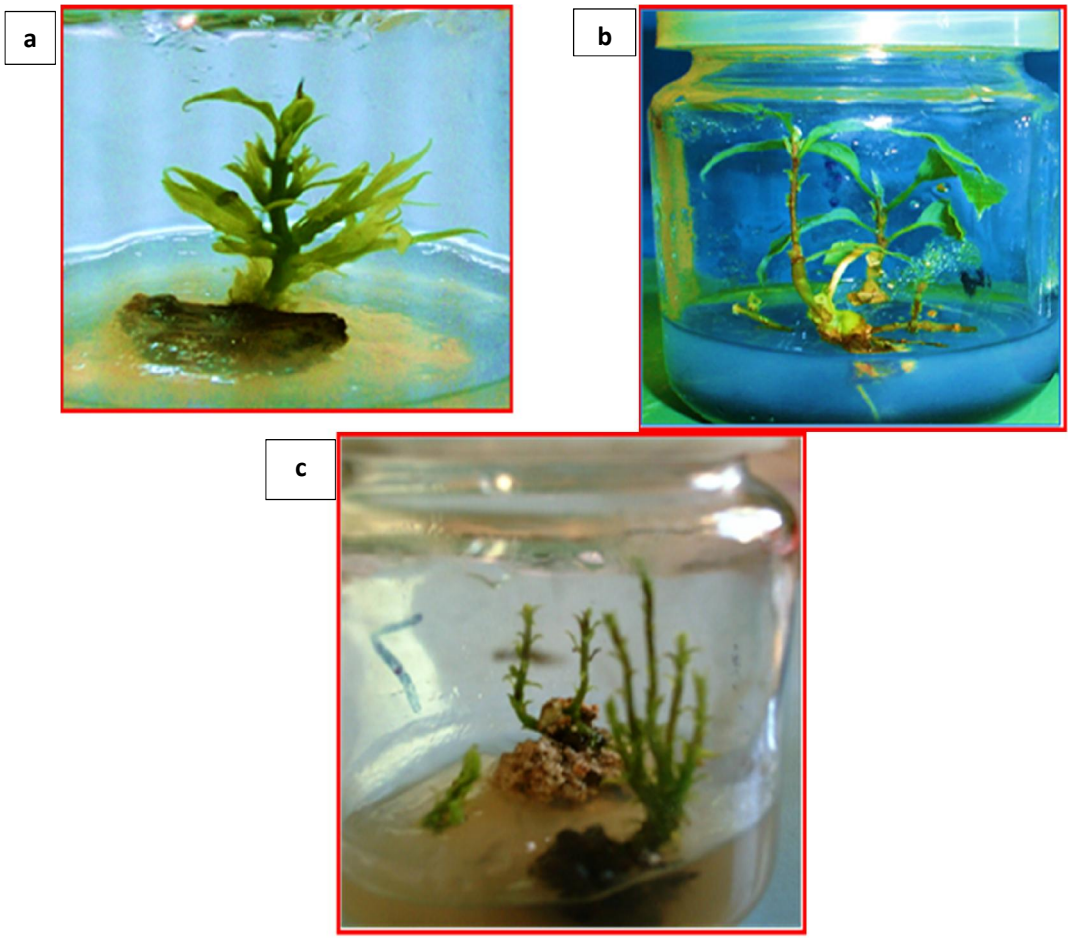

Plate 5: Effect of cytokinin treatments and number of subcultures on number of proliferated shoots/ explants of avocado cv. Fuerte during multiplication stage.

a. Shoots of avocado cv. Fuerte on MS medium with BA at 2.0mg/L in the 2nd subculture.
b. Shoots of avocado cv. Furete with MS medium with BA at 2.0mg/L in the 5th subculture.

c. Shoots of avocado cv. Furete with B5 medium with BA at 5.0mg/L in the 2nd subculture.

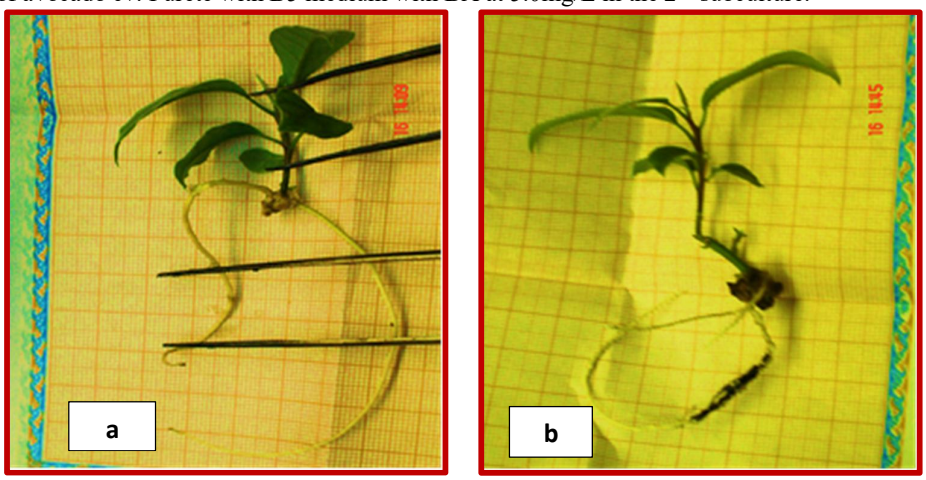

Plate 7: Effect of auxin type (IBA and NAA) and concentrations on rooting percentage, number of roots/ microshoot and root length (mm) of avocado cv. Fuerte during rooting stage.
a. Rooting of avocado cv. Fuerte microshoots on MS medium with IBA at 2 mg/L.
b. Rooting of avocado cv. Fuerte microshoots on MS medium with IBA at 4 mg/L.

4. Discussion

In Establishment stage, these results are in agreement with the finding of Peran- Quesada *et al.,* (2005) on avocado, Skene and Barlass (1983) on avocado and Sanchez-Romero *et al.,* (2002) on avocado. They found that *in vitro* embryo germination MS medium gave the higher average shoot length compared with B5 medium. In addition, Rai *et al.,* (2009) on guava, Fuentes *et al.,* (2003) on avocado and Nower (2013) on mango, they reveal et that increase of sucrose can cent rations resulted in lower germination percentage. Cultured on the medium with 30 g/L sucrose gave the highest significant average shoot length (Rohim *et al.,* 2013).

In general, during multiplication stage, MS Medium recorded the highest average shoot length of avocado cv. Hass as compared with B5 medium. In addition, BAP at different concentrations surpassed kinetin in increasing number of proliferated shoots/ explants. Also, kinetin surpassed BAP in increased average shoot length. However, the second subculture gave number of proliferated explants as compared with the other subcultures with *in vitro* multiplication of Avocado. These results are somewhat go in line with the findings of Peran- Quesade *et al.,* (2004) on acocado, Ahmed *et al.,* (2001) on avocado, Nookaragu (2007) on grapevine, Hassanien (2003) on olive and Mustafa and Rania (2012). They declared that, MS medium and BAP at different concentrations showed a good result during multiplication stage. The first, and second subculture gave maximum multiplication as compared with other subcultures.

In rooting stage, the above mentioned results concluded that addition of IBA at 20 mg/L to the culture medium increased *in vitro* rooting percentage, number of roots and root length of avocado cv. Fuerte. These results are in general agreement with the finding of Peran-Quesada *et al.,* (2004) on avocado, Ahmed *et al.,* (2001) on avocado, Natalia *et al.,* (2006) on prunus and Zamir *et al.,* (2007) on guava. They found that IBA at 2.0 mg/L was the most effective treatments during rooting stage. On the other hand, El-salem *et al.,* (2008) indicated that, the least values of rooting percentage, number of roots and root length of apricot were noticed with all NAA tested concentration compared with IBA.
In Accimatization stage, results clearly indicated that medium mixtures of sand: peat: vermiculite at 1: 2: 1 recorded the highest survival percentage of avocado cvs, Hass and Fuerte. This results are in accordance with those found by, AbdEl-Gawaad *et al.* (2006) indicated that the combination treatment (25% vermiculite + 25% Perlite + 25% sand + 25% loam) induced the highest survival percentage of banana shoots and improved shoot length, shoot thickness, leaf length, root length and roots number parameters. Moreover, Bondouk *et al.* (1989) found that the mixture of peat-moss, sand and clay at the rate of 1: 1: 1 was the best medium for banana acclimatization and plant growth which expressed as number of leaves.

According to Yepes and Adwinckle (1994) lack of vascular connections between roots and shoots was implicated in low survival of *in vitro* rooted apple plantlets after transfer to the soil. Similarly, indirect *in vitro* rhizogenesis through callus formation can be one of the reasons for low percentage of acclimatization in Gisela 5, as was shown in pear cultivar Bartlett (Bommineni *et al.,* 2001).

Corina, (2008) refers that the best results obtained within the acclimatization process of vitro plants belonging to pear cultivars are quantified from 79.0 – 70.0% acclimatized plants. The quince cultivars showed superior acclimatization degrees such as 82.0 – 80.0% for the best variants. Pear tree cultivars showed the highest acclimatization degree on nutritive mixture that contains perlite and red peat in proportion 2:1. The red peat assures an optimal humidity in the culture sub-layer. Rooted shoots of guava with 4-5 fully expanded leaves were planted in 7.5-cm in diameter plastic pots containing a mixture of sand and garden soil (3:1), covered with polyethylene bags for 15-20 days to prevent excessive water loss and watered twice a week. Approximately 90.0% plants successfully acclimatized after 4-6 weeks from transplantation to the mixture of sand and soil (Rai *et al.,* 2009).

5. Conclusion

Embryo culture is a good way to safe hybrids and rescue sexual embryo of avocado cultured in MS and B5 medium with 30g/l sucrose with best germination percentage at 180 and 210 days embryo age after full bloom. The best number of proliferated shoots/explant and average shoot length (mm) were occurred with MS and B5 medium supplemented by BA at 5.0 and 2.0 mg/l in first subculture in proliferation stage. In is clear that supplementation of culture medium with 2.0 mg/L IBA caused a significant increase of rooting percentage. While, addition of either IBA at 2.0 and 4.0 mg/L or NAA at 2.0 mg/L gave the highest significant number of roots in rooting stage. The medium mixture continuing sand: peat: vermiculite (1:2:1 by volume) recorded the highest survival percentage (53.33%) in acclimatization stage (Rohim *et al.,* 2013).

References

Abd El-Gawad, N.M.A., 2006. Studies on acclimatization of some tissue cultured monocotyledons of fruit plants. Ph. D. Dissertation, Department of Horticulture faculty of agriculture, moshtohor, Benha university.

Ahmed, M.F., A.S. Kantharajah and P. Holford, 2001. Factors Affecting Adventitious shoot and root formation from the avocado cultivar 'Hass' (*Persea americana*, Mill.) *in vitro*. Gartenbauwissenschaft, 66(6): 293–297 (CAB Abst.).

Barcelo-Munoz, A., C.L. Encina, E. Simon-Perez and F.P. Alfaro, 1999. Micropropagation of adult avocado. Plant Cell, Tissue and Organ Culture, 58: 11–17.

Bommineni, V.R., H. Mathews, S.B. Samuel, M. Kramer and D. R. Wagner, 2001. A new method for rapid *in vitro* propagation on apple and pear. HortScience, 36: 1102–1106.

Bondok, A. Z., S. Z. El- Agamy and H. Gomaa, 1989. *In vitro* propagation of Mariana 2624 plum rootstock. Egyptian journal of Horticulture, 16(1): 9-16.

Burgos, L. and C.A. Ledbetter,1993. Improved efficiency in apricot breeding: Effects of embryo development and nutrient media on *in vitro* germination and seedling establishment. Plant Cell Tissue Org. Cult. 35: 217–222.

Bushra, Z., N.A. Abbasi, T. Ahmad and I.A. Hafiz, 2009. Effect of explant sources and different concentrations of plant growth regulators on *in vitro* shoot proliferation and rooting of avocado (*Prsea americana* MILL.) cv. Fuerte. Pak. J. Bot., 41(5): 2333-2346.

Corina, G.A., 2008. Contributions to setting the *in vitro* multiplication biotechnology for some horticultural species – the pear tree (*Pyrus communis* L.) and the quince tree (*Cydonia oblonga* Mill.) Ph.D. THESIS. Faculty of Horticulture, Craiova University.

EL-Salem, M.G., A.M. El Hammady, Wafaa H. Wanas and A. Abdel-Hamid, 2008. Physiological studies on *in vitro* propagation of some fruit trees. Ph.D. Thiess, Faculty of Agric., Ain Shams University.

Fuentes-Lorenzo, J.L., 2003. Zygotic embryo culture and mutation breeding in avocado (*Persea americana* Mill). Proceedings V World Avocado Congress (Acta V Congreso Mundial del Aguacate) , 73-81.

Gamborg, O.L., R.A. Muller and K. Ojima, 1968. Nutrient requirements of suspension cultures of soybean roots cells. Exp. Cell Res., 50: 151–158.

Hassanien, S.A., 2003. Using tissue culture technique to propagate some Fruit Species of Hard to Root Cutting. Ph.D. Thiess, Faculty of Agric., Ain shams University.

Knight, R.J.J., 2002. History, distribution and uses. In: Whiley AW, Schaffer B, Wolstenholme BN (eds) The avocado: botany, Production and uses. CABI, Wallingford, 1–14.

Litz, R.E., R.S. Witjaksono, D. Efendi, F.P. Alfaro and A. Barcelo-Munoz, 2005. *Persea americana* avocado. In: Litz, R.E. (Ed.), Biotechnology of Fruit and Nut Crops. Biotechnology in Agriculture Series, no. 29. CABI Publishing, Wallingford, 326–347.

Litz, R.E., S. Raharjo and M. Gomez-Lim, 2007. Avocado. In: Pua EC, Davey MR (eds) Biotechnology in agriculture and forestry. Transgenic crops V. Springer, Berlin., 167–187.

Marquez-Martin, B., E.G. Garcıa, A. Barcelo-Munoz; F.P. Alfaro and C.S. Romero, 2009. Effects of an *in vitro* maturation treatment on plant recovery from avocado zygotic embryos. Scientia Horticulturae, 122: 532–539.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473–497.

Mustafa, N.S. and Rania A. Taha, 2012. Influence of plant growth regulators and subculturing on *in vitro* multiplication of some Fig (Ficus Carica. L) cultivars. Journal of Applied Sciences Research, 8(8): 4038- 4044.

Natalia, K. and K. Manana, 2006. Embryo rescue techniques in prunus l. breeding . journal of fruit and ornamental plant research, 14 (suppl. 1).

Nookaraju, A., M.S. Barreto, G.S. Karibasappa and D. C. Agrawal, 2007. Synergistic effect of CPPU and benzyladenine on embryo rescue in six stenospermocarpic cultivars of grapevine. Vitis, 46 (4): 188–191.

Nower, A.A., 2013. *In vitro* production of somatic embryos from nucellus of mango (Mangifera indica L.). Life Sci. J., 10(2):1164-1174.

Olaeta, J.A., 2003. Industrializacion del aguacate: estado actually Perspectivas futuras. Actas V Cong. Mundial Aguacate, 2:749–754.

Peran-Quesada, R., C.S. Romero, A. Barcelo-Munoz and F. Pliego-Alfaro, 2004. Factors affecting maturation of avocado somatic embryos. Scientia Horticulturae, 102(1): 61–73.

Peran-Quesada, R., C. Sánchez-Romero, F. Pliego-Alfaro and A. Barcelo-Munoz, 2005. Histological aspects of avocado embryo development and effect of developmental stages on germination. Seed Science Research, 15:125–132.

Raghavan, V., 1997. Molecular embryology of flowering plants. Cambridge University Press, Cambridge.

Rai, M.K., V.S. Jaiswal and U. Jaiswal, 2009. Shoot multiplication and plant regeneration of guava (*Psidium guajava* L) from nodal explants of *in vitro* raised plantlets. Journal of Fruit and Ornamental Plant Research, 17(1): 29-38.

Rohim, F.M., W.H. Wanas, A.B. Abou Aziz, A. Abd El-Hamid and Eman A. Abd El-Moniem, 2013. *In vitro* rescue and regeneration of zygotic embryos of Avocado (*Persea americana* Mill.) cv. Hass. Journal of Applied Sciences Research, 9(7): 4132-4141.

Sanchez-Romero, C., R. Peran-Quesada, A. Barcelo-Munoz and F. Pliego-Alfaro, 2002. Variations in storage protein and carbohydrate levels during development of avocado zygotic embryos. Plant Physiol. Biochem., 40(12): 1043–1049.

Sendecor, G.W. and W.G. Cochran, 1982. Statistical Methods. 7th Ed. The Iowa state Univ. Press. Ames, USA.

Sharma, D.R., R. Kaur and K. Kumar, 1996. Embryo Rescue in Plants. A review. Euphytica, 89: 325–337.

Simon, H.T. Raharjo and N.F.N. Witjaksono, 2008. Recovery of avocado (*Persea americana* Mill.) plants transformed with the antifungal plant defensin gene PDF1.2. *In vitro* Cell. Dev. Biol.-Plant, 44:254 – 262.

Skene, K.G.M. and M. Barlass, 1983. *In vitro* culture of abscised immature avocado embryos. Ann. Bot., 52: 667–672.

Yepes, L.M. and H.S. Adwinckle, 1994. Micropropagation of thirteen Malus cultivars and rootstocks, and effect of antibiotics on proliferation. Plant Growth Regulation, 15:55–67.

Zamir, R., N. Ali, S.T. Shah, T. Muhammad and S.A. Shah, 2007. *In vitro* re-generation of guava (*Psidium guajava* L.) from shoot tips of mature trees. Pak. J. Bot., 39(7): 2395-2398.