10th Conf. Agric. Dev. Res., Fac. Agric., Ain Shams Univ., Cairo, Egypt, 2006
Annals Agric. Sci., Sp. Issue, 1, 163-178, 2006

MICROPROPAGATION OF MEET-GHAMR PEACH AND AL-AMAR APRICOT

[13]

Wanas, Wafaa[1] H.; M.B. Abou-Rawash[1]; A. Abd el-Hamid[1] and A.A. El-Homosany[2]

ABSTRACT

Stem node explants of seedling peach (*Prunus persica*) tree from Meet-Ghamr and seedling apricot (*Prunus armeniaca*) tree from Al-Amar region were successfully established and proliferated *in- vitro*. During the establishment stage, the highest survival percentage of stem node explants and the longest shoots were obtained with MS or WPM medium contained BA at 0.5 mg l^{-1} plus IBA at 0.01 mg l^{-1} for both cultures. In the proliferation stage, using BA at 4.0 mg l^{-1} plus 0.1 mg l^{-1} IBA gave the highest number of proliferated shoots for Meet-Ghamr peach, while the best shoot length was recorded with MS medium plus BA at 2.0 mg l^{-1} and IBA at 0.1 mg l^{-1}. On the other hand, using BA at 2.0 mg l^{-1} plus 0.1 mg l^{-1} IBA gave the highest number of proliferated shoots for Al-Amar apricot, while the best shoot length was recorded with WPM medium plus BA at 1.0 mg l^{-1} and 0.1 mg l^{-1} IBA. The best proliferation medium was used for subculturing every four weeks up till seven subculures. The average number of new proliferated shoots increased as number of subcultures increased up to the 5th subculture then decreased during the 6th and 7th subcultures for apricot cultures, however it decreased significantly after the sixth subculture for peach cultures. The highest average shoot length occured during the 1st and 2nd subcultures then decreased during the 3rd up to the 7th subcultures for the two studied cultures. For Meet-Ghamr peach, the greatest rooting percentage (87.50%) and root length were obtained with half strength MS medium with IBA at 0.2 mg l^{-1} plus NAA at 0.2 mg l^{-1}, while the highest average number was obtained in half strength MS medium with NAA at 0.5 mg l^{-1}. For Al-Amar apricot, the greatest rooting percentage (81.25%) was obtained with half strength MS medium contained NAA at 0.2 mg l^{-1}, the highest average number of roots was obtained with IBA + NAA both at 0.5 mg l^{-1} and the highest average root length was obtained with IBA at 0.5 mg l^{-1}. The rooted shoots of apricot and peaches were succesfully acclimatized with 50.00 %, 66.66 % survival, respectively.

Keywords: Micorpropagtion, Peach, Apricot, Stem node, Multiplication, Rooting, Subcultures, Growth regulators.

1- Department of Horticulture, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt.
2- National Gene Bank, Agriculture Research Center (ARC), Giza, Egypt.

(Received May 30, 2006)
(Accepted August 21, 2006)

INTRODUCTION

Peaches and apricot are grown in temperate zones between latitudes 30° and 45° North and South, also grown through the subtropical and tropical zones but at high elevations (**Bajaj, 1986**). In Egypt, Meet-Ghamr peach (*Prunus persica*) is concentrated in Dakahlia and Al-Amar apricot (*Prunus armeniaca*) is concentrated in Qualubia; are considered the main local cultivars grown in Egypt.

These culivars have been used as a common rootstocks for peach, apricot and sometimes for other stone fruits. Their cuttings are difficult to root in spite of plant growth regulators application at high concentrations, so they alternatively, propagated by budding and grafting. Commonly, rootstocks are propagated by seeds, and thus grafting onto seedling rootstocks, has the potential disadvantage of genetic variation. Thus the growth and performance of the combined plants are variable (**Skirvin, 1984**).

Variations in necessity of media (MS or WPM), benzyl adinen (BA) and indol butric acid (IBA) or naphthaline acetic acid (NAA) used for peach and apricot cultures were obvious in the different reports. For example: in the establishment stage, **Miller *et al*** (1982) found that optimum growth of Nemagaurd peach rootstock with minimum necroses and callus formation occurred on MS medium supplemented with 0.2 (BA) and 0.01 to 1.0 IBA (mg l^{-1}). While, **Harada and Murai** (1996) and **Kandil** (2001) found that shoot proliferation from axillary buds of *Prunus mume* and Canino or Al-Amar apricot was dependent on basal WPM medium rather than MS medium.

In the proliferation stage, **Reeves *et al*** (1983) showed that overall growth of Nemaguard peach rootstock was better on MS medium supplemented with 1.0 mgl^{-1} (BA) and 0.01 mgl^{-1} (IBA). **Fouad *et al*** (1995) found that increasing BA concentration in culture mdium from 1.0 to 5.0 mgl^{-1} BA in combination with IBA increased average shoot number/explant of Nemaguard and Meet-Ghamre peach. Furthermore, **Marino *et al*** (1991) showed that shoot proliferation rate of apricot *Prunus armeniaca* cvs. San Castrese and Protici increased with increasing BA concentration (2.2, 4.4 and 8.8μM) combination with IBA (0.25 μM) and GA3 (0.29 μ M). The subcultures was found to be, also, effective in the proliferation rate by **Parra & Amo-Marco** (1998) and (**Wanas 1999**).

Different concentrations of NAA and IBA were also reported for the rooting of either apricot and peach. **Cos *et al*** (2004) reported that IBA at 1.0 mgl^{-1} was the best concentration for peach–almond hybrid "Mayor Reg" rooting precentage (20%). Also, **Touqeer *et al*** (2004) cleared that the maximum number of roots and length of peach rootstock GF 677 was obtained with IBA at 0.4 mgl^{-1}. While **Yonemitsu *et al*** (2003) found that root fromation of Japanese apricot was induced with 0.54 μM NAA.

This work aimed to improve the propagation of Meet-Ghamre peach and Al-Amar apricot through tissue culture technique by finding out the best simple nutrient media, some growth regulators and their concentration for shoot proliferation, rooting induction and the best subculures number after which the cultures must be renewed.

2-Materials and Methods

This work was carried out during two successive years; from 2003 to 2004 in

the Tissue Culture Laboratory, National Gene Bank, Agricultural Research Center (ARC), Giza, Egypt. The aim was the studying of several factors that affecting *in vitro* propagation of Meet-Ghamr peach and Al-Amar apricot.

Apical parts of shoots 10–15 cm long were taken from an adult selected tree from each seedling tree during the growing season (April–July). The tree of Meet-Ghamr peach was about 20 years old planted in Meet-Ghamr region in Dakahlia governorate, and 40 years old tree of Al-Amar apricot planted in Qualubia governorate.

2.1. Culture procedure

After removing the leaves, shoots were put under running tap water for about one hour, then sterilized under laminar flow hood condition. Sodium hypochlorite solution was prepared using commercial bleach "Clorox" (5.25% available chlorine) at 10 % concentration. The shoots were dipped for 10 minutes in such solution before rinsing three times in sterile distilled water, 5 minutes for each rinsing. After the streilization, stem nodes were cultured in establishment culture media. The pH of different media was adjusted to 5.7 before autoclaving at 100 K. pa (15 P.S.I) and 121°C for 20 minutes, then left to cool and harden for 24 hours before being used. The cultures of different experiments were incubated at temperatures almost maintained between 25 ± 2 °C and photoperiods of 16 hour day and 8 hour night supplied by fluorescent lamp (four lamps per shelf) to provide light intensity of 3000 lux at explants level (30 cm from light).

2.1.1. Establishment stage

Each stem node explant from each cultivar was separately cultured in jars 350 mm filled with 25 ml of full strength **Murashige and Skoog (1962)** (MS) or Woody Plant Medium (WPM) plus 3% sucrose, 0.7 % agar and supplemented with benzyl adenine (BA) at 0.0 (control), 0.2 or 0.5 mgl^{-1} in combination with indole butyric acid (IBA) at 0.0 or 0.01 mgl^{-1}. Survival percentages, and shoot length (cm) were determined after four weeks. The experiment was repeated three times, and was arranged in a completely randomized design with 6 treatments for each cultivar (3 growth regulators x 2 medium types x 1 cultivar) in a factorial experiment with four replicates (three explants for each replicate).

2.1.2. Proliferation stage

2.1.2.1. Effect of auxin / cytokinin levels and medium types

Approximately uniform growing shoots 1.5 cm in length from both seedling trees were aseptically transferred after four weeks to proliferation medium which was consisted of MS or WPM salts and vitamins supplemented with BA at 0.0, 1.0, 2.0 or 4.0 mg l^{-1}, 2iP or Kin at 0.0, 2.0 or 4.0 mg l^{-1} in combination with IBA at 0.1 mg l^{-1}.

The shoots were cultured into glass jars 350 mm filled with 25 ml of medium. The proliferated shoots were subcultured onto fresh medium three times, each of 4 weeks period. Survival percentage, average number and length (cm) of new proliferated shoots were recorded each subculture during the proliferation stage.

The experiment were arranged in a completely randomized design with 16 treatments for each cultivar of (8 growth regulators x 2 medium types x 1cultivar) in a factorial experiment with four replicates (three explants for each replicate).

2.1.2.2. Effect of number of subcultures on survival percentage, number and length (cm) of shoots

The best treatment which recorded the highest proliferation rate was used for this experiment in which the shoots were transferred into fresh medium every four weeks up to 7 subcultures. Survival %, average number and length (cm) of new proliferated shoots were recorded for each subculture for Meet-Ghamr peach and Al-Amar apricot.

2.1.3 Rooting stage

Uniform proliferated shoots about 2 cm in length from the 3rd subculture were transferred to glass jars 350 mm filled with 25 ml of rooting medium which consisted of half strength MS medium plus 3% sucrose, 0.7 % agar and supplemented with either IBA or NAA at 0.0, 0.2, 0.5 or 1.0 mg l^{-1} or their combinations (IBA + NAA both at 0.2, 0.5 and 1.0 mg l^{-1}). The following measurements were recorded after 8 weeks of cultures on rooting medium; rooting percentage, average root number and length (cm).

The experiment was arranged as factorial experiment in a completely randomized design of (10 treatments x 1 cultivar) with four replicates; three explants for each.

Duncan's multiple range test at 5% level was used to verify the differences between means of the treatments (Sendecor and Cochran, 1982) in all the experiments.

2.1.4. Acclimatization stage

Rooted shoots (taken from the best treatment after the 3rd subculture) were rinsed carefully with steril distilled water to remove adhering medium before transplanting to plastic 10 x 15 cm. pots filled with a mixture of peatmoss : sand (1 : 1 by volume) and covered with clear plastic bags then maintained in growth chamber (22 ° C ± 1) for 3 weeks before being transferred to the greenhouse with artificial lighting, and 80% relative humidity for 8 weeks after which success percent were recorded for both studied cultures.

3. RESULTS AND DISCUSSION

3.1. Effect of medium type and growth regulators (BA and IBA) levels on survival percentage and shoot length during establishment stage

Data in Table (1) revealed that the highest mean survival percentage for Meet-Ghamr peach was noticed with MS medium (88.89%) compared to WPM (77.78) without significant difference between them. Specific effect of growth regulator revealed that BA at 0.5 mg l^{-1} plus 0.01 mg l^{-1} IBA exhibited the highest survival percentage. Insignificant differences among all tested BA levels (control, 0.2 and 0.5 mg l^{-1}) percentage were existed. The interactions between the two studied factors showed that MS medium supplemented with BA at 0.5 mg l^{-1} plus IBA at 0.01 mg l^{-1} recorded the highest significant survival % (95.83).

Concerning shoot length there was significant difference between the studied media.

Table 1. Effect of medium type and (BA, IBA) combinations on survival % and shoot length (cm) for nodal explant cultures of Meet-Ghamr peach during establishment stage.

Medium types / Treatments (mg l^{-1})	MS	WPM	MEAN
	Survival %		
Control	83.33bc	75.00d	79.16A`
0.2 BA + 0.01 IBA	87.50 b	81.25bcd	84.37A`
0.5 BA + 0.01 IBA	95.83 a	77.08cd	86.46A`
Mean	88.89A`	77.78A`	
	Avg. shoot length (cm)		
Control	0.52 c	0.33 d	0.42C`
0.2 BA + 0.01 IBA	0.83 b	0.54 c	0.68B`
0.5 BA + 0.01 IBA	1.23 a	0.61 c	0.92A`
Mean	0.86 A`	0.49B`	

Means followed by the same letter (s) are not significantly different from each other at 5% level

The highest mean shoot length (0.86 cm) was obtained by MS medium compared with WPM medium. Specific effect of growth regulator revealed that BA at 0.5 mg l^{-1} plus 0.01 mg l^{-1} IBA exhibited the highest significant mean shoot length (0.92cm). The interactions showed that MS medium supplemented with BA at 0.5 mg l^{-1} plus IBA at 0.01 mg l^{-1} recorded the highest significant shoot length (1.23 cm).

Table (2) data revealed that the highest mean survival percentage (90.27 %) was noticed with WPM medium compared with MS medium without significant difference between them. As for BA and IBA concentrations, insignificant differences were noticed among all tested treatments in mean survival%. The interactions between the two studied variables showed the highest insignificant values with WPM medium plus BA at 0.5, 0.2 mg l^{-1} compared with control (93.75, 91.66 and 85.41).

Table 2. Effect of medium type and (IBA, BA) combinations on survival % and shoot length (cm) for nodal explant cultures of Al-Amar apricot during establishment stage.

Medium types / Treatments (mg l^{-1})	MS	WPM	Mean
	Survival%		
Control	77.08 b	85.41ab	81.25A`
0.2 BA+ 0.01 IBA	81.25 b	91.66 a	86.45A`
0.5 BA+ 0.01 IBA	81.25 b	93.75 a	87.50A`
Mean	79.88A`	90.27A`	
	Avg. shoot length (cm)		
Control	0.25 d	0.31 cd	0.28C`
0.2 BA+ 0.01 IBA	0.40 c	0.79 b	0.59B`
0.5 BA+ 0.01 IBA	0.66 bc	2.09 a	1.37A`
Mean	0.44 B`	1.06 A`	

Means followed by the same letter (s) are not significantly different from each other at 5% level

As for the average shoot length, WPM medium significantly affected mean shoot length increase compared to MS medium. While the specific effect of growth regulator revealed that (mg l^{-1}) BA at 0.5 plus 0.01 IBA exhibited the highest mean shoot length (1.37cm.) with significant

differences among all tested BA levels. The interactions cleared that the highest significant shoot length (2.09cm.) occurred with WPM medium supplemented with 0.5 BA plus 0.01 IBA (mg l^{-1}).

Presented data during the establishment stage showed that the highest survival percentages and shoot length were obtained with MS medium contained BA at 0.5 plus IBA at 0.01 (mg l^{-1}) for Meet-Ghamr peach (**Table 1**). In agreement with current results **Reeves *et al* (1983)** and **El-Deen (1998)** found that initiated shoot tip and nodal explants cultures of peach rootstock Nemaguard and Okinawa was better on modified MS medium with BA 0.5 to1.0 and IBA 0.01 to 0.1(mg l^{-1}). Al-Amar apricot single nodal explants showed highest survival and shoot length with WPM medium plus BA at 0.5 and IBA at 0.01 mg l^{-1} **Table (2)**. Similarly, **Harada and Murai (1996)** found that shoot proliferation from axillary buds of *Prunus mume* depended on basal WPM rather than MS medium. Furthermore, **Perez-Tornero *et al* (1999)** found BA necessary for meristem survival and development of Helena apricot cv. For most BA concentrations, survival percentage was greatest. Also, **Kandil (2001) and Yonemitsu *et al* (2003)** mentioned that WPM medium was the best on enhancing the activity of Canino and Al-Amar apricot and *Prunus mume* nodal cutting for established in culture.

3.2. Effect of medium type and different cytokinin concentrations on average number and length (cm) of shoots

Table (3) revealed that the highest significant mean number of new proliferated shoots for Meet-Ghamr peach was noticed with MS medium, while WPM medium gave the least value. Concerning the effect of (BA, 2iP and Kin) levels, there were significant differences between the different cytokinins and its concentrations. The highest significant mean number of shoots / explant (3.48) was achieved with 4.0 BA plus 0.1 IBA (mg l^{-1}). Meanwhile control (zero cytokinins) or 2iP and kin at 2.0 mgl^{-1} failed to induce shoots. The interactions between the two studied factors showed that MS medium supplemented with 4.0 BA plus 0.1 IBA at (mg l^{-1}) recorded the highest significant number of shoots / explant (4.75). It could, also, noticed that the highest mean shoot length (cm) occurred with MS medium which significantly differed from WPM medium on Meet-Ghamr peach. BA, 2iP and Kin levels showed significant differences. The highest mean shoot length (1.24 and 1.22 cm) was achieved with 4.0 or 2.0 BA plus 0.1IBA (mgl^{-1}). While, 2iP or kin at 4.0 mgl^{-1} showed the lowest insignificant mean shoot length. The interactions between the two studied factors showed that MS medium supplemented with 2.0 BA plus 0.1 IBA (mg l^{-1}) recorded the highest significant average length (1.73 cm).

The mean values of different cytokinins (BA, 2iP and Kin) in the present results generally showed that BA incorporated in MS medium was the most effective cytokinin for stimulating shoot proliferation for Meet-Ghamr peach, nodal cutting explants, followed by 2iP then Kin in descending order. In, addition BA was the most effective cytokinin for elongation of the new developed shoots.

These results were in agreement with **Hammerschlage (1987) and Loreti & Pasqualetto (1988)** who recommended

Table 3. Effect of medium type and different cytokinin concentrations on number and length (cm) of proliferated shoots/ explant of Meet-Ghamr peach during proliferation stage.

Medium types / Treatments (mg l^{-1})	MS	WPM	Mean
	Number of proliferated shoots /explant		
Control	0.00 h	0.00 h	0.00 E`
1.0 BA + 0.1 IBA	1.60 e	0.81 g	1.21 D`
2.0 BA + 0.1 IBA	2.65 b	1.89 d	2.27 B`
4.0 BA + 0.1 IBA	4.75 a	2.21 c	3.48 A`
2.0 2iP + 0.1 IBA	0.00 h	0.00 h	0.00 E`
4.0 2iP + 0.1 IBA	2.31 c	1.66 e	1.99 BC`
2.0 kin + 0.1 IBA	0.00 h	0.00 h	0.00 E`
4.0 kin + 0.1 IBA	2.39 c	1.17 f	1.78 C`
Mean	1.71 A`	0.97 B`	
	Avg. shoot length (cm)		
Control	0.00 h	0.00 h	0.00 D`
1.0 BA + 0.1 IBA	1.27 c	0.72 fg	0.99 B`
2.0 BA + 0.1 IBA	1.73 a	0.71 fg	1.22 A`
4.0 BA + 0.1 IBA	1.37 b	1.10 d	1.24 A`
2.0 2iP + 0.1 IBA	0.00 h	0.00 h	0.00 D`
4.0 2iP + 0.1 IBA	0.77 ef	0.66 gh	0.72 C`
2.0 kin + 0.1 IBA	0.00 h	0.00 h	0.00 D`
4.0 kin + 0.1 IBA	0.71 fg	0.73 fg	0.72 C`
Mean	0.73 A`	0.49 B`	

Means followed by the same letter (s) are not significantly different from each other at 5% level

MS salts supplemented with 8.8 µM BAP or 0.7 mgl^{-1} BA for "Nemaguard" peach rootstock, 8 scion peach cultivars, the rootstocks GF 655/2 (*Prunus insititia*) and GF 677 (*P. persica X P. amygdalus*) shoot proliferation. Furthermore, Allam and EL-Rayyes (1991) and Fouad *et al* (1995) found that increasing BA concen-

tration in MS culture mdium from 1.0 to 5.0 mgl^{-1} in combination with 3.0 mg l^{-1} IBA and charcoal increased average shoot number/ explant of Nemaguard and Meet-Ghamr peach. However, **Channuntapipat *et al* (2003)** mentioned MS medium with 10.0 µM BAP gave the best shoot proliferation for Titan x Nemaguard hybrid.

The effect of medium type and cytokinins (BA, 2iP and Kin) concentrations on average number and length (cm) of proliferated shoots of Al-Amar apricot were shown in **Table (4)**. The highest significant mean number of new proliferated shoots occurred in WPM medium. Concerning the effect of BA, 2iP and Kin levels, there were significant differences. The highest shoot number / explant (1.86) was achieved with 2.0 BA plus 0.1 IBA (mgl^{-1}), while control treatment and either 2iP or kin at 2.0 mg l^{-1} failed to induce shoots. Also, BA, 2iP and kin at 4.0 mg l^{-1} caused necrosis and all explants died.

The interactions between the two studied factors showed that WPM medium supplemented with BA at 2.0 plus IBA at 0.1 (mg l^{-1}) recorded the highest significant average number of proliferated shoots/ explant (2.89).

As for average shoot length, it was noticed that WPM medium lead to significant increase in mean shoot length, while MS medium gave the least value. Concerning the effect of BA, 2iP and Kin levels on mean shoot length, there were significant differences were obtained. The highest mean shoot length (0.89 cm) was achieved with BA at 1.0 mgl^{-1} plus IBA 0.1 mg l^{-1}. The interactions showed a significant differences in most cases, the highest significant average shoots length (1.04 cm) was recorded with WPM medium supplemented with 1.0 BA plus 0.1 IBA (mg l^{-1}). Meanwhile, BA 1.0, 2.0 mg l^{-1} in MS medium gave lower values.

The mean values of different cytokinins (BA, 2iP and Kin) with MS and WPM media in the present results generally showed that BA in WPM medium was the most effective cytokinin for stimulating shoots proliferation and elongation of Al-Amar apricot nodal cutting explants. While zero, 2.0 and 4.0 mgl^{-1} 2iP or kin were not effective in shooting. Similarly, **Marino *et al* (1991) and Murai *et al* (1997)** showed that shoot proliferation rate of apricot *Prunus armeniaca* cvs. San Castrese and Protici increased with increasing BA levels (2.2, 4.4 and 8.8µ M) with IBA (0.25 µM) and GA_3 (0.29 µ M). Also, results on Bakuoh junkyou cv. showed that BA was most effective than zeatin and 2iP. Furthermore, **Perez-Tornero and Burgos (2000)** added that the proliferation rate was significantly affected by benzyladenine levels and the optimum differed for each studied cultivar and the best was between 1.78 – 3.11 µM.

3.3. Effect of number of subcultures on survival percentage, number and length (cm) of proliferated shoots for Meet-Ghamr peach and Al-Amar apricot.

Data in Table (5) revealed that mean survival % was significantly higher for peach rathar than apricot.

Table 4. Effect of medium type and different cytokinin concentrations on number and length (cm) of proliferated shoots / explant of Al-Amar apricot during proliferation stage.

Medium types / Treatments (mg l^{-1})	MS	WPM	Mean
	Number of proliferated shoots /explant		
Control	0.00 e	0.00 e	0.00 C'
1.0 BA + 0.1 IBA	0.49 d	0.98 b	0.74 B'
2.0 BA + 0.1 IBA	0.83 c	2.89 a	1.86 A'
4.0 BA + 0.1 IBA	0.00 e	0.00 e	0.00 C'
2.0 2iP + 0.1 IBA	0.00 e	0.00 e	0.00 C'
4.0 2iP + 0.1 IBA	0.00 e	0.00 e	0.00 C'
2.0 kin + 0.1 IBA	0.00 e	0.00 e	0.00 C'
4.0 kin + 0.1 IBA	0.00 e	0.00 e	0.00 C'
Mean	0.16 B'	0.48 A'	
	Avg. shoot length (cm)		
Control	0.00 e	0.00 e	0.00 E'
1.0 BA + 0.1 IBA	0.75 d	1.04 a	0.89 A'
2.0 BA + 0.1 IBA	0.66 ef	0.97 b	0.81 B'
4.0 BA + 0.1 IBA	0.00 e	0.00 e	0.00 E'
2.0 2iP + 0.1 IBA	0.00 e	0.00 e	0.00 E'
4.0 2iP + 0.1 IBA	0.00 e	0.00 e	0.00 E'
2.0 kin + 0.1 IBA	0.00 e	0.00 e	0.00 E'
4.0 kin + 0.1 IBA	0.00 e	0.00 e	0.00 E'
Mean	0.18 B'	0.25 A'	

Means followed by the same letter (s) are not significantly different from each other at 5% level

Table 5. Effect of number of subcultures on survival percentage of peach and apricot cultures

Species. / No. of subcultures	Peach	Apricot	Mean
1st subculture	89.58 a	87.50 a	88.54 A`
2nd subculture	89.58 a	89.58 a	89.58 A`
3 rd subculture	89.58 a	89.58 a	89.58 A`
4th subculture	89.58 a	89.58 a	89.58 A`
5th subculture	89.58 a	87.50 a	88.54 A`
6th subculture	87.50 a	62.50 c	75.00 B`
7th subculture	70.83 b	18.74 d	44.79 C`
Mean	86.60 A`	74.99 B`	

Means followed by the same letter (s) are not significantly different from each other at 5% level

Also, the mean of subcultures showed similar high mean survival starting from the first up to the 5th then decreased significantly by the 6th and further by the 7th subculture. The interaction between studied species and the number of subcultures showed that peach cultivar showed similar survival % from the first till the 6th subculture then started significant decreased in survival by the 7th subculture. While apricot culutures showed decreased by the 6th subculture.

Data in Table (6) showed that the mean number of proliferated shoots was significantly higher for peach rather than apricot. The mean of subcultures signifiantly increased as number of subcultures increased up to the 5th then siginificantly decreased during the 6th and 7th subcultures. The interaction between studied species and the number of subcultures showed that apricot cultures showed the highest number of shoots during the 5th subculture (8.10). While the lowest number of shoots was obtained with peach cultures at the first subculture (2.66).

Table 6. Effect of number of subcultures on number of shoots/explant of peach and apricot cultures.

Species. / No. of subcultures	Peach	Apricot	Mean
1st subculture	2.66 j	2.89 ij	2.77 F`
2nd subculture	3.44 h	3.12 I	3.28 E`
3 rd subculture	6.25 d	4.77 f	5.51 C`
4th subculture	6.79 c	7.04 bc	6.92 B`
5th subculture	7.23 b	8.10 a	7.66 A`
6th subculture	5.94 e	2.33 k	4.13 D`
7th subculture	4.38 g	0.641	2.51 F`
Mean	5.24 A`	4.13 B`	

Means followed by the same letter (s) are not significantly different from each other at 5% level

Data in Table (7) revealed that the mean length of proliferated shoots was significantly higher for peach rather than apricot. The mean of subcultures showed that the highest shoot length was recorded under 1st and 2nd subcultures with no significant differences between them, then decreased significantly by the 3rd up to the 7th subcultures. The interaction between species cultivars and the number of subcultures showed that peach cultures showed the highest shoot length during the 1st and 2nd subcultures. While apricot cultures showed the lowest shoot length during the 7th subcultures.

Table 7. Effect of number of subcultures on average shoot length (cm) of peach and apricot cultures.

Species / No. of subcultures	Peach	Apricot	Mean
1st subculture	1.73 a	0.97 d	1.35 A`
2nd subculture	1.75 a	0.93 d	1.34 A`
3rd subculture	1.58 b	0.79 e	1.18 B`
4th subculture	1.31 c	0.70 f	1.01 C`
5th subculture	0.94 d	0.56 g	0.75 D`
6th subculture	0.75 ef	0.46 h	0.61 E`
7th subculture	0.63 g	0.09 i	0.36 F`
Mean	1.24 A`	0.64 B`	

Means followed by the same letter (s) are not significantly different from each other at 5% level

There is no available reports on the effect of different subcultures on survival and growth rate for peach and apricot cultures. However, this has been reported for other species. For example **Parra and Amo-Marco (1998)** found that shoot multiplication and elongation declined in the 3rd to 5th subcultures of *Myrtus communis* L. taken from either seedlings or adult plants. While, **Wanas (1999)** reported an increase in number of proliferated shoots of Hansen rootstock by the 3rd and 4th subcultures and did not mention what happened afterwards. About the effect of subculture frequency, **Grant and Hammatt (1999)** concluded that total time in culture is the most important factor bringing about physiological changes in micropropagated M9 apple and cherry F12/1 genotypes. Moreover, the lowest and highest shoot proliferation rates for carnation cultivars were observed in the first and second subcultures and gradually decreased in subsequent subcultures **(Salehi, 2006)**. The auther suggested that the reduction may be due to using the more terminally buds with too matured tissues.

3.4. Effect of auxin type and concentration on rooting %, average number and length (cm) of roots

Concerning the effect of auxin type and concentration on Meet-Ghamr rooting **Table (8)**, the first root appeared after 28 days from culturing and the greatest rooting percentage (87.50%) was obtained by half strength MS medium with IBA + NAA both at 0.2 mgl^{-1} followed by NAA at 0.5 mgl^{-1} (56.25%). Insignificant differences between IBA at 0.5 or 1.0 mgl^{-1}, NAA at 0.2 mgl^{-1} and IBA + NAA both at 1.0 mgl^{-1} were obvious. The lowest rooting percentage (12.5%) was recorded by NAA at 1.0 mgl^{-1} alone or IBA + NAA both at 0.5 mgl^{-1}.

Table 8. Effect of different auxins concentrations and combinations added to half strength MS medium on rooting %, number and length (cm) of roots of Meet-Ghamr peach during rooting stage.

Treatments ($mg\ l^{-1}$)	Rooting %	Avg. no. of roots	Avg. root length (cm)
control	0.00E	0.00 E	0.00 E
0.2 IBA	0.00E	0.00 E	0.00 E
0.5 IBA	31.25C	0.50 D	2.83 B
1.0 IBA	37.50C	0.81 C	4.52 A
0.2 NAA	31.25C	0.81 C	2.99 B
0.5 NAA	56.25B	2.19 A	3.14 B
1.0 NAA	12.50D	0.25 DE	0.29 C
0.2 IBA + 0.2 NAA	87.50A	1.50 B	5.20 A
0.5 IBA + 0.5 NAA	12.50D	0.50 D	1.99 B
1.0 IBA+ 1.0 NAA	31.25C	0.50 D	2.06 B

Means followed by the same letter (s) are not significantly different from each other at 5% level.

Meanwhile, zero (control) and 0.2 IBA failed to induce rooting. The highest number of roots occurred with NAA at 0.5 $mg l^{-1}$ followed by IBA + NAA both at 0.2 $mg l^{-1}$, then decreased with (IBA at 1.0 $mg l^{-1}$ and NAA at 0.2 $mg l^{-1}$) with insignificant differences between them. The lowest number of roots was recorded by IBA at 0.5 $mg l^{-1}$ alone, IBA + NAA both at 0.5 and 1.0 ($mg l^{-1}$).

The highest significant average root length was clear with IBA + NAA both at 0.2 $mg l^{-1}$ or IBA at 1.0 $mg l^{-1}$ alone (5.20, 4.52 cm) respectively, significant differences were noticed among IBA, meanwhile the lowest root length was obtained with IBA at zero and 0.2 $mg l^{-1}$.

Concerning the effect of auxins type and concentration on Al-Amar apricot rooting (**Table 9**), the first root appeared after 32 days from culture and the highest significant value of rooting percentage (81.25 %) was recorded by half strength MS medium with 0.2 $mg l^{-1}$ NAA, followed by IBA at 0.5 or 1.0, NAA at 0.5 and IBA+ NAA both at 0.2 ($mg l^{-1}$) with insignificant differences among them. The highest significant number of roots was showed by IBA + NAA both at 0.5$mg l^{-1}$. IBA at 0.5 mg l^{-1} caused higher root length followed by NAA at 0.2 mg l^{-1}. The shortest roots appeared considerably at IBA + NAA both at 1.0 $mg l^{-1}$.

Some reportes dealing with the previous subject were illustrated as follows, **Murai *et al*** (1997) mentioned that IBA promoted rooting of Bakouh and Junkyou apricot cvs. and the optimum concentration was 2.0 µM IBA. No rooting occurred without IBA treatment. **Yonemitsu *et al*** (2003) found that root formation of Japanese apricot was induced when shoots were transferred into WPM agar medium containing 0.54 µM NAA. **Cos *et al*** (2004) reported that the best IBA concentration 1.0 mg l^{-1} with a 20 % success rate of rooting explants of peach-almond hybrid Mayor Reg. **Touqeer *et al*** (2004) cleared that the maximum number of roots of peach rootstock GF 677 was five and roots of more than 1.5 cm in length was four obtained with IBA at 0.4

mg l^{-1} without callus. NAA and IAA affected the root growth negatively. Callus formation was strongly stimulated by NAA.

Table 9. Effect of different auxins concentrations and combinations added to half strength MS medium on rooting %, number and length of roots (cm) of Al-Amar apricot during rooting stage.

Treatments (mg l–1)	Rooting %	Avg. no. of roots	Avg. root length (cm)
control	0.00 E	0.00 E	0.00 E
0.2 IBA	43.75 C	1.75 C	1.89CD
0.5 IBA	62.50 B	1.69 C	6.29 A
1.0 IBA	68.25 B	2.75 B	3.82 B
0.2 NAA	81.25 A	2.56 B	4.63 B
0.5 NAA	62.50 B	1.06 D	2.61 C
1.0 NAA	43.75 C	1.50 C	1.99CD
0.2 IBA+ 0.2 NAA	68.75 B	2.69 B	2.66 C
0.5 IBA+ 0.5 NAA	50.00 C	3.63 A	1.82CD
1.0IBA+1.0NAA	25.00 D	0.50 E	1.14 D

Means followed by the same letter (s) are not significantly different from each other at 5% level.

3.5. Acclimatization stage

Survival percentage of peach and apricot plantlets after acclimatization for 8 weeks after rooting were presented in **Table 10,** it was clear that all *in vitro* rooted shoots of acclimatization in the greenhouse where survival percentage reached up to 66.66 % and 50.00 %, respectively.

Data declared that survival percentage of the best *in vitro* rooted shoots of peach (24 plantlets) and apricot (20 plantlets) after acclimatization reached up to 66.66 % and 50.00 %, respectively.

In conclusion, this investigation has optimaized the establishment, proliferation and rooting media for both Meet-Ghamr peach and Al-Amar apricot. In addition the number of subcultures of their which cultures should be renewed had been determined. Further invistigation are needed to proceed for acclimatization.

Table 10. Survival percentage of peach and apricot plantlets after acclimatization for 8 weeks

Species	Survival %
Peach	66.66
apricot	50.00

REFERENCES

Allam, A.M. and D.E. El-Rayyes (1991). *In vitro* propagation of "Nemaguard" peach rootstock. ***Minufiya J. Agric. Res. (16) (1): 573-578.***

Bajaj, Y.P.S., (1986): ***Biotechnology in agriculture and forestry. PP. 170-183.*** Springer-Verlag. Berlin. Heidelberg, New York.

Channuntapipat, C.; M. Sedgley and G. Collins (2003): Micropropagation of almond cultivars Nonpareil and Ne Plus

Ultra and the hybrid rootstock Titan x Nemaguard. *Scientia Horticulturae, 98 (4): 473-484.*

Cos, J.; D. Frutos; R. Garcia; J. Rodriguez and A. Carrillo (2004). *In vitro* rooting study of peach- almond hybrid Mayor Reg. *Acta- Horticulurae*, 658 (2): 623-627.

El-Deen, S.A.; W.T. Saeed and L.A. Hassabila (1998). Micropropagation of peach rootstocks. *Bulletin of Faculty of Agriculture, Cairo Univ.*, 49(4): 563-574.

Fouad, M.M.; A.H. Gomaa and M.H. A. El-Zaher (1995). Factors influencing *in vitro* establishment and multiplication stages of peach. *Acta Horticulturae. (409): 191-196.*

Grant N.J. and N. Hammatt (1999). Increased root and shoot production during micropropagation of cherry and apple rootstalks: effect of subculture frequency. *Tree Physiol. 19(13): 899-903 (cabi abst.)*

Hammerschlag, F.A.; G.R. Bauchan and R. Scorza (1987). Factors influencing *in vitro* multiplication and rooting of peach cultivars. *Plant Cell, Tissue and Organ Culture, 8: 235-242.*

Harada, H. and Y. Murai (1996). Micropropagation of *Prunus mume*. *Plant Cell, Tissue and Organ Culure*, 46: 265-267.

Kandil, E.A. (2001). *Studies on Vegetative Propagation of Apricot, pp. 159-161.* Ph.D. Thesis, Fac. of Agric., Cairo Univ., Egypt.

Loreti, F. and P.L. Pasqualetto (1988). Effect of alternating temperature during proliferation and rooting stages of GF 655/2 and GF 677 shoots cultured *"in vitro". Acta-Horticulturae. 227, 467-469; International Symposium on Vegetative Propagation of Woody Species, Pisa, Italy, 3-5 Sept., 1987.*

Marino, G.; E. Magnanini; S. Battistini and B. Righetti (1991). Effect of hormones and main carbon energy sources on *in vitro* propagation of apricot (*Prunus armeniaca* L.) cvs. San Castrese and Portici. *Acta Horticulturae (293): 355-362; IX International symposium on apricot culture, Caserta, Italy, 9-15 July, 1989.*

Miller, G.A.; D.C. Coston; E.G. Denny and M.E. Romeo (1982): *In vitro* propagation of Nemaguard peach rootstock. *HortScience, 17 (2): 194.*

Murai, Y.; H. Harada and Yamashita H. (1997). *In vitro* propagation of apricot (*Prunus armeniaca L.*) cv. 'Bakuoh junkyou'.Journal of the Japanese Society for *Horticultural Science, 66: 3-4, 475-480.*

Murashige, T. and Skoog F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant, 15: 473-497.*

Parra R. and J. B. Amo-Marco (1998): Factors affecting *in vitro* shoot proliferation of *Myrtus communis* L. a comparison of adult and seedling material. *In vitro Cell Dev. Biol-Plant, 34: 104-107.*

Perez-Tornero, Olaya and Burgos Lorenzo (2000). Different media requirements for micropropagation of apricot cultivars. *Plant Cell, Tissue and Organ Culture, 63(2): 133-141; 26 ref.*

Perez-Tornero, Olaya; Ortin-Parraga, F.; J. Egea and L. Burgos (1999). Medium-term storage of apricot shoot tips *in vitro* by minimal growth method. *HortScience., 34(7): 1277-1278; 7 ref.*

Reeves, D.W.; B.D. Horton and G.A. Couvillon (1983). Effect of media and media pH on *in vitro* propagation of "Nemaguard" peach rootstock. *Scientia Hort., 21: 353-357.*

Salehi. H. (2006). Can a general shoot proliferation and rooting medium be used for a number of carnation cultivars? *African Journal of Biotechnology.5(1):25-30.*
Sendecor, C.W. and W.G.Cochran (1982). *Statistical Methods. 7th Ed.* The Iowa State Univ. press. Ames. Iowa, USA.
Skirvin, R.M. (1984). The tissue culture of stone fruits. *In: Handbook of Plant Cell Culture, Vol. 3 p. pp. 402-452,* Ammirato, V.; D.A. Evans; W.R. Sharpe and Y. Yamada (eds.). Macmillan publishing Comp. New York.
Touqeer Ahmad; Hafeez-Rahman and M.H. Laghari (2004). Effect of different auxins on *in vitro* rooting of peach rootstock GF 677. *Sarhad Journal of Agriculture. 20 (3): 373-375.*
Wanas, H.W. (1999). Some factors affecting micropropagation of the peach rootstock "Hansen 536". *Annals of Agric. Sci. Moshtohor. 37(4): 2703-2714.*
Yonemitsu, H.; K. Nishi; S. Sagan; L. Tong and Y. Matsumura (2003). *In vitro* propagation of mature Japanese apricot (*Prunus mume*). *Horticultural Research (Japan), 2(2): 77-82.*

المؤتمر العاشر لبحوث التنمية الزراعية، كلية الزراعة، جامعة عين شمس، القاهرة، مصر، ٢٠٠٦
مجلد خاص، حوليات العلوم الزراعية، عدد خاص، ١، ١٦٣-١٧٨، ٢٠٠٦

الإكثار المعملى لخوخ ميت غمر ومشمش العمار

[١٣]

وفاء حسانين ونس[١]- محمد أبو رواش بدر[١]- أحمد عبد الحميد[١]- أحمد عبد الوهاب الحمصانى[٢]

١. كلية الزراعة- جامعة عين شمس- شبرا الخيمة - القاهرة- مصر
٢. البنك القومى للجينات- مركز البحوث الزراعية- الجيزة - مصر

أسست بنجاح وأكثرت فى المعمل العقد الساقية للأشجار البذرية من خوخ ميت غمر ومشمش العمار. أثناء مرحلة التأسيس كانت أعلى نسبة بقاء لمنفصلات العقد الساقية وأيضاً أطول الفروع تم الحصول عليها باستخدام بينات موراشيج وسكوج (١٩٦٢) وبيئة الاشجار الخشبية التى تحتوى على بنزيل أدنين بتركيز ٠,٥ ملليجرام/لتر وإندول حمض البيوترك بتركيز ٠,٠١ ملليجرام/ لتر.

فى مرحلة التضاعف إستخدم بنزيل أدنين عند تركيز ٤,٠ ملليجرام/ لتر بالإضافة ٠,١ ملليجرام/ لتر إندول حمض البيوترك حيث أعطى أعلى عدد من الفروع لخوخ ميت غمر بينما أفضل الأطوال للفروع سجل مع بيئة موراشيج وسكوج

بالإضافة إلى ٢,٠ ملليجرام/لتر بنزيل أدنين و ٠,١ ملليجرام/لتر إندول حمض البيوترك. ومن جهة أخرى إستخدام البنزيل أدنين بتركيز ٢,٠ ملليجرام/ لتر بالإضافة إلى ٠,١ ملليجرام / لتر إندول حمض البيوترك أعطى أعلى عدد من الفروع بالنسبة لمشمش العمار بينما أفضل طول للفرع سجل مع بيئة الأشجار الخشبية بالإضافة إلى ١,٠ ملليجرام/ لتر بنزيل أدنين و ٠,١ ملليجرام/ لتر إندول حمض البيوترك. بعد ذلك إستخدمت أفضل بيئة للتضاعف في عملية النقل كل أربعة أسابيع حتى تم النقل لسبع مرات ووجد أن متوسط الفروع المتكاثرة إزداد بزيادة عدد مرات النقل حتى النقلة الخامسة ثم تناقص أثناء النقلة السادسة والسابعة لمزارع المشمش، بينما تناقص معنوياً بعد النقلة السادسة لمزارع الخوخ.

بالنسبة للتجذير ففي خوخ ميت غمر كانت أعلى نسبة تجذير (٨٧,٥ %) وأعلى طول للجذور تم الحصول عليها في نصف تركيز أملاح بيئة موراشيج وسكوج المحتوية على إندول حمض البيوترك بتركيز ٠,٢ ملليجرام/ لتر بالإضافة إلى نفثالين حمض الخليك بتركيز ٠,٢ ملليجرام/ لتر، بينما أعلى عدد من الجذور تم الحصول عليه في نفس البيئة ولكن بإضافة ٠,٥ ملليجرام/لتر نفثالين حمض الخليك. بالنسبة لمشمش العمار كانت أعلى نسبة تجذير (٨١,٢٥%) تم الحصول عليها عند إستخدام أيضاً نصف تركيز بيئة موراشيج وسكوج المضاف إليها ٠,٢ ملليجرام/لتر نفثالين حمض الخليك فقط بينما كان أعلى عدد من الجذور تم الحصول عليها عند إضافة إندول حمض البيوترك و نفثالين حمض الخليك كلاً بتركيز ٠,٥ ملليجرام/لتر بينما أعلى طول كان عند إضافة إندول حمض البيوترك بتركيز ٠,٥ ملليجرام/لتر. تم بنجاح أقلمة نبيتات كل من الخوخ والمشمش لمدة ٨ أسابيع وكانت نسبة البقاء ٦٦,٦٦% و٥٠,٠% على التوالي.

تحكيم: ا.د زينب حسين بحيري

ا.د إبراهيم عبد المقصود