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IN VITRO PROPAGATION OF SOMATIC EMBRYOGENESIS-DERIVED PLANTLETS OF CITRUS

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ABSTRACT

Nodal stem segments of *in vitro* growing somatic embyogenesis and seedling-derived plantlets of sweet orange, *C. sinensis* (L.) of (Washington navel, shamouti and blood orange cvs.); grapefruit, *C. paradisi* (L.); Local mandarin, *C. deliciosa* (Tenor); lemon, *C. limon;* (L.); citron, *C. medica* (L.); lime, *C. aurantifolia* (Christm); rough lemon, *C. jambhiri* (Lush) and sour orange, *C. aurantium* (L.) were cultured on MS medium supplemented with different concentrations of BA (0.5,1.0 and 2.0 mg/L) alone or in combination with NAA (0.5 mg/L) for shoot multiplication. The results indicated that somatic embryogenesis-derived plantlets can be successfully propagated *in vitro* with a good vigor compared with germinated seedlings. A large number of proliferated shoots were produced when these explants were cultured on MS medium containing 0.5mg/L of BA combined with 0.5mg/L of NAA. Most obtained shoots were rooted on half-strength MS medium containing 0.5 mg/L of NAA. These *in vitro* grown plantlets were then successfully transferred to green house through acclimatization process.

Keywords: Citrus, *In vitro* propagation, Nodal segments, Multiplication, Rooting

INTRODUCTION

Citrus is an important fruit crop worldwide. The development of efficient tissue culture protocols is necessary for conservation and genetic improvement of citrus. Recently tissue culture, particularly micropropagation, is gaining popularity over conventional methods of propagation due to several advantages; it would be used to produce mass number of true to type plants and for long-term storage of the citrus germplasm. In citrus micropropagation, establishment stage has been described for a number of explants from different citrus species, including stem segments **Duran-Vila *et al*** **(1989); Ghorbel *et al* (1998); Kobayashi *et al* (2003); Begum *et al* (2004)**, epicotyle segments **Can *et al* (1992);**

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Moreira-Dias *et al* (2000); Almeida *et al* (2002), hypocotyles **Normah *et al* (1997)**, shoot tips **Singh *et al* (1994); Baruah *et al* (1996); Paudyal and Haq, (2000); Huang *et al* (2002); Rana *et al* (2002)** and axillary buds **Otoni and Teixeira (1991); Desai *et al* (1996); El-Wasel,(2001)**. Differences have been reported on the morphogenic response and the success in micropropgation on different citrus species according to the composition of the culture medium, incubation conditions **Duran-Vila *et al* (1989) & (1992)**, the effect of explant type and genotype response.

In shoot proliferation, cytokinins are widely used to enhance the growing of lateral buds. In general, Benzyladenine (BA) is the most effective cytokinin for shoot multiplication, followed by 6-furfurylamino purine (Kinetin) and N-isopentenylamino purine (2ip) as reported by **Otoni and Teixeira (1991); Can *et al* (1992); Baruah *et al* (1996) and Ghorbel *et al* (1998)**. The best concentration of BA varies according to the genotype and the type of explant. Although exogenous auxins do not promote shoot proliferation, their presence may improve shoot growth as mentioned by **Mas *et al* (1994)** when he used NAA at 0.5mg/L in combination with BA at 0.5mg/L for multiplication of Citremon (*Poncirus trifoliata* X *Citrus sinensis*) stem segments or using 0.1mg/L NAA+ 1mg/L BA for trifoliate orange **(EL-Wasel 2001)**. In the root formation stage, auxins are essential for root initiation ,NAA or IBA are the best effective auxins in many cases, NAA was superior to IBA for root induction with higher number of roots per shoot as reported by **Normah *et al* (1997); Paudyal and Haq (2000); EL-Wasel (2001) and Begum *et al* (2004)**.

The acclimatization of micropropagated plantlets is a critical stage for further implementation of plant tissue culture. In the first week after transfer to *ex vitro* conditions plants has to cope with different stresses and to adapt to the new environmental conditions. Tissue cultured plantlets are often difficult to establish in green- house conditions. They behave as very tender seedlings, so they must be acclimatized with the utmost care. Many workers succeeded to adapt the *in vitro* derived plantlets of citrus under continuous moist air as reported by **Grosser and Chandler (1986) and Omura and Hidaka (1992)**. Adaptation also succeeded by using different culture mixtures such as light volcanic soil and peatmoss **(Starrantino and Caruso 1988)**, granual volcanic ash soil, ceramic wool or polyester wool with survival rates (93-98%) **(Hidaka and Kajiura 1989)** or soil mixture with survival rate 83% as reported by **Normah *et al* (1997)**.

The present study aimed to investigate an efficient micropropagation protocol of some citrus species (*C. sinensis* (L.); *C. paradisi* (L.); *C. deliciosa* (Tenor); *C. limon* ;(L.) *C. medica* (L.) and *C. aurantifolia* (Christm) obtained from somatic embryogenesis-derived plantlets and *C. jambhiri* (Lush) and *C. aurantium* (L.) obtained from seedling-derived plantlets using nodal stem segments

MATERIAL AND METHODS

This study was carried out at the Tissue Culture Laboratory, Plant Biotechnology Department, National Research Center during the period from 2002 to 2005.

Nodal stem segments used as the explant for the micropropagation involved multiplication stage, rooting stage and acclimatization stage.

Multiplication stage

Source of tissue and preparation of explants

Two kinds of tissues were used as a source of explants in this stage:

(1) Somatic embryogenesis-derived plantlets through embryogenic callus from ovules, stigma, style and ovary explants growing *in vitro* of sweet orange (*C. sinensis* (L). Osbeck) varieties Washington navel, Shamouti and Blood orang; lemon (*C. limon* (L.) Osbeck); grapefruit (*C. paradisi* Macf.); local mandarin (*C. deliciosa* Tenor); lime (*C. aurantifolia L.)* and citron (*C. medica* L.). The shoots of these plantlets were cut into segments (0.5cm) each having a node. The nodal segments of the varieties were then aseptically cultured on the culture medium with 5 explants/ jar.

(2) Two months-old *in vitro* germinated zygotic seedlings (6-8cm) of sour orange (*C. aurantium* L.) and rough lemon (*C. jambhiri* Lush.). The shoots of these plantlets were cut into nodal stem segments (0.5 cm long) and then aseptically cultured on the culture media with 5 explants/ jar.

Culture media: Murashige and Skoog (1962) MS medium supplemented with various concentrations (0.5, 1.0 and 2.0 mg/L) of Benzyladenine (BA) alone or in combination with 0.5 mg/L Naphthalene acetic acid (NAA) in addition to 30 g/L sucrose and solidified with 7g/L Diffico Bacto agar for multiple-shoot regeneration. The pH was adjusted to 5.8±1 with 1N sodium hydroxide before autoclaving at 121°C for 25 min. The media were dispensed as 30 ml aliquots into 300ml-culture gar, capped with polypropylene caps.

Culture conditions

The culture jars were maintained at 26±1°C under 16/8 h light cycle provided by white fluorescent lamps with light intensity of 2000-3000 Lux.

The culture explants were subcultured on fresh medium at four-week intervals. Observations were recorded over 7 days following inoculation. All experiments were repeated twice.

Rooting stage

Microshoots with length of 0.5 cm regenerated from nodal explants were transferred into rooting medium containing half-strength Murashige and Skoog (MS) medium supplemented either with Naphthalene acetic acid (NAA) at 0.5 and 1.0 mg/L or Indole butyric acid (IBA), at 0.5 and 1.0 mg/L plus the free-growth regulators medium. All media also contained 30 g/L sucrose and 7g/L Diffico Bacto agar. The pH was adjusted to 5.8±1 with 1N sodium hydroxide before autoclaving at 121°C for 25 min. The media were dispensed as 30 ml aliquots into 300 ml-culture gar, capped with polyproylene caps.

Culture conditions

The culture jars were maintained at 26±1°C under 16/8 h light cycle provided by white fluorescent lamps with light intensity of 2000-3000 Lux.

Transplantation into soil

The obtained plantlets after root initiation were carefully separated from the medium of the culture vessels using forceps to avoid damaging them. The roots of the plantlets were carefully washed with tap water to remove agar adhering them. Then transplanted into 6 cm plastic pots containing a soil mixture (50% peat and 50% sand), fertilized with a solution of inorganic salts of MS medium at transplant time. The pots were enclosed in polyethylene bags which were removed gradually one week later to provide a gradual conditioning to green house environment.

Experimental design and data analysis

All experiments were set up on a completely randomized design with five replicates for each treatment; each replicate consists of 2 culture jars with at least 50 explants per treatment. All experiments were repeated two times.

Data on the average number of proliferated shoots per explant, shoot length and number of leaves per shoot were recorded after 5 weeks. Rooting percentage was evaluated after 8 weeks from incubation.

All data were subjected to analysis of variances (ANOVA) and statistically analyzed using completely randomized method according to **Snedecor and Cochran (1972)**. Significant differences among means were recorded using Least Significant Differences (LSD) values at 5% level.

RESULTS AND DISCUSSION

Proliferation stage

After 10-15 days in multiplication media, many stem cutting explants began to proliferate and axillary buds continued to develop to form shoots. Evaluations were done after 5 weeks in culture .When shoots were higher than 0.5-1.0 cm they were counted (**Fig. 1**).

The percentage of responsive explants (% of proliferation) showed significant effect of cultivars (**Table, 1**) from ovule-derived plantlets. Washington navel orange gave the best shoot proliferation percentage (95.8 %) with a significant difference to other cultivars. whereas both Shamouti orange and blood orange gave the same result (83.3%). In case of flower-derived plantlets, lemon and lime produced the highest shoot proliferation (100%) with a significant difference to citron (94.5%) Also, there were significant differences between rough lemon and sour orange (100% and 50%), respectively in case of seedling-derived plantlets.

Growth regulators showed a significant effect for ovule-derived plantlets or seedling-derived plantlets, as 0.5 mg/L of BA alone or in combination with 0.5 mg/L of NAA gave the best results with significant differences to other treatments but in case of flower-derived plantlets, it is not significant. Also, there was significant effect of interaction between growth regulators and cultivars for Washington navel orange as 2.0 mg/L BA gave the lowest proliferation percentage but BA at 0.5 mg/L or 2.0 mg/L gave the best response for Shamouti orange whereas 2.0 mg/L or 0.5 mg/L BA in addition to

Table 1. Effect of different BA levels alone or combined with 0.5 mg/L NAA on shoot proliferation percentage of some citrus species

* Shoot proliferation%											
Treatments		Ovule-derived plantlets						Flower-derived plantlets			
BA mg/L	NAA mg/L	Washington navel orange *C. sinensis*	Shamouti orange *C. sinensis*	Blood orange *C. sinensis*	Grapefruit *C. paradisi*	Local mandarin *C. deliciosa*	Mean	Lemon *C. limon*	Citron *C. medica*	Lime C. aurantifolia	Mean
0.5	0.0	100 a	100 a	75 b	50 c	50 c	75 A	100 a	100 a	100 a	100A
1.0	0.0	100 a	75 b	75 b	25 d	75 b	70 B	100 a	100 a	100 a	100A
2.0	0.0	75 b	100 a	100 a	25 d	25 d	65 C	100 a	100 a	100 a	100A
0.5	0.5	100 a	75 b	100 a	25 d	75 b	75 A	100 a	66.7 b	100 a	88.9B
1.0	0.5	100 a	75 b	75 b	25 d	25 d	60 D	100 a	100 a	100 a	100 A
2.0	0.5	100 a	75 b	75 b	25 d	25 d	60 D	100 a	100 a	100 a	100A
Mean		95.8 A	83.3 B	83.3 B	29.2 D	45.8 C		100 A	94.5 B	100 A	

Table 1. Cont.

* Shoot proliferation%				
Treatments		Seedling-derived plantlets		
BA mg/L	NAA mg/L	Rough lemon *C. jambhiri*	Sour orange *C.aurantium*	Mean
0.5	0.0	100 a	100 a	100 A
1.0	0.0	100 a	75 b	87.5 B
2.0	0.0	100 a	0.0 e	50 E
0.5	0.5	100 a	50 c	75 C
1.0	0.5	100 a	50 c	75 C
2.0	0.5	100 a	25 d	62.5 D
Mean		100 A	50 B	

* Recorded after 5 weeks *in vitro* cultures.

LSD (p=0.05) for treatment (ovule) = 0.6111; genotype= 0.5579; interaction (treatment × genotype) = 1.366
LSD (p=0.05) for treatment (flower) = 0.03023; genotype=0.02138; interaction (treatment × genotype) = 0.05237
LSD (p=0.05) for treatment (seedling) = 0.6876; genotype = 0.3962; interaction (treatment × genotype.) = 0.9724

0.5 mg/L of NAA were the best treatment for Blood orange and BA at 0.5 mg/L for grapefruit and 1.0 mg/L BA or 0.5 mg/L of BA.+ 0.5 mg/L of NAA for local mandarin. These treatments were significantly different with other treatments for each cultivar. In case of flower-derived plantlets, there was no significant difference among cultivars or treatments except for citron that BA at 0.5 mg/L + NAA at 0.5 mg/L gave the lowest result. Also, in rough lemon, there was no significant difference among treatments but in sour orange; BA at 0.5 mg/L was significantly higher than other treatments.

The analysis of the number of axillary shoots obtained per explant showed significant effect of BA alone or combined with NAA regardless of the source of explants. BA at 0.5 mg/L combined with 0.5 mg/L of NAA gave the highest values of shoot number (2.7, 2.7) for ovule, flower-derived plantlets, respectively and (2.5) with 2.0 mg/L of BA combined with 0.5 mg/L of NAA for seedling-derived plantlets as shown in **(Table, 2)**. On the other hand, the analysis showed that there was no significant difference among cultivars regardless the source of explant. The interaction between cultivars and growth regulators showed that 1 mg/L BA gave the highest number of shoots/ explant for Shamouti orange, Blood orange and local mandarin but without significant differences among them. Whereas, 0.5 mg/L of BA combined with 0.5 mg/L of NAA was the best for Washington navel orange and grapefruit. But, there was no significant differences among treatments for lemon and rough lemon. On the other hand, BA at 0.5mg/L alone and combined with 0.5mg/L of NAA was the best treatments for citron and lime, respectively whereas 2.0mg/L of BA combined with 0.5mg/L of NAA gave the highest number of shoots for sour orange.

Shoot length was significantly different among cultivars as shown in **Table (3)**, Washington navel orange produced the highest shoot length followed by Shamouti orange and grapefruit without significant differences among them but local mandarin produced the lowest shoot length. The effect of growth regulators showed that the best treatment was BA (0.5mg/L) + NAA (0.5mg/L) with significant difference to other treatments except BA at 1.0mg/L. The interaction between cultivars and growth regulators showed that 0.5mg/L of BA+0.5 mg/L of NAA gave the highest shoot length for Washington navel orange and grapefruit with significant difference to other treatments in grapefruit .whereas, 1.0 mg/L of BA was the best for Shamouti orange, but no significant differences were obtained in treatments for Blood orange and local mandarin.

In case of flower-derived plantlets, lemon and citron gave the highest shoot length. BA at 0.5mg/L alone or combined with 0.5 mg/L produced the highest shoot length with no significant differences to BA at 1.0mg/L or 2.0mg/L or in combination with NAA at 0.5 mg/L. The best treatment varied among cultivars, as BA at 0.5 mg/L was significantly the best for lemon and when combined with 0.5 mg/L of NAA for citron but no significant differences obtained in treatments for lime. On the other hand, in seedling-derived plantlets, rough lemon was superior to sour orange in shoot length. BA at 0.5 mg/L alone or combined with NAA gave a high value but with no significant differences to other treatments in each cultivar.

Table 2. Effect of different BA levels alone or combined with 0.5 mg/L NAA on number of shoots per explant of some citrus species

Number of shoots per explant											
Treatments		Ovule-derived plantlets						Flower-derived plantlets			
BA mg/L	NAA mg/L	Washington navel orange *C. sinensis*	Shamouti orange *C. sinensis*	Blood orange *C. sinensis*	Grapefruit *C. paradisi*	Local mandarin *C. deliciosa*	Mean	Lemon *C. limon*	Citron *C.medica*	Lime C. aurantifolia	Mean
0.5	0.0	2.3 abc	2.3 abc	1.3 c	1.7 bc	1.0 c	1.7 BC	1.3 de	4.0 ab	2.0 cde	2.4 AB
1.0	0.0	2.3 abc	3.0 ab	2.0 bc	2.3 abc	1.7 bc	2.3 AB	2.3 cde	2.3 cde	3.0 bc	2.5 AB
2.0	0.0	1.7 bc	2.0 bc	1.7 bc	2.0 bc	1.0 c	1.7 BC	1.0 e	2.3 cde	2.0 cde	1.8 BC
0.5	0.5	3.7 a	3.0 ab	1.7 bc	3.7 a	1.3 c	2.7 A	2.0 cde	1.7 cde	4.3 a	2.7 A
1.0	0.5	2.0 bc	1.7 bc	1.7 bc	2.0 bc	1.3 c	1.8 BC	2.3 cde	2.3 cde	2.7 cd	2.4 AB
2.0	0.5	1.7 bc	1.3 c	1.3 c	2.3 abc	1.0 c	1.5 C	1.0 e	1.3 de	2.0 cde	1.4 C
Mean		2.3 A	2.2 A	1.6 B	2.3 A	1.2 B		1.7 B	2.3 A	2.7 A	

Table 2. Cont.

Number of shoots per explant				
Treatments		Seedling-derived plantlets		
BA mg/L	NAA mg/L	Rough lemon *C. jambhiri*	Sour orange *C.aurantium*	Mean
0.5	0.0	1.7 bc	1.3 c	1.5 BC
1.0	0.0	1.7 bc	1.7 bc	1.7 BC
2.0	0.0	2.0 bc	0.i d	1.0 C
0.5	0.5	2.0 bc	1.5 bc	1.8 B
1.0	0.5	1.7 bc	2.5 ab	2.1 AB
2.0	0.5	2.0 bc	3.1 a	2.5 A
Mean		1.9 A	1.7 A	

Recorded after 5 weeks *in vitro* cultures.

LSD (p=0.05) for treatment (ovule) =0.5760; genotype=0.5259; interaction (treatment × genotype) = 1.288
LSD (p=0.05) for treatment (flower) = 0.7243; genotype=0.5122; interaction (treatment × genotype) = 1.255
LSD (p=0.05) for treatment (seedling) = 0.6146; genotype= 0.3541; interaction (treatment × genotype) = 0.8691

Table 3. Effect of different BA levels alone or combined with 0.5mg/L NAA on shoot length of some citrus species

* Average shoot length (cm)											
Treatments		Ovule-derived plantlets						Flower-derived plantlets			
BA mg/L	NAA mg/L	Washington navel orange *C. sinensis*	Shamouti orange *C. sinensis*	Blood orange *C. sinensis*	Grapefruit *C. paradisi*	Local mandarin *C. deliciosa*	Mean	Lemon *C. limon*	Citron *C.medica*	Lime C. aurantifolia	Mean
0.5	0.0	0.53 bc	0.8 bc	0.4 c	0.4 c	0.4 c	0.5 BC	0.9 a	0.6 bc	0.5 c	0.7 A
1.0	0.0	0.75 bc	1.0 ab	0.46 c	0.53 bc	0.4 c	0.6 AB	0.4 c	0.53 bc	0.4 c	0.4 BC
2.0	0.0	0.4 c	0.33 c	0.4 c	0.3 c	0.4 c	0.4 C	0.6 bc	0.6 bc	0.4 c	0.5 AB
0.5	0.5	1.0 ab	0.6 bc	0.5 bc	1.3 a	0.4 c	0.8 A	0.4 c	0.8 ab	0.5 c	0.6 AB
1.0	0.5	0.7 bc	0.5 bc	0.5 c	0.4 c	0.33 c	0.5 BC	0.6 bc	0.6 bc	0.4 c	0.5 AB
2.0	0.5	0.75 bc	0.53 bc	0.5 bc	0.4 c	0.4 c	0.5 BC	0.33 c	0.3 c	0.4 c	0.3 C
Mean		0.7 A	0.6 AB	0.5 BC	0.6 AB	0.4 C		0.6 A	0.6 A	0.4 B	

Table 3. Cont.

* Average shoot length (cm)				
Treatments		Seedling-derived plantlets		
BA mg/L	NAA mg/L	Rough lemon *C. jambhiri*	Sour orange *C.aurantium*	Mean
0.5	0.0	0.5 abc	0.5 ab	0.5 A
1.0	0.0	0.4 abc	0.4 abcd	0.4 A
2.0	0.0	0.33 cd	0.0 d	0.2 B
0.5	0.5	0.6 a	0.4 abc	0.5 A
1.0	0.5	0.53 ab	0.4 abcd	0.5 A
2.0	0.5	0.33 bcd	0.33 bcd	0.33 AB
Mean		0.4 A	0.3 B	

* Recorded after 5 weeks *in vitro* cultures.

LSD (p=0.05) for treatment (ovule) = 0.2040; genotype = 0.1862; interaction (treatment × genotype) = 0.4561
LSD (p=0.05) for treatment (flower) =0.1418; genotype =0.1003; interaction (treatment × genotype) = 0.2456
LSD (p=0.05) for treatment (seedling) = 0.1359; genotype = 0.0782; interaction (treatment × genotype) = 0.1921

The study demonstrates the use of different cytokinin treatments alone or in combination with auxin for multiple shoot formation from somatic embryogenesis-derived plantlets. MS medium fortified with BA (0.5 mg/L) alone or combined with NAA (0.5 mg/L) induced maximum shoot proliferation (75%, 100%, 100%), maximum number of shoots (2.7, 2.7, 2.5) and the highest shoot length (0.8, 0.5, 0.5 cm) when compared to other concentrations. The direct organogenic pathways of shoot regeneration require BA for optimal regeneration, but it differ in the primary effect of this hormone and in the response to concentration, the main effect of BA is on bud sprouting rather than bud formation. So, the number of shoots developed increased with concentration of BA till 0.5 mg/L. Otherwise, when the cytokinin /auxin ratio was varied, it exhibited frequent drop in induction of shoot formation. In this connection, **Edriss and Burger (1984)** reported that the increase in BA concentration increased number of shoots from root segments explants. Whereas **Gill and Gosal (2002)** noted maximum shoot regeneration in MS medium supplemented with BA (1.0 mg/L) and GA_3 (2 mg/L). **Karwa (2003)** used different hormones alone or in combination for multiple shoots formation from nucellar embryos and found that MS medium fortified with BAP (8.88 uM), NAA (2.6 uM) and Kin (2.32 uM) induced maximum shoot induction (8.8) when compared to other hormonal combinations and a maximum number of multiple shoots (16.8 ± 0.96) per explant were also regenerated in the same combination. **Begum *et al* (2004)**, found that a large number of shoot buds were produced when such four weeks old cultures of nodal segments of three varieties of *Citrus grandis* were subcultured on 1/2 MS medium containing 1.0 mg/L of BAP. In this study, BAP concentration above 1.0 mg/L caused an antagonist effect as the higher BAP concentration causing the lowest number of shoots per explant (1.4). This trend has also been reported by several authors, **Maggon and Singh (1995), Perez-Molphe-Balch and Ochoa-Alejo (1997), Ghorbel *et al* (1998) and Moreira-Dias *et al* (2000).**

Rooting stage

The root formation of citrus microshoots was affected by NAA and IBA and its concentrations, genotype and the explant source **(Table, 4)**. In general, there have been significant differences in response to the type of auxin and concentration. **Table (4)** also indicates that NAA at 0.5 mg/L gave the highest results of rooting. It does mean that Shamouti orange gave the highest rooting percentage compared with Washington navel orange for ovule-derived plantlets. Significant differences were obtained among treatments as NAA at 0.5 mg/L gave the highest rooting (90%). The interaction between cultivars and growth regulators showed that 0.5 mg/L of NAA was the best for Washington navel orange and Shamouti orange but with no significant difference to 1.0 or 0.5 mg/L of IBA for Shamouti orange. For flower-derived plantlets, citron was significantly higher than lemon for root formation and the best treatment was 0.5 mg/L of NAA for both cultivars with significant difference to other treatments .Also, NAA at 0.5 mg/L was the best treatment for rough lemon.

Table 4. Effect of IBA and NAA concentrations on rooting percentage of some citrus species

* Rooting percentage								
Treatments		Ovule-derived plantlet			Flower- derived plantlet			Seedling-derived plantlet
IBA mg/L	NAA mg/L	Washington navel orange *C. sinensis*	Shamouti orange *C. sinensis*	Mean	Lemon *C. limon*	Citron *C. medica*	Mean	Rough lemon *C. jambhiri*
0.0	0.0	20 d	50 c	35 E	0.0 h	50 e	25 E	0.0 B
0.5	0.0	0.0 e	100 a	50 C	40 f	60 d	50 C	0.0 B
1.0	0.0	20 d	100 a	60 B	40 f	20 g	30 D	0.0 B
0.0	0.5	80 b	100 a	90 A	100 a	100 a	100 A	20 A
0.0	1.0	0.0 e	80 b	40 D	80 b	75 c	77.5 B	0.0 B
Mean		24 B	86 A		52 B	61 A		4

* Data recorded after 8 weeks *in vitro* culture.

LSD (p=0.05) for treatment (ovule) =0.8792; genotype = 0.5572; interaction (treatment × genotype) = 1.243
LSD (p=0.05) for treatment (flower) = 1.031; genotype = 0.6533; interaction (treatment × genotype) =1.458
LSD (p=0.05) for treatment (seedling) =0.8420

Fig. 1. Multishoots on nodal cutting of somatic embryogenesis derived citrus plantlets after 3-months of culture on MS medium supplemented with 0.5 mg/L BA+0.5 mg/L NAA

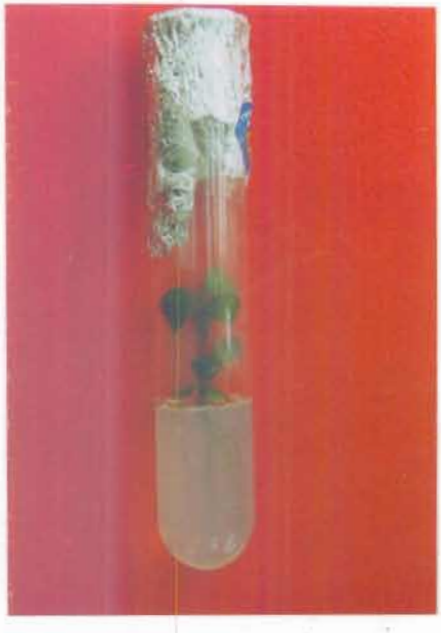

Fig. 2. Development of roots from the base of micro shoots on half-strength MS medium with IBA (0.5 mg/L).

Fig. 3. Adapted plantlet of citrus derived-tissue culture (4 week-old) grown in soil mixture (1 peat : 1 sand).

The important role of auxins upon root formation has been demonstrated by numerous studies (Torry (1965); **Parthasarathy and Nagaraju (1997) and Duran-Vila *et al* (1989). Table (4)** indicated that flower-derived plantlets were the best responsive shoots for root formation (56.5%). Whereas, shamouti orange gave the highest percentage of rooting with average (86%) in all treatments. But rough lemon was very poor in rooting (4%). NAA at 0.5mg/L gave the highest results of rooting. The findings are in agreement with those observed in *Citrus jambhiri* **(Rahman 1987)**, *Punica granatum* **(Kantharajah *et al* 1998)** and *Citrus grandis* **(Paudyal and Haq 2000). Begum *et al* (2004)** indicated that roots were induced when the isolated individual shoots of three pummelo (*C. grandis)* varieties were cultured on 1/2 MS medium containing 0.1 mg/L each of NAA, IBA or IAA.

Acclimatization

Rooted plantlets were successfully transferred into nursery pots containing soil mixture (1 peat: 1 sand) and grew normally developing of new leaves **Fig. (3)**.

Generally, mass propagation of some citrus species through *in vitro* culture is one of the best successful examples of commercial application of plant tissue culture technology. These plants may also be used for *in vitro* or *in vivo* grafting on healthy citrus rootstocks to produce true to type clones which are an important need for citrus improvement.

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الإكثار الدقيق لنباتات الموالح الناتجة من الأجنة الجسمية

[٤٦]

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أجـــرى هذا البحث بهدف الإكثار الدقيق باستخدام العقل الساقية الدقيقة لبعض أنواع الموالـــح الناتجـــة من الأجنـــة الجسمية في المعمل وكذلك من بادرات ناتجة من إنبات البـــذور معمليـــا واستنتاج افضل المعاملات لذلك ودراســـة مدى كفاءة هذة البادرات فى الإكثار الدقيق وقد تم اختيار الأنواع التالية: البرتقال ابو سرة صنف واشنطن ، البرتقال الشاموتى ، البرتقال الأحمر بدمه ، الجريب فروت ، اليوسفى البلدى ، الليمون الأضاليا،

الترنج ، الليمون المالح ، الليمون المخرفش والنارنج . وقد تم تقسيم البادرات الى عقل ساقية دقيقة ذات برعم واحد بطول ٠,٥ سم وزراعتها على بيئة موراشيج وسكوج مزودة بتركيزات مختلفة (٠,٥ ، ١,٠ ، ٢,٠ مجم/لتر) من بنزيل ادينين بمفرده او باضافة ٠,٥ مجم/لتر من نفثالين حمض الخليك. وتم الحصول على افضل تضاعف للنموات الخضرية باستخدام البنزيل ادينين بتركيز ٠,٥ مجم/لتر مع نفثالين حمض الخليك بتركيز ٠,٥ مجم/لتر وايضا أطول نموات خضرية. وتم تجذير هذة النموات الخضرية عندما تم نقلها منفردة على بيئة تحتوى ١/٢ تركيزات موراشيج وسكوج باضافة اندول حمض البيوتريك بتركيزات (٠,٥ أو ١,٠ مجم/لتر) او نفثالين حمض الخليك بتركيزات (٠,٥ أو ١,٠ مجم/لتر) حيث ادى استخدام نفثالين حمض الخليك بتركيز ٠,٥ مجم/لتر إلى أعلى نسبة تجذير (١٠٠%). وتم أقلمة النباتات الناتجة بنجاح باستخدام خليط من الرمل والبيتموس بنسبة (١:١). ويتضح من النتائج السابقة كفاءة النباتات الناتجة من الأجنة الجسمية فى الإكثار الدقيق بنجاح مقارنة بالبادرات الناتجة من انبات البذور.

الكلمات الدالة: الموالح ، العقل الساقية الدقيقة، الإكثار الدقيق.

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