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# PRODUCTION OF SALT-TOLERANT SOUR ORANGE PLANTS (*Citrus aurantium* L.) BY USING TISSUE CULTURE TECHNIQUE:I- *IN VITRO* CALLUS FORMATION

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## ABSTRACT

This study was conducted through 1996-1999 years in the Department of Pomology, Faculty of Agriculture, Alexandria University. The work involved *in vitro* callus formation from different seedling (grown *in vitro*) explants of sour orange.

**The main results can be summarized in the following points:**

1- On the MT (1969) medium containing 10 $mgl^{-1}$ NAA + 0.5$mgl^{-1}$ kinetin, the percentages of callus formation from shoot tip, epicotyl, hypocotyl and cotyledon explants (excised from seedlings of sour orange grown *in vitro*) were 66.67%, 80.00%, 66.67% and 93.33%, respectively. These results indicated that cotyledon segments were the best explants for callus formation.

2- Maintenance of continually growing for the produced callus by subculturing on the same medium without plant hormones.

## INTRODUCTION

*Citrus* is considered as the first economic fruit crop in Egypt. The total *Citrus* cultivated area in ARE reached 336822 feddans producing 2150256 tons of fruits according to the statistics of the Ministry of Agriculture, Cairo, 1998.

*Citrus* plants are among the most sensitive fruit crops to salinity (Furr *et al*., 1963). Therefore, it is of great importance to obtain plants capable of growing with elevated salt levels in the irrigation water (Ben-Hayyim and Kochba, 1983).

*Citrus* rootstocks adapted to difficult soil situations, such as high salt levels, are needed (Soost and Roose, 1996).

**Objectives of the present study**

1. *In vitro* callus formation from different seedling (grown *in vitro*) explants of sour orange.
2. Growth of the obtained callus.
3. Evaluation and selection of salt - tolerant callus.

## MATERIALS AND METHODS

The present investigation was carried out during four successive years (1996-1999), in the Department of Pomology, Faculty of Agriculture, Alexandria University, in order to study the possibility of using tissue culture technique for callus formation from different seedling (grown *in vitro*) explants of sour orange.

### 1. Plant Material

*In vitro* seedlings resulted from germinated seeds of sour orange (*Citrus aurantium* L.) were used as tissue sources (explants) for callus formation.

**2. Sterilization**

The seeds of sour orange were rinsed with running tap water for 10 min. Surface sterilization of the seeds was carried out in a laminar flow hood by immersing them for 3 min in 70% ethanol, dipping in 50% sodium hypochlorite for 30 min and three rinses in sterile redistilled water, followed by transferring to sterile petri dishes.

**3. Culture Media**

Each seed was stripped of its coat (outer and inner covers) and put on a sterile filter papers in sterile petri dishes by using a sterile scalpel blade and foreceps under aseptic conditions and cultured in test tubes (one seed per tube) on the basal, agar solidified medium of Murashige and Skoog (1962) (MS) at full strength plus 30 $gl^{-1}$ sucrose, 8$gl^{-1}$ agar and hormone free. The seeds germinated within two weeks and developed into 8 –12 cm long seedlings within a period of 3 to 4 weeks. The germinated seeds were used as the source of explants. These explants, comprised shoot tip, epicotyl, hypocotyl, cotyledon and root segments, were aseptically excised under sterile conditions from three week old seedlings.

Callus formation medium was either basal Murashige and Skoog (1962) (MS) or Murashige and Tucker (1969) (MT) salts supplemented with various concentrations of plant growth regulators included, ∝-naphthalene acetic acid (NAA) at 1, 5 and 10 mg $1^{-1}$, 2,4 – dichlorophenoxy acetic acid (2, 4 – D) at 1 and 2 mg $1^{-1}$, and 6-furfuryl amino purine (Kinetin) at 0.5 mg $1^{-1}$ were used for the culture of seedling explants. The pH of the media was adjusted to 5.7 before adding agar (Difco Bacto-agar, 8000 mg $l^{-1}$).

These media along with the different concentrations of growth regulators added were represented by 12 medium formulations as indicated in Tables (1 to 5)and took the medium code from C.F.M. 1 to C.F.M.12.

The media were dispensed in culture test tubes (20X150mm) with 10 ml (each), closed with cotton, capped with aluminum foil, and sterilized in an autoclave at 121°C for 20 min, then left to cool and harden for 24hrs before being used.

**4. Culture Conditions**

The cultures were incubated at 25 ± 2°C, under 16 hrs light period from fluorescent lamps (2 lamps per shelf), followed by 8hrs dark period. Callus formation was evaluated after one month from culturing date by the use of callus formation percentage and index which calculated as follows:

$$\text{Callus formation percentage} = \frac{\text{No. of cultured tubes with callus formation}}{\text{Total no. of cultured tubes}} \times 100$$

Callus formation index = fresh weight of the formated callus per explant: ≥100 mg.

**5. Statistical Analysis**

Percentages of sour orange seedling explants produced callus under the tested media, were determined after one month of explant cultured date. Each treatment consisted of 15 test tubes (five replicates). One explant cultured in test tube. Each three test tubes were considered a replicate. All the experiments were arranged in a completely randomized design and the statistical procedures were applied according to Steel and Torrie (1980).

## RESULTS AND DISCUSSION

**1. Callus Formation**

Data concerning the effect of various medium formulations on the percentages of callus formation from five different explants (shoot tip, epicotyl, hypocotyl, cotyledon and root segments) excised from three- week old seedlings (grown *in vitro)* of sour orange, are shown in Tables (1 to 5).

**1.1 Shoot Tip Explant**

The results represented in Table (1) indicated that, the percentage of callus formation from shoot tip explant was significantly the highest (66.67%) on the MT medium containing 10 $mgl^{-1}$ NAA + 0.5$mgl^{-1}$ kinetin (C.F.M.10). On the contrary, the lowest percentage (6.67%) was resulted in MS medium containing 5 $mgl^{-1}$ NAA +0.5 $mgl^{-1}$ Kinetin (C.F. M.3).

Keeping the kinetin concentration constant (0.5$mgl^{-1}$) in the MT medium, as the NAA concentration was increased from 1 $mgl^{-1}$ to 10 $mgl^{-1}$, the callus formation percentage was significantly increased from 20.00% to 66.67 % (C.F.M.8 to C.F.M.10). However, keeping the kinetin concentration constant (0.5$mgl^{-1}$) in MT medium, as the 2,4-D concentration was increased from 1 $mgl^{-1}$ to 2 $mgl^{-1}$, the callus formation percentage was significantly decreased from 40.00 % to 20.00 % (C.F.M.11 to C.F.M. 12). On the other hand, keeping the kinetin concentration constant (0.5$mgl^{-1}$) in the MS medium, as the NAA concentration was increased from 1 $mgl^{-1}$ to 10 $mgl^{-1}$, the callus formation percentage was significantly increased from 0.00 % to 40.00% (C.F.M.2 to C.F.M.4). However, keeping the kinetin concentration constant (0.5$mgl^{-1}$) in the MS medium, as the 2,4-D concentration was increased from 1 $mgl^{-1}$ to 2 $mgl^{-1}$, the callus formation percentage was decreased from 33.33 % to 20.00 % (C.F.M.5 to C.F.M.6).

Table (1) showed no callus formation occurred (0.00%) on the MS and MT basal media without the addition of growth substances (C.F.M.1) and (C.F.M.7), respectively. Also, a similar result was observed (0.00%) on the MS basal medium, supplemented with 1$mgl^{-1}$ NAA + 0.5$mgl^{-1}$ kinetin (C.F.M.2).

**Table (1): Effect of 12 medium formulations on the percentage of callus formation from shoot tip explant excised from three-week-old seedlings (grown *in vitro*) of sour orange.**

| Medium code | Basal medium | Growth regulators in $mgl^{-1}$ | | | % Callus formation[z] |
|---|---|---|---|---|---|
| | | NAA | 2,4-D | Kin | |
| C.F.M.1 | MS | 0.0 | 0.0 | 0.0 | 0.00 F* |
| C.F.M.2 | MS | 1.0 | 0.0 | 0.5 | 0.00 F |
| C.F.M.3 | MS | 5.0 | 0.0 | 0.5 | 6.67 EF |
| C.F.M.4 | MS | 10.0 | 0.0 | 0.5 | 40.00 BC |
| C.F.M.5 | MS | 0.0 | 1.0 | 0.5 | 33.33 CD |
| C.FM.6 | MS | 0.0 | 2.0 | 0.5 | 20.00 DE |
| C.F.M.7 | MT | 0.0 | 0.0 | 0.0 | 0.00 F |
| C.F.M.8 | MT | 1.0 | 0.0 | 0.5 | 20.00 DE |
| C.F.M.9 | MT | 5.0 | 0.0 | 0.5 | 53.33 AB |
| C.F.M.10 | MT | 10.0 | 0.0 | 0.5 | 66.67 A |
| C.F.M.11 | MT | 0.0 | 1.0 | 0.5 | 40.00 BC |
| C.F.M.12 | MT | 0.0 | 2.0 | 0.5 | 20.00 DE |
| L.S.D. $_{0.05}$ | | | | | 18.558 |

**C.F.M. = Callus formation medium.**

**[z] There were 15shoot tip explants (one per culture tube) per treatment (medium formulation).**

***Values followed by the same letters are not significantly different at the 0.05 level of probability.**

### 1.2 Epicotyl Explant

Table (2) indicated that, the percentage of callus formation from epicotyl explant was significantly the highest (80.00%) on the MT medium containing 10$mgl^{-1}$NAA+ 0.5$mgl^{-1}$ kinetin (C.F. M. 10) .In contrast, the lowest percentage (6.67%) was significantly resulted in the MS medium containing 1 $mgl^{-1}$ NAA +0.5 $mgl^{-1}$ Kinetin (C. F. M. 2).

**Table (2): Effect of 12 medium formulations on the percentage of callus formation from epicotyl explant excised from three- week- old seedlings (grown *in vitro*) of sour orange.**

| Medium code | Basal medium | Growth regulators in $mgl^{-1}$ | | | % Callus formation[z] |
|---|---|---|---|---|---|
| | | NAA | 2,4-D | Kin | |
| C.F.M.1 | MS | 0.0 | 0.0 | 0.0 | 0.00 F* |
| C.F.M.2 | MS | 1.0 | 0.0 | 0.5 | 6.67 F |
| C.F.M.3 | MS | 5.0 | 0.0 | 0.5 | 20.00 EF |
| C.F.M.4 | MS | 10.0 | 0.0 | 0.5 | 53.33 BCD |
| C.F.M.5 | MS | 0.0 | 1.0 | 0.5 | 40.00 CDE |
| C.F.M.6 | MS | 0.0 | 2.0 | 0.5 | 20.00 EF |
| C.F.M.7 | MT | 0.0 | 0.0 | 0.0 | 0.00 F |
| C.F.M.8 | MT | 1.0 | 0.0 | 0.5 | 53.33 BCD |
| C.F.M.9 | MT | 5.0 | 0.0 | 0.5 | 73.33 AB |
| C.F.M.10 | MT | 10.0 | 0.0 | 0.5 | 80.00 A |
| C.F.M.11 | MT | 0.0 | 1.0 | 0.5 | 60.00 ABC |
| C.F.M.12 | MT | 0.0 | 2.0 | 0.5 | 33.33 DE |
| **L.S.D. $_{0.05}$** | | | | | 20.476 |

**C.F.M. = Callus formation medium.**

**[z] There were 15 epicotyl explants (one per culture tube) per treatment (medium formulation).**

***Values followed by the same letters are not significantly different at the 0.05 level of probability.**

Keeping the kinetin concentration constant ($0.5mgl^{-1}$) in the MT medium, as the NAA concentration was increased from 1 $mgl^{-1}$ to 10 $mgl^{-1}$, the callus formation percentage was significantly increased from 53.33 % to 80.00% (C.F.M.8 to C.F.M.10). However, keeping the kinetin concentration constant ($0.5mgl^{-1}$) in the MT medium, as the 2,4-D concentration was increased from 1 $mgl^{-1}$ to 2 $mgl^{-1}$, the callus formation percentage was significantly decreased from 60.00 % to 33.33 % (C.F.M.11 to C.F.M.12).

On the other hand, keeping the kinetin concentration constant ($0.5mgl^{-1}$) in the MS medium, as the NAA concentration was increased from 1 $mgl^{-1}$ to 10 $mgl^{-1}$, the callus formation percentage was significantly increased from 6.67 % to 53.33% (C.F.M.2 to C.F.M.4). However, keeping the kinetin concentration constant ($0.5mgl^{-1}$) in the MS medium, as the 2,4-D concentration was increased from 1 $mgl^{-1}$ to 2 $mgl^{-1}$, the callus formation percentage was decreased from 40.00 % to 20.00 % (C.F.M.5 to C.F.M.6).

Table (2) showed no callus formation occurred (0.00%) on the MS and MT basal media without the addition of growth substances (C.F.M.1) and (C.F.M.7), respectively.

### 1.3 Hypocotyl Explant

Table (3) indicated that, the percentage of callus formation from hypocotyl explant was significantly the highest (66.67%) on the MT medium containing 10 $mgl^{-1}$ NAA + $0.5mgl^{-1}$ kinetin (C.F. M. 10). On the contrary, the lowest percentage of callus formation (6.67%) was significantly resulted in the MS medium containing 5 $mgl^{-1}$ NAA +0.5 $mgl^{-1}$ Kinetin (C. F. M. 3).

**Table (3): Effect of 12 medium formulations on the percentage of callus formation from hypocotyl explant excised from three-week-old seedlings (grown *in vitro*) of sour orange.**

| Medium code | Basal medium | Growth regulators in $mgl^{-1}$ | | | % Callus formation[z] |
|---|---|---|---|---|---|
| | | NAA | 2,4-D | Kin | |
| C.F.M.1 | MS | 0.0 | 0.0 | 0.0 | 0.00 F* |
| C.F.M.2 | MS | 1.0 | 0.0 | 0.5 | 0.00 F |
| C.F.M.3 | MS | 5.0 | 0.0 | 0.5 | 6.67 EF |
| C.F.M.4 | MS | 10.0 | 0.0 | 0.5 | 46.67 B |
| C.F.M.5 | MS | 0.0 | 1.0 | 0.5 | 26.67 CD |
| C.F.M.6 | MS | 0.0 | 2.0 | 0.5 | 20.00 DE |
| C.F.M.7 | MT | 0.0 | 0.0 | 0.0 | 0.00 F |
| C.F.M.8 | MT | 1.0 | 0.0 | 0.5 | 20.00 DE |
| C.F.M.9 | MT | 5.0 | 0.0 | 0.5 | 40.00 BC |
| C.F.M.10 | MT | 10.0 | 0.0 | 0.5 | 66.67 A |
| C.F.M.11 | MT | 0.0 | 1.0 | 0.5 | 40.00 BC |
| C.F.M.12 | MT | 0.0 | 2.0 | 0.5 | 20.00 DE |
| **L.S.D. $_{0.05}$** | | | | | 19.348 |

**C.F.M. = Callus formation medium.**

**[z] There were 15 hypocotyl explants (one per culture tube) per treatment (medium formulation).**

***Values followed by the same letters are not significantly different at the 0.05 level of probability.**

Keeping the kinetin concentration constant ($0.5mgl^{-1}$) in the MT medium, as the NAA concentration was increased from 1 $mgl^{-1}$ to 10 $mgl^{-1}$, the callus formation percentage was significantly increased from 20.00% to 66.67% (C.F.M.8 to C.F.M.10). However, keeping the kinetin concentration constant ($0.5mgl^{-1}$) in the MT medium,as the 2,4-D concentration was increased from 1 $mgl^{-1}$ to 2 $mgl^{-1}$, the callus formation percentage was significantly decreased from 40.00 % to 20.00 % (C.F.M.11 to C.F.M.12).

On the other hand, keeping the kinetin concentration constant ($0.5mgl^{-1}$) in the MS medium, as the NAA concentration was increased from 1 $mgl^{-1}$ to 10 $mgl^{-1}$, the callus formation percentage was significantly increased from 0.00 % to 46.67 % (C.F.M.2 to C.F.M.4). However, keeping the kinetin concentration constant ($0.5mgl^{-1}$) in the MS medium, as the 2,4-D concentration was increased from 1 $mgl^{-1}$ to 2 $mgl^{-1}$, the callus formation percentage was decreased from 26.67% to 20.00 % (C.F.M.5 to C.F.M.6).

Table (3) showed no callus formation occurred (0.00%) on the MS and MT basal media without the addition of growth substances (C.F.M.1) and (C.F.M.7), respectively. Also, a similar result was observed (0.00 %) on the MS basal medium supplemented with $1mgl^{-1}$ NAA+ 0.5 mg $l^{-1}$ kinetin (C.F.M.2).

**1.4 Cotyledon Explant**

Table (4) indicated that, the percentage of callus formation from cotyledon explant was significantly the highest (93.33%) on the MT medium

containing 10 $mgl^{-1}$ NAA + 0.5$mgl^{-1}$ kinetin (C.F. M. 10). In contrast, the lowest percentage of callus formation (20.00 %) was significantly resulted in the MS medium containing 1 $mgl^{-1}$ NAA +0.5 $mgl^{-1}$ Kinetin (C. F. M. 2).

Keeping the kinetin concentration constant (0.5$mgl^{-1}$) in the MT medium, as the NAA concentration was increased from 1 $mgl^{-1}$ to 10 $mgl^{-1}$, the callus formation percentage was significantly increased from 40.00 % to 93.33% (C.F.M.8 to C.F.M.10). However, keeping the kinetin concentration constant (0.5$mgl^{-1}$) in the MT medium, as the 2,4-D concentration was increased from 1 $mgl^{-1}$ to 2 $mgl^{-1}$, the callus formation percentage was significantly decreased from 66.67 % to 33.33 % (C.F.M.11 to C.F.M.12).
On the other hand, keeping the kinetin concentration constant (0.5$mgl^{-1}$) in the MS medium, as the NAA concentration was increased from 1 $mgl^{-1}$ to 10 $mgl^{-1}$, the callus formation percentage was significantly increased from 20.00 % to 73.33%(C.F.M.2 to C.F.M.4). However, keeping the kinetin concentration constant (0.5$mgl^{-1}$) in the MS medium, as the 2,4-D concentration was increased from 1 $mgl^{-1}$ to 2 $mgl^{-1}$, the callus formation percentage was decreased from 53.33% to 33.33% (C.F.M.5 to C.F.M.6).

Table (4) showed no callus formation occurred (0.00%) on the MS and MT basal media without the addition of growth substances (C.F.M.1) and (C.F.M.7), respectively.

**Table (4): Effect of 12 medium formulations on the percentage of callus formation from cotyledon explant excised from three-week-old seedlings (grown *in vitro*) of sour orange.**

| Medium code | Basal medium | Growth regulators in $mgl^{-1}$ | | | % Callus formation[z] |
|---|---|---|---|---|---|
| | | NAA | 2,4-D | Kin | |
| C.F.M.1 | MS | 0.0 | 0.0 | 0.0 | 0.00 E* |
| C.F.M.2 | MS | 1.0 | 0.0 | 0.5 | 20.00 DE |
| C.F.M.3 | MS | 5.0 | 0.0 | 0.5 | 53.33 BC |
| C.F.M.4 | MS | 10.0 | 0.0 | 0.5 | 73.33 AB |
| C.F.M.5 | MS | 0.0 | 1.0 | 0.5 | 53.33 BC |
| C.F.M.6 | MS | 0.0 | 2.0 | 0.5 | 33.33 CD |
| C.F.M.7 | MT | 0.0 | 0.0 | 0.0 | 0.00 E |
| C.F.M.8 | MT | 1.0 | 0.0 | 0.5 | 40.00 CD |
| C.F.M.9 | MT | 5.0 | 0.0 | 0.5 | 73.33 AB |
| C.F.M.10 | MT | 10.0 | 0.0 | 0.5 | 93.33 A |
| C.F.M.11 | MT | 0.0 | 1.0 | 0.5 | 66.67 B |
| C.F.M.12 | MT | 0.0 | 2.0 | 0.5 | 33.33 CD |
| L.S.D. 0.05 | | | | | 20.107 |

**C.F.M. = Callus formation medium.**

**[z] There were 15 cotyledon explants (one per culture tube) per treatment (medium formulation).**

***Values followed by the same letters are not significantly different at the 0.05 level of probability.**

### 1.5 Root Explant

Table (5) indicated that, the percentage of callus formation from root explant was significantly the highest (20.00 %) on the MS and MT media containing 10 $mgl^{-1}$ NAA + 0.5$mgl^{-1}$ kinetin (C.F. M. 4) and (C.F.M.10), respectively.

No callus formation occurred (0.00%) on the MS and MT basal media without the addition of growth substances (C.F.M.1) and (C.F.M.7), respectively. Also, the same result was observed on the MS basal medium supplemented with 1$mgl^{-1}$ NAA + 0.5 $mgl^{-1}$ kinetin (C.F.M.2).

Keeping the kinetin concentration constant (0.5 $mgl^{-1}$) in the MT medium, as the NAA concentration was increased from 1 $mgl^{-1}$ to 10 $mgl^{-1}$, the callus formation percentage was increased from 6.67% to 20.00 % (C.F.M.8 to C.F.M.10). However, keeping the kinetin concentration constant (0.5 $mgl^{-1}$) in the MT medium, as the 2,4-D concentration was increased from 1 $mgl^{-1}$ to 2 $mgl^{-1}$, the callus formation percentage did not affect.

On the other hand, keeping the kinetin concentration constant (0.5 $mgl^{-1}$) in the MS medium, as the NAA concentration was increased from 1 $mgl^{-1}$ to 10 $mgl^{-1}$, the callus formation percentage was significantly increased from 0.00% to 20.00% (C.F.M.2 to C.F.M.4). However, keeping the kinetin concentration constant (0.5 $mgl^{-1}$) in the MS medium, as the 2,4-D concentration was increased from 1 $mgl^{-1}$ to 2$mgl^{-1}$, the callus formation percentage did not affect (Table 5).

**Table (5): Effect of 12 medium formulations on the percentage of callus fromation from root explant excised from three-week-old seedlings (grown *in vitro*) of sour orange.**

| Medium code | Basal medium | Growth regulators in $mgl^{-1}$ | | | % Callus formation[z] |
|---|---|---|---|---|---|
| | | NAA | 2,4-D | Kin | |
| C.F.M.1 | MS | 0.0 | 0.0 | 0.0 | 0.00 B* |
| C.F.M.2 | MS | 1.0 | 0.0 | 0.5 | 0.00 B |
| C.F.M.3 | MS | 5.0 | 0.0 | 0.5 | 6.67 AB |
| C.F.M.4 | MS | 10.0 | 0.0 | 0.5 | 20.00 A |
| C.F.M.5 | MS | 0.0 | 1.0 | 0.5 | 6.67 AB |
| C.F.M.6 | MS | 0.0 | 2.0 | 0.5 | 6.67 AB |
| C.F.M.7 | MT | 0.0 | 0.0 | 0.0 | 0.00 B |
| C.F.M.8 | MT | 1.0 | 0.0 | 0.5 | 6.67 AB |
| C.F.M.9 | MT | 5.0 | 0.0 | 0.5 | 6.67 AB |
| C.F.M.10 | MT | 10.0 | 0.0 | 0.5 | 20.00 A |
| C.F.M.11 | MT | 0.0 | 1.0 | 0.5 | 6.67 AB |
| C.F.M.12 | MT | 0.0 | 2.0 | 0.5 | 6.67 AB |
| L.S.D. $_{0.05}$ | | | | | 17.303 |

**C.F.M. = Callus formation medium.**

**[z] There were 15 root explants (one per culture tube) per treatment (medium formulation).**

***Values followed by the same letters are not significantly different at the 0.05 level of probability.**

Regarding the forecited results in Tables (1 to 5), it was cleared that cotyledon explant of sour orange seedlings grown in vitro was the super to produce the highest callus formation percentage (93.33%) followed by epicotyl explant which tabulated 80%. The rest seedling explants were the lower in that respect with callus formation percentages of 66.67%, 66.67% and 20% for shoot tip, hypocotyl and root explants, respectively.

The same Tables also revealed that among the tested callus formation media, the MT one containing 10 $mgl^{-1}$ NAA + 0.5 $mgl^{-1}$ Kinetin (C.F.M. 10) was the best medium to produce the highest callus formation percentage from all seedling explants of sour orange tested.

The obtained results are in complete agreement with those reported by Gill *et al.,* (1994), who found that, different explants excised from *in vitro* seedlings of "Kinnow" mandarin initiated callus when cultured on the MT (1969) medium; cotyledon segments were the best explants for callus induction. The same authors (1991) stated that the maximum callus induction frequency in "Kinnow" mandarin (86.8%) was achieved on Murashige and Tucker (1969) medium with cotyledon segments.

Our findings also agreed with those obtained by Gill *et al.,* (1995). Who found that the best callus induction in "Local Sangtra" mandarin occurred on the MS (1962) medium containing NAA (10 $mgl^{-1}$) and kinetin (0.5$mgl^{-1}$).

The obtained results disagreed with those reported by Oh *et al.,* (1991). They found that the best combination of growth regulators for callus formation from shoot tips of *C. junos* was 0.5 to 1.0 $mgl^{-1}$ 2,4-D +1.0 $mgl^{-1}$ NAA. Also, Chaudhry *et al.* (1993) found that the Murashige and Skoog (1962) medium containing 1.0 $mgl^{-1}$ 2,4-D, 0.5 $mgl^{-1}$ BA and 0.5 $mgl^{-1}$ NAA was optimum for callus formation from cotyledons of "Kinnow" mandarin.

The best callus formation percentage occurred on the MT medium in the present study, similar results have also reported in *C. limon* (Murashige and Tucker, 1969), *C. sinensis* (Brunet and Ibrahim, 1973), *C. aurantifolia* (Raj Bhansali and Arya, 1978), *C. junos* (Song *et al.,* 1991), and *C*. *aurantium* (Koc and Can, 1992).

**2. Callus Growth Continuation**

Finally, the most obtained callus in this study was induced from cotyledon tissues of sour orange. Cotyledon callus was habituated by subculturing 8 times of 4 weeks each (monthly) on the MT basal medium containing some growth regulators. After these subcultures most of the callus was one more subcultured monthly on the MT basal medium without the addition of plant hormones. This procedure agreed with that used by Singh *et al.* (1992) and Gill *et al.* (1994).

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## "إنتاج نباتات نارنج متحملة للملوحة باستخدام تقنية زراعة الأنسجة":

## ١- تكوين الكالس معمليا

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أجريت هذه الدراسة خلال الفترة من بداية عام ١٩٩٦ وحتى نهاية عام ١٩٩٩ بقسم الفاكهة كلية الزراعة جامعة الإسكندرية وكان الهدف من إجراء هذه الدراسة هو تكوين الكالس معمليا من الأجزاء المختلفة لبادرات نارنج تم إنتاجها معمليا على بيئة صناعية.

ويمكن تلخيص النتائج الرئيسية لهذه الدراسة فى النقاط التالية:-

1. تم الحصول على أعلى نسب مئوية لتكوين الكالس من القمة النامية والسويقة الجنينية العليا والسويقة الجنينية السفلى والفلقات (هذه الأجزاء مفصولة من بادرات النارنج النامية على بيئة صناعية) ٦٦,٦٧% و ٨٠% و ٦٦,٦٧% و ٩٣,٣٣% على التوالى وذلك على بيئة موراشيج وتوكر لسنة ١٩٦٩ والمحتوية على ١٠ مليجرام فى اللتر نفثالين حمض الخليك + ٠,٥ مليجرام فى اللتر كينتين. هذه النتائج أوضحت أن الفلقات كانت افضل الأجزاء النباتية المختبرة لتكوين الكالس.
2. تم الحفاظ على استمرارية نمو الكالس الناتج وذلك بزراعته على نفس البيئة ولكنها خالية من الهرمونات النباتية.