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EFFECT OF MEDIA ON PROPAGATION OF *MAGNOLIA GRANDIFLORA* WITH TISSUE CULTURE TECHNIQUE

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ABSTRACT

The experimental trial was consummated throughout 2009 – 2010 years in Plant Tissue Culture laboratory at El-Zohria Botanical Garden, Horticulture Research Institute, Agriculture Research Center. It intended to find out the solve of the most important problem facing the propagation of *Magnolia grandiflora* plant under local conditions i.e. difficult to propagate traditionally. Accordingly, the study was designed to reach a well defined protocol for micropropagation of such plant. Shoot tips of *Magnolia grandiflora* were effectively surface sterilized with 30 % Clorox (sodium hypochlorite) as commercial bleaches for 15 min. The longest shoots and greatest number of leaves had been obtained at explants on MS establishment medium with (0.5 mg/l NAA). In the multiplication stage, 5.0 mg/l Kin formed not only the highest number of shoots but also the longest shoots. The shoots of *Magnolia grandiflora* successfully rooted when they cultured in MS medium supplemented with 1.5 mg/l IBA. Plantlets after root development exhibited 45% survival in plastic pots filled with peat moss and sand at a ratio of 1:1.

Key words: Micropropagation, *In vitro*, Tissue culture, Liquidambar, Shoot tips.

INTRODUCTION

Magnolia grandiflora Linn (Bull Bay) belongs to the family Magnoliaceae. It is an evergreen, broadly conical or rounded, dense tree, 10–24 m in length with oblong, glossy, mid to dark green leaves. Magnolia grandiflora bears large, very fragrant, bowl-shaped, white

flowers. They show intermittently from mid-summer to early autumn (Bailey & Bailey, 1960).

Magnolia grandiflora is a difficult-to-propagate evergreen tree through conventional vegetative methods. It is also difficult to establish its tissues *in vitro* from plant material taken from mature woody trees because of the loss of apparent regeneration ability and contamination occurring in mature plant tissues (Biedermann, 1987).

Previous results for sterilization of *Magnolia grandiflora* from shoot tips showed that 50% chlorox (NaOCl) combined with 70% ethanol was the most effective treatment for reducing the contamination of explants, but led to a low survival percentage (33.33%) compared with 40% chlorox alone which gave 64.0% survival (Sakr *et al*, 1999). Results for sterilization of *Magnolia grandiflora* shoots demonstrated that 4mg/l mercuric chloride (Hg_2Cl) and 2% sodium hypochlorite (NaOCl) solution (commercial bleach as 'Clorox') were the highest value of survived plants (El-Shamy *et al*, 2004).

Explants of *Magnolia delavayi* were cultured on quarter-strength MS medium or Vacin & Went (VW) medium with combinations of BA (0.50-5.00 mg/l) and NAA (0.05-0.50 mg/l) and different light and temperature regimes. VW medium was more successful than MS medium for shoot production. This was thought to be due to the lower nutrient concentrations in VW medium (Luo & Sung, 1996). Micropropagation cultures of *Magnolia X soulangiana* showed a decreasing growth rate over a 2-year period in Miller medium set with agar and supplemented with nicotinic acid, thiamine, pyridoxine, glycine, myo-inositol and sucrose. When the effect of addition of 0.2 mg IBA or 0.2, 0.5 and 1.0 mg/l BA to the medium was compared, explant growth was highest with 0.5 mg BA but IBA had no effect. There was a shift in dose-effectiveness with age of culture i.e. 0.2-0.5 mg BA was suitable for a 2-year-old culture whereas a 9-month-old culture required only 1.0 mg (Franc & Krejci, 1998).

Apical shoots from a 100-year-old *Magnolia soulangiana* plant were cultured on full- or half-strength modified S-medium or modified WP medium. All media were supplemented with 0.01-0.1 mg/l NAA, 0.5-0.3 mg/l BA [benzyladenine], 20 g/l sucrose and 7 g agar/litre. Tissue cultures were kept at 20-22 °C and 90% humidity with a light intensity of 35-40 mol m^{-2} s^{-1}. The full-strength S-medium with 0.3 mg/l BA and 0.1 mg/l NAA in a culture vessel was optimal

(Kamenicka & Takats, 1997). Two-year trials were carried out to determine the effect of different BA concentrations on multiplication *in vitro* of *Magnolia soulangiana*. The effect of addition to the medium of low concentrations (0.0, 0.2 or 0.5 mg/litre) of indolbutyric acid (IBA) was also investigated in relation to constant red or white light. Culture was performed on the Mil medium (7 g agar/litre + 2 mg nicotinic acid, 1 mg thiamine, 0.5 mg pyridoxine, 2 mg glycine, 100 mg myo-inositol and 25 g sucrose). In the first trial the best results were obtained with Mil medium + 1.0 mg IBA/litre (average number of shoots 4.6, average length of shoots 5.0 mm). In the second trial, addition of 0.2 mg IBA gave the best results in all illumination treatments, with best overall results in constant white fluorescent light at 40 μmol m^{-2} s^{-1} (Krejci & Franc, 1997). Using MS medium at full strength was more effective in increasing the number of shoots/explant and length of *Magnolia grandiflora* shoots than other medium strengths. Concerning the effect of medium type, explants grown on B5 medium showed promising results in terms of number of shoots and length of axillary shoots, whereas, SH medium and WP medium gave the lowest values. With regard to IBA concentration, the addition of IBA at 1 mg/litre to B5 medium resulted in the highest number of shoots. Raising the level of IBA to 2.0 mg/litre significantly decreased the degree of browning, induced callus formation and gave the longest axillary shoots (Sakr *et al*, 1999). The induction rate of *Magnolia officinalis* was 100% in Gamborg *et al* medium (B5) containing 4.0 mg 2,4-D and 1.0 mg NAA/litre. The highest proliferation rate and the lowest percentage of callus browning were recorded from B5 medium containing 1.2-2.0 mg BA and 1.0 mg NAA/litre (Tong *et al*, 2002).

On the other side, it was found that the lower the salt concentration, the more shoot elongation of *Magnolia* was hampered and the better the root formation. Higher Kin-levels (2.5 mg/l) in combination with high salt concentrations (1/1 and 1/2) allow fairly uniform elongation while rooting was poor. On low salt-media, rooting was prominent but the leaves were yellowing and the elongation was nil (Maene & Debergh, 1985). Microcuttings of *Magnolia soulangiana* were treated *in vitro* with 0,1.0, 2.0, 3.0 or 4.0 mg IBA/litre. Root number was greatest with 4.0 mg IBA and root length with 1.0 mg IBA (Kamenicka, 1996). In a later trial, the optimal rooting medium for *Magnolia* was half-strength. S-medium

with 4.0 mg IAA and 3.0 mg activated charcoal/litre giving 90% survival and 96% root production (Kamenicka & Takats, 1997).

MATERIALS AND METHODS

This study was carried out in the laboratory of Tissue Culture, Zohria Botanical Garden, Cairo-Egypt, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture. The experiments were carried out throughout 2009 – 2010 years. The objective of this study was to investigate the most suitable treatments for micropropagation of *Magnolia grandiflora* Linn. that are difficult to propagate traditionally. The mother plants were cultured in Zohria Garden. The parts used as explants were shoot tips.

1. Plant Material:

The mother plants were obtained from Zohria Botanical Garden grown naturally under the open field conditions. The parts taken as explants from *Magnolia grandiflora* were shoot tips from 6-meter tall trees (60 years old).

2. Incubation Conditions:

The cultures of *Magnolia soulangeana* were incubated at 23 ± 2 ^{0}C under a 16/8h photoperiod at about 18 µ mol m^{-2} S^{-1} provided by Philips TLD lamps (Maene & Debergh, 1985).

3. Culture Media:

The Murashige and Skoog (MS) medium and Gamborg (B5) medium were used for starting the explants of *Magnolia grandiflora.* All types of media were solidified and supplemented with 7.0 g/l agar. Sucrose at 30.0 g/l was added as a source of carbohydrate. The pH was adjusted to 5.7. Twenty ml medium were poured in 100 ml jars and sterilized by autoclaving under steam pressure 1.5 bar at 121°C for 20 min.

4. Experimental design and statistical analysis:

A complete randomized design was employed in all of the experiments. Analysis of variance was used to show statistical differences between treatments using the L.S.D. at probability level (Snedecor and Cochran, 1989).

5. Preparation of Explants:

Before being cultured onto the different media treatments, the shoot tips from *Magnolia grandiflora* were cut to 0.5-1.0 cm with a scalpel under sterile conditions

6. Experimental treatments:

Surface Sterilization of Explants:

The explants were excised from the mother plants and then washed by soapy water for 5 min followed by one h under running tap water. Then they were sterilized by immersion in a Clorox (commercial bleach) solution at the rate of 20, 30 and 40 % plus 3-5 drops of Tween 20 for 10, 15 or 20 min. Finally, they were washed 5 times with sterile distilled water. At the end of the experiments, the collected data included number of survived explants without contamination.

Establishment stage:

Each sterilized explant was cultured separately under sterile conditions in 100 ml jars filled beforehand with sterilized medium. The media employed at this stage were MS and B5. NAA were used for establishment stage, 4 treatments of NAA levels (0.0, 1.0, 2.0 or 4.0 mg/l) were combined with two types of media. Each treatment consisted of 9 jars. In each jar an individual sterilized shoot was cultured separately. Finally the shoot length (cm) and number of leaves were calculated.

Multiplication stage:

For multiplication stage, 48 treatments were initiated with either 2iP or Kin at different concentrations (0, 1, 2, 3 or 4 mg/l 2iP and 0, 1, 2, 3, 4, 5 or 6 mg/l Kin). This stage was repeated four times by subculturing on the same media treatments. . After four subcultures the number of shoot, shoot length (cm) and number of leaves were recorded.

Rooting stage:

In rooting stage, 36 treatments were initiated from the use of IBA and NAA (0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 mg/l). After 30 days on the rooting media the following data were confined recorded on number of roots and root length.

Acclimatization stag:

Rooted plantlets were pricked out singly into 10 cm plastic pots filled with 1:1, 2:1 and 3:1 (v/v) peatmoss and sand, respectively. To maintain cultures at high humidity, pots were covered with clear transparent plastic sheets for three weeks. The plastic covers were then gradually removed to reduce humidity and to adapt plantlets to greenhouse conditions, after that survival capacity was recorded.

Due to the difficulty in propagation and to the significance of *Magnolia grandiflora* as ornamental plants and their versatile uses in Botanical gardening, the aim of this study was to reach a well-defined protocol to easily *in vitro* propagate.

RESULTS AND DISCUSSION

1. Effect of different concentrations of Clorox and periods on surface sterilization explants of *Magnolia grandiflora*:

Results recorded in Table (1) show that Clorox (commercial bleach) at 30% for 15 min (i.e. not contaminated or died) gave the highest value of explants survival when compared to the other treatments on shoots.

On the other hand, the data indicated also that increasing the time of immersed explants decreased the survival percentage of explants at the high concentrations of Clorox (40%) while the best concentration (30%) increased the free contaminated explants (at 15 min) on shoots.

The interaction between Clorox and time were significant with the highest value of survived explants (9), 30% Clorox for 15 min was used.

Results obtained here are in harmony with similar results obtained elsewhere when 5.0% NaOCl was used on its own (Biedermann, 1987) or when used with other chemicals, i.e. 70% ethanol and Roccal (Merkle & Wiecko, 1990).

2. Influence of media and NAA during the establishment stage:

Results represented in Table (2) show that the survival of *Magnolia grandiflora* shoot tips was successfully achieved from culture on B5 medium. Regrettably, MS medium treatment gave no positive response with *Magnolia* shoot tips. As compared with

(control) treatment gave the highest shoots (1.9cm) and the best number of leaves (1.4) at B5 medium plus 2.0 mg/l NAA.

Table (1): Effect of different concentrations of Clorox and periods on surface sterilization explants of *Magnolia grandiflora*.

Clorox %	10	15	20	Mean
20	5.000	7.000	8.000	6.667
30	8.000	9.000	7.000	8.000
40	7.000	8.000	6.000	7.000
Mean	6.667	8.000	7.000	
LSD at 5% time (A)		0.641		
concent. (B)		0.141		
AxB		0.244		

Table (2): Effect of different concentrations of NAA and media type on establishment stage of *Magnolia grandiflora*:

Medium type	Number of leaves NAA (mg/l)				Mean (A)	Plant length (cm) NAA (mg/l)				Mean (A)
	0	1	2	3		0	1	2	3	
MS	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
B5	1.0	1.0	1.4	1.1	1.1	1.0	1.3	1.9	1.6	1.4
Mean (B)	1.0	1.0	1.2	1.1		1.0	1.2	1.4	1.3	
LSD media type (A)	= 0.1385					= 0.0616				
LSD NAA (B)	= 0.1958					= 0.0871				
LSD (A) X (B)	= 0.2937					= 0.1306				

In contrast, as pointed out earlier, when shoots where cultured on B5 medium they showed healthy signs and new leaves started to develop on them.

The interaction between the media and elapsed NAA showed that there were no significant differences between MS media and NAA. While, there were significant differences between B5 medium and NAA when compared with MS medium.

Although MS medium was reported earlier to satisfactorily form calli on *Magnolia* (Klimaszewska, 1981), results here showed that MS at full, half and quarter strength failed to form either calli or shoots on either shoot tips or seed explants. In contrast, results of this study clearly demonstrated that WP medium was a better choice and improved growth of shoot tip explants (the main cause of explant death in *Magnolia*) (Biedermann, 1987).

2. Effect of different concentrations of 2iP on multiplication stage of *Magnolia grandiflora*:

For shoot length, data calculated in Table (3) show that 2iP concentrations induced the decrease in elongation of shoot length. Here, it was found that the shoots were tallest (1.5) at zero-level of 2iP. Also, there were significant differences in shoot length between the different concentrations of 2iP.

When subculturing, it was found that the tallest shoots were recorded at the end of the first subculture and that shoot length was stable in the following subcultures.

For the interaction between 2iP concentrations and subcultures, results showed the tallest shoots was achieved when 2iP was at zero-level at the end of fourth subculture.

Results for number of leaves presented also in Table (3) show that 2iP decreased number of leaves probably due to a decrease in stem elongation and in number of internodes. Thus, it was found that the highest number of leaves was obtained and maintained at zero-level 2iP (3) when compared to the higher 2iP concentrations (1, 2, 3 and 4 mg/l). The number of leaves was constant when maintained at the higher levels of 2iP.

There was a steady increase in leaf numbers with subculturing. After the fourth subculture, the greatest number of leaves (2.7) were obtained when compared to the first, second and third subcultures (1.4, 1.9 and 2.2, respectively).

The interaction between 2iP concentrations and subcultures showed that there were not any differences at the high concentrations of 2iP (2, 3 and 4 mg/l 2iP) during the four subcultures; with all shoots having one leaf only. In contrast, there were significant differences between the zero-level and 1 mg/l 2iP in all of the four subcultures.

For number of shoots, results represented in Table (3) demonstrate that there was no new shoot formation at zero-level of 2iP during the four subcultures understudy. Increasing of shoot numbers was positively correlated with increasing of 2iP concentrations. There were significant differences between the different concentrations 1, 2 and 3 mg/l 2iP, in respect order (3.8, 4.6 and 7.2, respectively) but no significant differences were recorded between 2iP at 3 mg/l (7.2) and 2iP at 4 mg/l (7.5).

Table (3): Effect of different concentrations of 2iP on multiplication stage of *Magnolia grandiflora*:

2iP (mg/l)	Shoot length (cm)					Number of leaves					Number of shoots				
Subculture	1	2	3	4	Mean (A)	1	2	3	4	Mean (A)	1	2	3	4	Mean (A)
0	1.3	1.4	1.6	1.8	1.5	2.5	4.7	5.7	7.2	3.0	1.0	1.0	1.0	1.0	1.0
1	1.1	1.2	1.3	1.4	1.2	1.7	2.0	2.5	3.2	2.3	1.7	3.2	4.5	5.7	3.8
2	1.1	0.9	0.8	0.7	0.9	1.0	1.0	1.0	1.0	1.0	2.5	3.7	5.5	6.7	4.6
3	1.0	0.8	0.7	0.6	0.8	1.0	1.0	1.0	1.0	1.0	4.5	5.7	7.7	11.0	7.2
4	1.0	0.5	0.3	0.2	0.5	1.0	1.0	1.0	1.0	1.0	4.7	6.0	8.0	11.5	7.5
Mean (B)	1.1	0.9	0.9	0.9		1.4	1.9	2.2	2.7		2.9	3.9	5.3	7.2	
LSD 2iP (A)	= 0. 0500					= 0. 2122					= 0. 4053				
LSD subculture (B)	= 0.0446					= 0.1900					= 0.3624				
LSD (A) X (B)	= 0.1000					= 0.4247					= 0.8107				

Also, number of shoots were increased with subculturing. This was true and valid for the four subcultures understudy (2.9, 3.9, 5.3 and 7.2, respectively).

As for the interaction between 2iP concentrations and subcultures it was found that the greatest number of shoots were obtained at 3 or 4 mg/l 2iP in the fourth subculture (11.0 and 11.5, respectively).

However, in other occasions BA was reported to be not suitable for *Magnolia* elongation in the multiplication stage (Maene & Debergh, 1985; Biedermann, 1987; Kamenicka *et al*, 1996; Luo & Sung, 1996 and Kamenicka & Takats, 1997).

3. Effect of different concentrations of Kin on multiplication stage of *Magnolia grandiflora*:

Results illustrated in Table (4) indicate that shoot length of *Magnolia grandiflora* was increased due to both existence and increased dosage of Kin concentration. There were significant differences between almost all the different concentrations of Kin when compared with the zero-level control except at 1 mg/l.

Similarly, the subcultures showed persistent increases in shoot length in all four subcultures.

The interaction between Kin concentrations with time was significant in increasing shoot length, which was demonstrated clearly in almost all the different treatments.

Also results exhibited in Table (4) show that Kin caused an increase in number of *Magnolia grandiflora* leaves. This was true between the different concentrations of Kin used and also when compared with the zero-level control. The highest number of leaves was found when 6 mg/l Kin was used (10.6).

Similarly, as in shoot length, subculturing was significant and led to increases in number of leaves. The fourth subculture showed the highest number of leaves (13.7).

Also, the interaction between Kin concentrations and subculturing was significant in increasing number of leaves in almost all the different combinations. The only exception was between Kin 5 and 6 mg/l at the fourth subculture.

Moreover, results exhibited in Table (4) indicate that there were continuous additive increases in number of *Magnolia grandiflora* shoots due to the increase in the concentrations of Kin except in one case only, i.e. between 5 and 6 mg/l of Kin.

Table (4): Effect of different concentrations of Kin on multiplication stage of *Magnolia grandiflora*:

Kin (mg/l)	Shoot length (cm)					Number of leaves					Number of shoots				
Subculture	1	2	3	4	Mean (A)	1	2	3	4	Mean (A)	1	2	3	4	Mean (A)
0	1.3	1.4	1.6	1.8	1.5	2.5	4.7	5.7	7.2	5.0	1.0	1.0	1.0	1.0	1.0
1	1.3	1.4	1.7	1.9	1.6	2.7	5.2	6.2	7.5	5.4	1.5	2.5	3.7	4.5	3.0
2	1.4	1.5	1.7	1.9	1.6	3.0	5.7	6.5	8.0	5.8	2.0	3.0	4.2	5.5	3.6
3	1.5	1.6	1.8	1.9	1.7	4.0	6.2	7.2	8.7	6.5	3.2	4.2	5.7	7.0	5.0
4	1.6	1.6	1.8	2.0	1.7	5.2	6.5	7.7	9.0	7.1	4.0	5.0	6.5	7.7	5.8
5	1.8	1.9	2.1	2.4	2.0	6.7	9.5	11.0	13.2	10.1	5.0	6.2	9.5	11.7	8.1
6	1.8	1.9	2.1	2.4	2.1	7.5	10.0	11.5	13.7	10.6	5.0	6.5	9.7	12.0	8.3
Mean (B)	1.5	1.6	1.8	2.0		4.5	6.8	8.0	9.9		3.1	4.0	5.7	7.0	

LSD Kin (A)	= 0.0313	= 0.3856	= 0. 3480
LSD subculture (B)	= 0.0235	= 0.2912	= 0.2631
LSD (A) X (B)	= 0.0627	= 0.7716	= 0.6963

Following the same trend, as in number of leaves and numbers of shoots were increased as a result of subculturing. The best subculture was the fourth one (7), which led to the greatest number of shoots.

The interaction, between the different concentrations of Kin and the four subcultures, was in general significant for increasing number of *Magnolia grandiflora* shoots. Notably, the zero-level of Kin did not form any shoots with subculturing. There were enormous significant differences between 5 or 6 mg/l Kin and the zero level (control) at the fourth subculture (11.7, 12 and 1, in respect order).

On the other hand, *Magnolia grandiflora* also responded positively to form callus by application of Kin during the multiplication stage (Klimaszewska, 1981).

6. Effect of IBA and NAA during rooting stage of *Magnolia grandiflora*:

Data in Table (5) demonstrate that IBA clearly affected the rooting stage of *Magnolia grandiflora* plant. IBA was superior than NAA in the number of roots and root length.

For IBA level, it was found that 1.5mg/l IBA gave the highest number of roots and root length (6.89 and 9.50 cm, respectively) and there were significant differences between it and the different concentrations. Similarly for NAA level, it was noted that 2.5mg/l NAA gave the highest number of roots. NAA at 2.0 mg/l gave higher shoot length when compared to other concentrations used.

The interaction between IBA and NAA was significant for increasing number of roots and root length, which was demonstrated clearly in almost all the different treatments. IBA at 1.5 mg/l plus 0.0 mg/l NAA was the best for greater number of roots and root length when compared to the other combinations.

Noticeably, the use of IBA at 2.0 mg/l here seemed to promote elongation of shoots that was considered an added help for the survival and growth of shoots during the initial establishment stage. Similar results during the establishment stage were reported elsewhere on *Magnolia grandiflora* when IBA was employed (Franc & Krejci, 1998).

7. Effect of peatmoss and sand during Acclimatization stag of *Magnolia grandiflora*:

During this phase of culture the plantlets grew slowly and had healthy appearance. A high percentage of plant survival (45) was achieved by transplanting of plantlets in pots containing peatmoss and sand at a ratio of 1:1, v/v. The number and/or size of roots formed *in vitro* on shoots did not affect the acclimatization of plantlets to greenhouse conditions. After four weeks, no abnormalities in physical appearance and growth habits were observed on the transplanted plants.

Table (5): Effect of IBA and NAA during rooting stage of *Magnolia grandiflora*.

NAA(mg/L) / IBA (mg/L)	Number of roots							Root length						
	0.00	0.50	1.00	1.50	2.00	2.50	Mean	0.00	0.50	1.00	1.50	2.00	2.50	Mean
0.00	4.33	5.33	6.00	6.33	6.33	6.67	5.83	7.33	7.33	8.00	8.33	9.33	8.33	8.11
0.50	5.33	6.00	6.67	6.67	6.67	6.33	6.28	8.33	8.67	9.33	9.33	8.67	8.67	8.83
1.00	7.33	7.00	6.33	7.00	6.67	6.33	6.78	8.67	9.00	8.33	8.00	7.67	7.67	8.22
1.50	8.67	7.67	6.33	6.33	6.33	6.00	6.89	10.67	9.67	9.67	9.33	9.00	8.67	9.50
2.00	7.67	6.67	6.00	5.33	6.67	7.67	6.67	9.67	9.33	9.33	8.67	8.33	8.33	8.94
2.50	6.67	6.33	6.00	5.33	4.67	4.33	5.56	9.00	8.67	8.33	8.33	7.67	7.67	8.28
Mean	6.67	6.50	6.22	6.17	6.22	6.22		8.94	8.78	8.83	8.67	8.44	8.22	
LSD at 5% NAA (A)			0.394							0.378				
IBA (B)			0.394							0.378				
AxB			0.966							0.927				

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تأثير البيئات على إكثار نبات المانوليا بواسطة تكنيك زراعة الأنسجة

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فى محاولة للقضاء مشكلة من أهم المشاكل التى تواجه إكثار نبات المانوليا *Magnolia grandiflora* تحت الظروف المحلية فى مصر ألا وهى صعوبة إكثار النبات بالطرق التقليدية، تم إجراء هذه الدراسة بمعمل زراعة الأنسجة بحديقة الزهرية - معهد بحوث البساتين - مركز البحوث الزراعية خلال الفترة من الأعوام 2009- 2010 بهدف التوصل إلى أنسب المعاملات لإكثار هذا النبات عن طريق زراعة الأنسجة وذلك لوضع بروتوكول للإكثار الدقيق لهذا النبات.

ويمكن تلخيص أهم النتائج التى أمكن التوصل إليها فى الأتى:

بالنسبة لمرحلة التعقيم فقد استخدمت البراعم الطرفية لتعقيمها بالكلوركس وقد أعطى تركيز 30% لمدة 15 دقيقة أعلى نسبة حيوية (90%) وأقل نسبة تلوث (10%). أما بالنسبة لمرحلة التأسيس كان لأستخدام بيئة جامبورج (B5) المضاف اليها 2 ملليجرام/لتر نفثالين حمض الخليك (NAA) فى الحصول على أطول الافرع و أكبر عدد من الاوراق.

- أما فى مرحلة التضاعف فقد أستخدمت بيئة موراشيجى وسكوج المضاف إليها الكينتين بتركيزات 1 و 2 و 3 و 4 و 5 و 6 مجم / لتر. وكذلك تم استخدام الأيزوبنتيل أدينين بتركيزات 1 و 2 و 3 و 4 مجم / لتر وذلك خلال 4نقلات متتالية. و قد تبين من النتائج أن أفضل سيتوكينين للتضاعف هو الكينتين بتركيز هو 5 أو 6 مجم / لتر.

- مرحلة التجذير أستخدمت بيئة موراشيجى وسكوج المضاف إليهما إنول حمض البيوتريك و نفثالين حمض الخليك بتركيز صفر، 0.5، 1.0، 1.5، 2.0، 2.5 مجم/لتر.أوضحت النتائج أن أفضل معاملة هى بيئة موراشيجى وسكوج المضاف إليها 1.5مجم/لتر إنول حمض البيوتريك.

- مرحلة الأقلمةأستخدم فيها بيتموس ورمل بنسبة 1:1 و2:1 و 3:1 تحت أنفاق بلاستيكية داخل صوب بلاستيكية فكانت نسبة نجاحها 45% عند أستخدام البيئة المكونة من مخلوط البيت موس والرمل (1:1حجما).