ISSN 1110-0419

Annals Of Agric. Sci., Moshtohor,
Vol. 47(2): Ho. 339-347, (2009).

STUDIES ON MICROPROPAGATION OF *LIQUIDAMBER STYRECIFLUA*

BY

El-Shamy, M. A.*; Mohamed, A.K. and Gehan H. Abdel-Fattah*****

* Botanical Garden Res. Dept Hort. Res. Inst. Agric. Res. Center, Giza, Egypt.
** Horticulture Dept. Faculty of Agric. Ain Shams Univ. Shoubra El-Kheima. Cairo. Egypt.
*** Ornamental plants Res. Dept Hort. Res. Inst. Agric. Res. Center, Giza, Egypt.

ABSTRACT

The experimental trial was consummated in Plant Tissue Culture Laboratory at El-Zohria Botanical Garden, Horticulture Research Institute, Agriculture Research Center, throughout 2007 – 2009 year. It was intended to find out the well defined protocol easily *in vitro* propagation of *Liquidambar styraciflua* plant for its difficulty in propagation. So, shoot tips of the plant were effectively surface sterilized with a mixture of mercuric chloride (Hg_2Cl) and sodium hypochlorite (NaOCl) as commercial Clorox were used at 2% NaOCl plus 4 mg/l Hg_2Cl. Shoot tip explants of the plant cultured on MS medium managed to establish effectively. In the multiplication stage, 5.0 mg/l Kin formed not only the highest number of shoots but also in the extension lengths of shoots. Kin was better than BA during the multiplication stage. For *in vitro* rooting, 2.0 mg/l IBA was more suitable than 1.0 or 3.0 mg/l to form roots on *Liquidambar* shoots when added to ¼ MS medium. IBA was better than IAA for the rooting stage. Plantlets after root development exhibited 70% survival in plastic pots filled with peat moss and sand at a ratio of 1:1 under plastic tunnel at plastic house condition.

Key words: Micropropagation, *In vitro*, Tissue culture, Liquidambar, Shoot tips.
Abbreviations: MS = Murashige & Skoog medium, KIN= Kinetin
BA = BAP = 6-benzyladenine = 6- benzylaminopurine, IBA = Indolbutric acid

INTRODUCTION

Liquidambar from Latin liquidus, fluid, and the Arabic ambar, amber; in allusion to the fragrant juice which exudes from the tree. *Liquidambar styraciflua*, linn, tree, 60-120 ft. high; lvs. 5-7 lobed, with acuminate, finely serrate lobes, lustrous and dark green above, paler below and glabrous except tufts of pale rufoua hairs in the axils of the principal veins, 3-7 in. across; petioles 5-6 in. long; fr. 1- 1 ½ in across. March-May (Bailey and Bailey, 1960)..

El-shamy *et al.* (2004) concluded that both *Magnolia grandiflora* shoot tips and seeds explants were effectively surface sterilized by a mixture of mercuric chloride and sodium hypochlorite (NaOCl) (comercial Clorox) were used at 2% NaOCl plus 4 mg/l mercuric chloride and at 1.5 mg/l NaOCl plus 2 mg/l mercuric chloride respectively.

The success of tissue culture in propagation of ornamental plants is greatly influenced by the nature of culture media. The nutrient media has two major functions, the first, is to supply the basic nutritional ingredients for continued growth of isolated explants and subsequent propagules; the second function, is to direct growth and development through hormonal control (George and Sherrington, 1994).

At the proliferation stage the best proliferation was achieved on *Strelitzia reginae* with the presence of IBA and BA (at 0.25 and 2 mg/l, respectively). In order to eliminate apical dominance and to promote growth, several incisions were made on the apices (Bettaieb and Tissaoui, 1994).

The addition of activated charcoal (concentration of 0.2-3.0 % w/v) to the medium effectively suppressed browning and necrosis of Japanese Cycad (*Cycas revoluta*) calluses (Monnier & Norstog, 1986).

Tissues of *Magnolia soulangeana* formed callus when cultured on a modified free-hormone MS medium (Klimaszewska 1981).

For stem segment from cordyline placed with one or two nodes on agar- basal half strength Murashige and Skoog (MS) medium for shoot growth Sagawa and Kunisaki (1990) concluded that shoots should appear in 2-3 weeks.

MS medium at one-half the concentration, which contains 3% sucrose, 10 ppm 2,4-D and 0.1% activated charcoal, induced calluses from *Cycas revoluta* tissues in 2 weeks (Tadera *et al.*, 1995).

The decrease of macronutrient salts in the medium induced formation of lateral roots expressed both as the percentage of roots forming lateral roots and the number of laterals per shoot of Dracaena. Roots formed on medium supplemented with 1/10 MS macronutrient salts (Vinterhalter and Vinterhalter, 1992).

The formed roots of Dracaena were the best for the shoots cultured on 3/4 MS-strength. This was the case for using MS without activated charcoal (El-shamy *et al.*, 1999).

Liquidambar styraciflua is a difficult-to-propagate evergreen tree through conventional vegetative methods. Moreover, it is also difficult to establish *in vitro* its tissues from plant material taken from mature woody trees.

The aim of this study was to reach a well-defined protocol easily *in vitro* propagation *Liquidambar styraciflua*. So, the experimental trial was carried out mainly by using explant and nutrient culture media, and also by manipulating growth with the use of various concentrations of different growth regulators. Moreover, it was intended to determine the device simple and reliable methods for *in vitro* propagation through inducing growth by completely controlled of oxidative browning and preventing the detrimental effect of the brown exudates. So, the following steps were investigated: The effects of sterilization treatments, BA and Kin on multiplication stage, IBA, IAA and MS strength on rooting behavior.

MATERIALS AND METHODS

This study was carried out in the laboratory of Tissue Culture, Zohria Botanical Garden, Cairo, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture. The experiments were carried out throughout 2007 – 2009 years. The objective of this study was to investigate the most suitable treatments for micropropagation of *Liquidambar styraciflua*. The mother plants were cultured in Zohria Garden. The parts used as explants were shoot tips.

Culture room condition:

Cultures of *Liquidambar styraciflua* were incubated in a growth chamber under controlled conditions at 23 ± 2 °C. All cultures were exposed to a 16-h photoperiod/day (24 h cycle) at an intensity of 2000 lux from white fluorescent tube lamps.

Surface Sterilization of Explants:

The shoot tips of *Liquidambar styraciflua* were excised from the mother plants and then washed by soapy water for 10 min followed by 1 h under running tap water. Then they were sterilized by immersion in a mercuric chloride (Hg_2Cl) solution at the rates of 1, 2, 3 and 4 mg/l plus 3-5 drops of Tween 20 for 3 min, followed by rinsing three times in sterile distilled water. Subsequently, they were immersed in a sodium hypochlorite (NaOCl) solution (commercial bleach as 'Clorox' at 1.0, 1.5, 2.0 and 2.5 % NaOCl) plus 3-5 drops of Tween 20 for 20 min. Finally, they were washed 5 times with sterile distilled water.

Culture Media:

The Murashige and Skoog (MS) medium was used for explants of *Liquidambar styraciflua*. 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.

At the establishment stage:

Each sterilized explant was cultured under sterile conditions in 100 ml jars filled with MS medium free hormone supplemented with 3 g/l activated charcoal to prevent browning of the cultures.

At the multiplication stage:

For multiplication stage, 48 treatments were initiated with either BA or Kin at different concentrations (0, 1, 2, 3 or 4 mg/l BA 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.

At the rooting stage:

For rooting stage, 24 treatments were used with either IBA or IAA at different concentrations (0, 1, 2 or 3 mg/l IBA and 0, 1, 2 or 3 mg/l IAA). IBA or IAA and different MS strength (full, half and quarter strength) were used during rooting stage. After one month number of roots and Plantlet height (cm) were calculated.

At the Acclimatization stag:

Rooted plantlets were cultured singly into 10 cm plastic pots filled with 1:1 (v/v) peatmoss and sand under plastic tunnel at plastic house condition. The plastic covers were then gradually removed to reduce humidity and to adapt plantlets to greenhouse conditions.

Experimental design and data analysis

The layout of the experiment was designed in completely randomized design and the test LSD was used for comparison among means according to (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of different concentrations of sodium hypochlorite (NaOCl) and mercuric chloride (Hg_2Cl) on surface sterilization explants of *Liquidambar styraciflua*:

Results demonstrated in Table (1) indicate that surface sterilization by sodium hypochlorite (NaOCl) was positively significant on plant survival explants (i.e. explants neither contaminated nor dead) of *Liquidambar styraciflua* shoot tips. This effect increased with the increase of NaOCl concentration. The best concentration was 2.5 %, which gave 6.5 survived explants.

Furthermore, the use of mercuric chloride (Hg_2Cl) on surface sterilization of shoot tips of *Liquidambar styraciflua* was lower at 1.0 and 2.0 mg/l but still gave positive significant effects when high concentrations were used (3 and 4 mg/l) compared with the lower concentration (1.0 mg/l).

The interaction between NaOCl and Hg_2Cl was significant with the highest value of survived explants (9) at 2 % NaOCl plus 4.0 mg/l Hg_2Cl.

Results obtained here are in harmony with those obtained elsewhere when Clorox and mercuric chloride were used on its own at *Magnolia grandiflora* (El-shamy *et al.*, 2004).

Effect of different concentrations of BA on multiplication stage of *Liquidambar styraciflua*:

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

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.

Table (1): Effect of different concentrations of sodium hypochlorite (NaOCl) and mercuric chloride (Hg_2Cl) on surface sterilization explants of *Liquidambar styraciflua*:

NaOCl (%)	Hg_2Cl (mg/l)				Mean (A)
	1	2	3	4	
1.0	0.0	0.0	0.0	0.1	0.3
1.5	0.0	2.0	5.0	5.0	3.0
2.0	3.0	5.0	6.0	9.0	5.8
2.5	6.0	6.0	7.0	7.0	6.5
Mean (B)	2.3	3.3	4. 5	5.5	

LSD NaOCl (A) = 0.1342 LSD Hg_2Cl (B) = 0.1343 LSD (AXB) = 0.2692

Table (2): Effect of different concentrations of BA on multiplication stage of *Liquidambar styraciflua:*

BA (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 BA (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				

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

Results for number of leaves presented also in Table (2) show that BA 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 BA (3) when compared to the higher BA concentrations (1, 2, 3 and 4 mg/l). The number of leaves was constant when maintained at the higher levels of BA.

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 BA concentrations and subcultures showed that there were not any differences at the high concentrations of BA (2, 3 and 4 mg/l BA) 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 BA in all of the four subcultures.

For number of shoots, results represented in Table (2) demonstrate that there was no new shoot formation at zero-level of BA during the four subcultures understudy. Increasing of shoot numbers was positively

correlated with increasing of BA concentrations. There were significant differences between the different concentrations 1, 2 and 3 mg/l BA, in respect order (3.8, 4.6 and 7.2, respectively) but no significant differences were recorded between BA at 3 mg/l (7.2) and BA at 4 mg/l (7.5).

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

In the same trend, BA at 0.5 mg/l was superior than 1.0, 2.0 or 4.0 mg/l when compared to explants responded from leaves of *Gerbera* (El-shamy *et al.*, 2009).

Effect of different concentrations of Kin on multiplication stage of *Liquidambar styraciflua*:

Results illustrated in Table (3) indicate that shoot length of *Liquidambar styraciflua* 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 (3) show that Kin caused an increase in number of *Liquidambar* 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 (3) indicate that there were continuous additive increases in number of *Liquidambar* 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.

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 in increasing number of *Liquidambar* 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).

A comparison between the effect of BA and Kin on Liquidambar:

When results were compiled together, it was found that shoot length of *Liquidambar* was decreased with increasing of BA concentration while vice versa shoot length was increased with increasing of Kin concentration. Also, there were decreases in shoot length with subculturing when BA was used. In contrast, there were increases in shoot length in the following subcultures when Kin was used. For shoot length elongation, the best concentration of BA was zero-level while the best concentration of Kin was at 6 mg/l. So in general, for the sake of shoot length elongation, Kin at concentrations utilized were better than BA concentrations.

Similarly, number of *Liquidambar* leaves increased with increasing of Kin concentrations and vice versa for BA as in shoot length. Number of leaves was limited to 1 only when BA at 3 and 4 mg/l was used compared to 6.5 and 7.1 when Kin was used, in respect order.

It was found that number of shoots increased when either BA or Kin were used. BA showed a higher increase in shoots numbers when compared to Kin at 1, 2, 3 or 4 mg/l. Although BA was not compared at 5 and 6 mg/l with Kin, Kin was still preferred because it gave higher number of shoots with desirable features (i.e. in both elongation and number of leaves/ shoot) which was suitable for the rooting stage still to come.

On the other hands, Kin was better than BA for the multiplication stage of *Magnolia grandiflora* which could be used to increase the number of shoot formed per shoot (Hartmann *et al.*, 1990 and El-shamy *et al.*, 2004).

Effect of different concentrations of IBA and MS strength on rooting stage of *Liquidambar styraciflua*:

Results presented in Table (4) demonstrate that the MS strength under study clearly affected number of roots during the rooting stage of *Liquidambar styraciflua*. The MS medium at quarter was superior than other MS strength in the number of roots formed (1.3 and 1, respectively). Whereas, full and half MS strength did not affect rooting of *Liquidambar* shoots.

For IBA levels, it was noted that only 2 mg/l IBA induced the formation of roots on *Liquidambar* shoots (1.4) when compared with the remaining treatments (1).

The interaction between the different concentrations of IBA and MS strength treatments showed that the only combination which led to the formation of most roots (2.2) on *Liquidambar* shoots, was when 1/4 MS was used and supplemented with 2 mg/l IBA.

Also results presented in Table (4) show that only 1/4 MS gave positive elongated plantlet height during *in vitro* rooting of *Liquidambar* results. While full and half MS strength did not affect plantlet height at all.

Moreover, results in Table (4) indicated that plantlet height also increased when IBA concentration was increased from 0 to 2 mg/l with estimated values of 2.9, 3.5 and 4.2 cm at 0, 1 and 2 mg/l IBA, in respect order. At 3 mg/l IBA, plantlet height was stable (4.2 cm) and not significantly different than the lower concentration 2 mg/l (4.2 cm).

The interaction between the different concentrations of IBA and different MS strength on plantlet height elongation demonstrated that the highest results were obtained when 1/4 MS and 2 or 3 mg/l IBA were used in combination together (7.7 and 7.8 cm, respectively).

For successful *in vitro* rooting, shoots of *Magnolia grandiflora* were treated with IBA in the culture medium (El-shamy *et al.*, 2004).

Effect of different concentrations of IAA and MS strength on rooting stage of *Liquidambar styraciflua*:

Results exhibited in Table (5) indicate that MS strength did not affect number of roots. IAA was also not significant in inducing root formation when expressed as number of roots.

With regards to the interaction between the different concentrations of IAA and different MS strength on root formation, it was noted that 1/4 MS plus 2 mg/l IAA gave the only positive response (0.2) in number of roots when compared with the rest of the different other combinations.

Also, results calculated in Table (5) demonstrate that only 1/4 MS affected *Liquidambar* plantlet height and increased it (5.5 cm) when compared to full and half MS strength (2.5 cm).

As far as IAA was concerned, it was found, as in IBA, that increases in plantlet heights were found due to increases in IAA concentrations.

Table (3): Effect of different concentrations of Kin on multiplication stage of *Liquidambar styraciflua*:

Kin (mg/l)	Shoot length (cm)					Number of leaves					Number of shoots				
Sub-culture	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

Table (4): Effect of different concentrations of IBA and MS strength on rooting stage of *Liquidambar styraciflua*:

Medium type	Number of roots IBA (mg/l)				Mean (A)	Plantlet height (cm) IBA (mg/l)				Mean (A)
	0	1	2	3		0	1	2	3	
MS	1.0	1.0	1.0	1.0	1.0	2.5	2.5	2.5	2.5	2.5
1/2 MS	1.0	1.0	1.0	1.0	1.0	2.5	2.5	2.5	2.5	2.5
1/4 MS	1.0	1.0	2.2	1.0	1.3	3.8	5.6	7.7	7.8	6.2
Mean (B)	1.0	1.0	1.4	1.0		2.9	3.5	4.2	4.2	

LSD MS strength (A) = 0.1980 = 0.1382
LSD IBA (B) = 0.2290 = 0.1594
LSD (A) X (B) = 0.3963 = 0.2765

Table (5): Effect of different concentrations of IAA and MS strength on rooting stage of *Liquidambar styraciflua*:

Medium type	Number of roots IAA (mg/l)				Mean (A)	Plantlet height (cm) IAA (mg/l)				Mean (A)
	0	1	2	3		0	1	2	3	
MS	1.0	1.0	1.0	1.0	1.0	2.5	2.5	2.5	2.5	2.5
1/2 MS	1.0	1.0	1.0	1.0	1.0	2.5	2.5	2.5	2.5	2.5
1/4 MS	1.0	1.0	1.2	1.0	1.0	3.8	5.2	6.3	6.7	5.5
Mean (B)	1.0	1.0	1.0	1.0		2.9	3.4	3.7	3.9	

LSD MS strength (A) = NS = 0.1160
LSD IAA (B) = NS = 0.1336
LSD (A) X (B) = 0.2085 = 0.2320

The interaction between the different concentrations of IAA and different MS strength on plantlet height showed that the highest value for plantlet height was obtained when 1/4 MS was used plus 3 mg/l IAA (6.7 cm). While, no elongation was recorded when

full and half MS strength was used in combination with any IAA concentration (2.5 cm).

A comparison between the effect of IBA and IAA on Liquidambar:

When results were compiled and compared together, it was found that 2 mg/l IBA was better than 2 mg/l IAA in increasing number of roots and also in improving plantlet height.

Adaptation stage:

Successful adaptation (70%) to greenhouse conditions was obtained by transplanting of plantlets in pots containing sand and peatmoss in ratio of 1:1.

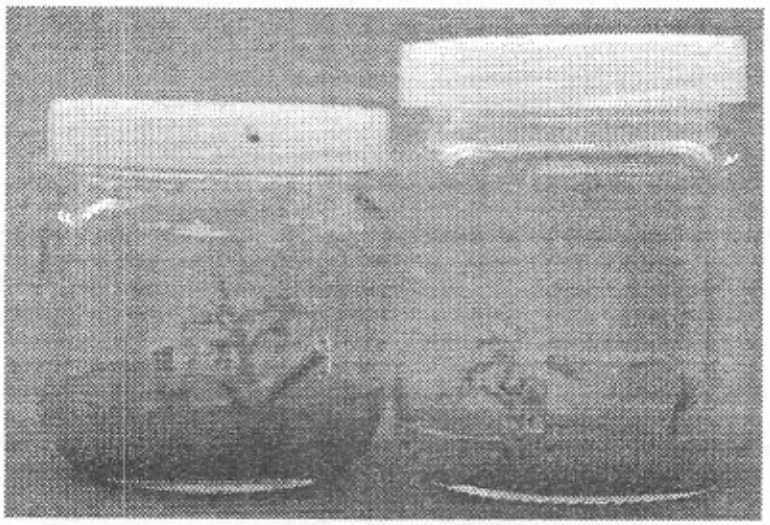

Plate (1): *In vitro* micropropagation of *Liquidambar styraciflua*

REFERENCES

Bailey, L.H. and Bailey, E.Z. (1960): Hortus third A Concise Dictionary of Plants Cultivated in the United States and Canada, 3rd Ed, Macmillan, New York, 120 pp.

Bettaieb, T. and Tissaoui, T. (1994): In vitro propagation of Strelitzia reginae Ait. Annales de Institut National de la Recherche Agronomique de Tunisie, 67(1/2): 125-131.

El-shamy, M.A.; Saker, S.S.; El-Shehey, U. and Abou-Dahab, T.A. (1999): Studies on micropropagation of Dracaena by tissue culture technique. M.Sc. Thesis Faculty of Agriculture Cairo University, 90 pp.

El-shamy, M.A.; El-Gendy, S.A.; Hosni, A.M. and Hosni, Y.S. (2004): Studies on micropropagation of some woody ornamental plants. Ph.D. Thesis Faculty of Agriculture Ain Shams University, 32 pp.

El-shamy, M.A.; Abdel-Sattar, M.M.; Amal S.A. El-Fouly (2009): Studies on Gerbera Jamesonii Bolus cv. aurantiaca, Under micropropagation condition. J. Agric. Sci. Mansoura Univ., 34 (3) 1939-1951.

George, E.F. and Sherrington, P.D. (1994): Plant propagation by tissue culture. Handbook and Directory of Commercial Laboratories. Edington. Westbury Wiis, England.

Klimaszewska, K. (1981): Plant regeneration from petiole segments of some species in tissue culture. Acta Agrobotanice, 34 (1): 5-28 (H. A. No.8068, 1982).

Monnier, M. and Norstog, K. (1986): Effect of in ovulo period on the differentiation and regulation of immature embryos of Zamia cultured in vitro. Journal of Experimental Botany, 37 (184): 1633-1642. (CAB Abstract No. 860340935, 1986).

Sagawa, Y. and Kunisaki, J.T. (1990): Handbook of Plant Cell Culture. Vol. 5. Ornamental Species, Micropropagation of Floriculture crops.: 2nd Ed, Mcg raw-Hill, New York: 25-34.

Steel, R.G.D. and Torrie, J.H. (1980): Principle of statistics. A biometrical approach. Second Ed., Mc Graw-Hill Kogakusha, L.T.D.

Tadera, K.; Ginya, H.; Sawada, R.; Motani, Y.; Aikawa, Y.; Nozaki, A.; Yagi, F. and Minami, Y. (1995): Cycasin formation in tissue culture of Japanese cycad. Phytochemistry, 38 (5): 1199-1201.

Vinterhalter, D. and Vinterhalter, B. (1992): Effect of inorganic nutrition on the formation of lateral roots in Dracaena fragrans ker- Gawl cultured in vitro. Plant Cell, Tissue and Organ Culture, 28 (3): 267-274.

دراسات على الإكثار الدقيق لنبات ليكودامبر

ممدوح أحمد إبراهيم الشامى* ، أيمن كمال إبراهيم محمد، جيهان حسن عبدالفتاح*****

* قسم بحوث الحدائق النباتية -معهد بحوث البساتين -مركز البحوث الزراعية -الجيزة-مصر .

** قسم البساتين – كلية الزراعة – جامعة عين شمس – شبرا الخيمة – القاهرة -مصر .

*** قسم بحوث نباتات الزينة -معهد بحوث البساتين -مركز البحوث الزراعية -الجيزة-مصر .

أجريت هذه الدراسة فى خلال الفترة من سنة ٢٠٠٧ – ٢٠٠٩ فى معمل زراعة الأنسجة بحديقة الزهرية التابعة لمعهد بحوث البساتين-مركز البحوث الزراعية-وزارة الزراعة-جمهورية مصر العربية.

كان الهدف من هذه الدراسة هو تحديد أنسب المعاملات لإكثار نبات الليكودامبر عن طريق زراعة الأنسجة لصعوبة إكثاره بالطرق التقليدية وكذلك بزراعة الأنسجة. ويمكن تلخيص أهم النتائج التى تم التوصل إليها فى الأتى:

أمكن إكثار نبات الليكودامبر بواسطة البراعم الطرفية كمنفصلات نباتية تم تعقيمها بواسطة كلوريد الزئبقيق بتركيز ١، ٢، ٣، ٤ ملجم/لتر لمدة ٣ دقائق. وكذلك هيبوكلوريد الصوديوم بتركيز ١، ١½، ٢، ٢½ % لمدة ٢٠ دقيقة. بالإضافة إلى التداخلات بينهما.

فتبين أن أفضل تركيز من الكلوركس للحصول على أعلى نسبة بقاء للنباتات وأقل نسبة تلوث هى ٤مجم/لتر كلوريد الزئبقيق بالإضافة إلى ٢ % هيبوكلوريد الصوديوم.

وفى مرحلة التأسيس أستخدمت بيئة موراشيجى وسكوج بدون هرمونات والمضافة إليها فحم نباتى نشط.

أما فى مرحلة التضاعف فقد أستخدمت بيئة موراشيجى وسكوج المضاف إليها الكينتين بتركيزات ١ و ٢ و ٣ و ٤ و ٥ و ٦ مجم / لتر. وكذلك تم استخدام البنزيل أدينين بتركيزات ١ و ٢ و ٣ و ٤ مجم / لتر وذلك خلال ٤نقلات متتالية.

و قد تبين أن أفضل سيتوكينين للتضاعف هو الكينتين بتركيز هو ٥ أو ٦ مجم / لتر.

مرحلة التجذير أستخدمت بيئة موراشيجى وسكوج بتركيز قوة كاملة ونصف قوة وربع قوة والمضاف إليهما إندول حمض البيوتريك بتركيز ١ و ٢ و ٣ مجم / لتر وكذلك إندول حمض الخليك بتركيز ١ و ٢ و ٣ مجم / لتر.

مرحلة الأقلمة أستخدم فيها بيتموس ورمل بنسبة ١:١ تحت أنفاق بلاستيكية داخل صوب بلاستيكية فكانت نسبة نجاحها ٧٠% فى كل المعاملات.