In Vitro Propagation and *Ex Vitro Acclimatization* of Magnolia (*Magnolia grandiflora,* Linn) Trees

EL-Gedawey*, M. E. H., A. I. A. Abido,M. G. EL-Torky*** , B. M. Waheda* and M. K. A. Gaber****

* Agriculture Research Center, Horticulture Research Institute, Deprt. of Ornamental & Landscape Gardening, EL-Montzah, Alex.

**Plant Production Dept., Faculty of Agric-Saba Basha, Alex.Univ.

***Floriculture, Ornamental and Landscape Gardening Dept., Fac. Agric., Alex. Univ.

ABSTRACT: This study was carried out in the tissue culture laboratory, Faculty of Agriculture, Saba basha, Alexandria University, Egypt during the period from 2013 to 2015. An efficient and reliable protocol for *in vitro* propagation of *Magnolia grandiflora*, Linn was optimized. However, nodal explants from field grown of magnolia were used during *in vitro* culture study for induction of multiple shoots. Nodal explants were effectively surface sterilized with 30% Clorox (sodium hypochlorite) as commercial bleaches for 20 min plus 1.5mg/l mercuric chloride for 5 min with few drops of Tween-20, also. Nodal explants were inoculated on various initiation or establishment media with different combinations of IBA and KIN and the neoformed shoots were cultured on proliferation (multiplication) media for the development of multiple shoots, and the elongation media to elongate of the neoformed shoot. The subsequent elongated shoots were rooted*, successfully*. The best medium for shoot initiation was Woody Plant Medium (WPM) supplemented with 2.0 mg/l KIN and 1.00 IBA. The favourable medium for multiplication was the tested medium augmented with 5.0 mg/l KIN and 1.00 mg/l IBA.. Furthermore, the *in vitro* shoots showed healthy root development when the tested medium was supplemented with combination of 1.00 mg/l IBA and NAA ,each in turn (rooting stage). The shoots of Magnolia grandiflora multiplication and rooted successfully when they cultured in WP medium supplemented with 1.00mg/l charcoal. The combination of sand: compost (1:3) was used as substratum for the hardening of the *in vitro* plantlets, as a potting mix, was the best suited mix for the acclimatization of plantlets.

Key words: *In vitro* culture, *Magnolia grandiflora*, nodal explants, initiation, multiplication, rhizogenesis, acclimatization

INTRODUCTION

Magnolia grandiflora, Linn which also called evergreen magnolia, bull boy, or large flower magnolia, is evergreen tree. It is pyramidal tree with creamy flowers belongs to family Magnoliaceae *(*Chaidaroon *et al*., 2004; Said, 2007*).* There are at least 100 species which share genus *magnolia*. It was first grown as an ornamental evergreen tree in the world in southern U.S. A. It is growen in Egypt in botanical and private gardens. It was quickly popularized for its glossy evergreen foliage, large beautiful flowers and elegant form and extensively planted as an ornamental plant (Said, 2007). *Magnolia grandiflora* bears large, very fragrant, bowl- shaped, white flowers.They show intermittently from mid-summer to early autumn (Bailey and Bailey ,1960) . It is, also, used a specimen plant, shade trees, screen or wind break, it can be grown as esplalier. It is valuable for garden or park planting in Japan. Its wood is used for furniture and various industrial arts, and the bark contains valuable medicinal compounds (Nakmura *et al*., 1995).

In Alexandria (Egypt), *Magnolia grandiflora* trees are grown in private and botanical gardens with a alimented. It is, known that *Magnolia grandiflora* is propagating by such vegetatively propagated methods as Averages seeds, but its lower germinability rate (*ca*.35%), or by cutting but it, also, difficult

because of its poor rooting ability (Nakmura *et al*., 1995) or by air layering, but it is labor extensive and wasting time- method, as well as very expensive (Mccracken *et al.,*1996) Further, rooting of this species is too -hard to achieve. Also, the demands of *Magnolia grangiflora* for rooting facilities are very much expensive and may be unavailable to root shoot cuts (Said, 2007). Therefore, propagation of *Magnolia* using plant tissue culture techniques may offer certain advantages over traditional method of propagation. It is one of the most promising and advanced applications of plant cell and tissue culture technology to propagate this species *in vitro.* This technique could be very useful to provide hundreds or thousands of this species withen a limited time.
Therefore, the present study was aimed to establish an efficient and reliable protocol for *in vitro* propagation and with focusing on rhizogenesis of this hard-to –root species.

MATERIALS AND METHODS

Plant material and explants sterilization

The plant material was collected from tree grown in garden of Ornamental and Landscape, of the Research Department of EL-Montazh, Alexandria, Egypt. The tree was sprayed with the fungicide and insecticide 2-3 week prior to start initiation and over head watering was strictly avoided. Freshly grown shoot tips, with two to three nodes, were selected as explants' source. The collected material was brought to the Plant Tissue Culture Laboratory of the Plant Production Department of the Faculty of Agriculture, Saba Basha, Alexandria University during 2013-2015 seasons and washed, thoroughly, with running tap water for 30 minutes to remove the dust or sand particles. The explants were cut to nodal segments (single node) as an explants' source (Bhattacharya *et al*., 1990).The excised explants were dipped in 70% ethanol, for 1 min. after treatment with ethanol the explants were rinsed with double distilled water twice, so as to lower the toxic effect of ethanol. Nodal segments of magnolia were surface sterilized with sodium hypochlorite (NaOCl) solution (commercial bleach as 'clorox') at 30% for 20 minutes followed by mercuric chloride $HgCl_2$ at 1.5mg/l for 5min with few drops of Tween-20, also, were added as a surfactant to the sterilized water . Finally, they were washed three times with sterile distilled water and became ready for culture.

Microprogation stages

Intiation stage

The explants were cultured on solidified woody plant medium coined as WPM (Lloyd and McCown, 1980) which contained different concentrations of kintine (KIN) at four concentrations:0.0(nil), 0.5,1.0 and 2.0 mg/l, in combinations with the auxin Indole butric acid (IBA) at four concentrations: 0.0(nil) ,1.0, 2.0 and 3.0 mg/l. Three explants were cultured in each jar which containing 30ml of medium and were placed, vertically. Each treatment was replicated three times and each has 3 explants (i.e.9 explants /treatment).The jars were capped with aluminum foil closures. The cultured jars were incubated in growth chamber at $25\pm1^{\circ}$ C temperature under 16 hr daily light and 8hr darkness illumination by a florescent light intensity of 2880 Lux (40μ mol $m^{-2}S^{-1}$) at 97%R.H.

Multiplication stage

The neoformed propagule of the initiation stage was sectioned into single leaflet node. The excised nodal cutting explants of the different positions were cultured, randomly, onto the multiplication medium (WPM) supplemented with KIN at four concentrations: 0.00(nil), 1.00, 3.00 and 5.00 mg/l, in combinations with IBA at four concentrations:0.00(nil) ,0.50, 1.00 and 2.00 mg/l.

Rooting (rhizogenesis) stage

The obtained shoots of magnolia from the multiplication stages were, individually, separated and cultured on a rooting medium for rhizogenesis to achieve this stage .This medium was augmented with two types of auxins which were used as Indole Butyric Acid (IBA) at four concentrations: 0.00(nil), 0.50, 1.00 and 2.00 mg/l, in combinations with NAA at four concentrations: 0.00, 1.00, 2.00 and 3.00 mg/l. Generally, the data were recorded per propagule at initiation, multiplication and rooting stages after 35 days in culture. The tested characters were as follows:

-Average shoots length (cm)/propagule.
-Average number of shoots formed/ propagule.
-Average number of leaflets formed/ propagule
-Average number of roots formed/ propagule.

Comparison between the effect of BA and KIN on explants of magnolia

The comparison between the effect BA(Benzyl adenine) and KIN(Kintine) on growth of *Magnolia* during multiplication stage was investigated in a 5x3 factorial experiment in which the treatment at 3.00mg/l , each in trun.

Effects of activated charcoal on shoots plantlets

Activated charcoal has been used previously to adsorb ethylene and other growth inhibitory substances produced in shoot cultures derived from the medium or from the plant tissues or both. Four main treatments were tested :no addition of activated charcoal(AC) to the medium ,and three concentrations of 0.25,0.50 and1.00g/l, (AC).The media pH was adjusted to 5.7, then gerlrite at 7g/l was added before autoclaving .Each treatments was replicated 3 times and each replication has 3 explants. The followed characters were recorded per propagule after 35 days in culture:

1-Average number of shoots formed per propagule.
2-Average shoots length (cm) per propagule.
3- Average number of roots formed per propagule.
4-Average number of leaflets formed per propagule.

Acclimatization stage

The new formed plantlets (rooted shoots) were then transferred to the greenhouse for hardening. The potting mix used in this study comprised of sand and compost (1:3) .The transferred plants were monitored weekly for at least 6 weeks.

Statistical analysis

A completely randomized design was used for all the experiments (Gomez and Gomez, 1984).Recorded data were analyzed, statistically, using analysis of variance technique (ANOVA) and averages were compared by the least

significant difference (L.S.D.) (Steel *et al*., 1997) and significance was determined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Achievement of optimal and reliable system for micropropagation of *Magnolia grandiflora* was urgent and in focus. Therefore, a set of experiments was conducted, and the obtained results were presented and discussed in the following section as follows:

Micropropagation

Initiation stage

Data outlined in Table (1) exhibit that both applied growth regulator, levels and their combinations exerted, highly, significant effects on the initiation stage characters of Magnolia. Single node explants were grown *in vitro* for 35 days as shown in Figure (1).

Concerning the main effect of studied cytokinin (KIN), in terms of the Average shoot length/propagule, supplementing the culture medium with at 0.5mg/l; resulted in the highest Average value (2.11cm), compare with the other treatments. On the other hand, augmenting the culture medium with IBA at 2.00 mg/l was concomitant with the highest Average value of the given trait (2.60cm).

In addition, the interaction between KIN at either nil level (0.00) or 0.5 mg/l of KIN with IBA at 2.00mg/l, brought about the highest Average values of the studied trait (i.e. 2.96 and 3.00 cm) each in turn.

Respecting the Average number of shoots formed/propagule, fortifying the culture medium with KIN, led to remarkable notes; where, as KIN levels increased the Average value of the given trait increased. Whereas, adding the highest level, 2.00mg/l KIN; gave rise to the highest Average value of the studied character (1.78) and *vice versa*. As for the main effect of IBA ,it is obvious that augmenting the culture medium with it at 1.00mg/l, contributed in achieving the highest Average value of this trait (1.68) compare to other treatments. Mean while, the interaction between KIN and IBA at 2.00 mg/l, and either of added levels of IBA led to the highest Average values, with siginificant difference. With reference to the Average number of leaflets formed/propagule, the main effect of KIN was obvious through adding it at 1.00mg/l, which recorded the highest Average value of the given trait (3.77) compare to the other treatments .On the other hand, the main effect of IBA declared that adding 2.00mg/l of IBA to the culture medium ; achieved the highest Average value (3.72) compare to the other tested levels. Also, the interaction between KIN and IBA at various combinations, especially at 1.00 and 2.00 mg/l, each in turn, achieved the highest Average value, but without significant differences . Refer to the Average number of roots formed /propagule, the KIN levels were in reverse relationship with this studied trait. Whereas, as KIN levels increased the given trait decreased. Hence, the absence of KIN in culture medium led to the highest Average number of roots formed/propagule (1.36) compare to the other treatments. On the other tank, the main effect of IBA was obvious especially at its presence in culture medium at 2.00 mg/l, recorded the highest

Table(1). Effect of different levels of KIN and IBA (mg/l) and their combinations on the initiation stage of *Magnolia grandiflora* nodal cuttings cultured *in vitro* for 35 days

Characters	IBA levels (mg/l)	KIN levels (mg/l) 0.00	0.50	1.00	2.00	Average IBA	Significance KIN	IBA	KINXIBA
(a)Average shoot length (cm)/propagule:									
	0.00	0.91	0.96	0.90	0.83	0.90	**	**	**
	1.00	1.93	1.96	1.83	1.73	1.86			
	2.00	2.96	3.00	2.30	2.13	2.60			
	3.00	2.16	2.53	2.26	2.13	2.27			
Average(KIN)		1.99	2.11	1.82	1.70				
L.S.D.(0.05)							0.05	0.05	0.10
(b) Average number of shoots formed /propagule:									
	0.00	0.67	1.20	1.26	1.56	1.17	**	**	**
	1.00	1.26	1.76	1.83	1.86	1.68			
	2.00	1.26	1.36	1.66	1.86	1.54			
	3.00	1.00	1.30	1.40	1.83	1.38			
Average (KIN)		1.05	1.40	1.54	1.78				
L.S.D.(0.05)							0.05	0.05	0.10
(c) Average number of leaflets formed /propagule:									
	0.00	1.70	3.81	3.83	3.93	3.32	**	**	**
	1.00	3.23	3.70	3.87	3.71	3.62			
	2.00	3.60	3.75	3.96	3.57	3.72			
	3.00	3.41	3.40	3.44	3.31	3.39			
Average (KIN)		2.98	3.66	3.77	3.63				
L.S.D.(0.05)							0.08	0.08	0.16
(d) Average number of roots formed /propagule:									
	0.00	0.01	0.33	0.33	0.00	0.16	**	**	**
	1.00	1.81	0.73	0.37	0.36	0.82			
	2.00	2.32	1.67	0.66	0.56	1.30			
	3.00	1.33	1.00	1.00	1.00	1.08			
Average (KIN)		1.36	0.93	0.59	0.48				
L.S.D.(0.05)							0.04	0.04	0.09

$L.S.D._{(0.05)}$= Least Significant Difference test at 0.05 level of probability.*, ** Significant of highly significant.

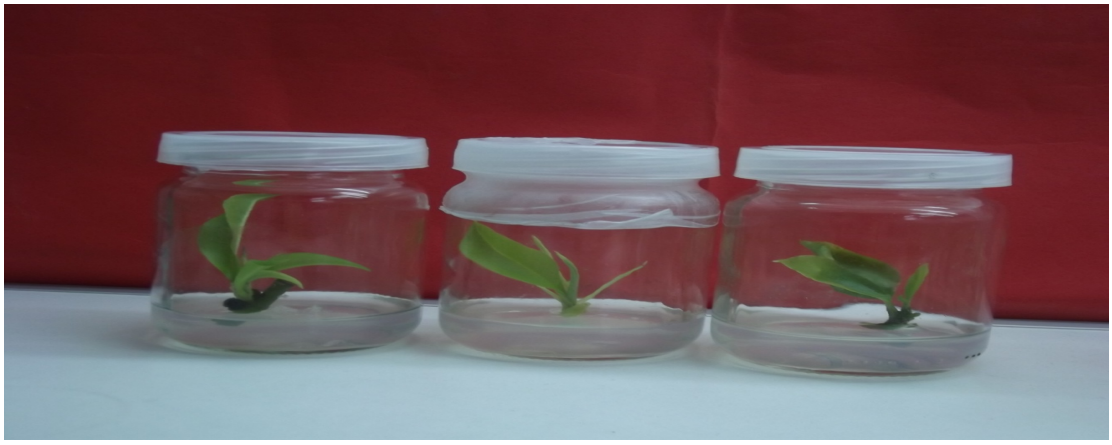

Fig.(1):Intiation of magnolia nodal explants cultured for 35 days on WP medium supplemented with KIN (2.00mg/l) and IBA at 1.00 mg/l.

average value (1.30). While, the interaction between KIN and IBA at nil level (0.00) and 2.00mg/l, consecutively, achieved the highest Average value (2.32). The obtained results in this respect are matching with the mode of actions of both applied growth regulators; whereas, auxin exerts significant roles in plant tissue culture and usually form an integral part of nutrient media. Auxin promotes either individually or in combination with cytokinins, the growth of calli, cell suspensions and organs and also regulatate the direction of morphogenesis. At the cellular level, auxins control basis processes such as cell division and cell elongation (George *et al*., 2008).Also, they play critical event in promoting rhizogenesis (Kim *et al*.,2003).

Cytokinins, together with auxin, take part in the regulation of the cell cycle in plant cells (i.e. stimulation of cell division, break apical dominance, enhance axillary shoot proliferation, and adventitious, inhibition root formation). Also, the interaction between auxin and cytokinin or their ratio other reperesents an important signal in the formation of cell phenotype and in the onest and maintenance of the process of cell division (Stickens *et al*., 1996).The ability of auxins (together with cytokinins) to mange key events in plant morphogenesis was documented (Skoog and Miller,1957) who discovered the regulation of organogenesis *in vitro* by Averages of the auxin: cytokinin ratio in culture media. It has been further supported by such other researches on the relationship between auxin and cytokinin levels and the morphogenetic response of various plants (Li *et al*., 1994; Centeno *et al*., 1996; Leyser *et al*., 1996).

The higher concentrations of the auxins as NAA is usually ineffective against shoot proliferation (Vijaya *et al.,* 1991, Waseem *et al*., 2011). Results of this study clearly demonstrated that WP medium was a better choice and improved growth of cultured explants (nodal segments) as reported by Biedermann (1987). It is noticeable that using of IBA at 2.0 mg/l here seamed to promote elongation of shoots that was considered an added help for the survival and growth of shoots. Similar results, during the establishment stage, were reported, elsewhere, on Magnolia when IBA was employed (Franc and Krejci, 1998). EL-Shamy (2004 and EL-Shamy et al. 2010) reported that subcultures on MS medium supplemented with 2.0 mg/l NAA led to increase the shoot length and number of leaves. With regard to IBA concentration, the addition of IBA at 1 mg/l to B5 medium resulted in the highest number of shoots. Raising the level of IBA to 2.0 mg/l, significantly, decreased the degree of browning induced callus formaition and gave the longest axillary shoots (Sakr *et al*.,1999). The induction rate of *Magnolia officinalis* was 100% on Gamborg medium (B5) containing 4.0 mg/l 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/l (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 concentration (1/1 and1/2) allow fairly uniform elongation shoot while rooting was poor. On low salt media, rooting was prominent but the leaves were yellowing and the elongation was nil (Maene and Debergh, 1985). The best results were obtained with mill medium + 1.0 mg IBA/ l (average number of

shoots 4.6, average length of shoots 5.0 mm. Krejci and Franc,1997). Qi *et al*. (2010) reported the terminal buds of *Magnolia officinalis* cultured on MS medium plus 6-BA, NAA and 2,4-D under different light conditions. The results showed that the optimum medium for callus indication of *Magnolia officinalis* terminal buds is MS+2.0 mg/l 2,4-D+ 0.5 mg/l BA+1.0 mg/l NAA +30 g/l sucrose+8 g/l agar at pH 5.8. Also, Li and Dong (2007) found that the terminal bud of the lower branches of *M.Simicum* should be used as explants in a medium consisting of ½ MS medium with 0.1- 1.5 mg/l IBA and 0.1- 1.0 mg/l IBA. Angsumalee *et al*. (2005) reported that all the explants formed viable shoots when MS medium supplemented with 0.1- 10 mg BA /l. Shooting was highest (2.72±0.37) on MS medium supplemented with 1 mg/l for 4 weeks. For successful *in vitro* rooting, shoots of *Magnolia grandiflora* were treated with 2.00 mg/l IBA in the culture medium. Whereas, lesser or higher IBA concentrations (i.e.1.00 mg/l or 3.0 mg/l) failed to form roots on shoots. Similarly in other trials, 2.0 mg/l IBA promoted *in vitro* rooting on Magnolia shoots (Maene and Debergh, 1985; Kamenicka *et al*., 1996, Sakr *et al*., 1999, El-Shamy *et al.,* 2004 and 2010). In some other cases, IAA was also used for *in vitro* rooting of Magnolia shoots (Kamenicka and Takats, 1997).

Multiplication stage

Results of Table (2) and Figure (2) display the effect of both applied growth regulator' levels and their combinations practiced highly, significant effects on the multiplication stage's characters of *Magnolia grandiflora* where single nodal explants were cultured and grown *in vitro* for 35 days . Respecting the shoot length formed per propagule, the main effect of KIN declared that increasing levels' concentrations within the range of1.00- 3.00mg/l with no significant difference, led to increase the shoot length at but increasing its level up to at 5.00mg/l, coused such significant decrease. On the other hand, the main effect of IBA, declared that there was a proportional relationship between it and the given trait. Whereas, the IBA at 1.0 mg/l and/or at 2.00mg/l, resulted in the highest Average values (2.44 and/or 2.48) without significant difference. Regarding the interaction between both applied growth regulators, the presence of two hormones KIN and IBA at 1.00 mg/l each, resulted in the highest number of shoots per propagule (2.85). Respecting the Average number of shoots formed/propagule, the main effect of KIN showed that its presence in the culture medium at 5.00mg/l achieved the highest Average value (2.88).On the other extreme, the presence of IBA in culture medium at either 1.00 or 2.00mg/l, brought about the highest Average values, i.e.2.44 or 2.48, without significant difference. The interaction between KIN and IBA at 5.00 and 1.00 mg/l, respectively recorded the highest Average value (3.40). In terms of Average number of leaflets formed/propagule, adding KIN to the culture medium at 5.00mg/l contributed to record the highest Average value of the studied trait (6.58). Respecting the main effect of IBA, augmenting the culture medium with either 1.00 or 2.00mg/l, resulted in similar finding; where achieved either 5.44 or 5.35 respectively, without siginificant difference. Meanwhile, the interaction between KIN and IBA at 5.00 and 1.00mg/l, led to the highest Average value of the given trait (7.33). With respect of the Average number of roots

formed/propagule, it is obvious that the absence of KIN from the culture medium (0.00mg/l), gave rise to the highest Average number of roots formed/propagule(1.18), then as its level increased in the culture medium, the given trait was in inverse relationship. On the other hand, augmenting the culture medium with IBA was in direct propontional relationship with the given trait, especially at 2.00mg/l which achieved the highest Average value (1.33). Meanwhile, the interaction between 0.00mg/l and 2.00mg/l of both KIN and IBA, each in turn, brought about the highest Average value (2.00 roots per propagule).

Table (2).Effect of different levels of KIN and IBA (mg/l) and their combinations on the multiplication stage of *Magnolia grandifloral* nodal cuttings cultured *in vitro* for 35 days.

Character	IBA levels (mg/l)	KIN levels (mg/l) 0.00	1.00	3.00	5.00	Average IBA	significance KIN	IBA	KIN X IBA
(a)Average shoot length(cm)/propagule:									
	0.00	1.06	1.26	1.23	1.20	1.19	**	**	**
	0.50	1.30	1.33	1.41	1.33	1.34			
	1.00	1.63	2.85	2.63	2.66	2.44			
	2.00	2.55	2.53	2.68	2.16	2.48			
Average (KIN)		1.63	1.99	1.99	1.83				
L.S.D.(0.05)							0.10	0.10	0.21
(b) Average number of shoots formed /propagule:									
	0.00	0.85	1.23	2.63	2.73	1.86	**	**	**
	0.50	1.00	1.57	2.06	2.85	1.87			
	1.00	1.32	1.91	2.33	3.40	2.24			
	2.00	1.56	1.67	2.13	2.55	1.98			
Average (KIN)		1.18	1.67	2.29	2.88				
L.S.D.(0.05)							0.14	0.14	0.29
(c) Average number of leaflets formed /propagule:									
	0.00	2.36	4.78	5.80	6.25	4.80	**	**	**
	0.50	2.93	4.16	5.25	6.66	4.75			
	1.00	3.50	4.58	6.36	7.33	5.44			
	2.00	3.83	5.50	6.00	6.08	5.35			
Average (KIN)		3.15	4.75	5.85	6.58				
L.S.D.(0.05)							0.19	0.19	0.39
(d) Average number of roots formed /propagule:									
	0.00	0.00	0.33	0.33	0.00	0.16	**	**	**
	0.50	1.00	0.56	0.44	0.00	0.50			
	1.00	1.72	0.70	0.67	0.55	0.91			
	2.00	2.00	1.33	1.00	1.00	1.33			
Average (KIN)		1.18	0.73	0.61	0.38				
L.S.D.(0.05)							0.06	0.06	0.11

L.S.D.$_{(0.05)}$= Least Significant Difference test at 0.05 level of probability.*, ** Significant of highly significant.

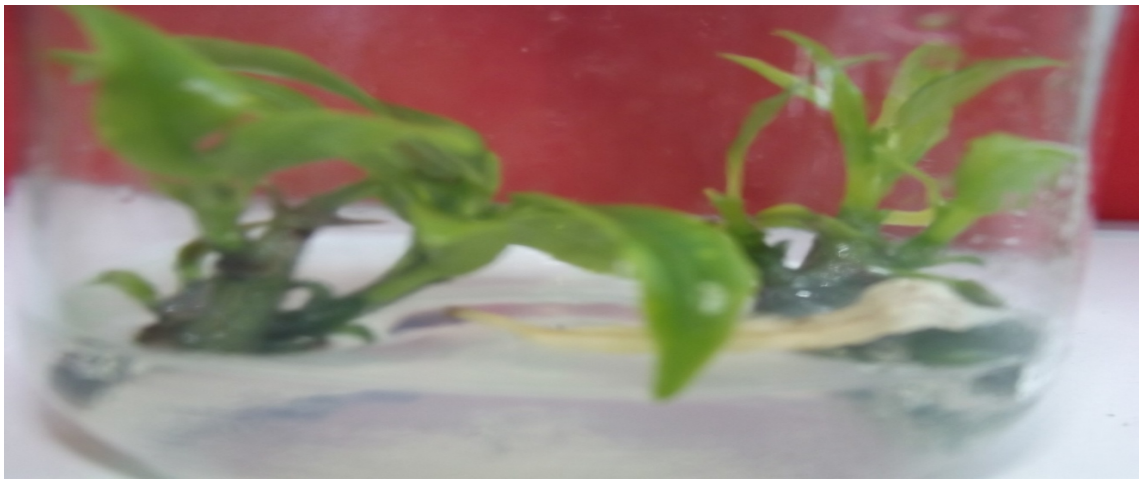

Figure (2): Multiplication of magnolia from newly nodal segments of initiation stage, upon cuturing for 35 days on WPM augmented with KIN and IBA at 5.00 and 1.00mg/l, consecutively

On the other side, *Magnolia grandiflora,* also, responded positively to form callus by application of KIN during the multiplication stage (Klimazewska, 1981). In the multiplication stage, adding 5.0 mg/l KIN culture medium the c formed the highest number of shoots as reported by EL-Shamy *et al*.(2010). This finding could be achieved due to the mode of action of auxin (IBA) within cultured tissues which many enhance, control various distinctive processes such as cell growth and elongation (George and Sherrington, 1984) and Wilkins (1989). Additionally, it has been stated that auxin induced number of response which involved cell division,cell enlargement, protein and nucleic acids synthesis which are concenation of auxin – induced growth and changes in wall plasticity of plant cell and increase the apical dominance as there are assential and rapid processes involved in growth and elongation. Howaever, the presence of auxin in the culture medium, positively, increased the Average shoot length of *Magnolia grandiflora* (Saker *et al*., 1999; Zaman *et al*., 2001; EL-Shamy *et al*., 2010). In this respect, also, Lemos and Black (1996) showed in *Annona muricata* that the addition of NAA promoted bud elongation. The more important multiplication stage, the use of KIN favoured not only proliferation of shoots, but also promoted plant height of magnolia shoots. Whereas, KIN at 5 mg/l led to the highest number of shoots and at 1.00 or 3.00 mg/l led to the tallest plant heights, number of leaves was restricted to leaf surrounding the formed bud only at 1.00 or 3.00 mg/l of KIN. When KIN was used in a lesser concentration (0.00mg/l or 1.00mg/l) number of leaves were dramatically decreased. However, MS supplemented with NAA or IAA was used successfully with magnolia for shoot proliferation purposes (Krejci and Franc,1997). However, in other occasions Magnolia, also, responded positively to form callus by application of KIN during the multiplication stage (Klimaszowska, 1981). This later auther reported that the obtained result was in harmony with *Magnolia grandiflora* results obtained here, which on the whole, seems to favour KIN for the multiplication stage of Magnolia. As an explanation for this phenomenon, its more likely that high levels of KIN- utilized in this study (i.e. 5.0 mg/l) and elsewhere too, may have caused the removal of apical dominance thus

enhanced shoot proliferation (Klimaszewska,1981). In this respect, in *Magnolia grandiflora*, it was, also, observed that shoot multiplication was developed using different concentration of cytokinin with auxin achieved the best results as reported by some investigators (Nakmura *et al*., 1995; Luo and Sung,1996; Tong *et al*., 2002; Zaikang *et al*., 2002;Rosas and Rodriques, 2006; Lina *et al*.,2006 and Parris *et al*., 2010).

Rooting (rhizogenesis) stage

Since the growth and development are correlated processes the recorded characters, here, should be presented as a whole.

Results of Table (3) and Figure (3) manifested that various levels of both applied growth regulators and their interactions had, highly significant effects on the rooting stage's traits of *Magnolia grandiflora*. Respecting the Average shoot length per propagule, results of IBA demonstrated that the presence of IBA in culture medium results in the highest shoot length. In total, the main effect of NAA showed similar performance that has been noticed as the above-mentioned characters. The interaction between IBA at 1.00mg/l and NAA at 2.00 mg/l gave the highest Average value of shoot length (2.31). It could be concluded from the above-mentioned results that the presence of IBA in the culture medium led to better performance of shoot length . Higher concentration of IBA had shown best results. This reason could be due to the fact that IBA as usually take an active role in the shoot proliferation and its effect is visible in callus or root formation. On the contrary, NAA at intermediate concentration (*viz* 2.00 mg/l), caused the longest shoot per propagule. The finding could be attributed to the mode of action of auxin (NAA) within cultured tissues is capable of controlling various distinctive processes such as cell growth and elongation (George and Sherrington, 1984; George *et al.,* 2008). Concerns for the Average number of shoots per propagule, the main effect of IBA .

Fig.(3):Rhizogenesis of magnolia microshoots of multiplication stage, upon culturing then for 35 days on WP medium fortifited with NAA and IBA at 1.00 mg/l, each in turn

Table (3). Effect of different levels of NAA and IBA(mg/l) and their combinations on the rooting stage of *Magnolia grandiflora* nodal cuttings cultured *in vitro* for 35 days.

Characters	IBA levels (mg/l)	NAA levels (mg/l) 0.00	1.00	2.00	3.00	Average IBA	Significance NAA	IBA	NAAXIBA
(a)Average shoots length (cm) /propagule:									
	0.00	1.00	1.41	1.60	1.76	1.44	* *	**	**
	0.50	1.65	1.80	1.90	1.98	1.83			
	1.00	1.95	2.03	2.31	1.91	2.05			
	2.00	1.95	2.00	2.00	1.60	1.86			
Average (NAA)		1.64	1.81	1.95	1.81				
L.S.D. (0.05)							0.03	0.03	0.05
(b)Average number of shoots formed /propagule:									
	0.00	0.66	1.64	1.33	1.03	1.16	**	**	**
	0.50	1.33	1.46	1.39	1.30	1.37			
	1.00	1.63	1.86	1.34	1.23	1.51			
	2.00	1.31	1.40	1.10	1.00	1.20			
Average (NAA)		1.23	1.59	1.29	1.14				
L.S.D. (0.05)							0.04	0.04	0.09
(c) Average number of leaflets formed /propagule:									
	0.00	2.32	3.18	3.23	3.16	2.97	**	**	**
	0.50	2.62	3.30	3.26	3.28	3.11			
	1.00	2.73	3.34	3.38	3.18	3.16			
	2.00	2.18	3.03	2.96	2.76	2.89			
Average (NAA)		2.62	3.21	3.21	3.09				
L.S.D.(0.05)							0.10	0.10	0.19
(d) Average number of roots formed /propagule:									
	0.00	0.20	1.00	1.60	2.33	1.28	**	**	**
	0.05	1.26	1.33	1.80	2.66	1.76			
	1.00	1.85	2.83	2.60	2.46	2.43			
	2.00	2.26	2.53	2.23	2.00	2.25			
Average (NAA)		1.39	1.92	2.05	2.36				
L.S.D.(0.05)							0.06	0.06	0.11

L.S.D $_{(0.05)}$= Least Significant Difference test at 0.05 level of probability*, ** Significant of highly significant.

and NAA, divulged that the presence of both growth regulators-into WP medium had significant effects of the given trait. The interaction between NAA and IBA exerted, highly, significant effects and at the highest levels of both the growth regulators brought about the lowest Average values. Concerning the effect of IBA on the rooting mass perpagule, it is obvious that, the fortifying WP-medium with IBA at 1.00 mg/l, results in the highest Average value. On the other way, the presence of NAA into culture medium at 3.00 mg/l, brought about the highest Average value. Likewise, the combinations between both IBA and NAA at 1.00 mg/leach, gave the highest Average values. These results showed that the medium fortified with 1.00 mg/l IBA, and NAA at 3.00 mg brought about the

highest rooting mass per propagule. These results cope with those of microcuttings of *Magnolia soulangiana* were treated *in vitro* with 0.1 , 1.0 , 2.0, 3.0 or 4.0 mg IBA . Root number was greatest with 4.0 mg IBA and root length with 1.0 mg IBA (Kamenicka, 1996). In the later, trial, the optimal rooting medium for Magnolia was half stermsth s-medium with 4 mg IAA/l (Kamenicka and Takats, 1997). Similar results were reported by Chaidaroom *et al*.(2004). Concenring, the main effect of IBA tested levels on the Average number of leaves per propagule, the presence of IBA at 1.00 mg/l, led to the highest Average values of above - mentioned traits. On the other hand, NAA main effect, augmenting WP- basal medium with 1.00 or 2.00 mg/l of it, brought about the highest Average value of the above - mentioned traits. However, the interaction between both added levels of IBA and NAA at 1.00 mg/l, resulted in the highest Average value.

Comparison between the effect of BA and KIN on explants of *Magnolia grandiflora*

As for data presented in Table (4) and Figure(4) declared that the effectiveness of KIN surpassed significantly its counterpart of BA (at 3.00mg/l =13.33μM each). Regarding shoot length and number of shoots/propagule (*viz*., 2.67 and 2.31viz. 1.90 and 1.20, each in turn). Meanwhile, there were insignificant differences respecting number of leaftlets and number of shoots/propagule. These results could be attributed to the mode of action of KIN which is more effective than BA and /or variations in their metabolism or to active forms or to differences in primary mechanism of action as reported earlier. Alternatively, responses of explants to both cytokinins are different due to various aspects. Also, this variation may be due to the degree of cell sensitivity towards both tested cytokinins, which depends on the endogenous levels of growth regulators. Likewise, in other occasions, BA was reported to be not suitable for Magnolia elongation in the multiplication stage (Maene and Debergh, 1985; Biedermann,1987; Kamenicka *el al*.1996; Luo and Sung,1996; Kamenicka and Takats,1997). Magnolia, also, responded positively to form callus by application of KIN during the multiplication stage (Klimaszewska, 1981). This later reported result is in harmony with *Magnolia grandiflora* results obtained here, which on the whole, seems to favour KIN for the multiplication stage of Magnolia .As an explanation for this phenomenon, it is more likely that high levels of KIN utilized in this study (3.00mg/l) and elsewhere, too, may have caused the removal of apical dominace thus enhancing lateral shoot proliferation (Klimazewska, 1981). Also, EL-Shamy(2004)reported that *Magnolia grandiflora* at the multiplication stage, the best medium was WP medium plus the growth regulators KIN (at 5.00 or 6.00mg/l) which increased plant height, number of leaves/shoot and number of shoot .Notably, KIN was better than BA for the multiplication stage of *Magnolia grandiflora*.

Table(4). Effect of both growth regulators (BA and KIN) at 3.00mg/l each on *Magnolia grandiflora* explants grown *in vitro* for 35 days/propagule during multiplication stage

characters	BA (3.00mg/l)	KIN (3.00mg/l)	t.cal.	t. tab . 0.05	0.01
Shoot length (cm)/propagule	1.90	2.667	8.712 **	2.776	4.604
Number of shoots/propagule	1.20	2.31	6.416 **		
Number of leaflets/propagule	4.167	5.50	3.204 n.s.		
Number of roots/propagule	0.33	0.36	0.189 n.s.		

Figure (4): Effect of BA (left) and KIN (right) at 3.00mgIL, each on growth performance of magnolia grown in vitro for 35 days

Effects of activated charcoal on Magnolia

Data presented in Table (5) and Figure (5) showed that adding activated charcoal to WP medium resulted in high significant effects on the given traits . However there were direct proportional relationships between concentrations of AC and the all given traits, especially at 1.00 g/l AC. The role of activited charcoal in tissue culture is interpreted by Fridborg *et al.*(1978) as being an adsorbent for inhibitory materials that may be persented in the medium or that might originate from the explants themeseleves .Also, charcoal is characterized by the adsorption of toxic brown or black pigments (phenol-like compounds and melanin) and absorption of all other organic compounds, auxin, cytokinin,

ethylene, vitamins Fe and Zn chelates (Pierik, 1987).Fridberg *et al*.(1978) found that compounds excreted from growing cells of *Daucus* and *Allium* could be adsorbed by activated charcoal to allow embryogenesis and root formation to occure. On *Ruscus hypoglossum* plants Abou-Dahab *et al.* (2005) decided that the highest number of roots was recorded with 5g /l sucrose and with activated charcoal. Parris *et al*.(2010) found that supplemented McCown woody plant medium (WPM) with charcoal produced elongated plantltes which more sutitable for increase rooting and *ex vitro* establishment on Magnolia "Ann" .Also, Thomas (2008) reported that increased shoot elongation *Acacia mearnii* and *Anacardium*. Gad (2011)reported that shootlets of *Populus alba* were elongated on medium supplemented with 3mg/l GA_3 +3g/l AC+0.5 mg/l BAP followed by highest rooting on medium with 3mg/l GA_3+3g/l AC+0.1mg/l NAA +0.5mg// IBA. Then, Charcoal can increase the capability of culture shoots to form more roots.

Table(5).Effect of activated charcoal mixed in the medium of *Magnolia grandiflora* shoots cultures grown on multiplication media for 35 days *in vitro*

Characters	Activated charcoal concentrations 0.00	0.25	0.50	1.00	significant	L.S.D.(0.05)
(a)Average shoot Length (cm)/propagule	1.39	1.93	2.60	3.31	**	0.20
(b)Average number of shoots formed propagule	1.50	1.62	2.00	2.96	**	0.16
(c)Average number of Leaflets formed/propagule	3.03	3.00	5.30	5.83	**	1.20
(d)Average number of rooting mass formed/propagule	0.52	1.13	1.86	2.06	**	0.45

L.S.D.(0.05)=Least significant different test at 0.05 level of probability*,: significant or highly significant**

(a) (b) (c) (d)

Figure (5): Effect of activated (AC) charcoal concentrations on the growth of magnolia, (a)control ; (b)0. 25 g/l ; (c)0.50g/l ,and (d)1.00 g/l

The fourth stage (acclimatization)

Acclimatization of *in vitro* grown plants is an important step in micropropagation (Smart, 2008; Rout *et al*., 2006).The *In vitro* grown plantlets with at least two to three roots were transferred to the greenhouse for the acclimatization *ex vitro*. The potting mix (sand and compost, 1:3), routinely used in the nursery of our institute, was found suitable for the hardening of the plants. The survival rate of the *In vitro* grown plants was 40% as shown in Fig. (6).

Figure (6): Acclimatization of Magnolia plantlets *ex vitro* in a mixture of compost and sand (3:1)

Further prospects (recommendations)

The present study declared the necessity for further studies to improve (1)proliferation or multiplication of neoformed shoots; (2) achieving both proliferation or multiplication and rooting at the same time *via* a dual stage culture medium for saving the time and efforts to achieve each stage indivivally ; (3) in addition find out the way through to overcome decline multiplication rates with heading the subculture via resting the multiplied shoots over subculture (culture on media without growth regulators or half concentration……ect., and(4) improving the survival of magnolia plantlets *ex vitro* and *in vivo, via* choosing the appropriate mixtures.

CONCLUSION

It could be concluded that there is a possibility to propagate magnolia trees by micropropagation. The protocol here in descried is very much efficient for the *in vitro* intiation (elongation of regenerated shoots), multiplication shoot prolifiration, rooting of nodels egements of this species (hard -to –root plants).

In the light of our results:

1- It can be suggested that enhanced shoots and buds formation can be achieved by using the WP media fortified with different concentrations of the cytokinin and auxin to evaluate their effects in this respect. The plant growth varied as the concentration of the growth regulators changed. The *in vitro* roots were successfully induced also.

2-The rooted plantlets were acclimatized in magnolia trees , but the present study declared the necessary for further studies to improve acclimatization to improve survival rate for the commmerical industry.

REFERENCES

AbouDahab, A.M., A.M.A. Habib, Y.A. Hosni and A.M.M. Gabr (2005). Effect of MS-salt strength, sucrose and IBA concentration and acclimatization media on *Ruscus hypoglossum*, L. micropropagation.Arab J. Biotech, 8 (1): 141-154.

Angsumalee,D.,W.Sukaeed and M. Janyawanichakul(2005). Micropropgati-on of plau-Yai (*Croton oblongifolius*).Proceeding of 43rdKasetsart University. Annual Conference, Thailand, 1-4, February, Subject: Plants, 658- 663.

Bailey, L.H. and E.Z. Bailey (1960): In :Hortus third A Concise Dictionary of Plants Cultivated in the United States and Canada, 3rd Ed., Macmillon, New York, pp.680-1969.

Bhattacharya, P., S. Dev, and B. S. Bhattacharya(1990). Rapid mass propagation of *Chysanthemum morifolium* by callus derived from stem and leaf explants.Pl.Cell Rep.,9:439-442.

Biedermann, L.E.G. (1987). Factors affecting establishment and development of *Magnolia* hybrids *in vitro*. Acta Horti., 212 (2): 628-629.

Centeno M.L., A. Rodriguez, I. Feito and B. Fernandez(1996). Relationship between endogenous auxin and cytokinin levels and morphogenic responses in *Actinidia deliciosa* tissue cultures. Plant Cell Rep. 16,58-62.

Chaidaroon, S., I. Ungvichian and K. Ratanathavornkiti (2004). In vitro root initiation of 'Champi Sirindhorn' (*Magnolia sirindhorniae*, Noot.&Chalemglin).AUJ.T. 129- 132.(Jan,2004).

EL-Shamy, A.A. (2004). Studies on micropropagation of some woody Ornamental Plants. Ph.D. Thesis, Fac. Agri., Ain Shams, Univ.

EL-Shamy, M.A. S.S. Ahmed and A. Ibrahim (2010). Effect of media on propagation of *Magnolia grandiflora* with tissue culture technique. J. Biol. Chem. Environ. Sci., 5 (4): 277- 291.

Franc, P. and P. Krejci (1998). Plant effects on two year micropropagation cultures of *Magnolia X soulangiana* Soul – Bod in vitro Zahradnictivi., 25 (2): 47-51

Fridborg, G., M. Pedersen, L.E. Landstrom and T.Eriksson(1978). The effect of activated charcoal on tissue cultures: adsorbtion of metabolites inhibiting morphogenesis. Physiol. Plant, 43: 104–106.

Gad, M.M.A.(2011). CLONAL Propagation and mass production of *Populus alba* mother- plus trees through tissue Culture Techniques. Egypt. J. of Appl. Sci., 26 (12B):390-416.

George, E.F.; M.A. Hall and G.J.D. Klerk (2008). Plant Propagation by Tissue Culture.3rdEdition.Springer.

George, E.F. and P.D.Sherrington (1984). Plant propagation by tissue culture.Exegeetic Ltd.,Basingtoke,U.K.709 P.

Gomez, K. and A.A.Gomez (1984). Statistical procedures for Agricultural Research (2nded.).An International Rice Research Institute Bok. A Wiley Interscience Publisher, New York.

Kamenicka, A. (1996). Rooting of *Magnolia* X *soulangiana* microcuttings. Biologia Bratislava, 51 (4): 435- 439.

Kamenicka, A. and J. Takats(1997). Direct regeneration of *Magnolia spp. via in vitro* propagation Magnolia, 32(1): 1-6 (CAB Abstract No. 970309040, 1996).

Kamenicka, A., M. Valova and M. Lanakova(1996). Effects of culture media on the formation of axillary shoots of *Magnolia* X *soulangiana* Soul Bod.*in vitro*. ActaAgron Hungarica, 44 (1): 53- 57.

Kim, Y. S., E.J. Hahn and K.Y. Paek(2003). Lateral root development and saponin accumulation as affected by IBA or NAA in adventitious root cultures of *Panax ginseng* CA Meyer. *In Vitro* Cell.Develop.Biol.,39: 245-249.

Klimaszewska, K. (1981). Plant regeneration from petiole segments of some species in tissue culture. Acta Agrobotan, 34(1): 5-28.

Krejci, P. and P. Franc (1997). Optimization of multiplication phase in *Magnolia* × *Soulangiana* Soul. Bed.in vitro. Zahradnictvi, 24(4): 133-137 .

Lemos, E.P. and J. Black (1996). Micropropagation of juvenile and mature *Annona muricata*, L. J. Horti. Sci. Biotech., 71: 395- 405.

Leyser H. M. O., F. B. Pickett, S. Dharmasiri, and M. Estelle(1996). Mutation in AXR3gene of Arabidopsis result in altered auxin response including ectopic expression from the SAUR-ACI promoter. Plant J., 10:403-413.

Li, L. J. and M. M. Dong(2007). Study on browing of endangered Manglietiastrumsinicum in tissue culture. (Chinese). Journal of Zhejiang Forestry Science and Technology, 27 (1): 20-23.

Li Y., X.Y.Shi, T.J. Strabala,G. Hagen andT.J. Guilfoyle(1994). Transgenic tobacco plants that over produce cytokinins show increased tolerance to exogenous auxin and auxin transport inhibitors. Plant Science, 100:9-14

Lina, L., W. Xiuli, L. Hui, Z. Gen Yu and L. Jianyne(2006). Study on culturing germ- free shoots from carnation stems and micro-propagation. Acta Agric. Shanghai, 22(3): 63- 67.

Lloyd, G. and B. McCown (1980). Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia* by use of shoot tip culture. Proc. Intl. Plant Prop. Soc., 30: 421-427.

Luo, G.F. and W.B. Sung (1996).A brief report on micropropagation of a rare ornamental shrub-the red form of *Magnolia delavayi*. Magnolia, 31 (1): 22-27.

Maene, L. and P. Debergh (1985). Liquid medium additions to established tissue cultures to improve elongation and rooting *in vitro*. Plant Cell, Tiss. & Org Cult., 5(1): 23-33.

Mccracken, T.P., J. Christoher and T.E. Bilderback(1996). Rooting of 'BrownVelvet' southern magnolia stem cutting as influenced by medium and auxin treatment.Journal of Environmental Horticulture, 14, (3) 158-159.

Nakmura, K., Y. Wakita and S. Yokota (1995). Induction of Multiple shoots by shoot Apex culture in *Magnolia obovata* Thumb. Plant Tissue Culture Letters, 12 (1): 34- 40.

Parris, J.K., D.H. Touchell and T.G. Ranneg (2010). Optimizing *in vitro* growth conditions for *magnolia* "Ann". SNA Res. Con., 55: 30-35.

Pierik, R.L.M. (1987). *In vitro* culture of higher plants. Kluwer Acedamic Publishers incorporates the programmes of D. Reidel. MartinusNijhoff, Dr W. Junk and MTP press.

Qi, H. P., F. Ping and Y. Yin (2010). Optimization of induction condition for callus of *Magnolia officinalis* (Chinese).Guizhou Agricultural Sciences, 7: 20- 21.

Rosas, M.M. and A.J. Rodrigues (2006). Somatic embroyogenesis and organogenesis in *Magnolia dealbata* Zucc.(Magnoliaceae), and Endangered, Endemic Mexican Species. Hort. Sci., 41 (5): 1325- 1329.

Rout, G.R.,A. Mohapatra and S. Mohan Jain (2006). Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects. Biotechnology Adv.,24: 531-560.

Said, M. (2007).Comparison of hormonal effect in different medium substrates and propagation methods on rooting of *Magnolia grandiflora*,L. M.S.Thesis, Fac. Agric. Sci. Alex. Univ.

Sakr, S. S., M. A. El-Khateeb and A.H. Abdel-Kareim (1999). Micropropagation of *Magnolia grandiflora* L. through tissue culture technique. Bulletin of Faculty of Agriculture, University of Cairo, 50 (2): 283- 298. (Horticultural Abstracts No. 7183, 1999).

Skoog, F. and C.O. Miller (1957). Chemical regulation of growth and organ formation in plant tissue cultured *in vitro*. Symp. Soc. Exp. Biol., 11: 118-130.

Smart, L. (2008). EFB 530 Plant Physiology, cytokinins and cell division, EFB 530 Plant Physiology- Syllabus with lecture notes- spring.

Steel, R. G. D.,J.H. Torrie and D. A. Dickie. 1997. Principles and procedures of statistics-a biometric approach. Thired edition. McGraw-Hill Publishing Company. Toronto.

Stickens D.,W. Tao, and J.P. Verbelen(1996).A single cell model system to study hormone singaltransduction.Plant Growth Regul., 18: 149-154.

Thomas, T.D. (2008). The role of activated chrocoal in plant tissue culture.Biotechnol. Adv., 26: 618-631.

Tong, Z.K., Y.Q. Zhu and Z.R. Wang (2002). Studies on tissue culture and the establishment of a high yield cell line of *Magnolia officinalis*. Journal of Nanjing Forestry University, 26 (4): 23-26. (CAB Abstract No. 30033066340, 2002).

Vijaya, N., G. Satyanarayana, J. Prakashand and R.L.M. Pierik (1991). Effect of culture media and growth regulators on *in vitro* propagation of rose. Horti. NewTech.Appl Proc. Inter. Sem. New Frontiers in Hort., organized by Indo –American Hybrid Seeds, Bangalore, Ind., Nov.25-28,209-214.

Waseem, K., M. S. Jilani, M. S. Khan, M. Kiran and G. Khan (2011). Efficient *in vitro* regeneration of chrysanthemum(*Chrysanthemum morifolium* L.)plantlets from nodal segments.Afri.J.Biotech.,10(8):1477-1484.

Wilkins, M.B. (1989). Advanced plant physiology.The Bath Press,Avon,13-15.

Zaman, M.S., A. Quershi, G. Hassan, R.U. Din, S. Ali, A. Khabir and N. Gul (2001). Meristem culture of potato (*Solanum tuberosum*, L.) for production of virus free plantlets. On line J. Bio. Sci. 1 (10): 898- 899.

Zaikang, T., Z. YuQui and W. ZhangRong (2002). Studies on tissue culture and the establishment of a high- yield cell line of *Magnolia officinalis*. Journal of Nanjing Foresty University, 26(4): 23-26.

الملخص العربي

الإكثار المعملي الدقيق وأقلمة لأشجار الماجنوليا

*** هدى اسماعيل محمد الجداوى ، **علي ابراهيم علي عبيدو ، ***محمد جمال محمد التركى، *بثينة محمد لبيب وحيدة ، **محمد قدري عبد الحفيظ جابر**

*مركز البحوث الزراعية – معهد بحوث البساتين – قسم بحوث الزينة بالمنتزة – الاسكندريه

** قسم الانتاج النباتى (تخصص البساتين) – كلية الزراعة سابا باشا – جامعة الأسكندرية

*** قسم الزهور و نباتات الزينة و تنسيق الحدائق – كلية الزراعة –الشاطبى– جامعة الأسكندرية

أجريت هذه الدراسه في معمل زراعة الانسجه – قسم الانتاج النباتى– كلية الزراعه – سابا باشا– جامعة الاسكندريه خلال السنوات ما بين ٢٠١٣– ٢٠١٥ لتطوير أو إيجاد بروتوكول فعال للأكثار المعملى الدقيق لأشجار الماجنوليا . ولقد تم أستخدام عقل ساقية من أشجار الماجنوليا النامية بحدائق قسم بحوث الزينه بقصر المنتزة(النباتات الأم) خلال دراسة معملية لأستحثاث إكثار (تضاعف) المجاميع الخضرية . تم زراعة الأجزاء النباتية العقدية على بيئات مغذية للتدشين أو البدء باستخدام توليفات مختلفة من الأوكسين (IBA) و السيتوكينين (KIN)، و تمت زراعة المجاميع الخضرية المتكونة خلال مرحلة البدء أو التدشين علي بيئات مختلفة للتضاعف أو الأكثار للحصول على أعداد كبيرة (متضاعفه) من تلك المجاميع الخضرية ، ثم أستطالتها و كذلك تجذيرها ، هذا بالأضافة الى أقلمة تلك النبيتات خارج المعمل. كانت أفضل بيئة لتدشين أو بدء المجاميع الخضرية تحت الظروف المعملية هى بيئة إكثار النباتات الخشبية (WPM) المزودة بالسيتوكنيين KIN بتركيز ٢ ملليجرام/ لتر بالأضافة ألى الأوكسين(IBA) بتركيز ١ مليجرام/لتر. و كانت بيئة التضاعف أو الأكثار هى نفس البيئة المزودة ٥ ملليجرام/لتر من السيتوكنين بالأضافة الى١ مللجرام/ لتر من الاوكسين IBA.. و الأكثر من ذلك، عند تعريض تلك المجاميع الخضرية النامية معمليا لتراكيز مختلفة من الأوكسين NAA و كذلك IBA اظهرت مجاميع جذرية قوية و سليمة ، خاصة عند تزويد البيئة بكلا الأوكسينيين عند تركيز ١ مللجرام/ لتر (مرحلة التجذير) وعند تزويد البيئة بتركيزات مختلفة من الفحم النباتى المنشط كان التركيز الأعلى المستخدم وهو امليجرام لكل لتر هو الأفضل فى أنتاج المجاميع الخضرية وكذلك تحسين أنتاج الجذور. كما أن الخلطة من الرمل : الكومبوست (٣:١) لأقلمة تلك النباتات ناتج زراعة الأنسجة كانت الأفضل في هذا الصدد .