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TISSUE CULTURE PROPAGATION OF LEMON VERBENA (*Aloysia triphylla*)

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ABSTRACT

The current research was conducted at the experimental station and tissue culture laboratory of Vegetable and Floriculture Department, Faculty of Agriculture, Mansoura University during the seasons of 2008 – 2010. A protocol is described for rapid and large-scale propagation of the woody aromatic and medicinal shrub lemon verbena (*Aloysia triphylla*) by *in vitro* culture of shoot tips or nodal segments from mature plants. Nodal segments were found to be more efficient than shoot tips for lemon verbena shoots regeneration on MS medium (Murashig and Skoog 1962) supplemented with BAP at 0.1 mg/L. Of the three different cytokinins ,6-benzyl aminopurine (BAP), kinetin (Kin) and thidiazrone (TDZ) evaluated as supplements to Murashige and Skoog medium (MS), BAP at concentration of 0.5 mg/L was found to be the most effective cytokinin in inducing multiple shoots. The frequency of shoot proliferation was markedly influenced by number of subcultures, since the highest percentage of shoot multiplication (100 %) as well as large the number of shoots/ explant (11.5 shoots) were obtained in the 4^{th} subculture, after which there was a declined in shoot development. Rooting was best induced (92 %) in shoots cultured on MS medium augmented with indole-3-butyric acid (IBA) at 0.5 mg/L. Mixture of soil: sand: peatmoss (1: 1: 1) was the most suitable planting substrate for hardening and its use ensured high survival frequency (100 %) of regenerated plants prior to outdoor transfer.

INTRODUCTION

Lemon verbena (*Aloysia triphylla*. L'her), which belongs to family *verbinaceae*, is a native medicinal semi-shrub largely distributed in Argentina (Botta, 1979). The leaves are used for medicinal purpose as digestive and carminative due to the content of carvone and its precursor limonene as the main components of *Aloysia*'s essential oil (Cabanillas *et al*., 2003; Figueiredo *et al*., 2004). Also, the high quality essential oil of *Aloysia*'s is used in perfumery, since the essence of lemon verbena is one of most expensive and rare in the essential oils market.

Recently, an increasing interest in cultivation and production of new aromatic plants has been recognized in Egypt to cover the increasing demands of the local industries as well as, for export. In order to expand cultivation of *A.triphylla*, the first step is the production of high quantities of genetically homogeneous plant material, since it is difficult to obtain seeds owing to Egyptian climate, usual multiplication procedure is vegetative.

The intensive multiplication by *in vitro* culture technique is a powerful tool for micro propagation and genetic improvement of the species. Hence, the aim of present work was to develop a methodology for *in vitro* propagation of lemon verbena (*A.triphylla*) and this paper describes the procedure used to induce direct regeneration of shoots and subsequently plantlets production from nodal segments of this plant.

MATERIALS AND METHODS

Plant material:

Young healthy shoots of *Aloysia triphylla* were collected from an adult plant growing in the farm of Medicinal and Aromatic plant, Faculty of Agriculture, Mansoura University. After removing the leaves, the shoots were cut into shoot tips and nodal segments with 3 auxiliary buds (about 1 – 1.5 cm long). Explants were thoroughly washed with tap water containing a few amount of household detergent for one hour. The surface sterilization was done with sodium hypochlorite at 3 % for 8 and 10 min for shoot tips and nodal segments, respectively and finally washed four times with sterile distilled water for 3 min each.

Media and culture conditions:

Murashig and Skoog medium (1962) nutrient medium was used in all experiments supplemented with 3% (w/v) sucrose. The medium was solidified with 7g agar /l (w/v) and the pH of the medium was adjusted to 5.8 before autoclaving at 121° C for 20 min. All the cultured jars (250 ml) contained 30 ml of medium were incubated in plant growth room at 25 ± 2°C under constant fluorescent light of 2500 Lux for 16/8 h (light/ dark) photoperiod.

Effect of explants type:

To study the effect of the two types of explant (shoot tips and nodal segments) on the production of multiple shoots, the surface sterilized shoot tips and nodal segments were cultured on Murashig and Skoog medium (MS) supplemented with 6-benzyl aminopurine (BAP) at 0.00, 0.05 and 0.10 mg/L. After 30 day of culture, data were recorded on shoot proliferation. A factorial experiment in a randomized complete block design was used with 4 replicates for each treatment.

Multiplicat of shoot cultures:

In vitro raised shoots were cut into pieces containing a single node along with 3 axillary buds and were cultured on MS medium supplemented with BAP (6-benzyl aminopurine), Kin (kinetin) or TDZ (Thidiazuron) at different concentrations (0.00, 0.10, 0.25, 0.50 and 1.00 mg/L) for each substance. A completely randomized design was used with 4 replicates for each treatment. Subsequently, subcultures were done at 30 day interval by transferring nodal segments explants to fresh medium without growth regulators or media containing the optimum concentration of BAP or Kin to study the effect of culture passages on the explants response for shoot induction and multiple shoot formation. A factorial experiment in a randomized complete block design was used with 4 replicates for each treatment.

Induction of rooting and acclimatization:

For root induction, individual *in vitro* raised micro-shoots (3 – 4 cm long) were excised from 4 weeks old shoot clusters and transferred to full strength MS basal medium supplemented with different auxin types, i.e., a-naphthalene acetic acid (NAA), indole-3-butyric acid (IBA) or indole-3-acetic acid (IAA) at different concentrations 0.0, 0.1, 0.25, 0.5, 1 and 2 mg/L in combination with active charcoal at 0.0 and 3.0 g/L. The *in vitro* rooted plants were taken out from the medium, washed under tap water to remove all

traces of media and then individual plants were transferred to plastic pots containing soil: sand: peatmoss (1: 1: 1). A factorial experiment in a randomized complete block design was used with 4 replicates for each treatment.

Statistical analysis:

Data of all experiments were subjected to analysis of variance (ANOVA) by the general linear models (GLMs) procedure using (SAS) Statistical Analysis System (2000). Mean comparisons were performed using the least significant difference (LSD) method according to (Gomez and Gomez, 1984). A significance level of 5 % was used for all statistical analyses.

RESULTS AND DISCUSSION

Multiplication stage:

Effect of explants type and BAP concentrations:

Concerning the effect of explant types, the obtained results in Table (1) indicated that culturing nodal segments of lemon verbena on MS basal medium supplemented with BAP at different concentrations (0.00, 0.05 and 0.10 mg/L) recorded the highest values of all studied characters (i.e., responded explant %, shoots number/explant, shoot length and leaves number/shoot) as compared to shoot tips

Table (1): Effect of explant type on the development of lemon verbena explants after 4 weeks of culture.

Treatments		Responded explants %	Shoots Number/ explant	Shoot Length (cm)	Leaves Number/ shoot
Explant type	BAP conc. mg/L				
Shoot tip	0.00	25.0	1.00	2.58	8.88
	0.05	41.7	1.25	2.25	7.33
	0.10	58.3	1.58	2.00	7.00
Nodal segment	0.00	66.7	1.63	3.88	11.92
	0.05	66.7	1. 75	3.63	11.50
	0.10	75.0	2.38	3.56	11.37
L.S.D. at 0.05		30.88	0.51	0.63	1.73

Concerning the effect of BAP concentration, it was observed that increasing BAP concentration from 0.00 mg/L up to 0.10 mg/L increased the values of responded explant percentage and shoots number for both of the two explants type. However, the highest values of response percentage (75 %) and shoots number/ explant (2.38 shoots) were obtained with nodal segments and BAP at 0.10 mg/L. Also, culturing nodal segments on MS medium supplemented with BAP at 0.10 m g/L recorded the highest values of leaves number per shoot (11.37 leaves) with average shoot length of 3.56 cm as compared to 7.00 leaves and 2.00 cm shoot length for shoot tips. Hence, it was proven that using nodal segments is more efficient than shoot tips for lemon verbena shoots regeneration.

This result may be due to the apical dominance in the shoot tip explants which decreases number of the grown auxiliary or adventitious buds. This finding agreed with the obtained results of Burdyn *et al*. (2006) on

Alloysia polystachya and Cecilia *et al*. (2006) on *Alloysia citriodora* who stated that nodal segment explants were found to be better than leaf or shoot tip explants in shoots regeneration on MS basal medium supplemented with different concentrations of BAP.

Effect of cytokinin types on nodal segments of lemon verbena after 4 weeks of culture:

Because of the superior response of the nodal segment explants compared to the apical shoot tips, only the former were used in subsequent experiments. In general shoot proliferation in cultures of nodal explants was a function of cytokinin activity as shown in Table (2).

Of the three cytokinins tested, BAP was most effective in inducing bud break and multiple shoot formation. One should be aware of the fact that, although a given cytokinin may not work well certain species; it may be quite effective in others. In the herein study, there was a correlation between the increasing in BAP concentration up to the optimal level of 0.5 mg/L and shoot development percentage (responded explants %) and number of shoots per explants. MS medium containing 0.5 mg/L BAP induced bud break in 100 % of the nodal explants. The explants cultured on this medium gave the highest significant number of new shoots (3.42 shoots/ explant) attained average shoot length of 3.92 cm and possessed 11.67 leaves per shoot. The more efficiency of BAP in induction of multiple shoot formation than Kin and TDZ has been identified in several medicinal plants (Hiregaudal *et al.* 2006; Usha *et al.* 2007; Kozomara *et al.* 2008 and Nassem and Mohammed 2010).

Table (2): Effect of cytokinin types at different concentrations on nodal segments of lemon verbena after 4 weeks of culture.

Treatment		Measurement				
Growth regulator	Conc. (mg/l)	Responded explants %	Shoots number/ explant	Shoot Length (cm)	Leaves number/ shoot	Callus formation
Control		75.0	1.83	3.96	11.04	50.0
BA	0.1	75.0	2.46	3.27	10.27	58.3
	0.25	83.3	2.58	3.69	10.71	83.3
	0.5	100	3.42	3.92	11.67	91.7
	1.0	91.7	2.33	3.71	11.42	58.3
Kin	0.1	100	2.75	4.40	13.00	41.7
	0.25	83.3	1.96	3.73	11.62	66.7
	0.5	83.3	2.17	3.65	11.92	58.3
	1.0	66.7	2.25	3.04	10.50	33.3
TDZ	0.1	58.3	2.00	2.94	10.21	50.0
	0.25	50.0	1.50	2.50	9.62	25.0
	0.5	33.3	1.00	2.50	10.50	8.3
	1.0	25.0	1.00	1.88	9.00	0.0
L.S.D. at 0.05		30.30	0.51	1.00	1.70	30.77

The response percentage and multiple shoot induction declined with the increase in BAP concentration beyond the optimal level (0.5 mg/L) and also a reduction in number of multiple shoots per explant (2.33 shoots/ explant) was attained at higher concentration of BAP (1.0 mg/L). Reduction in the number of shoots per explant obtained at BAP concentration higher than

the optimal level was also reported in many studies (Burdyn *et al.* 2006; Balaraju *et al.* 2008). Concerning the effect of Kin, it was noticed that MS containing 0.1 mg/L Kin induced bud break in 100 % of the nodal segments explants but with less significant number of multiple shoots per explant (2.75 shoots/ explant) compared to BAP.

TDZ at 0.1 - 1.0 mg/L was found to be the least effective of all tested cytokinins. Although multiple shoot were induced (58.3 %) on MS containing TDZ at 0.1 mg/L, but these shoots failed to elongate and were often fascinated at the higher concentrations. The formation of stunted shoots or fasciations of shoots on TDZ supplemented medium has been reported in several studies (Shoo and Chand 1998; Gabr 2004). The inhibition of shoot elongation may be due to the high cytokinin activity of TDZ where as the presence of a phenyl group in TDZ may be the possible cause of shoot bud fasciation (Huetteman and Preece 1993).

Effect of subculture number:

The effect of subculture number of nodal segments on improvement of multiplication rate of lemon verbena using two different cytokinin (i.e., BAP at 0.5 and Kin at 0.1 mg/L) in addition to the control (cytokinin free MS medium) was shown in Table (3). The results indicated that subculturing nodal segments on MS medium supplemented with 0.5 mg/L BA recorded the highest response percentage (100 %) till the fifth subculture as compared to the other two treatments (i.e., control and Kin at 0.1 mg/L) which recorded a reduction in response percentage after the second subculture.

There was a correlation between the increments in subculture number up to the fourth subculture and number of shoots per explants. MS containing 0.5 mg/L BAP recorded the highest number of multiple shoots per explants in the fourth subculture (11.5 shoots), while subsequent subculture (5^{th} subculture) had a lower rate (9.92 shoots/ explant). Also, there was a relationship between subculture number, shoot length and leaves number per shoot, since every increase in number of subculture was followed by an increase in shoot length and leaves number per shoot for all treatments. The maximum shoot length of 10.53 cm and leaves number per shoot of 24.22 leaves were obtained in the fifth subculture on MS medium supplemented with Kin at 0.1 mg/L.

Application of the cytokinin (BAP and Kin) to the subculture medium clearly induced callus formation percentage in all subculture, but BAP was more effective. The lowest callus formation percentage was obtained with the control treatment (cytokinin free MS medium) in the fifth subculture.

As clear in the obtained results, subcultures number significantly increased the multiplication rate. This may be due to that nodal segments which formed from the previous subculture included a little amount of BAP within its tissue that make them partly grow and develop till they uptake the proper amount of BAP from the fresh medium. Therefore, the multiplication rate gradually increases in the following subculture.

These obtained results are in agreements with the finding of Sumita and Satyesh (2001), Gisele and Thomas (2005), Nisha and Nair (2006) and Iyyakkannu *et al.* (2011) who concluded that the percentage of shoot development with number of of shoots per explants increased with increasing

of subculture number (the best subculture differed from one plant to another) and then there was a gradual decline.

Table (3): Effect of subculture number and cytokinin types on nodal segments of lemon verbena.

Treatment	Measurement				
Subcultures number / Growth regulator Conc. (mg/l)	Response %	Shoots number/ explant	Shoot Length (cm)	Leaves number/ shoot	Callus formation %
1st subculture					
Control	83.3	2.17	4.02	11.62	50.0
BA: 0.5	100	4.67	3.04	10.62	83.3
Kin: 0.1	100	3.42	4.29	12.35	58.3
2nd subculture					
Control	83.3	2.79	5.13	14.54	75.0
BA: 0.5	100	6.42	3.85	11.68	100
Kin: 0.1	91.7	4.21	4.75	14.15	83.3
3rd subculture					
Control	75.0	2.67	7.28	17.04	75.0
BA: 0.5	100	8.83	4.49	14.86	100
Kin: 0.1	83.3	4.92	5.75	16.05	91.7
4th subculture					
Control	66.7	2.50	7.95	17.60	75.0
BA: 0.5	100	11.50	5.59	16.36	100
Kin: 0.1	83.3	5.42	8.10	19.65	83.3
5th subculture					
Control	66.7	2.08	9.31	21.17	66.7
BA: 0.5	100	9.92	6.42	17.29	100
Kin: 0.1	75.0	4.63	10.53	24.22	75.0
L.S.D. at 0.05	33.38	0.795	0.748	1.40	35.03

Rooting of shoots:

Effect of auxin type, auxin concentration, active charcoal and their interactions on the rooting behavior of lemon verbena shoots after 4 weeks of culture:

This experiment was conducted to test the effect of different auxins (i.e., IAA, IBA and NAA) at various concentrations 0.00, 0.10, 0.25, 0.50, 1.0 and 2.0 mg/L and active charcoal at two concentrations (0.0 and 3.0 g/L) as well as their interactions on rooting percentage, roots number, root length and callus induction percentage of lemon verbena shoots.The results were recorded after 4 weeks of culture on MS medium and are shown in Tables (4, 5, 6 and 7).

Effect of auxin type (A) on rooting behavior:

Concerning the effect of auxin type on rooting percentage, roots number and length, data in Tables (4, 5 and 6) clearly indicated that IBA gave the highest rooting percentage (70.14 %), roots number per shoot (3.1 roots) along with the highest root length of 6.1 cm followed by IAA which recorded 57.64 % of rooting and 2.61 roots/ shoot with average root length of 3.46 cm. The lowest treatment in this regard was NAA.

The effect of auxin type on callus formation was also very clear as shown in Table (7), data obviously revealed that NAA was very effective in this concern and recorded the highest callus percentage of 66.7 % followed by IBA with 36.8 %.

Effect of auxin concentration (B) on rooting behavior:

Regarding the effect of auxin concentration on rooting percentage as shown in Table (4), the obtained results showed a positive relationship between auxin concentrations and rooting percentage, it was noticed that every increase in auxin concentrations from 0.10 mg/L up to 1.0 mg/L gradually and significantly increased rooting percentage. The highest recorded percentage (86.11 %) was obtained with auxin at 1.0 mg/L and increasing auxin concentration to 2mg/L significantly reduced rooting percentage to 70.83 %

Table (4): Effect of auxin type, auxin concentration, activated charcoal and their interactions on rooting percentage (%) of lemon verbena shoots after 4 weeks.

Auxin type (A)	Auxin conc. mg/L (B)	ACh conc. g/L (C)		Mean of (A)	Mean of (B)	Mean of (A× B)
		0.0	3.00			
NAA	0.00	0.00	0.00	52.08	0.00	0.00
	0.10	58.33	33.33			45.83
	0.25	66.67	41.67			54.17
	0.50	75.00	66.67		50.00	70.83
	1.00	83.33	75.00			79.17
	2.00	66.67	58.33			62.50
Mean of (A× C)		58.33	45.83			
IBA	0.00	0.00	0.00	70.14	72.22	0.00
	0.10	75.00	66.67			70.83
	0.25	91.67	83.33			87.50
	0.50	91.67	91.67		80.56	91.67
	1.00	91.67	91.67			91.67
	2.00	83.33	75.00			79.17
Mean of (A× C)		72.22	68.06			
IAA	0.00	0.00	0.00	57.64	86.11	0.00
	0.10	66.67	0.00			33.33
	0.25	83.33	66.67			75.00
	0.50	83.33	75.00		70.83	79.17
	1.00	91.67	83.33			87.50
	2.00	75.00	66.67			70.83
Mean of (A× C)		66.67	48.61			
Mean of (C)		65.74	54.17	LSD at 5%		
Mean of (B×C)	0.00	0.00	0.00	A 3.58	A×B 16.02	
	0.10	66.67	33.33	B 11.50	A×C 5.17	
	0.25	80.56	63.89	C 5.03	B×C 13.22	
	0.50	83.33	77.78	A×B×C 19.61		
	1.00	88.89	83.33			
	2.00	75.00	66.67			

Concerning the effect of auxin concentration on roots number per shoot and root length, the highest value of roots number (4.84 roots/ shoot) was obtained when MS medium was supplemented with auxin at 0.5 mg/L, while the highest value of the root length (7.6 cm) was recorded with auxin at 1.0 mg/L (Tables 5 and 6). It was a matter of importance to notice that MS medium free of auxin (control) failed to induce rooting.

As for callus induction, data presented in Table (7) revealed that callus formation percentage was significantly increased with increasing auxin

concentration, as the highest percentage (72.2 %) was recorded with the highest auxin concentration (2 mg/L). Also, no callus formation was detected with control (auxin free medium).

Table (5): Effect of auxin type, auxin concentration, activated charcoal and their interactions on roots number of lemon verbena shoots after 4 weeks.

Auxin type (A)	Auxin conc. mg/L (B)	ACh conc. g/L (C)		Mean of (A)	Mean of (B)	Mean of (A× B)
		0.0	3.00			
NAA	0.00	0.00	0.00	2.01	0.00	0.00
	0.10	1.25	1.00			1.13
	0.25	2.38	1.25			1.81
	0.50	4.63	3.31		1.32	3.97
	1.00	3.50	2.19			2.84
	2.00	2.75	1.81			2.28
Mean of (A× C)		2.417	1.59			
IBA	0.00	0.00	0.00	3.10	2.71	0.00
	0.10	2.88	1.31			2.09
	0.25	4.13	3.13			3.63
	0.50	6.25	4.94		4.84	5.59
	1.00	4.88	3.75			4.31
	2.00	3.75	2.25			3.00
Mean of (A× C)		3.65	2.56			
IAA	0.00	0.00	0.00	2.61	3.67	0.00
	0.10	1.50	0.00			0.75
	0.25	3.36	2.00			2.69
	0.50	5.81	4.13		2.89	4.97
	1.00	4.25	3.46			3.85
	2.00	3.63	3.17			3.40
Mean of (A× C)		3.09	2.13			
Mean of (C)		3.05	2.09	LSD at 5%		
Mean of (B×C)	0.00	0.00	0.00	A 0.169	A×B 0.279	
	0.10	1.88	0.77	B 0.132	A×C 0.190	
	0.25	3.29	2.13	C 0.062	B×C 0.200	
	0.50	5.56	4.13	A×B×C 0.381		
	1.00	4.21	3.13			
	2.00	3.38	2.41			

Effect of active charcoal (C) on rooting behavior:

As for the effect of active charcoal, data in Tables (4, 5, 6 and 7) indicated that adding active charcoal to MS medium significantly reduced the values of all studied characters (i.e., rooting percentage, roots number per shoot and root length and callus formation percentage) as compared to MS media without active charcoal.

Effect of the interaction between auxin type and auxin concentration (A×B) on rooting behavior:

As shown in Tables (4, 5 and 6), the interaction between auxin type and auxin concentration showed a highly significant differences in all cases. The best interaction effect on rooting percentage, roots number per shoot and root length was achieved when the MS medium was supplemented with IBA at 0.5 mg/L.

In regard to callus induction percentage as illustrated in Table (7), it was found that NAA at 2 mg/L recorded the highest significant value of 91.7 %.

Table (6): Effect of auxin type, auxin concentration, activated charcoal and their interactions on root length (cm) of lemon verbena shoots after 4 weeks.

Auxin type (A)	Auxin conc. mg/L (B)	ACh conc. g/L (C)		Mean of (A)	Mean of (B)	Mean of (A× B)
		0.0	3.00			
NAA	0.00	0.00	0.00	3.408	0.00	0.00
	0.10	3.81	2.25			3.03
	0.25	4.44	2.46			3.45
	0.50	4.94	3.38		2.90	4.16
	1.00	5.31	3.56			4.44
	2.00	5.88	4.88			5.38
Mean of (A× C)		4.063	2.753			
IBA	0.00	0.00	0.00	6.092	4.59	0.00
	0.10	4.88	3.31			4.09
	0.25	7.38	6.19			6.78
	0.50	9.25	7.94		5.80	8.59
	1.00	12.13	9.29			10.71
	2.00	6.81	5.94			6.38
Mean of (A× C)		6.74	5.44			
IAA	0.00	0.00	0.00	3.76	7.60	0.00
	0.10	3.17	0.00			1.58
	0.25	4.71	2.38			3.54
	0.50	5.85	3.44		5.63	4.65
	1.00	8.56	6.75			7.66
	2.00	6.17	4.13			5.15
Mean of (A× C)		4.74	2.78			
Mean of (C)		5.18	3.66	LSD at 5%		
Mean of (B×C)	0.00	0.00	0.00	A 0.093	A×B 0.300	
	0.10	3.95	1.85	B 0.245	A×C 0.161	
	0.25	5.51	3.67	C 0.138	B×C 0.288	
	0.50	6.68	4.92	A×B×C 0.414		
	1.00	8.67	6.54			
	2.00	6.29	4.98			

Effect of the interaction between auxin type and active charcoal (A×C) on rooting behavior:

Concerning the interaction between auxin type and active charcoal concentrations, the obtained results in Tables (4, 5 and 6) showed that the highest significant values of rooting percentage (72.22 %), roots number (3.65 roots/ shoot) and root length (6.74 cm) were recorded with IBA containing medium without active charcoal. On the other hand, the weakest interaction effect was recorded when shoots were cultured on MS containing NAA and active charcoal, since they were 45.83 %, 1.59 roots and 2.75 cm, respectively.

It appeared from data in the Table (7) that the highest percentage of callus formation (79.2 %) was recorded with NAA containing medium without active charcoal. Also, culturing the shoots on the MS medium containing IAA and active charcoal produced precisely the lowest values of callus formation percentage (16.7 %) when compared with the other treatments.

Table (7): Effect of auxin type, auxin concentration, activated charcoal and their interactions on callus induction (%) of lemon verbena shoots after 4 weeks.

Auxin type (A)	Auxin conc. mg/L (B)	ACh conc. g/L (C)		Mean of (A)	Mean of (B)	Mean of (A× B)
		0.0	**3.00**			
NAA	**0.00**	0.00	0.00	**66.7**	**0.00**	**0.00**
	0.10	83.3	41.7			**62.5**
	0.25	91.7	58.3			**75.0**
	0.50	100.0	66.7		**29.2**	**83.3**
	1.00	100.0	75.0			**87.5**
	2.00	100.0	83.3			**91.7**
Mean of (A× C)		**79.2**	**54.2**			
IBA	**0.00**	0.00	0.00	**36.8**	**40.3**	**0.00**
	0.10	25.0	0.0			**12.5**
	0.25	33.3	25.0			**29.2**
	0.50	58.3	33.3		**54.2**	**45.8**
	1.00	66.7	50.0			**58.3**
	2.00	83.3	66.7			**75.0**
Mean of (A× C)		**44.4**	**29.2**			
IAA	**0.00**	0.0	0.0	**25.7**	**62.5**	**0.00**
	0.10	25.0	0.0			**12.5**
	0.25	33.3	0.0			**16.7**
	0.50	41.7	25.0		**72.2**	**33.3**
	1.00	50.0	33.3			**41.7**
	2.00	58.3	41.7			**50.0**
Mean of (A× C)		**34.7**	**16.7**			
Mean of (C)		**52.8**	**33.3**	**LSD at 5%**		
Mean of (B×C)	**0.00**	**0.00**	**0.00**	**A** 5.25	**A×B** 13.61	
	0.10	**44.4**	**13.9**	**B** 10.10	**A×C** 5.80	
	0.25	**52.8**	**27.8**	**C** 2.95	**B×C** 10.60	
	0.50	**66.7**	**41.7**	**A×B×C** 16.98		
	1.00	**72.2**	**52.8**			
	2.00	**80.6**	**63.9**			

Effect of the interaction between auxin type and active charcoal (B×C) on rooting behavior:

Regarding the interaction between the auxin type and active charcoal, data in Tables (4, 5, 6 and 7) showed that the highest records of rooting percentage, roots number per shoot and root length were recorded with MS medium active charcoal free and the two higher concentration of 0.5 or 1.0 mg/L, while the highest percentage of callus formation (80.6 %) was recorded with the same medium and auxin at 2.0 mg/L.

Effect of the interaction between auxin type, auxin concentration and active charcoal (A×B×C) on rooting behavior:

As for the interaction among auxin type, auxin concentration and active charcoal on rooting percentage, data presented in Table (4) showed that rooting percentage of 91.67 % was obtained when MS medium was supplemented with IBA at 0.25, 0.5 and 1.0 mg/L with or without active charcoal and the same rooting percentage was achieved with IAA at 1.0 mg/L without active charcoal.

Concerning the effect of this interaction on roots number per shoot and root length (Tables 5 and 6), it was found that highest values of roots number

per shoot (6.25 roots) and root length (12.13 cm) were recorded with MS media supplemented with IBA at 0.5 and 1.0 mg/L, respectively.

As for callus formation, data in Table (7) showed that NAA at the three higer concentrations, i.e., 0.5, 1.0 and 2.0 mg/L recorded the highest value (100 %) followed with IBA at 2 mg/L (83.3 %) on active charcoal free MS medium.

Results obtained clearly indicated that of the three tested auxins (i.e., NAA, IBA and IAA), IBA at 0.5 mg/L was found to be most effective in inducing roots. About 92 % of the excised shoots developed (six to seven roots/ shoot) with root length averaging 9.25 cm. Similar results on different plants were obtained by Gemma and Krystyna (2001), Sumita and Satyesh (2001), Balaraju *et al.* (2008), Frabetti *et al.* (2009) and Iyyakkannu *et al.* (2011) who indicated that of the three auxiun tested (NAA, IBA and IAA) at different concentrations on the rooting of *in vitro*, IBA was found to be the most effective in inducing roots percentage and number of roots per shoot.

Callus formation was also affected by application of auxins to the nutrient medium, since NAA gave the highest significant percentage of callus formation with poor rooting as compared to IBA and IAA. These results are in general agreement with Shoo and Chand (1998), Chandramu *et al.* (2003) and Gisele and Thomas (2005).

The reduction effect of active charcoal on the rooting parameter may be due to the role of AC on adsorbing auxin on its services as mentioned by Pierik (1987). Also, Krajnakova *et al.* (2009) cleared that active charcoal is a common supplemented in tissue culture media to darken the immediate media surroundings and adsorb inhibitory or toxic substances and PGRs.

After 30 day of root induction, regenerated plantlets with fully expanded leaflets and well-developed roots were washed several times with water to remove all adhering culture medium. The rooted plants were transferred to plastic cups containing soil, sand and peat moss (1: 1: 1). About 100 % plantlets were surviving one month after transfer.

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اكثار نبات اللويزا باستخدام زراعة الانسجة

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اجري هذا البحث بمعمل زراعة الانسجة بقسم الخضر و الزينة- كلية الزراعة جامعة المنصورة في الفترة من ٢٠٠٨ – ٢٠١٠. يهدف البحث للوصول الى بروتوكول لاكثار سريع علي نطاق واسع لنبات شجيري طبي و عطري و هو اللويزا بواسطة تكنيك زراعة الانسجة باستخدام القمة النامية او قطع ساقية برعمية صغيرة و كانت اهم النتائج ما يلي:

استخدام القطع العقدية الساقية كان اكثر كفاءة عن القمة النامية علي بيئة موراشيج و سكوج و التي تحتوي علي ٠,١ مللجم / لتر من البنزيل امينوبيورين. وقد ادي استخدام البنزيل ادينين بتركيز ٠,٥ مللجم/ لتر الي الحصول علي اعلي النتائج بالمقارنة بالكينتين و الثيديازرون كماتزايد معدل تضاعف الافرع في العدد و الطول مع زيادة عدد مرات الزراعة و كان اعلي معدل للتضاعف (١٠٠%) و اعلي عدد للافرع (١١,٥) عند اعادة الزراعة للمرة الرابعة ثم تناقص بعد ذلك .

أما بالنسبة للتجذير كانت اعلي ما يمكن باستخدام بيئة موراشيج و سكوج مزودة باندول حمض البيوتيرك بتركيز ٠,٥ مللجم/لتر. وقد تم اقلمة النبيتات بزراعتها علي بيئة مكونة من التربة و البيتموس و الرمل بنسبة ١:١:١ و قد ادت الي نجاح الاقلمة بنسبة ١٠٠% قبل نقل النباتات لزراعتها خارج المعمل.

قام بتحكيم البحث

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