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IMPROVING AN *IN VITRO* PROPAGATION PROTOCOL FOR *Cestrum nocturnum* L.

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A b s t r a c t

The present study was carried out to assess the micropropagation of *Cestrum* (*Cestrum nocturnum* L.) by using single nodes and shoot tips excised from soft cuttings using MS salts, 30 g × l^{-1} sucrose, 7 g × l^{-1} agar, and different concentrations of plant growth regulators in culture medium. The results revealed that the use of mercuric chloride (0.05%, $HgCl_2$) for 7 minutes was very effective in preventing contamination and gave the highest survival percentage (99%). The highest response (100%) was gained at initiation stage from lateral bud explants on MS medium supplemented with 1.5 mg × l^{-1} of BA with most of NAA concentrations. However, in case of terminal buds, higher percentages of responses were resulted from the interaction of BA (1.5 mg × l^{-1}) with 0.2 mg × l^{-1} NAA. The lateral buds also produced more new shoots as well as a higher number of leaves and length of new shoots on the medium supplemented with 1.5 mg × l^{-1} BA as compared with those from terminal buds. Significant differences were observed at multiplication stage between the lateral buds and terminal buds, since the lateral buds produced a higher number of new shoots and leaves as well as longer new shoots. At rooting stage, the treatment with 1 mg × l^{-1} IBA gave the highest percentage of rooting (100%), the highest number of roots (13.2 root/explant), and the longest roots (8.44 cm), respectively, on half strength MS medium. Plantlets obtained were transferred to pots and acclimatized with 90% success.

Key words: Tissue culture, *Cestrum nocturnum,* BA, NAA.

INTRODUCTION

Cestrum nocturnum is a member of the family Solanaceae. Among *Cestrum* species, there are the following four: *C. aurantiacum*, *C. elegans (C. purpureum), C. parqui,* and *C. nocturnum*; all of them are evergreen shadow-loving shrubs and the fruit is poisonous (M a r u y a m a , 1995). *C. nocturnum* is a species of *Cestrum* that is most widespread in Iraq and it is commonly known under other names: Night blooming Cestrum, Lady of the Night, Queen of the Night, Night blooming Jessamine, and Night blooming Jasmine, because of its strongly scented flowers at night (S u l t a n a et al. 1992; W i k i p e d i a , 2006). It is widely naturalized in tropical and subtropical regions throughout the world, including Australia, Southern China and the Southern United States (M a r u y a m a , 1995). It is an evergreen shrub growing to 4 m tall. The leaves are simple, narrow lanceolate, 6.20 cm long and 2–4.5 cm broad, smooth and glossy, with an entire margin. Inflorescences drooping, many-flowered, axillary or terminal racemose panicles 7–10 cm. The flowers are greenish-white, with a slender tubular corolla 2–2.5 cm long with five acute lobes, 10–13 mm diameter when open at night; they are produced in cymosely inflorescences and are strongly scented (S u l t a n a et al. 1992; S h u S h u , 1994).

Tissue culture has found application in a number of areas of plant science, including basic physiology, production of natural and pharmaceutical compounds, plant pathology, germplasm preservation, breeding, recovery of transgenic plants, and propagation (H a r t m a n n et al. 2002). R o y et al. (2004) reported that by planting shoot tips and single nodes of *Rose* sp. on MS medium supplemented with 1.5 mg × l^{-1} BA a high number of shoots was obtained from single nodes (about 8 shoots/explant) compared with shoot tips (about 5 shoots/explant). N o d o y e et al. (2003) found that the best multiplication was obtained while culturing shoots (apical apices) at initiation stage for *Balanites aeggyptiaca* on MS medium supplemented with 2.5 mg × l^{-1} BA and 0.1 mg × l^{-1} NAA (about 3.13 shoots/explant). M u n s h i et al. (2004) recorded that *in vitro* propagation of *Ficus benghalensis* L. produced a high number of shoots from nodes cultured on MS medium supplemented with 1 mg × l^{-1} BA and 0.1 mg × l^{-1} NAA (about 5.7 shoots/explant). S a l a h a d d i n

et al. (2005) found that the best shoot rooting of *Peltophorm pterocarpum* was on MS medium supplemented with different concentrations of IBA (0.05, 0.1, 0.2, 1, 2 mg × l^{-1}), by obtaining the highest rooting percentage (91.66%) and the highest average length of roots (4.62 cm) in MS medium supplemented with 1 mg × l^{-1} IBA after 16 days of culture. Abdullah *et al.* (2003) revealed that the best shoot rooting of *Gardenia jasminoides* was on half strength MS medium supplemented with different concentrations of IAA. The best rooting was obtained while using IAA 1 mg × l^{-1} (98.33%). Hossain et al. (2003) recorded that the best rooting of *Z. jujube* was recorded on half strength MS medium supplemented with different concentrations of IAA.

The objectives of this study were to evaluate the effects of explant type, MS salt strength as well as different types and concentrations of cytokinins and auxins on *in vitro* propagation.

MATERIALS AND METHODS

Actively growing shoots, 10–20 cm long, were cut from 1-year-old *Cestrum nocturnum* grown in the greenhouse of the Department of Horticulture, Faculty of Agriculture and Forestry, University of Duhok.

Shoots were defoliated and washed with water for 60 minutes to remove soil and other superficial contamination, followed by tap water and liquid soap for 20 minuets, followed by three – five minute rinses in sterile distilled water. Then, they were cut into shorter sections 1.5 cm long, including the [terminal (apical) bud] and single nodes with an axillary bud (Tisserat and Zaid, 1983; Mohammed and Omer, 1990; Olivares et al. 1990). Shoot tips and nodes with axillary buds were removed and disinfected by immersion in the solutions of the Mercuric Chloride ($HgCl_2$), (0.05%) w/v for 7 minutes. The disinfested tissues [explants] were rinsed 3–4 times with sterilized distilled water, and the ends of explants exposed to sterilant were trimmed. The experiments were conducted with ten replicates and the explants were placed aseptically in 25×150 mm test tubes containing 15 ml of MS medium supplemented with different concentrations of growth regulators.

Benzyladenine (BA) with 0, 1.5, 3 and 4.5 mg × l^{-1} was added to the culture medium to observe the response of cultured explants at the initiation stage. Ten explants were cultured (an explant in each test tube for each concentration). They were incubated at 25±2 °C under light conditions of 16 light hours and 8 darkness hours. The results were recorded after 4–6 weeks from planting.

Different concentrations of BA and NAA were tested to find out their effect on culture initiation when combined together. BA was used at 0, 1.5, 3 and 4.5 mg × l^{-1} and NAA at 0, 0.2, 0.4 and 0.6 mg × l^{-1}. Ten test tubes were used for each treatment. On the basis of stage I results, the produced microshoots from the treatments were moved to MS medium (multiplication stage medium) from the best treatment. Number and length of shoots were recorded after 6 weeks from planting. Multiplication stage experiments included the effect of BA.

BA was tested at 0, 1.5, 3 and 4.5 mg × l^{-1} to discover its effects on number and length of new shoots as well as the effect of the interaction between BA and NAA on multiplication stage. BA was added at 0, 1.5, 3 and 4.5 mg × l^{-1}, while NAA at 0, 0.1, 0.2 and 0.3 mg × l^{-1}. GA_3 was added to MS medium at 3 mg × l^{-1} to all treatments, including the control.

The effects of IBA, NAA and IAA added to the culture medium on microshoot rooting were studied by carrying out several separate experiments by adding IBA, NAA and IAA (0, 0.5, 1 and 2 mg × l^{-1}). All these treatments were examined in half strength MS medium. As far as the rooting stage is concerned, features such as number of microshoots, number of roots/shoots and root length (cm) were recorded. These evaluations were performed on a weekly basis for 4–6 consecutive weeks. At the end of six weeks, the results were compiled, averaged and expressed as a percentage or number for each treatment.

After 6–8 weeks from *Cestrum nocturnum* shoot rooting, several microplants were selected from those that showed good vegetative growth. They were washed under tap water to remove agar from the roots, which might be a source of contamination. They were then put in Benlate fungicide solution (0.1%) and planted in plastic pots filled with a sterilized mixture of peatmoss and river soil (1:1). In order to maintain high humidity in the culture environment, the pots were covered with a light plastic cover which permits light penetration and contains many openings to permit air entrance. Microplants were watered and given a solution containing MS salts with 0.25 of original strength. The plastic cover was removed from time to time after two weeks from planting. After four weeks, the microplants were transplanted after being sprayed with Benlate fungicide (0.1%) as required.

Statistical Analysis: The experiments were carried out using Complete Randomized Design (CRD). Significant differences between mean values were separated by using Duncan's multiple range tests at $P \leq 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

Initiation stage: Effects of BA and NAA concentrations on explant establishment

Figure (1) displays the effect of different concentrations of BA, NAA and their interactions on the

percentage of response of lateral and terminal buds excised from soft cuttings of *Cestrum* cultured on MS medium. For lateral buds, it can be noticed that the concentration of BA (1.5 mg × l^{-1}) was significantly superior over the other concentrations for both lateral and terminal buds and gave the highest response percentage (100%) (Fig. 1, a). As regards the interaction between BA and NAA concentrations, this figure shows that in case of lateral buds the treatment of 1.5 mg × l^{-1}BA with most of NAA concentrations gave the highest response (100%). However, in case of terminal buds, higher percentages of responses resulted from the treatment of 1.5 mgl^{-1} BA with 0, 0.2 mg × l^{-1} NAA concentration (Fig. 1, c).

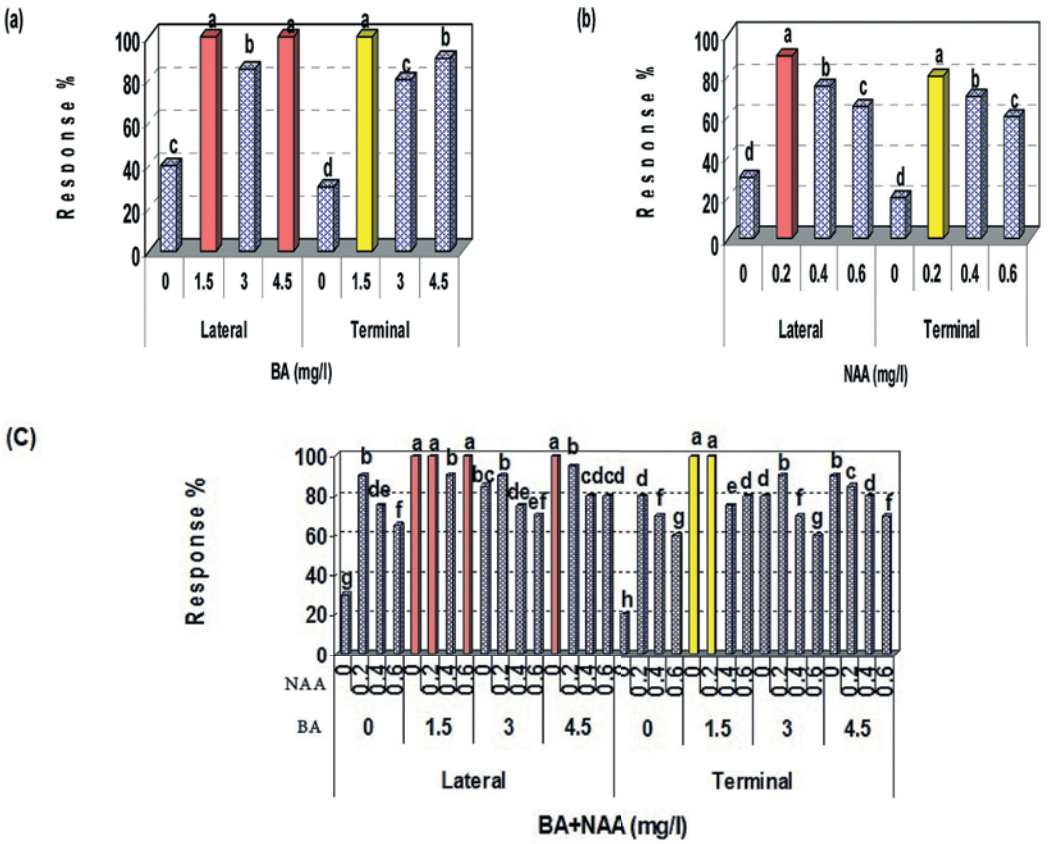

Fig. 1. The effects of different concentrations of BA (a), NAA (b) and their interaction (c) on the percentages of response of lateral and terminal buds at initiation stage.

Table (1) and Figure (2) show the effects of BA, NAA concentrations and their interactions as well as types of buds on the average number of shoots, leaves and the length of new shoots at initiation stage. It is clear that lateral buds produced more new shoots as well as a higher number of leaves and length of new shoots as compared with those from terminal buds. This may be due to cytokinin deficiency in the lateral buds (S t e r n et al. 2004). Using BA at 1.5 mg × l^{-1} resulted in obtaining the highest number of shoots and leaves as well as the highest shoot length in lateral and terminal buds (5.2, 4 shoots/explant, 19, 13.2 leaves/explant and 6.06, 5.54 cm; respectively). This indicates the necessity of cytokinin (BA) presence in initiation medium. This fact has been discussed in many published studies on tissue culture of many fruit trees like pear (H i r a b a y a s h i et al. 1987), plum (D r u a r t and G r u s e l l e , 1986), and walnut (P e n u e l a et al. 1987). As regards the effect of NAA, it is clear that the highest values of number of new shoots, number of leaves and length of new shoots were obtained at 0.2 mg × l^{-1} NAA for both lateral and terminal buds. Concerning the interaction between BA, NAA and types of buds, it is clear that the highest values of number of shoots, number of leaves and length of new shoots were obtained from the interaction between the low concentrations of both growth regulators for both lateral and terminal buds. The treatment resulted in a significant increase in the average number of new shoots, average number of leaves and average length of new shoots on lateral buds as compared with those from terminal buds. But the lowest number of new shoots, average number of leaves and average length of new shoots were produced from the treatment free of plant growth regulators (2.6, 2.2 shoots/explant,

7.2, 6.6 leaves/explant, and 4.78, 3.34 cm). These results are in agreement with what has been found by Pontikis and Sapoutzaki (1984), Pasqual and Audo (1989) and Singh et al. (1994); they found that using of cytokinins and auxins in this category is very important and the role of cytokinins at this stage is essential to break apical dominance in buds and to induce the subsidiary meristem grow into a shoot.

Table 1
The effects of BA, NAA concentrations and their interactions on the average number of new shoots, number of leaves and length of new shoots of lateral and terminal buds at initiation stage

Growth regulators mg × l^{-1}	Single Nodes (Lateral bud)			Shoot Tips (Terminal buds)		
	Average no. of new shoots	Average no. of leaves	Length of of new shoots (cm)	Average no. of new shoots	Average no. of leaves	Length of new shoots (cm)
			BA			
1.5	5.2	19	6.06	4	13.2	5.54
	a	a	ab	b-d	b-d	a-g
3	3.4	16.6	6	3.2	12	4.98
	c-g	ab	a-c	c-h	c-e	a-i
4.5	4.2	18.2	5.48	3	10.6	4.92
	bc	a	a-g	d-h	c-f	a-i
			NAA			
0.2	3.4	9.6	6.04	2.8	8	5.84
	c-g	d-f	ab	e-h	ef	a-e
0.4	3	8.6	5.18	2.4	7.8	4.12
	d-h	d-f	a-h	gh	ef	h-l
0.6	2.8	9	4.78	2.2	7.4	4.3
	e-h	d-f	b-j	h	ef	g-l
			BA+NAA			
1.5+0.2	4.6	18.2	6.16	3.8	14.6	5.62
	ab	a	a	b-e	a-c	a-f
1.5+0.4	3.2	10.2	4.6	2.8	8.6	3.8
	c-h	c-f	e-l	e-h	d-f	j-l
1.5+0.6	3.8	7.4	4.4	2.6	9	3.62
	b-e	ef	f-l	f-h	d-f	j-l
3+0.2	3.4	10.4	4.58	2.2	8.4	3.56
	c-g	c-f	e-l	h	d-f	j-l
3+0.4	3.2	10.2	4.06	2.4	9.2	3.42
	c-h	c-f	h-l	gh	d-f	kl
3+0.6	3	10	5.24	3.4	8.6	4.66
	d-h	c-f	a-h	c-g	d-f	d-k
4.5+0.2	4	11.8	5.94	3.2	11	5.08
	b-d	c-e	a-d	c-h	c-f	a-i
4.5+0.4	3.6	9.6	5.1	2.6	9.8	4.72
	c-f	d-f	a-h	f-h	c-f	c-j
4.5+0.6	3.6	11	5.84	3	10.6	5.42
	c-f	c-f	a-e	d-h	c-f	a-g
control	2.6	7.2	4.78	2.2	6.6	3.34
	f-h	ef	b-j	h	f	l
Effect of type of buds	3.5625	11.6875	5.265	2.8625	9.7125	4.55875
	a	a	a	b	b	b

* Means followed by the same letter within a column do not differ significantly (=0.05) according to Duncan's Multiple Range Test (Duncan, 1955).

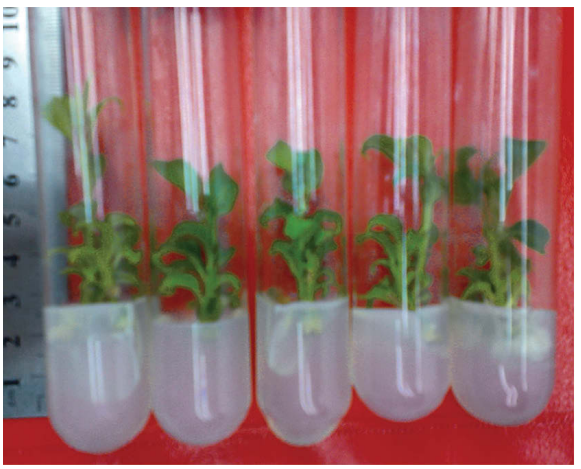

Fig. 2. Shoot initiation of *Cestrum nocturnum* on MS medium supplemented with BA+NAA at different concentrations after 4–6 weeks of culture.

Multiplication stage: Effects of BA and NAA on shoot proliferation

Table (2) and figure (3) reveals the effects of different concentrations of BA, NAA and their interactions and types of buds on the average number of shoots, average number of leaves and lengths of new shoots at multiplication stage. Significant differences were recorded between the lateral and terminals buds in which lateral buds produced higher numbers of new shoot, leaves and lengths of new shoots. It is thought that cytokinins promote the formation of woody tissues neighboring to the vascular tissues of the bud and stem, thus will make easy the translocation of water and nutrients which cause bud initiation (Mohammed and Al-Younis, 1991). It can be noticed that using low concentrations of BA (1.5 mg × l^{-1}) led to get the highest responses in number of shoots (4.4 and 4.2 shoots/explant), number of leaves (15.4 and 4.2 leaves/explant) and length of new shoots (5.36 and 4.86 cm) for lateral and terminal buds respectively. These results agree with those reported by Werner and Boe (1980); Hammerschlag (1982) and Brookner (1991) in their studies on the importance of cytokinins in shoot multiplication. Whereas the use of NAA (0.2 mg × l^{-1}) gave the highest values of number of new shoots (3.4, 3) shoots/explant, concerning the number of leaves and length of new shoots, the concentration of 0.1 mg × l^{-1} NAA was significantly superior upon the other concentrations for both lateral and terminal buds growth. This may be due to the effect of auxins on cell wall enlargement (Abdul, 1987). Regarding the interaction between BA, NAA and types of buds, it is clear that for average number of shoots, number of leaves and the length of new shoots for both lateral and terminal buds, the treatment of 1.5 mg × l^{-1} BA and 0.1 mg × l^{-1} of NAA resulted in higher values (5, 4.2 shoots/explant, 17.2, 13.4 leaves/ explant and 7.7, 5.44 cm respectively) as compared with control. The effect of interaction between cytokinins and auxins in vegetative multiplication and increasing growth lengths can be interpreted by the increase of cytokinins role in the presence of auxins as Mohammed and Al-Younis (1991) reported that movement of cytokinins is generally activated in the presence of auxins, so a larger number of buds will have a chance to grow and start to produce shoots (Tran Thanh ran, 1981 and Murashige, 1990). These results are in agreement with those reported by Roy et al. (2004); Nodoy et al. (2003) and Munshi et al. (2004), who emphasized the importance of the interaction between auxins and cytokinins in vegetative multiplication processes.

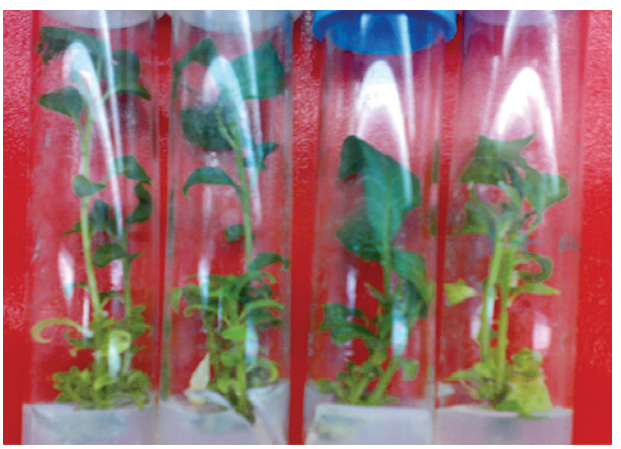

Fig. 3. Shoot multiplication of *Cestrum nocturnum* on MS medium supplemented with BA+NAA at different concentrations after 4–6 weeks of culture.

Table 2
The effect of BA, NAA concentrations and their interactions on the average number of new shoots, number of leaves and length of new shoots at multiplication stage. (3 mgl^{-1} of GA_3 were added to all the treatments).

Growth regulators mg × l^{-1}	Single Nodes (Lateral bud)			Shoot Tips (Terminal buds)		
	Average no. of new shoots	Average no. of leaves	Length of of new shoots (cm)	Average no. of new shoots	Average no. of leaves	Length of new shoots (cm)
			BA			
1.5	4.4	15.4	5.36	4.2	12.4	4.86
	a-c	ab	bc	a-d	b-d	c-e
3	4	11	5.16	3.2	10.8	4.72
	a-e	b-d	b-d	c-i	b-d	c-f
4.5	3.8	11.6	4.36	2.2	9	3.82
	b-f	b-d	c-h	h-j	cd	e-i
			NAA			
0.1	3.2	11.4	4.5	2.8	9.8	3.58
	c-i	b-d	c-g	e-j	cd	f-i
0.2	3.4	10	4.32	3	9.2	3.28
	b-h	c-d	c-h	d-j	cd	hi
0.3	2.8	13.2	4.06	2.4	8.8	2.9
	e-j	a-d	d-h	g-j	cd	i
			BA+NAA			
1.5+0.1	5	17.2	7.7	4.2	13.4	5.44
	a	a	a	a-d	a-c	bc
1.5+0.2	4.6	11.8	7.16	2.6	11	5.38
	ab	b-d	a	f-j	b-d	bc
1.5+0.3	3.8	11	6.08	2.4	9.8	3.66
	b-f	b-d	b	g-j	cd	f-i
3+0.1	3.8	12.2	4.52	3.6	10.8	4.1
	b-f	b-d	c-g	b-g	b-d	d-h
3+0.2	3.6	11	4.74	3.2	9.4	4.62
	b-g	b-d	c-f	c-i	cd	c-f
3+0.3	3.4	12	5.22	3	9.4	4.66
	b-h	b-d	b-d	d-j	cd	c-f
4.5+0.1	3	10.8	4.52	3	10	4.54
	d-j	b-d	c-g	d-j	cd	c-g
4.5+0.2	3	9.8	4.72	2.8	10.6	3.68
	d-j	cd	c-f	e-j	b-d	f-i
4.5+0.3	3.2	11.2	4.16	2.6	8.2	3.42
	c-i	b-d	d-h	f-j	d	g-i
control	2	8.4	3.5	1.8	4	3.34
	ij	cd	f-i	j	e	hi
Effect of type of buds	3.5625	11.75	5.01	2.9375	9.7875	4.125
	a	a	a	b	B	b

* Means followed by the same letter within a column do not differ significantly (=0.05) according to Duncan's Multiple Range Test (Duncan, 1955).

Rooting Stage: Microshoots were transferred from multiplication medium and placed in half strength MS macro- and microelements supplemented with different concentrations of IBA, NAA and IAA (0–2 mg × l^{-1}). The microshoots showed different responses to rooting after 4–6 weeks of culture (Figs 4 and 5). The highest percentage of rooting (100%) was obtained on half strength MS medium supplemented with 0.5, 1 mgl^{-1} NAA and IBA, respectively. On the other hand, in case of IAA, the highest rooting percentage of *Cestrum* shoots cultured in half strength MS (90%) were obtained at a concentration of 1mg × l^{-1} IAA.

Endogenous hormones might have a role in promoting plants to root (P e a k et al. 1987), until the hormonal balance reached its optimal level to push the roots to grow and develop in the presence of exogenous hormones, since increasing auxin concentration promotes root formation on shoot bases (G e o r g e and S h e r m i n g t o n , 1984).

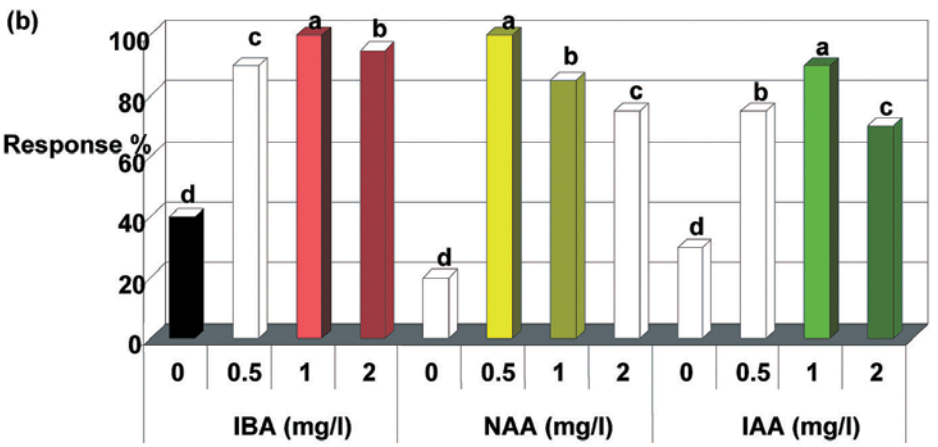

Fig.4. The effects of different concentrations of IBA, NAA and IAA on rooting percentages of *Cestrum* shoots cultured on half strength MS medium.

Table 3
The effect of IBA, NAA and IAA on root numbers and length in shoots planted in half strength MS medium

Auxin	Average no. of roots	Average length of roots (cm)	Auxin	Average no. of roots	Average length of roots (cm)	Auxin	Average no. of roots	Average length of roots (cm)
	IBA			NAA			IAA	
0	2.8	2.84	0	1.8	1.96	0	2.2	2.7
	d	c		c	c		b	b
0.5	7.8	6.9	0.5	7	5.46	0.5	3.4	3.08
	c	b		a	a		b	b
1	13.2	8.44	1	6.2	4.74	1	6.8	5.74
	a	a		a	ab		a	a
2	11.2	7.4	2	4.4	4.28	2	3	3
	b	b		b	b		b	b

Means followed by the same letter within a column do not differ significantly (=0.05) according to Duncan's Multiple Range Test (D u n c a n , 1955).

Table (3) shows the effect of IBA, NAA and IAA concentrations on the average number of roots per shoot and average root length. It can be noticed that IBA and IAA have a significant effect on root numbers per shoot and root length in half strength MS medium. At the concentration of 1 mgl^{-1}, IBA and IAA gave the highest number of roots (13.2, 6.8 roots/explant) and root length (8.44, 5.74 cm), respectively. Concerning the effect of NAA, it is clear that the highest values for root numbers per shoot (7 roots/explant) and root length (5.46 cm), respectively, were obtained at the concentration of 0.5 mgl^{-1} NAA in half strength MS medium.

These results prove that auxins have a role in the rooting process, since they promote adventitious root initiation in the bases of cultured shoots (A u d u s , 1959; A b d u l , 1987; S a l e h , 1990). These results are

in agreement with those found by Abdullah et al. (2003) and Hossain et al. (2003), Salahaddin et al. (2005), who observed that reducing the levels of MS salts in the medium to half increased rooting of many tree species. Decreasing the level of salts in the medium means decreasing the level of nitrogen in the medium to half or quarter; this will result in decreasing the nitrogen level in the shoots, which may cause the percentage of carbohydrates to be increased to the nitrogen level and this in turn may result in increasing the percentage of root primordia and root numbers (Gawel, 1990).

a b c

Fig. 5. Root initiation of *Cestrum nocturnum* on MS medium supplemented with (a) IBA, (b) NAA, and (c) IAA at different concentrations after 4–6 weeks of culture.

Acclimatization stage: (Figs 6, 7) The microplants of *Cestrum* were carefully removed from rooting media and transferred to the greenhouse in small plastic pots with medium consisting of peat moss and river soil (1:1). The plants were finally hardened by gradually reducing the humidity.

After four weeks from transplanting, the survival percentage reached 90% of plants. This protocol for vegetative micropropagation agrees with what has been found by many researches in the case of fruit plants that were moved to open air field like apples (Snir and Erez, 1980), peaches (Reeves et al. 1983), and chestnut (Preece and Sutter, 1991).

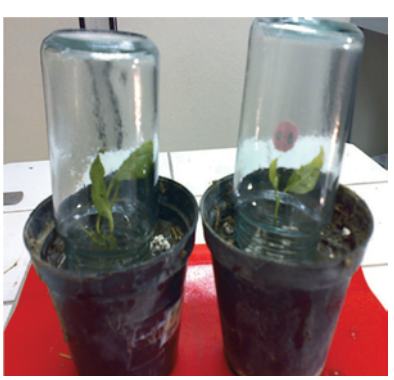

Fig. 6. Microplants established in pots after 6–8 weeks of transfer.

Fig. 7. Plant after 8–10 weeks ex vitro.

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Udoskonalenie metody rozmnażania *Cestrum nocturnum* L. w warunkach *in vitro*

Streszczenie

Badania przeprowadzono w celu oceny mikrorozmnażania *Cestrum* (*Cestrum nocturnum* L.) przy wykorzystaniu pojedynczych węzłów oraz wierzchołków pędów wyciętych z miękkich części roślin. Zastosowano sole MS, 30 g × l^{-1} sacharozy, 7 g × l^{-1} agaru oraz różne stężenia regulatorów wzrostu roślin w podłożu hodowlanym. Wyniki pokazały, że zastosowanie chlorku rtęci (0,05%, $HgCl_2$) przez 7 minut było bardzo efektywne w usuwaniu zanieczyszczeń oraz dało najwyższy procent przeżywalności (99%). Najwyższy procent reakcji (100%) uzyskano w fazie inicjacji eksplantatów przypadku eksplantatów z pąków bocznych na pożywce MS uzupełnionej 1,5 mgl^{-1} BA

i przy zastosowaniu większości stężeń NAA. Jednak w przypadku pąków wierzchołkowych wyższe procenty reakcji wynikały z interakcji BA (1.5 mg × l^{-1}) z 0,2 mg × l^{-1} NAA. Pąki boczne również wytworzyły więcej nowych pędów, większą liczbę liści oraz nowe pędy o większej długości na pożywce z dodatkiem 1,5 mg × l^{-1} BA w porównaniu z pąkami wierzchołkowymi. Istotne różnice pomiędzy pąkami bocznymi i pąkami wierzchołkowymi obserwowano w fazie namnażania, ponieważ pąki boczne wytworzyły większą liczbę nowych pędów i liści oraz dłuższe nowe pędy. W fazie ukorzeniania zastosowanie 1 mg × l^{-1} IBA dało najwyższy procent ukorzenienia (100%) oraz najwyższą liczbę korzeni na explantat (13,2) i najdłuższe korzenie (8,44 cm) na pożywce MS z zestawu soli. Uzyskane mikrosadzonki zostały przeniesione do doniczek i aklimatyzowano je z 90% skutecznością.