Micropropagation of *Hyoscyamus muticus* L. and *Datura metel* L. by Using Shoot Tips Explants

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Abstract: Shoot tips of *Hyoscyamus muticus* L and *Datura metel* L were excised and placed on B5 medium supplemented with BA at 0.0, 0.25 and 0.5 mgl^{-1} combined with NAA at 0.0, 0.125, 0.25 and 0.5 mgl^{-1}. After 4 weeks, the addition of 0.25 or 0.5 mgl^{-1} BA to B5 medium in the absence or presence of low concentration of NAA (0.125 mgl^{-1}) was the optimal for the production of shoots of *H. muticus*. The presence of NAA at 0.125 mgl^{-1} increased both shoot length and callus formation. Also, in *D. metel,* the addition of 0.25 mgl^{-1} BA and 0.125 mgl^{-1} NAA to B5 medium was optimal for increasing number of shoots. To study the effect of BA and medium type on shoot multiplication, shoot tips of *H. muticus* and *D. metel* were cultured on either MS or B5 media supplemented with different concentration of BA at 0.0, 0.05, 0.1, 0.2, 0.4 and 0.8 mgl^{-1}. After 4 weeks, the addition of 0.1 mgl^{-1} BA to either MS or B5 medium resulted in the highest number of *H. muticus* multiple shoots. However, in *D. metal,* the addition of 0.8 mgl^{-1} BA to MS medium was superior for increasing number of multiple shoots and formation of callus. In addition, using MS medium proved to be best choice because it produced quality shoots in both plants. After 24 days in culture, root induction was successfully occurred on MS medium supplemented with 1.0 mgl^{-1} IBA for *H. muticus* and 0.8 mgl^{-1} IBA for *D. metel.* Rooted *H. muticus* and *D. metel* plantlets were transferred to small plastic pots (6 cm) containing sterile mixture of sand: peat moss: vermiculite at ratio of 1:1:1 (by volume). The pots were kept in the greenhouse under fine mist of water for 3 weeks. The survival percentage reached 50 % and 80 % for *H. muticus* and *D. metel,* respectively, under the conditions of this experiment and the plants grown normally and well.

Keywords: *Hyoscyamus muticus – Datura metel*, Benzyl adenine, Murashing and Skoog medium, B5 medium, Naphthalene acetic acid, Indole butyric acid

INTRODUCTION

Hyoscyamus muticus L., (Egyptian henbane) is one of the most important medicinal plants belonging to family *Solanaceae* and represents an important commercial source of tropane alkaloids, where more than 20 alkaloid compounds were detected in intact plants of *H. muticus*. The main constituents are hyoscyamine and scopolamine (also known as hyoscine), Biondi *et al.* (2002).

Datura metal L (Thorn apple) belongs to the family *Solanaceae*. It is used economically as a main source of tropane alkaloids, particularly scopolamine and hyoscyamine (Loyola-Vargas, 1999 and Berkov *et al.* 2003). More than 30 alkaloids have been detected in *Datura* (Verzar-Petri and Bajaj, 1989)

The importance of *Datura* and *Hyoscyamus* plants for tropane alkaloid production decreased. By breeding for high tropane alkaloid – producing strains with interesting cultivation features, it should be possible to stimulate renewed interest in *Datura* and *Hyoscyamus* for tropane alkaloid production (Strauss, 1989).

Plant tissue culture is an alternative method of clonal propagation and is being used widely for the commercial propagation of a large number of uniform plant species, including many medicinal plants, while maintaining their genotypes. (Rout *et al.,* 2000 and Arikat *et al.* 2004). Micropropagation approaches are now routinely used to support clonal programmers. *In vitro* clonal propagation via meristem culture is one of the ways for producing large number of true-to-type healthy planting material. (Sanatombi and Sharma, 2007).

During the last few years, the interest in mass propagation of medicinal plants *in vitro* has distinctly increased. For various reasons many of these plants, when propagated by conventional methods, take a long time for multiplication, poor seed germination, and are often under protection of threatened with extinction. The alternative to this situation is the rapid *in vitro* multiplication.

Media type, growth regulators including cytokinins and auxins are probably the most important compounds for regulating growth and morphogenesis in plant organ culture. Maria *et al.,* (1990) studied the influence of balance between cytokinins and auxins on micropropagation of *D. insignis*, they placed single-node explants of 0.2-0.5 cm on MS medium supplemented with 2.4-D plus BA and IAA plus Kin and BA, they found that the best results were obtained in a medium supplemented with 1.0 mgl^{-1} of BA.

Muthukumar and Arockiasamy (2003) reported that the highest percentage of shoot regeneration of *D. metel* was obtained with MS medium supplemented with 2 mgl^{-1} BAP from hypocotyl explants. Also, Muthukumar *et al.,* (2004) on *D. metel* found that when the explants were cultured on MS medium containing BA at 3.0 mgl^{-1} and NAA at 0.5 mgl^{-1}, the nodal explants isolated from *in vivo* source exhibited a greater number of healthy multiple shoots.

The objective of this study is to develop a method for propagation of *Hyoscyamus* and *Datura* plants by using the tissue culture technique, as a recent and fast method to produce mass production of these plants.

MATERIALS AND METHODS

This study was carried out at the Tissue Culture Laboratory of the Horticulture Department, Faculty of

Agriculture, Suez Canal University during the years of 2005 to 2007.

Seeds of *Hyoscyamus muticus* L. (Egyptian henbane) and *Datura metel* L (Thorn-apple) were collected from wild plants grown in Ismailia. Seeds were rinsed in tap water for one hour, and surface sterilized by immersion in 70 % (v/v) ethanol for one minute, followed by soaking in 20% (v/v) Clorox (commercial bleach containing 5.25% sodium hypochlorite) supplemented with three drops of Tween-20 as wetting agent for 20 minutes. Seeds were then rinsed three times with sterile distilled water.

Sterilized seeds were germinated in culture tubes containing 10 ml of half strength B5 medium (Gamborg *et al.,* 1968) supplemented with 7.5 gl^{-1} agar and 20 gl^{-1} sucrose. Cultured were maintained in the light at 24±4 °C until germination occurred.

Effect of BA and medium type on shoot multiplication of *H. muticus* L. and *D. metel* L.

Five shoot tips of *H. muticus* and *D. metel* were cultured in jars (200 ml) containing either MS (Murashige and Skoog 1962) or B5 (Gamborg *et al.,* 1968) media supplemented with different concentrations of BA at 0.0, 0.05, 0.1, 0.2, 0.4 and 0.8 mgl^{-1}. All media containing 30 gl^{-1} sucrose and solidified with 7.5 gl^{-1} agar. Each treatment included twelve jars as replicates. After 4 weeks, number of proliferated shoots /shoot tip, shoot length (cm), number of leaves, and fresh weight of culture (callus and shoots) were recorded.

Effect of BA and NAA on shoot multiplication of *H. muticus* L. and *D. metel* L.

Five-shoot tips (meristem) of *H. muticus L.* and *D. metel* were cultured in jars (200 ml) containing 35 ml of B5 medium. The medium supplemented with 30 gl^{-1} sucrose and solidified with 7.5 gl^{-1} agar. The B5 medium supplemented with the combinations of BA at 0.0, 0.25 and 0.5 mgl^{-1} and NAA at 0.0, 0.125, 0.25, 0.5 mgl^{-1}. Each treatment included twelve jars as replicates. After 4 weeks, number of proliferated shoots / shoot tip, average shoot length (cm), number of leaves and fresh weight of culture (callus and shoots) were recorded.

Effect of IBA on *in vitro* rooting of *Hyoscyamus muticus L.* and *Datura metel L.*

The medium used for *in vitro* rooting of proliferated plants were MS medium supplemented with IBA at 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mgl^{-1} and 40 gl^{-1} sucrose and solidified with 7.5 gl^{-1} agar. Each treatment consisted of twelve replicates (jars) for both *Datura* and *Hyoscyamus*. After 24 days, rooting percentage, number of roots / plantlets, root length (cm), shoot length (cm) and fresh weight of plantlet (g) were recorded.

The pH of medium was adjusted to 5.7 before autoclaving in all experiments. Culture vessels were incubated in growth room at 25±1°C under a 16/8 photoperiod with light intensity of 1500 Lux.

For acclimatization, rooted shoots were carefully removed from the culture tubes, and washed with distilled water to remove residual agar around the roots. Shoots were immersed in fungicide (Rizolex) at 1% and the plantlets were planted in small plastic pots (6 cm) filled with growing medium consisted of a sterile mixture of sand: peat moss: vermiculite at ratio of 1:1:1(by volume). The pots placed under plastic tunnels where they irrigated with a fine mist of water for 3 weeks and transferred to grow under the greenhouse conditions. Survival percentage was determined after 4 week.

The design of the experiments was a complete randomized design. Data for each experiment subjected to analysis of variance (ANOVA) by the general linear models (GLMS) procedure using Statistical Analysis System (SPSS10) (Anonymous, 2005). Mean comparison was performed using Duncan's Multiple Range Test according to Snedecor and Cochran (1967). A significance level of 5% was used for all statistical analyses.

RESULTS AND DISCUSSION

Effects of BA and medium type on shoot multiplication of *H. muticus* L. and *D. metel* L.

Data in Table (1) show that incorporation of BA in the medium was essential for enhancing shoot multiplication. The B5 medium supplemented with 0.1 mgl^{-1} BA significantly produced the greatest number of multiple shoots (8.67 shoots/ plantlet) from shoot tips of *H. muticus,* followed by MS medium containing 0.1 mgl^{-1}BA which gave 7.58 shoots/ plantlet (Fig. 1-A).

On the other hand, in *D. metel,* the best multiplication rate was recorded form MS medium supplemented with BA at 0.8 mgl^{-1} (high level) which gave 8.25 shoots/ plantlet (Fig. 1-C), followed by MS medium containing 0.4 mgl^{-1} BA and B5 medium supplemented with 0.2 mgl^{-1} BA which gave 6.25 and 5.67 shoots/ plantlet, respectively.

Concerning shoot length of *H. muticus,* data presented in Table (1) indicated that using either MS or B5 medium supplemented with 0.05 and 0.1 mgl^{-1} BA or without BA produced the tallest shoots without significant differences between MS medium supplemented with 0.4 or 0.8 mgl^{-1} BA. These lengths ranged from 2.58 cm to 2.75 cm. The B5 medium supplemented with 0.8 mgl^{-1} BA produced the shortest shoot length as 1.73 cm.

Data presented in Table (1) illustrate that the MS medium supplemented with 0.8 mgl^{-1} BA produced the longest shoot (2.85 cm) of *D. metel,* with no significant differences than MS without BA. The B5 medium supplemented with 0.2 mgl^{-1} BA produced the shortest shoot length (1.79 cm). Arikat *et al.*, (2004) reported that shoot tips of *Salvia fruticosa* cultured on B5 medium produced shortest shoots 2 nodes with abnormal leaves as compared with MS medium.

As shown in Table (1), the highest number of leaves of *H. muticus* (34.5) was obtained with B5 medium supplemented with 0.1 mgl^{-1} BA. It was noticed that the increase in number of leaves was attributed to the increase in number of proliferated shoots. Whereas, in *D. metel* the highest number of leave (33) was obtained with MS medium supplemented with 0.8 mgl^{-1} BA.

Vitrification was observed with Egyptian henbane shoots on media containing B5 salt, where the stems were translucent and the leaves were thickened, turgid and brittle (Fig. 1-B).

Data of Table (1) show that both media (MS and B5) supplemented with different concentration of BA significantly affected the fresh weight of cultures. The heaviest fresh weights of cultures (3.57 and 2.85 g) were obtained on MS medium containing 0.8 mgl^{-1} BA for *H. muticus* and D. *metel,* respectively. Also, it was observed that the fresh weight of callus increased with increasing BA concentrations. This increase in fresh weight of cultures was due to the increase in number of shoots and the fresh weight of callus in the base of explants.

Similar results were obtained in leaf explants of *Datura metel* cultured on MS medium supplemented with 1.0 mgl^{-1} BA where Muthukumar and Arockiasamy (2003) reported that the highest percentage of shoot regeneration was obtained with MS medium supplemented with 2.0 mgl^{-1} BA.

In general, production of multiple shoots from the shoot-tip explants resulted in the regeneration of large number of plantlets from a single shoot tip excised from seedling. This technique, therefore, presents as an efficient system compared to seed propagation for conserving and mass multiplication of this important medicinal plants.

Effects of BA and NAA on shoot multiplication of *H. muticus* L. and *D. metel* L.

Data presented in Table (2) show that B5 medium devoid of growth regulators or with NAA at 0.250 mgl^{-1} had no influence on the shoots number of *H. muticus.* Both treatments produced the lowest number of shoots as 1.0 shoots/ plantlet. Also, there were no significant differences in the number of multiple shoots produced per plantlet in these treatments when compared with treatment containing 0.5 mgl^{-1} BA plus 0.5 mgl^{-1} NAA which gave 1.25 shoots/ plantlet.

In the same table data indicate that B5 medium supplemented with BA at 0.25 mgl^{-1} significantly increased number of multiple shoots (4.38 shoots/ plantlet), followed by B5 medium supplemented with 0.5 mgl^{-1} BA with 0.125 mgl^{-1} NAA or without NAA, where both gave 3.88 shoots/ plantlet (Fig. 2-A).

Similar trend was reported by Atta- Alla *et al.,* (2001) on *Gypsophila paniculata.* They showed that MS medium supplemented with BA at the 2.0 mgl^{-1} significantly produced the highest number of proliferated shoot. El-Shafey *et al.,*(1997) on *Acacia albida* reported that BA was the most effective on promoting shoot multiplication. Whereas, effect of BA

Table (1): Effects of BA and medium type on shoot multiplication of *H. muticus* L and *D. metel* L .

Treatments		No. of shoots/ plantlet	shoot length (cm)	No. of leaves/ plantlet	Fresh weight of plantlet (g)
Media	BA mgl^{-1}				
		H. muticus			
MS	0	1.00 f	2.65 a	10.58 f	0.74 f
	0.05	5.00 d	2.73 a	26.58 b	1.90 d
	0.1	7.58 b	2.73 a	29.42 b	2.2 cd
	0.2	4.75 de	2.19b	19.92 de	1.95 d
	0.4	4.17 e	2.63 a	17.42 e	2.38 bc
	0.8	4.67 de	2.75 a	28.83 b	3.57 a
B5	0	1.00 f	2.75 a	6.83 g	0.93 f
	0.05	6.00 c	2.67 a	23.00 cd	2.08 cd
	0.1	8.67 a	2.58 a	34.50 a	2.64 b
	0.2	5.08 d	1.79 c	23.42 c	1.45 e
	0.4	4.75 de	1.92 c	22.67 cd	2.02 cd
	0.8	1.25 f	1.73 c	8.83 fg	1.33 e
		D. metel			
MS	0	1.00 f	2.69 ab	7.67 f	0.60 ef
	0.05	1.67 f	2.58 bc	12.17 e	0.88 cde
	0.1	3.17 de	2.77 ab	18.25 d	0.94 bcd
	0.2	4.42 c	2.69 ab	25.83 b	1.14 bc
	0.4	6.25 b	2.79 ab	26.25 b	2.27 b
	0.8	8.25 a	2.85 a	33.00 a	2.82 a
B5	0	1.00 f	2.63 b	7.92 f	0.66 def
	0.05	2.67 e	2.04 d	15.92 d	0.46 f
	0.1	3.5 d	2.40 c	17.33 d	0.62 ef
	0.2	5.67 b	1.79 e	24.25 bc	0.69 def
	0.4	4.58 c	2.58 bc	22.00 c	1.22 b
	0.8	4.50 c	2.19 d	26.75 b	1.05 bc

Means with the same letter (s) in the same column are not significantly different according to Duncan's Multiple Range at 5%.

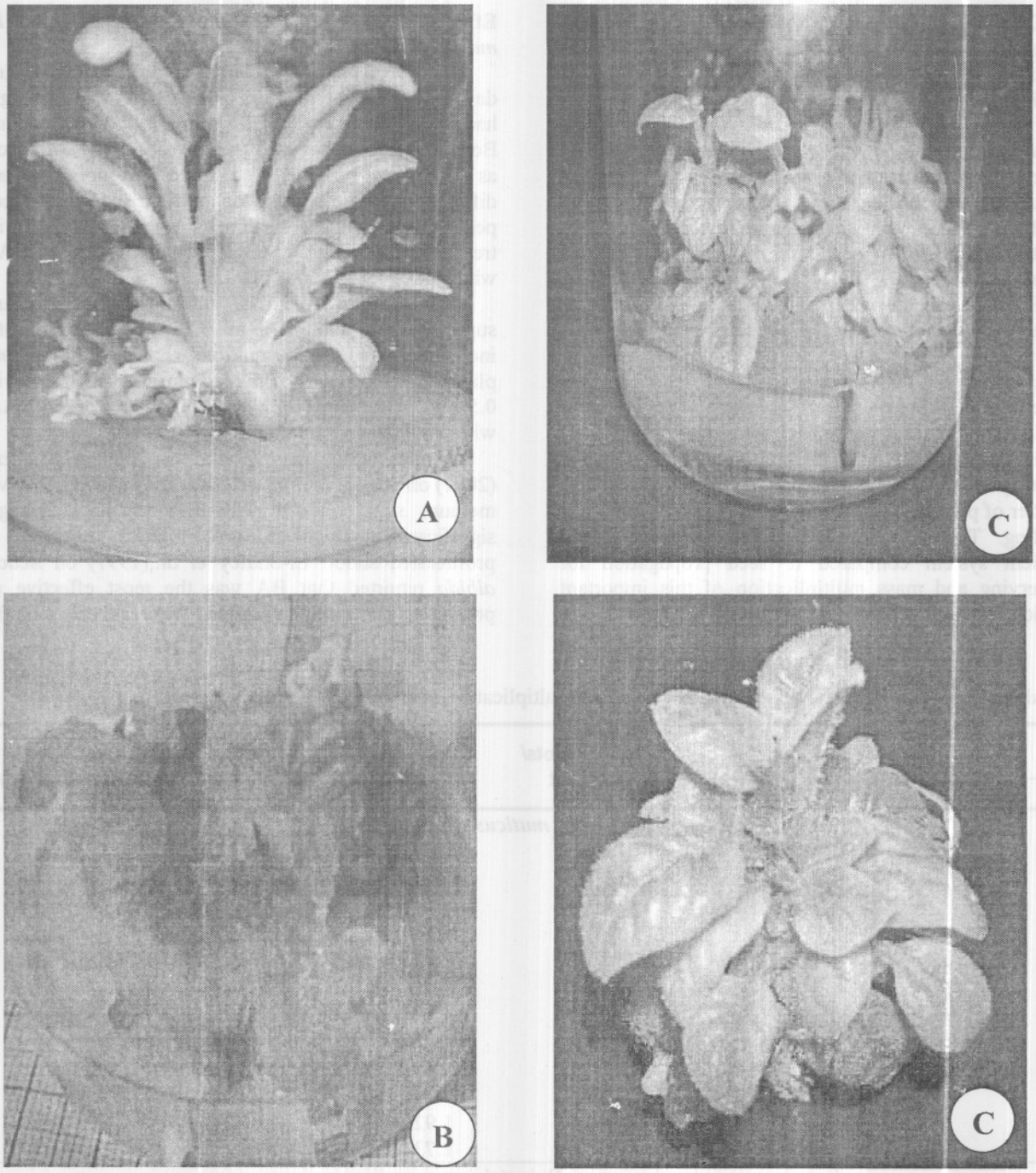

Fig. (1- A) - *In vitro* multiplication of *H. muticus* L on MS medium supplemented with 0.1 8 mgl^{-1} lBA)
(1- B)- Vitrification appears in B5 medium of *H. muticus* L.
(1-c) *In vitro* multiplication of *Datura metel* L on MS medium supplemented with 0.8 mgl^{-1} BA)

on decreasing the number of proliferated shoots was reported by Youssef (1994) on *Acacia saligna* and Rudud *et al*., (1997) on *Eucalyptus nitens.*

As for *D. metel*, data presented in Table (2) indicate that B5 medium supplemented with BA at 0.25 mgl^{-1} and NAA at 0.125 mgl^{-1} significantly increased number of multiple shoots (3.13 shoots/ plantlet). The least number of multiple shoots per plantlet produced on medium devoid of BA, but there was no significant differences in number of shoots per explant produced by treatments compared with treatment containing 0.25 mgl^{-1} BA plus 0.25 mgl^{-1} NAA or BA alone at 0.5 mgl^{-1}which gave 1.0 and 1.88 shoots/explant, respectively.

Similar findings reported on *D. metel* by Muthukumar *et al* (2004) who demonstrated that MS medium supplemented with BA at 3.0 mgl^{-1} and NAA at 0.5 mgl^{-1} was optimum for regeneration of shoots. Also, Carelli and Echeverrigaray (2002) on *Rosa hybrida cv.* Baronesse found that addition of NAA at 0.5 mgl^{-1} to the medium enhanced the production of multiple shoots.

Concerning the effect of BA and NAA on shoot length of *H. muticus,* data presented in Table (2) show that the tallest shoots (2.75 , 2.69 and 2.69 cm), were produced from B5 medium supplemented with 0.5 mgl^{-1} BA plus 0.125 mgl^{-1}NAA, control and NAA at 0.250 mgl^{-1} respectively. The shortest shoots were obtained

with medium containing 0.25 mgl^{-1} BA plus 0.25 mgl^{-1} NAA and medium containing 0.50 mgl^{-1} BA plus 0.50 mgl^{-1} NAA.

Whereas on *D. metel,* B5 medium containing 0.25 mgl^{-1} NAA produced the tallest shoot length (2.81 cm), but this treatment did not significantly difference than the treatments received 0.25 mgl-^{1} BA plus 0.0, 0.125 or 0.5 mgl^{-1} NAA.

As for, number of *H. muticus* leaves, data in Table (2) indicate that the greatest number of leaves (23.50 and 22.50) was obtained from plantlet grown on B5 medium supplemented with 0.25 and 0.5 mgl^{-1} BA, respectively, without any NAA concentrations. It was noticed that number of leaves was interrelated with number of proliferated shoots.

On *D. metel* the greatest number of leaves (18.38 and 15.13) was obtained in plantlets grown on medium supplemented with 0.25 mgl^{-1} BA plus 0.125 mgl^{-1} NAA and 0.50 mgl^{-1} BA plus 0. 25 mgl^{-1} NAA respectively, (Table 2).

Data of Table (2) show that B5 medium supplemented with a combination of NAA and BA slightly affected the fresh weight of cultures (shoots plus callus). The fresh weight of cultures increased with the increase of BA and NAA. The heaviest fresh weight of culture (2.71 g) was obtained from medium containing 0.25 mgl^{-1} BA and 0.125 mgl^{-1} NAA and 0.50 mgl^{-1}BA and 0.25 mgl^{-1} NAA. This increase is mainly due to formation of both callus and shoots.

In general, multiplication and characteristics of proliferated shoots of *H. muticus* were affected by auxins and cytokinins combinations. The presence of NAA at 0.125 mgl^{-1} increased shoot length. Addition of BA at 0.5 mgl^{-1} alone in the multiplication medium was the best choice. This concentration was superior over 0.25 mgl^{-1} BA as no undesirable responses such as callus formation were observed.

Similar trend was reported by Mujib and Pal (1995) who found that the highest number of shoots of carnation was obtained by culturing shoot tips on MS medium supplemented with low concentrations of BA (0.5 mgl^{-1}). They also observed that the addition of NAA did not promote shoot proliferation, but increased the shoot length. Similarly, Matu *et al.,*(2006) found that MS medium supplemented with 0.5 or 1.0 mgl^{-1} BA in the absence or presence of low concentrations of IAA or IBA (0.1 or 0.5 mgl^{-1}) was optimal for shoot induction and growth of *Maytenus senegalensis,* in terms of number of shoots produced per nodal explant. However, incorporation of 0.5 mgl^{-1} BA alone in the shoot multiplication medium was eventually chosen.

On *D. metel,* data in Table (2) show that the heaviest fresh weight of culture (1.06 g) was obtained on medium containing 0.5 mgl^{-1}BA and 0.125 mgl^{-1} NAA. In general, the fresh weight of culture increased with increasing BA level. This increasing may be due to the increase in number of multiple shoots and formation callus.

Table (2): Effects of BA and NAA on shoot multiplication of *H. muticus* L and *D. metel* L cultured on B5 medium

Treatments		No. of shoots/ plantlet	shoot length (cm)	No. of leaves/ plantlet	Fresh weight of plantlet (g)
BA mgl^{-1}	NAA mgl^{-1}				
			***H.* muticus L**		
0.00	0.000	1.00 f	2.69 ab	8.00 d	1.03 d
	0.125	1.00 f	2.28 bc	9.63 cd	1.21 cd
	0.250	1.00 f	2.69 ab	10.25 cd	1.16 cd
	0.500	1.38 ef	2.63 ab	11.75 cd	1.95 abc
0.25	0.000	4.38 a	2.06 c	23.50 a	1.48 bcd
	0.125	3.50 bc	2.56 ab	20.38 a	2.71 a
	0.250	2.88 cd	2.00 c	18.00 ab	2.21 ab
	0.500	2.63 d	2.38 abc	14.25 bc	2.13 ab
0.50	0.000	3.88 ab	2.25 bc	22.50 a	2.27 ab
	0.125	3.88 ab	2.75 a	21.50 a	2.46 a
	0.250	1.88 e	2.13 c	10.13 cd	2.71 a
	0.500	1.25 ef	2.06 c	9.25 cd	2.61 a
			D. metel		
0.00	0.000	1.00 c	2.19 cde	5.50 c	0.08 b
	0.125	1.00 c	2.06 de	5.75 c	0.09 b
	0.250	1.00 c	2.81 a	8.00 c	0.30 c
	0.500	1.00 c	2.16cde	5.50 c	0.52abc
0.25	0.000	1.75 bc	2.56 ab	7.63 c	0.44 bc
	0.125	3.13 a	2.63 ab	18.38 a	0.61abc
	0.250	1.00 c	2.41bcd	7.25 c	0.33 c
	0.500	2.63 ab	2.75 ab	13.63 b	1.00 ab
0.50	0.000	1.88 bc	2.50c abc	10.13 bc	0.45 bc
	0.125	2.50 ab	2.00 e	9.25 bc	1.06 a
	0.250	2.63 ab	2.50 abc	15.13 a	0.53abc
	0.500	2.13 ab	2.09 de	6.25 c	0.29c

Means with the same letter(s) in the same column are not significantly different according to Duncan's Multiple range at 5%.

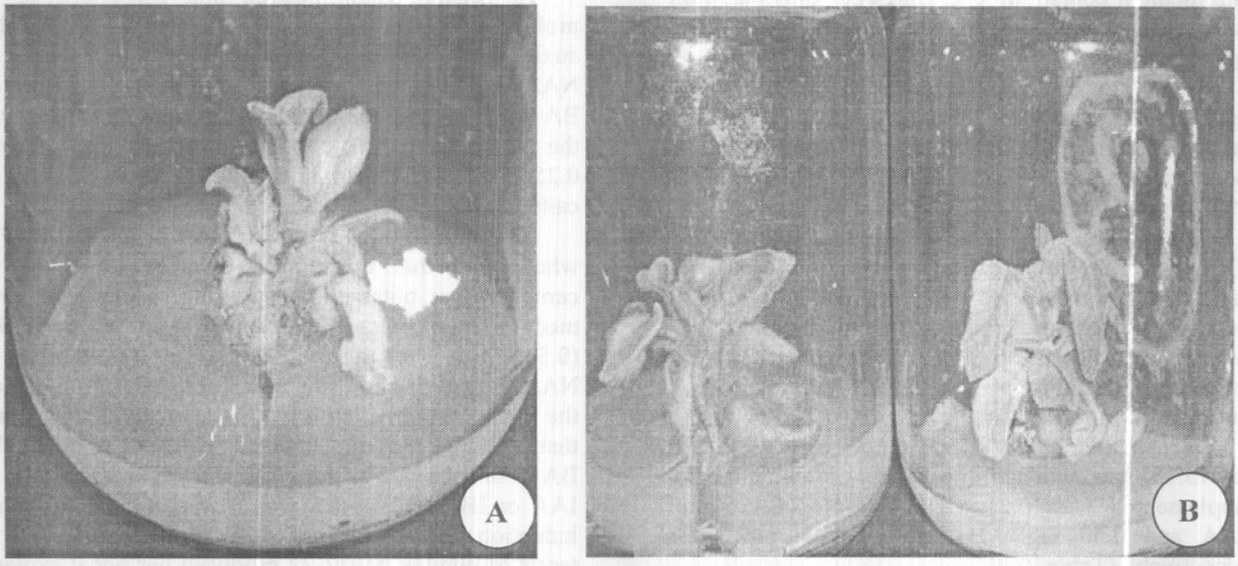

Fig. (2) A- *In vitro* multiplication of *H. muticus,* L. on B5 medium supplemented with 0.5 mgl^{-1} BA.

Fig. (2) B- *In vitro* multiplication of *D. metel,* L (B5 medium supplemented with 0.25 mgl^{-1} BA plus 0.125 NAA)

Effect of IBA on *in vitro* rooting of *H. muticus L.* and *D. metel L.*

The proliferated shoots were individually separated and cultured on MS medium supplemented with IBA to investigate the effect of IBA concentrations on *in vitro* rooting of *H. muticus*, and *D. metel.*

Concerning *H. muticus,* data presented in Table (3) show that MS medium supplemented with 1.0 mgl^{-1} IBA produced the highest percentage of rooting (50 %). Medium devoid of IBA or supplemented with low concentration of IBA (0.2 mgl^{-1}) were not able to induce roots. Meanwhile, in *D. metel,* all shoots cultured on MS medium with or without BA produced roots (100%) rooting

Similar trend was obtained by Missaleva *et al.,* (1993) who found that 50% of shoots of *Datura innoxia* were unable to form roots due to callus formation at the basal portion of the stem section. Slater *et al.*, (2003) concluded that IBA as auxin is necessary for the root formation.

On *H. muticus* , data presented in Table (3) show that MS medium supplemented with 1.0 mgl^{-1} IBA produced the greatest number of roots (1.33 roots / plantlet) but without significant differences than MS medium supplemented with either 0.4 or 0.6 mgl^{-1} IBA, where both produced 0.58 roots per plantlet. Meanwhile, on *D. metel,* MS medium supplemented with 0.4 mgl^{-1} IBA produced the highest number of roots (12.38 roots / plantlet) with no significant differences when compared with MS medium supplemented with 0.2 , 0.6 or 1.0 mgl^{-1} IBA, which produced 11.0 , 10.88 and 11.75 roots / plantlet, respectively.

The tallest roots of *H. muticus* (2.46 cm) was obtained on medium containing 0.6 mgl^{-1} IBA with no significant differences when compared with medium supplemented with 0.4, 0.8 or 1.0 mgl^{-1} IBA . On the other hand, the average longest roots of *D. metel* (12.25 cm) was obtained on MS medium containing 0.8 mgl^{-1} IBA, with no significant differences than control and when supplemented with 0.4 mgl^{-1} .

As shown in Table (3) the tallest shoot of *H. muticus* was obtained in MS medium containing 0.4 mg l^{-1}IBA. No significant differences were detected between different treatments, on shoot length of *D. metel*. The length of shoots ranged from 2.81 to 3.25 cm.

Concerning the fresh weight of *H. muticus* plantlets, data presented in Table (3) showed that MS medium supplemented with IBA at 0.4 mgl^{-1} significantly gave the heaviest fresh weight (1.59 g). The obtained results indicated that the presence of IBA as a kind of auxins was necessary for root induction in *Hyoscyamus muticus* since no root produced in the absence of IBA. The same trend was reported by Arikat *et al.*, (2004) who found that auxins were necessary for root formation in *Salvia fruticosa*. Also, Liu *et al.*, (2003) found that the addition of IBA promoted the induction of roots in *Artemisia judaica* .

Similar results were obtained by Muthukumar and Arockiasamy (2003) on *D. metel, who* reported that all the regenerated shoots rooted on MS medium supplemented with different concentrations of IBA, and 2 mgl^{-1} IBA was found to be the optimum concentration for root induction.

Table (3): Effect of IBA on *in vitro* rooting of *Hyoscyamus muticus* L and *D. metel* L shoot cultured on MS medium

Treatment IBA mgl^{-1}	Rooting %	No. of root/plantlet	Root length (cm)	Shoot length (cm)	F. W. of plantlet (g)
			H. muticus		
0.00	0.00	0.00 b	0.00 b	2.46 c	0.96 c
0.20	0.00	0.00 b	0.00 b	2.46 c	1.07 bc
0.40	16.6	0.58 ab	0.92 ab	3.08 a	1.59 a
0.60	16.6	0.58 ab	2.46 a	3.00 a	1.28 b
0.80	25	0.33 b	1.33 ab	2.73 b	1.27 b
1.00	50	1.33 a	1.83 ab	2.71 b	1.34 ab
			D. metel		
0.00	100	5.13 b	10.13 ab	3.13 a	0.9 b
0.20	100	11.0 a	10.88 a	3.25 a	1.76 a
0.40	100	12.38 a	10.0 ab	3.25 a	2.25 a
0.60	100	10.88 a	7.0 b	2.81 a	1.93 a
0.80	100	10.13 ab	12.25 a	3.0 a	2.37 a
1.00	100	11.75 a	10.38 ab	2.94 a	2.26 a

Means with the same letter(s) in the same column are not significantly different according to Duncan's Multiple range at 5%.

REFERANCES

Anonymous (2005) SPSS command syntax reference. SPSS inc. Chicago III.

Atta-Alla, H. K, Eman, I. Moghazy, Waly, A.K. and Abou El-Soad, I. H.(2001) Micropropagation of *Gypsophila paniculata* var perfecta. Annals of Agric. Sci. Moshtohor 39 (1): 629 – 644.

Arikat N.A., Jawad F.M., Karam N.S and Shibli R.A (2004) Micropropagation and accumulation of essential oils in wild sage (*Salvia fruticosa* Mill.). *Scientia Horticulturae* 100 :193–202.

Berkov, S., Pavlov, A., Kovatecheva, P., Staninirova, P, and Philipov, S. (2003) Alkaloid sepctrum in diploid and tetraploid hairy root cultures of *Datura sramonium*. Zeitschrift fur Naturforschung. Section C, Biosciences. 58 (1 2) 42 -46.

Biondi, S, Scaramagli S, Oksman-Caldentey K.M., and Poli F. (2002) Secondary metabolism in root and callus cultures of *Hyoscyamus muticus* L.: the relationship between morphological organization and response to methyl jasmonate. Plant Science. 163(3)563- 569

Carelli, B.P., and Echeverrigaray, S.(2002) An improved system for the *in vitro* propagation of rose cultivars. Sci Hortic . (92) pp 69 –74.

El-Shafey, Y. H, El-Saihy, O. M, Youssef, E. M, Ghallab, A. M. and Mervat, M. Gad. (1997) *In vitro* shootlets production of *Acacia albida*. Hort. Res. Inst. Agric. Res. Center (1): 227- 251.

Gamborg, O.L., Miller, R.A. and Ojima, K., (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50, 151–158.

Liu, C. Z., Murch, S. J., EL Demerdash, M., and Saxena, P. K. (2003) Regeneration of the Egyptian medicinal plant *Artemisia judaica* L. Plant Cell Rep. 21, 525–530.

Loyola-Vargas V.M. (1999) Genetic Transformation of *Datura* species In: Bajaja Y.P.S. (ed.) *Biotechnology in Agriculture and Forestry*, Vol. 45 Transgenic Medicinal Plants II. Springer-Berlin, Heidelberg, pp 103-116.

Maria, C. F. ,Maria, A. E. and Antonio, V. P. (1990). *In vitro* propagation of the alkaloid producing plant *Datura insignis* . Plant Cell Tissue and Organ Culture 21:75-78

Matu, E.N, Lindsey, K.L and Staden J.V. (2006) Micropropagation of *Maytenus senegalensis* . South African Journal of Botany 72:409–415

Missaleva, N, Petri G and Szoke E. (1993) Some morphological and biochemical peculiarities of *Datura innoxia* callus and regenerated cultures. Plant Cell Tissue and Organ Culture 35: 87-92

Mujib, A. and Pal A.K. (1995) Inter-varietal variation in response to *in vitro* cloning of carnation. Crop research 10 :190-194.

Murashige, T. and Skoog, F., (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473–497.

Muthukumar, B. and Arockiasamy, D.(2003) Micropropagation of *Datura metel*. Journal of Tropical Medicinal Plants. 4(2) 257-259.

Muthukumar, B. ,Arockiasamy,.D.I, and Natarajan,E.(2004) Direct organogenesis in *Datura metel* L. from *in vitro* and *in vivo* nodal explants. Indian Journal of Biotechnology. 3(3) 449-451.

Rudud, J. N, Churehill, K, Pepper, S, Altman, A. and Ziv, M. (1997) Somatic embryogenesis initiation in *Eucalyptus nitens*. Acta Hort. 447: 185 – 186.

Rout, G.R., Samantaray S. and Das P. (2000) *In vitro* manipulation and propagation of medicinal plants. Biotechnology Advances 18: 91–120

Sanatombi, K. and Sharma G.J. (2007) Micropropagation of *Capsicum frutescens* L. using axillary shoot explants. Scientia Horticulturae 113 (1): 96 – 99.

Slater, A., Scotee N.Wand Fowler M.R. (2003) Plant Biotechnology (The Genetic Manipulation of Plants. Oxford Univ. Press.

Snedecor, J. W. and Cochran, W. G. (1967) Statistical Method. Sixth Edition. Iowa State Univ. Press USA.

Strauss, A. (1989). *Hyoscyamus spp*: *In vitro* culture and the Production of Tropane Alkaloid. In: Bajaja Y.P.S. (ed.) Biotechnology in Agriculture and Forestry, Vol. 7 Medicinal and Aromatic Plants II. Springer- Berlin.

Youssef, E. M. A. (1994) Effect of cytokinins and repeated subcultures on *In vitro* micropropagation potentiality of *Acacia saligna* Lindi. First Conf. Ornamental Hort. Cairo Univ. (1): 30 – 34.

Verzar-Petri, G. and Bajaja Y.P.S.(1989) *Datura* spp: *In vitro* Regeneration and the Production of Tropanes. In: Bajaja Y.P.S. (ed.) Biotechnology in Agriculture and Forestry, Vol. 7 Medicinal and Aromatic Plants II. Springer- Berlin , pp 135-161.

الإكثار الدقيق لنباتى السكران المصرى والداتورة ميتل

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أجرى هذا البحث فى معمل زراعة الأنسجة بقسم البساتين – كلية الزراعة جامعة قناة السويس خلال الفترة ٢٠٠٥ – ٢٠٠٧ لدراسة تأثير كل من نوع البيئة والبنزيل أدنين و نفثالين حمض الخليك على إحداث تضاعف الأفرع على القمم النامية لنباتى السكران المصرى والداتورة ميتل. التجربة الأولى : إحداث التضاعف فى الأفرع عند زراعة القمة النامية على بيئة (B5) و التى تحتوى على البنزيل أدنين بتركيزات (صفر ، ٠,٢٥ ، ٠,٥٠ ملليجرام / لتر) مع نفثالين حمض الخليك بتركيزات (صفر ، ٠,١٢٥ ، ٠,٢٥ ، ٠,٥٠ ملليجرام / لتر). التجربة الثانية : بغرض دراسة تأثير البنزيل أدنين بتركيزات (صفر ، ٠,٠٥ ، ٠,١ ، ٠,٢ ، ٠,٤ ، ٠,٨ ملليجرام / لتر) ونوع البيئة (بيئة (B5) وبيئة موراشيج وسكوج) على إحداث التضاعف فى الأفرع لنباتى السكران المصرى والداتورة ميتل. التجربة الثالثة : بغرض إحداث وتكوين الجذور على الفروع الناتجة ، حيث زرعت الأفرع الناتجة من مرحلة التضاعف على بيئة موراشيج وسكوج والتى تحتوى على أندول حامض البيوتريك بتركيزات (صفر ، ٠,٢ ، ٠,٤ ، ٠,٦ ، ٠,٨ ، ١,٠ ملليجرام / لتر). وكانت أهم النتائج كما يلى : فى التجربة الأولى: بعد ٤ أسابيع من الزراعة أدى استخدام بيئة B5 مضافا إليها البنزيل أدنين بتركيز ٠,٢٥ ، ٠,٥٠ ملليجرام / لتر فى غياب أو وجود نفثالين حمض الخليك بتركيز منخفض٠,١٢٥ ملليجرام / لتر الى حدوث زيادة فى إنتاج الأفرع لنبات السكران المصرى ، أيضا وجود نفثالين حمض الخليك بتركيز منخفض٠,١٢٥ ملليجرام / لتر أدى الى زيادة كل من طول الأفرع وتكوين الكالس. أما فى نبات الداتورة ميتل وجد أن استخدام بيئة B5 مضافا إليها البنزيل أدنين بتركيز ٠,٢٥ ملليجرام / لتر و نفثالين حمض الخليك بتركيز ٠,١٢٥ ملليجرام / لتر أدى الى حدوث زيادة فى إنتاج الأفرع. فى التجربة الثانية : بعد ٤ أسابيع من الزراعة وجد أن إضافة البنزيل أدنين بتركيز ٠,١٠ ملليجرام / لتر الى أى من بيئة (B5) أو بيئة موراشيج وسكوج أدى الى حدوث زيادة فى إنتاج الأفرع لنبات السكران المصرى. أما فى نبات الداتورة ميتل وجد أن إضافة البنزيل أدنين بتركيز ٠,٨ ملليجرام / لتر الى بيئة موراشيج وسكوج أظهرت تفوقا فى عدد الأفرع. وجد كذلك أن استخدام بيئة موراشيج وسكوج كانت الأفضل لإنتاج أفرع جيدة فى كلا النباتين. فى التجربة الثالثة : بعد ٢٤ يوم وجد أن بيئة موراشيج وسكوج مضافا إليها أندول حامض البيوتريك بتركيز ١,٠ ملليجرام / لتر أعطت زيادة فى عدد الجذور المتكونة على الأفرع فى نبات السكران . أما فى نبات الداتورة وجد أن استخدام أندول حامض البيوتريك بتركيز ٠,٨ ملليجرام / لتر كانت الأفضل فى زيادة التجذير. تم نقل النباتات من بيئة التجذير وزراعتها فى أصص بلاستيكية تحتوى على خليط من الرمل : البيت موس : فرميكوليت بنسبة ١: ١: ١ ووضعت هذه الأصص فى الصوبة مع استخدام الرش بالرزاز لمدة ٣ أسابيع وكانت نسبة النباتات الناجحة ٥٠ % فى نبات السكران المصرى و ٨٠ % فى نبات الداتورة ميتل والنباتات نمت بشكل طبيعى وجيد.