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IN VITRO PROPAGATION METHODES OF SNAPDRAGON (*Antirrhinum majus*.L) PLANT.

Hamza, A. M; O. M. Abd El-Kafie; A. A. Helaly and M. S. EL-Mongy.
Veget and Flori Department., Fac. Of Agric., Mansoura Univ.

ABSTRACT

The present investigation was carried out to study the direct and indirect micropropagation of *Antirrhinum majus* In order to reduce the cost of production and to ensure the production of genetically identical ornamental plantlets for further field culture as well as for medium- term conservation for future use and research. For the direct micropropagation addition of 70.0 % Ethyl alcohol for 30 sec and 30.0% commercial Clorox (1.30% NaOCl) for 5.00 minute were the best treatment for seeds sterilization. Hypocotyl, cotyledon and cotyledonary node were excised from in vitro grown seedlings. These explants were cultured for adventitious shoot regeneration. Using MS medium supplemented with BAP at 0.50 and 1.00 mg/L produced the highest shoots number of 13.50 shoots for both of them. Microshoots were rooted on MS medium containing IBA, IAA or NAA at 0.0, 0.50, 1.00 or 2.00 mg/L. Ninety percent of the microshoots were rooted on MS medium supplemented with 0.50 and 1.0 mg/L IBA. The rooting which achieved on medium fortified with NAA at 0.50 mg/L significantly recorded the highest roots number of 12.83 roots. A total of 90% survival was achieved and an increase in shoots length (12.71cm) when rooted explants were acclimatized *ex vitro* using 1: 1 soil: vermiculite mixture. On the other hand, for indirect micropropagation, explants successfully formed callus by using MS medium supplemented with TDZ at 1.00, 2.00 or 4.00 mg/L. The highest shoots number derived from callus was recorded for callus cultured on MS medium fortified with 2.00 mg/L TDZ + 0.50 mg/L NAA.

Abbreviations: 2,4-D; 2,4-dichlorophenoxyacetic acid, BAP; 6-benzylamino purine, IBA; 3-indolebutyric acid, MS; Murashige and Skoog (1962) basal medium, NAA; Naphthaleneacetic acid, TDZ; Thidiazuron, Kin; N6-furfuryladenine (Kinetin), IAA; indole acetic acid

INTRODUCTION

Antirrhinum majus L. (snapdragon) family (Scrophulariaceae) is a herbaceous perennial plant, cultivated as ornamental plant for its attractive view. Snapdragons have right stems dressed with two-lipped tubular flowers that come in an almost endless palette of colors Blamey and Grey-Wilson(1989). Moreover *Antirrhinum majus* is important commercially as a cut flower, annual bedding plant, and potted plant, flower pigmentation.

Snapdragon if propagated by seeds cannot ensure genetic uniformity may produce undesired phenotypes and subsequently influence the quality and regeneration potential of the plants, as the plants may be randomly selected without taking necessary care. This will result in negative economic implications on mass production of this plant. Thus, the development of in vitro culture techniques would increase the knowledge about its germplasm with potential relevance as an ornamental crop. A rapid propagation system for Antirrhinum majus would assist breeding programs and commercial propagation of highly desirable cut-flower and nursery industries. This could

help develop a new type of ornamental that can be produced year round with a high quality / price ratio and distributed far more widely than classically propagated methods (Hall, 1999; Beyl, 2000).

Thus the use of tissue culture in the production of *A. majus* would alleviate these problems, aiding in the commercial success of the crop. The present study was undertaken to develop a protocol for mass propagation of *A. majus* through in vitro culture to produce a large number of plants with high quality.

MATERIALS AND METHODS

The current research was conducted at the experimental station and tissue culture laboratory of the Vegetable. and Floriculture Department, Faculty of Agriculture, Mansoura University during the seasons of 2011-2013.

Direct micropropagation method:

Seeds sterilization: The plant material was obtained from hybrid plants of *Antirrhinum majus* seeds (Apple Blossom) that were imported from France. Seeds were thoroughly washed with running tap water containing a few amount of house hold detergent for 20 min, and immersed in70% ethyl alcohol for 0.50 or 1.00 minute and 30% Commercial Clorox solution (1.3% sodium hypochlorite)for 3.0, 5.0 or 10.0 minutes. Then the seeds were rinsing four times in sterilized distilled water under laminar air flow cabinet. The seeds were cultured on 250 ml sterilized glass jars, contained 30 ml of Murashige and Skoog (1962) nutrient medium supplemented with sucrose (30 g/L). The medium was solidified with 7g agar/L (w/L) and the pH of the medium was adjusted to 5.8 with NaOH or HCl after adding growth regulators to the media and before agar was added. All media were autoclaved at 121 ^{o}C at 1.5 kg/cm2 for 20 min. Data were recorded after 2weeks for:

1- Survival %= (Number of the germinated seeds / All number of the cultured seeds) × 100.
2- Contamination%= (Number of the contaminated seeds / All number of the cultured seeds) × 100.
3- Mortality %= (Number of the mortality seeds / All number of the cultured seeds) × 100.

This experiment included 6 treatments each of which consisted of 5 replications, each replicate included 5 jars and each jar have 4 seeds**.**

Shoot multiplication: Hypocotyl, cotyledon and cotyledonary node explants were taken from the previous sterilized seeds at length of 1-2 cm approximately. Then, each explant was cultured on (MS) basal medium supplemented with thidiazuron (TDZ) at different concentrations (0.0, 0.1, 0.2, 1.0 and 2.0 mg/L) or benzylaminopurine (BAP) at (0.5 and 1.0 mg/L). Data were recorded after 4 weeks for:

1- Survival%= (Number of the grown explants / All number of the cultured explants) × 100.
2- Shoots number per explant. 3- Shoots length (mm).
4- Leaves number per explant. 5- Dry and fresh weight of shoots (g).
6- Callus formation % = (number of explants formed callus / All number of the cultured explants) × 100.

A simple experiment in randomize complete block design was used with 3 replicates included 9 jars for each treatment.

***In vitro rooting formation*:** Shootlets which were cultured on MS medium containing BAP at 0.50 and 1.00 mg/L and sucrose at 30 g/L (the best proliferation treatment) were grown on the nutrient medium supplemented with IBA, IAA and NAA at concentrations of 0.0, 0.5, 1.0, and 2.0 mg/L.

. Data were recorded after four weeks for:

1- Rooting %= (Number of the rooted shoots / All number of the cultured shoots) × 100.
2- Roots number per explant. 3- Roots length (mm).

This experiment included 12 treatments each of which consisted of 3 replicates of 3 jars per replicate.

***Ex vitro establishment*:** Well developed rooted plantlets were taken out of the culture jars and washed with warm water to eliminate any medium surplus, then transferred into plastic pots containing one of the following autoclaved media; [(Peat moss + vermiculite (1:1), (Peat moss + Loamy soil (1:1), Vermiculite + Loamy soil (1:1) and Peat moss + vermiculite + Loamy soil (1:1:1) by volume)]. Data were recorded after 4 weeks for; survival% and average increase in shoots length (cm)**.**

The experiment included 4 treatments (media) each of which consisted of 3 replicates of 3 pots for each. Every pot contained one plantlet and covered with transparent plastic bag to maintain high relative humidity around plants. The plastic bags were bored gradually starting from the second day of transplanting in order to get rid of excess humidity as well as exposing the plantlets to the normal atmosphere condition. On the fifth day from transplanting, these bags were removed completely. The plantlets were irrigated with tap water whenever needed.

Indirect micropropagation method:

Callus induction: Shoot tips of shootlets with length of 1-2 cm were taken as explants. Explants were cultured on MS solid medium supplemented with different hormones such as NAA at 1.0 mg/L, 2.4-D at 1.0 mg/L and 3.0 mg/L, TDZ at 1.0 mg/L, 2.0 mg/L and 4.0 mg/L, NAA at 1.0 mg/L+2.4-D at 2.0 mg/L, BAP at 5.0 mg/L+ NAA at 1.0 mg/L, 2.4-D at 1.0 mg/L+ kin at 2 mg/L+IAA at 2 mg/L and 2.4-D at 1.0 mg/L+ BAP at 5.0 mg/L+ NAA at 5.0 mg/L).

Data were recorded after four weeks for:

1- Callus formation % = (Number of explants formed callus / All number of the cultured explants) × 100.
2- Callus fresh and dry weight (g). 3- Callus volume (cm3)
4-Rooting percentage. 5- Shoots number per callus.

This experiment included 10 treatments each of which consisted of 3 replicates of 3 jars per replicate, and every jar contained 30 ml of of the nutrient media and 3 explants

Callus redifferentiation: The calluses derived from the best result in the previous experiment were examined, cut and piece of 0.5 × 0.5 cm^2 and used in this experiment. Calluses were cultured on MS solid medium fortified with different treatments such as BAP at 1.0 mg/L+ NAA at 0.5 mg/L, BAP at 2.0

mg/L+ NAA at 0.5 mg/L, TDZ at 2.0 mg/L+GA3 at 0.5 mg/L and TDZ at 2.0 mg/L+NAA at 0.5 mg/L.

Data were recorded after four weeks for:

1- Callus redifferentiation % = (Number of calluses formed shoots / All number of the cultured calluses) × 100.
2- Shoots number per callus.
3- Shoots length (mm). 4- Leaves number.

This experiment included 4 treatments each of which consisted of 3 replicates in 3 jars.

Incubation conditions: All the previous experiments were incubated at temperature of 25 Cº ± 2 Cº, photoperiod also was adjusted to 16 hours light and 8 hours dark, controlled automatically. Illumination intensity was 1500 lux at top culture vessels level from fluorescent lamps (60cm long).

Statistical analysis:

A complete randomize design for each experiment was 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. Data given in percentage were subjected to arcsine (√X) transformation according to (Snedecor and Cochran, 1967) before statistical analysis.

RESULTS AND DISCUSSION

Direct micropropagation method:

Seeds sterilization:

Effect of the interaction between sodium hypochlorite and Ethyl alcohol on contamination, survival and mortality % after arcsine √% transformation of Snapdragon seeds after 2 weeks from culturing.

Data presented in Table (1) cleared that using sodium hypochlorite for 3.00 min and Ethyl alcohol for 0.50 min produced the highest contamination percentage, as it was 66.00%, followed by 54.00% for using sodium hypochlorite for 3.00 min and Ethyl alcohol for 1.00 min. On the other hand, no contamination was observed when seeds sterilized by either Ethyl alchol for 0.5 or 1.00 min and sodium hypochlorite. Concerning the interaction effects it was observed from Table (1) cleared that the highest survival percentage were produced when the seeds treated with Ethyl alcohol for 0.50 min and sodium hypochlorite for 5.00 min, as it was 63%, followed by 57% when 5.00 ethyl alcohol and 1.00 sodium hypochlorite were used . On the other hand, using sodium hypochlorite for 3.00 min with Ethyl alchol for 0.50 and 1.00 min produced the lowest survival percentages, as they were 24.00 and 30.00 % respectively. Also, increasing sodium hypochlorite duration with Ethyl alchol for 0.50 and 1.00 min reduced the survival percentage to 51.00 and 36.00 % respectively.

As for the effect of interaction between sodium hypochlorite and Ethyl alchol on mortality %, data Table(1) showed that using sodium hypochlorite for 10 min with Ethyl alchol for 0.50 and 1.00 min produced the highest

mortality percentage, as they were 39.00 and 54.00 % respectively. But, it was observed that using the lowest duration of sodium hypochlorite (3 min) with the lowest duration of Ethyl alchol (0.50 min) significantly produced the lowest mortality percentage, as it was 0.00 %.

In general, a relationship was detected between the sodium hypochlorite and Ethyl alcohol and the mortality percentage, since increasing the sodium hypochlorite and Ethyl alcohol durations were followed by increasing in the mortality percentage with all treatments.

Table (1): Effect of the interaction between sodium hypochlorite and Ethyl alcohol on contamination, survival and mortality % after arcsine √% transformation of Snapdragon seeds after 2 weeks from culturing.

sodium hypochlorite (1.3 %) (A) min	contamination %		Survival %		mortality %	
	Ethyl alchol (70%) (B) Min					
	0.50	1.00	0.50	1.00	0.50	1.00
3.00	66.00	54.00	24.00	30.00	00.0	12.0
5.00	18.00	12.00	63.00	57.00	18.0	27.0
10.00	0.00	0.00	51.00	36.00	39.0	54.0
LSD at 5% (A×B)	18.75		25.65		23.58	

The obtained results may be due to the response of the explants tissues was vary with treatment duration depending on the type and physiological stage of the same plant resulting in establishment of aseptic culture. The morphogenetic pattern of cell changes according to the physiological age of the plant material resulting in direct correlation between the sensitivity of the tissue to sterilizing agent and the physiological age of the explant.

It is also important to be cautious that a surface sterilization is also toxic to the explants tissue, therefore concentration of the sterilizing agent and duration of the treatment should be optimum to minimize tissue mortality of the explants due to over sterilization.

The obtained results are in harmony with a number of reports (Betino et al 1996; Shimasaki and Fukumoto 1998; Yang *et al*. 1999; Minlong et al. 2000) for sterilization of seeds of *Antirrhinum majus* with ethanol and sodium hypochlorite at different concentrations. Also our results was similar to that recorded by Cui *et al*. (2004) on *Antirrhinum majus* cv, who revealed that seeds were surface sterilized by a brief rinse in 70.00% (v/v) ethanol and then in1.00% sodium hypochlorite for 6.00 min , followed by washing five times with sterile distilled water.

Shoot multiplication:

Effect of the BAP and TDZ concentrations on shoots [number, length (cm)], and leaves number of Snapdragon plantlets.

It is appeared from Table (2) and Figure (1) that adding BAP at concentration of (0.50 or 1.00 mg/L) gave the highest value for number of shoots, as it was 13.50 shoots for both of them. Incompartion to the control medium which resulted in the lowest shoots number value of 1.89 shoots. It

was also observed that increasing TDZ concentration to reach 1.00 or 2.00 mg/L significantly decreased the shoots number to 5.66 and 4.00 shoots respectively, but it was still higher than the control.

These results agreed with the results of (Özturk *et al*. 2004) *on Ludwiga repens* who revealed that a higher maximum number of new shoots was obtainded on MS medium supplemented with 0.5 mg/L BA and 0.1 TDZ.

Table (2): Effect of the BAP and TDZ concentrations on shoots [number, length (cm)], and leaves number of Snapdragon plantlets.

Measurements / Treatments (mg/L)		Shoot number	Shoot length (cm)	Leaves number
Control mg/L	0.00	1.89	7.22	5.77
TDZ Conc. mg/L	0.10	10.44	3.73	4.60
	0.20	11.44	2.77	3.37
	1.00	5.66	2.31	2.54
	2.00	4.00	2.12	2.42
BAP Conc. mg/L	0.50	13.50	4.89	4.17
	1.00	13.50	5.05	5.40
L.S.D at 5%		2.45	1.71	1.35

Moreover results were similar to results achieved by Abdl- Kafie (1988) on *Cassia fistula* and *Delonix regia* who observed an increase in shoot number which may be related to the known fact of cytokinins inhibiting apical dominance and stimulating axillary shoots.

As for shoots length, the results in Table(1) and Figure (1) revealed that the control medium (0.00 mg/L) significantly produced the tallest shoot length of 7.22 cm, followed by 5.05 cm for medium contained BAP at 1.00 mg/L. Also, it was observed that no significant difference between the media which contained BAP at (0.50, 1.00 mg/L) as it was 4.89 cm and 5.05 cm respectively.

The formation of stunted or fascinated of shoots on TDZ supplemented medium has been reported by (ÇÖÇÜ *et al*. (2004). The inhibition of shoot elongation may be due to the high cytokinin activity of TDZ were as presence of a phenyl group in TDZ may be the possible cause of shoot bud fasciations (Huetteman and Preece 1993).

Regarding the leaves number results in Table (2) revealed that using the control medium produced the highest number of leaves (5.77 leaves/shoot). No significant difference among the TDZ and BAP concentrations in comparison the control treatment (the hormone free medium). Also it was observed that the lowest number of leaves of 2.42 was produced when the medium was supplemented with TDZ at concentration 2.00 mg/L. This effect of BAP concentrations on leaves number is actually derived from the role of BAP on enhancing cell division, by extension the leaves number is increasing. In addition, when the leaves number is increasing, it stimulates the photosynthesis process and consequently the content of the microshoots from growth substances (like carbohydrate) is increasing. This result confirm

the results of Sheyab *et al*. (2010) on *Antirrhinum majus* who stated that in medium supplemented with BA, shoot length was taller than the control, and the number of leaves was increased in all treatments when compared with the control.

Figure (1): Effect of BAP and TDZ concentrations on average shoots number of Snapdragon plantlets, and indicated that BAP at 0.50 and 1.00 mg/L was considered the best proliferation medium for producing the highest shoots number.

In vitro rooting formation:

Effect of the interaction between auxin type and auxin concentration on rooting %, after arcsine√% transformation roots [number and length (cm)] of Snapdragon plantlets.

Examining the response of the interaction between auxin type and auxin concentration, the results in Table (3) showed that there were significant differences in most cases. The tremendous rooting percentage of 90.00% was achived with MS medium supplemented with IBA at 0.50 and 1.00 mg/L. The pre-mentioned treatments had an increment effect on rooting percentage than all the other treatments. The extremely low percentage of 43.10% was obtained when media had supplemented with NAA at concentrations of 2.00 mg/L. On the other hand auxin-free medium (control) produced 60.00 % with no significant difference than highest root percentage(90%) which obtained from IBA at 0.5 or 1.00 mg/L however it was not the lowest value. The same result was obtained by Sheyab *et al*. (2010) on *Antirrhinum majus* who declared that a small percentage of rooting produced in the absence of auxins, and the low concentration of auxins were

effective in including rooting. Also, data in Table(3) and Figure (2) revealed that adding NAA to the nutrient medium at concentration of 0.50 and 1.00 mg/L significantly recorded the highest roots number of 12.83 and 12.00 roots respectively comparing with all other treatments, followed by 7.00 roots for using free medium (Control). However, these roots get easily detached from the microshoots during transplantation comparing with IBA and IAA. On the other hand, the lowest roots number of 4.05 has been produced from media supplemented with IAA at concentration of 0.50 mg/L. The matter was differed with IBA one, as a positive relationship was found between IBA concentration and roots number. Since, using IBA at the concentration of 1.00 mg/L recorded value of 6.00 roots, which was higher than the other IBA treatments**.**

Regarding the interaction between auxin type and auxin concentration, a data in Table (3) and Figure (2) indicated that IBA in all concentrations increased the value of roots length. Since, adding IBA to the nutrient media at concentration of 0.50 and 1.00 mg/L significantly measured the tallest roots length of 4.00 and 4.58cm respectively when compared with other cases. It was also obvious that adding NAA at concentration of 2.00 mg/L significantly decreased the roots length to 1.56 cm, than other treatments. These results were in the same line with the findings of Shajiee (2007) on *Saintpaulia ionantha*, who found that increasing IBA caused rooting and regeneration, but increasing NAA led to dedifferentiation and eventually callus tissue. In general, it was noticed that IBA had the upper hand in that respect, since adding at any concentration gave higher values in most of cases, when compared with the corresponding NAA or IAA.

Table (3): Effect of the interaction between auxin type and auxin concentration on rooting %, after arcsine√% transformation roots [number and length (mm)] of Snapdragonr plantlets.

Auxin type (A)	Rooting %				Roots number				Roots length (cm)			
	Auxin conc. (mg/L) (B)											
	0.00	0.50	1.00	2.00	0.00	0.50	1.00	2.00	0.00	0.50	1.00	2.00
IBA	60.00	90.00	90.00	60.00	7.00	5.28	6.00	4.50	1.90	4.00	4.58	3.46
IAA	60.00	78.20	60.00	54.70	7.00	4.05	5.17	5.00	1.90	3.26	3.12	2.65
NAA	60.00	57.90	55.10	43.10	7.00	12.83	12.0	6.17	1.90	1.85	1.58	1.56
LSD at 5% (A×B)	32.0				2.34				0.73			

***Ex vitro establishment*:**

Effect of transplanting medium on survival % after arcsine√% transformation and increase in shoots length (cm) of Snapdragon transplants.

The benefit of any micropropagation system can be fully realized only by the successful transfer of plantlets from tissue culture vessels to the ambient conditions found *ex vitro*. Most species grown *in vitro* require an acclimatization process in order to ensure that sufficient number of plants survive and grow normally and vigorously when transferred to soil. So, the aim of this experiment was conducted to study the effect of transplanting media on survival % and shoot length. It was clear from data in Table (4) that

culturing the plantlets of Snapdragon on loamy soil mixed with vermiculite (1:1 by volume) increased the survival percentage, as it reached the highest value of 90.00%. This may be attributed to the occurrence and the balance of nutrients in this mixture, in addition to the more suitable physical properties as compared with other media. The following value of 78.20% was for using loamy soil mixed with peat moss and vermiculite (1:1:1 by volume). On the other hand, using peat moss mixed with vermiculite or loamy soil (1:1 V) resulted in less values of 60.00 and 66.50%, respectively and no significant difference was detected between them. These results were in agreement with these obtained by Faisal and Anis (2006) on *Psoralea corylifolia* transplantlets.

Figure (2): Effect of the interaction between auxin type and auxin concentration on roots (number and length). And showed that adding IBA at 1.00 mg/L onto MS medium gave the tallest roots length, but adding NAA at 0.5 mg/L produced the highest root number.

Reviewing data in Table (4) and Figure (3) showed that using the mixture of vermiculite and loamy soil stimulated the plantlets length, as it reached 9.67cm, followed by 9.00cm when the mixture of peat moss, vermiculite and loamy soil was used. But, no significant difference was found between them. Using the mixture of peat moss and vermiculite recorded the shortest plantlets of 3.67cm. These results may be attributed to the interaction effects of the two transplanting media, as presence of loamy soil and vermiculite saved nutrient elements (containing potassium and magnesium for the aforementioned one) in an available form. Thus, these factors acted on saving the suitable conditions for good growth and development of Snapdragon plantlets during this stage and consequently production of well adapted plant

Indirect micropropagation method:

Callus induction:

Effect of auxin and cytokine concentrations on callus formation % after arcsine√% transformation and callus (volume, fresh and dry weight) and shoot regeneration number.

Concerning callus formation percentage data in Table (5) cleared that all the media which were supplemented with 3.00 mg/L 2.4D and TDZ at (1.00,

2.00 and 4.00 mg/L) significantly produced the highest values (90 %). In the same trend , the same results were produced from media contained 5.00 mg/L BAP +1.00mg/L NAA, 1.00 mg/L 2.4D + 2.00 mg/L kin + 2.00 mg/L IAA and 1.00 mg/L 2.4D +5.00 mg/L BAP + 5.00 mg/L NAA.

Table (4):Effect of transplanting medium on survival % after arcsine√% transformation and increase in shoots length (cm) of Snapdragon transplants.

Transplanting media	Survival %	shoots length (cm)
Peat moss + Vermiculite (1:1)	60.00	3.67
Peat moss + Loamy soil (1:1)	66.50	4.33
Vermiculite + Loamy soil (1:1)	90.00	9.67
Peat moss + Vermiculite + Loamy soil (1:1:1)	78.20	9.00
LSD at 5%	37.65	3.32

Figure (3): Effect of the transplanting media on increase in shoots length (cm). and can be observed that Loamy soil augmented with vermiculite (1:1 by volume) was the best for shoot length

Table (5): Effect of auxin and cytokine concentrations on callus formation % after arcsine√% transformation and callus volume.

Treatments	Measurements		
	Callus formation %	Callus formation % after arcsine√% transformation	Callus volume cm3
NAA 1.0 mg/L	66.7	54.70	0.86
2.4 D 1.0 mg/L	77.8	66.50	0.66
2.4 D 3.0 mg/L	100	90.00	0.55
TDZ 1.0 mg/L	100	90.00	1.25
TDZ 2.0 mg/L	100	90.00	2.51
TDZ 4.0 mg/L	100	90.00	2.83
NAA 1.0 mg/L+2.4D 2.0 mg/L	77.8	66.50	0.55
BAP 5.0 mg/L+ NAA 1.0 mg/L	100	90.00	2.35
2.4D 1.0 mg/L+ Kin 2.0 mg/L+IAA 2 mg/L	100	90.00	0.78
2.4D 1.0 mg/L+BAP 5.0 mg/L+NAA 5 mg/L	100	90.00	0.77
L.S.D at 5%	----	15.51	0.49

On the other hand the lowest callus formation percentage were produced from media supplemented with 1.00 mg/L NAA , 1.00 mg/L 2.4D and 1.00 mg/L NAA +2 mg/L 2.4D, as they were 54.70, 66.50 and 66.5% respectively, and there were no significant differences between them.

This result was similar with the findings of (Tawfik (1992) who cleared that TDZ could alone induce nodular meristematic callus, and Perrini *et al*. (2009) who found that Callus of Helichrysum italicum were found to form on the basal medium only when it was supplemented with thidiazuron (TDZ) alone or in combination with naphthalene acetic acid (NAA). . Simon and Petrasek (2011) referred to the important roles of auxin in cell enlargement, stimulating cell division and elongation by increasing the plasticity of the cell wall. When extensibility of the wall is increased, the wall pressure around the cell decreases and the pressure caused by osmotic forces in the vacuolar sap causes water to enter the cell, resulting in cell enlargement.

Regarding the effect of auxin and cytokine concentrations, data in Table (5) and Figure (4) indicated that all media which contained TDZ at concentration of 2.00 and 4.00 mg/L produced the highest callus volume, as they were 2.51and 2.83 cm^3 respectively, followed by 2.35 cm3 for using 5.00 mg/L BAP +1.00 mg/L NAA as it reduced the callus volume, but there was no significant difference between them. On the other hand the lowest callus volumes (0.55) were significantly obtained from media supplemented with 3.00 mg/L 2.4D or 1.00 mg/L NAA +2 mg/L 2.4D. These results were mentioned by Simon and Petrasek (2011) who suggested that auxin may function by activating a messenger-type of RNA that induces the synthesis of specific enzymes. These enzymes would cause insertion of new materials into the cell wall, resulting in its extension. In addition, the concentrations of the used auxins may be in the optimal range. These results were in harmony with those obtained by Wang *et al*. (2007) on *Capparis spinosa.* Whenever the callus volume increases, the number of the derived shoots also

increases, so the callus volume is one of the most important parameter in the indirect micropropagation technique.

The concerned results in Table (6) and Figure (4) indicated that the media which were supplemented with 4.00 mg/L TDZ and 5.00 mg/L BAP +1.00 mg/L NAA significantly induced the highest shoot regeneration number, as they were 7.00 and 5.67 shoots respectively when compared with other values, followed by 3.33 and 2.00 shoots for using 2.00 and 1.00 mg/L TDZ. As the all media which supplemented with TDZ produced shoot regeneration. On the other hand the media which contained 1.00 mg/L NAA, 1.00 mg/L 2.4D, 3.00 mg/L 2.4D, 1.00 mg/L NAA +2 mg/L 2.4D and 1.00 mg/L 2.4D + 2.00 mg/L kin + 2.00 mg/L IAA did not produced shoots. These results were in harmony with those obtained by Xiaofang *et al*. (1992) on *Antirrhinum majus* that combinations of the concentrations of BA 7.00–9.00 mg / L and NAA 0.1–0.5 mg/ L were advantageous to the regeneration of buds and NAA was very important for callus growth. Data in Table (6) cleared that the heaviest callus fresh weight of 3.02 g was obtained when the media were supplemented with TDZ at concentration of 4.00 mg/L, followed by 2.99 g when the media were supplemented with 5.00 mg/L BAP +1.00 mg/L NAA, also it was observed that previous media significantly produced the heaviest callus fresh weight, and there was no significant difference between them. And all media which contained TDZ produced heavy callus fresh weight when compared with most of other weights. In contrast, the lighter callus formation (0.38 g) was performed from explants cultured on the medium supplemented with 2.4D at concentration of 1.00 mg/L. In this concern, medium supplemented with 4.00 mg/L TDZ presented the heaviest callus dry weight of 1.43g.

Photo (4): Effect of auxin and cytokinin concentrations on callus volume and shoot regeneration number of Snapdragon, and indicated that TDZ at 4.00 mg/L was the best for shoot regeneration number.

Table (6): Effect of auxin and cytokine concentrations on callus (fresh and dry weight) and Shoot regeneration number

Treatments	Measurements		
	Shoot regeneration number	Callus fresh weight (g)	Callus dry weight (g)
NAA 1.0 mg/L	0.00	0.84	0.07
2.4 D 1.0 mg/L	0.00	0.38	0.03
2.4 D 3.0 mg/L	0.00	0.51	0.03
TDZ 1.0 mg/L	2.00	2.69	0.88
TDZ 2.0 mg/L	3.33	1.80	0.61
TDZ 4.0 mg/L	7.00	3.02	1.43
NAA 1.0 mg/L+2.4D 2.0 mg/L	0.00	0.41	0.04
BAP 5.0 mg/L+ NAA 1.0 mg/L	5.67	2.99	1.12
2.4D 1.0 mg/L+ Kin 2.0 mg/L+IAA 2 mg/L	0.00	0.56	0.04
2.4D 1.0 mg/L+BAP 5.0 mg/L+NAA 5 mg/L	2.67	0.97	0.06
L.S.D at 5%	1.42	0.30	0.33

On the other hand, the media which fortified with 2.4D at concentration of 1.00 and 3.00 mg/L significantly produced the lightest callus dry weight (0.3 g) for each one**.**

Callus redifferentiation:

Effect of auxin and cytokinin concentrations on callus redifferentiation% after arcsine√% transformation, shoots [number per callus, length (cm)], and leaves number of Snapdragon.

It was obvious from data presented in Table (7) that media supplemented with 2.00 mg/L TDZ + 0.5 mg/L NAA promoted the highest callus redifferentiation %, since it was 90.00 %, followed by 70.07%, when medium was fortified with 1.00 mg/L BAP + 0.5 mg/L NAA. The lowest value 30.00% of callus redifferentiation was recorded for medium containing 2.00 mg/L TDZ + 0.5 mg/L GA_3. Visser *et al*. (1992) referred this result to the role of TDZ in saving the auxins and cytokinins requirements on geranium tissue culture**.**

Also it was clear from data Table (7) and shown in Figure (5) that nutrient medium which was fortified with 2.00 mg/L TDZ + 0.5 mg/L NAA significantly promoted the shoots number per callus, since it was 29.33 shoots when compared with adding other auxins and cytokinins to the medium. The next positive value of 7.67 shoots was recorded for callus cultured on medium augmented with 2.00 mg/L BAP + 0.5 mg/L NAA. While, it was clear that the media which contained 2.00 mg/L TDZ + 0.5 mg/L GA_3 resulted in the lowest values in these respects, as it was 1.33 shoots only. In the same line, Our results was similar with these recorded by ÇÖÇÜ *et al*. (2004) on *Calendula officinalis* who find that the highest number of shoots per explant was achieved on media supplemented with 0.75 mg /L TDZ alone or 0.75 mg /L TDZ and 0.05 mg /L NAA.

Table (7):Effect of auxin and cytokinin concentrations on callus redifferentiation% after arcsine√% transformation, shoots [number per callus, length (cm)], and leaves number of Snapdragon.

Treatments	Measurements				
	Callus rediffere ntiation %	Callus redifferentiation % after arcsine√% transformation	Shoot number per explants	Shoot length (cm)	Leaves number per shoot
BAP 1.0 mg/L+ NAA 0.5 mg/L	85.0	70.7	6.00	3.17	5.33
BAP 2.0 mg/L+ NAA 0.5 mg/L	75.0	65.0	7.67	3.67	6.67
TDZ 2.0 mg/L+GA_3 0.5 mg/L	25.0	30.0	1.33	6.83	2.00
TDZ 2.0 mg/L+ NAA 0.5 mg/L	100.0	90.0	29.33	2.33	2.00
L.S.D at 5%	------	26.72	4.416	2.07	3.60

Photo (5): Effect of auxin and cytokinin concentrations on shoots (number per callus and length cm) of Snapdragon callus, and revealed that2.00 mg/L TDZ + 0.5 mg/L NAA recorded the highest callus redifferentiation % and largest shoot number.

Concerning shoot length data in Table (7) and Figure (5) indicated that the tallest shoots length (6.83cm) was obtained from the media supplemented with 2.00 mg/L TDZ + 0.5 mg/L GA3, followed by (3.67 cm) when using 2.00 mg/L BAP + 0.5 mg/L NAA was added to the culture medium. The extremely shortest shoots length of 2.33 cm was recorded when the media was supplemented with 2.00 mg/L TDZ +.0.5 mg/L NAA

compared to other media. Also it was observed from data in Table (7) and Figure (5) that callus which was cultured on medium included 2.00 mg/L BAP + 0.5 mg/L NAA significantly counted the greatest leaves number (6.67 leaves), followed by 5.33 leaves for medium supplemented with 2.00 mg/L BAP + 0.5 mg/L NAA. But, 2.00 mg/L TDZ + 0.5 mg/L GA_3 and 2.00 mg/L TDZ + 0.5 mg/L NAA significantly possessed the weakest value of 2.00 leaves when compared with other media.

REFERENCES

Abdl-Kafie, O.M (1988) Physiological studies on some ornamental plants. Ph.D. Thesis, Fac. of Agric., Mansoura University.

Betino, G.E.M.; Tapia, J.; Rodrigues, N. and Iriondo, J.M. (1996). Micropropagation of commercial and wild genotypes of snapdragon (*Antirrhinum spp*). Journal of Horticulture Scince. 71 (1): 11-15.

Beyl, C. A. (2000). Getting started with tissue culture- media preparation, sterile technique and laboratory equipment. In: Trigiano, R. N. and Gray, D. J. (eds.), Plant tissue culture concepts and laboratory exercise, 2nd edition. USA: CRC Press, LLC, Ch 3.

Blamey, M. and Grey-Wilson, C. (1989). Flora of Britain and Northern Europe. ISBN 0-340-40170-2.

Çöçü, S.; Uranbey, S.; İpek , A.; Khawar, K.M.; sarihan, E.O.; Kayaparmaksiz, M.D. İ. and Özcan, S. (2004). Adventitious shoot regeneration and micropropagation in *Calendula officinalis* L. BIOLOGIA PLANTARUM 48 (3): 449-451.

Cui , M.; Takayanagi , K. and Handa ,T.(2004) High frequency of shoot regeneration from hypocotyls and stem segments of *Antirrhinum majus* (Snapdragon). Plant cell ,Tissue and organ culture.78: 51-53.

Faisal, M. and Anis, M. (2006). Thidiazuron induced high frequency axillary shoot multiplication in *Psoralea corylifolia* . Biologia Plantarum, Volume 50, Issue 3, pp 437-440.

Gomez, K.A. and Gomez, A.A. (1984). Statistical Procedures for the Agricultural Research. John Wiley & Sons; Int. Rice Res. Inst. Book 2 Ed.

Hall, R. (1999). Methods in Molecular Biology. Plant cell culture protocols. Springer Publishing Company, NY.

Huetteman, C.A and Preece, J.E.(1993): Thiadiazuron a potent cytokinin for woody plant tissue culture. - Plant Cell Tissue Organ Cult. 33: 105-119.

Minlong, C.; Takayanagi, K.;Kamada, H.; Nishimura, S. and Handa, T. (2000). Transformation of *Antirrhinum majus* L. by a rol-type multi-auto- transformation (MAT) vector system. Plant Science 159 :273 – 280.

Özturk, M.; Khawar, K.M.; Atar, H.H.; Sancak, C. and Ozcan, S.(2004). In vitro micropropagation of the aquarium plant Ludwiga repens. Asia Pacific Journal of Molecular Biology and Biotechnology ,12:21-25.

Perrini, R. ; Alba, V.; Ruta, C.; Morone-Fortunato, I .; Blanco, A. and Montemurro , C.(2009). An evaluation of a new approach to the regeneration of *Helichrysum italicum* (Roth) G. Don, and the molecular characterization of the variation among sets of differently derived regenerants. Cellular and Molecular Biology Letters, Volume 14, Issue 3, pp 377-394.

Shajiee, K. (2007). Comparison of rooting of African violet (*Saintpauliaionantha Wendl.*) shoots in vitro and in vivo culture conditions. Acta Horticulturae. 748: 115-120.

Sheyab, S.; Shatnawi , M.A.; Shibli, R.A.; Obeidat, M.; Al-Shadaideh, A.N.; Alhussaen, K.M. and Abu-Zahra, T. (2010). Micropropagation and Medium Term Conversation of *Antirrhinum majus*. Jordan Journal of Agricultural Sciences, Volume6, No.2,171-182.

Shimasaki, K. and Fukumoto, Y. (1998). Effects of B vitamins and Benzyelaminopurine on Adventitious Shoot Formation from Hybocotyl Segments of snapdragon (*Antirrhinum majus* L.) plant biotechnology 15(4). 239-240.

Simon, S. and Petrasek, P. (2011). "Why plants need more than one type of auxin". Plant Science. 180 (3): 454-460.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. The lowa State University press, lowa.

Tawfic, A.A. (1992). Factors affecting proliferation, essential oil yield and monoterpenoid constituents of rosemary and sage cultured in vitro. PhD dissertation, university of Nebraska, Lincoln, USA.

Visser, C.; Qureshi, J.A.; Gill, R. and Saxena, P.K. (1992). Morphoregulatory role thidiazuron: substitution of auxin and cytokinin requirements for the induction of somatic embryogenesis in geranium hypocotyl cultures. Plant Physiol. 99: 1704-1707.

Wang, Y.T.; Gan, L.; Liu, W.; Yu, L.J. and Li, M.T. (2007). Research on the callus inducing and the cell growth and metabolism characteristics of Capparis spinosa L. Progress in Modern Biomedicine. 7(12): 1779-1783.

Xiaofang, Z.; Yafen, X. and Hongwei, L.(1992). Regulation of phytohormons on the regeneration of shoot-tips of *Antirrhinum majus* into plantlet in vitro. Journal of Northeast Forestry University, Volume 3, Issue 1, pp 16-22.

Yang, G.; Shen, S.; Kobayashi, T.; Matsubayshi, Y.; Sakagami, Y. and Kamada, H. (1999) Stimulatory Effects of a Noval Peptidyl Plant Grotwth Factor ,Phytosulfokine , on the Adventitious Bud Formation from Callus of *Antirrhinum majus* . plant Biotechnology, 16(3)231-234.

طرق الاكثار المعملى لنبات حنك السبع

على منصور حمزة ، أميمة محمد عبد الكافى , أحمد عبد المنعم هلالى و محمد صلاح المنجى
قسم الخضر والزينة- كلية الزراعة- جامعة المنصورة

أجرى هذا البحث بمعمل زراعة الأنسجة بقسم الخضر والزينة – كلية الزراعة – جامعة المنصورة خلال الفترة من ٢٠١١– ٢٠١٣. بهدف اكثار نبات حنك السبع معمليا بواسطة تكنيك زراعة الانسجة. وذلك لتقليل تكلفة الانتاج وانتاج شتلات متاطبقة وراثيا والحفاظ عليها.وقد اتبعت طريقتان للإكثار الدقيق: اكثار دقيق مباشر واكثار دقيق غير مباشر. للإكثار الدقيق المباشر تم الوصول الى أفضل طريقة لتعقيم بذور حنك السبع وهى استخدام الكحول الإيثيلى بتركيز ٧٠% ولمدة نصف دقيقة بالإضافة الى إستعمال الكلوروكس التجارى بتركيز ٣٠% (هيبوكلوريت الصوديوم بتركيز ١.٣%) ولمدة ٥ دقائق. دراسة تأثير زراعة كلا من السويقة الجنينية العليا والعقدة والاوراق الفلقيةعلى بيئة موراشيج وسكوج لتقيم أعلى النسبة المئوية للبقاء، عدد الأفرع/الجزء النباتى، عدد الأوراق وطول النموات الناتجة. وجد من الدراسة انه للحصول على أعلى عدد للنموات الخضرية(١٣.٥نمو) الحامل لأكبر عدد من الأوراق استخدام بيئة موراشيج وسكوج المزودة بالبنزيل أمينو بيورين بتركيز ٠,٥٠و١.٠٠ ملليجرام/اللتر والسكروز بتركيز ٣٠ جرام/اللتر. للتجذير المعملى صممت تجربة لدراسة تأثير نوع الأكسين المستخدم (اندول حمض البيوتريك ، اندول حامض الخليك و نفثالين حمض الخليك) وتركيز كلا منهما (صفر، ٠,٥٠، ١,٠ و ٢,٠ ملليجرام/اللتر) على النسبة المئوية للتجذير ، عدد و طول الجذور. أظهرت النتائج ان بيئة موراشيج وسكوج والتى زودت باندول حامض البيوتريك (IBA) بتركيز ٠.٥ و ١,٠٠ ملليجرام/اللتر قد سجلت أعلى نسبة مئوية للتجذير(٩٠%). بينما استخدام نفس البيئة السابقة والتى زودت بنفس الاندول ولكن بتركيز ١.٠٠ ملليجرام/اللتر قد اعطت اطول الجذور (٤,٥٨ سنتيمتر) ولكن عند استخدام نفس البيئة السابقة والتى زودت نفثالين حامض الخليك (NAA) بتركيز ٠,٥ ملليجرام/اللتر قد أعطت أعلى عدد للجذور (١٢,٨٣ جذر). ووجد ان أقلمة النباتات الناتجة فى المخلوط (الفرميكيوليت + التربة الطميية) بنسبة (١:١ بالحجم) كان الأفضل فى اعطاء اعلى نسبة بقاء وكذلك اكبر زيادة فى طول الأفرع ولكن لم يكن هناك فروق معنوية بين الاربع مخاليط((بيت موس : فيوميكيوليت ، بيت موس : تربة طميية ، فيوميكيوليت : تربة طميية ، بيت موس : فيوميكيوليت : تربة طميية بنسبة ١:١ بالحجم للجميع). فى الصفة الأخيرة.. اما للإكثار الدقيق الغير مباشر وللحصول على الكالس تم زراعة القمم النامية على بيئات مغذية زودت بعدة انواع من الأكسينات والسيتوكينينات وذلك بتركيزات مختلفة. وللحصول على أفضل كالس يفضل الزراعة على بيئة مغذية مضاف اليها ثيدىازيورون بتركيز(١.٠٠ ,٢.٠٠ و ٤.٠٠ ملليجرام/اللتر). وتم الحصول على أعلى عدد للنموات الخضرية المتكشفة من الكالس، عندما نقل الكالس المتكون على بيئة مغذية مضاف اليها ثيدىازيورون بتركيز ٢,٠٠ ملليجرام/اللتر و بنفثالين حامض الخليك بتركيز ٠,٥ ملليجرام/اللتر.

قام بتحكيم البحث

أ.د / محمد نزية شرف الدين	**كلية الزراعة – جامعة المنصورة**
أ.د / عزة عبد العزيز توفيق	**كلية الزراعة – جامعة اسيوط**