Jordan Journal of Agricultural Sciences, Volume **6**, No.**2**, 2010

Micropropagation and Medium Term Conservation of *Antirrhinum majus* L.

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ABSTRACT

Antirrhinum majus is an attractive herbaceous plant, cultivated as ornamentals for its attractive view. An efficient *in vitro* protocol for *A. majus* was optimized. Shoot tips were excised from field grown mature plants and transferred to hormone-free Murashige and Skoog (MS) medium. The addition of 1.5 mg/L kinetin and 0.5 mg/L benzyl adenine (BAP) produced the highest multiplication rate (2.37) per explant. There was no callus at the bases of the microshoots. Micorshoots were rooted on MS medium containing indole-3-butyric acid (IBA), indole acetic acid (IAA) or naphthalene acetic acid (NAA) at 0.0, 0.4, 0.8, 1.2, 1.6 or 2.0 mg/L. Rooting did not occur in the absence of IBA, IAA or NAA. Ninety percent of the microshoots were rooted on MS medium supplemented with 0.4 mg/L IBA, IAA or NAA. A total of 90% survival was achieved when rooted explants were acclimatized *ex vitro* using 1 soil: 1 perlite: 1 peat mixture. In another experiment, *in vitro A. majus* explants were successfully stored without serious losses by using MS medium supplemented with an appropriate concentration of 3% sucrose, 3-6% sorbitol or 6% glucose at 24 ± 2 °C for up to 32 weeks.

Keywords: *Antirrhinum majus* L., Micropropagation, Medium-term conservation.

INTRODUCTION

Antirrhinum majus L. (snapdragon) is a herbaceous plant, cultivated as ornamentals for its attractive view (Neal, 1965; Oyama and Baum, 2004). Snapdragons have right stems dressed with two-lipped tubular flowers that come in an almost endless palette of colors, from white to yellow to orange to red to purple and almost black. Moreover, Snapdragon has been used in molecular studies of transposons, flower pigmentation, flower development and shoot regeneration (Sommer *et al*., 1988; Lister and Martin, 1989; Cui *et al*., 2001). Snapdragon if propagated by seeds can not 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 this germplasm with potential relevance as an ornamental crop. A rapid propagation system for *A. 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

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Received on 18/2/2009 and Accepted for Publication on 27/9/2009.

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production of *A. majus* would alleviate these problems, aiding in the commercial success of the crop.

Micropropagation can be used as a tool in plant breeding process. In this sense, the micropropagation would allow the propagation of selected traits within a specific genotype, besides the propagation rate, the space exploitation and the improvement of the sanitary. This tool also has the advantages that the results are obtained relatively fast and also that plant environment can be fully controlled (Singh *et al*., 2000; Cavagnaro *et al*., 2006). Acclimatization stage is considered as one of the important stages in the *in vitro* plants. Plantlets grown *in vitro* were exposed to a unique microenvironment that provides minimal stress and optimum conditions for plant multiplication. Plantlets were developed within the culture vessels under low level of light and in aseptic conditions, on a medium containing ample sugar and nutrients to allow for heterotrophic growth in an atmosphere with high level of humidity (Hazrika, 2003). Conservation of plant material via tissue culture has involved an immense research effort on many laboratories around the world. Slow growth techniques are strongly recommended for the storage of shoot cultures in many industrial laboratories as well as in many regional and international germplasm conservation centers (Shatnawi *et al*., 2006). The miniaturization of explants also allows the reduction of space requirements and consequently labor costs for the maintenance of germplasm collections. Moreover, this allows the storage of healthy germplasms with extended subculture intervals, thus reducing the time needed and costs of maintenance. Therefore, the present study was undertaken to develop a protocol for mass propagation of *A. majus* through *in vitro* culture 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 programs.

MATERIALS AND METHODS

Establishment of *in vitro* Culture

Shoot tips were collected from healthy plants grown in the field station of Al-Balqa Applied University, Al-Salt, Jordan. Snapdragon shoot tips were then sterilized in 5% chlorox (5.25 sodium hypochlorite) plus 0.1 Tween-20 (surfactant) for 10 min and then rinsed three times with sterile distilled water (for 5 min each time) under laminar air flow cabinet, followed by the inoculation on half strength MS medium (Murashige and Skoog, 1962). The medium was supplemented with 0.01 mg/L benzyl adenine (BA), 30 g/L sucrose and 8 g/L agar agar. Medium pH was adjusted to 5.8-5.9 prior sterilization. Shoot tips were kept in the dark for at least 2 d, then moved to the growth chamber (24 ± 2 ºC) under 16 h light (photosynthetic flux 40-50 µmol $m^{-2}S^{-1}$) / 8 h darkness. Microshoots were then transferred to a new MS medium supplemented with the same growth regulators. Four weeks later, microshoots were transferred to fresh media before starting *in vitro* multiplication. Figure 1 outlines the general experimental approach for this study. Subculturing was performed every four to five weeks in order to establish a massive mother stock culture before initiating the experiments.

Effect of Growth Regulators on Shoot Proliferation

Microshoots (15 mm in length) were subcultured to hormone-free MS medium for two weeks to eliminate any carry-over effects of growth regulators. Microshoots were then subcultured onto fresh MS medium. For shoot proliferation experiments, microshoots were subcultured on MS medium supplemented with different concentrations of benzyl adenine (BA) and kinetin; a) (Kin) (0.0 mg/L BA+ 0.0 mg/L Kin (control), b) 0.0 mg/L BA + 0.5 mg/L Kin, 0.5 mg/L BA + 0.5 mg/L Kin,

c) 1.0 mg/L BA + 0.5 mg/L Kin, d) 1.5 mg/L BA + 0.5 mg/L Kin, e) 2.0 mg/L BA + 0.5 mg/L Kin, f) 0.5 mg/L BA + 0.0 mg/L Kin, g) 0.5 mg/L BA + 1.0 mg/L Kin, h) 0.5 mg/L BA + 1.5 mg/L Kin and i) 0.5 mg/L BA + 2.0 mg/L Kin. For each replicate, an amount of 50 mL of the medium was dispensed into a 250 mL culture flask. Each treatment consisted of 4 replicates, each with 5 microshoots. Each experiment was repeated twice. Culture condition was maintained as described above. Following six weeks, data were collected on the number of shoots/explant and shoot height.

Effect of Auxins on Root Formation *in vitro*

Microshoots (15 mm in length) were subcultured on MS medium containing indole-3-butyric acid (IBA), indole acetic acid (IAA) or naphthalene acetic acid (NAA) at 0.0, 0.4, 0.8, 1.2, 1.6 and 2.0 mg/L. For each replicate, an amount of 50 mL of the medium was dispensed into 250 mL culture vessels. Each treatment consisted of ten replicates, each with three microshoots. Culture conditions were identical to those described previously. Six weeks later, data were collected on shoot length, number of roots/explant, root length and percentage of shoots developing roots.

Effect of Solidifying Agent on *in vitro* Growth

Explants were subcultured on MS medium free growth regulators for two weeks prior to the experiments. Microshoots (15 mm) were placed on MS medium supplemented with 0.5 mg/L BA with one of the following solidifying agents:

1. Agar agar at 0.2, 0.4 or 0.6% (Scharlu chemical company),
2. Nutrient agar at 0.2, 0.4 or 0.6% (Fluka chemical company),
3. Gelcarin at 0.2, 0.4 or 0.6% (Sigma chemical company) and
4. Phytagel at 0.2, 0.4 or 0.6% (Sigma chemical company).

After six weeks, data were collected on number of shoot and shoot height.

***Ex vitro* Acclimatization**

In vitro rooted plantlets were extracted from the medium, washed under a running tap water to remove agar and transplanted to 5 X 5 cm plastic pots filled with sterile mixture of 1 peat: 1 perlite: 1 sand. Shoots were grown under intermittent mist. Humidity was reduced gradually to ambient conditions over a period of about three weeks, until the plants produced new leaves, and to allow plantlets to develop large quantities of surface waxy layer to reduce water loss. Data were collected for survival percentage of the acclimatized plants.

Medium Term Conservation

Prior to the experiment, *in vitro* grown plantlets were transferred to hormone-free MS medium for 2 weeks to eliminate any carry-over effects of growth regulators. Plantlets of 15 mm length were then transferred to hormone-free MS medium supplemented with a series of concentrations of sucrose, sorbitol or glucose at 0, 3%, 6%, 9% or 12% (w/v). Subculturing was performed by plating the explants into different media under sterile conditions. The stored explants were incubated in the growth room under 16 h light/8 h darkness (50 μmol $m^{-2}s^{-1}$) or under dark conditions at 24 ± 2 °C. Survival data were collected after 16 and 32 weeks in storage, and for regrowth percentage after 32 weeks following subculture on hormone-free fresh MS medium.

Statistical Analysis

Data were subjected to one-way ANOVA using STATISTICA (StatSoft, 1995). Differences between individual means were determined by least significant differences (LSD) at 0.05 level of probability. Values presented were the means and standard errors (SE).

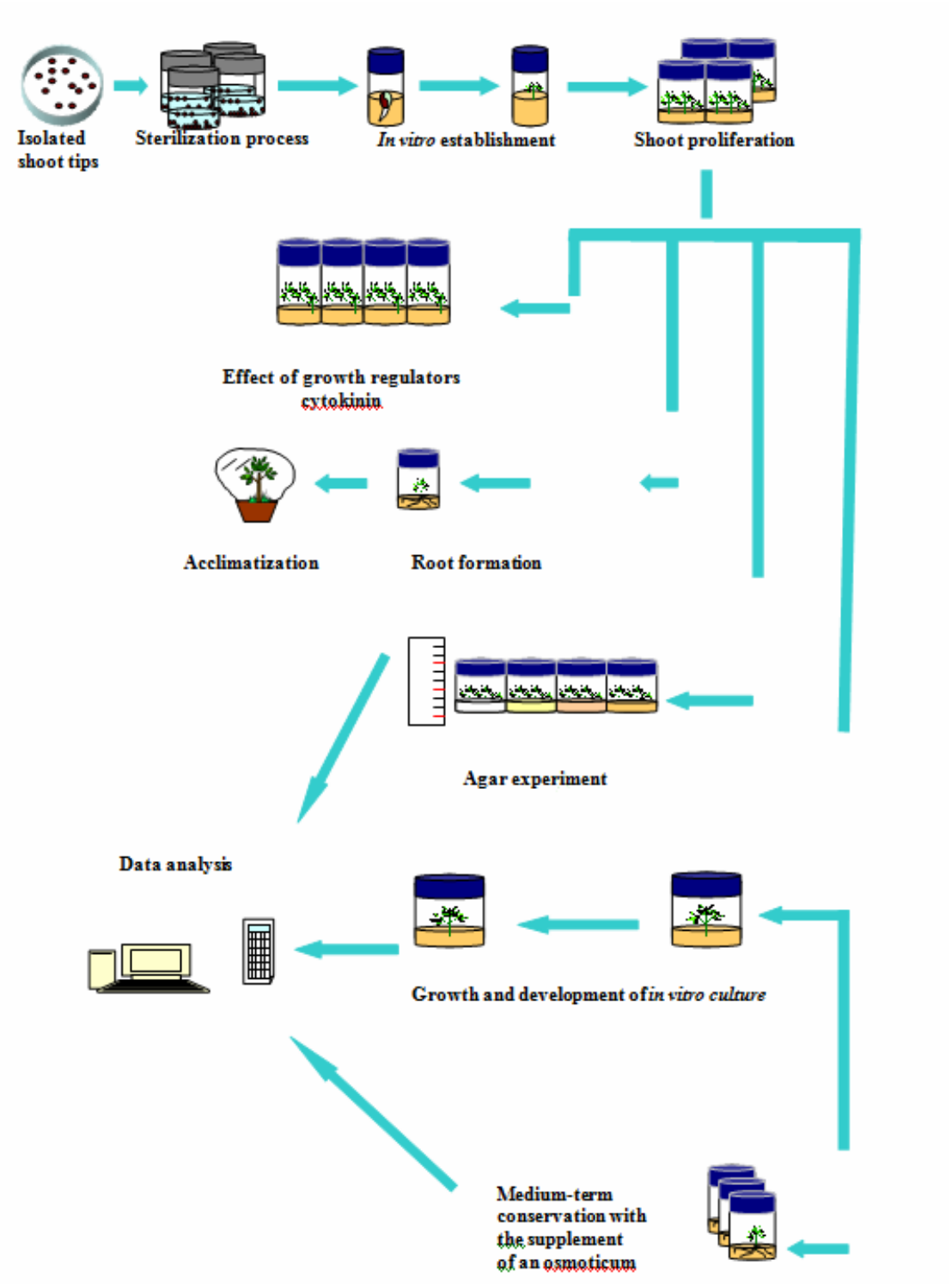

Figure 1: Outline of the research of this study.

RESULTS AND DISCUSSION

The influence of BA and Kin combination was different when compared to the control. The maximum number of new shoots formed was recorded when the medium was supplemented with 1.5 mg/L Kinetin and 0.5 mg/L BA (Table 1). This was not significant when compared to the medium supplemented with 2.0 mg/L BA and 0.5 mg/L Kin. Özturk *et al*. (2004) reported that a higher maximum number of new shoots of *Ludwigia repens* was obtained on MS medium supplemented with 0.05 mg/L BA and 0.1 mg/L TDZ (Table 1). A possible explanation of this observation would be the balance between the exogenous growth regulators and the endogenous hormones. As the culture progressed, the available exogenous cytokinin was consumed or degraded, changing BA/kinetin ratio to levels adequate for the proliferation of the shoot. In medium supplemented with BA and Kinetin, shoot length was higher than the control. The number of leaves was increased in all treatments when compared with the control. Plants grown with (BA + Kin.) had differentially higher fresh and dry weight, with the greatest reduction noted at BA (2.0 mg/L) + Kin (0.5 mg/L) and BA (2.0 mg/L) + Kin (2.0 mg/L). Higher proliferation rates were obtained on MS medium containing BA and Kinetin. A study by Balogun *et al*. (2007) showed that increased BA concentration enhanced multiple shoot formation from apical shoot bud of *Telfairia occidentalis*. The optimal shoot proliferations were recorded with BA and Kin due to the pulse treatment between BA and Kin. Any micropropagation system must produce a large number of uniform plants similar to the original plant from which they were propagated; this could be achieved using meristematic regions. Thus the presence of BA and Kin facilitated shoot proliferation. Similar results were also obtained in apple (Fasolo *et al.,* 1989). Thus the mass propagation of this species through *in vitro* culture is one of the best and most successful examples on commercial application on *in vitro* culture technology.

Root formation is one of the important issues on the *in vitro* culture. In this study, the results showed that microshoots were successfully rooted *in vitro* on MS medium supplemented with different concentrations of IBA, IAA or NAA (Table 2). Root initiation was first observed after 15 days in culture containing 0.8-2.0 mg/L IBA. Roots did not appear in the absence of auxin in any of the species. In these experiments, the number of shoots increased with increasing IBA, IAA and NAA. MS medium containing IBA, IAA or NAA increased the shoot lengths compared with the control. A maximum shoot height (59.8 mm) was obtained on MS medium supplemented with 1.2 mg/L IBA (Table 2). IBA concentration at 0.8-2.0 mg/L induced the maximum number (13.56-14.80) of roots, respectively. On the other hand, the maximum shoot length was obtained at 1.2 mg/L IBA, with an average of 33.8 mm root length. Rooting percentage increased up to 100% with 2.0 mg/L IBA or NAA, while with IAA reached 100% at 1.2 mg/L. Rooted shoots could be transplanted after 6 weeks into commercial soil mixture under greenhouse conditions.

Enhanced rooting was obtained in a range of treatments, whereas no rooting was found in the control (Table 2). With the supplement of auxin to the media, mostly single shoots were produced. Perez-Tornero *et al*. (2000) reported a small percentage of rooting in the absence of auxin; however, this was significantly lower than that obtained when auxin was present at any concentration in the medium. Low concentrations of NAA and IAA were effective in inducing rooting, and the development of roots was quite similar at the different concentrations. This has also been recorded in *Hadeoma multiflorum* with the supplement of 0.05 µM NAA (Koroch *et al*., 1997). Root induction was also

achieved, from the bases of excised shoots, in the presence of IBA, NAA or IAA, which is similar to previous findings by Shatnawi *et al*. (2004) in *Syzygium alternifolium*. Transfer of sterile rooted plantlets to greenhouse conditions is a critical operation, particularly for woody plant species. Rooted plants, when moved to acclimatization conditions, showed 90% survival. Plantlets resumed normal growth in the greenhouse, developing new leaves within 20 days.

The solidifying agent had a significant effect on the formation of new shoots and on shoot length. The formation of new shoots was significantly affected by the concentration and the type of the solidifying agent (Table 3). Increasing agar agar concentration influenced significantly the number of new microshoots produced from the explants. Moreover, increasing agar agar up to 0.6% produced higher shoot length compared to 0.2 and 0.4%. Increasing the concentration of phytagel or gelcarin decreased significantly the number of shoots produced per explant. Shoot length decreased with increased concentration of nutrient agar, phytagel and gelcarin. In this experiment, agar showed some superiority over the other solidifying agents, which is similar to previous findings on *Ceratopetalum gummiferum* (Armstrong *et al*., 1999).

The addition of sucrose to the medium increased survival when compared to media without sucrose for cultures stored without subculturing for 32 weeks. A total of 95.6 % explants that were stored on a medium supplemented with 3% sucrose survived after 32 weeks, while 84.3% survived in light conditions. Moreover, 44% were able to regrow after 32 weeks if stored in dark conditions on a medium supplemented with 3% sucrose. Regrowth and survival after 32 weeks also declined significantly with increased sucrose concentration from 3% to 12% (Table 4). A total of 40% of those stored on medium supplemented with 3% sorbitol were able to regrow after 32 weeks in light conditions, while only 22% were able to regrow after 32 weeks if stored in the dark. In addition, 45% were able to regrow after 32 weeks of storage on a medium supplemented with glucose if stored in light conditions. A total of 22% were able to regrow after 32 weeks, if stored in dark conditions in a medium supplemented to 3% glucose (Table 4). Sucrose was able to play an important role in the conservation of explants compared with the controls. Bertrand-Desbrunais *et al*. (1992) found that the elongation of *in vitro* stored shoot tips of C*offea* spp. was maximized at a sucrose concentration of 2% and that shoot tip elongation decreased as the sucrose concentration was increased in the medium. With respect to the other osmotica used, Dekker *et al*. (1991) found that the optimum mannitol concentration for *in vitro* storage of gingers was 2.5%. There was, however, much variation in the responses among the different osmoticums tested. In the current study, the addition of sorbitol to the medium increased survival at low concentrations. This is in agreement with previous findings by Zandvoort *et al*. (1994). Survival and regrowth percentages declined severely when cultures were stored in the dark on a medium supplemented with glucose, compared to those stored under light conditions with the supplemented of sucrose. This study has shown that microshoots of snapdragon can be stored without serious losses by using an MS medium supplemented with an appropriate concentration of sucrose, sorbitol or glucose in the growth chamber under light conditions for up to 32 weeks.

Table 1: Influence of BA and kinetin combination in growth parameters of snapdragon (*A. majus*) after six weeks growth period *in vitro.*

Growth regulators (mg/L)	*Number of new shoots per explant*	*Shoot height (cm)*	*Number of leaves*	*Fresh weight (gm)*	*Dry weight (gm)*
Control	1.00±0.0d	5.95±0.27bc	11.4±1.04d	0.12±0.01c	0.02±0.002b
BA(0.0)+Kin(0.5)	1.00±0.0d	6.07±0.27bc	12.4±1.04d	0.12±0.01c	0.02±0.002b
BA(1.0)+Kin(0.5)	1.80±0.27c	6.83+0.48b	12.4±1.20d	0.158±0.02b	0.02+0.001b
BA(1.5)+Kin(0.5)	1.73±0.27c	8.27±0.43a	16.3±0.75b	0.165+0.01b	0.02±0.001b
BA(2.0)+Kin(0.5)	2.27±0.25ab	6.43±0.70b	19.8±1.48c	0.170±0.03b	0.03±0.003a
Kin (0.0)+BA (0.5)	1.26±0.19d	5.43±0.64cd	12.8±1.16d	0.21±0.03a	0.035±0.004a
Kin (0.5)+BA (0.5)	1.80±0.26c	5.47±0.67cd	15.9±1.40c	0.21±0.03a	0.03±0.004a
Kin (1.0)+BA (0.5)	1.71±0.19c	6.63±0.76b	19.9±1.60a	0.17±0.01b	0.02±0.002b
Kin (1.5)+BA (0.5)	2.37±0.37a	7.94±0.82a	12.9±0.92d	0.17±0.02b	0.02±0.002b
Kin (2.0)+BA (0.5)	1.20±0.14d	5.83±0.65c	10.1±0.63e	0.23±0.03a	0.035±0.003a

Values represent means; each treatment consisted of 4 replicates, each with 5 microshoots and each experiment was repeated twice. The means within a column with the same letter are not significantly different based on least significant difference (LSD) at 0.05 level of probability.

Table 2: Influence of IBA, IAA and NAA on number of shoots, shoot length, number of roots and root length of *in vitro* grown snapdragon (*A. majus*) after six weeks growth period.

Growth regulator (mg/L)	***Number of shoots per explant***	***Shoot height (mm)***	***Number of roots per explant***	***Root length (mm)***	***Root formation (%)***
			IBA		
0.0	1.20+0.1a	27.30+5.6a	0.00+0.0a	0.00+0.0a	0.0
0.4	1.39+0.2ab	50.75+3.5c	8.24+1.3b	35.5+2.3b	90
0.8	1.29+0.3ab	51.70+3.5c	13.56+1.6c	33.4+3.3b	93
1.2	1.34+0.4ab	59.80+3.6d	15.78+0.9df	33.8+1.9b	93
1.6	1.33+0.3ab	48.80+3.7c	14.84+1.2d	30.3+2.1b	93
2.0	1.34+0.3ab	56.40+53.3d	9.80+1.4d	30.9+1.9b	100
			IAA		
0.4	1.30+0.2ab	41.3+3.6b	6.34+1.2b	54.5+4.8c	90
0.8	1.30+0.1ab	49.40+2.4c	8.45+1.7b	56.9+5.9c	90
1.2	1.35+0.2ab	49.76+2.3c	7.96+1.8b	57.7+2.4c	100
1.6	1.35+0.15ab	45.7+2.3b	6.20+3.3b	59.7+3.5c	100
2.0	1.30+0.15ab	49.58+2.4c	7.25+2.9b	57.8+3.5c	100
			NAA		
0.4	1.29+0.2ab	39.59+4.9b	9.20+0.4b	44.5+2.7b	90
0.8	1.30+0.3ab	50.67+4.9c	8.30+1.2b	53.2+4.6c	95
1.2	1.34+0.15ab	49.85+5.7d	7.27+1.3b	59.83+3.9d	95
1.6	1.32+0.1ab	49.70+2.8c	3.30+0.9b	60.7+5.9d	95
2.0	1.32+0.3ab	50.38+3.6c	3.35+0.9b	49.8+4.8c	100

Values represent means; each treatment consisted of 4 replicates, each with 5 microshoots and each experiment was repeated twice. The means within a column with the same letter are not significantly different based on least significant difference (LSD) at 0.05 level of probability.

Table 3: Effect of solidifying agent and concentration on number of new shoot and shoot length after six weeks growth period of *in vitro* snapdragon (*A. majus*) on medium supplemented with 2.0 mg/L Kinetin and 0.5 mg/L BA.

Solidifying agent	*Number of new shoots*	*Shoot length*
	Agar agar	
0.2%	1.52±0.35c	4.80±1.6 b
0.4%	1.91±0.26b	5.2±2.5 b
0.6%	2.35±0.3a	5.3±0.5 b
	Nutrient agar	
0.2%	1.41±0.20 c	3.5±1.2c
0.4%	1.52±0.22 c	2.6±0.5cd
0.6%	1.49±0.3e	1.9±0.4d
	Gelcarin	
0.2%	2.2±0.19 b	6.2±0.9 a
0.4%	2.3±0.2b b	6.4±1.3 a
0.6%	1.9±0.2 c	6.3±1.3 a
	Phytagel	
0.2%	1.7±0.2d	6.7±0.4 a
0.4%	2.36±0.17 a	6.9±1.2 a
0.6%	1.9±0.2 c	7.1±0.8a

Values represent means; each treatment consisted of 4 replicates, each with 5 microshoots and each experiment was repeated twice. The means within a column with the same letter are not significantly different based on least significant difference (LSD) at the 0.05 level of probability.

Table 4: Effects of different concentrations of sucrose, sorbitol or mannitol on survival and regrowth percentage of *in vitro* grown snapdragon (*A. majus*) stored under light/dark conditions at 24 ± 2 ºC after 16 or 32 weeks.

Osmotic agents % (w/v)	*Light condition*								
	Sucrose			**Sorbitol**			**Glucose**		
	Survival after 16 weeks	**Survival after 32 weeks**	**Regrowth after 32 weeks**	**Survival after 16 weeks**	**Survival after 32 weeks**	**Regrowth after 32 weeks**	**Survival after 16 weeks**	**Survival after 32 weeks**	**Regrowth after 32 weeks**
0	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
3	97.6e	95.6g	84.3d	68.6d	67.0d	40.3b	50.3d	33.3c	33.3d
6	68.3d	64.0f	33.6c	67.6c	66.6d	39.6b	50.3d	48.3d	45.6e
9	58.3cd	39.6cde	28.3bc	55.6d	0.0a	0.0a	50.0d	31.6c	23.0c
12	45.6bc	35.0bcd	19.6ab	45.6c	0.0a	0.0a	38.3bc	3.6bc	11.7b
				Dark condition					
0	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
3	70.3d	46.6de	44.6c	60.6d	53.7c	22.6c	28.3b	26.3bc	22.6c
6	59.0cd	46.3de	40.3c	55.6d	55.0c	30.0b	44.6cd	33.3c	15.0c
9	59.0cd	48.3def	29.3bc	66.6d	35.0b	13.3a	38.6bc	18.2bc	10.7b
12	29.0a	9.6abc	2.6a	40.6bc	11.6a	0.0a	32.0bc	0.0a	0.00a

Values represent means; each treatment consisted of 15 replicates, with 3 microshoots in each treatment and each experiment was repeated twice. The means within a column of the three hormones tested with the same letter are not significantly different based on least significant difference (LSD) at 0.05 level of probability.

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الإكثار الدقيق والحفظ المتوسط لنبات فم السمكة

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ملخص

فم السمكة (*Antirrhinum majus*) أحد النباتات البيتية الجذابة، ويزرع كنبات زينة من أجل منظره الجذاب. إن هدف هذه التجربة كان تأسيس نظام إكثار دقيق لنبات فم السمكة والحفظ متوسط الامد.إن أفضل النتائج تم الحصول عليها من التجارب في الوسط المعروف باسم Murashige and Skoog الذي يحتوي على 1.5ملغم/لتر من الكاينتين و 0.5 ملغم/لتر من البنزيل ادنين مع نسبة تضاعف اغصان دقيقة قدرها 2.37 لكل نبات خارجي. إن هذه الأغصان الدقيقة قد جذرت في وسط MS الذي يحتوي على حامض الإندول بيوترك أو الإندول اسيتك أو النفثالين اسيتك في تراكيز مختلفة (صفر، 0.4، 0.8، 1.2، 1.6 أو 2 ملغم/لتر). إن الجذور لم تتكون بغياب الايوكسينات. وإن 90% من الأغصان الدقيقة قد جذرت في وسط MS المضاف له 0.4 ملغم/لتر من أحماض الاندول بيوترك، أو الاندول اسيتك أو النفثالين اسيتك. وقد نجحت نسبة 90% من النباتات التي تم الحصول عليها من النباتات المجذرة بعد عملية التقسية في الوسط الذي يحتوي على 1 تربة: 1 برلايت : 1 مواد دبالية. وفي تجارب أخرى فإن نباتات فم السمكة تم تخزينها بنجاح من دون فقد يذكر باستخدام وسط MS المميز بتراكيز مناسبة من مواد مثل: 3% ستروز، 3–6% سوربيتول ، 6% غلوكوز وفي درجة حرارة 24 °م لمدة تصل إلى 32 أسبوعاً. إن الطرق المطورة في هذه الدراسة يمكن استخدامها قاعدة للحصول على معلومات عن نبات فم السمكة.

الكلمات الدالة: نبات فم السمكة، الإكثار الدقيق، الحفظ المتوسط.

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تاريخ استلام البحث 2009/2/18 وتاريخ قبوله 2009/9/27.