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STUDIES ON THE MICROPROPAGATION OF LILY "*Lilium longiflorum* Thunb." BULBS II. MULTIPLICATION

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ABSTRACT

Explants of *Lilium longiflorum* produced *in vitro* in the first part of this study were used in the current work.

The highest shoots weight grown *in vitro* was scored by applying BA at 5 ppm. Plantlet weight, leaves number and weight, roots number and length reached their significant highest values at 30 g of sucrose/l. At sucrose concentrations of 60 g/l, roots weight and bulb diameter, length and weight were significantly the greatest.

INTRODUCTION

The genus *Lilium*, of the family Liliaceae, consists of about 80 species and a considerable number of varieties and hybrids. Easter lily (*L. longiflorum*) is undoubtedly the most commonly used as container plant **(Everett, 1981)**

The need to import lily bulbs of good strains every year make it necessary to adopt the tissue culture technique in order to provide the needed amount of bulbs and flowers instead of getting them from abroad.

The aim of this study was to establish an applicable protocol for the rapid micropropagation of *Lilium longiflorum* in order to get both plants for flowering and bulbs for planting.

Cytokinins play an important role in shoots proliferation in the tissue culture technique. **George (1993)** stated that the requirement for a particular cytokinin was sometimes noted for the promotion of adventitious shoots formation. For example, BA promoted axillary bud proliferation of *Castanea*, whereas kinetin was without effect. Similarly, kinetin produced only single shootss from Prunus shoots cultures. It was necessary to use BA to obtain multiple shootss. Level of cytokinins represents an important factor that affects the cultured explants. A big range of concentrationss was mentioned in the literature. **Gangaprasad *et al* (2003)** working on *Utleria salicifolia* (Fam. Asclepiadaceae) found that the multiplication and bud formation occurred with BAP at low concentrationss (0.1 ppm). **Thao *et al* (2002)** stated that shootss of *Alocasia* x *amazonica* and *A. cucullata* (Fam. Araceae) developed directly on

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MS medium supplemented with 10 mg/l BA (benzyladenine). **Zhu *et al* (2003)** showed that length of the apple rootsstock M26 shoots explants decreased with an increase in the concentrations of benzyladenine (and IBA) in the multiplication medium. Similar results were obtained with the addition of kinetin in the elongation medium.

However, in a few cases the presence of any kind of a cytokinin was not necessary, while failure of cytokinins to induce proliferation was reported in other ones. **Bertsouklis *et al* (2003)** ascertained that node explants of *Globularia alypum* (Globulariaceae) gave 2 shootss per explant when cultured on solid MS medium without plant growth regulators.

Sucrose concentrations

The level of sucrose in media influenced to a great extent the outcome of the tissue culture process. **Faria *et al* (2004)** studied the *in vitro* growth of *Dendrobium nobile* (Fam. Orchidaceae) using different sucrose concentrationss (0, 5, 10, 20 and 60 g/litre), in a modified ½ MS medium. They mentioned that sucrose is a very important component in the *in vitro* culture media, serving as a source of carbon and energy. A high multiplication rate was observed in plants cultured in medium supplemented with 60 g sucrose/litre, even without the addition of plant hormones.

MATERIALS AND METHODS

This is the second part of a study carried out in the Tissue Culture Laboratory of the Transplant Production Project, Faculty of Agriculture, Ain Shams University through three successive years (2002-2004).

Glass jars of 11.5 cm height x 6.5 cm diameter with their polypropylene caps were used. **Murashige and Skoog 1962** (MS) basal medium was prepared. This medium contained, in addition to the prescribed salts and vitamins, 30 g/l sucrose, 7 g/l agar and either different concentrationss of 2 cytokinins in experiment 1 or different concentrationss of sucrose in experiment 2. It was adjusted to pH 5.8. Jars were filled with 40 ml of the MS medium, and autoclaved at 121°C for 20 minutes under 1.05 kg/cm^2 pressure, left to cool and stored at 25±2°c for one week before being used in order to exclude contaminated jars.

Basal parts of leaves of *Lilium longiflorum* grown *in vitro* (in the first part of this study) were inocubated in these jars in aseptic conditions. After inoculation, jars were incubated at 25/20°C (day/night) ±2°C, 70% relative humidity. Two fluorescent tubes/shelf were installed at 30 cm above explants to provide light intensity of 2200-2400 lux at explants level.

Experiment 1

A completely randomized experiment in a factorial design was carried out to study two factors affecting multiplication process, i.e. the types of cytokinin as a main plot and the concentrations used as a subplot, with 6 replicates (jars) in each subplot. The two cytokinins used were:

1 - Benzyladenine (6-benzylaminopurine)

2 - Kinetin (6-furfuryl aminopurine),

referred to for short as BA or BAP and KI or Kin, respectively. These two cytokinins were used at concentrationss of 0, 0.10, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0, 7.5, and 10 ppm.

Experiment 2

Six treatments of MS media were prepared. Each treatment contained a certain level of sucrose. These levels were: 10, 20, 30, 60, 90 and 120 g sucrose/l. *In vitro* grown shootss, each comprised 3 leaves, were inocubated on these different treatments, with 6 replicates in each treatment. These treatments were arranged in a completely randomized design.

Total chlorophyll content was determined in shootss using the method described by **Moran (1982)** and all weights measured were fresh ones.

N.B.: all weights measured were fresh ones.

Data of these experiments were statistically analyzed using SAS 1995 computer program, and means were compared by L.S.D. method according to **Snedecor and Cochran (1980)**.

RESULTS AND DISCUSSION

Experiment 1. Effect of cytokinin types and concentrations on *Lilium* explants

1-1- Effect of cytokinin types and concentrations on shoots number Table (1)

a– There was no significant effect of cytokinin types on shoots number.

This result contradicts with the findings of some authors. **Mendes (1999)** observed that cultures of *Thymus mastichina* were multiplied in Collet modified medium. BAP was the best cytokinin during multiplication phase. **Mishra and Sreenath (2003)** mentioned that among the various growth regulators evaluated, BA was the most effective in the induction of shoots buds of *Coffea bengalensis* (Fam. Rubiaceae). **Pirinc *et al* (2003)** mentioned that the number of shootss of the diploid Diyarbakir watermelon cultivar "Surme" per explant was approximately more than 50% higher when BA was used compared to kinetin. **YanJu *et al* (2004)** carried out a study to determine the best condition for the multiplication of *Elaeagnus angustifolia*. They found that benzyladenine was the most effective growth regulator among the cytokinins involved (benzyladenine, kinetin, thidiazuron and zeatin).

b– Shoots number was affected significantly by cytokinin concentrations. The highest shoots number was induced by using cytokinins at 5 ppm. However, this record was not significantly different from those resulted by using cytokinins at 2.5, 7.5 and 10 ppm. The significant lowest shoots numbers resulted from using the lower range of concentrationss, i.e. 0, 0.10, 0.25 0.50 and 0.75 ppm. At 1 ppm concentrations, the resultant number was not significantly different from either the first group or the second one.

A lot of workers noticed the effect of cytokinin concentrations on shoots number. **Mishra and Sreenath (2003)** reported that shoots buds of *Coffea bengalensis* (a wild coffee species) were induced from explants incubated in (MS) medium supplemented with BA (2.5-10 mg/litre). The frequency of shoots regeneration was positively correlated with the concentrations of BA. **Zhu *et al* (2003)** showed that multiplication rate of the apple rootsstock M26 increased with an increase in the concentrations of benzyladenine (and IBA) in the multiplication medium. Similar results were obtained with the addition of kinetin in the elongation medium.

c – There was no significancy in the interaction between cytokinin typess and concentrationss effect on shootss number. Despite this fact, as the concentrations of either BA or Kin rose, shoots number increased to its utmost with BA at 2.5 ppm or with Kin at 5 ppm. Using higher levels of these growth regulators had a negative effect on this character.

The effect of a certain types of cytokinins at a definite level was shown by many researchers. The highest number of shoots was recorded for explants cultured in media containing 0.5 mg BAP/litre as mentioned by **Mendes (1999)** on *Thymus mastichina*, **Pirinc *et al* (2003)** on the diploid Diyarbakir watermelon cultivar "Surme", **Sudhakaran and Sivasankari (2003)** on *Ocimum basilicum*, explants maintained that kinetin at 1.0 - 1.5 mg/l gave the highest shoots number.

On the contrary, **Palai and Das (2002)** stated that concentrations of BAP higher than 6 mg BA/litre inhibited shoots multiplication from apical meristem explants of *Musa balbisiana* cv. Muguni. **Sharma *et al* (2003)** claimed that kinetin at all levels (0-5 mg/litre) failed to induce shoots regeneration of wheat genotypess DI 9, UP 2338, Raj 3765, WH 147, PBW 343 and WH 542

1-2- Effect of cytokinin types and concentrations on shoots weight (g) Table (1)

a – The effect of cytokinin types and concentrations on shoots weight was found to be significant. In average, using BA resulted in shootss being heavier than those grown in the presence of Kin.

b – The cytokinin concentrations exerted a significant influence on shoots weight. Values of shoots weight increased gradually with the rise in cytokinin level. However, this increase was not always significant, especially between each two adjacent levels used. In general, the heaviest shootss were a resulted of applying cytokinins at 5 ppm, while the lightest shootss were those treated with cytokinins at 0, 0.10, and 10 ppm.

c – The interaction between cytokinin types and concentrations proved to be significant. In both typess of cytokinin used, shoots weight increased gradually with the concentrations to a certain peak, to decrease again with the continuous increase in concentrations. With Kin, the highest shoots weight, achieved at 1 ppm, was significantly lower than the highest shoots weight scored by BA at 5 ppm.

The effect of cytokinins on shoots weight was studied by many scientists **Brum *et al* (2002)** reported that the highest values for fresh matter weights of aerial parts of fig (*Ficus carica*) were

obtained in the culture media supplemented with BAP at 2.0 mg/litre. **Gollagunta *et al* (2004)** stated that BAP increased the biomass in the shootss of *Hosta tokudama* 'Newberry Gold' during stage II (multiplication phase).

1-3- Effect of cytokinin types and concentrations on shoots length (cm) Table (1)

a – The influence of cytokinin types on shoots length was significant. In average, kin application resulted in longer shootss compared to those grown in the presence of BA.

This result is in accordance with that obtained by **Sudhakaran and Sivasankari (2003)** who mentioned that shootss of *Ocimum basilicum* were longer in kinetin-supplemented media whereas BAP-supplemented media showed reduced shoots length. However, **GenLin *et al* (2003)** reported that BA was found to be suitable for the height growth of adventitious shootss of *Ilex latifolia* from nodal segments.

b – There was a significant effect of cytokinin concentrations on shoots length. The more the concentrations increased from 0 to 1 ppm the longer the shootss were, with that at 1 ppm being significantly the longest. Higher cytokinin levels resulted in deterioration in shoots length, with that at 10 ppm being significantly the shortest.

In this concern, **Bhalla and Mulwa (2003)** stated that inclusion of 2 mg benzyladenine/litre in the medium produced the highest shoots length of macadamia (*Macadamia tetraphylla* cv. Johnson, a highly valued nut tree crop). Levels above 2 mg benzyladenine/litre in the medium produced stunted shootss. **Zhu *et al* (2003)** showed somewhat similar results as mentioned before.

c – A significant effect of the interaction between cytokinin typess and concentrationss on shootss length was detected. In case of BA, shootss, in general, got longer as concentrationss increased from 0 to 1 ppm. As BA concentrationss increased to 10 ppm, shoots length decreased to its significant lowest level. Situation with Kin was somewhat similar, although the significant longest shootss were obtained at lower Kin level.

The effect of cytokinins types and concentrations on shoots length was investigated by a lot of workers. **Sebastian *et al* (2002)** reported that the shootss of *Rotula aquatica* (Fam. Boraginaceae) were transferred for elongation to woody plant medium fortified with 0.5 mg kin/l.

Brum *et al* (2002) stated that the highest values for the lengths of aerial parts of fig (*Ficus carica*) were obtained in the culture media supplemented with BAP at 2 mg/litre. **Benniamin *et al* (2004)** stated that nodal segments of *Crateva magna*, Fam. Capparidaceae (a medicinal plant) cultured in MS medium supplemented with 2 ppm BAP produced multiple shootss with the greatest length (63.2+0.92 mm). **Beura *et al* (2003)** mentioned that MS medium containing 4 mg BAP/litre gave the greatest shoots length from single elongated buds of *Gladiolus* cv. American Beauty.

1-4- Effect of cytokinin typess and concentrationss on leaves number Table (1)

a – Leaves number was not significantly influenced by the types of cytokinin. However, BA induced more number of leaves than those induced by Kin.

b – Cytokinin concentrations affected leaves number significantly. As long as the concentrations increased, this character increased, though inconsistently, to reach its significant highest level at 7.5 ppm. Further increase in cytokinin concentrations to 10 ppm affected leaves number negatively.

c – The interaction between cytokinin typess and concentrations on leaves number was found to be statistically significant. The highest leaves number was achieved by incorporating BA at 7.5 ppm in the media. The corresponding record in case of Kin, though lower was a result of using only 5 ppm.

In this respect, **Nagaraju *et al* (2003)** concluded that supplementation of BA (0.5-2.0 mg/litre) in the media increased leaves number of *Cymbidium* Lunavian Atlas explants.

1-5- Effect of cytokinin types and concentrations on shoots content of total chlorophyll (mg/g fresh weight) Table (1)

a – The effect of cytokinin types on shoots content of total chlorophyll was not significant. However, this content was higher when using BA than when Kin was the cytokinin used.

Table 1. Effect of cytokinine types and concentrations on vegetative growth and chlorophyll content.

Conc. (ppm)	Shoots number/ explant			Shoots weight/ explant			Shoots length (cm)/ explant			Leaves number / explant			Total chlorophyll (mg/g)		
	Cytokinin type														
	BA	Kin	Mean of conc.	BA	Kin	Mean of conc.	BA	Kin	Mean of conc.	BA	Kin	Mean of conc.	BA	Kin	Mean of conc.
0.00	3.29	2.96	3.13b	1.00fg	1.01e-g	1.01c	7.76d-f	7.51 f	7.64 cd	8.63 f	10.54d-f	9.58 f	1.90	1.69	1.79a
0.10	3.17	3.21	3.19b	1.07e-g	1.10e-g	1.08 c	8.25d-f	11.80a-c	10.03ab	10.92d-f	10.54d-f	10.73ef	2.06	1.70	1.88a
0.25	3.50	4.13	3.81b	1.30b-g	1.09e-g	1.20bc	8.18d-f	12.39a-c	10.28ab	12.04d-f	11.08d-f	11.56d-f	2.14	1.76	1.95a
0.50	3.58	3.92	3.75b	1.35b-g	1.15d-g	1.25a-c	7.57ef	13.53a	10.55ab	11.67d-f	9.17ef	10.42ef	1.94	1.67	1.80a
0.75	3.50	3.79	3.65b	1.38b-f	1.23c-g	1.30a-c	8.14d-f	12.87ab	10.50ab	13.00c-e	9.25 ef	11.13d-f	2.17	1.77	1.97a
1.00	3.92	5.25	4.58ab	1.54b-d	1.45b-e	1.49ab	10.86bc	12.25a-c	11.56 a	11.46d-f	13.63b-d	12.54c-e	3.47	1.78	2.63a
2.50	7.38	5.17	6.27a	1.74ab	1.07e-g	1.40ab	9.98c-e	8.18d-f	9.08bc	13.33cd	13.63b-d	13.48cd	2.74	2.27	2.51a
5.00	6.67	6.21	6.44a	2.01a	1.05e-g	1.53a	10.00cd	7.70d-f	8.85bc	13.17cd	17.25 b	15.21bc	2.60	2.26	2.43a
7.50	6.71	6.00	6.35a	1.62a-c	1.01fg	1.31a-c	7.84d-f	7.93d-f	7.89cd	26.33 a	16.75bc	21.54 a	1.52	2.52	2.02a
10.0	5.79	5.96	5.88a	0.92g	1.10e-g	1.01 c	6.43 f	7.35 f	6.89 d	16.83bc	15.96bc	16.40 b	1.48	2.27	1.87a
Mean of type	4.75a	4.66a		1.39 a	1.12 b		8.50 b	10.15 a		13.74a	12.78a		2.20a	1.97a	
LSD at 5% of Types	N.S.			0.14			0.76			N.S.			N.S.		
LSD at 5% of Conc.	2.00			0.31			1.71			2.73			N.S.		
LSD at 5% of Inter.	N.S.			0.44			2.41			3.86			N.S.		

b – Cytokinin concentrations did not affect shoots content of total chlorophyll significantly. However, highest content was obtained at the concentrations of 1 ppm.

c – Effect of the interaction between cytokinin types and concentrations on shoots content of total chlorophyll was not a significant one. However, highest shoots content of total chlorophyll in general was a result of using BA at 1 ppm. The highest record with Kin was found with the 7.5 ppm treatment.

The influence of BA and kinetin on chlorophyll content varied among different plant materials. **Gusev *et al* (1989)** remarked that reducing the BA concentrations in MS medium increased the contents of chlorophylls a and b in explants of both *Ficus elastica* and *Scorzonera spp* (Fam. Compositae). However, **Saadawy (2000)** working on two orchid species found that the highest content of total chlorophyll was achieved by adding BA in MS multiplcation medium at 1 ppm for *Laelia anceps* or at 5 ppm for *Cymbidium devonianum*.

1-6- Effect of cytokinin types and concentrations on roots number Table (2)

a – Cytokinin types had a significant influence on roots number. The presence of BA in the culture media gave significantly lower number of roots compared to the corresponding number given by Kin.

b – Cytokinin concentrations exerted a significant negative effect on roots number. Significant highest roots number was found in explants grown in media free of cytokinins. The more the cytokinin concentrations increased, the less the rootss were produced. Significant lowest roots numbers were those found in media supplemented with cytokinins at 7.5 or 10 ppm.

c – The effect of the interaction between cytokinin types and concentrations on roots number was significant. The presence of BA in the media seemed detrimental to roots growth rendering it nil at almost all BA concentrationss. On the contrary, the use of Kin was not so drastic. Roots number decreased gradually as Kin increased from 0 ppm to 10 ppm.

Cytokinins are generally not in favor of roots induction. BA has a notorious reputation in this concern more than that of kinetin. **Muniswamy *et al* (2002)** reported that plantlets of *Coffea liberica* produced rootss on media supplemented with different levels of benzyladenine (0.1, 0.2, 0.5, 1.0 and 2.0 mg/l), however, increasing the concentrationss of benzyladenine inhibited rooting. On the contrary, **Kumar *et al* (2004)** mentioned that the regenerated shootss of *Gerbera jamesonii* were rootsed on MS medium containing 1 mg/l BAP (+ 0.1 mg/l IAA).

Table 2. Effect of cytokinin types and concentrations on roots growth / explant.

Conc. (ppm)	Roots number/ explant			Roots length / explant		
	Cytokinin types					
	BA	Kin	Mean of conc.	BA	Kin	Mean of conc.
0.00	2.33a	2.46a	2.40a	1.76a-c	1.74a-c	1.75a
0.10	0.04c	2.38a	1.21b	0.03 e	1.82a-c	0.92b
0.25	0.04c	2.54a	1.29b	0.02 e	2.04ab	1.03 b
0.50	0.00c	2.42a	1.21b	0.00 e	2.08 a	1.04 b
0.75	0.00c	2.17a	1.08b	0.00 e	1.60bc	0.80 b
1.00	0.08c	2.00a	1.04b	0.04 e	1.41 c	0.72 b
2.50	0.00c	1.21b	0.60c	0.00 e	0.79 d	0.40 c
5.00	0.00c	0.50c	0.25cd	0.00 e	0.21 e	0.11cd
7.50	0.00c	0.17c	0.08d	0.00 e	0.15 e	0.07 d
10.0	0.00	0.29c	0.15d	0.00 e	0.07 e	0.03 d
Mean of types	0.25b	1.61a		0.18 b	1.19 a	
LSD at 5% of Types	0.17			0.14		
LSD at 5% of Conc.	0.39			0.32		
LSD at 5% of Inter.	0.55			0.45o		

1-7- Effect of cytokinin types and concentrations on roots length (cm) Table (2)

Roots length was affected by cytokinin types, concentrations and their interaction in almost the same way as roots number was affected. In this connection. **Brum *et al* (2002)** mentioned that the highest values for the lengths of rootss of fig (*Ficus carica*) were obtained in the culture media supplemented with BAP at 2 mg/litre.

Experiment 2- Effect of sucrose concentrations in the medium

2-1- Effect of sucrose concentrations on plantlet weight (g) Table (3)

Sucrose concentrations affected plantlet weight significantly. Increasing sucrose concentrations from 10 to 20 g/l increased plantlet weight significantly. At 30 g of sucrose/l plantlet reached its highest significant weight. Higher concentrationss of sucrose, i.e. 60, 90 and 120g/l, significantly affected plantlet weight inversely.

The effect of sucrose concentrations in the tissue culture media on plantlet weight was studied by many workers. **Gollagunta *et al* (2004)** investigated the effects of sucrose concentrations (10-70 g/l) in the media on the growth of *Hosta tokudama* 'Newberry Gold'. They remarked that shootss cultured in 50 and 70 g/l media sucrose had less leaves chlorosis than those cultured in 10 and 30 g/l media sucrose. Endogenous concentrationss of soluble sugars (glucose, fructose, and sucrose) in the shootss increased linearly as the sucrose concentrations in the media increased from 10 to 70 g/l during stage II (multiplication phase). As a result of that, shoots biomass increased with increasing media sucrose concentrations. Increased media sucrose levels during the multiplication cycle has a positive, long-term effect on plant morphology and quality.

Table 3. Effect of sucrose concentrations on vegetative growth / plantlet.

Sugar concentrations (g/l)	Plantlet weight (g)	Shoots length (cm)	Leaves number/ plantlet	Leaves weight (g)/ plantlet
10	0.72c	11.35 b	8.58 c	0.44 c
20	1.55b	14.61 a	10.96 b	0.89 b
30	1.94a	14.58 a	15.79 a	1.01 a
60	1.67b	10.08 c	5.13 d	0.33 d
90	0.75c	5.83 d	4.86 d	0.10 e
120	0.42d	4.29 e	3.81 d	0.06 e
LSD at 5%	0.18	1.02	1.45	0.10

2-2- Effect of sucrose concentrations on shoots length (cm) Table (3)

Shoots length increased significantly as sucrose concentrations increased from 10 to 20 g/l. At 30 g sucrose/l the increase in shoots length was not significant. Further increase in sucrose concentrations to 60, 90 and 120 g/l caused a significant decrease in shoots length.

Faria *et al* (2004) studied the *in vitro* growth of *Dendrobium nobile* (Fam. Orchidacease) using different sucrose concentrationss of 0, 5, 10, 20 and 60 g/litre in a modified ½ MS medium. They reported that an increase in plant height was observed in plants cultured in medium supplemented with 60 g sucrose/litre, even without the addition of plant hormones. **Rafique *et al* (2004)** found that sucrose at 60 g/l in MS medium containing BA at 0.23 ppm had a significant effect on shoots length of 4 cultivars of potato (*Solanum tuberosum*). **Pounders and Nyochembeng (2005)** mentioned that tissue culture propagation efficiency for colored callas (*Zantedeschia sp.*) cvs. Florex Gold, Garnet Glow, Majestic Red, Mango, Pink Persuasion, Pot of Gold and Rubylite Pink Ice was improved, as reflected in propagule size, by increasing sucrose levels in production media from 30 g/liter up to 120 g/liter.

2-3- Effect of sucrose concentrations on leaves number Table (3)

Leaves number/plantlet was significantly influenced by sucrose concentrations. Raising sucrose concentrations from 10 to 20 g/l was reflected in a significant increase of this number. The highest significant leaves number was a resulted of 30 sucrose g/l. Further increase of sucrose concentrations to 60, 90 and 120 g/l had a detrimental impact on leaves number.

2-4- Effect of sucrose concentrations on leaves weight (g) Table (3)

Sucrose concentrations in the media affected leaves weight significantly. As long as this concentrations increased from 10 to 20 and 30 g/l, leaves weight increased significantly. Higher concentrationss, i.e. 60, 90 and 120 decreased this character significantly.

2-5- Effect of sucrose concentrations on roots number Table (4)

Roots number/plantlet increased as sucrose concentrations increased in the media from 10 to 20 g/l. At 30 g/l of sucrose, roots number reached its significant highest level. Higher sucrose concentrationss (60, 90 and 120 g/l) suppressed rhizogenesis significantly.

Faria *et al* (2004) studied the *in vitro* rooting of *Dendrobium nobile* (Fam. Orchidacease) using different sucrose concentrationss (0, 5, 10, 20 and 60 g/litre), in a modified ½ MS medium. They mentioned that sucrose concentrations in the culture medium did not influence the *in vitro* plant rooting. **Fatima *et al* (2005)** studied microtuber induction in an indigenous potato cultivar PARS-70 at different sucrose levels (30, 60, 90 and 120 g/l) supplemented in MS medium. They remarked that number of rootss was better at 60 g/l sucrose concentrations in MS medium.

2-6- Effect of sucrose concentrations on roots weight (g) Table (4)

Roots weight/plantlet was affected by sucrose concentrations significantly. As sucrose concentrations increased from 10 to 20, 30 and 60 g/l, roots weight increased significantly. At higher sucrose concentrationss (90 and 120 g/l), roots weight decreased significantly.

In this regard **Gollagunta *et al* (2004)** investigated the effects of sucrose concentrations (10-70 g/l) in the media on the growth of *Hosta tokudama* 'Newberry Gold'. They remarked that roots biomass increased with increasing media sucrose concentrations.

2-7- Effect of sucrose concentrations on roots length (cm) Table (4).

Roots length was significantly influenced by sucrose concentrations. The significant longest rootss were those produced in media supplemented with sucrose at 30 g/l. All changes in this concentrations, either negative or positive, induced shorter rootss.

Rafique *et al* (2004) found that sucrose at 60 g/l in MS medium containing BA at 0.23 ppm had a significant effect on roots length of 4 cultivars of potato (*Solanum tuberosum*).

Table 4. Effect of sucrose concentrations on roots growth / plantlet.

Sugar concentrations (g/l)	Roots number / plantlet	Roots weight (g)/plantlet	Roots length (cm)
10	3.29 e	0.01 c	0.80 e
20	11.46 c	0.15 c	2.59 c
30	33.00a	0.46 a	5.36 a
60	18.79b	0.52 a	4.40 b
90	12.86 c	0.22 b	2.89 c
120	6.31 d	0.09 d	1.59 d
LSD at 5%	2.99	0.06	0.74

2-8- Effect of sucrose concentrations on bulb number Table (5)

Sucrose concentrations had a significant effect on bulb number/plantlet. This number increased significantly as concentrationss increased from 10 to 20 g/l. Applying sucrose at 30 g/l gave rise to the significant highest value. More sucrose in the medium, i.e. 60, 90 and 120 g/l produced less number of bulbs.

In this respect, **Rafique *et al* (2004)** found that sucrose at 60 g/l in MS medium containing BA at 0.23 ppm recorded the maximum number of microtubers of 4 cultivars of potato (*Solanum tuberosum*). **Staikidou *et al* (2005)** stated that with Narcissus cv. St. Keverne and cv. Hawera, increasing the carbon supply (sucrose) stimulated bulblet production.

2-9- Effect of sucrose concentrations on bulb weight (g) Table (5)

Bulb weight/plantlet was influenced significantly by sucrose concentrations. Bulb weight increased significantly as sucrose concentrations increased from 10 to 20 g/l. At 30 g sucrose/l, bulb weight decreased insignificantly. Adding 60 g sucrose to one litre of medium resulted in the significant heaviest bulb weight. At higher sucrose concentrations, i.e. 90 and 120 g/l bulb weight decreased significantly. This might be attributed to plasmolysis imposed on bulb cells due to high sucrose concentrations.

In this connection, **Staikidou *et al* (2005)** stated that with Narcissus, increasing the carbon

supply (sucrose) stimulated total dry matter accumulation. In *Narcissus* cv. St. Keverne total dry matter accumulation and dry weight per bulblet were stimulated by increasing the sucrose supply from 19.0 to 37.9 g C/l (45 to 90 g sucrose/l). The *Narcissus* cv. Hawera showed similar responses to sucrose at 37.9 g C/l (90 g sucrose/l). **BongHee *et al* (2005)** stated that sucrose improved bulblet growth on *in vitro* grown shootss of *Lilium oriental* hybrid 'Casablanca'. Bulblet growth was effectively performed on MS medium containing 60 g/l sucrose, where bulblet weight reached an average of over 1100 mg.

2-10- Effect of sucrose concentrations on bulb height (cm) Table (5)

Bulb height was significantly influenced by sucrose concentrations. Significant longest bulbs were produced on media supplemented with sucrose at 60 g/l. Lower sucrose concentrations, i.e. 10, 20 and 30 g/l, produced bulbs measuring significantly less than the above-mentioned value. Higher sucrose levels (90 and 120 g/l) resulted also in significantly shorter bulbs.

2-11- Effect of sucrose concentrations on bulb diameter (cm) Table (5)

Sucrose concentrations exerted a significant effect on bulb diameter. Sucrose at 30 and 60 g/l resulted in producing the significant widest bulbs. Both lower sucrose concentrationss (10 and 20 g/l) and higher ones (90 and 120 g/l) produced thinner bulbs. The significant thinnest bulbs were those grown on media supplemented with sucrose at 120 g/l.

2-12- Effect of sucrose concentrations on scale number/bulb Table (5)

A significant effect was detected for sucrose concentrations on scale number/bulb. At sucrose concentrationss of 10, 20 and 30 g/l, scale number/bulb scored the highest significant records. Although there was no significant difference between these three values, scale number at 20 g sucrose/l was the greatest. Further increase in sucrose concentrationss to 60, 90 and 120 g/l, affected this character significantly.

2-13- Effect of sucrose concentrations on number of days to leaves yellowing and bulb formation Table (5)

Number of days to leaves yellowing and bulb formation was influenced significantly by sucrose concentrationss. As 10, 20 or 30 g of sucrose were added to one liter of medium, more days were needed for the bulbing process, with the later value being significantly the greatest. At higher sucrose concentrationss (60, 90 and 120 days), these days decreased significantly.

The above-mentioned effects of sucrose could be interpreted on the light of work done by **Fuentes *et al* (2005)** who reported that coconut (*Cocos nucifera* L.) plantlets grown *in vitro* with high exogenous sucrose (90 g/l) had reduced photosynthetic activity that resulted in a poor photosynthetic response. These plantlets also had low amounts of ribulose 1,5-bisphosphate carboxylase (Rubisco) protein, low Rubisco activity, and reduced growth despite showing high survival when transferred to the field. Decreasing the medium's sucrose concentrations from 90 to 22.5 g/l or 0 g/l resulted in increased photosynthetic response to light and CO2 along with increased Rubisco and phosphoenolpyruvate carboxylase (PEPC) activities and proteins. However, plantlets grown *in vitro* without exogenous sucrose died when transferred *ex vitro*, whereas those grown with intermediate exogenous sucrose showed intermediate photosynthetic response, high survival, fast growth, and *ex vitro* photosynthesis. Thus, exogenous sucrose at moderate concentrations decreased photosynthesis but increased survival, suggesting that both *in vitro* photosynthesis and exogenous sucrose reserves contribute to field establishment and growth of coconut plantlets cultured *in vitro*.

Table 5. Effect of sucrose concentrations on bulb growth/ plantlet.

Sugar concentrations (g/l)	Bulb number/ plantlet	Bulb weight (g)/ plantlet	Bulb height (cm)	Bulb diameter (cm)	Scale number/ bulb	No. of days to leaves yellow and bulb formation
10	1.33 c	0.22 d	1.03bc	0.91 c	11.50a	86.29d
20	1.63 b	0.34 b	1.07 b	1.03 b	12.54a	125.04c
30	3.54 a	0.31bc	1.06bc	1.26 a	11.67	23a.83a
60	1.71 b	0.65 a	1.26 a	1.28 a	9.96 b	168.50b
90	1.14cd	0.35 b	1.08 b	0.94 c	8.06 c	87.36d
120	1.06 d	0.24cd	0.98 c	0.83 d	7.83 c	67.50e
LSD at 5%	0.20	0.08	0.10	0.07	1.14	2.72

SUMMARY AND CONCLUSION

1– Multiplication

The cytokinin types had a significant effect on shoots weight, shoots length, roots number and roots length. Using BA resulted in shootss being significantly heavier than those grown in the presence of Kin. However, Kin application resulted in longer shootss compared to those grown in the presence of BA. The presence of Kin in the culture media gave significantly greater number of rootss and longer rootss compared to the corresponding number and length given by BA.

Cytokinins concentrations exerted a significant negative effect on roots number and length. Significant highest roots number and length were found in explants grown in media free of cytokinins. Shootss produced with cytokinins at 1 ppm were significantly the longest. Higher cytokinin levels resulted in deterioration in shoots length. The heaviest shootss and greatest number of them were a result of applying cytokinins at 5 ppm. However, shoots number at 5 ppm was not significantly different from those resulted by using cytokinins at 2.5, 7.5 and 10 ppm. As long as cytokinin concentrations increased, leaves number increased, to reach its significant highest level at 7.5 ppm. On the contrary, cytokinin concentrations did not significantly affect shoots content of total chlorophyll.

In case of BA, shootss got longer as concentrations increased from 0 to 1 ppm. Situation with Kin was somewhat similar, where the significant longest shootss were obtained with Kin at 0.5 ppm. With Kin, the highest shoots weight, achieved at 1 ppm, was significantly lower than the highest shoots weight scored by BA at 5 ppm. The highest leaves number was achieved by incorporating BA at 7.5 ppm in the media. The corresponding record in case of Kin was lower and was a result of using it at 5 ppm.

2 - Sucrose concentrations

Sucrose concentrationss of 20 and 30 g/l produced significantly the longest shootss. At sucrose concentrationss of 10, 20 and 30 g/l, scale number/bulb scored the significant highest records. Although there was no significant difference between the abovementioned values, shoots length and scale number at 20 g sucrose/l were the greatest. At 30 g of sucrose/l, plantlet weight, leaves number and weight, roots number and length, bulb number and the number of days to leaves yellowing and bulb formation reached their significant highest values. Higher concentrationss of sucrose significantly affected these characters inversely. At sucrose concentrations of 60 g/l, roots weight and bulb diameter were significantly the greatest. The same parameters at 30 g of sucrose /l were lower but not significantly different.

Significant longest and heaviest bulbs were produced on media supplemented with sucrose at 60 g/l.

RECOMMENDATIONS

Using BA at the multiplication stage at 2.5 ppm was suitable to get the highest number of shootss. The presence of sucrose in the medium at 30 g/l is better for the best shoots production, while using it at 60 g/l suits bulb formation to a great extent.

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حوليات العلوم الزراعية
جامعة عين شمس، القاهرة
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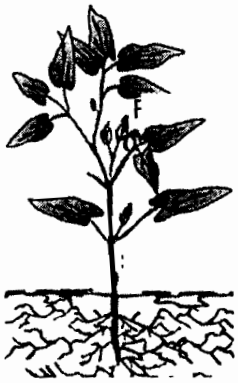

دراسات على الإكثار الدقيق لأبصال الليليم

٢- الإكثار

[٣٣]

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هذا هو الجزء الثانى من دراسة أجريت فى معمل زراعة الأنسجة الخاص بمشروع إنتاج الشتلات بقسم البساتين بكلية الزراعة-جامعة عين شمس خلال المدة من ٢٠٠٢ حتى ٢٠٠٤. وتهدف هذه الدراسة لوضع بروتوكول صالح للتطبيق للإكثار الدقيق السريع لأبصال الليليم. وقد إستعملت الأجزاء القاعدية من منفصلات الأوراق النامية فى الأنابيب على بيئة موراشيج وسكوج.

١- التجربة الأولى : تأثير السيتوكينين

تسبب البنزايل أدينين فى الحصول على أفرع أثقل بدرجة معنوية مقارنة بتلك النامية فى وجود الكينتين . ومع ذلك فقد تسبب الكينتين فى إنتاج أفرع أطول مقارنة بتلك النامية فى وجود البنزايل أدينين . كما أن وجود الكينتين فى بيئة الزراعة أعطى جذورا أكثر عددا وطولا بدرجة معنوية مقارنة بالعدد والطول المناظرين فى وجود البنزايل أدينين.

نمت أكثر الجذور عددا وطولا على المنفصلات النامية فى بيئة خالية من السيتوكينين (تركيز صفر جزء فى المليون). ونتجت أطول الأفرع عند إستعمال السيتوكينين بتركيز ١ جزء فى المليون. أما التركيزات الأعلى فقد تسببت فى تدهور طول الأفرع.

نتجت أثقل الأفرع وأكثرها عددا عند إستعمال السيتوكينين بتركيز ٥ جزء فى المليون. ومع ذلك فإن عدد الأفرع عند هذا التركيز لم يختلف معنويا عن تلك الناتجة بإستعمال السيتوكينين بتركيز ٢,٥ ، ٧,٥، ١٠ جزء فى المليون.

بإرتفاع تركيز السيتوكينين فإن عدد الأوراق كان يزداد ليصل إلى أقصاه معنويا عند تركيز ٧,٥ جزء فى المليون . وعلى العكس ، فإن تركيز السيتوكينين لم يؤثر معنويا على محتوى الأفرع الكلى من الكلوروفيل.

وفى حالة البنزايل أدينين ، إزداد طول الأفرع بزيادة التركيز من صفر إلى ١ جزء فى المليون . وكان الأمر مشابها تقريبا فى حالة الكينتين ، حيث نتجت أطول الأفرع من الناحية المعنوية بإستعمال الكينتين بتركيز ٠,٥ جزء فى المليون . وكانت أثقل الأفرع الناتجة بإستعمال الكينتين بتركيز ١ جزء فى

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المليون أقل وزنا بدرجة معنوية من أثقل الأفرع التى نتجت عند إستعمال البنزايل أدينين بتركيز ٥ جزء فى المليون. وقد تم إنتاج أكبر عدد من الأوراق بإستعمال بيئة تحتوى على البنزايل أدينين بتركيز ٧,٥ جزء فى المليون . أما العدد الأكبر الذى نتج عند إستعمال الكينتين بتركيز ٥ جزء فى المليون فكان أقل من السابق.

٢- التجربة الثانية : تأثير تركيز السكر فى البيئة

تسبب وجود السكر بتركيز ٢٠ و ٣٠ جم/لتر فى إنتاج أطول الأفرع . ووصل عدد الحراشيف/البصلة إلى الحد الأعلى معنويا عند تركيز سكر ١٠ ، ٢٠ ، ٣٠ جم/لتر. وبالرغم من عدم وجود فروق معنوية بين الصفتين المذكورتين فى التركيزات السابقة، فإن أكبرها ما كان عند تركيز ٢٠ جم/لتر. وأثر تركيز السكر بدرجة معنوية فى وزن النبيتات، وعدد ووزن الأوراق، وعدد وطول الجذور، وعدد الأبصال وعدد الأيام حتى إصفرار الأوراق وتكوين الأبصال. وقد وصلت هذه الصفات إلى أعلى المستويات معنويا عند تركيز ٣٠ جم/لتر.

عند وجود السكر بتركيز ٦٠ جم/لتر وصل وزن الجذور وقطر الأبصال إلى أعلى قيمة من الناحية المعنوية. ورغم أن هاتين الصفتين كانتا أقل قيمة عند تركيز ٣٠ جم/لتر، إلا أن الفرق لم يكن معنويا. وتأثر طول الأبصال ووزنها بدرجة معنوية بتركيز السكر. وكانت أطول الأبصال وأثقلها وزنا تلك الناتجة عند تركيز ٦٠ جم/لتر.

وتوصى هذه الدراسة بإستعمال البنزايل أدينين بتركيز ٢,٥ جزء فى المليون لإنتاج أكبر عدد من الأفرع . وكان وجود السكروز بتركيز ٣٠ جم/لتر فى البيئة هو الأفضل لإنتاج الأفرع ، بينما كان وجوده بتركيز ٦٠ جم/لتر أكثر ملائمة لتكوين الأبصال.