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STUDIES ON THE MICROPROPAGATION OF LILY "*Lilium longiflorum* Thunb" BULBS III – ROOTING AND ACCLIMATIZATION

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ABSTRACT

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

The significant highest number of roots was achieved with IBA at 5 ppm. Highest roots number in case of NAA was significantly lower than the previous one, and was induced at 0.50 ppm. The significant longest roots were obtained with IBA at 10 ppm. The longest roots with NAA (at 0.25 ppm) were shorter than those obtained with IBA.

The "Peat moss + Perlite" medium surpassed other media significantly in inducing the longest and heaviest plantlets, longest roots, the highest number of green leaves and shoots content of total chlorophyll.

INTRODUCTION

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

Rooting

Auxins, either endogenous or synthetic, are welknown rooting promoters. **Aswath *et al* (2003)** mentioned that in the absence of NAA, the initiation of a single roots was observed on shoots of gerbera (cvs. GJ-1, GJ-2 and GJ-3). The presence of NAA in the media induced the formation of multiple adventitious roots. As auxins vary in their chemical configuration, the types of auxin used might have a great effect on the plant material. The overall difference between IBA and NAA in their effects might be attributed to the fact that IBA is heat liable and decomposed during autoclaving while NAA is not denatured as mentioned by **George (1993)**. **Karwa (2003)** stated that the MS medium without auxins resulted in very poor or no rooting of *Citrus reticulata* Blanco (Nagpur mandarin). In some instances, however, it dosen't matter whether the auxin used was IBA or NAA as they behave similarly. **Zobayed and Saxena (2003)** stated that IBA or NAA effectively induced new roots (32 roots/explant) of St. John's

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wort (*Hypericum perforatum*, Fam. Clusiaceae) cv. 'New Stem'.

Auxin level in the media exerted a great effect on the cultered shoots and the expected roots. **Aloufa *et al* (2003)** on *Ximenia americana* (Fam. Oleaceae) mentioned that a low concentrations of IBA (0.1 ppm) was effective to promote roots formation on shoots of these plants. **WeiMei and FengYu (2003)** mentioned that in the 1/4-strength MS medium supplemented with IBA at 0.4 ppm, the rooting rate of *Actinidia macrosperma* shoots (Fam. Actinidiaceae) was 100%. The presence of IBA at 1 mg in the media gave the best rooting induction as mentioned by **LiJun (2001)** on *Spathiphyllum floribundum*, **TingHao *et al* (2002)** *Sinningia speciosa*, **Hosoki *et al* (2003)** on chocolate cosmos (*Cosmos atrosanguineus*). In the same regard, **LiJun (2001)** on *Spathiphyllum floribundum* and **Singh *et al* (2003)** on *Petunia hybrida* nana reported that media containing NAA at 1 mg were the best for rooting. **YanJuan *et al* (2002)** stated that the most suitable medium for rooting of *Michelia* (Fam. Magnoliaceae) was that contained IBA at 3 ppm. However, some plant material can do without auxins in the media. **Khan *et al* (2004)** on local and exotic garlic cultivars ascertained that the micro shoots of these plants were roots on media without auxins. On the other hand, auxins sometimes affected rooting negatively, particularly at certain levels. **Mederos-Molina (2003)** stated that the use of either IBA or NAA at 0.2 ppm, decreased the rooting capacity of *Chamaecytisus prolifer* (Fam. Fabaceae) shoots.

Acclimatization

Plantlets developed *in vitro* are very susceptible to the environmental conditions *ex vitro*. The stage of acclimatization is a very important procedure for the tissue culture process to be a profitable one. Components of the potting mixture play a big role in rendering acclimation a success. **XinPing *et al* (2003)** stated that the transplanting substrate for the *in vitro* produced *Rhododendron simsii* was composed of one part of peat soil and one part of perlite. **KwangSoo and GiWon (2004)** found that when planted in plastic pots filled with 1:1 peat moss and sand, plantlets of *Drosera peltata* (a tuberous sundew), a carnivorous plant, survived and exhibited normal development. **Vaidya and Bansal (2004)** stated that plantlets of *Bombax ceiba* were hardened and transferred to pots containing sand and soil (1:1).

MATERIALS AND METHODS

This is the third 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 different concentrations of one of 2 auxins in experiment 1. 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.

Experiment 1: Rooting

A completely randomized experiment in a factorial design was carried out to study two factors affecting rooting process, i.e. the types of auxin as a main plot and the concentrations used as a subplot, each subplot comprised 5 replicates (jars), with one shoots in every jar. The two auxins used were 1-naphthalene acetic acid and 3-indolebutyric acid, referred to for short as NAA and IBA, respectively. These two auxins were used at concentrations of 0. 0.10, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0, 7.5, and 10 ppm.

Shoots of *Lilium longiflorum* grown *in vitro* (in the second 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 explant level.

Data obtained in this experiment were: plantlet weight (g), shoots length (cm), leaves number, roots number, roots weight (g), roots length (cm), roots diameter (mm) and shoots content of total chlorophyll (mg/g fresh weight).

Experiment 2: Acclimatization

The acclimatization process started by subculturing plantlets produced in the previous rooting stage into ½ MS medium (sucrose and salts at half strength) supplemented with IBA at 0.1 ppm for growth enhancement. One month later jar caps

were loosened gradually for 1 week before removing plantlets to small plastic cups containing sterilized acclimatization media according to 4 regimes or treatments i.e.:

1 - Peat moss.

2 - Peat moss + sand (at 1:1 v/v)

3 - Peat moss + perlite (at 1:1 v/v)

4 - Peat moss + ¼ MS (plantlets were put with their roots immersed in ¼ MS liquid medium in plastic cups (7 x 8 cm) as a transient step for 1 week before being planted in peat moss).

All these treatments were replicated 4 times and arranged in a completely randomized design. These plastic cups were covered with polyethylene bags, which were perforated after one week before being removed ultimately. Total chlorophyll content was determined in shoots using the method described by **Moran (1982)**.

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 auxin types and concentrations on *Lilium* explants

1-1- Effect of auxin types and concentrations on plantlet weight (g) Table (1)

a– Auxin types had a significant influence on plantlet weight. Plantlets grown in the presence of NAA were significantly heavier than those grown in media supplemented with IBA.

b– Plantlet weight was significantly affected by auxin concentrations. As this concentrations increased from 0 to 7.5 ppm, plantlet weight increased from the lowest level to the highest one. When the concentrations was raised to 10 ppm, a decrease occurred insignificantly.

c– A significant interaction effect was detected between auxin types and concentrations on plantlet weight. In media supplemented with IBA, plantlet weight increased as the concentrations rose from 0 ppm to 7.5 ppm. At 10 ppm of IBA this weight decreased insignificantly. The case with NAA was somewhat different. Plantlet weight increased to the significant highest weight by raising NAA level from 0 to 2.5 ppm, respectively. More increase in NAA level from 5 to 7.5 and 10 ppm reduced this character gradually, though insignificantly.

In this concern, **Saadat and Hennerty (2002)** stated that for rooting of Persian walnut (*Juglans regia*) shoots, media containing IBA at 0.01 or 0.1 mg/litre were significantly better than media containing IAA for shoots fresh weight, but neither of them was significantly different from media containing NAA at the same concentrations. **Aloufa *et al* (2003)** reported that the medium supplemented with 0.1 ppm IBA improved number of roots/shoots of *Ximenia Americana* (Fam. Oleaceae).

1-2- Effect of auxin types and concentrations on shoots length (cm) Table (1)

a– NAA was significantly the causative agent for producing longer shoots compared to those grown in the presence of IBA.

b– Auxin concentrations affected shoots length significantly. Auxin free media produced the significant shortest shoots. The more the auxin level increased from 0.10 to 2.50 ppm the more the shoots length increased. More auxin levels up to 10 ppm, resulted in shorter shoots.

c– There was a significant effect for the interaction between auxin types and concentrations on shoots length. IBA in the media at concentrations from 0 to 2.5 ppm induced a gradual increase in shoots length. Higher IBA levels, from 5 to 10 ppm, affected shoots length negatively. When the growth regulator used was NAA, the same trend was adopted with some alterations. The significant longest shoots were obtained at 1 ppm. Higher levels of NAA had also the same negative effect.

Mendes (1999) stated that the best results for shoots length of *Thymus mastichina* plantlets were observed with 1 mg/l NAA. **Aswath *et al* (2003)** stated that the shoots height of gerbera (cvs. GJ-1, GJ-2 and GJ-3) was highest (4.9 cm) in media containing NAA at 2 mg/litre. **Bekheet *et al* (2004)** mentioned that IBA at a concentrations of 2 mg/litre gave the best results of shoots height of onion explants.

Table 1. Effect of auxin types and concentrations on vegetative growth / plantlet and chlorophyll content .

Conc. (ppm)	Plantlets weight (g)			Shoot length (cm)			Leaves number/ plantlet			Total chlorophyll (mg/g)		
	Auxin type											
	IBA	NAA	Mean of conc.	IBA	NAA	Mean of conc.	IBA	NAA	Mean of conc.	IBA	NAA	Mean of conc.
0.00	0.59 k	0.72 jk	0.65 e	8.41 h	10.92g	9.66 f	4.85	4.20	4.53 c	2.67 cd	2.26c-f	2.46bc
0.10	0.95 i-k	1.38 hg	1.17 d	9.47h	13.14ef	11.30 e	6.85	9.50	8.18ab	5.04 a	1.72d-g	3.38 a
0.25	0.90 i-k	1.78 ef	1.34 d	9.38h	14.88b-d	12.13 de	6.65	9.20	7.93 b	4.90 a	1.58e-g	3.24 a
0.50	1.00 h-j	2.24 cd	1.62 c	10.87 g	14.86b-d	12.90cd	7.05	9.65	8.35 ab	4.27ab	1.35fg	2.81ab
0.75	1.07 h-j	2.52 b-d	1.80 bc	10.94g	16.04ab	13.45 c	6.45	10.60	8.53 ab	3.25bc	1.25fg	2.25b-d
1.00	1.28 hi	2.61 bc	1.95 b	12.61f	16.37a	14.49 ab	6.31	9.80	8.06 ab	3.27bc	1.34fg	2.32bc
2.50	1.70 fg	3.16 a	2.43 a	14.31c-e	15.75ab	15.03 a	6.75	9.85	8.30 ab	2.60c-e	1.18 g	1.89c-e
5.00	2.17 de	3.01 a	2.59 a	13.83d-f	15.31a-c	14.57 ab	7.60	9.85	8.73 ab	1.85d-g	1.01 g	1.43 e
7.50	2.31 cd	3.02 a	2.66 a	13.86d-f	13.79d-f	13.82 bc	8.35	12.45	10.40 a	1.92d-g	1.15 g	1.54de
10.0	2.19 d	2.89 ab	2.54 a	13.70d-f	13.70d-f	13.81 bc	6.80	7.40	7.10	1.62d-g	1.14 g	1.38 e
Mean of type	1.42 b	2.33 a		11.73b	14.50a		6.77 b	9.25 a		3.14 a	1.40 b	
LSD at 5% of Types	0.12			0.44			1.10			0.33		
LSD at 5% of Conc	0.28			0.98			2.46			0.74		
LSD at 5% of Inter.	0.39			1.39			N.S.			1.05		

1-3- Effect of auxin types and concentrations on leaves number Table (1)

a- Auxin types affected leaves number significantly. NAA effect was significantly superior to that of IBA, as leaves number was higher with NAA compared that with IBA.

b– Auxin concentrations exerted a significant influence on leaves number. The presence of auxins at concentrations from 0.1 to 5 ppm raised this number to a somewhat higher level. At the concentrations of 7.5 ppm there was an abrupt significant increase in leaves number. Further increase in auxin concentrations reduced this number significantly.

c– The interaction between auxin types and concentrations did not have a significant effect on leaves number. However, lowest significant leaves number was found in the media free of auxins. At 7.5 ppm the highest leaves number in the IBA treated plants was lower, while the significant highest leaves number in the NAA treated plants was higher.

1-4- Effect of auxin types and concentrations on shoots content of total chlorophyll (mg/g shoots fresh weight) Table (1)

a - Shoots content of total chlorophyll was significantly influenced by auxin types. Media incorporated with IBA induced significant higher shoots content of total chlorophyll than those supplemented with NAA.

b - Auxin concentrations had a significant effect on shoots content of total chlorophyll. Auxin concentrations of 0.10 ppm resulted in the significant highest content. At 0.25 ppm this content decreased, though insignificantly. As auxin concentrations rose more and more to 10 ppm, shoots content of total chlorophyll decreased more and more to the smallest significant level.

c - The interaction between auxin types and concentrations exerted a significant influ-

ence on shoots content of total chlorophyll. The presence of IBA at the lowest concentrations in the media, i.e. at 0.1 ppm, increased content of total chlorophyll to its significant highest level. The more the IBA concentrations rose after the previously mentioned level, the more the deterioration in this content. On the other hand, supplementing NAA to the media had a negative effect on shoots content of total chlorophyll. From 0 to 10 ppm of NAA this content decreased to the significant lowest level.

In this respect, **Pudake and Dhumale (2003)** found that among the different combinations involving auxins (IBA or NAA) tested for rooting of *Chlorophytum borivilianum* shoots, IBA (3 mg/litre) was relatively better. **Ghanti *et al* (2004)** transferred *in vitro* shoots of *Phyllanthus amarus* (*P. niruri*) into rooting medium containing different concentrations of IBA or NAA. They reported that the highest number of roots was induced in the medium containing 0.5 mg IBA/litre. **Maurya *et al* (2004)** working on *Rosa hybrida* cv. Raktagandha found that the highest values for number of primary roots per plantlet were higher with IBA than with NAA supplementation.

1-5- Effect of auxin types and concentrations on roots number Table (2)

a – Roots number was significantly influenced by auxin types. Roots of plantlets grown on media supplemented with NAA were greater in number than those induced by the presence of IBA.

b – Auxin concentrations affected roots number significantly. As auxin concentrations rose from 0 to 2.5 ppm, roots number increased significantly. Although the greatest roots number was that achieved at 5 ppm, all values resulted by auxin concentrationss from 2.5 to 10 ppm were not significantly different.

Table 2. Effect of auxin types and concentrations on roots growth / plantlet.

Conc. (ppm)	Roots number / plantlet			Roots weight (g) plantlet			Root length (cm)			Root diameter (mm)		
	Auxin type											
	IBA	NAA	Mean of conc.	IBA	NAA	Mean of conc.	IBA	NAA	Mean of conc.	IBA	NAA	Mean of conc.
0.00	4.85 h	4.45 h	4.65 e	0.07 h	0.07 h	0.07 f	2.0 f-h	2.09fg	2.05 c	0.92 i	1.00 i	0.96 f
0.10	10.65 g	10.40 g	10.53 d	0.15 h	0.12 h	0.14 ef	2.71 b-e	2.28 ef	2.49ab	1.21hi	1.40 ih	1.31 ef
0.25	10.05 g	17.20ef	13.63 c	0.24 f-h	0.23 f-h	0.24 de	2.65 c-e	2.74 b-e	2.70 ab	1.31 hi	1.90 f-h	1.61 de
0.50	13.50fg	23.25b-d	18.38 b	0.21 gh	0.41e-g	0.31 cd	2.83 b-d	2.28 ef	2.55 ab	1.28 hi	2.73 c-e	2.00 cd
0.75	12.02 g	21.95b-d	16.98 b	0.23 f-h	0.49 c-e	0.36 cd	3.13 a-c	2.40 d-f	2.76 a	1.63 g-i	3.15 bc	2.39 bc
1.00	13.05fg	21.40c-e	17.23 b	0.22 f-h	0.69 bc	0.46 c	3.15 a-c	2.34 d-f	2.74 a	1.39 f-h	3.85 b	2.89 b
2.50	21.35c-e	22.25c-e	21.80 a	0.45 d-f	1.37 a	0.91 b	3.21 ab	1.69 g-i	2.45 ab	2.20 e-g	5.38 a	3.79 a
5.00	27.70 a	21.15c-e	24.43 a	0.86 b	1.41 a	1.13 a	3.19 ab	1.52 hi	2.35 bc	3.00 cd	5.50 a	4.25 a
7.50	25.85 ab	22.00c-e	23.93 a	0.66 b-d	1.35 a	1.00 ab	3.46 a	1.66 g-i	2.56 ab	2.53 c-f	5.58 a	4.05 a
10.0	25.00a-c	19.40de	22.20	0.66 b-d	1.49 a	1.07 a	3.56 a	1.29 i	2.43 ab	2.29 d-g	5.85 a	4.07 a
Mean of types	16.40 b	18.35 a		0.37 b	0.76 a		2.99 a	2.03 b		1.83 b	3.63 a	
LSD at 5% of Type	1.38			0.07			0.17			0.25		
LSD at 5% of Conc	3.08			0.16			0.37			0.55		
LSD at 5% of Inter.	4.36			0.23			0.53			0.78		

In this concern, **Atta-Alla *et al* (2003)** stated that rooting of proliferated shoots of *Bombax malabaricum* (*B. ceiba*) was on MS medium containing 0, 0.5, 1, 2 or 3 mg IBA or NAA/litre. IBA and NAA at low concentrations resulted in the highest number of developed roots.

c – There was a significant effect for the interaction between auxin types and concentrations on roots number/plantlet. In media supplemented with IBA, the significant greatest number of roots was achieved at 5 ppm. Greatest roots number in case of NAA was significantly lower than the previous one, and was induced by the presence of only 0.5 ppm. Higher levels of either IBA or NAA, up to 10 ppm, affected roots number adversely.

Auxins types and concentrations affected roots number to a great extent as mentioned in a lot of reports. However, the effective range of concentrations may vary according to auxin types and plant material. **Wynne and McDonald (2002)** remarked that maximum rooting densities (roots number) of *Betula pendula* were achieved *in vitro* using relatively low levels of IBA (0.08–0.15 ppm). **Owies *et al* (2004)** studied the *in vitro* rooting of *Gundelia tournefortii* (Fam. Asteraceae). They reported that the best result in terms of roots number was achieved at 0.5 ppm NAA or 2 ppm IBA.

Mendes (1999) stated that the best results for roots number of *Thymus mastichina* plantlets were observed with 1 mg/l NAA. **Prashantha *et al* (2004)** reported that IBA and NAA promoted rooting of the microcutting of sweet carambola (*Averrhoa carambola*) regardless of concentrations, with IBA at 1 mg/litre promoting the highest number of roots.

1-6- Effect of auxin types and concentrations on roots weight (g) Table (2)

a– Auxin types had a significant effect on roots weight. NAA application resulted in a significant heavier roots compared to those produced by means of IBA.

b– A significant effect on roots weight was achieved by auxin concentrations. When auxin concentrations increased from 0 to 5 ppm, roots weight increased significantly to the highest significant level. Further increase in auxin concentrations from 7.5 to 10 ppm, resulted in an insignificant decrease in roots weight.

c– There was a significant interaction between auxin types and concentrations affecting roots weight. Incorporating IBA in the media at concentrationss from 0 to 1 ppm induced increments in roots weight, significant in some steps. When IBA level was raised from 2.5 to 5 ppm, roots weight increased significantly. At higher levels of IBA, i.e. 7.5 and 10 ppm, roots weight decreased, though insignificantly the two levels. With NAA at concentrationss from 0 to 1 ppm, roots weight increased gradually, sometimes significantly. A significant and abrupt increase occurred by using NAA at 2.5 ppm where roots weight soared up. At higher NAA concentrationss, from 5 to 10 ppm, roots weight fluctuated insignificantly to the highest record of roots weight at 10 ppm NAA.

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

a – Roots length was affected significantly by auxin types. IBA in the media resulted in roots being significantly longer than those produced in the presence of NAA.
Bekheet *et al* (2004) mentioned that IBA at a concentrations of 2 mg/litre gave the best results of roots length of onion plantlets. **Prashantha *et al* (2004)** reported that IBA and NAA promoted rooting of the microcutting of sweet carambola (*Averrhoa carambola*) regardless of concentrations, with IBA at 1 mg/litre promoting the highest elongation of roots.

b – Auxin concentrations exerted a significant effect on roots length. Roots became longer as auxin concentrations increased fro 0 ppm to 0.1 ppm. Roots length at concentrationss of 0.25 and 0.50 ppm did not differ significantly from the later record. At concentrationss of 0.75 and 1.00 ppm roots length reached the significant highest level with no significant difference between the last two figures. As concentrationss increased more from 2.5 to 10 ppm, this character decreased, sometimes significantly.

A significant effect for auxin types and concentrations was detected on roots length. Roots got longer as IBA concentrations increased from 0 to 10 ppm, when the

significant longest roots were obtained. On the other hand, roots length increased significantly as NAA concentrations increased from 0 to 0.25 ppm. At higher NAA concentrationss, roots length decreased significantly to its lowest level.

Mendes (1999) stated that the longest roots of *Thymus mastichina* plantlets were observed with 1 mg/l NAA. **Maurya *et al* (2004)** working on *Rosa hybrida* cv. Raktagandha found that the highest values for length of longest roots (5.22 cm) were recorded on half-strength MS medium supplemented with 0.5 mg IBA/litre, followed by half-strength MS medium supplemented with 1.0 mg IBA/litre. These parameters were higher with IBA than with NAA supplementation. **Owies *et al* (2004)** studied the *in vitro* rooting of *Gundelia tournefortii* (Fam. Asteraceae). The best result in terms of number and length was achieved at 0.5 ppm NAA or 2 ppm IBA.

1-8- Effect of auxin types and concentrations on roots diameter (mm) Table (2)

a- Auxin types affected roots diameter significantly. NAA in the medium resulted in the roots being thicker compared to those induced by means of IBA.

b- Auxin concentrations exerted a significant influence on roots diameter. As auxin concentrations rose from 0 to 5 ppm, roots became significantly thicker. Although the concentrations of 5 ppm produced the significant thickest roots, higher concentrations (7.5 and 10 ppm) resulted in insignificantly thinner roots.

c- The interaction between auxin types and concentrations had a significant effect on roots diameter. As the concentrations of NAA rose from 0 to 2.5 ppm, roots diameter increased. The significant thickest roots were those induced by using NAA at 10 ppm, with no significant difference between this value and values resulted by using NAA at levels from 2.5 to 7.5 ppm. Situation with IBA was different. Using IBA at concentrations from 0 to 5 ppm increased roots diameter significantly. Higher concentrations of IBA, i.e. 7.5 and 10 ppm had a negative effect on this character by reducing it. The thinnest roots were found in media free of either IBA or NAA.

Ahmad *et al* (2003) stated that the best roots system was developed on shoots peach rootstock GF 677 grown on half strength MS media supplemented with 3 mg IBA/l. Higher level of IBA (4 mg/l) induced callus and inhibited normal roots development.

Experiment 2 - Acclimatization stage

2-1- Effect of acclimatization media on survival % Table (3)

Acclimatization media had no effect on survival percentage. All plantlets survived the acclimatization process; hence, survival percentage was 100% in all media tested. No significant difference could be detected among the different acclimatization media.

However, **Bekheet *et al* (2004)** mentioned that the highest percentage of survival in free-living conditions was obtained when *in vitro* produced onion plantlets were transplanted into pots containing peatmoss and perlite (1:1). **Ipekci and Gozukirmizi (2004)** reported that *in vitro* produced plantlets of *Paulownia elongata* were transferred to pots with a mixture of peat and perlite in a 3:1 ratio and showed a survival rate of 70-80%.

2-2- Effect of acclimatization media on plantlet weight (g) Table (3)

Plantlet weight was significantly influenced by the acclimatization media. The significant heaviest plantlet was that grown on "peat moss + perlite". Media composed of "peat moss" or "peat moss + sand" produced plantlets that were significantly lighter than the previous one, with no significant difference between the last two values. Plantlets grown on "peat moss + ¼ MS" were significantly the lightest. The supremacy of the "peat moss + perlite" mixture may be explained by its less compactness and better aeration which allowed for better roots growth and functioning.

2-3- Effect of acclimatization media on plantlet length (cm) Table (3)

Plantlet length was significantly influenced by acclimatization media. Significant longest plantlets were those grown on "peat moss + perlite". Plantlets grown on other media were significantly shorter than the abovementioned ones.

Table 3. Effect of acclimatization media on vegetative growth/ plantlet and chlorophyll content.

Acclimatization media	Survival %	Plantlet of weight (g)	Plantlet length (cm)	Number of green leaves/ plantlet	Number of yellow leaves/ plantlet	Total chlorophyll (mg/g)
Peat moss	100	1.78 b	14.95 b	2.92 a	0.92 b	2.10 a
Peat moss + sand	100	1.74 b	14.27 b	2.25 b	2.08 a	1.41 b
Peat moss + perlite	100	2.22 a	16.02 a	3.25 a	1.21 b	2.45 a
Peat moss + ¼ MS	100	1.30 c	14.13 b	1.54 c	1.75 a	1.01 b
LSD at 5%		0.05	0.83	0.57	0.42	0.46

2-4- Effect of acclimatization media on number of green leaves/plantlet Table (3)

The "peat moss + perlite" medium surpassed other media significantly in inducing the highest number of green leaves on plantlets grown on it. However, plantlets grown on "Peat moss" produced green leaves/plantlet, insignificantly fewer compared to the former value. "Peat moss + sand" and "peat moss + ¼ MS" resulted in significantly lower numbers.

2-5- Effect of acclimatization media on number of yellow leaves Table (3)

The highest significant number of yellow leaves belonged to plantlets acclimatized on "peat moss + sand". This record was followed, without any significant difference by the number of yellow leaves of plantlets grown on "peat moss + ¼ MS". The other two media, i.e. "peat moss" and "peat moss + perlite" resulted in lower number of yellow leaves.

2-6- Effect of acclimatization media on shoots content of total chlorophyll (mg/g fresh weight) Table (3)

The effect of acclimatization media on shoots content of total chlorophyll was found to be significant. The significant highest content was a result of using "peat moss + perlite" as an acclimatization medium. Chlorophyll content of plantlets grown on "peat moss" was lower than the previous record, however, the difference was not significant. Both "peat moss + sand" and "peat moss + ¼ MS" resulted in significant lower contents.

2-7- Effect of acclimatization media on roots number Table (4)

Acclimatization media affected roots number significantly. "Peat moss + sand" resulted in plantlets having the significant highest number of rootss. However, this value was not significantly different from those of plantlets grown on "peat moss + perlite". The remaining two media, i.e. "peat moss" and "peat moss + ¼ MS" had rootss less n number than the previous two records.

2-8- Effect of acclimatization media on roots weight (g) Table (4)

Acclimatization media had a significant influence on roots weight. Plantlets grown on "peat moss + sand" had the significant heaviest roots, followed without any significant difference with "peat moss + perlite". Both "peat moss" and "peat moss + ¼ MS" gave roots that were not significantly different and were lighter than the first two records.

2-9- Effect of acclimatization media on roots length (cm) Table (4)

The significant longest rootss were a result of using "peat moss + perlite" as an acclimatization medium. "Peat moss + sand" induced rootss that came significantly in the second order descendingly. Other two media, "peat moss" and "peat moss + ¼ MS" produced the shortest rootss.

Table 4. Effect of acclimatization media on roots growth/ plantlet

Acclimatization media	Roots number/ plantlet	Roots weight (g)	Roots length (cm)
Peat moss	10.69 b	0.26 b	4.64 c
Peat moss + sand	14.75 a	0.42 a	6.28 b
Peat moss + perlite	14.56 a	0.38 a	7.79 a
Peat moss + ¼ MS	12.00 b	0.22 b	4.75 c
LSD at 5%	1.48	0.07	1.01

2-10- Effect of acclimatization media on bulbs weight (g) Table (5)

There was no significant influence of the acclimatization media on bulb weight. Irrespective of this fact, "peat moss + sand" encouraged the production of the heaviest bulbs. On the other hand, "peat moss + perlite" produced the lightest ones.

2-11- Effect of acclimatization media on bulbs height (cm) Table (5)

Acclimatization media did not affect bulb height significantly. Despite this finding, the longest bulbs were produced by plantlets grown on "peat moss + sand" medium, while the shortest ones were a result of using "peat moss" medium.

2-12- Effect of acclimatization media on bulbs diameter (cm) Table (5)

Acclimatization media did not exert any effect on bulb diameter. However, "peat moss + sand" was the cause of producing the widest bulbs. Thinnest bulbs belonged to plantlets acclimatized on "peat moss + ¼ MS".

2-13- Effect of acclimatization media on scale number Table (5)

The effect of acclimatization media on scale number was not significant. Despite this insignificancy, "peat moss + ¼ MS" resulted in producing the greatest number of scales/bulb (8.19), while "peat moss" gave the least one (7.19).

Table 5. Effect of acclimatization media on bulbs growth/ plantlet.

Acclimatization media	Bulbs weight (g)/plantlet	Bulbs height (cm)	Bulbs diameter (cm)	Scale number/ bulbs
Peat moss	0.52 a	1.26 a	1.12 a	7.19 a
Peat moss + sand	0.57 a	1.33 a	1.13 a	7.94 a
Peat moss + perlite	0.45 a	1.28 a	1.12 a	7.69 a
Peat moss + ¼ MS	0.54 a	1.28 a	1.11 a	8.19 a
LSD at 5%	N.S	N.S	N.S	N.S

SUMMARY AND CONCLUSION

Rooting

Auxin types had a significant influence on all characters under study. The use of NAA resulted in significant higher values of many characters compared to IBA. These include plantlet weight, shoots length, leaves number, roots weight, roots number and roots diameter.

On the other hand, IBA in the media resulted in rootss being significantly longer than those produced in the presence of NAA. Media incorporated with IBA induced significant higher shoots content of total chlorophyll than those grown in media supplemented with NAA.

Auxin concentrations significantly affected all the variables under study. Using auxins at 0.10 ppm resulted in the significant highest shoots content of total chlorophyll, with no significant difference between this content and those resulted by using auxins at 0.25 and 0.50 ppm. Auxins at 2.5 ppm produced the longest shootss significantly. At auxin concentrations of 5 ppm, roots weight increased significantly to the highest significant level. Although the greatest roots number and diameter were those achieved at 5 ppm, all values resulted by auxin concentrationss from 2.5 to 10 ppm were not significantly different. With auxins at 7.5 ppm, plantlet weight and leaves number increased to the highest level.

Apart from leaves number on which the interaction between auxin types and concentrations had no significant effect, all other characters were significantly influenced by this interaction.

NAA was more powerful than IBA in inducing the highest values in certain variables. With IBA in the media at 2.5 ppm longer shootss were induced. When the growth regulator used was NAA, the significant longest shootss were obtained at 1ppm.

However, IBA was more efficient than NAA in some cases. On the other hand, NAA had a negative effect on shoots content of total chlorophyll.

In media supplemented with IBA, the significant highest number of rootss was achieved at 5 ppm. Highest roots number in case of NAA was significantly lower than the previous one, and was induced at 0.50 ppm.

The significant longest rootss were obtained with IBA at 10 ppm. The longest rootss with NAA (at 0.25 ppm) were shorter than those obtained with IBA.

Acclimatization

Acclimatization media significantly influenced plantlet length and weight and roots length. Significant longest and heaviest plantlets and longest rootss were those grown on "peat moss + perlite".

Acclimatization media affected number of yellow leaves and roots number and weights significantly. On the other hand, acclimatization media had no effect on survival percentage, bulb height, diameter and weight and scale number/bulb.

RECOMMENDATIONS

IBA in the rooting stage at 5 ppm is recommended to get the highest number of roots. Potting mixture of peat moss and perlite at 1:1 (v/v) gave the best results in the acclimatization stage.

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حوليات العلوم الزراعية
جامعة عين شمس، القاهرة
مجلد(٥١)، عدد (٢)، ٤٨٥-٤٩٦، ٢٠٠٦

دراسات على الإكثار الدقيق لأبصال الليليم
٣- التجذير والأقلمة

[٣٤]

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هذا هو الجزء الثالث 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 إستعمل فى هذا العمل الأفرع shoots النامية فى الأنابيب على بيئة موراشيج وسكوج فى الجزء الثانى من هذه الدراسة.

١ - التجربة الأولى : تأثير الأكسين

فى البيئة المزودة بحمض إندول بيوتيريك ، نتج أكبر عدد من الجذور معنويا عند تركيز ٥ جزء فى المليون. أما عدد الجذور فى حالة إستعمال حمض نفثالين أسيتيك والذى نتج عند تركيز ٠,٥٠ جزء فى المليون فكان أقل معنويا مقارنة بالعدد السابق.

كانت أطول الجذور من الناحية المعنوية تلك التى نتجت فى وجود حمض إندول بيوتيريك عند تركيز ١٠ جزء فى المليون. أما أطول الجذور فى حالة إستعمال حمض نفثالين أسيتيك والتى نتجت عند تركيز ٠,٢٥ جزء فى المليون فكان أقصر من الناتجة فى وجود حمض إندول بيوتيريك.

٢ - التجربة الثانية : تأثير بيئة الأقلمة

أثرت بيئة الأقلمة بدرجة معنوية على طول ووزن النبيتات وطول الجذور. وكانت أطول وأثقل النبيتات وأطول الجذور تلك النامية على بيئة "بيت موس+ بيرلايت". وقد تفوقت بيئة "بيت موس+ بيرلايت" على البيئات الأخرى بدرجة معنوية فى تشجيع إنتاج العدد الأكبر من الأوراق الخضراء ومحتوى الأفرع من الكلوروفيل الكلى.

وتوصى هذه الدراسة بإستعمال حمض إندول بيوتيريك بتركيز ٥ جزء فى المليون لإنتاج أكبر عدد من الجذور. وكذلك بإستعمال مخلوط الزراعة فى الأصص المكون من "بيت موس+ بيرلايت" بنسبة ١: ١ حجما الذى أعطى أفضل النتائج فى مرحلة الأقلمة.

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