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RAPID VEGETATIVE PROPAGATION OF *LILIUM ASIATICUM*,L. *IN VITRO*

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ABSTRACT

For the expensive agriculture land and the high production cost, in Egypt, this investigation was performed *in vitro* throughout 2009 and 2010 years at tissue culture and Germplasm Conservation Research Laboratory, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt, aiming to study the propagation technique to achieve producing profuse number of plantlets and bulblets of *Lilium asiaticum,*L .plant with reducing the cost, of propagation methods which are used under local conditions. Data reveal that soaking the explants (shoot tip, bulb scales and discoid stem) on clorox at 30% for 15 min. immersion in ethanol for five second,significantly increased survival percentage and significantly reduced the contamination percentage. Meanwhile, a significant increments in shoot tip and callus formation percentage was occurred by addition of 1.00mg/l NAA plus 1.00 mg/l IBA to culture medium. Treating the plantlet product from shoot tip and bulb scales with kinetin at " 2.00,4.00 and 6.00mg/l"displayed the maximum amounts of roots number and length ,while kinetin at 6.00mg/l plus 1.00gm/lpollen grains of date palm significantly increased shoot length , shoot number and leaves number .The maximum values of rooting percentage ,root number and length were achieved by using 2.00mg/lNAA plus 1.00 gm/l charcoal. Moreover, using sucrose at 60.00 gm/l recorded the maximum values of bulblet formation, (number and diameter) comparing to the control and other glucose treatments .

So, it could be recommend to culture shootlets on MS medium containing 2.00mg/l KI +1.00gm/l pollen grains of date palm and

sucrose at 60.00gm/l to obtain a large amount of bulblets formation with good quality *of Lilium asiaticum,*L . .

Key word: *Lilium asiaticum,*L -Clorox -Auxin- Kinetin- Pollen grains of date palm- Sucrose- Glucose- Bulblet production.

INTRODUCTION

The genus Lilium are herbaceous flowering plants growing from bulbs.Most species are native to the temperate northern hemisphere. They comprise a genus of about 110 species in the lily family (Liliaceae).They are important as large showy flowering garden plants.Additionally, they are important culturally and in literature in much of the world. Some species are sometimes grown or harvested for the edible bulbs.The species in this genus are the true lilies. Many other plants exist with "lily" in the common English name, some of which are quite unrelated to the true lilies.(Asiaticum hybrids)These are plants with medium sized, upright or outward facing flowers, mostly unscented. They are derived from central and East Asian species. The *Lilium asiaticum* 'Lennox',is pure and spotless white. It grows to a height of 24" Nothing compares to a mass of colorful lilies in full bloom. The lily is a symbol of majesty and these regal carefree blooms produce up to 12 beautiful colour. The flowers of all lilium ,with the exception of a few ill-smelling species ,are excellent for cutting. Only the upper part of the stem should be cut off ,however, leaving the foliage on the lower part ,so the bulb may complete its growth. The stem is cut off right down to the ground when in active growth, the bulb will be injured or perhaps destroyed .Most lilium will succeed in any light, sandy or loamy soil. In cold climates, the bulbs of all liliums should be protected from freezing during winter by a heavy covering of leaves, hay or straw. The best time to plant lily bulbs is soon after the flowers fade or seeds ripen.,(Bailey, 1976). On the contrary, *in vitro* propagation can produce large numbers of healthy plants, homogenous, and identical to their mother plants in a short and exact time. Also, reduces losses in plant materials with the lowest expenses which maximize the profitability of the propagation techniques. Meantime, *in vitro* propagation encouraged many researchers to study the behavior of the plant and finding out solutions of the problems faced the plants in open climate, *i.e.* diseases and

different stresses. In this concern,Zhang YanLon, *et al*.,(2004)indicated that the optimal medium for the callus inducing of *Lilium brownie* was MS + 6-BA [benzyladenine [1.0 mg/l] + NAA at 0.1 mg/1 in which the inducing rate was 76%. MS+6-BA at 0.5 mg/1 + NAA 0.1 mg/1 was the proper medium for subculture. The best rooting medium was 1/2 MS + IBA at 0.3 mg/1. Along with culture time ،the rooting rate increased gradually. Goo DaeHoe, *et al*.,(2004)reported that treatment with 0.01 mg benzyladenine/litre + 0.1 mg NAA/l.resulted in a higher number of *L. hansonii* bulblets formed (3.4). Addition of 6% glucose in the culture media resulted in the enlargement of bulblets and hence an increase in their fresh weight. In this concern, Han BongHee, *et al*.,(2005) on *Lilium oriental* hybrid 'Casablanca' found that normal bulblet growth was stimulated more by the culture of shoots than that of bulb scaless. Bulblet weight from shoots reached to an average of over 1100 mg of a bulblet after 3 months in culture on MS medium containing 60 g/L sucrose and 2 g/LAC(activated charcoal). ZhangYiPing,*et al*.,(2006)studied the adventitious shoot formation, plant regeneration and micropropagation from lily leaves.Sterilization of the explant in 0.10% aqueous mercuric chloride for 5 min resulted in only 36% contamination rate, 75% germination rate and 68.8% induction rate. Optimum adventitious shoot formation was recorded in MS + 2.0 mg 6-BA + 0.5 mg NAA/litre. Shoots grew well in MS + 1.0 mg 6-BA + 0.1 mg NAA/litre ،whereas root regeneration was optimum in MS + 0.1 mg NAA/litre.

Similarly revert the results of, Joshi, and Uppeandra Dhar (2009) on *in vitro* study of the propagation protocol for production *Lilium oxypetalum*. Moreover, Wang Yue, *et al*., (2009) concluded that young leaves of *Lilium oriental* hybrids were used as explant materials in tissue culture. The results showed that the appropriate medium to induce roots was 1/2 MS + 0.20 mg NAA/litre. The concentration of sucrose in the medium can affect the increase of bulblet weights as 60.0 g sucrose/litre being most suitable.

MATERIALS AND METHODS

This study had been carried out on *Lilium asiaticum*,L. at Tissue Culture and Germplasm Conservation Research Laboratory, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt ,during two successive years 2009 and 2010 in order to

investigate the propagation technique to produce a large number of plantlets and bulblets *in vitro* culture.

Plant materials

In vitro plantlets of *Lilium asiaticum*,L. resulting from "shoot tip explants (1.0 cm long), basic leaves "bulb scales" and discoid stem (0.5 cm long)" were obtained from Mahmouad Helmy Farm in Giza city . Homogenous plants were selected as plant material source and healthy plants of *Lilium asiaticum*,L. after that shoot tips ,bulb scaless and discoid stem were subjected to running water for 5 minutes and immersing in 0.0, 20, 30 and 50%clorox "a commercial bleach" which contained two drops of Tween-20 for 15 minutes ,with or without ethanol "ethyl alcohol "70% for five seconds then, immersed in a sterilized distilled water 3 times for 5 minutes each.

Culture medium:

The used MS culture medium was the formulation of Murashig and Skoog (1962)enriched with some growth regulators and addition of agar (0.7%).The medium was adjusted to PH of5.7, then poured at 25 ml capacity glass jars before being autoclaved at 121°C and 15 lb/in2 for 15 minutes.

Culture conditions :

The cultures were incubated in a growth chamber at 24±1°C under 16 hr photoperiod (day light fluorescent tube)at 3K lux.

Establishment stage

NAA (Naphthalene acetic acid),IBA (Indole –3-butyric acid); and NAA+IBA at the rates of 0.00 &1.00 mg /l were done to investigate the suitable auxin type and concentration which encouraged the highest callus formation and shoot initiation of *Lilium asiaticum*,L..

Multiplication stage:

The plantlets resulted from establishment stage of *Lilium asiaticum*,L. "shoot tips and bulb scales" were used to study the effect of kinetin at different levels i,e.0.0,2.00, 4.00 and 6.00 mg/l with or without pollen grains of date palm at 1.00 gm /l dry weight to detect the suitable concentration which induced the highest multiplication.

Rooting stage:

The proliferated shoots were used as explants and cultured on MS medium and treated with NAA at 1.00 and 2.00 mg/l and activated charcool at 1.00 gm/l to study their effects on rooting percentage ,number of roots and root length.

Bulblets production stage:

In this stage, an experiment was carried out on shootlets of *Lilium asiaticum*,L. cultured on MS medium supplemented with 2.00mg/l KI +1.00gm/l pollen grains of date palm analyzed in **Table** (a)to study the effect of sucrose and glucose at the rates of 0.00,30.00,60.00 and 90.00 gm/l on percentage of bulblets formation ,bulblets number and diameter .

Table (a) The contents of date palm pollen grains (D. W.) according to Bukhaev, et *al.*, (1983):

Ash	**5.50%**
Coir pure	**9.90%**
Proteins pure	**27.20%**
Total sugars	**18.10%**
Reducing sugars	**2.20%**
Non reducing sugars	**15.10%**
Total lipids	**12.10%**

The parameters recorded for the three consecutive micro propagation stages (i.e., shooting, rooting and bulblets production stages)were as follows:

a) For shooting stage , at the end of the first cycle of subculture "4.00 weeks" the survival capacity of explants were established as percentage, the contaminated plants %, the number of formed shootlet per explant , the length of shootlet (cm) and number of developed leaves per shootlet were recorded.

b) For rooting stage, at the end of second cycle of subculture, the rooting response of explants as percentage, the number of the

initiated roots per explant and the length of the deviled root (cm) were determined.

c) Bulblets production stage, included bulblet formation, bulblets number and diameter.

Statistical analysis: -

The layout of such factorial experiment was a randomized complete design (Das and Giri,1986) each treatment included five jars was performed containing four explants and each experiment was replicated three times. Data were subjected to analysis of variance by MSTAT-C (1990) Computer statistical analysis program. By using Duncan, Multiple range test (1955) to verify the significance level among means of various treatments.

RESULTS AND DISCUSSION

Survival percentage:

It is evident from the obtained data (Table 1) that soaking all explants in colorax at 30%concentration for 15 min followed by immersion in ethanol "ethyl alcohol" for 5 seconds induced the highest survival percentage of explants and reduced contamination in all explants**.**

Table (1) Effect of Colorax and ethanol alcohol treatments on survived and contaminated explants of *Lilium asiaticum,* L.explants.

Explants / Treatments		Survived explants (%)				Contaminated explants (%)			
Ethanol(%)	Colorax(%)	Shoot tip	Bulb scales	Discoid stem	Mean	Shoot tip	Bulb scales	Discoid stem	Mean
0.00	0.00	0.00H	0.00 H	0.00 H	0.00 H	0.00 B	0.00 B	0.00 B	0.00 B
	20	60 E	80 C	40 G	60.00 D	5.0 A	10 A	10 A	8.33A
	30	70 D	90 B	40 G	66.66 C	0.00 B	0.00 B	0.00 B	0.00 B
	50	60 E	70 D	50 F	60.00 D	0.00 B	0.00 B	0.00 B	0.00 B
70	0.00	0.00H	0.00 H	0.00 H	0.00 H	0.00 B	0.00 B	0.00 B	0.00 B
	20	80 B	90 B	50 F	73.33B	0.00 B	0.00 B	0.00 B	0.00 B
	30	90 B	100 A	60 E	83.33A	0.00 B	0.00 B	0.00 B	0.00 B
	50	70 D	80 C	50 F	66.66C	0.00 B	0.00 B	0.00 B	0.00 B
Mean		53.75B	63.75A	36.25C		0.625B	1.25A	1.25A	

Means within column having the same letters are not significantly different according to Duncan's multiple range test (DMRT)

Callus formation and shootlets percentage per explants:

From data record in Table (2) it could be concluded that callus and shootlets percentage per explants of shoot tip, bulb scales and discoid stem were significantly affected by various auxin "NAA and IBA" treatments. Culture shoot tip, or discoid stem on MS medium contained NAA at 1.00 mg/l significantly increased callus% .Meanwhile addition NAA at 1.00 mg/l plus 1.00 mg/l IBA significantly increased shootlets percentage .However, the maximum callus and shootlets percentage achieved by culture bulb scales on MS medium contained NAA at 1.00 mg/l plus 1.00 mg/l IBA. Generally, the aforementioned results recommended culturing of shoot tip explant on modified Murashige and Skoog medium for the best direct regeneration parameters.These results may be due to the structure of shoot tip which include meristimic cells and number of leaf primordia with axillary buds which developed directly into plantlets.These results in general agreement with the findings of **Podwysyska (1992)** on *Aglaonema cv*. Silver Queen.

Table (2) Effect of NAA and IBA treatments on callus formation and shootletes percentage of *Lilium asiaticum,* L.explants :

Explants / Treatments	Shoot tip		Bulb scales		Discoid stem	
	Callus formation(%)	Shootlets (%)	Callus formation(%)	Shootlets (%)	Callus formation(%)	Shootlets (%)
Control	0.00D	0.00 D	10 D	0.00 D	10 D	0.00 C
0.1mg/l NAA	60 A	40 C	50 B	30 C	100 A	30 B
1.00mg/l IBA	40 C	60 B	30 C	70 B	30 C	30 B
0.1mg/l NAA+1.00mg/l IBA	50 B	70A	60A	80 A	60 B	40 A

Means within column having the same letters are not significantly different according to Duncan's multiple range test (DMRT)

Multiplication stage:

Effect of kinetin and pollen grains of date palm on plantlets:

Data in Table(3) show that addition of Kinetin at 6.00 mg/l plus 1.00 gm/l pollen grains of date palm to the medium of shoot tip of *Lilium asiaticum*,L. significantly increased shoot length "12.66 cm ", shoot number"9.00" and leaves number "5.66" comparing with "9.33, 4.00 and 2.33 0f untreated plantlets. Concerning the number of roots, the highest value 2.66 was gained due to using kinetin treatment at 2.00mg/l level comparing with 0.00 control. The maximum root

length values "0.66, 0.53 and 0.36cm were obtained from Kinetin levels at 2.00, 4.00 and 6.00 mg/l respectively while untreated explants record 0.00.

Regarding the effect of Kinetin and pollen grains of date palm on plantlet product from bulb scales, the length of shootlets was significantly increased by various concentration of Kinetin and pollen grains of date palm examined ,the tallest shootlets "17.00 and 14.00 cm" were record after culture bulb scales on MS medium containing 2.00 and 4.00 mg/l Kinetin plus pollen grains of date palm respectively .The highest number of leaves and shootlets formed were obtained from all other explants culture on 6.00 mg/l Kinetin plus 1.00 gm/l pollen grains of date palm .Meanwhile, using Kinetin at 2.00 mg/l significantly increased roots number to the maximum values "7.66" comparing to control and all other treatments. However ,the highest length of the initiated roots 4.00 and 3.33 cm were recorded from Kinetin treatment(2.00 mg/l) and control respectively illustrated in Table (3) and Fig (1).These findings are in accordance with the data recorded by Zhang JianHua, *et al*.,(2006) on Lily *in vitro* reported that the highest frequency of adventitious bud differentiation was obtained from MS medium supplemented with 1.5 mg benzyladenine/litre, 0.1 mg kinetin/litre and 0.1 mg NAA/litre, in which 93.33% callus explants differentiated into adventitious buds after 8 days.

Table (3) Effect of kinetin and pollen grains of date palm on multiplication stage of *Lilium asiaticum,* L.explants:

Treatments	Shoot tips explants					Bulb scales explants				
	Shoot length (cm)	Shoot number	Leaves number	Root number	Root length (cm)	Shoot length (cm)	Shoot number	Leaves number	Root number	Root length (cm)
Control	9.33 D	4.00 E	2.33 D	0.00 C	0.00 B	7.66 E	2.00 D	2.33 B	3.33 B	3.33 A
2.0mg/lKI	7.66 E	6.66 C	3.33 CD	2.66 A	0.66 A	12.00 CD	3.66 CD	4.00 AB	7.66 A	4.00 A
4. 0mg/lKI	8.00 E	7.00 BC	4.66 AB	1.66 B	0.53 A	13.66 BC	7.33 B	4.66 AB	2.66 B	1.83 BC
6. 0mg/lKI	10.33 CD	5.33 D	5.00 AB	1,33 B	0.36 A	8.33 E	3.66 CD	5.66 A	1.66 BC	1.66 C
1.0gm/LPollen grains	10.66 C	4.33 E	6.00 A	0.00 C	0.00 B	15.33 AB	8.66 AB	3.33 AB	2.33 BC	1.83 BC
2.0mg/lKI+1.0gm/LPollen grains	11.33 BC	7.00 BC	5.00 AB	0.00 C	0.00 B	17.00 A	7.00 B	4.00AB	2.66 B	2.66 AB
4.0mg/lKI+1.0gm/LPollen grains	12.00 AB	7.66 B	4.00 BC	0.00 C	0.00 B	14.00 B	4.66 C	4.66 AB	1.66 BC	1.83 BC
6.0mg/lKI+1.0gm/LPollen grains	12.66 A	9.00 A	5.66 A	0.00 C	0.00 B	11.00 D	9.66 A	5.66 A	0.66 C	0.66 C

Means within column having the same letters are not significantly different according to Duncan's multiple range test (DMRT)

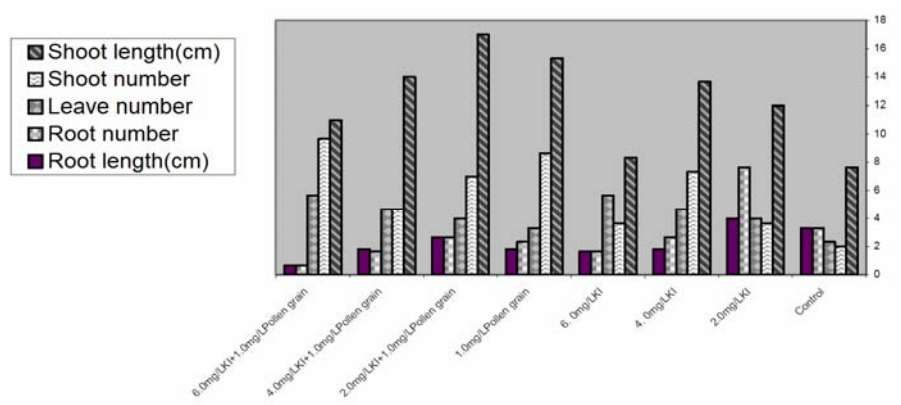

Fig (1): Effect of kinetin and pollen grains of date palm on multiplication of *Lilium asiaticum,* L. plantlet product from bulb scales.

Rooting stage:

Effect of NAA and charcoal treatments on rooting parameters:

From the data shown in Table (4) it is noticed that the rooting percentage ,root number and root length per shootlet explants were significantly affected by either the different NAA or NAA plus charcoal treatments tested. Addition of 2.00 mg/l NAA plus 1.00gm/l charcoal significantly increased rooting percentage, root number and the length of initiated roots.Similarity matrices were recorded according Zhang YanLon, *et al*.,(2004) on *Lilium brownie*.

Table (4) Effect of NAA concentration and charcoal on rooting parameters of *Lilium asiaticum,* L.explants:

Treatments	Rooting (%)	Root number	Root length(cm)
Control	20.00 E	2.00E	1.66 D
1.00mg/lNAA	50.00 D	11.66D	3.16 C
2.00mg/lNAA	70.00 B	12.33 C	5.33 B
1.00mg/lNAA+1.00gm/l charcoal	60.00 C	17.00 B	6.16 AB
2.00mg/lNAA+1.00gm/l charcoal	100 .00A	20.33 A	6.66 A

Means within column having the same letters are not significantly different according to Duncan's multiple range test (DMRT)

Bulblet production stage:

In this stage ,data scored in Table (5)and Photo(1&2)indicate that using either sucrose or glucose at ,30.00,60.00 and 90.00 gm/l significantly increased bulblet formation from 0.00"control" to 100% bulblet at 60.00 gm/l sucrose concentration comparing with control and glucose treatments. However, adding sucrose at 90.00 gm/l to culture medium gave highly significant values in diameter of bulblet and bulblet number comparing with glucose .Whereas the addition of sucrose at 60.00gm/l to MS medium gave the highest significantly increaments in all parameters in this stage. These results are in agreement with the findings of Han BongHee, *et al*.,(2005) on *Lilium oriental* hybrid 'Casablanca' and Wang Yue, *et al.,* (2009) on *Lilium oriental* as hybrids they found that the concentration of sucrose in the medium can affect the increase of bulblet weights with 60.0 gm sucrose/litre being most suitable.

Table (5) Effect of sucrose and glucose treatments on bulblet production of *Lilium asiaticum* ,L.explants:

Treatments	Bulblet formation(%)					Bulblet diameter(cm)				Bulblet number			
	0.00 gm/l	30.00 gm/l	60.00 gm/l	90.00 gm/l	Mean	30.00 gm/l	60.00 gm/l	90.00 gm/l	Mean	30.00 gm/l	60.00 gm/l	90.00 gm/l	Mean
Sucrose	0.00E	80 C	100A	80 C	8.66A	1.00B	1.2AB	1.5A	1.23A	16B	25A	25A	22A
Glucose	0.00E	70 D	90 B	90 B	83.33B	0.8BC	1.00B	0.9B	0.9B	9D	11C	12C	10.66B
Mean	0.00E	75C	95 A	85B		0.9B	1.1A	1.2A		12.5B	18AB	18.5A	

Means within column having the same letters are not significantly different according to Duncan's multiple range test (DMRT)

Photo (1) Stage of bulblets formation by using sucrose at 60 gm/l.

Photo (2) Bulblets after two months from formation

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الإكثار السريع لنبات الليليم عن طريق زراعة الأنسجة

بشرة عبد الله السيد- حنان محمد أحمد يوسف

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نظراً لارتفاع ثمن الأرض الزراعية وكذلك تكاليف الإنتاج في مصرأجري هذا البحث بمعمل زراعه الأنسجة- معهد بحوث البساتين-مركز البحوث الزراعية بهدف التوصل إلى البروتوكول المناسب لإنتاج أكبر عدد من النبيتات والبصيلات لنبات الليليم ***Lilium asiaticum* L.** من خلال زراعة الأنسجة خلال عامي 2009-2010. **وأوضحت النتائج مايلى**:

- نقع كل من" القمة النامية والأوراق الحرشفية والساق القرصية" في محلول الكلوروكس بتركيز 30 % لمدة 15 دقيقة ثم غمسها في كحول الايثايل لمدة 5 ثواني أدى لزيادة معنوية في النسبة المئوية للنبيتات الحية وكذلك إلي تقليل التلوث معنوياً .

- بينما إضافة 1مليجرام /لتر من كل من أندول حمض البيوتريك وألاندول اسيتك أسيد(NAA & IBA) لبيئة الزراعة "مورا شيج وا سكوج "أدى لحدوث تأثيرات معنوية في النسبة المئوية لكل من القمة النامية والكالس المتكون .

- معاملة النبيتات الناتجة من زراعة القمة النامية والأوراق الحرشفية بالكينتين بتركيز" 2 ،4، 6 مليجرام/لتر أدى إلي زيادة عدد و طول الجذور المنتجة، في حين وجد إن اضافة1جرام /لترمن طلع النخل و6مليجرام /لتر كينتين أدى لزيادة طول وعدد النبيتات وكذلك عدد الأوراق المتكونة معنوياً.

- ووجد أن أقصي نسبة مئوية للتجذير و عدد وطول الجذور المتكونة نتجت من إضافة 2 مليجرام/لترمنNAA مع 1جرام /لتر فحم نباتي لبيئة الزراعة.

-إضافة " السكروز بتركيز60 جرام/لتر " أدى لتكوين أقصي عدد من البصيلات المتكونة وزيادة سمكها مقارنة بالكنترول والجلوكوز.

وعلي ذلك فأنة يوصي من النتائج السابقة:

- زراعة النبيتات علي بيئة مورا شيج وا سكوج تحتوي علي 2مليجرام /لتر كينتين و1جرام /لترمن طلع النخل و60 جرام/لتر سكروزهو انسب بيئة لتكوين ا كبر عدد من البصيلات جيدة الصفات لنوع الليليم ***Lilium asiaticum* L.**