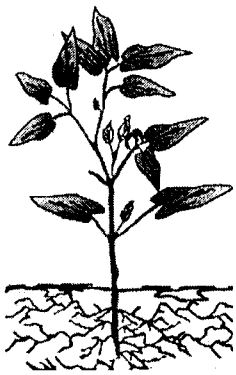

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STUDIES ON THE MICROPROPAGATION OF EASTER LILY "*Lilium longiflorum* Thunb" BULBS I- ESTABLISHMENT

[13]

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Keywords: *Lilium longiflorum*, Establishment, Bulbs, Murashig & Skoog strength (MS).

ABSTRACT

On taking explants from field grown lily plants, no growth response was detected for any of the bulb explants and they eventually died. On the contrary, scales of the aerial bulbils grown on the flowering stem, survived and positively responded exhibiting growth and proliferation. Only the basal part of the *in vitro* grown leaf explants survived excision and after inoculation on MS medium showed a positive response by growth and proliferation. Strength of MS medium did not significantly affect shoot number/explant. On the contrary, shoot weight was significantly increased as the medium strength increased. Using MS medium at ¾ and full strength gave the significantly longest shoots, the highest leaf number and the highest total chlorophyll content of shoots. The full strength MS resulted in the heaviest shoots.

INTRODUCTION

The genus *Lilium*, of the family Liliaceae, consists of about 80 species and a considerable number of varieties and hybrids. Plants of this genus are confined in the wild to the northern hemisphere, with very few exceptions to temperate parts. Almost all of these plants are deciduous or in rare cases in mild climates semi-evergreen herbaceous perennials. Lilies have erect leafy stems and scaly bulbs, i.e. the bulbs are composed of overlapped scales. The white trumpet lily or Easter lily (*L. longiflorum*) is a native of Japan. Plants of this species have white, sub-spherical bulbs about 5 cm long and stems up to 90 cm tall that root from their bases. The leaves are scattered, pointed-lanceolate, 10-20 cm long by 1-1.5 cm wide. The pure white, waxy blooms, green-tinged towards their bases, 15-20 cm in length and trumpet-shaped are carried nearly horizontally in racemes of few to several flowers. In garden landscapes, lilies do not lend themselves to formal arrangements. They are better planted somewhat irregularly as the chief feature in the season on either side of a straight path. Groups of strong-growing lilies make fine accents in mixed flowerbeds, but may not be very permanent there and may need replacing from time to time. Lilies are at their best in informal or semiformal surroundings. They are good for planting informally among and in front of low shrubs, particularly those of slow growth. Evergreen shrubs supply rich background that complements the trumpets or bells of the lilies. As cut flowers, lilies lend themselves well, chiefly for large arrangements. Easter lily is undoubtedly the most commonly used as a container plant **Everett (1981)**.

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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.

Because they are relatively free of contamination compared to the ordinary bulbs of lily plants, aerial bulbils that grow on the flowering stem were used by many workers as a means of starting an *in vitro* culture. **Chung *et al* (1981)** cultured *Lilium lancifolium* bulbil scales on Murashige and Skoog medium with NAA, IAA or IBA for bulblets formation. **Paek and Shin (1983)** used mature bulbil segments of *Lilium lancifolium* for bulb regeneration *in vitro*. **Lee *et al* (1994)** stated that bulbil scales of *Lilium elegans* (*L. maculatum*) hybrid Odasetsuka x Inferno seemed promising as explant sources.

In vitro grown leaves of many plants were employed as explants in several works. However, different portions of the leaf are characterized by different degrees of regeneration ability, with the basal part being more active. **Yang and Chi (1979)** stated that plantlets were successfully obtained following bud formation in leaf explants of *Pelargonium domesticum*, *Begonia semperflorens*, *Chrysanthemum* sp., *Petunia hybrida*, *P. hortorum* and *Brassica oleracea* var. acephala. **Firoozabady and Moy (2004)** mentioned that leaf bases of *in vitro* shoots (produced from culture of crown tip meristem) of pineapple plants were used as explants for plant regeneration on MS medium. Leaf explants cultured on MS medium with 27 µM (5 ppm) NAA and 1 µM (0.23 ppm) BA produced shoots via direct organogenesis. **KyungHee *et al* (2004)** used leaf base segments of Korean oat cultivars Malgwiri and Samhangwiri as materials in an experiment to check plant regeneration efficiency on MS medium. **Newell and Gray (2005)** investigated the regenerability of *Lolium perenne* and *L. multiflorum* using leaf-base sections excised from plantlets grown *in vitro*. When they cut young leaf bases into three sections each 2 mm in length, rooted, green plantlets from the 0-2 mm sections of *L. perenne* cv. Limes and from leaf-base sections of 0-2, 2-4 and 4-6 mm of *L. multiflorum* were obtained

Strength of the medium used affects to a great extent shoot production as mentioned by a lot of workers. **Debnath (2004)** mentioned that cultures of dwarf raspberry (*Rubus pubescens*) were initiated from shoot tip and nodal explants on 1/2 strength MS containing 8.9 µM (2 ppm) BA and 0.98 µM (0.2 ppm) IBA. **Fraguas *et al* (2004)** found that the regular strength of woody plant medium (WPM) in combination with 0.5 mg kinetin litre-1 was the best condition for shoot proliferation of *Ficus carica* cv. Roxo de Valinhos. **JongHee *et al* (2004b)** found that shoot growth and microtuber formation of Chinese yam (*Dioscorea opposita*) were affected by medium strength. **Enrico *et al* (2005)** found that the development of multiple buds on explants of *Alnus acuminata* at the initiation step was obtained with MS at 1/2 strength with 1-2 µM (0.23-0.45 ppm) of BAP.

MATERIALS AND METHODS

This work is the first part of a series of experiments that were carried out in the Tissue Culture Laboratory of the Horticulture Department, Faculty of Agriculture, Ain Shams University through the years of 2002-2004. The aim of this study was to establish an applicable protocol for the rapid micropropagation of *Lilium longiflorum* in order to get bulbs for planting and plants for flowering.

Murashige and Skoog (1962) basal medium was prepared. This medium contained, in addition to the prescribed salts and vitamins, benzyladenine (6-Senzylaminopurine), referred to for short as "BA or BAP" at 3 ppm, 30g/l sucrose and 7g/1 agar. It was adjusted to *p*H 5.8, poured in the jars 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.

Explants were excised from different parts of lilium bulbs (buds, scales and pieces of the basal plate) in addition to scales of aerial bulbils grown on the floral peduncle. These explants were surface sterilized by mercuric chloride ($HgCl_2$ or MC for short) at 500 ppm for 5 min., followed after rinsing with a distilled sterilized water, by clorox at 20% (1% a.i.) for 15 min. Final rinsing with the distilled sterilized water for three times preceded explant inoculation on MS medium supplemented with BA at 5 ppm.

Experiment 1

Shoots grown *in vitro* were used as a source of explants. Leaves excised from these shoots were horizontally cut into three parts, terminal, middle and basal. The last part contained the excision edge at its base. Types of these explants represented three treatments.

Each treatment comprised 6 replicates. These treatments were arranged in a completely randomized design. Data obtained in this experiment were: percentage of explant survival, shoot number, shoot weight (g), shoot length (cm) and leaf number.

Experiment 2

Four strengths of MS medium salts, i.e. 0.25, 0.50, 0.75% and full strength were prepared. Explants consisting of the basal part of leaves excised from *in vitro* grown shoots mentioned above, were inoculated on these media. Each one of these MS strengths (treatments) were replicated 8 times. These treatments were arranged in a completely randomized design. Data obtained in this experiment were: shoot number, shoot weight (g), shoot length (cm), leaf number and shoot content of total chlorophyll (mg/g fresh weight).

Data were analyzed statistically due to the methods described by **Snedecor and Cochran (1980).** Using L.S.D. for comparing between means of treatments.

RESULTS AND DISCUSSION

Preliminary experiment

On taking explants from field grown lily plants, no growth response was detected for any of the bulb explants and they eventually died. On the contrary, scales of the aerial bulbils survived and positively responded exhibiting growth and proliferation. This gave rise to a lot of shoots through successive subculturing. The great number of shoots enabled the us to carry out six consecutive experiments.

Bulbils that grew on the flowering stem of lily plants were used as explants in the tissue culture technique since long. **Allen (1975)** used vegetative propagation on tissue culture media to free 10 lily cvs. of viruses. He used stem bulbils from virus-free field-grown plants of cv. Enchantment to produce plants.

Exp. 1- Establishment stage

1. Effect of explant type on *Lilium* explants

The type of explant used showed a critical and decisive effect. It was only the basal part of the *in vitro* grown leaf that survived excision and inoculation on MS medium and showed a positive response exhibiting growth and proliferation. The other two parts of the leaf gradually became brown and died eventually. Using the lower third of the leaf with the excision edge immersed in the medium, gave rise to 5.04 shoots that weighed 0.79 g and comprised 11.21 leaves that were 6.31 cm high as shown in **Table (1).**

The positive response of leaf bases when used as explants in the tissue culture technique was mentioned by many authors. **Landby and Neiderwieser (1992)** studied various factors known to affect adventitious shoot initiation of *Ornithogalum* cv. Rollow (a yellow-flowered cultivar) from leaf explants *in vitro*. They mentioned that optimization of *in vitro* shoot initiation on existing culture media was dependent on the explant source, with explants from the leaf base being most prolific. **Nair and Seeni (2001)** cultured that leaf bases (approx. equal to 1 cm), excised from young vines of the flowering woody climber, *Celastrus paniculatus* subsp. *paniculatus* (Fam. Celastraceae) in Murashige and Skoog (MS) medium. All the explants were regenerative. Leaf bases of *in vitro* derived shoots responded better. **HsiuJane *et al* (2002)** found that when different explants of trailing *Petunia integrifolia* and *P. hybrida* cv. Surfinia were cultured on basal MS medium, the highest growth index was obtained from the leaf base. **Mishra and Khurana (2003)** developed a simple and reproducible protocol for regeneration of plantlets from leaf base cultures of agronomically important Indian *Sorghum bicolor* genotypes (296 B and RS 585). Cultures were raised from leaf bases, excised from *in vitro* raised plantlets. **KyungHee *et al* (2004)** used leaf base segments of Korean oat cultivars Malgwiri and Samhangwiri as materials in an experiment to check plant regeneration efficiency on MS media. They stated that regeneration from leaf base segments showed high frequency of shoot in medium containing 1 mg tri-iodobenzoic acid and 1 mg BA/l.

Exp. 2- Effect of MS medium strength on some vegetative growth parameters and total chlorophyll content

2.1. Effect of MS medium strength on shoot number/explant (Table 2 and Fig. 1-1)

Strength of MS medium did not affect shoot number/explant. However, a slight increase was observed in this character from 1.28 to 1.66 and

Table 1. Effect of explant type on survival and some growth parameters of *Lilium* explants during 2002 seasons

Explant type	Explant survival (%)	Shoot No.	Shoot weight (g)	Shoot length (cm)	Leaf No.
Basal part	100.00	5.04	0.79	6.31	11.21
Medium part	0.00	0.00	0.00	0.00	00.00
Terminal part	0.00	0.00	0.00	0.00	00.00
L.S.D. at 5%	0.00	0.87	0.20	0.96	1.07

Table 2. Effect of medium strength on some vegetative parameters and total chlorophyll content of *Lilium* explants (during 2002 seasons)

MS strength	Shoot No.	Shoot wt. (g)	Shoot length (cm)	Leaf No.	Tot. chlorophyll (mg/g) f.w.
¼	1.28	0.33	5.20	3.28	0.39
½	1.66	0.45	5.85	4.22	0.59
¾	2.00	0.65	6.88	5.59	0.71
full	2.00	0.83	7.53	6.06	0.86
L.S.D. at 5%	N.S.	0.10	0.95	0.95	0.19

further to 2.00 shoots as the medium strength was increased from ¼ to ½ and further to ¾. At full strength MS medium no more increase was induced.

2.2. Effect of MS medium strength on shoot weight (Table 2 and Fig. 1-2)

Shoot weight was influenced by medium strength. This weight increased progressively from 0.33 g to 0.45, 0.65 and 0.83 g as medium strength was increased from ¼ to ½, ¾ and full strength, respectively.

2. Effect of MS medium strength on shoot length (cm) (Table 2 and Figs. 1-3)

Strength of medium exerted a significant effect on shoot length. However, this significancy was not consistent. Although the increase of medium strength from ¼ to ½ was accompanied by an insignificant increase in shoot length from 5.20 to 5.85 cm, respectively, another increase in this variable to ¾ strength was reflected in a significant increase in this on character to 6.88 cm. Final increase in MS strength to a full one yielded an insignificant increase in shoot length to 7.53 cm compared to the previous value, though it was significant when compared to the results of ¼ and ½ strengths.

2.4. Effect of Ms medium strength on leaf number (Table 2 and Fig. 1-4)

Although there were non significant differences between leaf number from using MS medium at ¼ and ½ strengths (3.28 and 4.22, respectively), or due to using the same medium at ¾ and full strengths (5.59 and 6.06, respectively), there were a significant differences between results of the two groups, with the later one being higher.

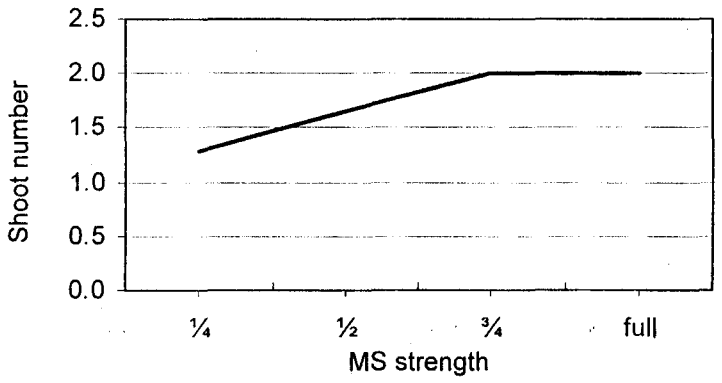

Fig. 1.1. Effect of medium strength on shoot number

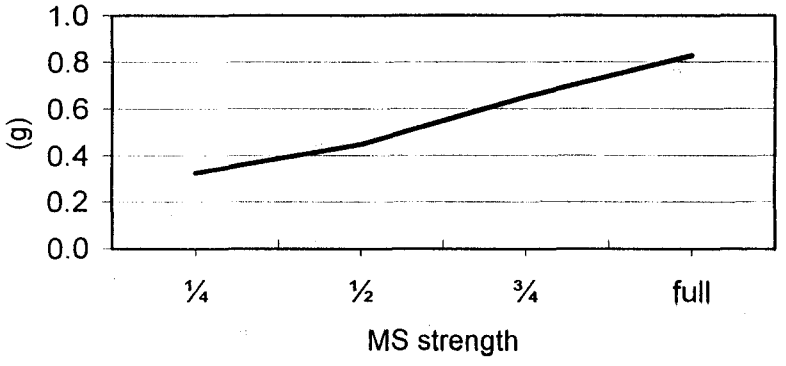

Fig. 1.2. Effect of medium strength on shoot weight

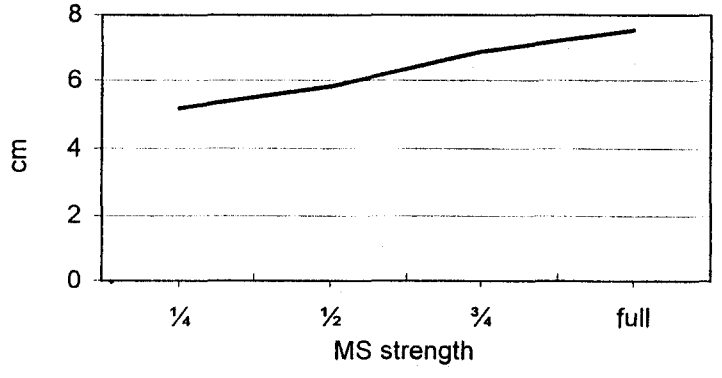

Fig. 1.3. Effect of medium strength on shoot length (cm)

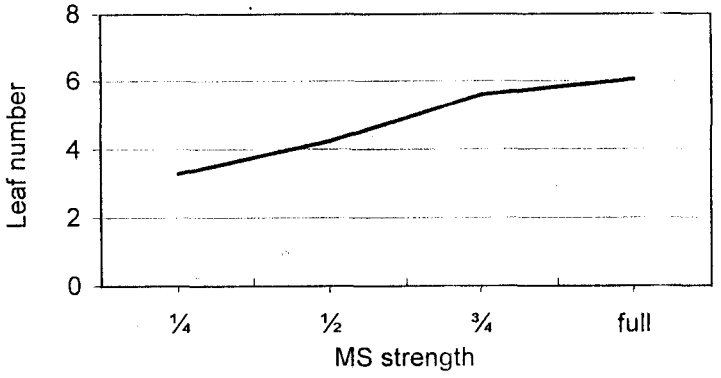

Fig. 1.4. Effect of medium strength on leaf number

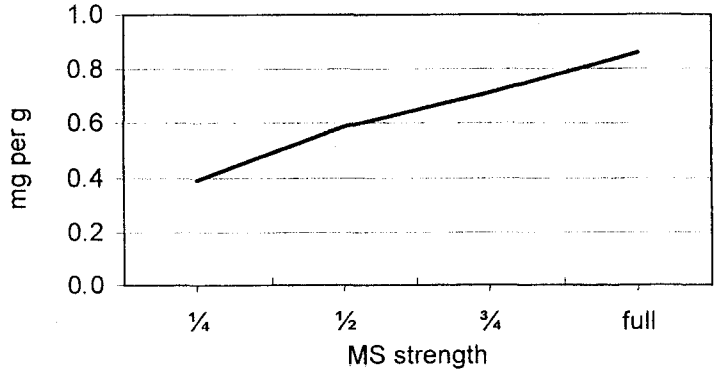

Fig. 1.5. Effect of medium strength on total chlorophyll content (mg/g. f.w.)

2.5. Effect of MS medium strength on total chlorophyll content (mg/g). (Table 2 and Fig. 1-5)

MS strength exerted a significant effect on the total chlorophyll content of shoots. The increase in MS strength from ¼ to ½ strength was reflected on a significant increase in this content from 0.39 to 0.59 mg/g of leaf fresh weight, respectively. Another increase to ¾ strength of MS resulted in a content of 0.71 mg f.w. of total chlorophyll which was insignificantly different from the previous or the next record. The later one, resulted from using the full strength of MS was 0.86 mg/g, was the highest record at all, although it was significantly higher than those of using either ¼ or ½.

Medium strength influenced growth and multiplication of the *in vitro* explants to a great extent. **JongHee *et al* (2004a)** studied the effect of inorganic salts concentration of the culture medium on bud induction and shoot growth of three different genotypes of the Chinese yam (*Dioscorea opposita*) cv. Jangma, Danma and Dunggunma. They found that lower salt strength of the medium inhibited shoot elongation but did not have much effect on the shoot and bud induction from the shoot apices. **JunWen *et al* (2004)** ascertained that the basic synthetic medium used for anther culture of wheat (*Triticum aestivum*) was W14 medium, with the inorganic salts (except iron) and the vitamins reduced to three-fourths of the original strength of the W14 medium. **Kalia *et al* (2004)** reported that to raise cultures of *Dalbergia sissoo*, surface sterilized explants were inoculated on full strength MS medium. **Nagira and Ozeki (2004)** remarked that leaf discs prepared from torenia plantlets grown under sterile conditions were grown on solidified half-strength MS medium. **Tamas *et al* (2004)** found that the most noticeable effect on regeneration frequency of some wheat cultivars was achieved by reducing the concentration of macroelements in the

regeneration medium to half-strength. **Enrico *et al* (2005)** stated that the development of multiple buds of *Alnus acuminata* ssp. *acuminata* explants at the initiation step was obtained with MS at 1/2 strength with either 1 or 2 µM (0.23-0.45 ppm) of BAP. Multiplication gave up to 15 elongating shoots by explant.

CONCLUSION

It is recommended to use scales of the aerial bulbils that grow on the flowering stem of lilium plants as preliminary explants to start a culture. The basal parts of the *in vitro* grown leaves were the only explants that showed a positive response exhibiting growth and proliferation. Using MS medium at ¾ strength was more economical than the full strength one as results of both were not significantly different.

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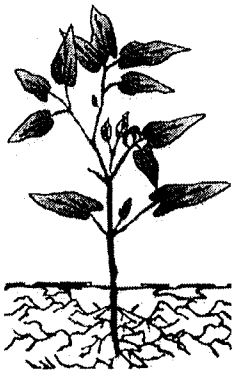
حوليـــات العلـــوم الزراعية
جامعة عين شمس ، القاهرة
مجلد(٥٤)، عدد (١)، ١٦٥-١٧٣، ٢٠٠٩



دراسات على الإكثار الدقيق لأبصال اليليم عيد الفصح
١ – إنشاء المزرعة

[١٣]

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٢– قســم البساتين– كليــة الزراعــة – جامعة عين شمس– شبــرا الخيمة– القاهرة– مصر

الموجـــز

أجريت هذه الدراسة فى معمل زراعة الأنسجة الخاص بمشروع إنتاج الشتلات بقسم البساتين بكلية الزراعة – جامعة عين شمس خلال الفترة من ٢٠٠٢ حتى ٢٠٠٤. وتهدف هذه الدراسة لوضع بروتوكول صالح للتطبيق للإكثار الدقيق السريع لأبصال اليليم عيد الفصح . وقد أوضحت النتائج أنه عند أخذ منفصلات من نباتات ليليم نامية فى الحقل، لم تستجب أى من منفصلات الأبصال وماتت فى النهاية. وعلى العكس ، فإن حراشيف البلابل النامية على الشمراخ الزهرى ظلت حية وأظهرت إستجابة من حيث النمو والتكاثر. وقد ظلت الأجزاء القاعدية فقط من منفصلات الأوراق النامية فى الأنابيب حية بعد فصلها وزراعتها على بيئة موراشيج وسكوج حيث إستجابت للنمو والتكاثر.

ولم يؤثر تركيز أو قوة بيئة موراشـــيج وســـكوج على عدد الأفرع لكل منفصل بشكل معنـــوى. وقـــد تسبب إستعمال بيئة موراشيج وسكوج بتركيز ثلاثـــة أرباع أو بالقوة الكاملة فى إنتاج أطول الأفرع وأكبر عدد مـــن الأوراق وأعلـــى محتـــوى للأفـــرع مـــن الكلوروفيل الكلى. وتسببت القوة الكاملة فقط فى إنتاج أثقل الأفرع.

تحكيـــم: أ.د سهير السيد حسن
أ.د إمـــــام نوفـــل