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IN VITRO PLANT REGENERATION OF SOME ORNAMENTAL FLOWERING BULBOUS PLANTS AND ESTABLISHMENT IN SOIL

Mohamed-Yasseen, Y.
Genetic Engineering and Biotechnology Research Institute, Sadat City, Minufiya University

ABSTRACT

A rapid and efficient method for plant regeneration and establishment in soil for three ornamental flowering bulbous plants from different explants was conducted. Corm segments, twin scales and immature flower explants of Freesia, spider lily and amaryllis were excised and cultured *in vitro*. Explants were cultured on Murashige and Skoog medium (MS) supplemented with different combinations of cytokinins: benzyladenine (BA) and thidiazuron (TDZ) and auxins: 2,4 dichlorophenoxyacetic acid (2,4-D) and naphthaleneacetic acid (NAA). Multiple shoots were produced from primary explants or derived callus. Regenerated shoots were then transferred into shoot-proliferation medium for further multiplication. Nature and concentration of the growth regulator as well as type and genotype of explants affected shoot regeneration. Produced shoots were transferred into corm and bulb-induction medium containing 90 g/l sucrose and 2 mg/l paclobutrazol (PP333). Corms were stored for the next culture season in soil while bulbous shoots of spider lily and amaryllis were preserved *in vitro* for 40 month. Corms, bulbs and bulbous shoots were transferred directly, without acclimatization, into soil and normal phenotypic plants were effectively established in soil. This study described a simple method for rapid propagation, corm and bulb formation, *in vitro* preservation and successful establishment of regenerated plants in soil.

Abbreviations: ABA: abscisic acid; BA: benzyladenine; MS: Murashige & Skoog,s (1962) medium; CCC: chlormequat chloride; GA_3: gibberellic acid; NAA: naphthaleneacetic acid; PP333: [paclobutrazol: 1-(4-chlorophenyl)-4,4- dimethyl-2-(1H-1,2,4 triazol-1-ly) pentan-3-ol]; SA: salicylic acid; TDZ: thidiazuron: [N-phenyl-N-(1,2,3 thiadiazol-5-yl)urea)]; 2,4-D: 2,4 dichlorophenoxyacetic acid.

Keywords: Amaryllidaceae, Iridaceae, bulbous plants, callus, ornamental plants, propagation, tissue culture.

INTRODUCTION

Freesia (*Freesia hybrida*), spider lily (*Hymenocallis speciosa* L.) and amaryllis (*Hippeastrum X hybridum* Hort,) are ornamental flowering geophytes bulbous plants which appertain to the family Iridaceae and Amaryllidaceae, respectively. Freesia is distributed in many parts of the world due to its pleasant scented flowers. Spider lily is a summer flowering bulbous herb produces white fragrant flower on a solid scape. Amaryllis species have significant floricultural importance and are one of the major bulb crops in the commercial market in many countries in the world (Ilczuk *et al*., 2006). There are numerous reports on the medicinal effects of many members of Amaryllidaceae which are prominent for high content of alkaloids. Furmanowa and Oledzka, (1990) had reviewed several features of the alkaloids in this family. It was confirmed that certain species of Hippeastrum and Hymenocallis

are effective in treating viral and cancer diseases (Backhaus *et al*., 1992; Petit *et al*., 1995, Griffin *et al*., 2007 and Saidi *et al*., 2007).

These plants are usually propagated by corms and bulbs however, number of daughter corms and bulbs produced from one mother plant is a rather small number for commercial propagation or releasing new varieties. Furthermore, spider lily and amaryllis are difficult to propagate by conventional methods for ornamental bulbous plants such as twin scaling because of low propagation rate and viral diseases. In addition, the plants are good host for many insects, pests and bacterial, fungal and viral diseases, which encumber propagation and plant breeding. Further development demands optimization of the propagation methods and a shortening of the growing period.

Various explants of these bulbous plants were studied for the purpose of producing high number of propagules in a short period of time using plant biotechnology to satisfy growing demand in markets (Zhu *et al*., 2005). Tissue culture of Freesia is carried out using different explants including: meristem, shoot tip, corm segments, flower buds, pedicel, ovaries, leaf base and root explants (Bajaj, 1989; Wang and Huang, 1995; Kim *et al*., 1997, Wang *et al*., 1998 and Mohamed-Yasseen and Abass, 2003). Twin scales are commonly used for propagation of spider lily (Yanagawa and Ito,1988) and amaryllis (Takayama and Yokokawa, 1996; Arteaga, *et al*., 1998; Okubo *et al.*1999; Ilczuck *et al*., 2005 and 2006). Nevertheless, inflorescence derived explants are employed occasionally in tissue culture of spider lily (Mohamed-Yasseen, 2002) and amaryllis (Mujib *et al*., 1991; De Bruyn, 1997).

Establishment of a rapid and efficient system for plant regeneration and successful establishment, of newly formed plants, in soil, would be a good asset towards mass propagation and production of pathogen-free plants with desirable traits. This study described a rapid and efficient protocol for plant regeneration, shoot proliferation, induction of storage organs, *in vitro* preservation and establishment of plants in soil of three bulbous ornamental plants.

MATERIALS AND METHODS

Source of explants. All experiments were conducted from 2005 to 2007 at the Genetic Engineering and Biotechnology Research Institute at Sadat City, University of Minufiya. Corms of Freesia, bulbs of spider lily and amaryllis as well as flower bud of Freesia and immature inflorescence of spider lily and amaryllis (local varieties) were used in this study as source of explants.

Explant preparation and surface sterilization for underground organs. Leaves and roots were removed from bulbs of spider lily and amaryllis and dried scales were discarded. Basal plate was trimmed and outer scales were removed then one third of distal part of the bulb was discarded. Corms and bulbs were consequently thoroughly washed with tap water and a detergent, surface sterilized with 0.1% (w/v) mercury chloride for 10 min and rinsed three times with a sterile distilled water. Corms were cut into segments (approximately 10 to 15 mm^3 in size) and bulbs were cut into pieces of two

adjacent scales joined by a piece of the basal plate, herein called twin scales, (15-20 mm in length and 12- 15 mm in width). Corm segments and twin scales were surface sterilized with 0.8 % (v/v) sodium hypochlorite for 20 min and rinsed three times in a sterile distilled water.

Explant preparation and surface sterilization for inflorescence. Flower bud of Freesia and inflorescence of spider lily and amaryllis were prepared prior surface sterilization. The spathe was removed from inflorescence then each individual flower was trimmed 4-8 mm above the receptacle. Each receptacle was cut longitudinally into 10-16 fragments depending on the size of the inflorescence. In some instances, whole small inflorescence were longitudinally quartered into four sections. Whole bud flower of Freesia, receptacle explants , perianthes, style filaments, quarter inflorescence and ovaries were surface sterilized in 70% ethanol for 3-5 sec. They were then rinsed with a sterile distilled water, surface sterilized with 0.8 % (v/v) sodium hypochlorite for 10 min and rinsed three times in a sterile distilled water.

Direct shoot regeneration from primary explants. Corm segments, twin scales and receptacle explants were placed on 25 ml of culture medium contained in 55-ml culture tubes and sealed with plastic polypropylene lids (Sigma, Saint Louis, MO). Whole bud flower of Freesia, receptacle explants , perianthes, style filaments, quarter inflorescence and ovaries were placed on 25 ml of culture media contained in 118 or 177-ml glass jars and sealed with clear plastic polypropylene lids. Culture medium was composed of MS containing 30 g/l sucrose, 2 g/l gelrite, (Phytagel, Sigma, Saint Louis, MO) and supplemented with different combinations of growth regulators. Corm segments were cultured on medium supplemented with 0.0, 0.5, 1.5, 3, 5 or 7 mg/l BA alone or with 0.0, 0.1 or 0.5 mg/l NAA. Twin scales, leaf base, perianthes and style filaments of spider lily were cultured on medium supplemented with 0.5, 1, 2, 3, 4, 5 or 6 mg/l BA and 0.1, 0.2, 0.5 or1 mg/l NAA. Receptacle, ovary, perianth, quarter inflorescence and twin scales explants of amaryllis were cultured on medium supplemented with 0.0, 0.5, 1, 1.5, 2 or 3 BA mg/l with 0.1, 0.2 or 0.5 mg/l NAA.

Callus induction and shoot regeneration from callus. Shoots were also regenerated from corm segments and shoot tip of Freesia; twin scales of amaryllis through callus formation. Callus-induction medium was composed of MS containing 30 g/l sucrose, 2 g/l gelrite, 200 mg/l casein hydrolysate, 300 mg/l glutamine and supplemented with 1.2 mg/l 2,4-D, 5mg/l NAA and 1 mg/l kinetin. Embryonic callus was produced after ten to twelve weeks from culture. Callus was transferred into MS containing 30 g/l sucrose, 2 g/l gelrite, 200 mg/l casein hydrolysate, 300 mg/l glutamine and supplemented with 0.2 mg/l BA.

Shoot proliferation. Produced shoots were transferred into shoot-proliferation medium for further multiplication. Shoots were cultured on 25 ml of culture media contained in 118 or 177-ml glass jars and sealed with clear plastic polypropylene lids. Shoot-proliferation medium was composed of MS supplemented with different combinations of growth regulators. Shoot-proliferation medium for Freesia shoots was containing 0.3, 1, 2, 3, 5 BA or 3 mg/l TDZ with 0.1, 0.2 or 0.5 mg/l NAA. Shoots of both spider lily and amaryllis were cultured on proliferation medium containing 0.5 mg/l BA and

0.1 mg/l NAA. Plantlets produced from shoot-proliferation media were transferred into root-induction medium prior transferring to soil or into corm and bulb-induction medium.

Corm and bulb induction. Proliferated shoots of Freesia were transferred into corm-induction medium composed of MS containing 2 g/l gelrite and supplemented with 30 or 90 g/l sucrose with 2 mg/l PP333, 10 mg/l ABA, 500 mg/l CCC, 25 mg/l SA or 10 mg/l GA3. Cultures were then kept at 15ºC for ten weeks prior transferring to 26ºC for additional eight weeks. Shoots of spider lily and amaryllis were cultured in bulb-induction medium composed of MS containing 2 g/l gelrite, 90 g/l sucrose and 2 mg/l PP333.

***In vitro* preservation.** Spider lily and amaryllis shoots produced bulb in bulb-induction medium. Bulbous plants were then, preserved *in vitro* for 40 months in the same medium. Plants were maintained in the growth room under the same temperature and light settings, described below. Data were recorded at the end of experiment and viable plants were thereafter, trimmed, quartered and cultured into shoot-proliferation medium for further multiplication or transferred into soil.

Media and culture conditions. Media pH was adjusted to 5.7 with 1N KOH after adding growth regulators and before gelling agent. Media were solidified with 2 g/l gelrite. Growth regulators, with the exception of GA_3 and CCC, were added before sterilization in an autoclave at 121ºC and 98 KPa for 20 min. GA_3 and CCC were dissolved in 70 % ethanol and were added to media after sterilization. Unless otherwise stated, cultures were maintained at 26 ± 2ºC and under an 18-hr photoperiod (cool white fluorescent light, 40 $\mu mol.m^{-2}.s^{-1}$). Explants cultured on callus-induction media were maintained in the dark until formation of embryonic callus. Cultures of amaryllis derived from initial explants were all incubated in the dark during the first four weeks of culture, in order to reduce browning, prior transfer to light.

Rooting and transfer to soil. Produced shoots from all plants were separated and transferred for rooting and elongation in MS supplemented with 30 g/l sucrose, 2 g/l gelrite with 0.1 mg/l NAA dispensed on 300-ml jars. Rooted shoots were planted into 165 cm^3 plastic pots filled with autoclaved mixture of soil, peat moss, vermiculite (1:1:1, v:v:v) and covered with a transparent polyethylene bag for acclimatization in a greenhouse. Corms of bulbs formed *in vitro* were planted in soil without acclimatization.

Experimental design. All experiments were conducted using a completely randomized design. Twenty replicates were used in each treatment and each experiment was repeated three times. Data were evaluated by analysis of variance and means between treatments were compared according to Duncan, (1955).

RESULTS AND DISCUSSION

Establishment of aseptic culture. Surface sterilization of underground organs require rigorous procedure for elimination of rhizosphere-inhabited microorganism (Cassells, 1991 and Arteaga *et al*., 1998). Disinfection is considered a determining factor for mass propagation with commercial aim for many ornamental bulbous plants (Arteaga *et al*., 1998). Despite this

precaution, contamination was high in explants derived from corms and bulbs. Contamination reached sixty five percent with twin scales and fifty percent with corm segments. Contaminations from flower and inflorescence-derived explants was as low as five percent. Plantlets production from inflorescence explants has several advantages over plantlets production from corm or bulb segments. One advantage is to avoid high losses usually encountered during surface sterilization of underground parts. In addition, excising inflorescence is not terminal to the mother plant. Nonetheless, corms and bulbs are present and available all over the year while inflorescence are not accessible except during certain and limited time of the year.

Table 1: Effect of different concentrations of BA and NAA on shoot regeneration from corm segments of *Freesia hybrida*

Concentrations (mg/l)	Shoot/ explant	Responding explants (%	Shoot length (cm	Root/ explant
MS alone	1.2 f [z]	65d	12.5 b	4.8 b
0.5 mg/l BA	4.2 d	88b	14.2 a	10.2 a
1.5 mg/l BA + 0.1 mg/l NAA	6.8 b	96ab	11.8 c	3.4 c
3 mg/l BA + 0.5 mg/l NAA	8.9 a	100a	9.6d	0.0 d
5 mg/l BA + 0.5 mg/l NAA	5.5 c	100a	8.8 e	0.0 d
7 mg/l BA+ 1 mg/l NAA	3.4 e	93b	5.3 f	0.0 d
1mg/l BA +1 mg/l TDZ +1mg/l NAA	9.5 a	100a	10.6 dc	0.0 d
2 mg/l BA + 0.2 mg/l NAA [y]	6.2 b	85 c	8.9 e	0.0 d

[z] Mean separation within column by Duncan's multiple range test at P = 0.05. [y]Explants used on this treatment were whole flower bud of Freesia.

Morphogenesis of Freesia explants. Corm segments of Freesia produced multiple shoots which evolved from somatic embryogenesis (Fig.1a, b) upon culture on shoot-regeneration medium. Shoot regeneration attained its maximum on medium containing 3 mg/l BA and 0.5 mg/l NAA (Table, 1). Number of regenerated shoots was reduced with decreasing or increasing cytokinin concentrations above or under 3 mg/l BA. Shoot length and number of roots per explant were decreased generally with increasing cytokinin concentration. Flower bud of Freesia proved to be an excellent explant for shoot regeneration and gave rise to multiple shoots.

Regenerated shoots of Freesia from corm segments were then transferred into shoot-proliferation medium for further multiplication. Shoot proliferation increased with increasing concentration of cytokinins (Fig.1c) and reached its highest number using 3mg/l BA and 0.5 mg/l NAA (Table, 2) then decreased with increasing concentration of cytokinins. Shoot length and root formation were typically reduced with increasing concentration of cytokinins. Callus was produced from shoots cultured on callus-induction medium and shoots were then recovered from somatic embryos. Direct regeneration and regeneration from callus could be employed for clonal propagation and breeding purposes, respectively.

Figure 1. Shoot regeneration and corm formation from Freesia. (a). Somatic embryogenesis produced on corm segments. (b). Shoot initiation and root formation (c). Shoot proliferation. (d). Typical corm formation *in vitro*.

Table 2. Effect of different concentrations of cytokinins and auxins on shoot proliferation from shoot tip explants of *Freesia hybrida*

Concentrations (mg/l)	Shoot/ explant	Responding explants(%)	Shoot length (cm)	Root/ explant
3 mg/l BA	1.8 f [z]	95 b	13.8 a	2.2 b
1mg/l BA + 0.1 mg/l NAA	2.5e	100 a	12.5b	3.5 a
2 mg/l BA + 0.2 mg/l NAA	5.4 b	100a	10.6 c	1.5 c
3 mg/l BA + 0.5 mg/l NAA	6.2 a	100a	8.5 e	0.0 d
5 mg/l BA + 0.5 mg/l NAA	4.0 cd	95 b	5.8 f	0.0 d
3 mg/l BA +3 mg/l TDZ +1mg/l NAA	3.8 d	95 b	6.4 f	0.0 d
1mg/l TDZ + 0.5 mg/l NAA	2.9 e	100b	11.8 b	2.0 b
1.2 mg/l 2,4-D +5 mg/l NAA + 1 mg/l kinetin[y]	4.5 c	85 c	8.8 d	0.0 d

[z]Mean separation within columns by Duncan's multiple range test at P = 0.05. [y] Shoots produced in this treatment were derived from callus which was consequently transferred into shoot-induction medium for shoot formation.

Morphogenesis of spider lily explants. Twin scale explants produced multiple shoots on MS containing BA and NAA (Table, 3). Contamination rate with twin scales was too high to upset other combinations of growth regulators and as a result another alternative source of explants such as perianth and

style filament was used. Perianth and filament explants formed multiple shoots on the presence of BA and NAA. Shoots production from perianthes and filaments was a slow process. This later may be due to the small size and fragile nature of these two explants. Nevertheless, the totipotencey of these explants was high and multiple shoots were regenerated following callus formation (Table, 3). It is worthy to note that filament explants produced assortment of colorful callus white, yellowish, reddish and green callus (Fig. 2a). This callus turned progressively into green color during the process of shoot regeneration. All shoots were transferred into shoot-proliferation medium and resulted shoots were used as a source for leaf base explants. Leaf base explants produced multiple shoots on different combinations of BA and NAA. Leaf base produced small whitish callus on leaf surface prior shoot formation (Fig. 2b). Number of regenerated shoots was increased with increasing concentration of BA until 2-3 mg/l then decreased with increasing its concentration (Table, 3). Shoot regeneration was the highest from leaf base than other explants which may due to the fact that leaf-base explants are derived from *in vitro* plants.

Table 3. Effect of different concentrations of BA and NAA on shoot regeneration from different explants from spider lily

Concentrations (mg/l)	Shoot/explant	Responding explants (%)	Shoot length (mm)	Root/ explant
Twin scale explants				
1 mg/l BA + 0.2 mg/l NAA	1.9 b [z]	85 a	55 a	2.5 a
3 mg/l BA+ 0.2 mg/l NAA	4.8 a	85 a	43 b	0.0 b
5 mg/l BA + 0.5 mg/l NAA	2.2 c	85 a	32 c	0.0 b
Perianth explants				
2 mg/l BA + 0.2 mg/l NAA	2.3 a	80 b	45a	0.2 a
3 mg/l BA+ 0.2 mg/l NAA	2.8 a	85 a	35 b	0.0 b
5 mg/l BA + 0.5 mg/l NAA	1.4 b	85 a	29 b	0.0 b
Filament explants				
2 mg/l BA + 0.2 mg/l NAA	3.9 a	80 b	42 a	0.5 a
3 mg/l BA+ 0.2 mg/l NAA	4.2 a	85 a	32 a	0.0 b
5 mg/l BA + 0.5 mg/l NAA	2.9 b	85 a	25 b	0.0 b
Leaf base explants				
MS alone	0.5 f	20 f	35 d	0.6 d
0.5 mg/l BA	3.4 d	78 c	45 c	4.3 a
1 mg/l BA + 0.1mg/l NAA	4.2 b	85 b	58 b	2.4 b
2 mg/l BA + 0.2mg/l NAA	5.3 ab	90 a	64 a	1.2c
3 mg/l BA + 0.2mg/l NAA	6.5 a	82 b	42 c	0.0 e
4 mg/l BA+ 0.5mg/l NAA	4.3 b	75 c	25 e	0.0 e
5 mg/l BA+ 0.5mg/l NAA	2.5 e	60 d	18 f	0.0 e
6 mg/l BA+ 1mg/l NAA	1.8 e	40 e	15 f	0.0 e

[z] Mean separation within columns for each type of explants by Duncan's multiple range test at P = 0.05.

Figure 2. Callus formation, shoot regeneration and establishment in soil of spider lily. (a). Embryonic callus of spider lily produced from style filament. (b). Callus initiation on leaf base (right), followed by shoot regeneration on leaf base explant (left). (c). Establishment of regenerated plants in soil.

Morphogenesis of amaryllis explants. Receptacle explants of amaryllis were excised from inflorescence at the primordial stage produced numerous shoots (Fig. 3a) on BA and NAA containing media. It appeared that shoot regeneration was better with relatively low concentrations of BA (Table, 4). Shoot regeneration was high with 1-1.5 mg/l BA and then decreased with increasing BA concentration. Shoot length and root formation decreased with increasing BA concentrations. Similar results were obtained with perianth and ovary explants. Shoot regeneration from quarter-inflorescence and twin-scale explants cultured on 2,4-D containing medium was higher than explants cultured on BA-containing medium. However, shoot regeneration from BA-containing medium was faster since shoots were formed directly without passing through callus formation followed by shoot regeneration from produced callus. Recent researchs demonstrated that amaryllis required low concentrations of cytokinins (Ilczuk *et al*., 2006) for multiplication *in vitro*.

Table 4. Effect of different concentrations of cytokinins and auxins on shoot regeneration from different explants derived from inflorescence of amaryllis and twin scales

Concentrations (mg/l)	Shoot/ explant	Responding explants (%)	Shoot length (mm)	Root/ explant
Receptacle explants				
MS alone	0.0 f [z]	0.0 d	0.0 f	0.0 C
0.5 mg/l BA + 0.1mg/l NAA	5.2 b	88 b	67 a	2.3 a
1 mg/l BA + 0.1mg/l NAA	5.9 b	95 a	54 b	1.5 b
1.5 mg/l BA + 0.1mg/l NAA	6.5 a	98 a	25 c	0.0 c
2 mg/l BA + 0.1mg/l NAA	4.1 c	85 b	15 d	0.0 e
3 mg/l BA + 0.5 mg/l NAA	2.2 d	80 c	13 d	0.0 e
Perianth explants				
1 mg/l BA+ 0.1 mg/l NAA	2.2 b	75 a	14a	0.8 a
1.5 mg/l BA+ 0.1 mg/l NAA	3.8 a	78 a	19 a	0.0 b
2 mg/l BA+ 0.2 mg/l NAA	1.7 c	45 b	5 b	0.0 b
Ovary explants				
1.5 mg/l BA+ 0.5 mg/l NAA	3.3 a	75 a	23 a	0.4 a
2 mg/l BA+ 1mg/l NAA	2.5 b	70 b	15 b	0.0 b
Quarter inflorescence				
1mg/l BA and 0.2 mg/l NAA	4.8 b	100 a	74 a	1.3 b
1.2 mg/l 2,4-D +5 mg/l NAA + 1 mg/l kinetin	6.9 a	100 a	58 b	2.4 a
Twin scale explants				
1mg/l BA and 0.2 mg/l NAA	3.3 a	70 b	63 a	2.2 b
1.2 mg/l 2,4-D +5 mg/l NAA + 1 mg/l kinetin	5.4 b	75a	70 a	3.4 a

[z] Mean separation within columns for each type of explants by Duncan's multiple range test at P = 0.05.

Corms and bulbs induction. High sugar concentration and cold treatment increased frequency of corm formation (Fig. 1d), corm weight and size (Table, 5). The inclusion of PP333 with high sugar concentration (90 g/l sucrose) showed no supplementary effect, nevertheless the addition of this antigibberelin-biosynthesis with 30g/l sucrose showed its stimulatory effect on corm formation. The inductive role of high sugar concentration, PP333 (Dantu and Bhojwani, 1995; Ilczuck *et al*., 2005) and chilling treatment (Doi *et al*., 1992) is well established in several crops. SA did not induce corm formation though it was reported that it induced tuber formation (Xiong *et al*., 1999) yet, Davis (2004) revealed stated that SA has no direct promotive effects on formation of storage organs. ABA appeared to be slightly inductive to corm formation and CCC has no significant effect, while GA proved to be inhibitory for corm formation (Dantu and Bhojwani, 1995). Spider lily and amaryllis produced bulbous shoots and bulbs (Fig. 3 b, c) on bulb induction medium.

Table 5. Effect of different cold treatments, sucrose and growth regulators on corm formation from plantlets of *Freesia hybrida*

Treatment	Temperature of incubation	Responding plant (%)	Corm fresh weight (mg)	Corm diameter (mm)
30 sucrose	26ºC	25 e[z]	125 e	**5 e**
90 g/l sucrose	26ºC	80 b	240 b	**18 b**
90 g/l sucrose + 2 mg/l PP333	26ºC	80 b	250 b	16 b
30 sucrose	15ºC	60 d	135 e	7 d
90 g/l sucrose	15ºC	100 a	835 a	23 a
90 g/l sucrose + 2 mg/l PP333	15ºC	100 a	850 a	20 a
30 g/l sucrose + 2 mg/l PP333	15ºC	100 a	230 b	12 c
30 g/l sucrose + 10 mg/l ABA	15ºC	75 c	170 c	10 c
30 g/l sucrose + 500 mg/l CCC	15ºC	60 d	130 e	5 e
30 g/l sucrose + 25 mg/l SA	15ºC	65 cd	145 d	7 d
30 g/l sucrose + 10 mg/l GA3	15ºC	0.0 f	0.0 f	0.0 f

[z] Mean separation within columns by Duncan's multiple range test at P = 0.05.

***In vitro* Preservation.** Eighty percent of stored plants were viable after 40 month. Low temperature, reduced light intensity, growth retardants and osmotic stressants are fundamentally used for *in vitro* preservation (Golmirzaie and Toledo, 1999). Nevertheless, utilizing of low temperature and reduced light intensity, would require special space and certain equipments for preservation which append additional costs. It was possible to store bulbous plants at the same conditions of growth without of temperature and light intensity, thus reducing labor and space costs. *In vitro* preservation was achieved in, this experiment, using PP333 as a growth retardant and high sugar concentration as osmoticum. Several reports utilized shoot tip, plantlets (Golmirzaie and Toledo, 1999) and callus for preservation (Toshinari and Masahiro, 2001). In this work bulbous plants were used. Bulbous plants appeared to be more tolerant for storage. This non-cryogenic preservation method presented herein, is simple, not expensive and has potential applications for other geophyte plants.

Rooting and transfer to soil. Shoots from Freesia, spider lily and amaryllis formed roots and elongated in MS medium supplemented with 0.1 mg/l NAA. All shoots easily produced multiple roots in root-induction medium. Rooted shoots were transferred into soil and ninety percent of these plants were successfully established in soil... Rooting and acclimatization steps were not required for corms of Freesia and bulbs of spider lily and amaryllis. These corms and bulbs were transferred directly into pots filled with a soil and hundred percent of plants were effectively established in the soil (Fig. 2c, 3d) and normal phenotypic flowering plants were produced.

Figure 3. Shoot regeneration, bulb formation and establishment in soil of amaryllis. (a). Typical inflorescence of amaryllis at the primordial stage (right) and shoot regeneration from receptacle explant (left). (b). Shoot proliferation and initiation of bulb formation. (c). Typical bulb produced *in vitro*. (d). Establishment of regenerated plants of amaryllis in soil.

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إكثار بعض أبصال الزينة وتكوين كورمات وأبصال باستخدام تقنيات زراعة الأنسجة ونقل النباتات الناتجة إلى التربة

يس محمد محمد يس

معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية - مدينة السادات - جامعة المنوفية

أجرى هذا البحث على ثلاثة من أبصال الزينة وهى الفريزيا و الهيمينوكالس و الأمريللس وأستخدم في هذا البحث أجزاء مختلفة وتشمل أجزاء نباتية من الكورمات و الأبصال والنورات الزهرية. تم زراعة الأجزاء النباتية المختلفة على بيئة ميراشيج وسكوج المحتوية على البنزيل أدينين والثاى داى أزيرون والتو فوردى و نفثالين حمض الخليك وتم الحصول على العديد من النباتات من الأجزاء المختلفة بعد عدة أسابيع من الزراعة. و قد وجد أن نوع منظمات النمو وتركيزه وجنس النبات وكذلك طبيعة الجزء النباتي المنزرع له تأثير على عدد النباتات الناتجة وطولها وعدد الجذور. تم حث النباتات المتكونة على تكوين كورمات بزراعتها على بيئة ميراشيج وسكوج المحتوية على 90 جرام/لترمن السكروز او 2 مليجرام/لتر باكلوبيوترازول أو حمض الأبسيسيك أو السيكوسيل أو حمض السلسليك و وضعها لمدة 10 أسابيع على درجة حرارة 15ºC ثم نقلها إلى درجة الحرارة 26ºC أو تكوين أبصال بزراعتها على بيئة ميراشيج وسكوج المحتوية على 90جرام/لترمن السكروز و 2 مليجرام/لتر باكلوبيوترازول. تم زراعة بعض النباتات أيضا على بيئة محتوية على 1,. مليجرام/لتر من نفثالين حمض الخليك لتكوين الجذور. تم حفظ النباتات التى كونت أبصال على نفس البيئة لمدة 40 شهر و أقلمة النباتات التى كونت جذور أما الأبصال و الكورمات فنقلت مباشرة إلى التربة دون حاجة إلى أقلمة. يوضح هذا البحث طريقة سريعة لإكثار ثلاثة من نباتات الزينة وتكوين كورمات وأبصال وحفظ النباتات المكونة لأبصال لعدة شهور ثم نقل النباتات بنجاح إلى التربة.