

## **PLANT REGENERATION FROM PERIANTHES AND DIVERSE EXPLANTS FROM GLADIOLUS INFLORESCENCE**

**Mohamed-Yasseen, Y.**

**Genetic Engineering and Biotechnology Research Institute Minufiya University, Sadat City, Egypt**

### **ABSTRACT**

A procedure for plant regeneration from perianthes and different explants of gladiolus inflorescence is described. Perianthes from different stages of development were cultured on Murashige and Skoog medium (MS) supplemented with 2 mg/l benzyladenine (BA) and 0.1 mg/l naphthaleneacetic acid (NAA). Small perianthes were cultured on MS containing 0.0, 1, 2, 3, 4, 5, 6 mg/l BA and 0.1 mg/l NAA. Inflorescence stem, inflorescence nodes, bracts and ovaries were cultured on MS supplemented with 2 mg/l BA and 0.1 mg/l NAA. Inflorescence explants and anthers were also cultured on callus-induction medium composed of MS supplemented with 1.5 mg/l dichlorophenoxyacetic acid (2,4-D), 0.5 mg/l NAA, 0.5 mg/l kinetin, 200 mg/l casein hydrolysate and 300 mg/l glutamine. Multiple shoots were regenerated from all types of inflorescence explants cultured on media containing BA and NAA. Shoot regeneration from perianthes was greatly affected by flower age and concentration of BA and NAA. Callus was produced from inflorescence explants on callus-induction medium; however, callus was not produced from anthers. Plantlets were regenerated from callus upon transfer to MS containing 0.2 mg/l BA. Produced shoots were transferred to root-induction medium composed of MS containing 0.1 mg/l indolebutyric acid (IBA). Rooted plantlets were transferred to soil and normal phenotypic plants were successfully established in soil.

### **INTRODUCTION**

Gladiolus is an herbaceous geophyte plant which belongs to the family Iridaceae. It is originated in the Mediterranean area and in South Africa (Buch, 1972). Gladiolus is a top selling cut flower which is cultivated the world over, however, it is highly susceptible to fungal and viral diseases and its conventional propagation is slow (Mohamed-Yasseen, 1999a). The new development in plant biotechnology presented by tissue culture and genetic engineering could have a tremendous role in improving plant characteristics. Using these approaches it would be possible to achieve improvements in gladiolus. Tissue culture technique has been used in propagation of several bulb and corm producing plants, including several members of Liliaceae (Mohamed-Yasseen *et al.*, 1994); Amaryllidaceae (Mohamed-Yasseen, 1999b, 2001, 2002) and Iridaceae (Hussey, 1975; Mohamed-Yasseen, 2000).

Plantlets were regenerated from different explants from gladiolus (Ziv and Lilien-Kipnis, 1990; Mohamed-Yasseen, 1999a) such as meristem (Takatsu, 1982); apical buds (Aminuddin and Singh, 1993); corm slices and young leaf bases (Hussey, 1975; Bajaj *et al.*, 1983; Remotti and Loffler, 1995) and root explants (Mohamed-Yasseen, 2000).

However few attempts were made to regenerate plants from explants derived from inflorescence. Inflorescence nodes were used to produce plants from callus (Zhuo and Sun, 1986; Bajaj *et al.*, 1983). Plantlets were

regenerated from compact callus derived from inflorescence stem (Kamo *et al.*, 1990; Bajaj *et al.*, 1983; Hussey, 1975). Bajaj *et al.*, (1983) referred to callus production from bract, perianth and anthers, however, plant regeneration from these explants was not reported.

This paper describes the establishment of an efficient system for plant regeneration from perianthes of several genotypes of gladiolus. Moreover, it describes a developed method for callus formation and plant regeneration from inflorescence stem, inflorescence node, bract and ovary derived from gladiolus inflorescence.

## **MATERIALS AND METHODS**

*Source of explants.* Inflorescences of *Gladiolus X grandiflorus*, cultivars 'White Prosperity', 'Rose Supreme', 'Eurovision' and 'Victor Borge' were used as a source of explants. All experiments were conducted at Genetic Engineering and Biotechnology Research Institute from 1999 to 2002.

*Surface-sterilization of explants.* Inflorescences, with two to three mature opened flowers, were washed thoroughly with tap-water and detergent. Opened flowers along with inflorescence stem and inflorescence node were discarded. Intact unopened flowers (approximately 12 to 40 mm in length), were surface sterilized with 0.79% (v/v) sodium hypochlorite for 20 min. They were then rinsed three times in sterile distilled water. Bracts, perianthes ovaries and anthers were separated from surface-sterilized flower prior culture *in vitro*.

*Preparation of explants.* Perianthes were excised from flowers at different stages of development. Flowers with length less than 10 mm were considered very small flowers, flowers with length ranging from 12-20 mm were considered small flowers, flowers with length ranging from 20-30 mm were considered medium flowers, while flowers whose length exceed 30 mm were considered large flowers. Perianthes were separated from each other prior culture. Ovaries were obtained from medium and large flowers, bracts were excised from small flowers, while samples of anthers were assayed from all types of flowers. All explants; inflorescence stem, inflorescence node, bract, perianthes, ovaries and anthers, were placed on 25 ml of culture media contained in 55-ml culture tubes and sealed with plastic polypropylene lids (Sigma, Saint Louis, MO). Culture medium was composed of MS supplemented with 30 g/l sucrose, 8 g/l agar and supplemented with different growth regulators.

*Composition of culture medium.* Direct shoot formation was accomplished on MS containing different concentrations of BA with NAA. Perianthes from different stages of maturity were cultured on MS supplemented with 30 g/l sucrose, 8 g/l agar, 2 mg/l BA and 0.1 mg/l NAA. Small perianthes, were cultured on MS containing 0.0, 1, 2, 3, 4, 5, or 6 mg/l BA with 0.1 mg/l NAA. The effect of gibberellin, kinetin and silver nitrate on shoot regeneration from perianthes was examined. Perianthes were cultured on MS supplemented with 30 g/l sucrose, 8 g/l agar, 2 mg/l BA and 0.1 mg/l NAA with 1mg/l kinetin, gibberellin or 5 mg/l silver nitrate. Inflorescence stem, inflorescence node, bracts and ovaries were placed on MS

supplemented with 30 g/l sucrose, 8 g/l agar (Sigma, Saint Louis, MO), 2 mg/l BA and 0.1 mg/l NAA.

Shoots were also produced from callus induced on MS containing 2,4-D. Inflorescence stem, inflorescence node, bracts, perianthes, ovaries and anthers were placed on MS containing 30 g/l sucrose, 2 g/l gelrite (Phytigel, Sigma, Saint Louis, MO), 200 mg/l casein hydrolysate, 300 mg/l glutamine, and supplemented with 1.5 mg/l 2,4-D, 0.5 mg/l NAA and 0.5 mg/l kinetin. Shoots were regenerated from produced callus on MS supplemented with 0.2 mg/l BA.

*Media and culture conditions.* Media pH was adjusted to 5.7 with 1N KOH after adding growth regulators. Growth regulators were added before sterilization in an autoclave at 121°C and 98 KPa for 20 min. Explants cultured on BA containing-medium were maintained under an 18 hr photoperiod (cool white fluorescent light, 40  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and 28°C, while callus induction cultures were kept in the dark.

*Rooting and transfer to soil.* All produced shoots were separated and transferred for rooting and elongation in MS supplemented with 30 g/l sucrose, 8 g/l agar with 0.1 mg/l IBA. Rooted shoots were planted in 165 cm<sup>3</sup> plastic pots filled with autoclaved commercial potting soil (Agro Mix no. 2; Conard Fafard, Springfield, Mass) and covered with glass beakers for 7-10 days.

*Experimental design.* All experiments were conducted using a completely randomized design (Duncan, 1955). Twenty explants at least were used in each treatment and each experiment was repeated at least twice.

## RESULTS AND DISCUSSION

Shoot regeneration from perianthes was greatly influenced by the ontogenetic state of explants (Table 1) and concentration of BA and NAA (Table 2). Perianthes excised from mature flower did not produce shoots, very small perianthes produced low number of shoots, while medium and small flowers produced the highest number of shoots. The highest number of shoot regeneration and highest percentage of shoot regeneration was obtained from small perianthes.

Shoot regeneration from perianthes was successful from several genotypes (Fig 1), such as 'White Prosperity', 'Rose Supreme', 'Eurovision' and 'Victor Borge'. Many perianthes-derived shoots were having the same color of perianthes and had the shape of petal, nevertheless they become like normal shoots with growth.

**Table 1: Effects of stage of maturity on shoot regeneration from perianthes cv. "Rose Supreme" cultured on MS containing 2 mg/l BA and 0.1 mg/l NAA.**

Stage of maturity	explants producing shoots %	shoot per explant
large flower	0.0	0.0 <sup>zc</sup>
medium flower	40	3.8 a
small flower	80	4.1 a
very small flower	10	2.3 b

Mean separation by Duncan's multiple range test at P = 0.05.

**Fig. 1: Typical shoot regeneration from perianthes of different genotypes: a: "White Prosperity", b: "Rose Supreme", c: "Eurovision" and d: "Victor Borg" (each square corresponds to 10 mm).**

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The addition of BA and NAA was critical for shoot regeneration from perianthes (Table, 2), moreover, shoot regeneration was affected by concentration of BA and NAA. Shoot regeneration from perianthes reached its maximum on MS containing 2 or 3 mg/l BA and 0.1 mg/l NAA.

**Table 2. Effects of concentrations of BA and NAA on shoot regeneration from perianthes cv. 'Rose Supreme'. Explants were cultured on MS alone or containing 0.1 mg/l NAA with different concentrations of BA.**

Medium	explants producing shoots %	shoot per explant
MS alone	0.0	0.0 c
MS + 1 mg/l BA	60	3.1 b
MS + 2 mg/l BA	80	3.8 a
MS + 3 mg/l BA	60	4.3 a
MS + 4 mg/l BA	60	2.9 bc
MS + 5 mg/l BA	40	2.2 bc
MS + 6 mg/l BA	15	1.8 c

<sup>z</sup> Mean separation by Duncan's multiple range test at P = 0.05.

High concentration of BA (4 to 6 mg/l BA) reduced the number of regenerated shoots. Cluster of shoots initiated from perianthes explants required transfer into fresh medium after four weeks, otherwise they would become necrotic and lose viability. The addition of 1mg/l kinetin, 1mg/l gibberellin or 1mg/l silver nitrate did not increase viability of shoots. Perianthes are known to have high contents of phenolic compounds. It is possible that phenolic compounds are implicated in the inhibition of shoot growth. Transfer of explants into fresh medium alleviated this phenomena. Callus formation and shoot regeneration was restricted to the distal section of perianthes (Fig. 1).

Inflorescence stem, inflorescence node and bract produced callus in BA-containing medium after six weeks from culture (Table 3). This callus produced shoots after transfer to fresh medium. Ovaries produced shoots directly without callus formation. Shoot regeneration from ovary and inflorescence node was higher than shoot regeneration from inflorescence stem and bract.

**Table 3. Shoot regeneration from inflorescence stem, node, bracts and ovary cv. "Rose Supreme" cultured on MS containing 2 mg/l BA and 0.1 mg/l NAA.**

Explant	% explants producing shoots	shoot per explant	shoot length (mm)
inflorescence stem	40	3.4 b <sup>z</sup>	10.2 a
inflorescence node	70	15.0 a	9.8 a
bract	60	4.1 b	5.6 b
ovary	70	4.8 b	4.3 b

<sup>z</sup> Mean separation by Duncan's multiple range test at P = 0.05.

Inflorescence stem produced dark exudates into culture medium which may cause low percentage of explants producing shoots. Although, inflorescence node produced shoots in BA-containing medium after callus formation (Fig. 2a), young explants produced shoots directly without callus formation (Fig. 2b). Produced shoots from ovary were regenerated from the ovary wall and not from ovule. Shoots produced from ovary had the shape and color of petals of the mother plants (Fig. 2c); however they turned into normal shoots after several weeks from growth.

Inflorescence stem, inflorescence node, bract, ovary and perianthes produced regenerable callus in 2,4-D containing medium (table 4). Perianthes and ovaries produced small callus. Some perianthes produced small callus which become necrotic. Ovary produced small compact callus from the outer tissues.

**Table 4. Shoot regeneration from inflorescence stem, inflorescence node, bract, ovary and perianth cv. "Rose Supreme" cultured on MS containing 1.5 mg/l 2,4-D, 0.5 mg/l NAA and 0.5 mg/l kinetin.**

<b>Explant</b>	<b>explants producing callus %</b>	<b>shoot /explant</b>
Inflorescence stem	50	3.6 c <sup>z</sup>
Inflorescence node	60	5.4 a
bract	50	4.8 b
ovary	10	3.1 d
perianth	40	2.8 d

<sup>z</sup> Mean separation by Duncan's multiple range test at P = 0.05.

Hussey ( 1975) reported that ovary wall tissues produced callus in the presence of 2,4-D alone. Attempts to induce callus from anthers was unsuccessful, nevertheless anther filaments produced callus. Bajaj *et al.* (1983) were the only to report callus formation from anther, however; since this date, there is no available information about callus or shoot formation from gladiolus anther.

Bajaj *et al.* (1983) investigated callus formation from several explants derived from gladiolus inflorescence. They obtained callus notably from inflorescence stem. However; produced callus mostly underwent rhizogenesis and occasionally (in 5 % cases) differentiated shoots. This is the first paper to report successful protocol with high frequency for shoot regeneration from perianthes of several genotypes. Moreover, it describes two methods for shoot regeneration from several explants derived from gladiolus inflorescence. The first method relies on direct shoot regeneration on BA and NAA containing medium, while the other method depends on callus formation on 2,4-D containing medium followed by shoot regeneration from callus on shoot-induction medium. Shoot regeneration from inflorescence explants was generally, higher through direct shoot formation method.

**Fig. 2. Morphogenesis from inflorescence node and ovary. a: Typical shoot regeneration from inflorescence node callus (each square corresponds to 10 mm). b: Typical direct shoot regeneration from inflorescence node. c. Typical shoot regeneration from ovary, cv. "Rose Supreme". Regenerated shoots had the color and shape of petals.**

Shoot regeneration from inflorescence explants has the potential for both shoot proliferation from explants containing pre-existing buds such as inflorescence node and shoot regeneration from adventitious buds. Plantlets production from inflorescence explants has several advantages over plantlets production from corm segments. One advantage is to avoid high losses usually encountered during surface sterilization of underground parts. In addition, excising inflorescence is not detrimental to the mother plant.

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### إنتاج نباتات فى المزارع النسيجية من البتلات و أجزاء مختلفة من النورة الزهرية لنبات الجلادولوس

يس محمد محمد يس

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

يعرض البحث طريقة لإنتاج نباتات فى المزارع النسيجية من البتلات فى مراحل مختلفة من النمو وكذلك من أجزاء عديدة من النورة الزهرية وقد تم زراعة البتلات وأجزاء من الساق والعقد والقنابة والمبيض على بيئة ميراشيخ وسكوج محتوية على ٢ ملليجرام/لتر بنزيل أدينين و ١, ٠ ملليجرام/لتر نفتالين حمض الخليك ٠ كما زرعت البتلات الصغيرة على بيئة ميراشيخ وسكوج بدون منظمات النمو او محتوية على ١ أو ٢ أو ٣ أو ٤ أو ٥ أو ٦ ملليجرام/لتر بنزيل أدينين ١, ٠ ملليجرام/لتر نفتالين حمض الخليك ٠ وقد زرعت الأجزاء النباتية السابق ذكرها بالإضافة الى المتك على بيئة ميراشيخ وسكوج محتوية على ١, ٥ ملليجرام/لتر من التوفوردى و ٥, ٠ ملليجرام/لتر نفتالين حمض الخليك و ٥, ٠ ملليجرام/لتر كينين و ٢٠٠ ملليجرام/لتر كازين هيدوليزات و ٣٠٠ ملليجرام/لتر جلوتامين ٠ وقد تم الحصول على نباتات من البتلات وقد تأثر عدد النباتات المتكونة بعمر البتلات وتركيز كل من البنزيل أدينين ونفتالين حمض الخليك و تم إنتاج كالوس من الأجزاء النباتية المنزرعة على بيئة ميراشيخ وسكوج محتوية على التوفوردى فيما عدا المتك وتكونت نباتات من الكالوس بعد زراعته على بيئة ميراشيخ وسكوج محتوية على ٢, ٠ ملليجرام/لتر بنزيل أدينين ٠ وتم نقل النباتات إلى بيئة ميراشيخ وسكوج محتوية على ١, ٠ ملليجرام/لتر بيوتريك حمض الخليك لتشجيع تكوين الجذور ونقلت النباتات التى كونت جذور الى الصوبة وتم الحصول على نباتات مزهرة.

**Mohamed-Yasseen, Y.**