

Effect of Gibberellic Acid (GA₃) and Sugar on *In Vitro* Cormlet Formation, Multiplication and *Ex Vitro* Sprouting of *Gladiolus hybrida* Variety Princess Lee

P.A.I.U. Dharmasena, D.P. Karunananda¹ and J.P. Eeswara^{2*}

Postgraduate Institute of Agriculture
University of Peradeniya
Sri Lanka

ABSTRACT. Calli were initiated from corms of *Gladiolus hybrida* variety Princess Lee by culturing on Murashige and Skoog (MS) medium supplemented with 1.0 mg/l naphthalene acetic acid (NAA) and 20 g/l sucrose. *In vitro* shoots were regenerated from the calli by transferring into MS medium containing 30 g/l sucrose without growth regulators. Regenerated shoots produced cormlets at the base of the shoot on full or half strength MS medium supplemented with 40-60 g/l sucrose. Highest number of large corms (diameter > 1.5 cm) was obtained in the half strength MS medium containing 60 g/l sucrose. Multiplication of *in vitro*-grown cormlets was achieved by transferring them on to half strength MS medium containing 60 g/l sucrose with 5 or 10 mg/l gibberellic acid (GA₃). Multiplied corms were successfully acclimatized on a medium containing soil: sand: brick pieces at 1:1:1 ratio. Acclimatized cormlets were established on two different potting mixtures i.e. sand: leaf mould: brick pieces (1:1:1) or sand: compost: brick pieces (1:1:1) after pre-treating with or without 100 mg/l GA₃ for 24 hours. Early sprouting (13 days) was observed in cormlets treated with 100 mg/l GA₃ compared to cormlets without any GA₃ treatment (28 days) and, the highest sprouting percentage (83.3 %) was observed on potting mixture containing sand, leaf mould and brick pieces (1:1:1) under greenhouse conditions.

Keywords: *Gladiolus hybrida*, *in vitro* cormlets, *ex vitro* sprouting

INTRODUCTION

Gladiolus is a member of monocot family Iridaceae and native to South Africa. It is semi-hardy in temperate climates and grown in flowerbeds and containers. Gladioli are used mainly as cut flowers due to their wide range of colour, size, flower shape and long blooming season.

Gladioli are propagated by using underground stems or corms, cormlets and true seed. The severe loss and damage of corms and cormlets due to the infection by soil-borne fungus, *Fusarium oxysporum* f. sp. *gladioli* and corm rot during storage are the major problems for its mass propagation (Roy *et al.*, 2006). Therefore, *in vitro* techniques have potential use in gladiolus to produce quality planting materials and prevent transfer of diseases from one generation to the next. Enhanced plant regeneration through *in vitro* techniques has been

¹ Horticultural Research and Development Institute, Gannoruwa, Peradeniya, Sri Lanka

² Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka

* Author for correspondence: jpe@pdn.ac.lk

reported in *Gladiolus primulinus* (Sinha & Roy, 2002; Roy *et al.*, 2006) and gladiolus variety Hakgala Orange (Karunananda *et al.*, 2011). However, different cultivars respond differently depending on the culture medium and culture environment (Ziv, 1989). Therefore, this study was conducted with the aim of producing high quality gladioli planting materials through *in vitro* cormlet formation for *Gladiolus hybrida* (variety Princess Lee).

MATERIALS AND METHODS

Planting materials

Gladioli corms of variety Princess Lee were dipped in 0.5 % (v/v) Clorox™ (commercially available NaOCl) solution for 1 hr. Thereafter, corms were dipped in 0.2 % (w/v) HgCl₂ for 1 min and washed thoroughly with distilled water before transferring to Topsin (1 %v/v Thiophenate methyl solution) and kept for 10 min. Then, the corms were washed thoroughly with 1.5 % (v/v) Clorox solution for 10 min followed by washing 3-4 times in sterilized distilled water. Sterilized corms were dried on a filter paper and excised the growing bud was excised with a portion of corm and placed on full strength MS medium (Murashige & Skoog, 1961) supplemented with 20 g/l sucrose and 1 mg/l NAA and solidified with 8 g/l agar. Then the cultures were maintained at 1000 lux light intensity with 16 hr photoperiod at 25±1 °C. Calli initiated were transferred to MS medium supplemented with 30 g/l sucrose without plant growth regulators and maintained for 16 weeks by culturing at 4 weeks intervals. *In vitro*-derived shoots from these calli were used for the formation of cormlets.

Effect of different sucrose concentrations and the strength of MS medium on *in vitro* cormlet formation of gladiolus

In vitro grown shoot clumps were subdivided into individual shoots with 8-10 cm long leaves and cultured onto full strength or half strength MS medium supplemented with different concentration of sucrose (20, 30, 40, 50, and 60 g/l). The cultures were maintained at 25±1 °C and kept under diffused light (approximately 1000 lux). Cormlets formed at the base of the *in vitro* grown shoots were categorized as small (diameter <1 cm), medium (diameter 1-1.5 cm) and large (diameter >1.5 cm). This experiment was arranged as a two factor factorial experiment in a completely randomized design (CRD). One treatment composed of 20 culture vessels where each vessel contained five plants. Data were analyzed by Chi-square test with statistical analyzing system (SAS) and using the log linear model with CATMOD procedure.

Effect of different concentrations of gibberellic acid (GA₃) on growth and multiplication of *in vitro*-grown cormlets

In vitro-grown cormlets on half strength MS medium supplemented with 60 g/l sucrose without plant growth regulators were used for this experiment. Cormlets were separated and shoots and roots were removed in individual cormlets before culturing on half strength MS medium containing 60 g/l sucrose with 3 different concentrations of GA₃ (0, 5, and 10 mg/l) and solidified with 8 g/l agar. The cultures were maintained at 25±1 °C and kept under diffused light (16 hr photoperiod). The experiment was arranged as a CRD. One treatment composed of 20 culture vessels where each vessel contained five plants.

Acclimatization of *in vitro*-regenerated gladioli plantlets with corms

In vitro-formed plants with cormlets generated from different media (MS medium supplemented with 40, 50 and 60 g/l sucrose) were removed from the culture vessels and the plantlets were gently washed with the warm sterile water to remove any gelling agent adhered to them. The bases of the plantlets were dipped in thiophenate methyl 1 % (v/v) solution.

Soil mixture containing sand, coir dust and brick pieces at the rate of 1:1:1 ratio (v/v) was placed in glass jars and autoclaved at 120 °C and 1.03 kg/m³ for 20 min. At the time of planting, the soil mixture was moistened with sterilized distilled water and four plantlets were transplanted in each jar under aseptic condition. Then all the jars were covered with transparent polythene sheet to conserve moisture required for corm acclimatization. Acclimatization was done by gradually opening the polythene cover and exposing the plants to the external environment. Number of survived plantlets was counted 4 weeks after planting.

Effect of different potting mixtures and GA₃ on sprouting and growth of *in vitro*-formed corms

Acclimatized cormlets were planted in two different potting mixtures *i.e.* sand: leaf mould: brick pieces (1:1:1 ratio, by volume) or sand: compost: brick pieces (1:1:1 ratio by volume) in pots following dipping in 100 ml of 100 mg/ GA₃ solution for 24 hr or without dipping in GA₃. Pots were kept in the greenhouse and watering was done as required. One treatment was composed of 3 replicates where each replicate contained 8 plants. The experiment was arranged as a two factor factorial experiment in a CRD.

RESULTS AND DISCUSSION

Effect of sucrose concentration and the strength of MS medium on *in vitro* cormlet formation of *Gladiolus hybrida* (variety Princess Lee)

Statistical analysis showed that there was no significant interaction effect ($p>0.05$) between the strength of the medium and sucrose concentration. However, with the increase of sucrose concentration in the medium, increase of cormlet formations was observed (Fig. 1, Fig. 4(ii)). Media supplemented with 20 and 30 g/l sucrose did not produce cormlets at all. Therefore, these two concentrations were not considered for the statistical analysis of the results. Cormlet formation was started when the sucrose concentration of the medium was above 40 g/l and the highest number of cormlets was observed in the medium supplemented

with 60 g/l sucrose. Even though there was no significant difference between full and half strength MS media, higher number of cormlets were observed on half strength MS medium (Fig. 1). Furthermore, most of the cormlets formed in full strength MS medium were small (diameter <1 cm). Higher number of large cormlets (diameter >1.5 cm) formation was observed in the half MS medium (Fig. 2) and this was significantly different from the full MS medium.

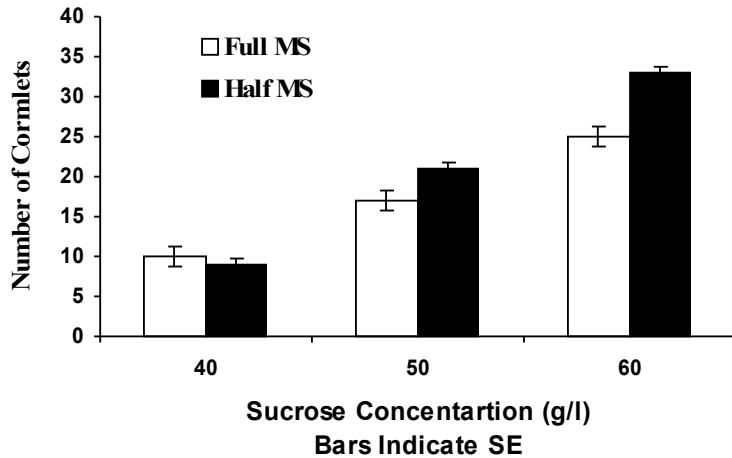


Fig. 1. Effect of sucrose concentration and the strength of MS medium for cormlet formation

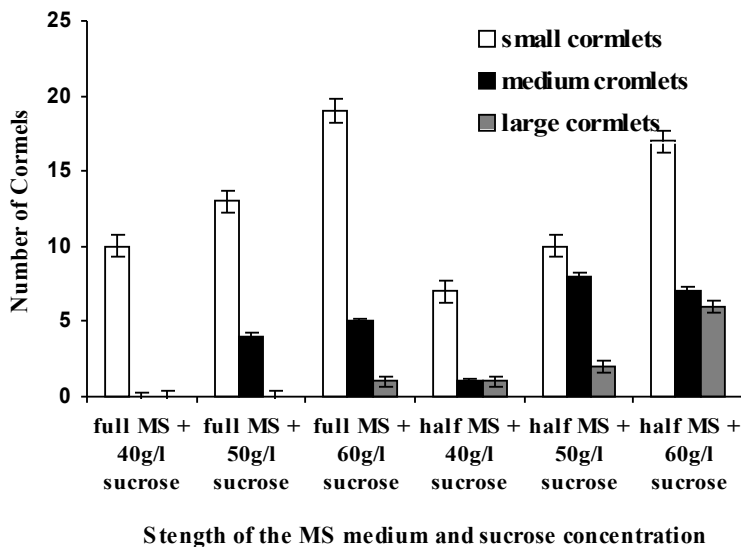


Fig. 2. Effect of sucrose concentration and the strength of MS medium for size of cormlets

Effect of concentration of gibberellic acid (GA₃) on *in vitro* multiplication of gladiolus cormlets

Addition of GA₃ significantly increased the multiplication of cormlets compared to the control. The cormlets transferred to half strength MS medium supplemented with 60 g/l sucrose and gibberellic acid multiplied very quickly. The multiplication rate was 80 and 90 cormlets per 1 cormlet after 8 weeks of culturing on media containing 5 and 10 mg/l GA₃ concentrations, respectively (Fig. 3, Fig. 4(iii)). The corms formed in both media were very small.

The addition of sucrose to the medium plays an important role in corm formation *in vitro*. In *Sparaxis*, with the increase of sucrose concentration (2 %, 3 %, 4 %, 6 %, 8 % and 10 %), an increase in corm induction and corm mass has been observed (Hauser & Horn, 1991). Sucrose is considered to be stored in the form of starch in the storage tissue and increasing carbohydrate levels results in the abundant availability of energy that can be used for induction and growth (Sinha and Roy, 2002). In most plants, sucrose has been found to promote formation of various storage organs such as bulb, corm, tuber and rhizomes.

Even though, there was no significant difference between full- and half-strength MS media for cormlet formation, sizes of cormlets could be increased by decreasing the strength of MS medium (Fig. 2). Half MS media produced a higher number of large- and medium-sized cormlets when compared to full MS media. An effect of mineral salts on *in vitro* bulblet formation has also been reported in *Ornithogalum virens* (Naik & Nayak, 2005). Half MS medium has a lower nitrogen concentration, and as a result, half MS medium has a higher C: N ratio than the full MS medium. It promoted vegetative growth, which competed more efficiently for carbohydrates from the medium (Dielen *et al.*, 2001). Thus, using half-strength MS mineral medium or reduced nitrogen levels, size of the *in vitro* cormlets could be enhanced.

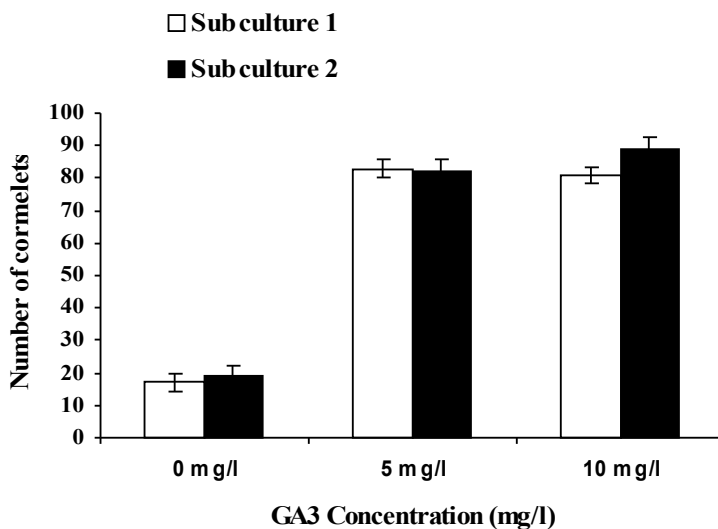


Fig. 3. Effect of gibberellic acid on multiplication of cormlets

Plant hormones play major roles in most plant growth responses and storage organ induction. By changing the composition of endogenous hormone, induction and promotion of growth of storage organ can be achieved. These storage organs enable the plant to survive in adverse environmental conditions by becoming 'dormant' for a period of time. Thus, when attempting to induce storage organs *in vitro*, either inductive environmental conditions or plant hormones are required.

In the present study multiplication of cormlets could be achieved by incorporating GA₃ into the half-strength MS medium containing 60 g/l sucrose. In contrast, in most plants of Iridaceae that have been examined, corm induction was promoted by anti-gibberellin agents (Ziv, 1989; Slabbert *et al.*, 1993; Piqueras *et al.*, 1999 and Madubanya *et al.*, 2006). Although it is more frequent for GA₃ to inhibit storage organ formation (Le Guen-Le *et al.*, 2002), this promotion of corm induction by GA₃ is not unique to gladiolus. GA₃ had a positive effect on *in vitro* corm formation in *Watsonia vanderspuyiae* (Ascough *et al.*, 2008) and a 15 hr-soak in GA₃ stimulated bulblet formation in tulip shoot explants (Pierik & Steegmans, 1975). GA₃-promoted corm induction observed in the present study indicates that GA₃ may be involved in transmitting storage organ-inducing signals in *Gladiolus hybrida* variety Princess Lee as observed in *Watsonia vanderspuyiae* (Ascough *et al.*, 2008) and tulips (Pierik & Steegmans, 1975).

Acclimatization of *in vitro*-regenerated gladioli with corms

A proper acclimatization technique is required for better survival of corms in the soil. Ziv, *et al.* (1970) reported a poor survival rate of *in vitro* produced plantlets of gladiolus. In contrast, *in vitro* produced cormlets were successfully acclimatized in the present study. With increase of the sugar levels in the *in vitro* media, significant increase in survival and growth of the corms during acclimatization was observed (Table 1, Fig. 4).

The highest survival (70±2.0 %) during the acclimatization was observed for the cormlets originated from, the medium with 60 g/l sucrose (Table 1). Furthermore, plants with longest leaves (6.8±1.2 cm) were observed at the same sucrose concentration. Thus, it may be possible that with the increase of sugar concentration in the medium, more carbohydrates are stored in cormlets and provide them with a better chance to survive during the acclimatization process. In agreement with the results of the present study, sucrose levels from 6-9 % have been found suitable for good size gladioli corms production (Ahmad *et al.*, 2000). Dantu and Bhojwani (1987) reported that high sucrose levels at 9-12 % did not improve the gladioli corm size, whereas lowering the level of sucrose to 3 % lead to smaller corms.

Table 1. Effect of sugar concentration in the culture medium on survival of *in vitro*-formed cormlets during acclimatization

Sucrose concentration in MS basal medium (g/l)	Survival during acclimatization (%) \pm SE	Length of the longest leaf after 4 weeks (cm) \pm SE
40	50.1 \pm 5.1	1.0 \pm 0.8
50	40.5 \pm 6.2	2.5 \pm 0.7
60	70.0 \pm 2.0	6.8 \pm 1.2

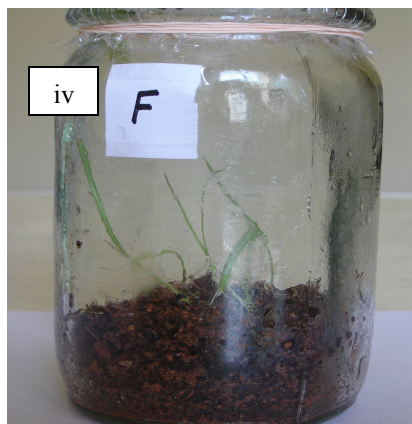
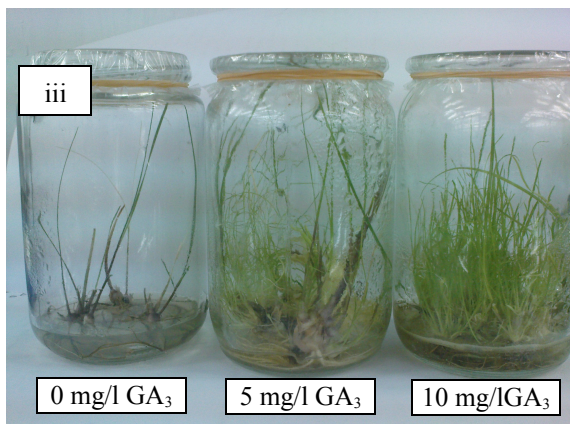
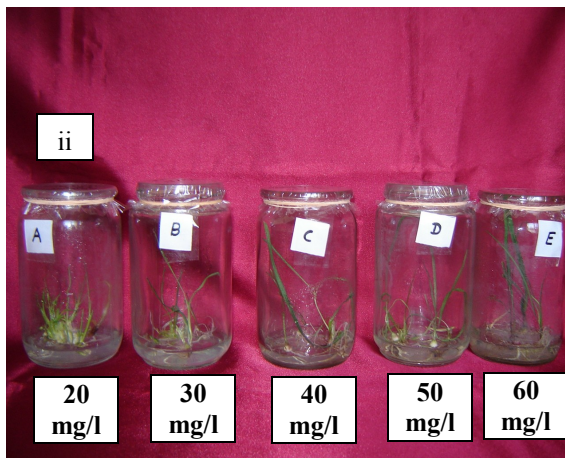
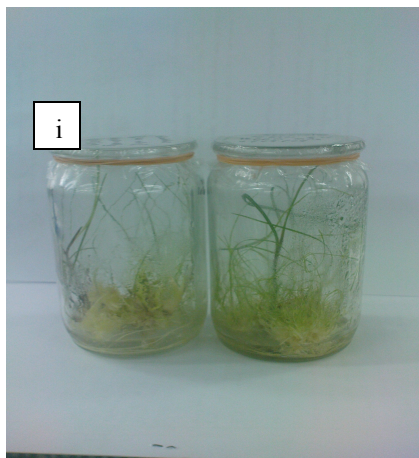




Fig. 4. Different stages of *in vitro* multiplication of gladiolus variety Princess Lee: i. Shoot initiation on MS medium without hormones ii. *In vitro* formation of cormlet on half MS medium supplemented with different levels of sucrose, iii. Effect of GA₃ on multiplication of gladiolus cormlets, iv. Acclimatization of cormlets, v. Cormlets after acclimatization, vi. Growth of acclimatized cormlets treated with 100 ppm GA₃ in greenhouse conditions

Effect of different potting mixtures and GA₃ on sprouting and growth of *in vitro*-formed cormlets

An early sprouting (13–14 days) was observed in cormlets treated with 100 mg/l GA₃ irrespective of the potting mixture. Furthermore, percentage of sprouting (70–80 %) was higher in cormlets treated with 100 mg/l GA₃ than the untreated cormlets (50-60 %). Even though, there was no significant difference, percentage of sprouting was slightly higher in the potting mixture containing sand: leaf mould: brick pieces than the potting mixture containing sand: compost: brick pieces (Table 2, Plate 1(vi)). Thus, it may be possible that the mixture containing the leaf mould could provide anchorage and moisture required for the growth of cormlets resulting in high percentage of sprouts. Due to compactness and lack of aeration in compost, it may not provide protection to the seedling and may result in poor survival of cormlets. Hence, potting mixture containing sand: leaf mould: brick pieces at 1:1:1 ratio can be recommended for sprouting of *in vitro* formed propagation of corms.

Table 2. Effect of potting mixture and GA₃ on sprouting of *in vitro*-formed cormlets

Potting mixtures	GA ₃ levels (mg/l)	Sprouting (Days) ± SE	Sprouting (%) ± SE
Sand: leaf mould: brick pieces (1:1:1 ratio)	0	27.9 ± 2.5	62.5 ± 2.4
	100	13.3 ± 3.6	83.3 ± 5.1
Sand: compost: brick pieces (1:1:1 ratio)	0	28.8 ± 2.8	50.1 ± 3.9
	100	13.9 ± 2.5	70.8 ± 4.5

Early sprouting and percentage sprouting under *ex vitro* conditions in gladiolus cormlets increased significantly with the application of 100 mg/l GA₃ than with the control treatment (0 mg/l GA₃). The results of the present investigation were similar to the findings of Singh *et al.* (2002) in which application of GA₃ increased the sprouting of gladiolus cormlets.

Gladiolus corms show dormancy and take a long time (2-6 months) to sprout. Due to dormancy, corms are sprouted unevenly which affect plant production and flowering. Therefore, various techniques for breaking dormancy of gladiolus corms have been tested by several workers, including cold storage and chemical treatment. The inhibitors which affect dormancy of gladiolus have been reported by Ginzburg (1973). Abscisic Acid (ABA) was identified as the major growth inhibitor present in the corms. Benzyl adenine (BA) and gibberellic acid (GA₃) have the ability to break dormancy in several crops by inhibiting the action of abscisic acid (Panday & Gaur, 1980). Thus, it may be possible that GA₃ promoted the sprouting of gladiolus, by counteracting with abscisic acid. The results of this study clearly showed that GA₃ promote growth and sprouting of gladiolus corms under *ex vitro* conditions as well as under *in vitro* conditions.

CONCLUSIONS

In vitro techniques can be used as an ideal tool for rapid multiplication of gladiolus. *In vitro* cormlet formation of gladiolus could be achieved by manipulating the strength and the concentrations of sucrose and gibberellic acid in the MS basal medium. Dipping in 100 mg/l GA₃ prior to transferring to the potting mixture can be used to accelerate sprouting of corms.

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