Floriculture and Ornamental Biotechnology ©2012 Global Science Books

Effects of Explant Position and Polarity on Callus Induction and Shoot Regeneration of *Gladiolus* (*Gladiolus hybridus* Hort.)

Faouzi Haouala* • **Emna Chaïeb**

Département des Sciences Horticoles et du Paysage, Institut Supérieur Agronomique, 4042 Chott Mariem, Sousse, Tunisia

Corresponding author: * **faouzi.haouala@laposte.net**

ABSTRACT

Different types of explants were used for callus induction in *Gladiolus* tissue culture of cultivars 'ChaCha' and 'Priscilla'. Different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) or α-naphthaleneacetic acid (NAA) were tested. Explant polarity was studied for leaves by placing fragments of these organs horizontally and vertically on the culture medium. Also, explants were taken from the apical, middle and basal parts of leaves and petals. Apical buds, leaves and flower stalks showed excellent callus formation (100%). However, petals were characterized by a low callus formation ability (10%) while floral stems, bracts and floral spikes showed no callus formation. The rate of callus development was significantly higher for horizontally cultured leaves and decreased from basal explants to middle and apical ones. The highest rate of callus formation was obtained on media containing either 2,4-D (1 or 3 mg l^{-1}) or NAA (2 or 5 mg l^{-1}). Callus budding was significantly higher on medium supplemented with 1 mg l^{-1} BA. The budding rate of callus obtained from apical buds was 93.3 and 100%, respectively, for cultivars 'Priscilla' and 'ChaCha'. For callus derived from leaf fragments, the rate of budding was 100% for both cultivars.

Keywords: callus formation, budding, *in vitro*, polarity
Abbreviations: BA, N6-benzyladenine; **2,4-D**, 2,4-dichlorophenoxyacetic acid; KIN, kinetin; **MS**, Murashige and Skoog basal medium; **NAA**, α-naphthaleneacetic acid

INTRODUCTION

Species of *Gladiolus* (Iridaceae) are of great horticultural value. Modern cultivated gladioli evolved through successive natural and artificial hybridization of *Gladiolus* species originating from the Mediterranean area and South Africa (Buch 1972; Wilfret 1980) including over 180 species with more than 10,000 cultivars. Approximately 20 cultivars are grown for commercial flower production (Nhut *et al.* 2004).

This genus has been subject of *in vitro* propagation experiments (Ziv *et al.* 1970; Hussey 1977; Bajaj *et al.* 1983; Kamo *et al.* 1990; Ziv and Lilien-Kipnis 1990; Sen and Sen 1995). *Gladiolus* micropropagation was achieved from axillary buds (Begum and Haddiuzaman 1995; Boonvanno and Kanchanapoom 2000), apex (Hussain *et al.* 2001; Aftab *et al.* 2008), corms (Nagaraju and Parthasarathy 1995; Emek and Erdag 2007) and inflorescence (Ziv and Lilien-Kipnis 2000).

Many authors (Bajaj *et al.* 1983, 1992; Kamo 1995; Remotti and Löffler 1995; Torabi-Giglou and Hajieghrari 2008; Pragya *et al.* 2012) studied *Gladiolus* callus formation from different explants: inflorescence, flower stems, early flower, bract, perianth, corm, cormel and leaf fragments. The best callus formation was observed in segments of flower stems and the best shoot production was achieved on medium containing 0.5-1 mg l^{-1} N6-benzyladenine (BA) and 6-9% sucrose, and maintained at 15°C.

In this paper we study the effects of the position and the polarity of leaf explants on callus formation in *Gladiolus* using different culture media. Shoot regeneration is also studied from different explants derived callus.

MATERIALS AND METHODS

Plant material

The starting material was gladioli (*Gladiolus hybridus* Hort. 'ChaCha' and 'Priscilla') corms having 10/12 cm circumference. Explants used were:

- apical buds 4-5 mm long taken from bulbs,
- leaf fragments of 25 mm^2 taken from *in vitro* plants 4-weeks old; explants were taken from the apical, middle and basal parts of leaves,
- flower stalk sections 5 mm long and a diameter of 2-3 mm,
- petals of 25 mm^2 taken from apical, middle and basal parts,
- floral stems 5 mm long and a diameter of 4-6 mm,
- bracts of 25 mm^2,
- floral spikes 5 mm long and 2-4 mm in diameter.

All floral part explants were obtained from plants cultivated in an open field in the region of Sousse (latitude 35°49' North), Tunisia.

Explants were disinfected with 70% ethanol. After washing them in sterile distilled water, they were soaked in 10% hydrogen peroxide (H_2O_2) for 10 min, rinsed twice (15 min each) with sterile distilled water then soaked again in 0.1% $HgCl_2$ for 10 min, rinsed four times (15 min each) with sterile distilled water and used for culturing. Explant polarity was studied for leaves: fragments were laid horizontally or stuck vertically with the basal end into the medium at 2 mm depth.

Culture media and experimental conditions

Culture medium was Murashige and Skoog (1962; MS) which was supplemented with 3% sucrose and 8% agar (Bacto Difco, Panreac Quimica) and the pH was adjusted to 5.8. Explants were cultured individually in tubes of 24 mm diameter and 150 mm length, containing 15 ml of MS medium. Cultures were conducted in a growth chamber at 24 ± 1°C, under 16-h photoperiod under cool white light at 35 µmol m^{-2} s^{-1} provided by white fluorescent tubes (18W/54 Philips). Callus formation was conducted in darkness (Bettaïeb 2003).

Induction of callus

Callus formation media were MS supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) at different concentrations (0, 1, 2, 3

Received: 16 September, 2010. Accepted: 20 December, 2012.

Original Research Paper

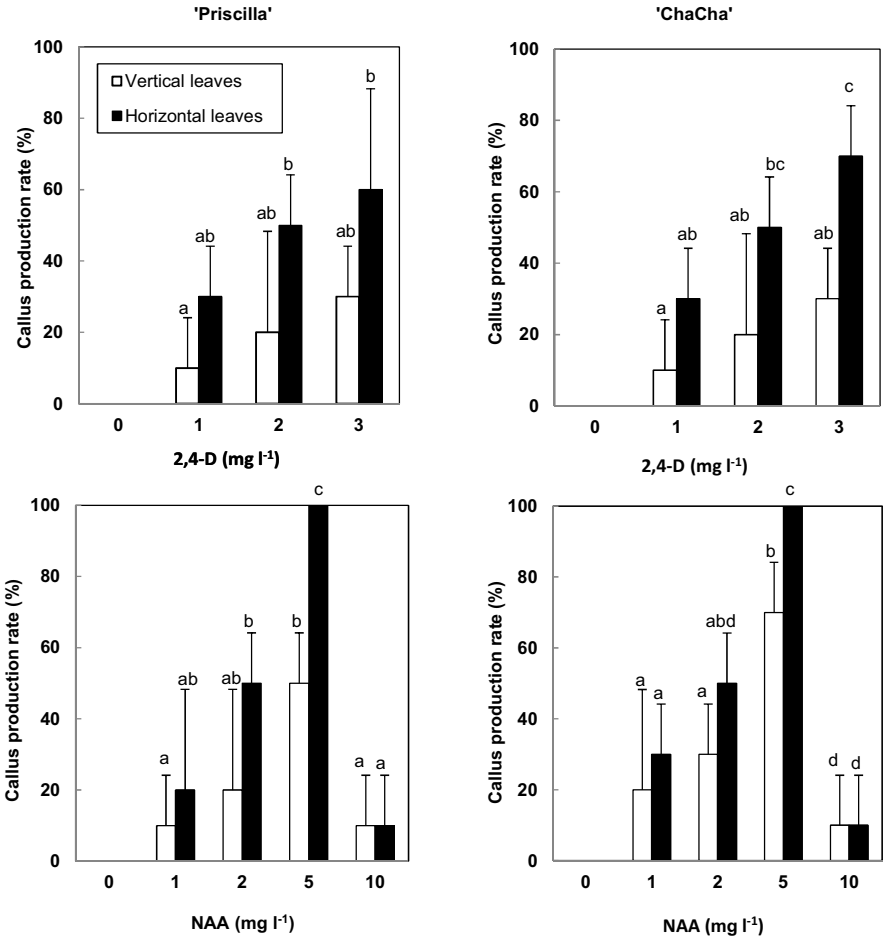

Fig. 1 Polarity differences in callus production from leaf fragments of gladiolus (*Gladiolus hybridus* Hort. 'Priscilla' and 'ChaCha') when placed horizontally or vertically on MS medium with 2,4-D or NAA. Duration of culture: 8 weeks. Number of explants per treatment: 24. Different letters indicate significant differences at $P = 0.05$ (Duncan's multiple range test).

mg l^{-1}) (Kim *et al.* 1988; Ziv and Lilien-Kipnis 2000; Giglou and Hajieghrari 2008), or α-naphthaleneacetic acid (NAA) at different concentrations (0, 1, 2, 5, 10 mg l^{-1}) (Bettaïeb 2003; Aftab *et al.* 2008). Cultures were maintained for 8 weeks with a subculture after 4 weeks.

Shoot regeneration

Eight weeks old callus was put on regeneration media; MS with different concentrations of BA (0, 0.5, 1, 2 mg l^{-1}). Each medium treatment (BA concentration) contained 15 explants.

Data analysis

Several parameters were recorded: induction of callus (+/-); rate of budding: number of calli which formed buds/total number of calli; bud (number of newly-formed buds per callus) and shoot proliferation (number of shoots per callus); morphological appearance of calli and shoots (structure, color, etc.).

Analysis of variance (ANOVA) was performed for all data using SPSS software. Statistically significant differences between means were determined using Duncan's multiple range test at α = 0.05.

RESULTS

Callus formation

1. Induction of callus from leaves

In cultures without plant growth regulators (control), there was no callus formation in either cultivar for leaves laid horizontally or vertically on the culture medium (**Fig. 1**). Callus formation rate increased with the concentration of auxin and was the highest using 3 mg l^{-1} 2,4-D: 60 and 70% for horizontal explants, respectively, for 'Priscilla' and 'ChaCha', 30% for vertical explants of both cultivars. For NAA, the best concentration was 5 mg l^{-1}, which had a callus formation rate of 100% for horizontal leaves and 50, 70% for vertical leaves, respectively, for 'Priscilla' and 'ChaCha' (**Fig. 1**). At a higher concentration of NAA (10 mg l^{-1}), induction of callus was very low and did not exceed 10% for both cultivars. Explants laid horizontally were better for callus formation than those laid vertically, but differences were significant only for the concentration of 3 mg l^{-1} for 2,4-D and 5 mg l^{-1} for NAA.

Calli formed were nodulous or crumbly with some cells

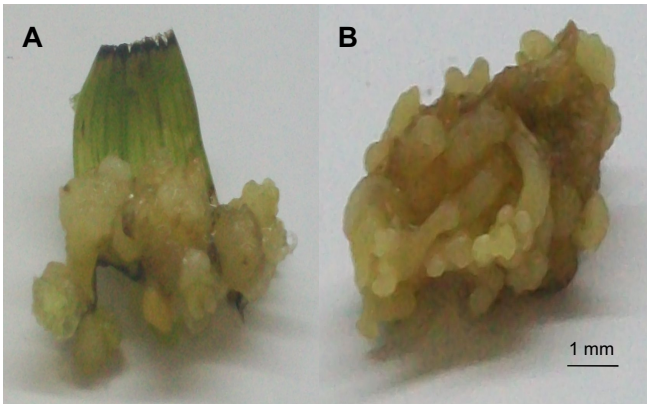

Fig. 2 Formation of callus on leaf fragments of gladiolus (*Gladiolus hybridus* Hort. 'Priscilla') cultured for 4 (A) or 8 weeks (B) on MS medium with 3 mg l^{-1} 2,4-D.

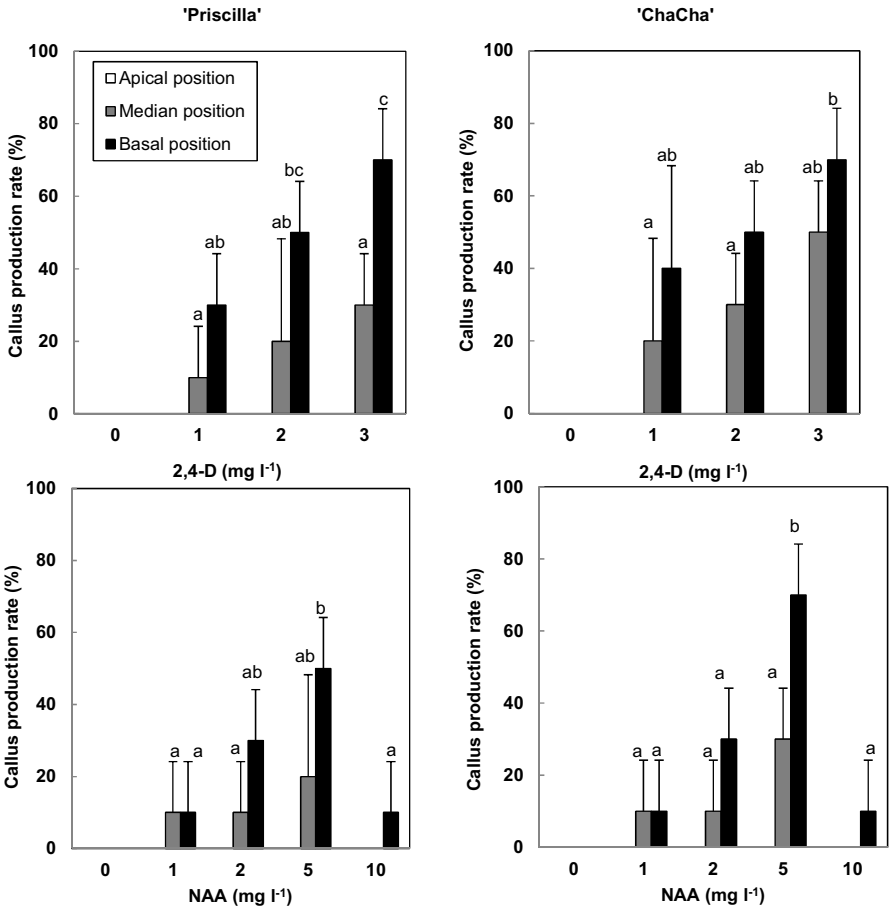

Fig. 3 Effect of explant position on callus production from leaf fragments of gladiolus (*Gladiolus hybridus* Hort. 'Priscilla' and 'ChaCha') cultured on MS medium with 2,4-D or NAA. Duration of culture: 8 weeks. Number of explants per treatment: 24. Different letters indicate significant differences at $P = 0.05$ (Duncan's multiple range test).

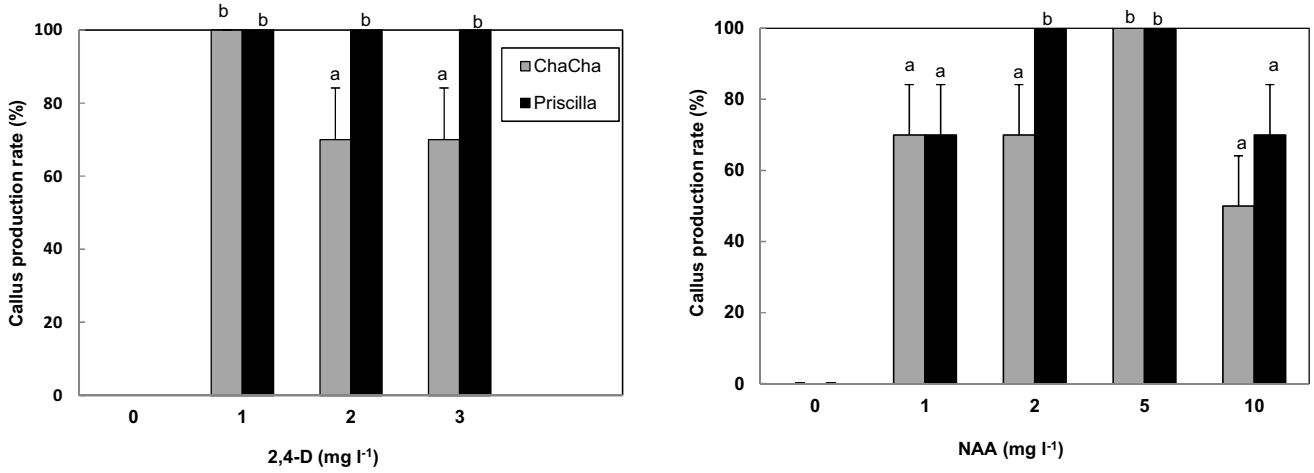

Fig. 4 Callus production from flower stalks of gladiolus (*Gladiolus hybridus* Hort. 'Priscilla' and 'ChaCha') cultured on MS medium with 2,4-D or NAA. Duration of culture: 8 weeks. Number of explants per treatment: 24. Different letters indicate significant differences at $P = 0.05$ (Duncan's multiple range test).

containing chlorophyll (yellow-greenish color: **Fig. 2**). For explant position on the leaf, induction of callus was possible only with basal and middle explants. Basal explants had the highest rate of callus formation which was 70% for 'ChaCha' and 50 to 70% for 'Priscilla', according to the type of auxin. There was no callus formation with apical explants (**Fig. 3**).

2. Induction of callus from flower stalks

Without hormones, there was no callus formation on flower stalks (**Fig. 4**). The presence of 2,4-D was very favourable for callus formation and its rate reached 100% for both cultivars using 1 mg l^{-1} 2,4-D. On media containing higher concentrations of 2,4-D, callus formation rate was unchanged for 'Priscilla' (100%) but decreased to 70% for 'ChaCha'. Induction of callus increased with NAA concentration reaching its maximum (100%) with 2 or 5 mg l^{-1} NAA, respectively, for 'Priscilla' and 'ChaCha'. Using 10 mg l^{-1} NAA, callus formation rate decreased to 50 and 70%, respectively, for 'ChaCha' and 'Priscilla'. Calli were globular, whitish and characterized by a compact structure (**Fig. 5**).

Fig. 5 Formation of callus on flower stalks of gladiolus (*Gladiolus hybridus* Hort. 'Priscilla') cultured for 8 weeks on MS with 1 mg l^{-1} 2,4-D.

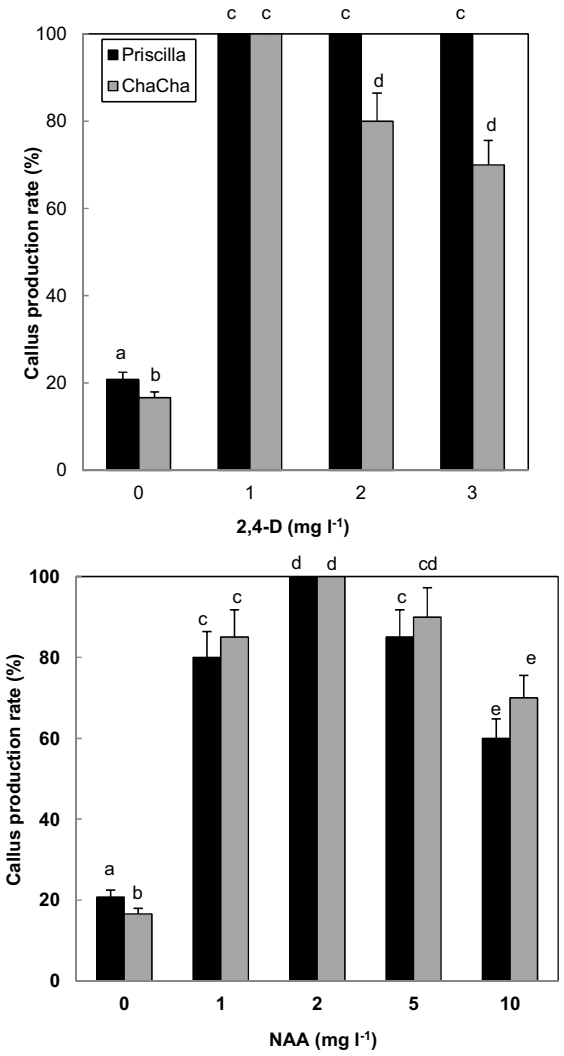

Fig. 6 Callus production from apical buds of gladiolus (*Gladiolus hybridus* Hort. 'Priscilla' and 'ChaCha') cultured on MS with 2,4-D or NAA. Duration of culture: 8 weeks. Number of explants per treatment: 15. Different letters indicate significant differences at $P = 0.05$ (Duncan's multiple range test).

3. Induction of callus from apical buds

With these types of explants, medium without hormones allowed callus formation but its rate was low: 16.6 and 20.8%, respectively, for 'ChaCha' and 'Priscilla' (**Fig. 6**). Using either 1 mg l^{-1} 2,4-D or 2 mg l^{-1} NAA permitted maximal (100%) callus induction for both cultivars. On media supplemented with higher concentrations of 2,4-D, callus formation rate was unchanged for 'Priscilla' (100%) but decreased sensibly for 'ChaCha' to 80 and 70% with 2 and 3 mg l^{-1} 2,4-D, respectively. Using higher concentrations of NAA, this parameter decreased for both cultivars but did not fall under 60%.

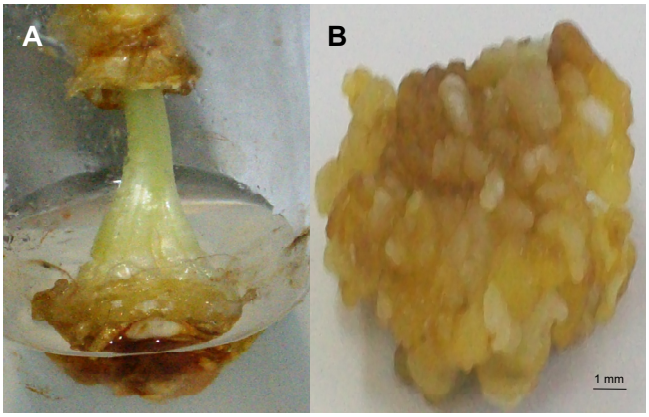

Fig. 7 Formation of callus from apical buds of gladiolus (*Gladiolus hybridus* Hort. 'Priscilla') cultured on MS with 1 mg l^{-1} 2,4-D. Swelling and early callus formation (**A**), callus after 4 weeks of culture (**B**).

Callus was nodulous, crumbly, not chlorophyllous and yellowish for both cultivars (**Fig. 7**).

4. Induction of callus from petals

Petals were taken from apical, middle and basal parts of these organs. Apical and middle explants of both cultivars did not develop callus on any culture media. On medium containing 1 mg l^{-1} 2,4-D, only basal explants showed swelling after 4 weeks while spongy, dark brown callus was formed after 8 weeks. The callus formation rate was very low (≤10%) for both cultivars. For the other types of explants (floral stems, bracts, floral spikes), there was no callus initiation at all.

Shoot regeneration

1. From leaf fragments derived callus

On the control medium (without BA), callus budding occurred at 20 and 47%, respectively, for 'ChaCha' and 'Priscilla' (**Table 1**). Rate of budding increased with BA concentration and was the highest on medium containing 1 mg l^{-1} BA reaching 100% for both cultivars after 4 weeks of culture. The number of newly-formed buds was significantly the best on the same medium and was 2.4 and 2.6 buds per callus, respectively, for 'ChaCha' and 'Priscilla'. Using a higher concentration of BA (2 mg l^{-1}), the number of buds per callus was reduced to 1.3 and 1 buds, respectively, for 'ChaCha' and 'Priscilla'.

The number of shoots per callus increased with concentration of BA and was the highest with 1 mg l^{-1} BA (**Table 1**): 1.9 and 2.5 shoots per callus for 'ChaCha' and 'Priscilla', respectively. On medium containing 2 mg l^{-1} BA, shoot regeneration decreased to 0.94 for both cultivars.

Shoot regeneration from leaf callus was generally better for 'Priscilla' but results were not significantly different (**Table 1**).

2. From apical buds derived callus

The number of newly-formed shoots per callus depended on cultivar and BA concentration. Indeed, on control medium without BA, shoot regeneration was possible, but its rate did not exceed 13.3 and 26.7%, respectively, for 'ChaCha' and 'Priscilla', significantly lower than the treatments with BA (**Table 2**). Budding rate of apical buds derived callus was the highest on medium containing 1 mg l^{-1} BA (**Table 2**). In addition, budding on this medium was the fastest and reached for both cultivars 53.3% after 10 days of culture and 93.3 and 100% after 4 weeks of culture, respectively, for 'Priscilla' and 'ChaCha' (**Table 2**).

Table 1 Rate of budding (%), number of buds/callus and number of shoots/callus from gladiolus 'ChaCha' and 'Priscilla' leaf fragment derived calli. Culture conditions: regeneration medium = Murashige and Skoog (MS) ± BA; 16-h photoperiod; light intensity 35 µmol m^{-2} s^{-1}; 24 ± 1° C. Number of explants per treatment: 15.

Regeneration medium	Cultivar	Rate of budding (%)	Number of buds per callus	Number of shoots per callus
MS (control)	ChaCha	20.0	0.26 ± 0.19 a	0.26 ± 0.11 a
	Priscilla	46.7	0.67 ± 0.31 a	0.40 ± 0.20 a
MS + 0.5 mg l^{-1} BA	ChaCha	60.0	1.00 ± 0.54 b	0.90 ± 0.50 b
	Priscilla	73.3	1.40 ± 0.80 b	1.06 ± 0.63 b
MS + 1 mg l^{-1} BA	ChaCha	100	2.40 ± 1.10 c	1.90 ± 0.90 c
	Priscilla	100	2.60 ± 1.30 c	2.50 ± 1.20 c
MS + 2 mg l^{-1} BA	ChaCha	80.0	1.30 ± 0.76 b	0.94 ± 0.50 b
	Priscilla	60.0	1.06 ± 0.66 b	0.94 ± 0.48 b

Values in the same column followed by different letters are significantly different ($P \leq 0.05$) according to DMRT.

Table 2 Rate of budding (%), number of buds/callus and number of shoots/callus from gladiolus 'ChaCha' and 'Priscilla' apical bud derived calli. Culture conditions: regeneration medium = Murashige and Skoog (MS) ± BA; 16-h photoperiod; light intensity 35 µmol m^{-2} s^{-1}; 24 ± 1° C. Number of explants per treatment: 15.

Regeneration medium	Cultivar	Rate of budding (%)	Number of buds per callus	Number of shoots per callus
MS (control)	ChaCha	13.3	0.13 ± 0.09 a	0.27 ± 0.12 a
	Priscilla	26.7	0.40 ± 0.23 a	0.20 ± 0.11 a
MS + 0.5 mg l^{-1} BA	ChaCha	53.3	1.20 ± 0.61 b	1.07 ± 0.51 b
	Priscilla	60.0	1.13± 0.55 b	0.87 ± 0.43 b
MS + 1 mg l^{-1} BA	ChaCha	100	2.20 ± 1.06 c	1.67 ± 0.91 c
	Priscilla	93.3	2.50 ± 1.02 c	2.40 ± 1.34 c
MS + 2 mg l^{-1} BA	ChaCha	66.7	1.06 ± 0.53 b	0.73 ± 0.46 b
	Priscilla	60.0	1.0 ± 0.61 b	0.93 ± 0.48 b

Values in the same column followed by different letters are significantly different ($P \leq 0.05$) according to DMRT.

Table 3 Rate of budding (%) and number of buds/callus from gladiolus 'ChaCha' and 'Priscilla' flower stalk derived calli. Culture conditions: regeneration medium = Murashige and Skoog (MS) ± BA; 16-h photoperiod; light intensity 35 µmol m^{-2} s^{-1}; 24 ± 1° C. Number of explants per treatment: 15.

Regeneration medium	Cultivar	Rate of budding (%)	Number of buds per callus
MS (control)	ChaCha	0	0
	Priscilla	0	0
MS + 0.5 mg l^{-1} BA	ChaCha	0	0
	Priscilla	0	0
MS + 1 mg l^{-1} BA	ChaCha	0	0
	Priscilla	0	0
MS + 2 mg l^{-1} BA	ChaCha	0	0
	Priscilla	0	0

The number of buds per callus was significantly higher than all other treatments on the medium with 1 mg l^{-1} BA: 2.2 and 2.5 buds, respectively, for 'ChaCha' and 'Priscilla' (**Table 2**). For higher concentrations of BA (2 mg l^{-1}), the number of buds/callus was reduced to only 1 bud for both cultivars.

The number of shoots/callus increased with the concentration of BA and was significantly higher at 1 mg l^{-1}. For higher concentrations of BA (2 mg l^{-1}), the number of shoots decreased by more than half.

Analysis of variance showed significant differences between the control and all BA concentration, however, differences between the two concentration of BA (0.5 and 2 mg l^{-1}) were not significant. Differences between cultivars were not also significant.

3. From flower stalks derived callus

For calli derived from flower stalks, shoot regeneration was completely inhibited, and degeneration affected all calli after four weeks of culture (**Table 3**). There was no shoot regeneration from petals derived callus.

DISCUSSION

Callus formation

Callus induction was achieved on MS medium supplemented with various concentrations of 2,4-D or NAA, similar to previous results using the same auxins (Bettaieb 2003; Emek and Erdag 2007; Aftab *et al.* 2008).

For increased callus induction, cultures were placed in darkness at 24 ± 1°C (Bettaieb 2003).

Callus could be induced from various explants: leaves, apical buds, flower stalks and petals, but not other types of explants (floral stems, bracts and floral spikes). These results are not in agreement with those of Bajaj *et al.* (1983) who reported that callus formation was obtained for *Gladiolus* 'Snow Princess' and 'Oscar' from various explants: inflorescences, floral stems, bracts and perianths on 10 mg 1^{-1} NAA and 0.5 mg.1^{-1} KIN. These differences could be attributed to the callus induction media and/or genotype. Callus could be obtained also from corm sections in MS medium with 8.5 mg 1^{-1} NAA (Emek and Erdag 2007) and from cormels on 4.44 µM BA and 2.32 µM KIN and 2.68 µM NAA (Pragya *et al.* 2012).

For leaf explants, media containing 3 mg l^{-1} 2,4-D or 5 mg l^{-1} NAA appeared most favourable to callus formation and both cultivars were comparable (**Fig. 1**). Aftab *et al.* (2008) showed that induction of callus from leaf explants was possible on MS with 3 mg l^{-1} 2,4-D or 2 mg l^{-1} NAA. Lower or higher NAA concentrations did not permit callus induction. However, Bettaieb (2003) reported that leaf explants of *Gladiolus* 'Peter Pears' and 'White friendship' on different media containing 2,4-D or NAA did not induce callus. Clearly, cultivar (genotypic) differences warrant testing of varying plant growth regulators concentrations to maximize callus formation.

The explants of both cultivars placed horizontally on the media showed a better ability to callus induction than those placed vertically (**Fig. 1**). This is probably related with the surface of the explant in contact with the culture medium. Explants taken from the basal part of the leaf gave the best results (**Fig. 3**). This is in agreement with Kasumi *et al.* (1999) on *Gladiolus* 'Topaz', where leaf explants taken from the basal part and cultivated in darkness for 60 days on MS with 5 mg l^{-1} NAA produced 100% callus. Wernicke and Milkovits (1987) showed for young leaves segments of wheat (*Triticum timopheevi* Zukh) that the most basal cells

divided readily in culture and complete the mitotic cycle.

Flower stalks showed a strong ability for callus production, reaching 100% on most media (**Fig. 4**). Petals did not generate callus, being inhibited for explants derived from apical and middle parts of the petal. For basal explants, induction of callus was very low (≤10%) on medium containing 1 mgl^{-1} 2,4-D. Kasumi *et al.* (2001) indicated that callus was not induced for the apical parts of the perianth, but it was possible for the basal parts (56% on MS supplemented with 5 mg l^{-1} NAA).

Shoot regeneration

The formation and number of buds on calli was possible in presence of BA (**Table 1**). After 2 weeks of culture, meristematic protuberances appeared on the calli and evolved in shoots (**Fig. 2**). Shoot formation was also possible without BA at a significantly lower rate than 1 mg l^{-1} BA (**Tables 1, 2**). Indeed, Margara (1984) noted that the formation of new buds in monocots from calli on a medium without plant growth regulators was found. According to our results, shoot regeneration from callus was possible on media with or without BA (0, 0.5, 1, 2 mg l^{-1}) (**Tables 1, 2**).

There were significant differences between BA concentrations but not between cultivars for callus production (**Tables 1, 2**). The highest number of shoots per callus was obtained on medium containing 1 mg l^{-1} BA (**Tables 1, 2**). De Bruyn and Ferreira (1992) indicated that the best production of shoots per explant was achieved on a medium containing 0.5 to 1 mg l^{-1} BA. The highest shoot induction (98.3%) and number of shoots was obtained from cormel sprouts on MS with 4 mg l^{-1} BA (Memon *et al.* 2010). Shaheenuzzaman *et al.* (2011) reported that the highest percentage of shoot regeneration (91.6%), for calli derived from leaf discs, was observed in MS medium containing 3 mg l^{-1} BA and 0.5 mg l^{-1} KIN. However, Bettaieb (2003) showed that the best formation of buds on callus was obtained using 0.5 mg l^{-1} BA and 10 mg l^{-1} $AgNO_3$. Thus, the concentration of BA could depend on cultivar and the addition of metal ions such as Ag^+ is needed in some cases to stimulate the formation of buds (De Block 1988; Hannachi 1996; Haouala 1999). $AgNO_3$ acts by inhibiting ethylene, unfavourable factor for regeneration (Rethmeier *et al.* 1991).

CONCLUSIONS

Callus production in *Gladiolus* depends on type of explants, polarity and position, plant growth regulators type, concentration and genotype. Indeed, callus induction was possible from leaves, flower stalks, apical buds and petals. However, floral stems, bracts and floral spikes did not permit callus initiation at all. Leaf explants laid horizontally were more favourable for callus formation than those laid vertically. A basipetal gradient was observed in leaf explants and callus formation was possible only with basal and middle ones while only basal petal explants formed callus. Induction of callus was possible either with 2,4-D or NAA at various concentrations according to type of explants. However, only 2,4-D produced callus from petals. Genotype-specific callus production was not significantly different in this experiment. Shoot regeneration was possible for calli derived from leaf fragments and apical buds but completely inhibited from that of flower stalks. The rate of budding, number of newly-formed buds and number of shoots per callus were highest on medium containing 1 mg l^{-1} BA. Shoot regeneration from leaf callus and apical buds did not differ between genotypes.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Messaoud Mars for language revision.

REFERENCES

Aftab F, Alam M, Afrasiab H (2008) *In vitro* shoot multiplication and callus induction in *Gladiolus hybridus* Hort. *Pakistan Journal of Botany* **40 (2)**, 517-522

Bajaj YPS, Sidhu MMS, Gill APS (1983) Some factors affecting the *in vitro* propagation of *Gladiolus*. *Scientia Horticulturae* **18**, 269-275

Bajaj YPS, Sidhu MMS, Gill APS (1992) Micropropagation of *Gladiolus*. In: Bajaj YPS (Eds) *Biotechnology in Agriculture and Forestry 20, High-Tech and Micropropagation IV*, Springer Verlag, Berlin, pp 135-143

Begum S, Hadiuzzaman S (1995) *In vitro* rapid shoot proliferation and corm development in *Gladiolus grandiflorus* cv. Redbrand. *Plant Tissue Culture* **5**, 7-11

Bettaieb T (2003) Régénération *in vitro* de variants somaclonaux de glaïeul (*Gladiolus grandiflorus* Hort.) tolérants aux basses températures. PhD thesis, Tunis, Tunisia, 136 pp

Boonvanno K, Kanchanapoom K (2000) *In vitro* propagation of *Gladiolus*. *Suranaree Journal of Science and Technology* **7**, 25-29

Buch PO (1972) The species. In: Koenig N, Crowely W (Eds) *The World of Gladiolus*, North American Gladiolus Council, Edgerton Press, MD, pp 2-7

De Block M (1988) Genotype-independant leaf disc transformation of potato (*Solanum tuberosum*) using *Agrobacterium tumefaciens*. *Theoretical and Applied Genetics* **76**, 767-774

De Bruyn MH, Ferreira DI (1992) *In vitro* corm production of *Gladiolus dalenii* and *G. tristis*. *Plant Cell, Tissue and Organ Culture* **31**, 123-128

Emek Y, Erdag B (2007) *In vitro* propagation of *Gladiolus anatolicus* (Boiss.) Stapf. *Pakistan Journal of Botany* **39 (1)**, 23-30

Giglou M, Hajieghrari B (2008) *In vitro* study on regeneration of *Gladiolus grandiflorus* corm calli as affected by plant growth regulators. *Pakistan Journal of Biological Sciences* **11**, 1147-1151

Hannachi C (1996) Amélioration de la tolérance de la pomme de terre (*Solanum tuberosum* L.) à la salinité (NaCl) par voie biotechnologique. PhD thesis, Gand, Belgium, 152 pp

Haouala F (1999) Régénération *in vitro* et tolérance à la salinité de l'œillet (*Dianthus caryophyllus* L.). PhD thesis, Tunis, Tunisia, 171 pp

Hussain I, Mohamed H, Rashid Quraishi A (2001) *In vitro* multiplication of *Gladiolus* (*Gladiolus crassifolius*). *Plant Tissue Culture* **11**, 121-126

Hussey G (1977) *In vitro* propagation of *Gladiolus* by precocious axillary shoot formation. *Scientia Horticulturae* **6**, 287-296

Kamo K (1995) A cultivar comparison of plant regeneration from suspension cells, callus and cormel slices of *Gladiolus*. *In Vitro Cellular and Developmental Biology – Plant* **31**, 113-115

Kamo K, Chen J, Lawson R (1990) The establishment of cell suspension culture of *Gladiolus* that regenerated plants. *In Vitro Cellular and Developmental Biology – Plant* **26**, 425-430

Kasumi M, Takatsu Y, Tomotsune Sakuma T, Iida S (1999) The isolation of varied flower color plants by ovary culture of sectorial chimera of *Gladiolus*. *Journal of the Japanese Society for Horticultural Science* **68 (1)**, 195-197

Kasumi M, Takatsu Y, Manabe T, Hayachi M (2001) The effect of irradiating gladiolus (*Gladiolus grandiflorus* Hort.) cormels with gamma rays on callus formation, somatic embryogenesis and flower color variations in the regenerated plants. *Journal of the Japanese Society for Horticultural Science* **70 (2)**, 126-128

Kim KW, Choi JB, Kwan KY (1988) Rapid multiplication of *Gladiolus* plants through callus culture. *Journal of the Korean Society for Horticultural Science* **29**, 312-318

Margara J (1984) Néoformation de méristèmes et embryogenèse somatique. In: INRA (Eds) *Bases de la Multiplication Végétative. Les Méristèmes et l'Organogenèse*, Paris, pp 75-175

Memon N, Qasim M, Jaskani MJ, Ahmad R (2010) *In vitro* cormel production of *Gladiolus*. *Pakistan Journal of Agricultural Sciences* **47 (2)**, 115-123

Murashige I, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-497

Nagaraju V, Parthasarathy VA (1995) Effect of growth regulators on *in vitro* shoots of *Gladiolus hybridus*. *Folia Horticulturae* **7/2**, 93-100

Nhut DT, Teixeira da Silva JA, Huyen PX, Paek KY (2004) The importance of explant source on regeneration and micropropagation of *Gladiolus* by liquid shake culture. *Scientia Horticulturae* **102**, 407-414

Pragya R, Singh SK, Misra RL, Ranjan JK (2012) *In vitro* shoot regeneration from cormel derived callus of gladiolus and bio-hardening of plantlets. *Indian Journal of Biotechnology* **11**, 99-104

Remotti PC, Löffler HJM (1995) Callus induction and plant regeneration from gladiolus. *Plant Cell, Tissue and Organ Culture* **42**, 2, 171-178

Rethmeier NOM, Jansen CE, Snel EAM, Nijkamp HJJ, Hille J (1991) Improvement of regeneration of *Lycopersicon pennellii* protoplasts by decreasing ethylene production. *Plant Cell Reports* **9**, 539-543

Sen J, Sen S (1995) Two-step bud culture technique for a high frequency regeneration of *Gladiolus* corms. *Scientia Horticulturae* **64**, 133-138

Shaheenuzzaman M, Haque MS, Karim MM, Noor ZU (2011) *In vitro* shoot proliferation and development of micropropagation protocol from leaf disc of Gladiolus. *Journal of Bangladesh Agricultural University* **9 (1)**, 21-26

Torabi-Giglou M, Hajieghrari B (2008) *In vitro* study on regeneration of *Gladiolus grandiflorus* corm calli as affected by plant growth regulators.

Pakistan Journal of Biological Sciences **11 (8)**, 1147-1151

Wernicke W, Milkovits L (1987) Effect of auxin on the mitotic cell cycle in cultured leaf segments at different stages of development in wheat. *Physiologia Plantarum* **69**, 16-22

Wilfret GJ (1980) *Gladiolus*. In: Larson RA (Eds) *Introduction to Floriculture*, Academic Press, New York, pp 166-181

Ziv M, Halevy AH, Shilo R (1970) Organ and plantlet regeneration of *Gladiolus* through tissue culture. *Annals of Botany* **34**, 671-676

Ziv M, Lilien-Kipnis H (1990) *Gladiolus*. In: Ammirato PV, Evan DA, Sharp WR, Bajaj YPS (Eds) *Handbook of Plant Cell Culture* (Vol 5), MacGraw-Hill, New York, pp 461-478

Ziv M, Lilien-Kipnis H (2000) Bud regeneration from inflorescence explant for rapid propagation of geophytes *in vitro*. *Plant Cell Reports* **19**, 845-850