



## SHORT COMMUNICATION

### ***IN VITRO* MICROPROPAGATION OF *GLADIOLUS GRANDIFLORA* (VAR. SNOW PRINCESS) FLOWER FROM CORMEL EXPLANT**

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#### SUMMARY

**Protocol for micropropagation of *Gladiolus grandiflora*, an economically important horticultural crop has been developed using aseptically grown cormel slices as explants. Better proliferation and multiplication of calli were obtained on Murashige and Skoog (MS) basal medium supplemented with 2.0 mg l<sup>-1</sup> NAA and 1.0 mg l<sup>-1</sup> BAP. Regenerable calli were formed from the basal region of *in vitro* cultured cormel slices in approximately three weeks after explants were placed on callus induction medium. For most prolific shoot induction one gram (fresh weight) of callus was placed on shoot induction medium containing MS basal salt and 0.5 mg l<sup>-1</sup> BAP. Shoot formation occurred one month after inoculation of callus on shoot induction medium. The standardized regeneration protocol will help to impart desirable traits in *Gladiolus* cultivar 'Snow Princess' for further transformation and genetic modification.**

**Key words:** Callus, cormel slice, gladiolus, regeneration

The colourful spikes of *Gladiolus* are delight to eye and it is cultivated worldwide as commercial horticultural crop. Every year the number of cultivated varieties is rising through hybridization, with prolonged vase life, introduction of new colors in spikes, floret arrangement on the spikes and extension of flowering period. New desired traits are being introduced horticultural crops through *in vitro* regeneration. Different medias have been tried in present study to identify the media in which maximum regeneration occurs. Cultivar specific protocols for plant regeneration are available indicating a requirement of hormones in cereals like corn, wheat, barley and sorghum. 2,4-dichlorophenoxyacetic acid (2,4-D) is known to induce callus and its removal leads to differentiation of callus into plants (Bhaskaran and Smith 1990). Callus of *Hemerocallis* was initiated from buds

cultured on a medium supplemented with kinetin, 6-benzylaminopurine (BAP), naphthyl acetic acid (NAA) or BAP and 2,4-D (Krikorian and Kann 1981). Cell suspensions of *Hemerocallis* initiated from this callus regenerated plants on the differentiating and regenerating medium. The callus of *Crocus sativus* was induced by culturing bulbs on a medium supplemented with 2,4-D and zeatin, and plants were regenerated from the callus on a medium containing NAA and BAP (Isa and Ogasawara 1988).

*Gladiolus* plants have been regenerated from callus (Isa and Ogasawara 1988, Simonsen and Hildebrandt 1971, Bajaj et al. 1982, 1992, Kamo et al. 1990, Stefaniak 1994), suspension cells (Kamo 1994) and cormel slices (Bajaj et al. 1982, 1992, Kamo 1994). Direct

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**Different combination of NAA and BAP with standard MS medium**

Medium number	1-Naphthyl acetic acid-NAA (mg <sup>l</sup> <sup>-1</sup> )	6-Benzylaminopurine-BAP (mg <sup>l</sup> <sup>-1</sup> )
1	4.0	2.0
2	0.5	-
3	2.0	1.0
4	0.1	-
5	0.1	0.2
6	-	0.5
7	-	0.2
8	-	-

**Fig. 1. Regeneration efficiency of different media combination of NAA and BAP with standard MS medium. Callus initiation in at least 50% (6 culture vessels per medium) of the culture where total number of culture in each case was twelve, representing a total of 96 culture vessels.**

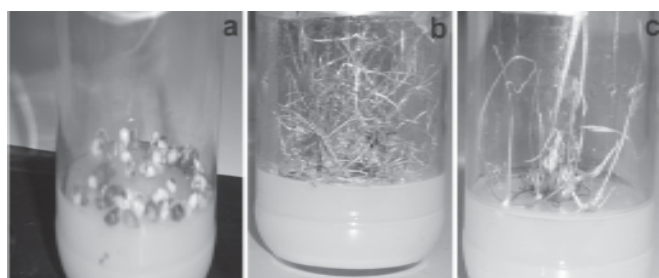
micropropagation system through enhanced axillary bud development and organogenesis has been reported for different species of *Gladiolus* (Begum and Hadiuzzaman 1995, Grewal et al. 1995, Sen and Sen 1995, Churvikova and Barykina 1995, Gupta and Sehgal 1997). Plant regeneration from callus initiated from different organs of *Gladiolus* such as inflorescence stalk (Kamo et al. 1990), apical meristem (Logan and Zettler 1985), axillary buds (Kamo 1995), basal leaves and cormel slices (Kamo 1994) require cytokinins. The callus initiation and regeneration is cultivar, explant and growth regulator specific (Grewal et al. 1995). In the present

study, the role of cytokinins and auxins in regeneration of *Gladiolus* has been investigated.

Corms of *Gladiolus grandiflora* var. Snow Princess were collected locally. For cormel formation corms were inoculated in autoclaved sand with essential moisture, after 4-5 weeks small cormels emerge. Before inoculation the cormels were surface sterilized with running tap water for 30 minutes, washed with Tween 20 for 15 minutes and with 0.1% antiseptic savlon 5% (v/v) for 10 minutes. These corms were then sterilized with 0.1% HgCl<sub>2</sub> for 10 minutes in a laminar air flow cabinet followed by washing thrice with autoclaved double distilled water to remove any traces of HgCl<sub>2</sub>. The cormels were blotted dry on autoclaved sterile filter paper for 5-10 minutes. MS medium (pH 5.8) containing standard salts and vitamins, 3% sucrose and 0.7% (w/v) agar was used with various combinations of auxins and cytokinins. The small cormels were sliced approximately 1-2 cm with sharp sterilized scalpel and placed on media (20 slices on each plate of media), cultures were incubated at 10,000 lux with cool fluorescent light and temperature at 24±2°C. Explants were maintained *in vitro* on MS (Murashige and Skoog 1962) and basal salts medium (Logan and Zettler 1985).

Regenerable calli were formed from basal region of *in vitro* cultured cormel slices approximately 3 weeks after the explant were transferred on callus induction medium (Fig. 2a). Eight different callus inducing media containing MS Basal salts, 3% (w/v) sucrose, 0.7% agar with mg<sup>l</sup><sup>-1</sup> of glycine 2.0, thiamine 1.0, pyridoxine 0.5, nicotinic acid 0.5, myo-inositol 100.0 and two plant hormones 1-Naphthyl acetic acid (NAA) and 6-Benzylaminopurine (BAP), individually and in combination were tried. Hard compact calli were initiated from cormel slices cultured on MS medium supplemented with 2.0 mg<sup>l</sup><sup>-1</sup> NAA + 1 mg<sup>l</sup><sup>-1</sup> of BAP.

Axillary buds, nodal segments and shoot tips have been successfully used for shoot regeneration at higher concentrations of BAP (Grewal et al. 1995, Dantu and Bhojwani 1995). Higher dose response of BAP is attributed to genotypic differences (Hussain et al. 2001). In our cultures we got good response at lower concentration of BAP (0.5 and 1.0 mg<sup>l</sup><sup>-1</sup>).



**Fig. 2.** Plant regeneration through *in vitro* callus formation from *Gladiolus* cormels.

**a.** Three-month-old callus initiated from cormels slices cultured on MS media

**b.** Shoot formation from 8-9 months old callus

**c.** *In vitro* corms formation from 2-3 month old shoots

For shoot induction one gram of callus was placed on MS medium supplemented with twelve different combinations of BAP and NAA. Shoot induction occurred in all the twelve media after one month of callus inoculation. Maximum shoot induction was observed in MS media supplemented with 0.5 mg/L BAP (Table 1; Fig. 2b). Shoots from calli obtained from cormel explants were generally healthy. A linear relationship between the shoot induction and multiple shoot formation was observed.

**Table 1.** Effect of different media on regenerated calli from cormel slices for multiple shoot induction. Comparison of number of shoots is based upon the data collected after 8 weeks of inoculation of both explants. Results are mean (S.E) from ten replicates.

Medium composition (PGRs in mgL <sup>-1</sup> )	Multiple shoot induction from calli
MS+ BAP (0.3)	12 (2.8)
MS + BAP (0.4)	17 (4.1)
MS + BAP (0.5)	21 (6.2)
MS + BAP (1.0)	19 (3.2)
MS + BAP (2.0)	6 (2.5)
MS + BAP (3.0)	4 (2.1)
MS + BAP (4.0)	2 (1)
MS + NAA (0.4) + BAP (0.5)	15 (4.6)
MS + NAA (0.4) + BAP (1.0)	11 (2.1)
MS + NAA (0.4) + BAP (3.0)	3 (1.9)

Plant regeneration occurred from calli of *Gladiolus* derived from inflorescence stalks (Isa and Ogasawara 1988), cormel stem tips (Simonsen and Hildebrandt 1971) or suspension cells (Bajaj *et al.* 1982). Plants have been regenerated from callus of *Gladiolus* by many workers (Isa and Ogasawara 1988, Simonsen and Hildebrandt 1971, Bajaj *et al.* 1982, 1992, Kamo *et al.* 1990, Stefaniak 1994). Regeneration for Indian *Gladiolus* cultivar 'Snow Princess' has been successfully shown for the medium containing BAP 0.5 mgL<sup>-1</sup> which gave maximum shoot regeneration. Our results were in accordance with others laboratory observations (Kamo 1995). Media containing NAA (2 mgL<sup>-1</sup>) and BAP (1 mgL<sup>-1</sup>) promoted callusing. Both hormones in combination gave the best results.

The protocol highlights the usefulness of auxin and cytokinin for enhancing the callus formation that can be exploited for mass propagation of *Gladiolus* cultivar which can be used for introducing desirable characters into the plant through genetic engineering and transformation approach.

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