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In-vitro Multiple Shoots Production from Cormel Shoot Buds in Gladiolus (*Gladiolus hybrida*)

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A B S T R A C T

Gladiolus (*Gladiolus hybrida*) is an important ornamental plant cultivated world over for its attractive spikes. The commercial cultivation of *Gladiolus* is based on natural multiplication of corms and cormels. *In vitro* propagation techniques, is superior to conventional propagation and produces disease free plants in huge quantities. *In vitro* regeneration of gladiolus cultivars, *Sylvia*, *White Prosperity* and *Amsterdam* was achieved using shoot bud of cormels as an explant. Sylvia variety recorded the best for callus induction and regeneration than other two varieties. The concentration of plant growth regulators affects the regeneration response differently. The highest callus induction rate of 76% explants was recorded on Murashige and Skoog (MS) medium supplemented with 2 mgl^{-1} 2,4-D (2,4-Dichlorophenoxyacetic acid), after 28 days. MS medium supplemented with BAP at 2.0 mgl^{-1} exhibited higher shoot proliferating efficiency, i.e shoots per explant in Sylvia variety.

Introduction

Gladiolus (*Gladiolus hybrida*) is a bulbous ornamental plant. It has a great commercial value in cut flower industry all over the world as well as in India due to its magnificent and colourful spikes. The genus *Gladiolus* belongs to the family Iridaceae. United States (Florida and California), Holland, Italy, France, Poland, Bulgaria, Brazil, India, Australia and Israel are the major gladiolus producing countries. The major gladiolus producing states in the India are Uttar Pradesh, West Bengal, Odisha, Chhattisgarh, Haryana and Maharashtra. Gladiolus is also grown in states like Uttarakhand, Karnataka, Andhra Pradesh and Sikkim (Kadam *et al.*, 2014). Commercial production of corms and cormels of gladiolus is affected by *Fusarium* corm rot during storage. Besides, about 25 cormels produce from one mother corm in each season (Sinha and Roy, 2002). These cormels were not able

to be directly forced to flower, since gladiolus plant was naturally grown as biennial. Thus its commercial cultivation is limited by low rate of multiplication and does not fulfill the local demand of planting material which eventually affects the final cost of corms.

Therefore novel cultivars need to be rapidly mass multiplied by using modern *in vitro* technologies in order to fulfill the supply gap of huge demand of our local market which is of course not possible through conventional methods. Through modern technologies Mass propagation of corms and cormels such as tissue culture techniques have adopted at commercial level. Advanced countries are using highly sophisticated modern technologies for the commercial production of desired varieties in order to compete in the international markets. *In-vitro* plant tissue culture makes it possible to produce disease free and true to type planting material of Gladiolus.

In-vitro propagation techniques, prove significance especially for securing rapid multiplication of the novel cultivars. Although there are several research on *in vitro* propagation of gladiolus varieties using shoot bud, root, leaf and other different parts of plant as explant, and various plant growth regulators such as 2,4-D, IAA, NAA and BAP (Misra *et al.,* 1999; Pathania *et al.,* 2001; Kumari *et al.,* 2005 and Roy *et al.,* 2006). *In-vitro* micropropagation of gladiolus has been reported by using axillary buds (Begum *et al.,* 1995; Boonvanno *et al.,* 2000), shoot tip (Hussain *et al.,* 2001), cormels (Nagaraju *et al.,* 1995) and inflorescence axes (Ziv *et al.,* 2000). Successful protocols for *in vitro* corm formation (Dantu and Bhojwani, 1995; Sen *et al.,* 1995; Al-Juboory *et al.,* 1995), organogenesis and somatic embryogenesis (Remotti *et al.,* 1995; Kumar *et al.,* 2002) have been achieved also. However, there is a clear scope for further refinement through *in vitro* culture methodology to acquire a higher number of shoots to complement traditional nursery methods in Gladiolus (Hussain *et al.,* 2001). In present study has been undertaken for standardization of plant growth regulators in MS (Murashige and Skoog, 1962) medium supplemented with sucrose and agar for *in vitro* differentiation and regenraration of three popular varieties of gladiolus.

Materials and Methods

Procurement and preparation of explants

The healthy corms of gladiolus cv. *Sylvia, White prosperity* and *Amsterdam* were obtained from Department of Horticulture of Sardar Vallabh Bhai Patel University of Agriculture and Technology (SVPUA&T), Modipuram, Meerut. Plants were raised in the field laboratory of Department of Agriculture Biotechnology of SVPUA&T for collecting the cormels of gladiolus. All explants were taken from these cormels for the research work. The outer scale of cormels was removed and buds of cormels cut with the help of surgical blade. Then buds were washed with 3-4 drops of Twin-20 (liquid detergent) along with sodium hypochlorite solution. Then 1% bavistin for 10 min followed 0.1 % $HgCl_2$ for 2 minutes. After each treatment, the bud were washed with sterile distilled water, 4 to 5 times. Buds were dried using the blotting paper before inoculated on the media.

Culture media

Media for callus induction

MS (Murashige and Skoog, 1962) medium supplemented with various concentrations of plant growth regulators was used for the present study. Callus induction initiated when surface sterilized explants were inoculated on MS medium containing 2,4-D, NAA and IBA**.** Sucrose 3% (w/v) was added as carbon source.

The pH was adjusted to 5.8 by 1N NaOH or 1N HCl. Agar 0.7% was added as a gelling agent in the medium.

Media for shoot regeneration

After Callus initiation the explants were placed on MS medium supplemented with various concentrations of BAP, KIN for shoot regeneration. Sucrose 3% (w/v) was added as carbon source. The pH was adjusted to 5.8 by 1N NaOH or 1N HCl. Agar 0.7% was added as a gelling agent in the medium. All cultures were maintained at 25 ± 2°C with 16/8 h (light/dark) photoperiod.

Results and Discussion

In shoot bud culture, plantlet were regenerated via indirect organogenesis, In indirect approach, plantlets were regenerated from callus mass. Cormels shoot bud were cut and transferred into MS medium with varying hormonal concentrations of 2,4-D (0.5 to 4 mgl^{-1}), NAA (0.5 to 4 mgl^{-1}) and IBA (0.5 to 4 mgl^{-1}). Callus induction was successfully observed in the shoot bud explants of *Sylvia* variety. In *Sylvia* variety excellent callus formation was observed while in *White Prosperity* and *Amsterdam* poor callus formation was observed (Table 1). After four weeks of inoculation, Sylvia variety showed better callus formation as compared to other two varieties. Maximum mass of callus was recorded at the concentrations of auxin 2 mgl^{-1} 2,4-D followed by 2 mgl^{-1} IBA on full strength of MS medium. Kamo (1994) and Grewel *et al.*, (1995) also documented similar findings for diverse explant cultures in gladiolus.

Effect of PGRs on *in vitro* shoot differentiation from callus of *gladiolus*

Shoot regeneration from callus was done by transferring it to MS medium containing various concentration of growth regulators. Shoot induction was observed after 10-20 days after the transfer on shoot development media (Fig. 1B). Incubated in MS medium supplemented with BAP (1 mgl^{-1} and 2 mgl^{-1}). The result of mean value showed that among all the varieties studied, number of shoots was maximum with the concentration of BAP (1 mgl^{-1} and 2 mgl^{-1}) in Sylvia variety. While *White Prosperity* and *Amsterdam* variety showed poor number of shoot regeneration (Fig. 1C, D and Table 2). Earlier studies reported that cytokinin is required in shoot organogenesis (Remotti, 1995; Kumar *et al.*, 2002; Aslam *et al.*, 2012).

Table.1 MS basal medium treatments with different concentration of plant growth regulators for callus induction in gladiolus cultivars, *Sylvia*, *White prosperity* and *Amsterdam*

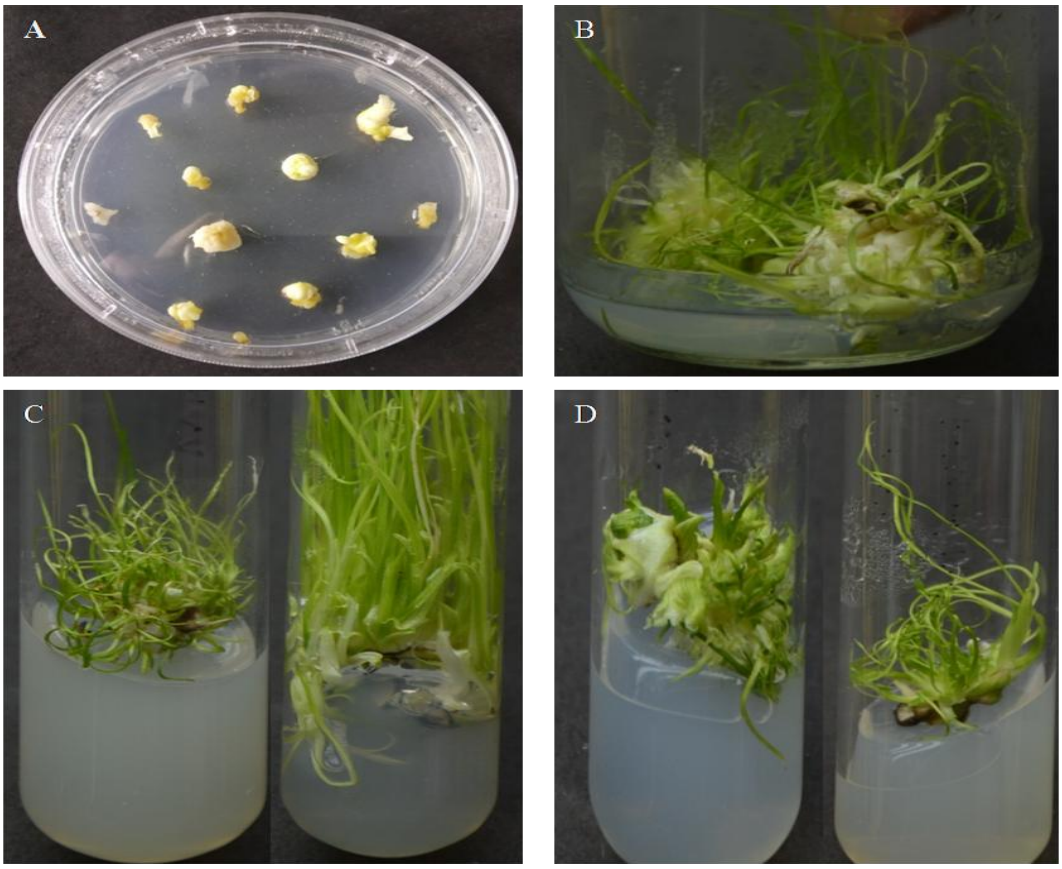
S. No.	Treatments	Media	Varietal response to callus formation		
			Sylvia	White prosperity	Amsterdam
1	T1	MS+0.5 mg/l 2,4-D	+++	++	-
2	T2	MS+1.0 mg/l 2,4-D	+++	+	-
3	T3	MS+2.0 mg/l 2,4-D	++++	++	+
4	T4	MS+4.0 mg/l 2,4-D	++	+	++
5	T1	MS+0.5 mg/l NAA	+	-	-
6	T2	MS+1.0 mg/l NAA	+	-	-
7	T3	MS+2.0 mg/l NAA	++	-	-
8	T4	MS+4.0 mg/l NAA	+++	+++	+
9	T1	MS+0.5 mg/l IBA	+	-	-
10	T2	MS+1.0 mg/l IBA	+++	+	-
11	T3	MS+2.0 mg/l IBA	++++	++	+
12	T4	MS+4.0 mg/l IBA	++	+	++

Abbreviation use in this table: ++++ very good, +++ good, ++ poor, + very poor and –No callus

Table.2 MS basal medium treatments with different concentration of plant growth regulators for regeneration from callus in gladiolus cultivars, *Sylvia*, *White prosperity* and *Amsterdam*

S. No.	Treatments	Media	Varietal response to micro shoots production (number)		
			Sylvia	White prosperity	Amsterdam
1	T1	MS+0.5 mg/l BAP	3.60 ± 0.24	2.11 ± 0.5	5.40 ± 0.12
2	T2	MS+1.0 mg/l BAP	9.00 ± 0.55	1.00 ± 0.25	4.70 ± 0.37
3	T3	MS+2.0 mg/l BAP	9.20 ± 0.86	2.30 ± 0.50	3.40 ± 0.81
4	T4	MS+4.0 mg/l BAP	4.60 ± 0.40	5.90 ± 0.21	3.11 ± 0.71
5	T1	MS+0.5 mg/l KIN	5.60 ± 0.75	10.90 ± 0.56	6.12 ± 0.87
6	T2	MS+1.0 mg/l KIN	4.20 ± 0.58	9.81 ± 0.67	4.23 ± 0.56
7	T3	MS+2.0 mg/l KIN	5.80 ± 0.73	7.23 ± 0.66	5.20 ± 0.57
8	T4	MS+4.0 mg/l KIN	6.40 ± 0.51	6.42 ± 0.23	6.60 ± 0.68
		Mean ± Std. error	6.05 ± 0.07	5.67 ± 1.27	4.85 ±0.98

Fig.1 A: Callus induction from cormel *Sylvia* variety of gladiolus inoculated in full strength MS medium supplemented with 2,4-D (2 mgl^{-1}) after twenty eight days of incubation. **B:** Shoot induction in eight week old callus of *gladiolus* inoculated in MS medium supplemented with BAP (1 mgl^{-1} and 2 mgl^{-1}) after ten-twenty days of incubation. **C:** Shoot induction *White Prosperity* variety of gladiolus inoculated in MS medium supplemented with BAP (1 mgl^{-1} and 2 mgl^{1}) after ten and twenty one days of incubation. **D:** Shoot induction *Amsterdam* variety of gladiolus inoculated in MS medium supplemented with BAP (1 mgl^{-1} and 2 mgl^{-1}) after ten and twenty one days of incubation.



For gladiolus tissue culture experiments, mostly MS basal medium was used by scientists (Hussey, 1977; Longan and Zettler, 1985; Kamo *et al.*, 1990; Dantu and Bhojwani, 1992). During the present study, basal MS medium was used throughout the experiment as this has been found more responsive than other medium. Various types and concentrations of plant growth regulators in different combinations were supplemented into basal MS medium. During present investigation, three variety of gladiolus were regenerated by supplementing different auxins (alone), diverse cytokinins (alone) to basal MS media.

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