Towards Development of a Suitable Protocol for Regeneration of Gladiolus (*Gladiolus grandiflorus* L.) *in-vitro*

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Abstract

The effect of different plant growth regulators on growth and development of gladiolus *in-vitro* using cormlets as explants was studied in two consecutive years. MS medium supplemented with Kinetin had greater efficiency on regeneration of gladiolus cormlets at the concentration of 2 mg L^{-1}. About 90% regeneration of cormlets was obtained by this medium, which started 6.50 days after inoculation to this medium. Among the eight different regeneration media, cormlets cultured on MS medium containing GA_3 @ 2 mg L^{-1} showed the earliest sign of proliferation (5.90 days after inoculation) followed by the medium containing GA_3 @ 1 mg L^{-1} and Kinetin @ 2 mg L^{-1}. After 15 days of culture, the maximum shoot length (5.22 cm) was obtained form the cormlets cultured on MS medium supplemented with GA_3 @ 2mg L^{-1}. But IBA proved inhibitory for shoot regeneration of gladiolus from cormlet explants. On the other hand IBA had an effective role on rooting of well-sprouted cormlets and NAA showed a synergistic effect on it. MS medium supplemented with NAA (2 mg L^{-1}) + IBA (2 mg L^{-1}) initiated rooting earliest (6.92 days after transfer). At the time of transfer to the soil, root number and length was found highest with that particular medium (7.43 roots of 48.48 mm/culture). So this medium is recommended for rooting of gladiolus plantlets grown *in-vitro*.

1. Introduction

Worldwide floriculture industry is expected to grow almost double by the turn of a decade, also recognize technological innovations, which are required to remove certain barriers that can be crucial. Gladiolus (*Gladiolus grandiflorus* L.) is one of the most demanded cut flowers of the world better known for its long lasting colourful cut spikes. It is commercially propagated vegetatively through corm and cormlets – which is based on the unique aspect of the plant in developmental biology. But, field planting of cormlets for the development of a large lot of planting materials (corms) revealed the idea that the smaller be the size of cormlets, the lesser would be the germination percentage. Therefore, sizeable quantities of smaller cormlets do not get sprouted. Besides *Fusarium oxysporium* f sp. *gladioli* is the important addition that hinders the way for successful cultivation of gladiolus (Elewa et al., 2001; Ramachandran, 2004). The conventional systems have been harnessed by the plant biotechnologists and exploited the method of plant tissue culture as an alternative method of commercial propagation. Tissue culture technique has opened up new possibilities in agro-industry, however there remains the task of establishing suitable cultural conditions for plant species, which are least investigated or have been investigated for the first time.

2. Materials and Methods

In this experiment attempts were made to develop a suitable tissue culture protocol for *in-vitro* regeneration of gladiolus from cormlet using different plant growth regulators in two consecutive years. Tiny cormlets of gladiolus (*Gladiolus grandiflorus* L.) cv. Sylvia having a diameter of 2.5-3 mm were used as explants. Murashige and Skoog (1962) semisolid agar based nutrient medium with varying concentration and

combination of plant growth regulators were used for *in-vitro* regeneration of gladiolus. The composition of eight different growing media and five different rooting media used are presented in Table 1. For gladiolus cormlets surface sterilization/ decontamination of the explants plays a major role for successful regeneration of explants because the disease causing

Table 1: Composition of the media used for *in-vitro* culture of gladiolus (concentration in mgL^{-1})

Media used	Basal medium	Kinetin	NAA	GA_3	IBA
For regeneration					
G_1	MS	-	-	1.0	-
G_2	MS	-	-	2.0	-
G_3	MS	1.0	-	-	-
G_4	MS	2.0	-	-	-
G_5	MS	-	1.0	-	-
G_6	MS	-	2.0	-	-
G_7	MS	-	-	-	1.0
G_8	MS	-	-	-	2.0
For rooting					
GR_1	MS	-	-	2.0	2.0
GR_2	MS	2.0	-	-	2.0
GR_3	MS	-	2.0	-	2.0
GR_4	MS	2.0	-	2.0	2.0
GR_5	MS	2.0	2.0	-	2.0

organisms may live either in the soil or in the plant part or in both. Surface sterilization was done by dipping the cormlets in a solution of copper oxychloride (2 g L^{-1}) for half an hour and then in fresh running water for 15 minutes. The cormlets were then treated with Teepol (0.5%) solution (a commercial detergent) for 10 minutes and rinsed in fresh running water carefully. Finally the explants were treated with $HgCl_2$ (0.1%) for 7 minutes and washed thoroughly in sterile distilled water for 5-7 minutes. The explants were then inoculated in the regeneration media.

The cultures were maintained in a culture room at 23±2 ^{0}C under white fluorescent lamps providing a light intensity of 2000 LUX under a 16h : 8h :: light : dark period at a relative humidity of 50-60%.

The experiment was laid in completely randomized design (CRD) with 8 treatments replicated thrice and CRD with 5 treatments replicated for four times for regeneration and rooting respectively. Collected data were subjected to standard statistical procedures.

3. Results and Discussion

The efficiency of different culture media on regeneration of gladiolus cormlets *in-vitro* was found to be statistically significant in both the individual years and in the pooled effect too. In the first year cormlets cultured on MS medium supplemented with kinetin @2mg L^{-1} (G_4) showed the highest percentage of regeneration (95.55) followed by the cormlets cultured on the medium(G_3) containing kinetin @1 mg L^{-1} (76.55%). MS medium containing IBA @ 1 mg and 2 mg L^{-1} (G_7 & G_8) were proved inhibitory regarding the shoot regeneration from gladiolus cormlets. Results of the second year also followed the same trend. The pooled effect represented G_4 as the highly effective regeneration medium for gladiolus regarding the percentage of proliferating cultures producing 90.00% regeneration followed by G_3 (75.78%). Growing medium containing NAA @ 2 mg L^{-1} (G_6) was found somewhat less effective (52.52% regeneration). Culture media G_7 & G_8 were found inhibitory regarding direct shoot regeneration from gladiolus cormlets *in-vitro* (Table 2). Plate 1 shows the different stages of *in-vitro* regeneration of gladiolus.

The impact of different culture media on the time period required for sprouting of gladiolus cormlets *in-vitro* was also

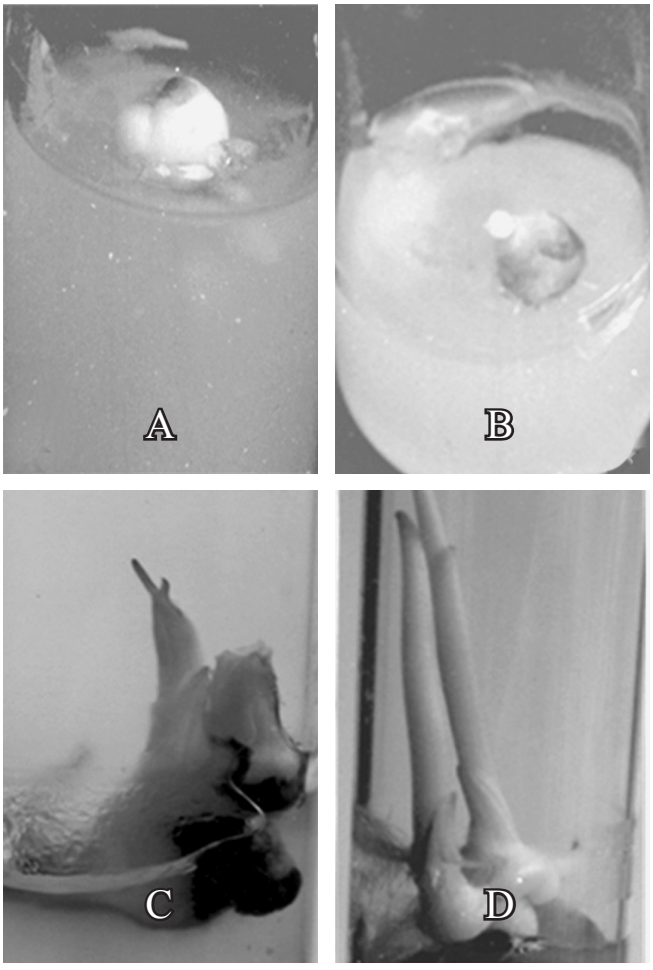

Plate 1: *In-vitro* regeneration of gladiolus from cormlets.
A. Inoculated cormlet on culture media, B. *In-vitro* Sprouting, C. Sprouting of multiple shoots, D. Growth and development of multiple shoot

Table 2: Effect of culture media on the regeneration of gladiolus (cv. Sylvia) from cormlet explants *in-vitro*

Treatments	Days required for sprouting			% of proliferating cultures			Plant height (cm) after 15 days		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled
G_1	6.15	6.70	6.43	65.00 (54.06)	68.33 (56.10)	66.67 (55.08)	4.67	4.17	4.42
G_2	5.75	6.05	5.90	68.33 (56.08)	71.54 (58.08)	69.94 (57.08)	5.31	5.12	5.22
G_3	7.80	8.23	8.02	76.55 (61.39)	75.00 (60.33)	75.78 (60.86)	3.48	3.75	3.62
G_4	5.50	7.50	6.50	95.55 (80.41)	84.45 (67.27)	90.00 (73.84)	4.43	4.42	4.43
G_5	10.65	10.58	10.61	66.67 (55.11)	62.22 (52.39)	64.45 (53.75)	2.88	3.03	2.96
G_6	9.81	10.75	10.28	51.11 (45.93)	53.33 (47.20)	52.52 (46.56)	3.36	3.55	3.45
G_7	-	-	-	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00	0.00	0.00
G_8	-	-	-	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00	0.00	0.00
SEm ±	0.29	0.18	0.17	2.05	0.97	1.17	0.08	0.06	0.05
CD (*p*=0.05)	0.86	0.53	0.49	6.13	2.92	3.37	0.24	0.19	0.15

Figures in the parenthesis are the angular values

found to be statistically significant after the data had been analyzed for both the years and when pooled. The longest time period required for sprouting of gladiolus cormlets (10.65 days) when cultured on MS medium containing NAA 1 mg L^{-1} (G_5) followed by G_6 (9.81 days). The other media showed moderate effects except G_4 showed earliest sign of regeneration (5.50 days after inoculation) in the first year. Results of the second year showed that the cormlets cultured on the MS medium containing GA_3 @2 mg L^{-1} (G_2) required shortest time period for regeneration (6.05 days). G_6 recorded the most delayed sprouting (10.75 days). The pooled effect revealed that the culture medium G_2 was the most effective resulted earliest sprouting of gladiolus cormlets (5.90 days). Plant height (after 15 days of transfer) was found highest with the cormlets cultured on G_2 (5.22 cm) and shorter plant height was obtained from the media G_5 (2.96 cm). MS media containing IBA (G_7 and G_8) proved inhibitory for sprouting and shoot development of gladiolus cormlets *in-vitro* (Table 2).

IBA had an effective role on *in-vitro* rooting of well sprouted cormlets and NAA showed a synergistic effect on it. MS medium supplemented with NAA (2 mg L^{-1}) + IBA (2 mg L^{-1}) initiated rooting earliest. Number of roots per plant and root length was also found highest with that particular medium in both the individual years and in pooled effect also. On the other hand kinetin and GA_3 showed delayed effect on rooting of gladiolus *in-vitro*. Rooting medium containing GA_3 and IBA showed the most delayed rooting started 18.17 days after transfer to the rooting medium. MS medium supplemented with GA_3 and IBA produced the lowest number of roots/plantlet. A combination of Kinetin and IBA showed the minimum root length (Table 3).

The investigation on tissue culture as a tool for propagation of gladiolus was initially demonstrated by Ziv (1979) with inflorescence stalk as explant. With the gradual advancement of the tissue culture techniques and formulation of different nutrient media the present investigation has been initiated to develop protocol for *in-vitro* propagation of a cultivar (Sylvia) of gladiolus of commercial importance in the plains of West Bengal. During present investigation proliferation and development of microplants from cormlet explants has been

Table 3: Effect of culture media on the rooting of gladiolus (cv. Sylvia) from cormlet explants *in-vitro*

Treatments	Days required for root initiation			Number of roots/culture at the time of transfer to soil			Root length (mm)		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled
GR_1	18.39	17.95	18.17	1.52	2.00	1.76	34.36	35.11	34.73
GR_2	15.05	14.14	14.59	4.20	3.55	3.88	17.53	17.29	17.41
GR_3	7.88	6.76	6.92	7.55	7.30	7.43	47.72	49.24	48.48
GR_4	13.98	12.70	13.34	3.43	3.10	3.26	25.26	25.29	25.27
GR_5	9.23	8.85	9.04	6.05	5.85	5.95	43.32	42.51	42.92
SEm ±	0.24	0.23	0.17	0.20	0.25	0.16	1.73	0.72	0.94
CD (*p*=0.05)	0.73	0.70	0.48	0.60	0.77	0.47	5.20	2.18	2.70

tried on basal MS nutrient medium. A number of workers (Mitrofanova and Vasil'eva, 1974; Takatsu, 1982; Aminuddin and Singh, 1985) suggested meristem culture to obtain virus-free gladiolus. In this experiment cormlets were used as explants because of the presence of immature meristematic tissues. Dickens et al. (1986) also used cormlets as explant. Medium is another absolute requirement for any successful regeneration. Among the different auxin used in the regeneration media none of the concentration of IBA was found suitable for establishment of explants. Though NAA was found to have some effect but kinetin showed remarkable impact that might be due to the fact that corms did not become non-dormant. Lilien and Kochba (1987), Dantu and Bhojwani (1987) and Duong et al. (2004) reported the use of cytokinin group of plant growth regulators for the growth and development of gladiolus from axillary bud explants. Dickens et al. (1986) also used kinetin for regeneration of gladiolus. Medium containing NAA and IBA showed successful rooting for the establishment of the plantlets. Zhuo and Sun (1986) reported the use of auxins for rooting of gladiolus. Similar observations were reported by several workers (Bajaj et al., 1983; Ziv, 1979; Sutter, 1986). The present study thus confirms the earlier findings and provides a new media combination for regeneration of gladiolus *in-vitro*.

4. Conclusion

Gladiolus cormlets when cultured *in-vitro* recorded maximum percentage of regeneration [90.00 (73.84)] using MS medium supplemented with kinetin @ 2 mg L^{-1}. The time period requirement for regeneration (6.50 days after inoculation) and plant height after 15 days (4.43 cm) were also found satisfactory under this particular regeneration medium. Hence this medium may be employed for shoot regeneration of gladiolus from cormlet explants *in-vitro*. Better rooting of regenerated cormlets *in-vitro* was obtained under the MS medium supplemented with NAA (2 mg L^{-1}) + IBA (2 mg L^{-1}). This medium initiated rooting earliest (6.92 days after transfer) and produced higher number of longer roots (7.43 roots of 48.48 mm culture^{-1}) at the time of transfer to the soil. So this medium may be used for rooting of gladiolus plantlets grown *in-vitro*.

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