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Plant Regeneration through *In vitro* Cormel Formation from Callus Culture of *Gladiolus primulinus* Baker

Pinaki Sinha and Shyamal K. Roy

Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh

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Abstract

Callus capable of plant regeneration was initiated at a higher frequency from slices of cormel sprouts of *Gladiolus primulinus* cv. Golden Wave, cultured in MS with 4.0 mg/l NAA, 2.0 mg/l BAP or 1.0 mg/l 2,4-D. Callus was maintained in callus initiation medium. Shoots regenerated from the callus in MS with 0.5 - 2.0 mg/l Kn or/and 0.5 - 2.0 mg/l BAP. Highest number of shoots regenerated in MS with 2.0 mg/l BAP from one-month-old callus which was initiated in the medium with NAA. Shoot regeneration capacity of callus decreased with the age and after fifth month no shoot was found to be regenerated. The shoots produced roots in half strength of MS with 2.0 mg/l IBA and 6 % sucrose. Rooted shoots produced cormels at the base of shoots in the same nutrient medium. About 80 - 90 % cormels germinated when sown in the field and 20 % of them blossomed in the first year.

Introduction

Gladiolus primulinus Baker is an herbaceous ornamental. The genus *Gladiolus* includes 180 species with more than 10,000 cultivers of which about 20 are grown for commercial perposes. The plant is developed annually from axillary buds on the corm which is a compressed thickened stem and as the resting perpetuating organ. A new corm is formed annually by swelling of the basal internodes of the inflorescence stalk before and during flowering. A large corm is capable of producing 25 - 200 cormels depending on cultivars and culture conditions. Flowering is terminal and occurs after all leaf primordials have differentiated. The inflorescence stalk starts elongation after the fourth leaf has fully expanded. The inflorescence is a spike, bearing up to 30 florets. Cultivated gladioli are propagated vegetatively by corms and cormels (Ziv and Lilien-Kipnis 1990).

As ornamental cut flower gladioli have great demand and are cultivated all over the world. Sometimes gladioli production declines due to incidence of viral and other diseases which cause to a great extent of commercial losses. Meristem culture for freeing plants from viruses and other pathogens and subsequent rapid in vitro propagation, have made tissue culture techniques the basis of "clean stock" programme. Breeding programmes for new hybrids specifically with desirable traits are also being hastened by tissue culture techniques (Ziv and Lilien-Kipnis 1990).

Direct 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, Ziv et al. 1997 and Hussain et al. 2001). Various results have also been reported for the role of cytokinins in plant regeneration from callus initiated from different organs of *Gladiolus* such as inflorescence stalk (Ziv et al. 1970), apical meristem (Logan and Zettler 1985), axillary buds (Lilien-Kipnis and Kochba 1987), basal leaves and cormel slices (Kamo 1994). Since the callus initiation and regeneration depend variously on cultivated varieties, explants and growth regulators used in culture media (Kamo 1994, 1995), the present investigation was undertaken to determine the proper concentratons of growth regulators for callus initiation and regeneration of a locally cultivated *Gladiolus* cv. Golden Wave, taking young cormel sprout as explant.

Materials and Methods

Cormels of *Gladiolus primulinus* cv. Golden Wave were collected from Globe Nursery, Calcutta, India and preserved in the refrigerator at 4_C, in the tissue culture laboratory, Jahangirnagar University, until culture.

For sprouting the cormels were aseptically cultured in half strength of MS (Murashige and Skoog 1962) with 1,0 mg/l BAP. Before culture the cormels were surface sterilized with continuous stream of running tap water for 30 min, washed with liquid detergent for 15 min and then with a solution of the antiseptic savlon 5 % (v/v) for 10 min. The cormels were then washed repeatedly with distilled water and finally treated with $HgCl_2$ (0.2 %) for 10 min in a laminar air flow cabinet and washed for two times with autoclaved double distilled water to remove any trace of $HgCl_2$. MS containing standard salts and vitamins, 3 % (w/v) sucrose and 0.7 % (w/v) agar was used. The pH of the medium was adjusted to 5.8 before adding agar. The medium was autoclaved at 1.1 kg/cm^2 for 20 min at 120_C. Cultures were incubated at 10,000

lux with cool white fluorescent light and maintained at 24 ± 2_C. Callus was initiated from seven-day-old sprouts of cormels cultured *in vitro.* Each sprout was sliced into 2 mm thick transverse sections and they were placed with a cut surface in contact with culture medium. The medium for callus initiation was BMS B basal salts, 3 % (w/v) sucrose, 0.7 % agar (BDH), and the followings in mg/l : glycine, 2.0; thiamine, 1.0; pyridoxine, 0.5; nicotinic acid, 0.5; mesoinositol, 100.00 and various hormones, like 2,4-D, BAP and NAA, individually at different concentrations.

One gram (approx.) of each fresh weight samples of callus were placed on shoot induction medium. Each treatment consisted of five callus samples. Medium for shoot induction was MS supplemented with Kn and/or BAP in different concentrations. When the regenerated shoots were 2 - 3 cm tall, they were separated from the base and implanted in half strength of MS with IBA (0.5 - 2.5 mg/l) and sucrose (3 - 10 %). Regenerating cultures were maintained under a 16-h light photoperiod at 24 ± 1_C.

Results and Discussion

Regenerable callus formed from the basal region of *in vitro* cultured sprout slices approximately three weeks after the explants was placed on callus induction medium. The explants formed regenerable callus when cultured on either NAA (1, 2 or 4 mg/l), 2,4-D (0.5, 1 or 2 mg/l). Callus initiated on the various concentrations of these three hormones was friable and proliferated over the next subculture (Fig. 1). As observed by Kamo (1994) that only actively growing *in vitro* plants of *Gladiolus* formed regenerable callus our results also reveal the fact in forming the callus. Callus was maintained on callus inducing medium and alicots of approximately one gram fresh weight were taken and cultured on regeneration medium.

For shoot induction one gram (fresh weight) of callus, maintained in callus initiating medium, was cultured on regeneration medium at one month interval up to fifth month. The results show (Table 1) that regeneration potential decreased with the age of callus and at the fifth month no regeneration of shoot was found on any medium. The highest number of shoots (Fig. 2) was found to be regenerated in medium with 2 mg/l BAP from one-month-old callus maintained in medium with 4 mg/l NAA. Callus initiated and maintained in medium containing 2,4-D was found to be less efficient than that in NAA.

Table 1. Effect of cytokinin on shoot regeneration from *Gladiolus primulinus* cv. Golden Wave callus of different age, induced in different hormonal supplements. Data were taken after two months of inoculation.

MS + auxins	Age of callus (month)	Number of shoots regenerated per culture on MS supplemented with cytokinin (mg/l)							
		Kn			BAP			Kn + BAP	
		0.5	1.0	2.0	0.5	1.0	2.0	0.5 + 0.5	1.0 + 1.0
NAA	1	16 (3.2)	12 (4.1)	22 (6.3)	17 (4.1)	21 (6.2)	28 (6.2)	13 (4.1)	19 (3.1)
(4 mg/l)	2	12 (2.8)	10 (3.2)	16 (4.3)	19 (3.2)	17 (6.5)	20 (6.5)	11 (2.1)	16 (3.6)
	3	4 (1.5)	2 ((1.6)	7 (3.1)	10 (3.0)	12 (4.2)	15 (4.6)	9 (3.2)	10 (2.8)
	4	2 (1.2)	1 (0.8)	3 (1.6)	7 (2.2)	6 (2.5)	11 (4.2)	3 (1.9)	5 (1.7)
	5	0	0	0	2 (1.0)	2 (1.0)	4 (2.1)	1 (0.6)	2 (1.2)
2,4-D	1	8 (2.1)	10 (1.8)	13 (2.6)	14 (2.1)	15 (3.2)	15 (3.4)	11 (2.2)	16 (3.2)
(1 mg/l)	2	7 (2.3)	6 (2.0)	8 (1.6)	7 (2.3)	6 (2.1)	8 (2.6)	7 (1.9)	6 (1.3)
	3	1 1.0)	2 (1.3)	2 (1.2)	5 (1.8)	6 (1.3)	8 (2.1)	3 (1.3)	2 (1.0)
	4	0	0	0	1(0.5)	2 (1.2)	4 (1.6)	0	2 (0.9)
	5	0	0	0	0	0	2 (0.8)	0	0

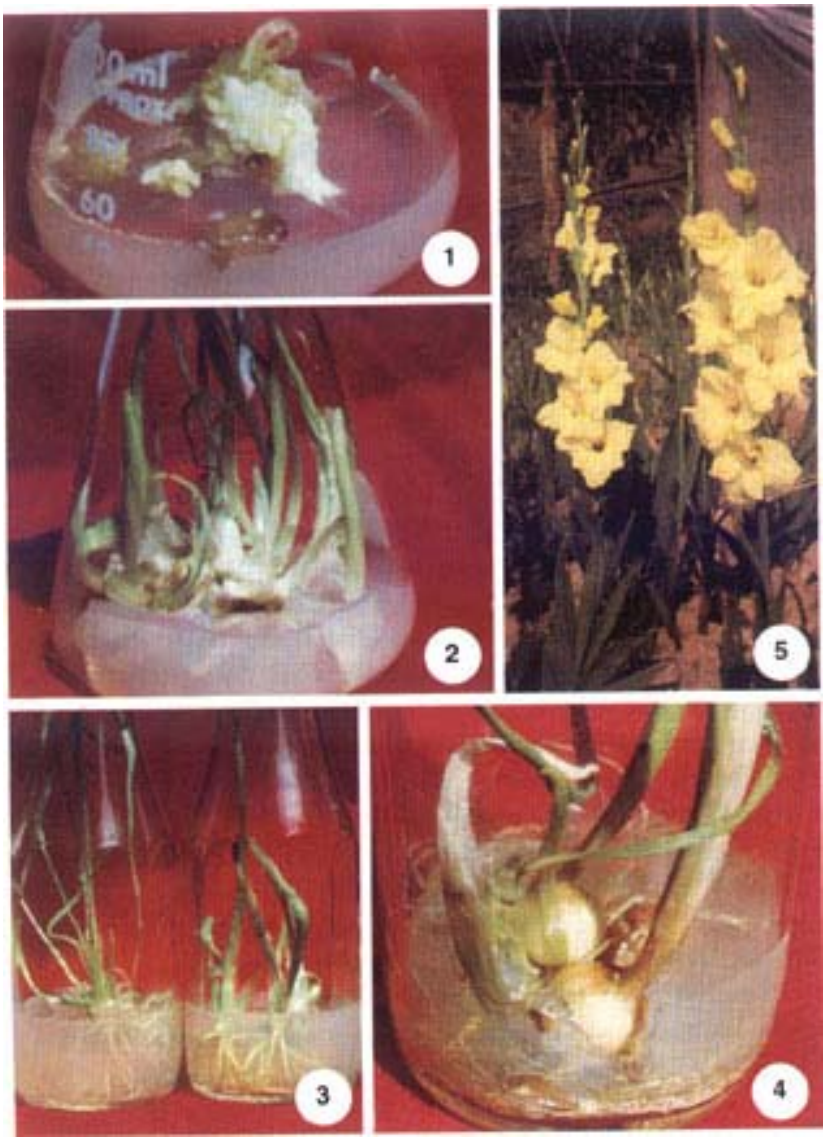
Each value is the mean of three replicates with 15 cultures in each. Figures in parenthesis indicate standard error.

Table 2. Effects of IBA and sucrose concentrations on root and cormel formation from shoots cultured in half strength MS.

Sucrose (%)	IBA mg/l)	Root formation		Cormel formation	
		Time required (days)	Shoot rooted (%)	Time[a] required (days)	Number[b] of cormel/shoot
3	0.5	22	80.5	78.5	6.4 (2.2)
	1.0	22	80.0	70.8	6.0 (3.0)
	2.0	21	79.5	69.8	5.9 (2.2)
	2.5	22	77.8	62.5	4.8 (2.0)
4	0.5	20	72.5	73.2	6.1 (4.2)
	1.0	15	80.0	69.5	7.2 (2.1)
	2.0	22	80.6	62.6	6.9 (3.1)
	2.5	17	80.3	67.8	6.8 (2.0)
6	0.5	12	95.3	56.8	11.6 (4.1)
	1.0	12	95.6	52.2	12.2 (4.2)
	2.0	12	100	52.5	12.5 (5.1)
	2.5	12	100	56.3	11.6 (3.2)
8	0.5	15	86.3	64.2	10.5 (2.2)
	1.0	14	76.4	68.3	10.0 (2.6)
	2.0	14	62.2	69.5	9.6 (2.3)
	2.5	16	71.5	58.9	9.9 (1.3)

[a]Each value is the mean of 15 cultures. Time was noted from the date of inoculation.

[b]Each value is the mean SE of 15 cultures.



Figs. 1 - 5 : 1. One-month-old callus initiated from sprout slices cultured on MS with 4 mg/l NAA. 2. Shoot regeneration in MS with 2.0 mg/l BAP, from one-month-old callus initiated in MS with 4mg/l NAA. 3. Rooted shoots in half strength MS with 2 mg/l IBA + 6 % sucrose. 4. Cormel formation in rooting medium two months after inoculation. 5. Blossomed *Gladiolus primulinus* cv. Golden Wave from *in vitro* formed cormels sown in the first year.

Kamo (1994) reported that callus initiated from explant in cytokinin redifferentiated shoots readily. Earlier researchers reported that plant regeneration occurred from callus of *Gladiolus* derived either from inflorescence

stalk (Ziv et al. 1970), cormel tips (Simonsen and Hildebrandt 1971) or cormel slices and basal leaf region (Kamo 1994) but in our results we observed shoot regeneration from callus initiated from slices of cormel sprouts. Individual shoot of shoot clump regenerated from callus was separated and cultured individually in medium with half strength MS salts with IBA (0.5 - 2.5 mg/l) and sucrose (3 - 8 %). Shoots cultured on half strength MS medium with 2 mg/l IBA and 6 % sucrose produced roots (Fig. 3) in 100 % culture within two weeks (Table 2). Rooted shoots produced cormels (Fig. 4) within eight weeks in the same medium and maximum number (12) of cormels was found to be produced in MS medium with 2 mg/l IBA + 6 % sucrose. After two months the cormels were taken out from the culture vessels. According to size (diam.) the cormels produced in vitro were of three categories, large (16 - 22 mm), medium (10 - 15) and small (5 - 10 mm). The cormels were stored at 4_C for two - three months. Afterwards, they were sown in the field and 80 - 90 % cormels were found to germinate and among them 20 % exhibited flowering in the first year (Fig. 5). Calli from some *Gladiolus* cultivers were capable of rhizogenesis or further callus proliferation (Bajaj et al. 1983). Calli from several other cultivers differentiated into shoots (Simonsen and Hildebrandt 1971, Kamo 1994). Kamo (1995) reported shoot regeneration from callus but did not mention about the formation of cormel *in vitro*. Kim et al. (1991) reported the differentiation of shoots, roots and cormels from *Gladiolus* callus. In the present experiment it is observed that the regeneration potential of callus decreased with the age of callus. Kamo (1994) also noted the same observation in his experiment but he observed regeneration in medium with 1 mg/l dicomba. Sucrose plays an important role for cormel formation *in vitro* in *Gladiolus* (Dantu and Bhojwani 1987. We observed highest number of cormel formation in medium with 6 % sucrose. This result agrees with that of obtained by Arora et al. (1996). The protocol described here is promising for propagation of disease free *Gladiolus primulinus* cultiver and screening of somaclonal variants. Moreover, it also provides with a source of cells for genetic transformation.

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