

# *In vitro* clonal propagation of locally cultivated pink color *Gladiolus* var. Neelima through Cormel-sprout culture

M. H. Kabir<sup>1\*</sup>, A. N. K. Mamun<sup>1</sup>, S. U. Talukder<sup>1</sup>, Sreeramanan Subramaniam<sup>2</sup>

<sup>1</sup>Institute of Food and Radiation Biology, Atomic Energy Research Establishment, G. P. O. Box 3787, Dhaka 1000, Bangladesh,

<sup>2</sup>School of Biological Sciences, Universiti Sains Malaysia, Georgetown, 11800, Penang, Malaysia

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## \*Address for correspondence:

M. H. Kabir, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, G. P. O. Box 3787, Dhaka 1000, Bangladesh. E-mail: mithu\_my@yahoo.com

## ABSTRACT

Micropropagation provides an economic advantage for the propagation of a particular crop like *Gladiolus*, a beautiful flowering plant which emits expression of love. The propagation by the conventional method is a slow process, and pathogen keeps on accumulation generation after generation which reduces yield and quality of flower and also generates insufficient propagules. An efficient propagation system could overcome those variabilities and meet the increasing demand of propagules production for the growing of *Gladiolus* in the country while it is an exporting plant in Bangladesh. Moreover, the establishment of a plant regeneration system through direct organogenesis or via callus is also a prerequisite to further *in vitro* genetic manipulation of the cultivar. Demand for disease-free planting materials is increasing day by day and crop like vegetatively propagated plant is an appropriate means to generate propagules through *in vitro* techniques. Production of sufficient numbers of plants of a unique genotype is possible using *in vitro* culture system. In this study, the effect of various concentrations and combinations of plant growth regulators for *in vitro* regeneration of *Gladiolus* was described using cormel-sprout as explants. However, an efficient *in vitro* plant regeneration protocol in locally cultivated pink color *Gladiolus* var. Neelima was established on MS media with various hormonal supplements using cormel-sprout as explants. 90% of the explants responded for shooting on 0.5 mg/L N-benzyl adenine + 0.5 mg/L Kin within the culture initiation period of 90-day. The average number of shoot per explants was  $8 \pm 1.20$  and the average shoot length of  $12.40 \pm 2.15$  cm was observed in this medium. Shoots are rooted well when they were excised individually and implanted on half strength of MS medium supplemented with 1.0 mg/L indole-3-butyric acid, in which 90% of the shoot induced roots. The average number of root per shoot was  $10 \pm 1.20$ , and the average root length of  $8.50 \pm 1.25$  cm was observed in this medium after culture of 30 days. 80% of the *in vitro* raised plantlets were survived in the natural environment.

**KEY WORDS:** Cormel-sprout explants, *in vitro* regeneration, pink color *Gladiolus*

## INTRODUCTION

Flowers relate to us a beautiful story of reproduction and bringing something sweet and tender into this world. *Gladiolus* is an important cut flower in many countries including Bangladesh mainly because of its many different colorful spikes which can be used in different floral arrangements at the hotel discussion, meeting, function, festival, celebration, religious ceremony, wedding, expression of love, etc. *Gladiolus* belonging to the family Iridaceae and over 180 known species with more than 10,000 cultivars of which about 20 are grown for

commercial purposes (Sinha and Roy, 2002; Kamo *et al.*, 1990). *Gladiolus* is mainly grown from corms which consist of one or more buds. It is also propagates by seeds but takes four seasons for blooming and seed set limited to genotypes and climate.

Once corms are planted the buds on the corms develop into leaves and flowering spikes. A new corm is formed annually at the base of the leaves while cormels are grown at the union of the parent and daughter corm which are used for propagation (Bushman, 1990; Ziv and Lilien, 1990). Cormels are not very effective for a traditional

method of propagation as they do not always germinated into plantlets. Moreover, corms are also needed to store at low temperature for vernalization. A large corm is capable of producing 25-200 cormels depending as cultivars and propagation method (Sinha and Roy, 2002). Cut flowers are not only offer aesthetical beauties but also have become a commercial object and contributing national economics by providing millions of dollars through exporting overseas (Akpinar and Bulut, 2011).

*Gladiolus* stands fourth in the international cut flower trade after carnation, rose and chrysanthemum. The propagation by corm may transmit several viral, fungal and bacterial diseases such as *Fusarium* corm rot, Botrytis blight, bacterial leaf rot, etc. which cause crop damage and commercial loss (Aftab *et al.*, 2008). Plant tissue culture offers a potential scope to deliver large quantities of disease free true to type healthy propagules within a short span of time (Hussain *et al.*, 2001). *In vitro* techniques have also been used for plants which present particular problems in conventional horticulture (Fay, 1992). The *in vitro* multiplication of *Gladiolus* has been reported using axillary bud (Boonvanno and Kanchanapoom, 2000; Begum and Haddiuzaman, 1995), shoottip (Hussain *et al.*, 2001) cormel (Nagaraju and Parthasarathy, 1995) and inflorescence axes (Ziv and Lilien, 2000; Ziv *et al.*, 1970). In addition, a successful protocol for *in vitro* corm formation (Dantu and Bhojwani, 1995; Sen and Sen, 1995; Aljuboory *et al.*, 1997), organogenesis and somatic embryogenesis (Remotti, 1995; Kumar *et al.*, 2002) have also been reported.

Since the regeneration of *Gladiolus* depends on cultivated varieties, explants and growth regulators used in culture media (Kamo, 1994; Kamo, 1995), thus the present study was attempted to determine the optimum concentrations of plant growth regulators for maximum multiplication of shoots and regeneration into plantlets using cormel-sprout as explants. This study also might be helpful for improvement by mutation breeding, creation of somaclonal variants and genetic engineering.

## MATERIALS AND METHODS

The corm of pink color *Gladiolus* cv. Neelima was collected from the farmers field at Jesore and planted at the experimental field of Plant Biotechnology and Genetic Engineering Division, Atomic Energy Research Establishment, Savar, Dhaka. The explants of cormel-sprouts were obtained from the plant adjacent to the corms planting after 3 months. The explants were grown from the surface of the protective brown color of the corms

and cormels and were collected for surface sterilization. The cormel-sprouts were washed with liquid detergent "Trix" and surface sterilized with 1% bavestin for 10 min and again washed with running tap water for 20 min. The cormel-sprouts were then treated with 0.1% HgCl<sub>2</sub> accompanied with 2 drops of Tween 20 for 10 min in the laminar airflow cabinet under aseptic conditions. Rinsing was done 3 times with sterile distilled water.

Sterilized cormel-sprouts were then cultured onto media containing MS supplemented with different concentrations of N-benzyl adenine (BA) and Kin alone or in combinations with BA + Kin, BA + naphthalene acetic acid (NAA), BA + Ads and BA + urea for multiple shoot induction. Subculture was done 30 days interval for promoting strong and healthy multiple shoots. Healthy shoots were excised individually and transferred to half strength of MS media supplemented with different concentrations of indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and NAA for root induction. The sucrose (table sugar) concentration was used 30 g/L and the pH of the media adjusted to 5.8 prior to autoclaving.

Cultures were incubated at 26 ± 2°C with 16 h illumination of 10,000 lux provided by cool white fluorescent tubes. Data were collected on different characters at day 90 for multiple shooting and at day 30 for rooting of *in vitro* raised shoots. Observations on cultures were carried out every alternative day. The experiments were arranged in a completely randomized design with three replications for each treatment and five explants per replication. Each experiment was repeated twice. A descriptive analysis was performed using the recorded data. Each value represents the mean ± standard errors. *In vitro* raised plantlets were removed from culture vessels, washed thoroughly to remove traces of nutrient medium, transferred to polybags and placed outdoor condition for acclimatization.

## RESULTS AND DISCUSSION

The investigation was carried out to establish a suitable protocol for large scale *in vitro* propagation of locally cultivated pink color *Gladiolus* cv. Neelima using cormel-sprout as explants with the interaction of different concentrations and combination of plant growth regulators and other supplements. The multiple shoot regeneration potential from cormel-sprout explants was found at all media type studied, but most satisfactory results of 90% explants produced multiple shoots on medium containing MS + 0.5 mg/L, BA + 0.5 mg/L Kin within the culture period of 90 days. The average number of shoot formed

per explants was  $8 \pm 1.20$  and the average shoot length of  $12.40 \pm 2.15$  cm was observed in this medium (Table 1).

Actively growing and maximum number of multiple shoot formation from cormel explants were observed in white and yellow color *Gladiolus* using BA singly at the concentrations of 0.75 mg/L and 1.0 mg/L respectively (Nagaraju and Parthasarathy, 1995; Aftab et al., 2008). Our results reveal that maximum shoot multiplications occurred in the combinations of BA + Kin and at the concentrations of 0.5 mg/L each, which might be due to the effect of genotypes. The combination of two growth regulators other than using single was found better toward multiple shoot formation also reported by many authors with other plants (Roy et al., 2011; Roy and Kabir, 2006; Roy and Kabir, 2007; Roy and Kabir, 2007; Rahman et al., 1999; Munshi et al., 2007; Kabir et al., 2006). These indicate that genotypes, type of explants, growth regulators and their concentrations and combinations

**Table 1: Effect of different concentrations and combinations of plant growth regulators on MS media for shoot formation of cormel-sprout explants in *Gladiolus* var. Neelima at 90 days**

Different concentrations and combinations of plant growth regulators (mg/L)	Percentage explants forming shoots	Mean±SE	
		Average number of shoots formed/explants	Average shoots length/explants (cm)
<b>BA</b>			
0.5	60	$4.0 \pm 0.03$	$5.50 \pm 0.65$
1.0	60	$6.0 \pm 0.45$	$10.20 \pm 0.85$
1.5	65	$5.0 \pm 0.30$	$7.50 \pm 0.45$
2.0	50	$5.0 \pm 0.40$	$5.20 \pm 0.40$
<b>Kin</b>			
0.5	60	$4.0 \pm 0.60$	$4.50 \pm 0.30$
1.0	60	$4.0 \pm 0.20$	$5.60 \pm 0.40$
1.5	50	$3.0 \pm 0.10$	$3.25 \pm 0.10$
2.0	40	$3.0 \pm 0.20$	$3.10 \pm 0.20$
<b>BA+Kin</b>			
0.5+0.5	90	$8.0 \pm 1.20$	$12.40 \pm 2.15$
1.0+0.5	70	$6.0 \pm 0.75$	$8.50 \pm 1.80$
1.5+0.5	65	$5.0 \pm 0.40$	$5.40 \pm 0.90$
2.0+0.5	60	$4.0 \pm 0.10$	$5.20 \pm 0.45$
<b>BA+NAA</b>			
0.5+0.1	50	$4.0 \pm 0.35$	$4.20 \pm 0.20$
1.0+0.1	70	$5.0 \pm 0.65$	$8.50 \pm 0.75$
1.5+0.1	45	$3.0 \pm 0.20$	$5.10 \pm 0.85$
2.0+0.1	40	$3.0 \pm 0.10$	$2.50 \pm 0.10$
<b>BA+Ads</b>			
0.5+60	45	$3.0 \pm 0.10$	$3.45 \pm 0.20$
1.0+60	45	$3.0 \pm 0.30$	$3.65 \pm 0.10$
1.5+60	20	$2.0 \pm 0.40$	$2.50 \pm 0.60$
2.0+60	20	$2.0 \pm 0.20$	$2.40 \pm 0.40$
<b>BA+Urea</b>			
0.5+1.0	40	$3.0 \pm 0.25$	$2.90 \pm 0.30$
1.0+1.0	40	$2.90 \pm 0.20$	$3.50 \pm 0.40$
1.5+1.0	10	$3.40 \pm 0.60$	$2.50 \pm 0.20$
2.0+1.0	10	$3.0 \pm 0.40$	$2.60 \pm 0.10$

Variables given are mean±SE. BA: N-benzyl adenine, NAA: Naphthalene acetic acid, SE: Standard errors

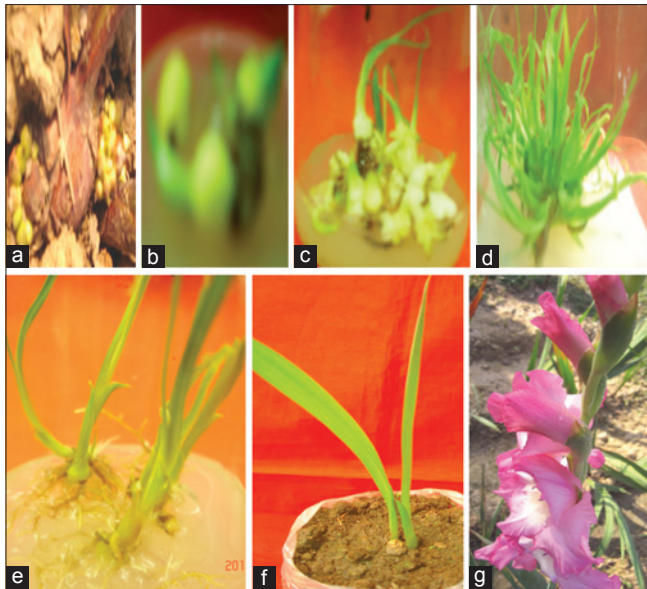
and also genetic make-up of the explants influenced greatly in plant micropropagation. From the study, it was obvious that all concentrations of BA and Kin alone and in combinations of BA + NAA, BA + Ads and BA + urea showed the decreased trend of explants responding for shooting, average number of shoot production and the performance of average shoot length induction compared to the combinations of BA + Kin. These suggest that the combinations of BA + Kin performed excellent for shoot proliferation and shoot multiplication in pink color *Gladiolus* cv. Neelima and 0.5 mg/L BA + 0.5 mg/L Kin were found to be the most optimum.

This study proved again that together with two growth regulators sometimes works well. The rooting response differed according to the concentrations of different auxins used in the study (Table 2). Among the auxins used, IBA was found to be best for root induction and 1.0 mg/L BA showed better performance for root induction, in which 90% shoots rooted within 30 days of culture. The average number of root-induced a shoot was  $10 \pm 1.20$  and the average root lengths of  $8.50 \pm 1.25$  cm were observed in this medium. Best rooting response was obtained from shoots using 2 mg/L IBA (Aftab et al., 2008) whilst using 0.5 mg/L IBA also found better rooting in *Gladiolus* genotypes (Begum and Haddiuzaman, 1995), but in our study most efficient rooting response was observed using 1.0 mg/L IBA which might be due to the fact that *in vitro* rooting also genotype dependence. This also indicates that root induction potential also varies with the IBA concentrations due to genotypes and shoots derived from the explants (Figure 1).

**Table 2: Effect of IBA, IAA and NAA on half strength of MS media in root induction of *in vitro* raised shoots of *Gladiolus* cv. Neelima at 30 days**

Name of the hormones	Concentrations (mg/L)	Percentage of shoot inducing roots	Mean±SE	
			Average number of root induced/shoot	Average root length (cm)
No hormones (MSO)		50	$6.0 \pm 0.65$	$4.20 \pm 0.45$
IBA	0.5	60	$8.0 \pm 0.60$	$6.25 \pm 0.50$
	1.0	90	$10.0 \pm 1.20$	$8.50 \pm 1.25$
	1.5	60	$8.0 \pm 0.75$	$6.20 \pm 0.90$
	2.0	50	$5.0 \pm 0.10$	$4.50 \pm 0.20$
IAA	0.5	40	$5.0 \pm 0.45$	$4.45 \pm 0.40$
	1.0	40	$4.0 \pm 0.20$	$3.90 \pm 0.20$
	1.5	-	-	-
	2.0	-	-	-
NAA	0.5	-	-	-
	1.0	40	$5.0 \pm 0.45$	$3.20 \pm 0.10$
	1.5	30	$3.0 \pm 0.10$	$3.0 \pm 0.20$
	2.0	-	-	-

Variables given are mean±SE. -: No response, SE: Standard errors, IBA: Indole-3-butyric acid, IAA: Indole-3-acetic acid, NAA: Naphthalene acetic acid



**Figure 1:** *In vitro* plant regeneration of locally cultivated pink color *Gladiolus* L. var. Neelima through cormel-sprout culture: (a) Cormel-sprout explants, (b) shoot initiation on MS + 0.5 mg/L BA + 0.5 mg/L Kin after 1 month of culture, (c) multiple shoot formation in the same medium after 3 months of culture, (d) healthy shoot formation and shoot elongation in the same medium after 3 months of culture, (e) root induction on half strength MS + 1.0 mg/L indole-3-butyric acid after 1 month of culture, (f) *in vitro* raised plant resumed new growth in the polybag, (g) 4 months old *in vitro* raised plant at flowering state

Poor performances for rooting were observed on media containing IAA and NAA. This might be due to the fact that IAA and NAA is toxic for rooting tissue or incompetent media type that inhibited root induction of pink color *Gladiolus* cv. Neelima. The superiority of IBA for *in vitro* rooting over other auxins has been reported (Jaiswal and Amin, 1987; Amin *et al.*, 1992; Amin and Akhter, 1993; Grewal *et al.*, 1994).

Comparatively, healthy rooted shoots were taken out from the culture vessels and washed gently under running tap water to get rid of agar. The *in vitro* rooted plantlets were then transferred into polybags containing a mixture of soil and compost (2:1) and covered with transparent polythene to maintain high humidity and after 1 week the polythene was removed. About 80% of the plantlets were resumed new growth within 30 days of acclimation period. A total number of 40 plantlets were survived in the field out of 50 *in vitro* regenerants. *Gladiolus* is a good consumer preference cut flower due to its attractive, colorful and showy florets. Therefore, the development of efficient tissue culture protocol is necessary for commercial cultivation, conservation, the creation of new variants, mutation breeding, and genetic improvement of this flowering plant. Thus, the protocol described in this study is repeatable, long-term *in vitro* regeneration and future

genetic improvement of this plant using cormel-sprout as explants and also useful for other crop variety.

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