http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (8): 1147-1151, 2008
ISSN 1028-8880

In vitro Study on Regeneration of *Gladiolus grandiflorus* Corm Calli as Affected by Plant Growth Regulators

Mousa Torabi-Giglou and Behzad Hajieghrari
Department of Plant Production, Moghan Junior College of Agriculture,
University of Mohaghegh-Ardabili, Ardabil, Iran

Abstract: In this study, *in vitro* organogenesis of *Gladiolus grandiflorus* cultivar pink corm segments were evaluated by culturing corm calli in modified MS medium supplemented with 3% sucrose and 0.7% agar with different concentration of BAP (0, 1, 2 and 4 mg L^{-1} medium) and NAA (0, 0.5, 1 and 2 mg L^{-1} medium) in factorial experiment of Completely Randomized Design (CRD). In order to obtain *Gladiolus* calli, corm segments (Aprox. 5×5×1 mm in size) were kept in modified MS medium (Murashige and Skoog, 1962) that was supplemented with 1 mg L^{-1} 2, 4-D, 3% sucrose and 0.7% agar. The results showed that increasing the concentration of BAP from 0 to 2 mg L^{-1} medium simulated plantlet regeneration but no significantly effect was obtained on shoot and cormel organogenesis between 2 and 4 mg L^{-1} BAP concentration in medium. Increasing of NAA content in media without BAP developed rootlet significantly. Interaction results showed that increasing BAP content against decreasing of NAA concentration stimulates the shoot and cormel proliferation.

Key words: BAP, *Gladiolus grandiflorus*, MS medium, NAA, organogenesis

INTRODUCTION

Gladiolus, an important bulbous ornamental plant, is one of the most important and popular herbaceous cut flowers with remarkable commercial value in Iran as well as in other countries. It has long lasting flower stalks, attractive colors and numerous forms that these make its an all-time favorite for the flower lovers and is said to be the queen of bulbous flowers (Ahmad *et al*., 2000; Nagaraju *et al*., 2002).

The species and varieties of the genus *Gladiolus* are varying because it includes 180 species with more than 10,000 cultivars (Sinha and Roy, 2002; Roy *et al*., 2006). However about 20 of them are grown for commercial purposes (Sinha and Roy, 2002). Annually cultivated gladioli are propagated vegetatively by corms and cormlets. The cormlet is auxiliary bud on the corm which is a compressed thickened stem and as the resting perpetuating organ (Steinitz *et al*., 1991; Remotti and Loffler, 1995; Sen and Sen, 1995; Ahmad *et al*., 2000; Nagaraju *et al*., 2002; Sinha and Roy, 2002). This kind of propagation is time consuming because most cultivars have a very slow rate of multiplication, also disease factors such as fungi, bacteria and viruses that exist in corms usually result in decrease of growth and flowering in this plant (Darvishi *et al*., 2006). Therefore there are highly desirable exist to develop methods to increase rate of multiplication as well as disease-free stocks (Singh *et al*., 2007). During the last two decades, biotechnologies have been adapted to agricultural practices and have opened vistas for plant utilization. Tissue culture technique is one of the most important components of plant biotechnology and being widely used for producing of high quality large-scaled plant multiplication, can play a major role to achieve a large number of planting material with genetically identical, physiological uniform and developmentally normal plants with the ability to survive upon transfer to *ex vitro* conditions in a relatively short period of time with the added advantage of maintaining disease-free stock.

It is demonstrated that callus regeneration mainly depends on cultivated varieties, explants and growth regulators used in culture media (Sinha and Roy, 2002). Organogenesis refers to *de novo* differentiation of callus or other tissues into adventitious meristems that can form stems, leaves, roots and cormlets and ultimately plants (Frey and Janick, 1991; Zaidi *et al*., 2000). Growth regulator concentrations such as auxin and cytokinin

Corresponding Author: Behzad Hajieghrari, Department of Plant Production, Moghan Junior College of Agriculture, University of Mohaghegh-Ardabili, Ardabil, Iran Tel: +989143186861 Fax: +984527463417

have been added as promoting factors in the culture medium, are critical to the plant regeneration and control of growth and morphogenesis (Zaidi *et al*., 2000).

In vitro propagation has been developed to accelerate the multiplication efficiency. In this regards, growing and multiplication of cells, tissues and organs on defined solid or liquid media can play a major role with the added advantages of maintaining clean stocks (Frey and Janick, 1991). Recently there are many attempts to *in vitro* propagation of *Gladiolus* (Sen and Sen, 1995; Dantu and Bhojwani, 1995; Kumar *et al*., 1999; Ahmad *et al*., 2000; Zaidi *et al*., 2000; Prasad and Gupta, 2006; Roy *et al*., 2006) under aseptic and controlled conditions developing a small cormlets at the base of shoot (Ahmad *et al*., 2000; Darvishi *et al*., 2006).

The presenting study has been undertaken to examine the effect of different concentrations of 6-benzylaminopurine (BAP) and Naphthalene Acetic Acid (NAA) in modified MS medium supplemented with sucrose, on organogenesis of *Gladiolus grandiflorus* cv. pink corm calli.

MATERIALS AND METHODS

Experiment was carried out at tissue culture laboratory of the Junior College of Agriculture, Mohaghegh Ardabili University, Ardabil, Iran, at 2007. Healthy *Gladiolus grandiflorus* cormlets cv. pink (10-20 mm in diameters) at the end of dormancy were used as source of material in this study. The cormlets were soaked in water for 24 h and then washed thoroughly under running water for 60 min. The cormlets were sterilized by immersion for 15 min in sodium hypochlorite solution (containing; 2.5%w/v NaClO, 0.2% v/v Tween20, H_2O) for 10 min and finally the explants were washed in sterile distilled water for 3 times in a laminar air flow cabinet.

For establishment, following surface disinfection and washing, cormlets were placed on autoclaved MS basal medium (Murashige and Skoog, 1962) containing 2% sucrose. The pH of the media was adjusted 5.7±0.1 with 0.1 N HCl and NaOH prior to adding with 0.7% (w/v) agar (Difco Bacto) and autoclaving at 121°C for 20 min. Culture bottle were kept in 25±1°C, in growth chamber for 10 days.

For callus induction, established cormlets were excised (5×5×1 mm aprox.) using a sharp surgical knife in a laminar air flow cabinet and subcultured in 150 mL glass flask containing 25 mL MS basal medium supplemented with 1 mg L^{-1} 2, 4-D, 3% sucrose and 0.7% agar. The pH of medium was adjusted to 5.7±0.1 before autoclaving for 20 min at 121°C. Cultures were incubated in temperature 25±1°C and relatively humidity of 60% in dark for 4 weeks. To obtain extra calli, they were subcultured in same media frequently. The friable calli were then transferred to regeneration medium.

In order to evaluate organogenesis (Shoot and root, cormel inducing), the MS medium was supplemented with two plant regulators, benzylaminopurine (BAP) and Naphthalene Acetic Acid (NAA) for checking their role and the best concentrations in development organogenesis. Various concentrations of BAP and NAA ranging from 0, 1, 2 and 4 mg L^{-1} and 0, 0.5, 1 and 2 mg L^{-1}, respectively were prepared in basal MS medium supplemented with 3% sucrose and 0.7% agar. Both individual and combined effects of BAP and NAA were tested. Corm calli (had a mean fresh weight of 6±2 g) cultured in treated medium and were placed at 25±1°C, continuous light (3000 Lux) with a photoperiod of 16 h daily and 60-70% relatively humidity. Their simple and interactive effects were analyzed in factorial experiment (with 2 factor, BAP in 4 levels; 0, 1, 2 and 4 mg L^{-1} NAA in 4 levels; 0, 0.5, 1 and 2 mg L^{-1}) of Completely Randomized Design (CRD). There were 5 replications per treatment combination and 20 samples per replicate. After three months culturing data were collected through parameters such as production proto corms-like bodies, number of shoots and number of roots per culture. The means were analyzed by analysis of variance (ANOVA) and Least Significant Difference (LSD) test at 5% significant level with SAS (1985) software (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

The pathways of regeneration, in tissue culture, depend on the media and the material used. Many substances have been added to culture media to enhance plant regeneration. Growth regulators concentrations in the culture medium are critical to the control of growth and morphogenesis. Several workers using various methods and plant parts for regeneration of *Gladiolus* (Hussey, 1977; Ziv, 1979; Bajaj *et al*., 1983) as well as Kim *et al*. (1991) reported the differentiation of shoots, roots and cormels from *Gladiolus* calli. In this study we evaluated regeneration of *Gladiolus grandiflorus* cultivar pink corm segments callus. For this, MS medium with 3% sucrose was supplemented with two plant growth regulators, BAP and NAA in order to check their role in the development of the shoot, cormel and root of *Gladiolus* callus.

Table 1: Effect of 6-benzylaminopurine (BAP) and naphthalene acetic acid (NAA) on shoot regeneration, cormel formation and root formation

Treatment combination No.	Treatment combination		Means		
	BAP	NAA	Mean root formation per explant	Mean cormel formation per explant	Mean shoot regeneration per explant
1	0	0.0	12.2c	0.2f	0.0d
2		0.5	14.4bc	1.6ef	0.4d
3		1.0	15.6b	2.0ef	0.6d
4		2.0	20.8a	1.8ef	0.4d
5	1	0.0	1.0g	3.2ef	2.6d
6		0.5	4.2ef	5.6de	8.6c
7		1.0	5.8de	12.0c	16.0b
8		2.0	8.8d	12.8c	15.6b
9	2	0.0	0.0g	9.6cd	16.2b
10		0.5	0.4g	12.6c	18.0b
11		1.0	2.6fg	18.2ab	25.4a
12		2.0	5.8de	19.4a	28.4b
13	4	0.0	0.2g	13.6c	19.0b
14		0.5	0.2g	13.8bc	17.8b
15		1.0	0.0g	22.0a	28.0a
16		2.0	0.0g	18.4a	24.6a
LSD value ($\alpha = 0.05$)			3.2	4.4	4.3

*Mean values followed by the same letter(s) are not significantly different at the 5% level (Least Significant Difference (LSD))

Prasad and Gupta (2006) demonstrated that a combination of NAA and BAP is necessary to induce multiple shoot buds. Also Ziv and Lilen-Kipnis (1990) reported similar requirement of a growth regulator combination of NAA/BAP for *in vitro* shoot regeneration of *Gladiolus* in medium. However a difference in optimum combination of NAA and BAP was evidence with different culture type and media composition. Furthermore the optimum concentration of BAP/NAA required for shoot multiplication varies from cultivar to cultivar (Hussey, 1977). In our work, BAP and NAA at various concentrations were tested for their effects on *in vitro* organogenesis (Table 1). Callus inducing medium, MS medium with 1 mg L^{-1} 2, 4-D and 3% sucrose, showed significantly good response in production of extra calli (Fig. 1C). When the corm calli were transferred to the different media supplemented with tested concentrations of BAP and NAA, they started proliferation after 4-5 weeks (Fig. 1D). In media without BAP and NAA no shoot regeneration were observed. This result agrees with that of obtained by Prasad and Gupta (2006) that without combination of NAA and BAP calli have not induced shoot regeneration (Table 1). However *in vitro* shoot production of *Gladiolus* from corm calli in media with different concentration of BAP can be occurred without NAA (Table 1). Therefore the results confirm that BAP is necessary for shoot induction. Similar trend in shoot formation was reported by De Bruyn and Ferriera (1992). Multiple shoot formation between tested concentrations was observed in combination of 2/1 and 4/2 or 4/1 mg L^{-1} BAP/NAA concentrations in culture medium. Also our study indicated that BAP in 2 or 4 mg L^{-1} concentrations in the medium plays same role and increasing BAP concentration from 2 to 4 have no significantly effects on shoot forming. In addition, 2 mg L^{-1} BAP in combination with 1 mg L^{-1} NAA were evaluated suitable result as compared to all other media used for shoot and cormel proliferation (Fig. 1D, E, F and G). Kumar *et al.* (1999) showed that BAP at lower concentrations (<10 mg L^{-1}) promoted shoot differentiation whereas higher concentration (>10 mg L^{-1}) resulted in callus proliferation and inhibited shoot differentiation.

The best cormel formation achieved in culture growing on high BAP and NAA medium. Media containing 2 or 4 mg L^{-1} BAP injunction with 1 or 2 mg L^{-1} NAA were high effective in cormel inducing and no significant influence were detected these media.

About root formation, the results indicated that NAA concentration plays an important role in *in vitro* root multiplication in *Gladiolus*. The best result was obtained on media without BAP (Fig. 1H). It was established that 2 mg L^{-1} concentration of NAA was optimal for root formation in callus cultures without BAP in MS medium supplemented with 3% sucrose (Table 1).

In addition based on the present study, high BAP and low NAA concentrations in the medium results in the formation of shoot morphogenesis whereas NAA alone or with a very low concentration of BAP is important in the induction of root (Fig. 1I).

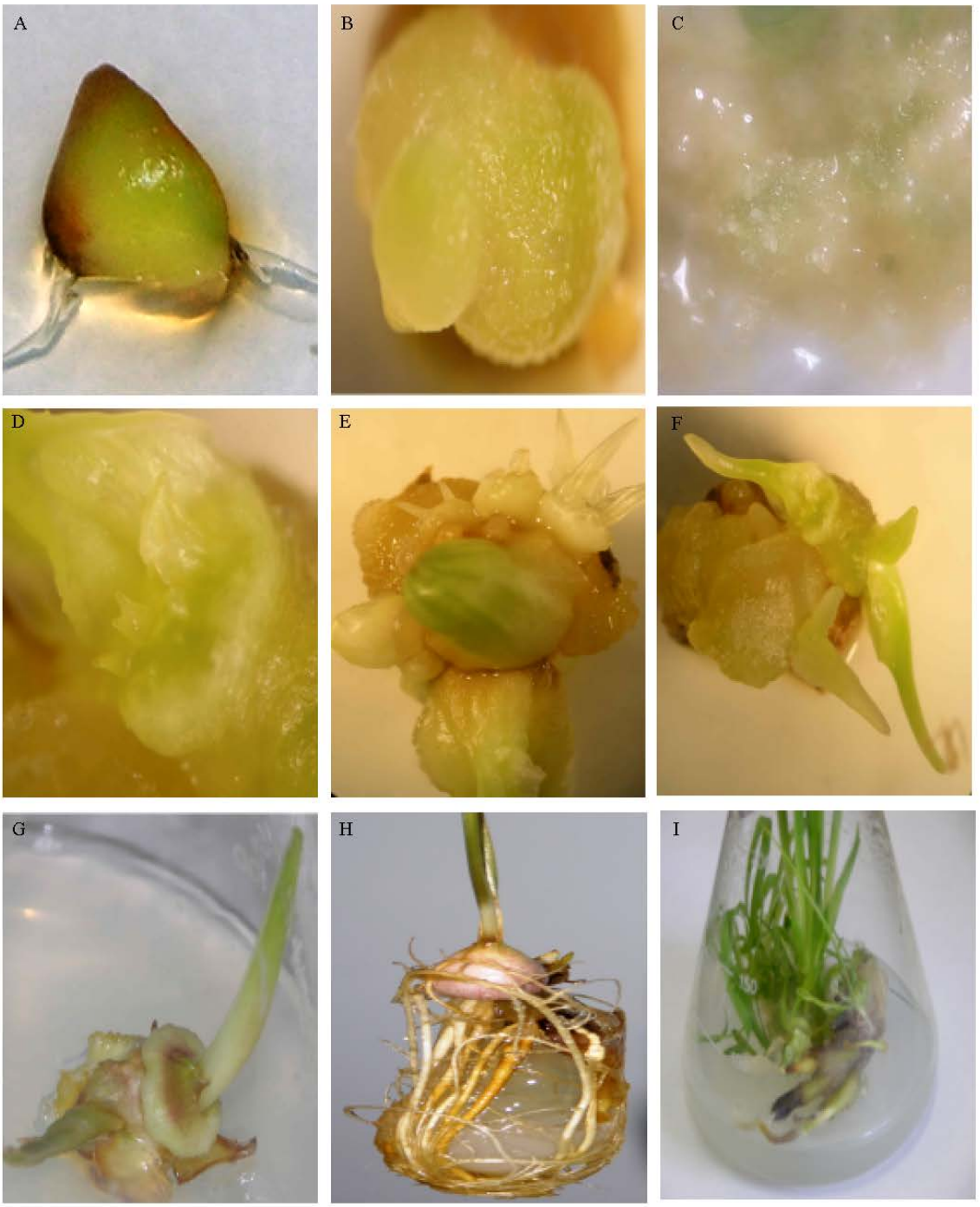

Fig. 1: (A): Established cormlet segment. (B): Initiation stage of callus. (C): Friable callus developed from established cormlet in MS medium with 1 mg L^{-1} 2, 4-D and 3% sucrose after 8 weeks planting on medium. (D, E, F and G): Shoot and cormlet differentiation on MS medium with 3% sucrose containing BAP (2 mg L^{-1}) and NAA (1 mg L^{-1}), 6, 8, 10 and 12 weeks after culturing respectively. (H): Root, Cormel and shoot formation in rooting medium with 3% sucrose containing 2 mg L^{-1} NAA without BAP. (I): Clumps of regenerated plantlets after 14 weeks on MS medium with 3% sucrose containing BAP (2 mg L^{-1}) and NAA (1 mg L^{-1})

ACKNOWLEDGMENT

I would like to express our special thanks to University of Mohaghegh Ardabili for its financial support of the present research through a grant offered for supporting scientific research.

REFERENCES

Ahmad, T., M.S. Ahmad, I.A. Nasir and S. Riazuddin, 2000. *In vitro* production of cormlets in *Gladiolus*. Pak. J. Biol. Sci., 3 (5): 819-821.

Bajaj, Y.P.S., M.M.S. Sidhu and A.P.S. Gill, 1983. Some factors affecting *in vitro* propagation of *Gladiolus*. Sci. Hortic., 18 (3): 269-275.

Dantu, P.K. and S.S. Bhojwani, 1995. *In vitro* corm formation and field evaluation of corm derived plants of *Gladiolus*. Sci. Hortic., 61 (1-2): 115-129.

Darvishi, E., R. Zarghami, C.A. Mishani, M. Omidi and A. Sarkhosh, 2006. *In vitro* production of pathogen-free plantlets via meristems culture in saffron (*Crocus sativus* L.). Biotechnology, 5 (3): 292-295.

De Bruyn, M.H. and D.I. Ferreira, 1992. *In vitro* corm production of *Gladiolus dalenii* and *G. tristis*. Plant Cell Tissue Organ Cult., 31 (2): 123-128.

Frey, L. and J. Janick, 1991. Organogenesis in carnation. J. Am. Soc. Hort. Sci., 116 (6): 1108-1112.

Hussey, G., 1977. *In vitro* propagation of Gladiolus by precocious axillary shoot formation. Sci. Hortic., 6 (4): 287-296.

Kim, K.W., M.S. Kang and D.H. Goo, 1991. The external and histological characteristics of organogenesis from *Gladiolus callus*. Korean Soc. Hort. Sci., 32 (1): 124-129.

Kumar, A., A. Sood, L.M.S. Palni and A.K. Gupta, 1999. *In vitro* propagation of *Gladiolus hybridus* Hort. synergistic effect of heat shock and sucrose on morphogenesis. Plant Cell Tissue Organ Cult., 57 (2): 105-112.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassys with tobacco tissue culture. Physiol. Plant., 15 (3): 473-497.

Nagaraju, V., G. Bhowmik and V.A. Parthasarathy, 2002. Effect of paclobutrazo/=and sucrose on *in vitro* cormel formation in gladiolus. Acta Bot. Croat., 61 (1): 27-23.

Prasad, V.S.S. and S.D. Gupta, 2006. *In vitro* shoot regeneration of Gladiolus in semi-solid agar versus liquid cultures with support systems. Plant Cell Tissue Organ Cult., 87 (3): 263-271.

Remotti, P.C. and H.J.M. Loffler, 1995. Callus induction and plant regeneration from Gladiolus. Plant Cell Tissue Organ Cult., 42 (2): 171-178.

Roy, S., G. Gangopadhyay, T. Bandyopadhyay, B.K. Modak, S. Datta and K.K. Mukherjee, 2006. Enhancement of *in vitro* micro corm production in Gladiolus using alternative matrix. Afr. J. Biotechnol., 5 (12): 1204-1209.

Sen, J. and S. Sen, 1995. Two-step bud culture technique for a high frequency regeneration of Gladiolus corms. Sci. Hortic., 64 (1-2): 133-138.

Singh, B.R., V.K. Dubey and A. Aminuddin, 2007. Inhibition of mosaic disease of Gladiolus caused by Bean yellow mosaic and Cucumber mosaic viruses by virazole. Sci. Hortic., 114 (1): 54-58.

Sinha, P. and S.K. Roy, 2002. Plant regeneration through *in vitro* cormel formation from callus culture of *Gladiolus primulinus* baker. Plant Tissue Cult., 12 (2): 139-145.

SAS, 1985. Users Guide: Statistics version 5 Edn. SAS Institute Inc. Cary. NC, pp: 956.

Steinitz, B., A. Cohen, Z. Goldberg and M. Cochba, 1991. Precocious Gladiolus corm formation in liquid shakes cultures. Plant Cell Tissue Organ Cult., 26 (2): 63-70.

Zaidi, N., N.H. Khan, F. Zafar and S.I. Zafar, 2000. Bulbous and cormous monocotyledonous ornamental plants *in vitro*. Q. Sci. Vis., 6 (1): 58-73.

Ziv, M., 1979. Transplanting Gladiolus plants propagation *in vitro*. Sci. Hortic., 11 (3): 257-260.

Ziv, M. and H. Lilen-Kipnis, 1990. Gladiolus. In: Hand Book of Plant Cell Culture, Ammirato, P.A., D.A. Evans, W.R. Shark and Y.P.S. Bajajl (Eds.). Vol. 5. McGraw Hill Publishing Co., New York, pp: 461-478.