Development of Protocol for *In Vitro* Propagation of Gladiolus

L.P.H. Liyanage, H.A.S.L. Jayasinghe *and* L.M.H.R. Alwis
Uva Wellassa University, Badulla, Sri Lanka

Introduction

Gladiolus, the queen of bulbous flowers belonging to family Iridiaceaeis one of the most important and popular cut flower. Gladiolus occupies the fifth place in the international floriculture trade (Ghani*et al.* 2008). Gladiolus has long lasting flower stalks, attractive colors and numerous forms which make its higher consumer demand in the market. In Sri Lanka, only few farmers have tended to cultivate gladiolus as a commercial crop. Due to high vulnerability to the diseases and pests and unavailability of high quality disease free planting materials, flower growers dis-incline to cultivate gladiolus in their fields. Therefore, mass production of high quality planting materials and continuous supply are essential to promote the gladiolus cultivation in Sri Lanka. Gladiolus is vegetatively propagated by corms and cormlets, which is a time consuming method due to very slow rate of multiplication and disease factors (Torabi-Giglou and Hajieghrari. 2008). *In vitro* propagation methods become a viable alternative to the conventional propagation methods and are widely used for producing high quality disease free planting materials. Therefore, present study was undertaken to develop a protocol for *in vitro* propagation of gladiolus by studying the effect of sterilants and different hormone combinations for direct and indirect organogenesis.

Methodology

The research was conducted in tissue culture laboratory at Uva Wellassa University. The corms (2 to 3 mm pieces) and cormlets were used as explants. In the first experiment, to determine sterilization method effect of two different time durations of soaking explants in 1% NaOCl (5 min. and 10 min.) and three different time durations of soaking explants in 70% Alcohol (5 min., 10 min, and 15 min.) were evaluated. Numbers of contamination vessels were observed after one week from explant establishment in hormone free MS medium. In second experiment, effect of different hormone combinations (MS medium (without PGR), MS medium +NAA 4 mg l^{-1} and MS medium + 2,4-D2 mg l^{-1} + BAP 1 mg l^{-1}) for callus induction of corms were evaluated. Number of days to initiate callus, diameter and fresh weight of callus were recorded after 8 weeks from explant establishment in MS medium. In third experiment, effect of hormone combinations (MS medium + NAA 4 mg l^{-1}, MS medium + NAA 4 mg l^{-1} + BAP 1 mg l^{-1} and MS medium + 2, 4-D 4 mg l^{-1} + BA 2 mg l^{-1}) for shoot induction and (Half strength MS medium + IBA 1 mg l^{-1} + Sucrose 5%, Half strength MS medium + IBA 2 mg l^{-1} + Sucrose 5% and MS medium + NAA 2 mg l^{-1} + Sucrose 3%) for root induction of corms were evaluated. Number of days to initiate shoots and roots, shoot length, shoot fresh weight, number of shoots per culture and root initiation percentage was recorded after 8 weeks from explant establishment in MS medium.

Results and Discussion

The six experiments conducted to determine the best sterilization method were significantly different at 5% significance level. The lowest contamination percentage of corms and

cormlets was found when it was treated with 1% NaOCl for 5 min. and 70% Alcohol for 15 min. which was the same method reported by Memon*et al.* 2010.
In present study also revealed that there were significant difference between treatments on callus fresh weight and callus diameter at 5% significance level. The results revealed that the highest fresh weight (0.07513 g) and maximum diameter (0.8267 cm) were recorded from corms in MS medium supplemented with 4 mg l^{-1} NAA within minimum days (16) (Table 1).

Table 1: Number of days to initiate callus and mean values of fresh weight and diameter of callus in treatments

Treatment	Mean of fresh weight	Mean of diameter	Number of days
MS medium (without PGR)	0.00000^{b}	0.0000^{b}	0
MS medium + NAA 4 mg l^{-1}	0.07513^{a}	0.8267^{a}	16
MS medium + 2,4-D2 mg l^{-1} + BAP 1 mg l^{-1}	0.05060^{a}	0.5433^{a}	30

Use of higher levels of NAA (8.5 and 10 mg l^{-1})was reported for callus induction of cormel slices by Emek and Erdag(2007). Kamo (1994) reported a greater number of regenerants from the callus cultured on medium having NAA than from callus cultured on medium containing 2,4-D. Boonvanno and Kanchanpoom (2000) considered that the best auxin for callus initiation is NAA. In the present study, it was revealed that NAA was effective for callus initiation from corm slices which is in accordance with the findings of Emek and Erdag (2007).

Table 2: Mean values of shoot length (cm), fresh weight (g), number of shoots and number of days to initiate shoots in treatments

Treatment	Number of Days	Shoot Length	Fresh Weight	Number of shoots
MS medium + NAA 4 mg l^{-1}	15	2.7533^{a}	0.03440^{ab}	1.3333^{b}
MS medium + NAA 4 mg l^{-1} + BAP 1 mg l^{-1}	4	1.3083^{b}	0.06857^{a}	4.3333^{a}
MS medium + 2,4-D 4 mg l^{-1} + BA 2 mg l^{-1}	10	1.1667^{b}	0.02823^{b}	2.3333^{b}

Significant difference can be observed between treatments for shoot weight, shoot length and number of shoots per culture at 5% significance level in the study. The highest shoot weight (0.06857 g) resulted from corms in MS medium supplemented with 4 mg l^{-1} NAA and 1 mg l^{-1} BAP within 4 days. The highest shoot length (2.7533 cm) showed from corms in MS medium supplemented with the 4 mgl^{-1} NAA (Table 2).

The induction, regeneration and proliferation of multiple shoots from any explant source are much dependent upon the level and kind of cytokinins added to MS medium. The present research indicated that shoot induction of corms was more dependent on BAP and more number of shoots were stimulated by 1 mg l^{-1} BAP, which is confirmed that cytokinin is a potent plant growth regulator for regeneration of multiple shoots in bulbous plants as reported by Dewir*et al.* (2006). Torabi-Giglou and Hajieghrari (2008) reported shoot regeneration in the presence of BAP (2 mg l^{-1}) and NAA (1 mg l^{-1}).

Root initiation percentage showed significantly different among the treatment at 5% significant level ($p = 0.045$). Earlier root induction resulted from corms in MS medium supplemented with 2 mg l^{-1} IBA and 5% sucrose level within 15 days at higher percentage (86.667%) (Table 3).

Table 3: Number of days to initiate roots and mean values of root initiation percentage

Treatment	Days to initiate roots	Root initiation percentage
Half strength MS medium + IBA 1 mg l^{-1} + Sucrose 5%	19^{ab}	77.333^{b}
Half strength MS medium + IBA 2 mg l^{-1} + Sucrose 5%	15^{b}	86.667^{a}
MS medium + NAA 2 mg l^{-1} + Sucrose 3%	22^{a}	76.000^{b}

Efficient methods for developing roots are equally important for better cormel formation. In the present study, it revealed that the corms produced roots at each PGR combination. Begum and Haddiuzaman (1995) obtained rooting even on low levels of IBA (0.5 mg l^{-1}). Priyakumari and Sheela (2005) produced the earliest and longest roots on IBA (2 mg l^{-1}).

Conclusion

According to protocol developed for *in vitro* propagation of gladiolus, the best sterilization method is corms and cormlets treated with 70% Ethanol for 15 minutes and 1% Sodium hypochlorite for 5 minutes. MS supplemented with the 4 mg l^{-1} NAA is the best hormone combination for earlier callus induction. MS medium supplemented with 4 mg l^{-1} NAA and 1 mg l^{-1} BAP is the best medium for shoot induction. MS medium supplemented with 2 mg l^{-1} IBA and 5% sucrose level is the best for earlier root induction at higher frequency.

References

Begum, S. and S. Haddiuzaman. 1995. *In vitro rapid shoot proliferation and corm development in glaiolusgrandiflorus cv. Redbrand.* Plant Tissue Culture. 5(1):7-12.

Boonvanno, K. and K. Kanchanapoom. 2000. *In vitro propagation of gladiolus.* Suranaree, Journal of Science and Technology, 7:25-29.

Dewir, Y.H., D. Chakrabarthy, E.J. Hahn and K.Y. Paek., 2006. *A simple method for mass propagation of Spathiphyllumcannifolium using an airlift bioreactor.In vitro* Cell Dev. Biol. Plant. 42:291-297.

Emek Y. and Erdag B., 2007. *Somatic Embryogenesis from Leaf Explants of Gladiolus anatolicus (Boiss.)Stapf.* Pakistan Journal of Biological Sciences, 10: 1190-1194.

Ghani, S., Jabeen, M., Hussain, F., Ejaz, G. Ghauri and Fatima, A. 2008. *Heterogeneity in the micropropagation of dicot and monocot cultured under same conditions in vitro.* International Journal of Biotechnology and Biochemistry

Kamo, K. 1994. *Effect of phytohormones on plant regeneration from callus of gladiolus cultivar "Jenny Lee".* In vitro Cell Dev. Biol. 30:26-31.

Memon, N., Qasim, M., Jaskani M.J. and Ahmad R. 2010. *In Vitro Cormel Production of Gladiolus.* Pakistan Journal of Agricultural Science, 47(2):115-123.

Priyakumari, I. and Sheela, V.L. 2005.*Micropropagation of gladiolus cv. 'Peach Blossom'through enhanced release of axillary buds.* Journal of Tropical Agriculture 43 (1-2): 47-50.

Torabi-Giglou, M. and Hajieghrari, B., 2008.*In vitro Study on Regeneration of Gladiolusgrandiflorus Corm Calli as Affected by Plant Growth Regulators.* Pakistan Journal of Biological Sciences, 11: 1147-1151.