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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 3 (5): 819-821, 2000

In vitro Production of Cormlets in Gladiolus

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Abstract: The ability of explants of *gladiolus* cv White Prosperity to form plantlets and cormlets was investigated *in vitro* studies. Maximum production of shoots per explant was obtained on basal medium of Murashige and Skoog (1962) containing 18 µM 6-Benzylaminopurin (BAP) and 36 µM Indole-3-butyric Acid (IBA). Callus formation was observed when *in vitro* cultures were maintained for 16-18 weeks. When different explants were used for their response to regenerate, maximum number of shoots per culture was achieved from nodal buds. In order to allow initiation of root formation, the shoots were transferred to one half strength Murashige and Skoog medium containing 2.5 µM, Naphthalenacetic Acid (NAA) or 19 µM Indole -3-butyric Acid (IBA). Roots developed readily within 4-5 weeks on either of the two media. However, all plantlets grew with better survival in medium containing NAA as harmonal supplement than in media containing IBA. Further MS medium containing 6 percent sucrose facilitated maximum growth of cormlets to allow their use as propagation material *in vivo*.

Key words: Gladiolus, multiple shoots, cormlet production, *in vitro*

Introduction

Gladiolus is one of the most popular cut flower. It has long lasting flower stalks, attractive colors and numerous forms, therefore there is high consumer demand in Pakistan as well as in foreign countries. The plant is propagated mainly through vegetative reproduction of cormlets and cormels. To boost economical production, it is highly desirable to develop methods to increase rate of multiplication. Clonal propagation through tissue culture has been commercially exploited in a large number of plant species, (Hoidgate, 1977). Hussey (1977), Ziv (1979) and Logan and Zettler (1984) reported *in vitro* propagation of gladiolus. *In vitro* cormel production from nodal buds and cormel tip has been achieved successfully (Arora *et al*., 1996). It has been also successfully tried from inflorescence stalks (Ziv *et al*., 1970), axillary buds of corm, (Dantu and Bhojwani, 1987) and nodal buds, (Grewal *et al*., 1990). De Bruyn and Ferreira (1992) reported *in vitro* production of cormlets from flowering species. Stefaniak (1994) studied the response of different explants for plant regeneration from Gladiolus. Nasir *et al*. (1996) investigated *in vitro* plant regeneration and cormlet production. The present studies report the effect of various media supplemented with different growth hormones either in combination or alone and high sucrose content level for multiple shoot formation, root induction and cormlet production of cv white prosperity.

Materials and Methods

Healthy gladiolus cv White Prosperity, was used as source material for terminal and axillary buds of cormlets at the end of dormancy and nodal buds. The cormlets were soaked in water for one day, followed by treatment with Dithane M-45 for 25 minutes at 56°C. The large cormlets, (Diameter above 8 mm), were sterilized for 30 minutes in 2 percent (w/v) NaOCl + 0.05 percent (v/v) Tween-20 as a surfactant while small cormlets (diameter less than 8mm) were sterilized in 1.5 percent (w/v) NaOCl solution containing 0.05 percent (v/v) Tween-20. Nodal buds were excised when the spikes were at slipping stage and surface sterilized with mercuric chloride (0.1 percent). After sterilization the cormlets were divided in such a way that each explant contained an axillary bud and a piece of corm tissue. One corm tip was also obtained from each corm. The basal medium Murashige and Skoog (1962) was modified by decreasing the strength of MS salts to one half and by increasing the sucrose concentration from 3 to 9 percent. For shoot formation the explants were transferred to S1, S2 and S3 media (Table 1). Multiple shoots were transferred to growth regulator free MS medium to enhance shoot elongation. Shoots 4-6 cm long were transferred to R1 and R2 media for root induction (Table 2). After adjusting the pH of media to 5.8, gelrite was added at 2.5 g/l prior to autoclaving. Culture conditions were adjusted for 16 hr photoperiod at 23 ± 2°C in accordance with the recommendations (Ziv *et al.*, 1970; Bajaj, 1983; Lilien-Kipnis and Kochba 1987; Steinitz *et al.*, 1991). Each treatment consisted of 25 replications. All the chemicals used were from Sigma Chemical Co. USA.

Table 1: Shoot Proliferation Media for Cv. White Prosperity

Media	Composition
S1	MS + 18uM BAP + 36uM IBA
S2	MS + 18uM BAP
S3	MS + 9uM kinetin

Table 2: Root/cormlet Induction and Development Media

Media	Composition
R1	1/2 MS + 19uM IBA
R2	1/2 MS + 2.5uM NAA

Results and Discussion

When different media along with various combinations and concentrations of cytokinin and auxin were used, S1, S2 and S3 media (Table 1) for multiple shoot formation, R1 and R2 media (Table 2) for root induction and MS with 6 percent sucrose level

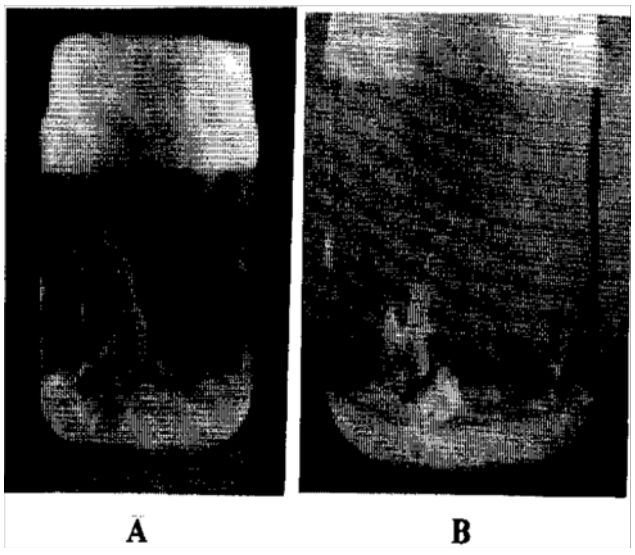

Fig. 1: Multiple shoot formation on S1 medium from (a) Cormel tip (b) Axillary bud

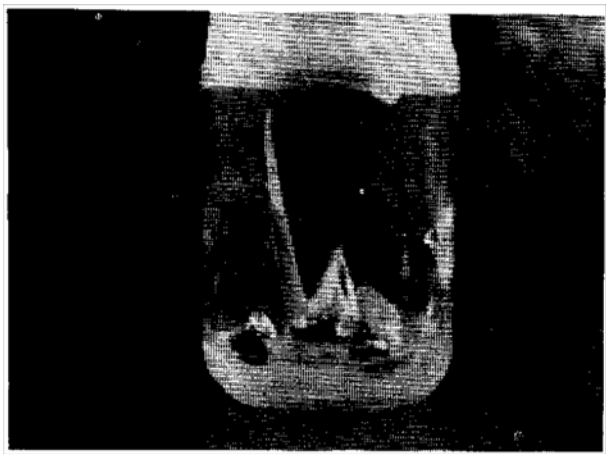

Fig. 2: Callus formation on S1 medium after 18 weeks

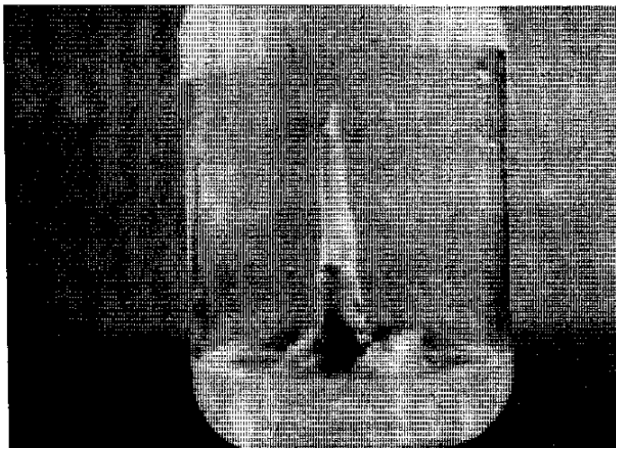

Fig. 3: Shoot elongation in harmone free medium

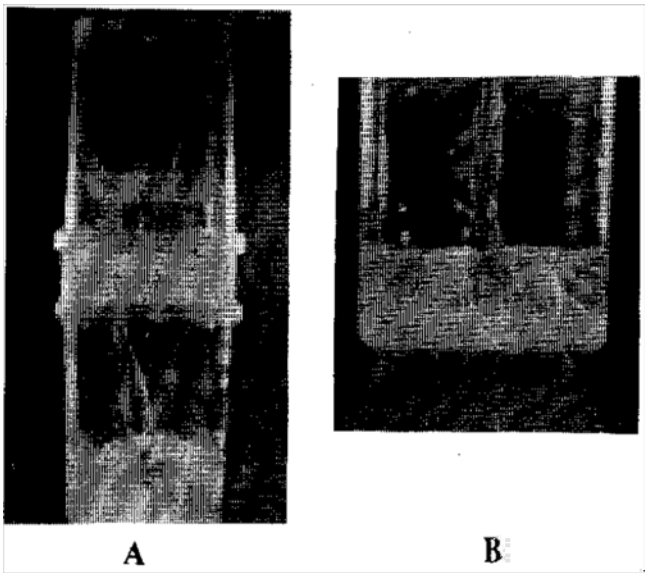

Fig. 4: Root induction after 4 weeks from (a) R1 medium (b) R2 medium

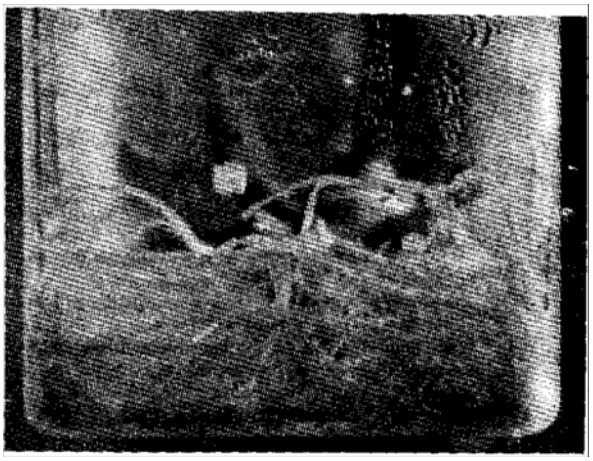

Fig. 5: Cormlet formation on basal medium containing 6 percent sucrose

for cormlets production were found most suitable. *In vitro* response of axillary buds, cormel tips and nodal buds is summarized (Table 3). When the explants were transferred onto the different media, they started proliferation after 2-3 weeks. Cormel tips gave better response as compared to axillary buds (Fig.1a, b) whereas average number of shoots per culture was more in case of nodal buds. A combination of 18uM BAP and 36uM IBA as shown in S1 medium (Table 1) produced better results as compared to all other media used for shoot proliferation. In S1 medium however callus from corm pieces was also observed after 16-18 weeks (Fig. 2). Multiple shoot formation was observed in S1 and S3 media (Table 1) after 14-16 weeks. Nodal buds were found better explant as compared to other types of explants, which started growing within a week and developed multiple shoots after 4-6 weeks in media (Table 1). Multiple shoot formation was greater in S3 medium than S1 and S2 media. De Bruyn and Ferreira (1992) also reported *in vitro* shoot production of Gladiolus from corm pieces on MS media containing BA at different concentrations. The best results were achieved on media containing 2.2-4.4uM BA with sucrose concentration 6-9 percent for winter flower Gladiolus. It has been reported that the optimum concentration of BAP required for shoot multiplication varies from cultivars to cultivars (Hussey, 1977; Dantu and Bhojwani 1987, Grewal *et al.,* 1990). This may be attributed to difference endogenous levels of harmones among different cultivars. Shoots produced in S1, S2 and S3 media were separated and cultured on harmone free basal medium, which showed elongation (Fig. 3). Arora *et al.* (1996) reported that buds cultured on hormone free basal media showed elongation. Dantu and Bhojwani (1987) also observed that shoot elongation occurred only when cytokinins were omitted or reduced to low level. For better induction of roots, elongated shoots were cultured on R1 and R2 media (Table 2). Root induction was observed within 2-4 weeks (Fig.4a, b). Medium containing NAA exhibited improved and early response as compared to medium supplemented with IBA. The poor survival of transplanted plants could have been the result of poorly developed roots (Ziv *et al.*, 1970). However these plantlets exhibited swelling at their basal ends and induced cormlets (Fig. 5) within 10-12 weeks in MS media containing 3 percent, 4 percent, 6 percent and 9 percent sucrose level. The sucrose levels from 6-9 percent were found suitable for good size cormlet production upto 10 mm diameter. Dantu and Bhojwani, 1987; Grewal *et al.* (1990) reported that high sucrose level at 9-12 percent did not improve the cormlet size whereas lowering the level of sucrose to 3 percent led to smaller cormlet. The survival of transplants was better in case of R2 medium containing NAA as compared to R1

Table 3: *In Vitro* Response of Axillary Buds, Cormel Tips and Nodal Buds on Different Media

Media	Status	Axillary Bud	Cormel Tip	Nodal Bud
S1	No. of cultures raised	25	25	25
	No. of cultures response	20	21	13
	Percentage response	80	84	52
	Average no. of shoots per culture	1	3	6
S2	No. of cultures raised	25	25	25
	No. of cultures response	17	20	15
	Percentage response	68	80	60
	Average no. of shoots per culture	1	2	9
S3	No. of cultures raised	25	25	25
	No. of cultures response	17	18	15
	Percentage response	68	72	60
	Average no. of shoots per culture	1	2	12

medium containing IBA (Table 2). Whereas *in vitro* produced cormlets showed 90-95 percent germination after 8 weeks of storage at 4°C i.e., necessary for breaking corm dormancy.

The production of twelve plants from nodal bud and five plants from one cormel is significant increase as compared with *in vivo* production of one plant per cormel and no plant from nodal bud, this therefore, allows rapid disease free cormlet production on a commercial scale.

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