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Micropropagation of gladiolus cv. 'Peach Blossom' through enhanced release of axillary buds

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Abstract

Gladiolus grandiflorus L. cv. 'Peach Blossom' was micropropagated through enhanced release of axillary buds. The cultures were established using intact cormels in Murashige and Skoog (MS) medium. Shoot proliferation was maximum in MS medium fortified with BA 4 mg L^{-1}+NAA 0.5 mg L^{-1}. Low concentrations of BA (1 or 2 mg L^{-1}) were, however, suitable for further shoot multiplication. IBA (2 mg L^{-1}) produced earliest rooting (7 days) and longest roots (5 cm). *In vitro* raised plantlets were successfully planted in sterile media consisting of sand: soil (2:1) in plastic pots.

Keywords: *In vitro* propagation, MS medium, cormel, multiple shoot proliferation

Introduction

Gladiolus (*Gladiolus grandiflorus* L.) is an important bulbous ornamental plant valued for its attractive spikes. Commercial gladiolus cultivation, however, is limited by the low multiplication rate of corms. Physiological dormancy of the corms and cormels, which usually last for about 4 to 5 months and corm rot during storage are other problems in this respect. They generally result in inadequate availability of quality planting materials. *In vitro* propagation techniques, therefore, assumes significance, especially for securing rapid multiplication of the novel cultivars. Although several reports on *in vitro* propagation of gladiolus varieties (Misra and Singh, 1999; Pathania et al., 2001) are available, such reports generally underscore differential cultivar responses. Therefore, a study was undertaken with the objective of evolving an *in vitro* protocol for 'Peach Blossom', a gladiolus variety suitable for cultivation in southern Kerala (KAU, 1996).

Materials and methods

Cormels of gladiolus cv. 'Peach Blossom' were collected from field-grown plants during June 2000 and were treated with 0.4% Mancozeb (Indofil M-45) for 30 min. Dehusked intact cormels of uniform size (0.8 to1.2 cm) were kept in 1000 times diluted 'Labolene' solution for 30 min. and washed thoroughly in running tap water (5 min.), followed by glass-distilled water. The cormels were subjected to final surface sterilization in the laminar airflow chamber with 0.08 % $HgCl_2$ (10 min.) and were washed with sterile distilled water, 4 to 5 times.

The culture medium used was MS (Murashige and Skoog, 1962) fortified with sucrose (3%) and agar (0.7%). Its pH was adjusted to 5.6 to 5.8 before autoclaving at 1.08 kg cm^{-2} for 15 min. Different levels of BA and kinetin (1, 2 and 4 mg L^{-1})—alone and in combination with NAA (0.1 and 0.5 mg L^{-1}) and IAA (1 and 2 mg L^{-1})— were tried for culture establishment and shoot multiplication. Bisected cormel halves obtained after cutting the elongated shoot and roots were used for shoot multiplication. *In vitro* rooting studies were conducted in MS medium supplemented with auxins like IBA, NAA and IAA (0.5, 1 and 2 mg L^{-1}) using micro shoots of 2.5 to 3.5 cm long. Rooted plantlets were taken

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out from culture flask after addition of distilled water and keeping it for about 15 min. After gently removing the agar particles adhering to the roots, the plantlets were treated with carbendazim 0.1% (Bavistin 50 WP) for 5 min., and planted in sand, *soil-rite* and sand-soil mixture (2:1 ratio). All the cultures were incubated at 26±1°C and 15h photoperiod at 50 µmol m^{-2} s^{-1} light intensity provided by cool white fluorescent tubes. The experiment was laid out in a completely randomized design, wherein each treatment was replicated thrice and the data were analysed following the ANOVA technique.

Results and discussion

The cormels cultured in establishment medium supplemented with growth regulators as per the treatment protocol showed considerable variations (Table 1). The time for bud initiation ranged from 3.7 to 35 days. The earliest bud initiation was noticed in kinetin 2 mg L^{-1} + NAA 0.1 mg L^{-1} combination (Fig. 1a). The highest shoot number (4) however, was obtained for the treatment with BA 4 mg L^{-1}+NAA 0.5 mg L^{-1} and was superior to all other treatments. Pathania et al. (2001) also reported that BAP 2 to 4 mg L^{-1}along with GA_3 was optimal for culture establishment of gladiolus cvs. 'Eurovision' and 'Wine and Roses'. Although kinetin and its combinations with auxin induced early bud break compared to BA and its combinations with auxin, they generally depressed shoot number and shoot growth. The treatments involving auxin alone also delayed bud-break and inhibited shoot growth, which is consistent with the findings of Hussain (1995).

A comparison of the data in Table 1 also indicates significant differences among the treatments with respect to shoot number. The highest rate of multiple shoots (33.7) was obtained in MS medium containing BA 4 mg L^{-1}+NAA 0.5 mg L^{-1} (Fig. 1b). This was, however, statistically on par with the medium supplemented with BA 4 mg L^{-1}+IAA 2 mg L^{-1} producing 27 shoots on an average. With regard to shoot length, the treatments supplemented with 'auxin alone' produced the longest shoots. It seems that higher levels of BA (e.g., 4 mg L^{-1}) combined with auxin stimulated multiple axillary bud production, but length of the individual shoots was lower. Higher levels of BAP presumably promoted secondary axillary bud production. Beura and Singh (1998) also reported a higher rate of bud proliferation for gladiolus in MS medium supplemented with high concentration of BAP (4 mg L^{-1}). To obtain shoots of ideal size (2.5 to 3.5 cm) for *in vitro* rooting, therefore, lower

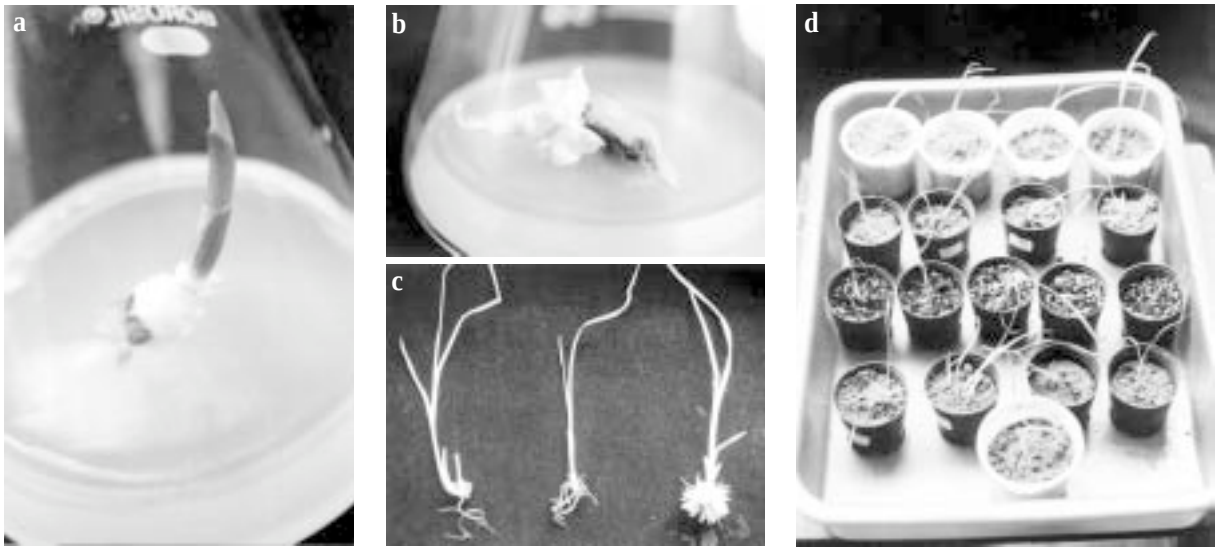

Fig. 1. a: Bud initiation in culture establishment medium with kinetin 2 mg L^{-1}+NAA 0.1 mg L^{-1}; b: multiple shoot proliferation in MS medium supplemented with BA 4 mg L^{-1}+NAA 0.5 mg L^{-1} (3-week culture period); c: *in vitro* rooted plantlet; d: hardening of *in vitro* rooted plantlets

Table 1. Effect of plant growth substances on culture establishment and multiple shoot proliferation of gladiolus

Treatments[1] (mg L^{-1})	Establishment[2]		Multiple shoot proliferation[3]		
	Days[4]	Shoots[5]	Days[4]	Shoots[5]	Longest shoot (cm)
NAA 0.1	23.0	1.0	13.0	1.0	17.7
NAA 0.5	33.0	1.0	12.0	1.0	19.6
IAA 1	19.7	1.0	8.7	1.7	20.6
IAA 2	35.0	1.0	8.0	1.0	19.4
BA 1	7.0	1.0	9.7	1.0	4.5
BA 1+NAA 0.1	17.0	1.0	5.0	4.3	6.2
BA 1+NAA 0.5	12.3	1.0	9.0	4.3	2.1
BA 1+IAA 1	12.0	1.0	15.0	2.7	5.8
BA 1+IAA 2	8.3	1.3	11.0	7.3	3.8
BA 2	20.7	1.0	8.3	1.3	2.7
BA 2+NAA 0.1	10.7	1.7	5.3	4.0	3.2
BA 2+NAA 0.5	19.3	1.3	9.0	13.3	2.8
BA 2+IAA 1	13.3	1.0	2.0	8.7	2.7
BA 2+IAA 2	9.0	1.0	5.0	8.3	0.6
BA 4	10.0	1.0	7.3	14.3	0.3
BA 4+NAA 0.1	7.0	1.0	3.7	14.3	3.9
BA 4+NAA 0.5	10.3	3.7	3.3	33.7	0.2
BA 4+IAA 1	17.3	1.0	4.0	26.3	1.1
BA 4+IAA 2	8.0	1.0	2.7	27.0	0.2
Kinetin 1	9.3	1.0	10.3	1.3	1.7
Kinetin 1+NAA 0.1	15.0	1.0	7.0	2.3	9.3
Kinetin 1+NAA 0.5	11.3	1.0	6.0	1.3	6.0
Kinetin 1+IAA 1	9.3	1.0	9.7	2.0	3.6
Kinetin 1+IAA 2	14.0	1.0	4.0	7.3	4.3
Kinetin 2	6.0	1.0	5.0	8.0	1.4
Kinetin 2+NAA 0.1	3.7	1.0	4.0	8.7	2.6
Kinetin 2+NAA 0.5	5.7	1.0	5.0	7.0	8.7
Kinetin 2 +IAA 1	7.7	1.0	6.0	8.3	1.9
Kinetin 2+IAA 2	8.0	1.0	4.0	7.3	5.7
Kinetin 4	30.0	1.0	4.7	7.0	2.1
Kinetin 4+NAA 0.1	16.3	1.0	10.3	5.3	5.3
Kinetin 4+NAA 0.5	13.3	1.0	6.7	8.0	3.8
Kinetin 4+IAA 1	4.3	1.0	5.7	6.3	2.3
Kinetin 4+IAA 2	7.7	1.0	3.0	2.0	2.5
GA3 1	12.0	1.0	-	-	-
GA3 2	16.3	1.0	-	-	-
Control	32.0	1.0	14.3	1.0	11.2
CD (0.05)	6.35	0.77	5.27	6.97	4.55

[1]numerical values following the treatment codes are concentrations in mg L^{-1}; [2]four-week culture period; [3]six-week culture period; [4]for bud initiation; [5]number per culture

concentrations of BA (1 and 2 mg L^{-1}) in combination with NAA (0.1 and 0.5 mg L^{-1}) are appropriate.

The time for root initiation, number of roots and the length of the longest root were also significantly influenced by differential concentrations of IBA, NAA and IAA (Table 2 and Fig. 1c). MS medium supplemented with IBA 2 mg L^{-1} recorded earliest rooting (7 days) and produced the longest root (5 cm). Likewise, the highest number of roots (24) was produced by the treatment having NAA 1 mg L^{-1}. MS medium supplemented with 2 mg L^{-1} IBA induced extensive root growth of *in vitro* raised shoots for cv. 'Friendship' (Hussain et al., 1994). The rooted plantlets transferred to plastic pots containing sand: soil (2:1) also recorded cent percent survival at 15 days after planting (Fig. 1d). Earlier, Jager et al. (1998), also observed successful hardening of *in vitro* plantlets in 2:1, sand: soil mix. In summary, clonal propagation of gladiolus (cv. 'Peach Blossom') through enhanced release of axillary buds has the potential to ensure large-scale production of desirable cultivars within a short time.

Table 2. Effect of plant growth substances on *in vitro* rooting of gladiolus

Treatments[1] (mg L^{-1})	Days for root initiation	Number of roots	Length of longest root (cm)
IBA 0.5	10.3	1.7	2.4
IBA 1	10.0	7.0	4.4
IBA 2	7.0	21.3	5.0
NAA 0.5	18.7	11.3	2.0
NAA 1	14.3	24.0	3.3
NAA 2	11.0	1.3	2.6
IAA 0.5	8.7	5.7	2.5
IAA 1	13.7	1.3	0.8
IAA 2	12.7	2.0	4.1
Control	24.7	1.3	0.5
CD (0.05)	6.40	11.23	3.96

[1]numerical values following the treatment codes are concentrations in mg L^{-1}

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