

IN VITRO PROPAGATION OF *GLADIOLUS ANATOLICUS* (BOISS.) STAPF

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Abstract

An *In vitro* propagation method was developed for *Gladiolus anatolicus* (Boiss.) Stapf (Iridaceae) using corm sections. Calli were obtained from longitudinally shaped corm sections in Murashige & Skoog medium supplemented with different concentrations of NAA and without growth regulators. The highest rate of callus formation (75 %) occurred in MS medium containing 8.5 mg l^{-1} NAA. Eight weeks after the culture was initiated, calli in 1 cm diameter were taken into regeneration experiments. In these experiments, shoot regeneration was promoted using NAA, BA only, and their combinations. The highest number of shoots per explant was obtained in MS medium containing 0.2 mg l^{-1} BA and 2 mg l^{-1} NAA (4.7 shoots per explant). Corm formation in the base of shoots were observed in the medium with 0.1 mg l^{-1} BA. Additionally, 5-6 cormel per shoot occurred during subculturing. Rooting was obtained in the same medium, but rooting rates in shoots were very low (% 20).

Introduction

The species of *Gladiolus* are of great horticultural value and are used in gardening and cut-flower production. It is also known that most geophytes have a very slow rate in propagation via seeds. *Micropropagation* and other *In vitro* techniques have been used for plants which present particular problems in conventional horticulture (Fay, 1992). The study of micropropagation on bulbous and cormous plants, have contributed to commercial production of geophytes and will continue to do so in the future (Ziv, 1997). Micropropagation of bulbous and cormous plant have significant advantages compared to conventional propagation methods. Micropropagation techniques increase multiplication rates (Novak & Petru, 1981; Takayama & Misawa, 1982; Takayama & Misawa, 1983; Van Aartrijk *et al.*, 1990). It has been also provided the materials free from viruses and other pathogens (Blom-Barnhoorn & Van Aartrijk, 1985, Van Aartrijk *et al.*, 1990).

Additionally, plant cells or tissue cultured *In vitro* are capable of regenerating whole plants affording biotechnology a novel method for mass propagation (Murashige, 1977). But, monocots have been more difficult to regenerate *In vitro* than dicots. The hormonal requirements for callus initiation and subsequent plant regeneration from the monocotyledonous bulb crops in the floral industry are largely unknown (Kamo, 1994). There are a number of reports pertaining to *In vitro* propagation of *Gladiolus* (Ziv *et al.*, 1970; Lilien-Kipnis & Kochba, 1987; Rao *et al.*, 1991; Dantu & Bhojwani, 1992; Sen & Sen, 1995).

Gladiolus anatolicus (Boiss.) Stapf, an endemic of Turkey is in VU category in Red Data Book of Turkey (Ekim *et al.*, 2000). There is no report on micropropagation of *Gladiolus anatolicus*. We reported an efficient method for plantlet regeneration from the callus using corm slices of *Gladiolus anatolicus* having great potential as a ornamental plant with its attractive flowers. This report might be helpful for improvement by mutation breeding, somaclonal variation and genetic engineering.

Materials and Methods

In this study, fresh corms of *Gladiolus anatolicus* were used as explant. The plants were collected from natural habitat before flowering period. For pre-treatment, the corms were cleaned in running tap water for 24 hour and outer scales were removed. Corms were surface-sterilized with 70% EtOH for 17 min (Ziv, 1989) and with 4.5% Sodiumhypochlorite for 20 min, consecutively, and washed three times with sterilized distilled water.

All the experiments were maintained on solidified basal medium. Basal medium contained Murashige & Skoog (Murashige & Skoog, 1962) mineral salts, 100 mg l⁻¹ myo-inositol, 2 mg l⁻¹ glisin, 0.5 mg l⁻¹ nicotinic acid, 0.5 mg l⁻¹ pyridoxine HCl, 0.1 mg l⁻¹ thiamine HCl, 30 g l⁻¹ sucrose, 8 g l⁻¹ agar and various concentrations of plant growth regulators. The pH of media were adjusted to 5.8 before autoclaving at 121°C for 15 min.

For callus cultures, sterilized corms were divided into two group. First group of corms was cut into 4 mm transverse slices, and the second group was cut into 4 mm longitudinal slices. Each corm slice was placed with a cut surface in contact with MS basal medium containing various concentration of NAA (2.5, 5.0, 7.5, 8.5 and 10.0 mg l⁻¹) or without growth regulators. Callus cultures were grown in the dark 24 ± 2°C and subcultured two times and subculturing was periodically carried out at 4 weeks intervals. The experiments were conducted with a minimum of four replicates consisting of five explants per flask and repeated three times. The data were subjected to analysis of variance and means were compared using Tukey HSD multiple-range test.

For shoot differentiation, approximately 1 cm callus was placed on regeneration medium. MS basal medium supplemented with BA (0.2 and 1 mg l⁻¹), NAA (1, 2 and 4 mg l⁻¹) and their combinations (BA: NAA, 0.2:1, 0.2:2, 0.2: 4, 1:1, 1:2 ve 1:4 mg l⁻¹) were used for plant regeneration.

The cultures were maintained at 24 ± 2°C with illumination provided by cool white florescent lamps at 40 µE m⁻²s⁻¹ with a 16-h light period. Five callus lumps were inoculated in each 250 ml flask, three flasks in each treatment. All experiments were repeated three times. The data were subjected to analysis of variance and means were compared using Tukey HSD multiple-range test.

The shoot proliferating stock was subcultured two times at 28 days intervals. Each shoot (4-6 cm) was separated from the stock and transferred on MS basal medium with BA (0.1, 0.5, 1 and 2 mg l⁻¹) or without growth regulators for corm induction.

After four weeks, the shoots with a basal corm were transferred on MS basal medium supplemented with 0.5 and 2.0 mg l⁻¹ NAA for root induction.

Results and Discussion

First group of explants (transversely sliced corms) become dark within first days of inoculation and died following the darkening (Fig. 1). Relatively large wound surface may be the cause death of explants and therefore, in a massive wounding reaction involving a possible oxidative stress-response (Halliwell & Gutteridge 1996). In this process free radicals are produced and peroxidative damage can occur, leading to activation of peroxidases, catalase and SOD (Lehsem, 1988, Olmos *et al.*, 1994). There was no darkening problem in second group of explants (longitudinally sliced corms) (Fig. 2). These corm slices formed callus within one month on MS basal medium supplemented with various concentrations of NAA. In all experiments auxin concentrations produced callus. The greatest amounts of callus was observed on MS

medium with 8.5 mg l^{-1} NAA. Although, the highest NAA concentration (10 mg l^{-1}) resulted in less callus production than concentration of 8.5 mg l^{-1} NAA where significantly more callus production was observed than other NAA levels (Table 1). For this reason, 8.5 mg l^{-1} of NAA concentration is optimum for maximum callus production.

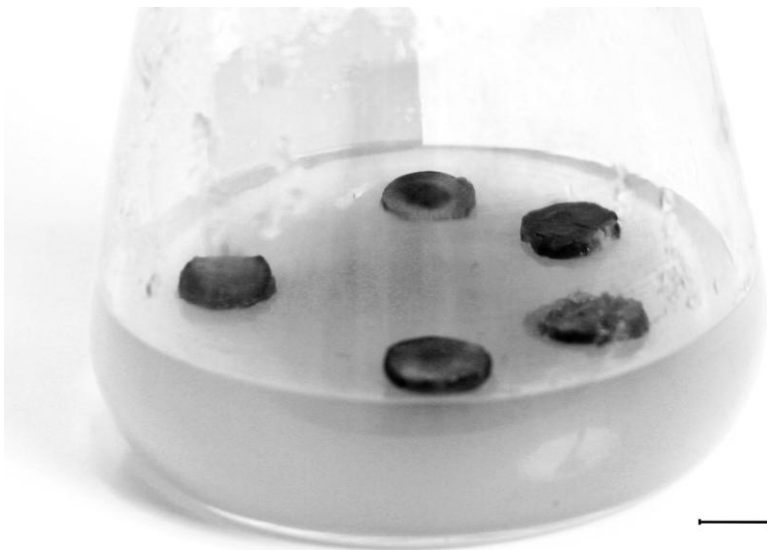


Fig. 1. Transversely sliced corm explants. Scale Bar: 1cm.

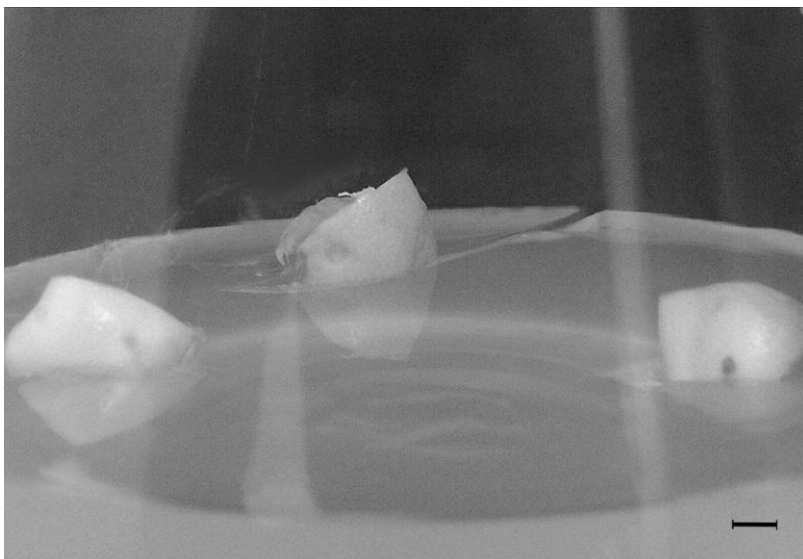


Fig. 2. Longitudinally sliced corm explants. Scale Bar: 0.2 cm.

Table 1. The effect of different NAA concentration in MS medium on callusing of longitudinally sliced corm segments.

NAA (mg l ⁻¹)	% callus formation rate (% ± SD)
0	17 ± 0.73 e
2.5	25 ± 0.92 de
5.0	33 ± 1.00 cd
7.5	41 ± 0.86 bc
8.5	75 ± 0.67 a
10.0	50 ± 0.78 b

Table 2. Effect of different BA and NAA combinations on shoot regeneration from callus.

Plant growth regulators (mg l ⁻¹)		Shoot number/ explant (Mean ± SD)
BA	NAA	
0.0	0.0	0.40 ± 0.54 hi
0.0	1.0	2.11 ± 0.83 c
0.0	2.0	1.40 ± 0.84 de
0.0	4.0	0.20 ± 0.40 i
0.2	0.0	0.60 ± 0.62 ghi
0.2	1.0	2.80 ± 0.92 b
0.2	2.0	4.71 ± 1.12 a
0.2	4.0	1.71 ± 0.97 cd
1.0	0.0	0.80 ± 0.73 fgh
1.0	1.0	1.11 ± 0.91 efg
1.0	2.0	1.20 ± 0.92 def
1.0	4.0	0.78 ± 0.70 fgh

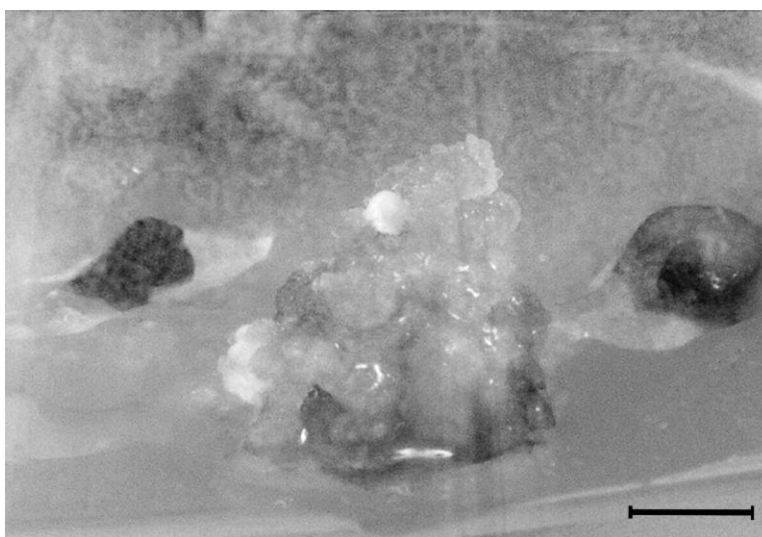


Fig. 3. Creamy-white callus from longitudinally sliced corm explants on MS medium containing 8.5 mg l⁻¹ NAA after 8 weeks from culture initiation. Scale bar: 0.5 cm



Fig. 4. Shoots from callus on MS medium containing 0.2 mg l^{-1} BA and 2 mg l^{-1} NAA Scale Bar: 2 cm.

Although, Kamo *et al.*, (1990) obtained compact calli in all concentration of NAA using various cultivars of *Gladiolus*, in our study, all experimented NAA concentrations were formed creamy-white and friable callus (Fig. 3).

Calli which were subcultured two times with four week intervals and then were taken onto regeneration media. Where NAA was used alone, number of shoots per explant decreased with increasing NAA amount (Table 2). Dantu & Bhojwani (1987) have reported BA as effective cytokinin for shoot multiplication. In our study, BA showed low regeneration rate. The highest number of shoots per explant was obtained in MS medium supplemented with 0.2 mg l^{-1} BA and 2 mg l^{-1} NAA (Fig. 4). These results are similar with the findings by Kumar *et al.*, (1999) working with *Gladiolus hybridus* Hort.

Shoot regeneration was observed at low frequencies from callus on MS media without growth regulators. Saunders & Shin (1986) reported a hormone-autonomous (habituated) callus which did not require plant growth regulators for growth and subsequent regeneration.

Elongated multiple shoots (4-6 cm) were transferred on MS basal medium with BA (0.1 , 1 and 2 mg l^{-1}) or without growth regulators for corm formation. Although Kumar *et al.*, (2002) reported corm formation on MS basal media without growth regulators, in our study, corm formation was only observed on MS media supplemented with 0.1 mg l^{-1} BA (Fig. 5). Optimum BA level is required for corm initiation may be dependent on endogenous cytokinin level. After four weeks, a part of shoots with corm were separated from stock cultures and taken onto media containing 0.5 and 2 mg l^{-1} NAA to promote rooting. But rooting were not observed in that medium. Another part of remaining shoots were transferred to media containing 0.1 mg l^{-1} BA for corm development. After two weeks, shoots rooted and formed 5-6 cormlets in this medium, (Fig. 6), but rooting rates in shoots were very low (% 20). It was interesting to note that rooting was stimulated by BA. This may be related to endogenous growth regulators levels of explant. Initial auxin level of explant was probability higher than cytokinin level and root formation was stimulated by high auxin level.



Fig. 5. Multiple shoots with corm on MS medium containing 0.1 mg l^{-1} BA. Scale Bar: 1 cm.

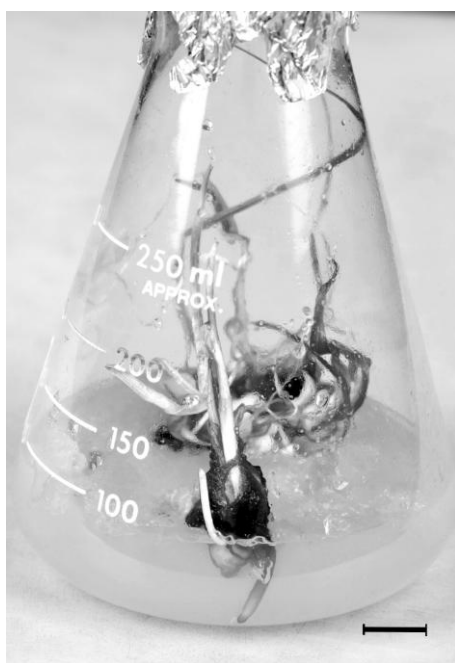


Fig. 6. Cormel formation and rooting on MS medium containing 0.1 mg l^{-1} BA. Scale Bar: 1 cm.

In this report, we report an efficient method for plantlet regeneration from callus using corm slices of *Gladiolus anatolicus* (Boiss.) Stapf., which report might be helpful for commercial propagation of contaminant free plant, new varieties and useful selections.

The experiments were conducted with four replicates consisting of five eksplant per flask and all experiments were repeated triplicate. The $(\%) \pm$ Standard deviation (SD) of three replicates. Values followed by different letters are significantly different at 0.05 probability level using Tukey's HSD multiple- range test.

The experiments were conducted with 3 replicates consisting of five eksplant per flask and all experiments were repeated triplicate. The Mean \pm Standard deviation (SD) of three replicates.

Values followed by different letters are significantly different at 0.05 probability level using Tukey's HSD Multiple- range test.

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