Propagation of *Gladiolus* by Somatic Embryogenesis

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Suspension callus was induced from gladiolus cormels after sprouting under aseptic conditions. Suspension callus was induced on a culture medium containing NAA with the best results obtained using a medium with NAA at 10 mg litre-1. A good rate of regeneration was obtained from suspension callus induced in culture medium containing 5 mg litre-1 NAA when regenerated in a hormone-free culture medium and also with suspension callus induced in a culture medium containing 10 mg litre-1 NAA and regenerated in a culture medium containing 0.1 mg litre-1 BA. Suspension callus was subcultured for more than a year and maintained its embryogenic ability.

INTRODUCTION

Gladiolus is an iridaceous plant grown for ornamental and cut flower uses. In the "old days" selective breeding was done by hobbyists. Now new plant cultivars are introduced by seed companies. Propagation by peduncle, lateral bud, cormel apex, a leaf piece, ovary, virus-free explants, and cormel cultivation has been reported (Ziv et al., 1970; Simonsen et al., 1971; Hussey, 1975; Hussey, 1977; Takatsu, 1982; Logan et al., 1985; Sutter, 1986; Dantu et al. 1987; De Bruyn and Ferreira 1992). However, regeneration by suspension callus has not been reported. We report on the development of a suspension callus micropropagation method of high regeneration ability with gladiolus. This programme is effective for the propagation of plants following induced somatic cell variation.

MATERIAL AND METHODS

Plant Material. Explants were obtained from cormels of *Gladiolus* 'Traveller'. The cormels were sterilized with 70% ethanol for several sec after the dry papery covering of the cormel was peeled away with tweezers. These were next sterilized in Antiformin solution of 1% chlorine for 20 min, and then washed in sterile water three times for 5 min each time. Explants were placed on Murashige and Skoog (MS) (1962) culture medium containing 3 g litre^{-1} sucrose and 2 g litre^{-1} Gelan Gum. The medium was adjusted to pH 5.8 before autoclaving. Cultures were incubated at 25C, and light was provided by warm white fluorescent tubes for 16 h daily at 4000 lux. Any cormel whose leaves grew 5 to 10 cm was used for suspension callus induction.

Induction of Suspension Callus. The suspension-callus induction medium consisted of MS basal salts and vitamins supplemented with 3 g litre^{-1} sucrose and 0, 5, 10, 15, or 20 mg litre^{-1} NAA. The medium was adjusted to pH 5.8 before autoclaving. For each cormel explant the leaf was cut 1 cm above the cormel and the cormel halved horizontally (Fig. 1) . The upper parts of the cut cormels were put in flasks containing 30 ml of medium with one explant per flask and incubated on

a shaker at 120 rpm at 25C. Light was provided by warm white fluorescent tubes for 16 h daily at 4000 lux. They were incubated for 8 weeks.

Regeneration Condition. Quantities of suspension callus tissue equivalent to 0.1 ml packed cell volume were placed uniformly on sterilized filter paper (55 mm in diameter). The medium consisted of MS basal salts and vitamins supplemented with 3 g litre^{-1} sucrose and 0 to 1.0 mg litre^{-1} BA. The medium was adjusted to pH 5.8 before autoclaving. The filter paper with suspension callus was placed on the medium in disposable petri dishes. These were incubated at 25C, and light was provided by warm white fluorescent tubes for 16 h daily (4000 lux).

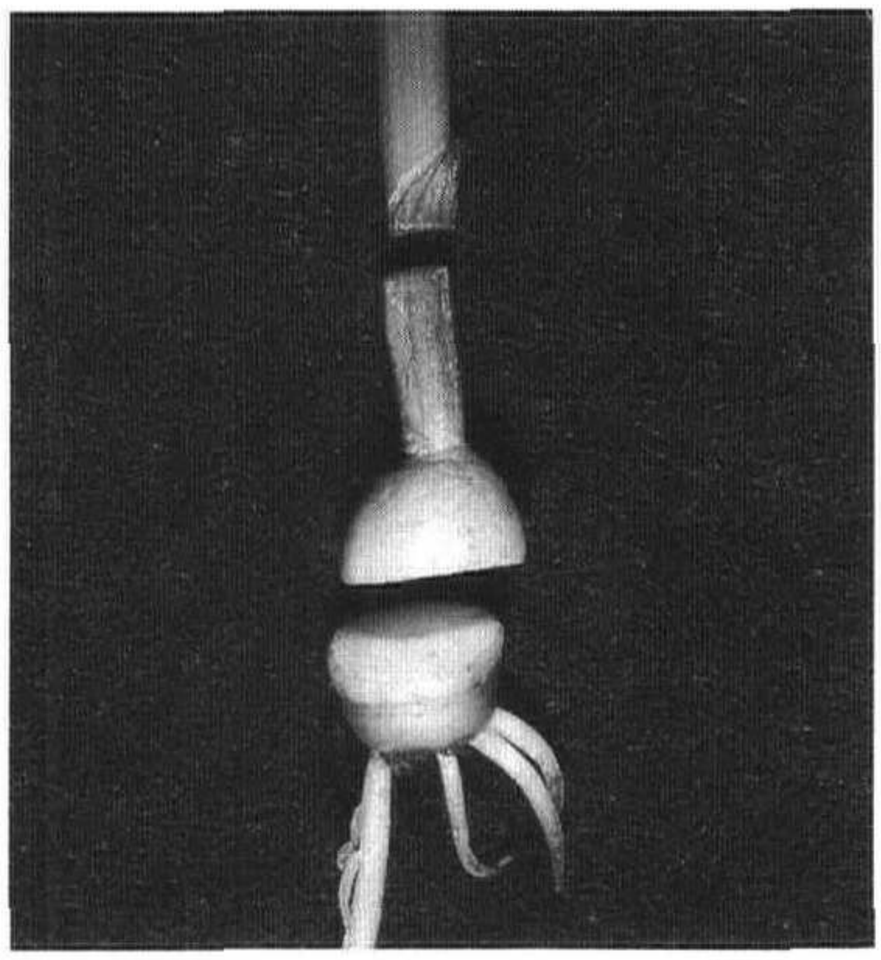

Figure 1. Explant preparation for callus induction.

Examination of Subculture. Suspension callus was washed in new induction medium. Flasks containing 30 ml of medium with 0.1 to 0.5 g of suspension callus were incubated on a shaker at 120 rpm at 25C. Light was provided by warm white fluorescent tubes for 16 h daily (4000 lux). These were investigated regularly for signs of increase.

RESULTS AND DISCUSSION

Suspension callus was induced at the apex of the cormel, at the junction of cormel and leaf (Fig. 2). The greatest amount of suspension callus induced was on the

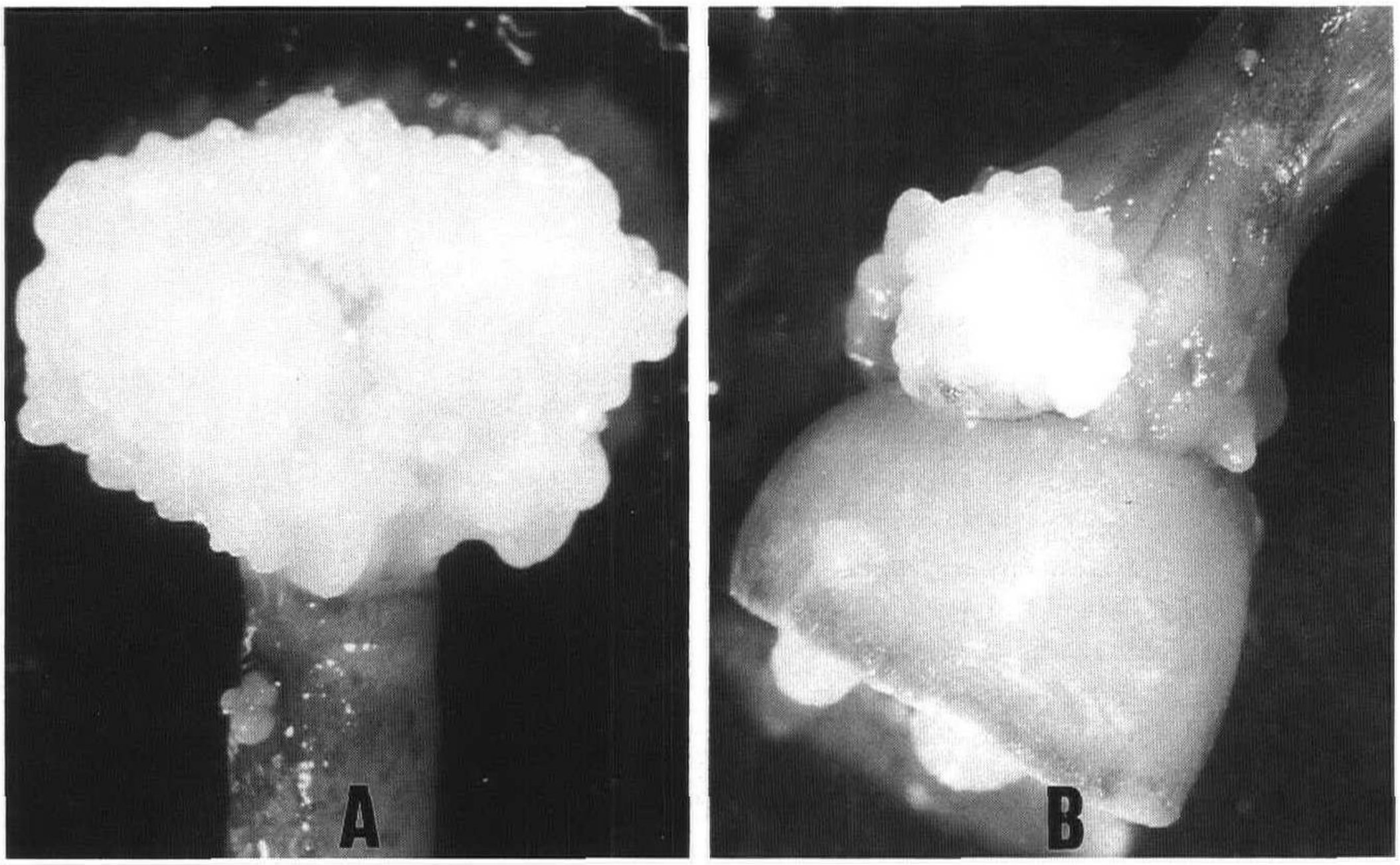

Figure 2. Development of callus on explant: (A) Callus induced in the region of the leaf; (B) Callus induction in the leaf stem and cormel region.

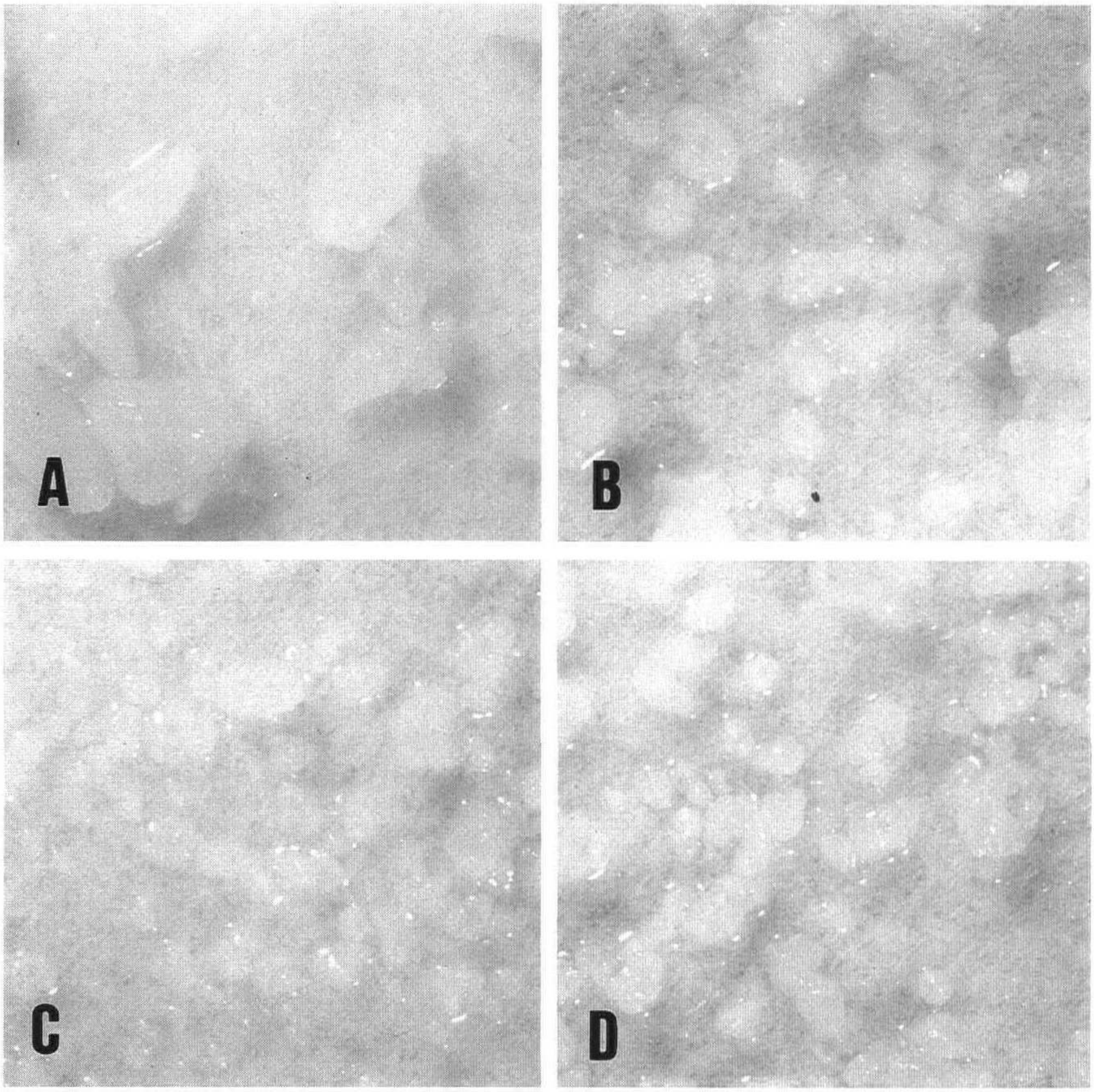

Figure 3. Suspension callus induced on media containing: (A) 5 mg litre^{-1} NAA; (B) 10 mg litre^{-1} NAA; (C) 15 mg litre^{-1} NAA; and (D) 20 mg litre^{-1} NAA.

medium with 10 mg litre^{-1} NAA (Table 1). The medium with 20 mg litre^{-1} NAA produced an uneven rate of increase. Suspension callus induced in the culture medium with 5 mg litre^{-1} NAA was largest in amount, with suspension callus induced in culture medium with 10 and 20 mg litre^{-1} NAA smaller (Table 1, Fig. 3). In the culture medium without added hormone, there was no suspension callus produced and rooting of the apical bud was noted. Suspension callus was regenerated in all regeneration culture medium. Regeneration was obtained through somatic embryos like those described by Stefaniak (1994) (Fig. 4). Good rates of regeneration of the suspension callus were obtained from the induction culture medium containing 5 mg litre^{-1} NAA and regenerated on medium with no hormone in the regeneration medium. Good rates were also achieved in the culture medium containing 10 mg litre^{-1} NAA and regeneration culture medium containing 0.1 mg litre^{-1} BA (Table 2). The rate of increase of suspension callus when 0.1 g was used as the cell mass produced the best results (Table 3). When a cell mass of 0.5 g was subcultured, it turned brown. Suspension callus was subcultured in this way for more than a year and maintained its regeneration ability.

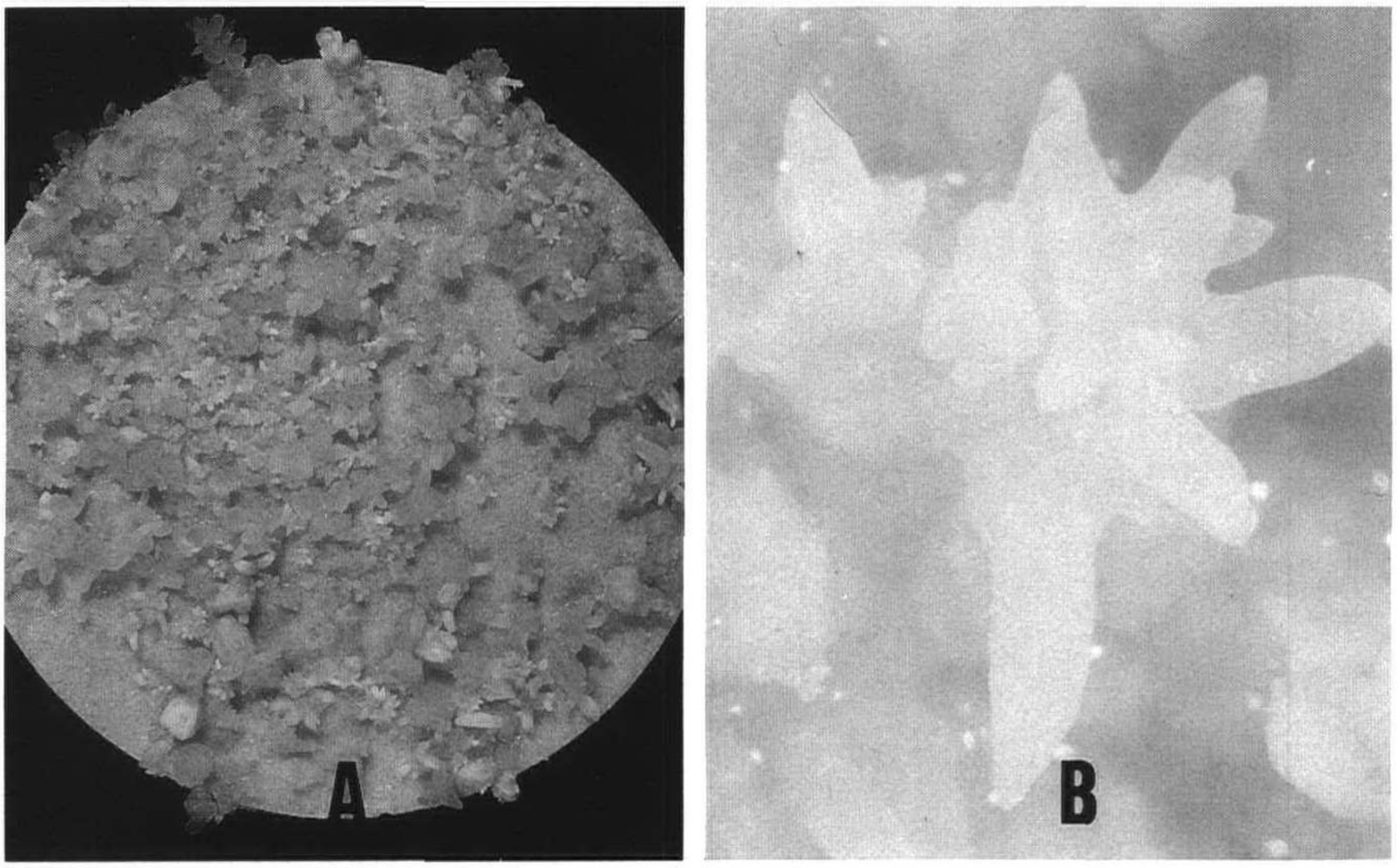

Figure 4. Regeneration of suspension callus: (A) After 3 weeks on regeneration culture medium; (B) Closeup of embryo formation.

Table 1. Effect of NAA concentration on induction of suspension callus from cormel explants[a].

NAA (mg litre^{-1})	Weight of suspension callus (g per flask)	Average size of suspension callus (mm)	Note
0	0.00	-	Rooting of apical bud
5	1.08	2.31	
10	1.49	0.87	
15	1.23	0.67	
20	0.75	0.99	

[a] 100 ml flask, medium 30 ml, 8 weeks after culture.

Table 2. The effect of NAA concentration in the induction medium and BA concentration of regeneration culture medium on somatic embryogenesis[a].

Induction medium NAA(mg litre^{-1})	Regeneration medium BA(mg litre^{-1})	Somatic embryos per g
5	0	224.1
	0.1	120.0
	0.5	102.0
	1.0	132.0
10	0	102.0
	0.1	198.0
	0.5	156.0
	1.0	111.0
15	0	88.9
	0.1	104.2
	0.5	110.9
	1.0	49.0
20	0	60.0
	0.1	90.0
	0.5	60.0
	1.0	60.0

[a] 3 weeks after culture on regeneration medium.

Table 3. Effect of suspension callus inoculation amount on suspension callus production 3 weeks after subculture[a].

Suspension callus inoculum (g)	Weight of suspension callus 3 weeks after subculture (g)	Increase rate (b/a)
0.1	1.61	161
0.3	2.97	99
0.5	3.40	68

[a] Inoculum from MS + 10 mg/l NAA, medium 30 ml per flask (100 ml), suspension callus induced from corm with MS +10 mg litre^{-1} NAA.

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