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In Vitro Morphogenesis through Leaf Explants of *Gypsophila paniculata* L.

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ABSTRACT

Callus cultures from leaf explants of *Gypsophila paniculata* L. cv. 'Bristol Fairy' have been tested their growth and morphogenic capacity on Murashige and Skoog medium supplemented with 0.1, 0.5, 1 and 3 ㎎/L 2,4-D. The frequency of callus formation ranged from 43.3% to 100%. The optimal 2,4-D concentration for promoting callus formation and growth was 0.5 to 3 ㎎/L. 4.2~5.6% of adventitious roots were obtained with the use of 0.1 and 0.5 ㎎/L 2,4-D. Calli grown well on 1.0 ㎎/L 2,4-D was the heaviest among the calli grown in various concentrations.

Key Words : Gypsophila, callus, tissue culture, 2,4-dichlorophenoxy acetic acid(2,4-D)

INTRODUCTION

Callus is initiated and maintained on nutrient media *in vitro*. It serves the dual purpose of study on plant growth and development and promotion for plant production and propagation. The formation of callus with an explant, an excised and isolated piece of tissue placed on nutrient medium makes the beginning of successful plant cell culture. Previous papers reported the successful micropagation from shoot tip of *Gypsophila paniculata*(Han et al., 1991, Hao et al., 1996). Somatic embryogenesis induced from mature somatic tissue is a desirable means of rapid vegetative propagation(Janick et al., 1989). However, the efficient system for callus production of this crop is still not available.

This study was carried out to examine the effects of 2,4-D concentration on callus formation from leaf explants of *Gypsophila paniculata* L. cv. 'Bristol Fairy'.

MATERIALS AND METHODS

Plant material

Plant materials used in this experiment were leaf explants(4~6 ㎜ long), obtained from *in vitro* regenerated plantlet through shoot tip culture of *Gypsophila paniculata* L. cv. 'Bristol Fairy'.

Callus induction medium

Callus induction medium(CIM) consisting of Murashige and Skoog(1962) salts, vitamins, 30 g/L sucrose, 2,4-D(0.1, 0.5, 1 and 3 ㎎/L) were prepared, and solidified with Gelrite 2 g/L after adjustment of pH

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to 5.8 . The media were autoclved for 15 min at 1.2kg/ cm^2, and then 30 *ml* was dispensed into petri dishes(ø 87 × 15 mm).

Culture conditions

The culture condition in the growth chamber was maintained at 25±2℃ air temperature, a 60 μmol · m^{-2} · s^{-1}, photosynthetic photon flux(PPF) with a 16 h photoperiod using white fluorescent lamps.

Determination of dry weight

Dry weight of callus were investigated on every three days after dry at 85℃.

Data analysis

The experiments were performed with ten replications per treatment. Mean values and the test criteria for Duncan's multiple range test were calculated, and the significant differences between mean values were determined.

RESULTS AND DISCUSSION

This study was carried out to examine the effects of various 2,4-D concentration of callus induction from leaf explants of *Gypsophila paniculata* L. cv. 'Bristol Fairy'. The number, percentage and length of callus and adventitious root formed from leaf explants were measured for four months after culture (Table 1). The frequency of callus formation per leaf explants ranged from 43.3 % to 100 % while adventitious root formation ranged from 4.2 % to 5.6 %. The callus length per leaf explants ranged from 6.0 mm to 11.2 mm while adventitious root length ranged from 8.8 mm to 26.2 mm.

The greatest production of callus on CIM was obtained from 1.0 mg/L 2,4-D treatments. However, the longest adventitious root length(26.2 mm) was induced by 0.1 mg/L 2,4-D. The frequency of callus formation was getting increase with increase of the 2,4-D concentration. But the frequency of adventitious root formation was getting decreased with increase of the 2,4-D concentration. At higher concentrations, 2,4-D stimulated excessive growth of callus and inhibited adventitious shoot formation.

The synthetic auxin 2,4-D concentrations equal or greater than 0.5 mg/L promoted callus formation in *Digitalis obscura* L. and *Spinacia oleracea* L. cv. Fall Green leaf cultures(Pérez-Berm dezúet. al., 1984; Al-Khayri et al., 1991). In the same way, we observed that 0.5～3.0 mg/L 2,4-D promoted the callus formation. The calli were induced from leaf explants on MS medium with all concentrations of 2,4-D tested, with 0.5～3.0 mg /L giving high frequency of callus formation(Figure 1).

The fresh weight, dry weight, and percentage of dry matter of callus were investigated on four months after leaf explants culture(Table 2). The fresh weight of callus per leaf explants ranged from 81.3 mg to 364.6 mg while dry weight of that ranged from 54.9 mg to 181.9 mg. Dry matter rate of calli ranged from 44.1% to 67.4%. The greatest callus weight of fresh and dry was obtained on MS medium supplemented with 1.0 mg/L

Table 1. Effect of 2,4-D on callus and adventitious root formation from the leaf explants after 4 months of culture

2,4-D	No. of	Callus formation			Adventitious root		
(mg/L)	explants	No.	%	Length(mm)	No.	%	Length(mm)
0.1	90	39	43.3	6.0b[z]	5	5.6	26.2a
0.5	120	113	94.2	10.5a	4	4.2	8.8b
1.0	110	105	95.5	10.5a	0	0	0c
3.0	90	90	100.0	11.2a	0	0	0c

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

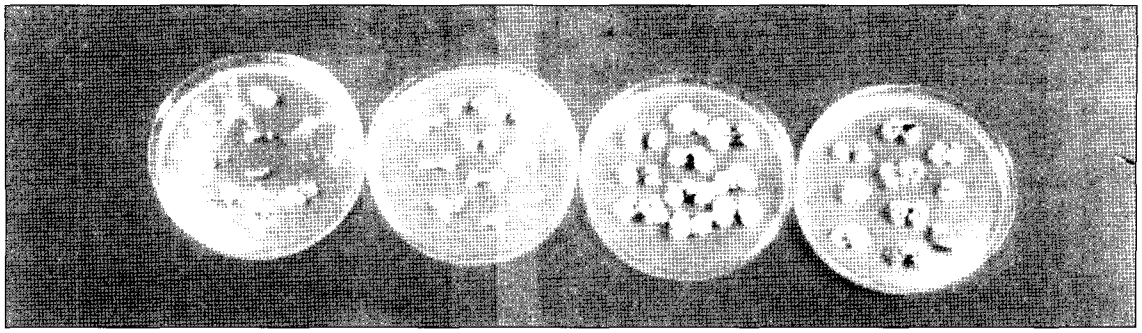

Fig 1. Four-month-old compact calli from leaf explants of *Gypsophila paniculata* L. cv. 'Bristol Fairy' on MS medium supplemented with 0.1, 0.5, 1.0 and 3.0 mg/L 2,4-D (left → right).

2,4-D. However, the highest rate of dry matter was induced on MS supplemented with 0.1 mg/L 2,4-D. We found that supplement of 0.1 mg/L 2,4-D may not be suitable for *Gypsophila paniculata* L. cv. 'Bristol Fairy' because of its inhibitory effect on callus formation, but it is helpful to the growth of adventitious roots.

Compact calli were induced from leaf explants on MS medium supplemented with 0.1, 0.5, 1.0 and 3.0 mg /L of 2,4-D. Callus has been maintained on MS medium with 1.0 mg/L of 2,4-D more than one year.

Table 2. Comparison of dry matters with various 2,4-D concentrations on the callus formation of *Gypsophila paniculata* L. cv. 'Bristol Fairy' after 4 months of culture

2,4-D	Callus weight (mg)		Dry matter
(mg/L)	Fresh	Dry	(%)
0.1	81.3b[z]	54.9b	67.4
0.5	349.1a	153.8a	44.1
1.0	364.6a	181.9a	49.9
3.0	336.7a	176.5a	52.4

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

The compact calli were not showed ability to regenerate into plantlets(data not shown). Further research should be required to understand various factors associated with the plant regeneration through embryogenic callus in *Gypsophila paniculata* L.

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