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Rapid Propagation of *Pelagonium Inquinans* Via Organogenesis from Mature Leaf Explants

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ABSTRACTS : A method for plant regeneration via organogenesis from *Pelagonium inquinans* leaf disc has been developed. Mature leaf explants were collected from field-grown plants and used for the induction of adventitious shoot regeneration on Murashige and Skoog (MS) medium supplemented with 3% (w/v) sucrose plus plant growth regulators. Maximum shoot organogenesis, with 11.8 ± 1.5 shoots (98.6%) per leaf disc, was obtained with 2 ㎎/ℓ N^6-benzyladenine (BA) and 0.5 ㎎/ℓ α-naphthyleneacetic acid (NAA) in 30 days. For rooting, the *in vitro* proliferated and elongated shoots were excised into 1.5-2 ㎝ in length microcutting, which were plated individually on an half-strength MS (1/2MS) medium supplemented with 2% (w/v) sucrose plus various concentrations of indole-3-butyric acid (IBA). Shoots rooted with a frequency of 100% following culture on 1/2MS medium containing 0.5 ㎎/ℓ IBA.

Key words : *Pelagonium inquinans*, mature leaf, organogenesis, plant regeneration

INTRODUCTION

Pelagonium inquinans, belonging to the family *Geraniaceae*, is a commercially important essential oil yielding aromatic and medicinal plant. It is an excellent relaxant for nervous tension, colds, bronchitis, larynigitis, PMT and menopausal problems (Lis-Balchin, 1996). It is propagated vegetatively through rooted terminal-stem cuttings. However, the transplanted cuttings have a characteristic initial slow growth and are susceptible to weed competition during this lag phase leading to yield losses (Rajeswara Rao and Bhattacharya, 1997).

Plant tissue regenerated shoot buds or embryos *in vitro* either directly from the explant or indirectly through callus phase (George and Sherrington, 1984). *In vitro* organogenesis in seedling and adult tissue in different plant species was principally controlled by plant growth regulators and other physiochemical factors (Doerschug and Miller, 1967; Webb and Torres, 1984) and systematic manipulation of conditions for differentiation in callus and cell cultures of various plant species (Sharma *et al.*, 1990; Bonneau *et al.*, 1994). In general, advances in the induction of organogenesis in *Pelagonium* spp. have been slow since the the requirements for the type and concentration of plant growth regulators used to induced regeneartion varies widely with the cultuvar and species. A variety of factors have been investigated for induction of shoot organogenesis in *Pelagonium* spp. Somatic embryogenesis in *Pelagonium* spp. was obtained from hypocotyls (Marsolais *et al.*, 1991; Visser-Tenyenhuis *et al.*, 1994; Wilson *et al.*, 1996; Hutchinson *et al.*, 1997; Senaratna *et al.*, 1999) or hypocotyls and cotylrdons (Murthy *et al.*, 1996). Shoot regeneration of *Pelagonium* spp. was also obtained using hypocotyls and cotyledons of seedling (Chang *et al.*, 1996) and genetic transformation was carried out using the same explants (Robichon *et al.*, 1995; KrishnaRaj *et al.*, 1997; Bi *et al.*, 1999). Agarwal and Ranu (2000), used mature explants of *Pelagonium x hortorum* and found a higher regeneration ability of petiole compared to leaves. The most of plant regeneration in *Pelagonium* spp. has been reported from very young explants. However, little information was available on organogenesis and plant regeneration of *Pelagonium* spp. using mature explants.

The present report described a procedure for high-efficiency shoot regeneration via organogenesis from mature leaf explants of mature *P. inquinans* plants.

MATERIALS AND METHODS

Plant material and medium preparation

Shoot cultures were established by culturing leaf explants obtained from 1-year-old plant of *Pelagonium inquinans*. The excised leaf with petiole were surface sterilized in 70% (v/v) ethanol for 5 min followed by 2% (v/v) mercuric chloride for 5 min. They were rinsed five times in sterile distilled water with 3 min duration each. The basal medium consisted of Murashige and Skoog (1962) medium supplemented with 3%

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(w/v) sucrose, 0.3% (w/v) phytagel (Sigma-Aldrich, USA). Medium was adjusted to pH 5.8 with 1N NaOH prior to autoclaving at 121 ℃ for 20 min. The different combinations of auxins and cytokinins as filter sterilized solutions were added to media after autoclaving and media were dispensed as 30 ㎖ aliquots per 90×20 mm petri-dish (SPL Co., Korea).

Adventitious shoots differentiation

Leaf discs (1.0 ㎝ diameter) were aseptically excised from sterilized leaf and cultured on basal MS medium supplemented with cytokinin, N^6-benzyladenine (BA), or kinetin, and auxin, α-naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), or indole-3-actic acid (IAA), individually or in combinations, plus 3% (w/v) sucrose. All cultures were maintained at 25 ± 1 ℃ under a 16h photoperiod provided by cool-white fluorescent tubes, at a light intensity of 50 μmol $m^{-2}s^{-1}$. The same conditions were used for all experiments. There were 20 explants in each treatment, and each treatment was replicated three times.

Rooting of shoot

Healthy single shoots (1.5-2 ㎝) were excised from multiple shoot cultures and cultured in different concentrations of indole-3-butyric acid (IBA; 0.1, 0.5, 1.0, and 2.0 ㎎/ℓ, respectively) along with a control (no auxin) for rooting. The auxins were included individually on 1/2MS medium containing 2% (w/v) sucrose (pH 5.8). Data recorded were for the percentage of rooting after 4 weeks of culture.

RESULTS AND DISCUSSION

Leaf explants cultured on MS medium with different combinations of auxin and cytokinin demonstrated adventitious shoots regeneration after 6 weeks of incubation. Contamination with microorganisms (bacteria and/or fungi) was very low (less than 1% of the explants). Shoot organogenesis was promoted only in the presence of both exogenous cytokinin and auxin. Of the combinations, MS medium supplemented with 2 ㎎/ℓ BA and 0.5 ㎎/ℓ NAA was the most effective, providing shoot regeneration for 98.6% of explants associated with a high number of shoots per explant (11.8 ± 1.5 mean shoots per explant) (Table 1). Explants cultured on this medium for 2 weeks formed callus at the cut surface, and after 3 weeks the callus began to produce multiple shoot primordia, which developed into adventitious shoot (Fig. 1, 2, 3, and 4). However, the other combinations of cytokinin/auxin failed to induced shoot buds in *P. inquinans* leaf exlants. In previous reports, plant regeneration from petiole explants of ivy *Pelagonium* was achieved by using TDZ and NAA (Robichon *et al.*, 1997), whereas, in the present study, BA in combination with NAA was most effective in inducing adventitious shoot buds from mature leaf explants. This difference could be a reflection of probable difference of tissue sensitivities to plant growth regulators or genotype.

Table 1. Effects of cytokinin/auxin on adventitious shoots formation from mature leaf explants of *P. inquinans*.

Plant growth regulators (㎎/ℓ)			No. of explants	% of explants producing shoot	No. of shoots per explant (Mean ± S.E)
Kinetin	1	2,4-D	0.5	20	0.18	0.7 ± 0.5
			1.0	20	3.41	1.2 ± 0.5
	2	2,4-D	0.5	20	C***	0.0 ± 0.0
			1.0	20	C***	0.0 ± 0.0
Kinetin	1	IAA	0.5	20	ND**	0.0 ± 0.0
			1.0	20	ND**	0.0 ± 0.0
	2	IAA	0.5	20	ND**	0.0 ± 0.0
			1.0	20	ND**	0.0 ± 0.0
Kinetin	1	NAA	0.5	20	C***	0.0 ± 0.0
			1.0	20	C***	0.0 ± 0.0
	2	NAA	0.5	20	C***	0.0 ± 0.0
			1.0	20	C***	0.0 ± 0.0
BA	1	2,4-D	0.5	20	ND**	0.0 ± 0.0
			1.0	20	ND**	0.0 ± 0.0
	2	2,4-D	0.5	20	ND**	0.0 ± 0.0
			1.0	20	ND**	0.0 ± 0.0
BA	1	IAA	0.5	20	C***	0.0 ± 0.0
			1.0	20	R*	0.0 ± 0.0
	2	IAA	0.5	20	C***	0.0 ± 0.0
			1.0	20	R*	0.0 ± 0.0
BA	1	NAA	0.5	20	61.3	2.3 ± 1.4
			1.0	20	48.7	3.7 ± 1.3
	2	NAA	0.5	20	98.6	11.8 ± 1.5
			1.0	20	73.1	6.4 ± 1.5

Data were collected after 6 weeks in culture.
Explants were performed three times.
*R, Adventitious root differentiation; **ND, not detected; ***C, Callusing.

In general, the balance of auxin to cytokinin is a determining morphogenic factor. Report of auxin and cytokinin combinations supporting organogenic differentiation in other species have been well documented for several species (Lisowska and Wysonkinsta, 2000; Vengadesan *et al.*, 2000; Khanam *et al.*, 2000; Makunga *et al.*, 2005). Results presented here were in

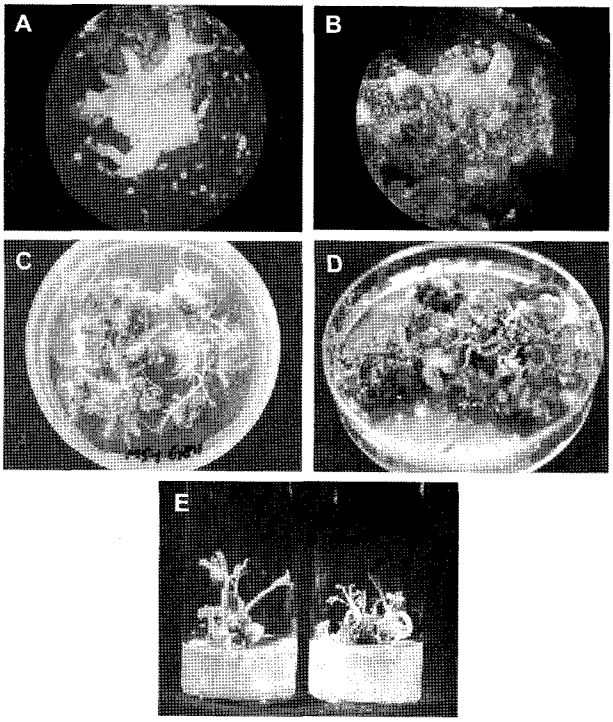

Fig. 1. Shoot organogenesis from mature leaf explants of *Pelagonium inquinans*. (a) Induction of adventitious shoot buds. (b) Growth of adventitious shoots. (c) Elongation of adventitious shoots. (d) Proliferation of adventitious shoots. (e) Regenerated plantlets.

agreement with these studies, where it was observed that low concentrations of NAA in combination with BA were successful for the induction of callus and differentiation of adventitious shoots from such callus.

All shoots longer than 1.5-2 ㎝ were transferred to an half-strength MS (1/2MS) media supplemented with various concentrations of IBA. Root initial formed 2 weeks which develpoed good root system within 4 weeks of culture. Low concentration of salts triggered root induction compared to that of higher concentrations (data not shown). Low ionic strength medium like 1/2MS was frequently used for *in vitro* rooting (Drake *et al.*, 1997; Vengadesan *et al.*, 2000; Schween and Schwenkel, 2002). This may be due to the need for only a small amount of total nitrogen for rooting (George and Sherrington, 1984). The highest percentage of elongated shoots forming roots (100%) was induced at concentration of 0.5 ㎎/ℓ IBA (Table 2). Among the auxins tested, IBA was more effective than IAA for *in vitro* root induction and proliferation of shoot in *P. inquinans* (data not shown). Earlier studies supported this conjecture in *Albizia procera* (Swamy *et al.*, 2004) and *Morus alba* (Kathiravan *et al.*, 1997). IBA is more resistant to catabolism, including peroxidase oxidation, where as

Table 2. Effects of auxin concentrations on adventitious rooting of *in vitro* regenerated shoots of *P. inquinans*.

IBA (㎎/ℓ)	No. of shoot inoculated	Rooting frequency (%)
0.0	24	13.1
0.1	24	43.7
0.5	25	100
1.0	24	67.3
2.0	25	8.3C*

Data were collected after 4 weeks in culture.
Explants were performed three times.
*C, Callusing

IAA rapidly undergoes photodegradation and forms IAA oxidase, which inhibits the formation of roots. The study also showed that higher concentration of IBA inhibited rooting and encouraged callus formation at the base of the shoot. These were in agreement with Kumar *et al.* (1998) and Kathiravan *et al.* (1997).

These results demonstrated that mature leaves of *P. inquinans* had a great organogenic potential for shoot formation, however the response is highly sensitive and directly resulted to the combinations of exogenouse growth regulators in the culture medium. The system described here provided an efficient method for adventitious shoot regeneration from leaf explants of *P. inquinans* that would be useful for both genetic transformation studies and for mass propagation of elite ornamental or chemotype selections of this medicinal plant species.

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