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Comparison of Adventitious Shoot Formation in Petiole Explant Cultures of 20 Cultivars of *Catharanthus roseus*

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Abstract

Petiole explants from 20 cultivars of *Catharanthus roseus* were cultured on various shoot-inducing media to assess their competence for adventitious shoot formation. After eight weeks of culture on Murashige and Skoog's medium supplemented with 4.4 μM 6-benzyladenine and 0.5 μM α-naphthaleneacetic acid, petiole explants from 'Cooler Icy Pink' exhibited the greatest frequency of adventitious shoot formation at 40%, which was followed by 'Little Bright Eye'. By comparing with a previous study on assessment of competence for adventitious shoot formation in hypocotyl explant cultures of various cultivars, it is indicated that the relative degree of their competence among cultivars varies to the organ used for the source of explant. Excised adventitious shoots were readily rooted on half-strength MS basal medium. Regenerated plantlets were successfully transplanted to potting soil and grown to maturity in a greenhouse.

Key words: Apocynaceae, indole alkakoids, Madagascar periwinkle, organogenesis

Introduction

Catharanthus roseus (Madagascar periwinkle) is a tropical and subtropical plant belonging to the family Apocynaceae. The plant has drawn attention due to the production of useful alkaloid compounds such as vinblastine and vincristine, which are used for blood cancer treatment (Lounasmaa and Galambos 1989).

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The establishment of a high frequency plant regeneration system in a species of interest may lead to reliable genetic transformation. Plant regeneration from leaf explant-derived callus via organogenesis has been reported in *C. roseus* (Constabel et al. 1982). However, the frequency of plant regeneration is too low for practical genetic transformation. We previously established an alternative plant regeneration system based on somatic embryogenesis in this species. Anther-derived embryogenic calluses yielded cell suspension cultures with a high competence for plant regeneration (Kim et al. 1994). However, in a preliminary experiment, we failed to regenerate somatic embryos into plantlets that were developed from *Agrobaterium*-mediated-transformed suspension cultures of *C. roseus*. Therefore, an attempt was made to develop a high frequency plant regeneration system via adventitious shoot formation for subsequent use with genetic transformation. Previously, we also assessed competence for adventitious shoot formation in hypocotyl explant cultures of 20 cultivars of *C. roseus* (Choi et al. 2002). Three cultivars exhibited greater competence than the other cultivars. However, we have raised a question as to whether the same three cultivars will exhibit greater competence for adventitious shoot formation in tissue cultures of other sources of explant such as petiole. In this study, petiole explants from 20 cultivars of *C. roseus* were cultured to compare their competence for adventitious shoot formation.

Materials and Methods

Plant materials

Seeds of 20 cultivars of *Catharanthus roseus* (L.) G. Don (Table 1) were purchased from companies of AustraHort Pty

Table 1. Frequency (%) of adventitious shoot formation and number of adventitious shoots per explant in petiole explant cultures of *C. roseus* [a, b].

Cultivar	F/N[c]	ZI	BN	TI	BI
Cascade Appleblossom	F	0	0	0	16.7±14.4
	N	0	0	0	0.3±0.3
Cooler Coconut	F	0	13.3±23.1	6.7±11.5	0
	N	0	0.5±0.8	0.2±0.3	0
Cooler Grape	F	0	0	6.7±11.5	0
	N	0	0	0.1±0.1	0
Cooler Icy Pink	F	0	40.0±20.0	0	0
	N	0	1.5±1.3	0	0
Cooler Orchid	F	0	6.7±11.5	0	0
	N	0	0.1±0.1	0	0
Cooler Strawberry	F	0	6.7±11.5	0	0
	N	0	0.2±0.3	0	0
Little Bright Eye	F	0	28.3±10.4	0	0
	N	0	0.8±0.7	0	0
Stardust Mix	F	13.3±11.5	6.7±11.5	0	0
	N	0.9±0.8	0.1±0.2	0	0

[a]Data from 'Cooler Mix Improved', 'Cooler Peppermint', 'Cooler Pink', 'Cooler Raspberry Red', 'Cooler Red', 'Cooler Rose', 'Ecuata Grape', 'Little Linda', 'Pacifica Red', 'Raspberry Cooler', 'Stardust Orchid', and 'Stardust Pink' were not listed because none of them produced adventitious shoots.

[b]Data were collected after eight weeks of culture. Each treatment consisted of 10 explants per dish with three replicates. MS medium supplemented with 14.5 μM zeatin and 2.4 μM IBA (ZI); 4.4 μM BA and 0.5 μM NAA (BN); 14.5 μM thidiazuron and 2.4 μM IBA (TI); or 14.2 μM BA and 2.4M μM IBA (BI) was used.

[c]F/N: Frequency of adventitious shoot formation (±SD); N: Number of adventitious shoots per explant (±SD).

(Australia), Ball Seed (USA), Sakata Seed (Japan), and Takii Seed (Japan). Seeds were surface-sterilized, in 70% (v/v) ethanol for 30 sec followed by a 0.4% (v/v) sodium hypochlorite solution for 20 min with occasional agitation. They were then rinsed four times with sterile distilled water. After surface-sterilization, seeds were placed onto half-strength Murashige and Skoog (MS) (1962) basal medium for germination. Approximately, 5-mm-long petiole explants were excised from 8-week-old seedlings and used as explants.

Culture medium and culture conditions

The basal medium used throughout the experiments consisted of MS inorganic salts, 100 mg/L myo-inositol, 0.4 mg/L thiamine HCl, 3% (w/v) sucrose, and 4 g/L Gelrite. The pH of all media was adjusted to 5.8 before autoclaving. Twenty-five mL of medium was dispensed into plastic Petri dishes (87 × 15 mm). Unless mentioned otherwise, all cultures were maintained under light (approximately 3 W/m^2 from cool-white fluorescent lamps with 16-h photoperiods) at 25°C.

Induction of adventitious shoots

To induce adventitious shoots, hypocotyl explants were placed onto MS medium supplemented with 14.5 μM zeatin and 2.4 μM indole-3-butyric acid (IBA), 4.4 μM 6-benzyladenine (BA) and 0.5 μM α-naphthaleneacetic acid (NAA), 14.5 μM thidiazuron and 2.4 μM IBA, or 14.2 μM BA and 2.4 μM IBA. Each treatment consisted of 5 explants per dish with three replicates. After eight weeks of culture, the numbers of explants producing adventitious shoots and adventitious shoots produced per explant were determined to assess competence for adventitious shoot formation. Adventitious shoots formed on explants were excised with a scalpel and transferred to a rooting medium (half-strength MS basal medium). Regenerated plantlets were subjected to acclimation, transplanted to potting soil, then maintained in a greenhouse.

Results and Discussion

After two weeks of culture, most petiole explants formed calluses at the cut edges (Figure 1A). A few petiole explants produced adventitious buds after four weeks of culture. The buds appeared to be derived directly from either the epidermal layer of the explants or from intervening calluses (Figure 1B). Adventitious shoots were elongated after five to eight weeks of culture (Figure 1C, D). After eight weeks of culture at 4.4 μM BA and 0.5 μM NAA, petiole explants from 'Cooler Icy Pink' exhibited the greatest frequency of adventitious shoot formation at 40%. Lower frequencies were obtained from 'Little Bright Eye' (Table 1). Adventitious shoots were readily rooted in a rooting medium at a frequency of 100% (Figure 1E). Regenerated plantlets were grown to maturity in a greenhouse (Figure 1F).

In this study and our previous study (Choi et al. 2003), competence for adventitious shoot formation was cultivar/genopype-dependent. In our previous study (Choi et al. 2003), 'Cooler Raspberry Red', 'Cooler Orchid', and 'Cooler Treated' exhibited the greatest frequencies of adventitious shoot formation in hypocotyl explant cultures. However, 'Cooler Icy Pink' and 'Little Bright Eye' were two of the cultivars that exhibited the greatest frequencies of adventitious shoot formation when petiole explants were cultured in this study. Comparison of these results with those of our previous study indicates that the relative degree of their competence among various cultivars varies to the organ used for the source of explant. In an extreme instance, 'Little Bright Eye' exhibited one of the greatest fre-

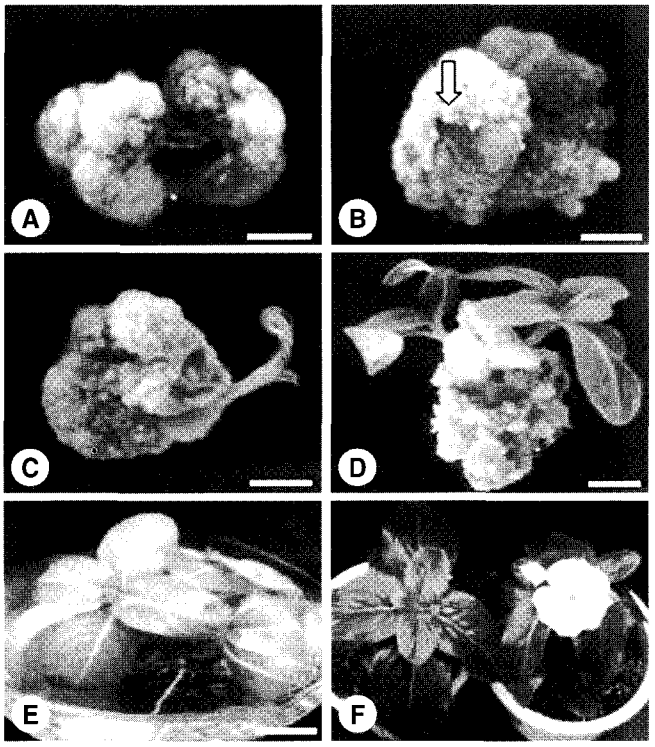

Figure 1. Adventitious shoot formation and plant regeneration in tissue cultures of *C. roseus*. A: Petiole explants after two weeks of culture; B: Adventitious bud (arrow) formation on petiole-derived callus after four weeks of culture; C, D: Adventitious shoots formed on petiole explant; E: Plantlet developed from adventitious shoot; F: Regenerated plant with flowers. Bars indicate 5 mm (A, B, C, D) and 10 mm (E), respectively.

quencies of adventitious shoot formation in petiole explant cultures of 20 cultivars in this study, but it ranked lowest frequency in hypocotyl explant cultures of our previous study. In other words, cultivars with the greatest frequencies of adventitious shoot formation in petiole explant cultures observed in this study ('Cooler Icy Pink' and 'Little Bright Eye') are different from those with the greatest frequencies of adventitious shoot formation in hypocotyl explant cultures of our previous study ('Cooler Raspberry Red', 'Cooler Orchid', and 'Cooler Treated'). It remains to be determined why the same cultivar that has greater competence for adventitious shoot formation in an organ for the source of explant than other cultivars that has lower competence in another organ.

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