In Vitro Propagation of Pandoreas

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Abstract. Pandoreas, Australian natives of horticultural significance, were successfully propagated using tissue culture. A protocol for rapid in vitro multiplication of commercial cultivars was developed using nodal segments cultured on Murashige and Skoog medium containing either BA or kinetin. Maximum shoot induction and number of shoots per explant for *P. pandorana* (Andrews) Steenis and *P. jasminoides* (Lindley) Schumann were on 8.8 μM BA and 4.6 μM kinetin. Higher levels of cytokinin in the medium inhibited shoot formation. Tissue-cultured shoots were rooted with IBA. This study demonstrates that Pandoreas can be successfully micropropagated. Chemical names used: 6-benzylaminopurine (BA); 3-indole butyric acid (IBA).

The genus *Pandorea* (family Bignoniaceae) consists of six species of ornamental plants. *Pandorea jasminoides*, *P. pandorana*, *P. baileyana* (Maiden & E. Betcher) Steenis, and *P. nervosa* Steenis are endemic to Australia (van Steenis, 1977); *P. jasminoides* and *P. pandorana* are commercially grown for their aesthetically appealing flowers and green foliage (Stewart, 1995); and *P. jasminoides* is highly valued for its large flowers and long flowering period, which extends through the summer when many other plants are not flowering. The flowers of *P. jasminoides* are either purple or white, whereas *P. pandorana* possesses horticulturally desirable polymorphism, including different growth forms, foliage, and flower color. The flowers of *P. pandorana* range in color from white, pink and yellow to brown, but the flowers are small. Popular commercial cultivars of *P. jasminoides* include ‘Lady Di’, ‘Bower of Beauty’, and ‘Charisma’, while important *P. pandorana* cultivars are ‘Golden Shower’, ‘Lemon Bells’, and ‘Ruby Belle’.

The ornamental plant industry relies on the introduction of new products with novel attributes. Pandoreas have significant genetic potential as a resource for the production of new cultivars for the local and the international ornamental plant industry. Evaluation of genetic diversity and genome fingerprinting of *Pandorea* by random amplification of polymorphic DNA (RAPD) and inter-simple sequence repeat (inter-SSR) polymerase chain

reaction (PCR) showed that taking advantage of existing genetic variability of *P. pandorana* to enhance the narrow genetic base of *P. jasminoides* by interspecific crosses should be possible (Jain et al., 1999). Such crosses would have the potential to generate novel, commercially important, elite cultivars of pandoreas. Our ongoing breeding program has indicated that it is possible to obtain interspecific hybrids of pandoreas.

Pandoreas are propagated by shoot-tip cuttings (Stewart, 1995). Micropropagation would facilitate the introduction of a new cultivar. The objective of this study was to establish a rapid micropropagation system for commercial cultivars of pandoreas.

Materials and Methods

Plant material. *Pandorea pandorana* cv. Golden Shower and *P. jasminoides* cv. Lady Di were used for these studies. The plants were obtained commercially from a local nursery and maintained under glasshouse conditions with a day temperature of $20 \pm 3^\circ\text{C}$ and night temperature of $16 \pm 3^\circ\text{C}$.

Explants and surface sterilization. Shoot segments (one node cuttings of *P. pandorana*; two to three node cuttings of *P. jasminoides*) were used as explants. Leaves were removed and explants were washed first under running tap water for 5 min before transferring to a 0.1% sodium dodecyl sulphate (SDS) solution and agitating vigorously for 10 min. After rinsing five to six times with distilled water, the explants were surface sterilized using 1% sodium hypochlorite for 45 to 50 min, followed by five to six rinses in sterile distilled water.

Tissue culture medium and conditions. Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 2% sucrose was used for tissue culture. The medium was gelled with 0.6% agar (A1296; Sigma Co., Castle Hill, NSW, Australia) and the pH was adjusted to 5.8 prior to autoclaving at 121°C for 20 min. The explants (five/cup) were cul-

tured in 240-mL sterile, transparent, disposable plastic cups (Polarcup Australia, Melbourne, Victoria, Australia) containing ≈ 40 mL of medium. The cultures were incubated under $40\text{--}50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light provided by OSRAMTM wattsaver cool-white tube lamps (Sherriff electrical, W. Melbourne, Victoria, Australia) at a photoperiod of 16 h at $25 \pm 2^\circ\text{C}$. All the culture vessels were sealed using micropore tape (Micropore surgical; 3M, Melbourne, Victoria, Australia).

Effects of plant growth regulators on shoot initiation and multiplication of *P. pandorana* and *P. jasminoides*. The effects of BA, kinetin, and 2,4-dichlorophenoxyacetic acid (2,4-D) on shoot formation were evaluated by adding filter-sterilized plant growth regulators to the autoclaved medium. In initial experiments, 2.2 μM of BA, kinetin, and 2,4-D were added separately to the medium; in subsequent experiments, the medium was supplemented with either BA (1.1, 2.2, 4.4, 6.6, 8.8, and 11.0 μM) or kinetin (1.1, 2.3, 4.6, 6.7, 9.2, and 11.6 μM).

Each experiment had three to five replicate cultures of five explants per treatment and was repeated three times. The number of shoots produced per explant and percentage of explants showing growth response were counted after 4 weeks in culture.

Root initiation. To study the effect of an auxin on root initiation from micropropagated shoots, filter sterilized IBA, 3-indole butyric acid, (1.2, 2.4, 4.9, 7.4, 9.8, and 12.3 μM) was added to the autoclaved medium. Shoots 2 to 3 cm long were separated from the explants and transferred to root initiation medium containing IBA. The percentage of shoots forming roots was quantified and the number of roots/explant was counted after 3 weeks.

Transfer to glasshouse conditions. Rooted shoots were rinsed in water to remove tissue culture medium and then planted using commercially available potting mix (Debco, Victoria, Australia) in 13-cm (2-L volume) plastic pots. These plantlets were acclimatized by placing in an intermittent mist chamber set to spray for 10 s at intervals of 15 min and with bottom heating set at 25°C for 2 weeks before transferring to a glasshouse maintained at $24 \pm 3^\circ\text{C}$ temperature. Observations were made 4 weeks after transfer to glasshouse conditions.

Statistical analysis. A completely randomized design was used in all experiments. Data on percentage of explants producing shoots and mean shoots/explant (Table 1) were subjected to analysis of variance (ANOVA) and means were separated by Tukey’s least significant difference (LSD) test at $P = 0.05$ using the Minitab computer package (University Computer Supplies, The Univ. of Melbourne, Victoria, Australia). Data on effects of BA and kinetin concentrations on shoot multiplication (Fig. 1) and IBA on root formation (Fig. 2) were analyzed using regression analysis.

Results and Discussion

Pandorea pandorana and *P. jasminoides* showed differential response to the growth

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Table 1. Effects of BA, kinetin, and 2,4-D on initiation and multiplication of *P. jasminoides* and *P. pandorana* stem explants.

Growth regulator (2.2 µM)	<i>P. jasminoides</i>		<i>P. pandorana</i>	
	Explants producing shoots (%)	Mean no. shoots/ explant	Explants producing shoots (%)	Mean no. shoots/ explant
None	0 c ^z	0.00 d	0 d	0.00 d
BA	96 a	2.08 b	93 a	3.88 a
2,4-D	83 b	3.38 a	88 b	2.46 b
Kinetin	100 a	1.61 c	76 c	0.71 c

^zMean separation within columns by Tukey's LSD, $P \leq 0.05$.

Fig. 1. Effects of cytokinins on shoot multiplication. BA was used for *P. pandorana* and kinetin for *P. jasminoides*. (A) Percentage of explants producing shoots. R^2 for *P. pandorana* and *P. jasminoides* = 0.66. ($P \leq 0.001$). (B) Mean number of shoots produced per explant. R^2 for *P. pandorana* = 0.75 and *P. jasminoides* = 0.51 ($P \leq 0.05$).

Fig. 2. Effect of IBA on root formation of *P. pandorana* and *P. jasminoides*. (A) Percentage of explants producing roots. R^2 for *P. pandorana* = 0.49 ($P \leq 0.5$) and *P. jasminoides* = 0.73 ($P \leq 0.05$). (B) Mean number of roots produced per explant. R^2 for *P. pandorana* = 0.51 ($P \leq 0.5$) and *P. jasminoides* = 0.77 ($P \leq 0.05$).

regulators tested. Axillary shoots of nodal stem cuttings on medium containing plant growth regulators started to grow after 3 weeks, and shoots were elongated enough to be subcultured by 4 weeks. Of the plant growth regulators tested, BA was effective for culturing *P. pandorana* and kinetin was more suitable for *P. jasminoides* (Table 1). The explants cultured on MS medium without growth regulators died after 1 week. Addition of 2,4-D in the medium was least effective on proliferation of *Pandorea* shoots (Table 1). There was a gradual increase in the number of explants producing shoots and number of shoots produced per explant of *P. pandorana* as cytokinin concentration increased (Fig. 1). Optimum explant response and number of shoots per explant was 8.8 to 11.0 µM BA for *P. pandorana* and 2.3 to 4.6 µM kinetin for *P. jasminoides* (Fig. 1).

The role of cytokinins in inducing axillary bud development in tissue culture of Australian native plants is well recognized (Bunn and Dixon, 1996). *Pandorea jasminoides* and *P. pandorana* are endemic to Australia and commercially exploited as climber plants for their beautiful flowers and green foliage. In vitro propagation studies using other softwood type ornamental Australian native plants such as *Scaevola* (Bhalla and Sweeney, 1998) and *Grevillea* (Bunn and Dixon, 1992) also indicate a requirement for high cytokinin concentration in the multiplication medium.

More than 80% of the explants of *P. pandorana* and *P. jasminoides* produced roots on root initiation medium containing 2.4 µM and 4.9 µM IBA, respectively (Fig. 2). No root formation was observed in the medium without IBA. Root initiation in *P. pandorana* was first observed after 8 d in the 2.4 and 4.9 µM medium while in *P. jasminoides* root initials were observed after 12 d in 4.9 µM IBA. No root formation of *P. pandorana* was observed in medium containing 9.8 µM and 12.3 µM IBA or of *P. jasminoides* in <2.4 µM IBA (Fig. 2). Our results showed that IBA at 2.4 µM is ideal for rooting of *P. pandorana* and 4.9 µM for *P. jasminoides*. The survival rate of tissue-cultured *pandorea* plants was >90% (46/50 for *P. jasminoides*; 48/50 *P. pandorana*) under glasshouse conditions.

The requirement of auxin in the rooting medium differs for softwood type ornamental Australian native plants. A number of commercial cultivars of *Scaevola* were propagated in hormone-free medium (Bhalla and Sweeney, 1998) while *Lechenaultia* and *Dampiera* required IBA and NAA (α-naphthaleneacetic acid) for rooting (Johnson, 1996; Worrall, 1996).

Australian native plants are recognized for their uniqueness and beauty, but have not been widely exploited for ornamental horticulture (Stewart, 1995). The use of new technologies will greatly accelerate their development. Tissue culture is being actively investigated as a propagation method for Australian native plants (Bhalla and Sweeney, 1998, 1999; Bunn and Dixon, 1996; Johnson, 1996; Mulwa and Bhalla, 2000) because many native species are difficult to propagate via conventional tech-

niques, the supply of starting material is often limited, seeds either do not develop or are recalcitrant, plants grow very slowly under field conditions, and hence are unsuitable for vegetative propagation and long-term germplasm storage. The present study enabled the successful in vitro multiplication of two commercial species, *P. pandorana* and *P. jasmminoides*. The micropropagation system reported here can be used to produce several hundred plants from a single mother plant in a short period of time.

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