Micropropagation of Australian native ornamental grevilleas©

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Abstract

Australian native grevilleas are very diverse in their habit and habitat. There are up to 350 species of *Grevillea* reported, although the number of species varies among authors. *Grevillea* species range in habit from small, prostrate shrubs, less than 50 cm (20 in.) to trees over 35 m (114 ft) tall. Their habitat extends from the wet tropics of Australia through temperate southern Australia and dry arid zones in Western Australia. Grevilleas are valued for their beautiful form, range of foliage, and diversity of flower colors, sizes, and shapes. Apart from their aesthetic value, they are also great garden plants, attracting native bees, butterflies, and birds, thus enriching the natural habitat. Many *Grevillea* species are propagated by seeds, but valuable, ornamental hybrids are propagated by cuttings, layering, or grafting. This paper will focus on the rapid cloning of some difficult-to-propagate cultivars of these beautiful ornamentals.

INTRODUCTION

The genus *Grevillea* is named after the eminent botanist Charles F. Greville (Olde and Marriott, 1994), is reported to contain 360 species, and is distributed throughout Australia and nearby islands. *Grevillea* belongs to the family *Proteaceae*. Plant habit ranges from small shrubs under 50 cm to tree forms, like *G. robusta*, which grows up to 35 m tall (Olde and Marriott, 1994). The OzNativePlants (2016) website provides a quick introduction to the diversity of ornamental grevilleas. Based on my readings, the three volumes of "The Grevillea Book" by Olde and Marriott (1994) provide the best reference on grevilleas.

Aboriginal Australians used various species of *Grevillea* for food (e.g., *G. annulifera* and *G. heliosperma*), medicine (e.g., *G. striata* and *G. pyramidalis*), and tool making (*G. pteridifolia* and *G. striata*) (Olde and Marriott, 1994). Their use in landscaping, owing to their diversity of growth habits, suitability to different habitats, plant architecture, variation in leaves, flowers, flower color, and nectaries attracting native bees, butterflies, and birds, are well documented by Olde and Marriott (1994).

Propagation of *Grevillea* spp. by seeds is common practice in nurseries for straight species. However, to maintain the characteristics of the hybrids for commercial applications, *Grevillea* hybrids are vegetatively propagated. Many of these hybrids are amenable to propagation by cuttings. *Grevillea* hybrids and cultivars that are difficult to propagate by cuttings are either grafted or tissue cultured in Australia. Grafting onto a species with wide adaptability to a larger geographic area is also practiced to increase the adaptability of some of the hybrids to a wider geographical area, thus enhancing their commercial potential. *Grevillea robusta* is considered to be a good rootstock for grafting grevilleas. This paper covers a brief review of the tissue culture of some cultivars of Australian native *Grevillea*.

Micropropagation of *Grevillea* has been reported for a few species (Bunn and Dixon, 1992; Watad et al., 1992; Rajasekaran, 1994; Leonardi et al., 2001; Evenor and Reuveni, 2008). Adventitious rooting of cuttings was also recently studied (Newell et al., 2003; Krisantini et al., 2006). Kennedy and De Filippis (1999) also studied response of in vitro cultures of *Grevillea* spp. to NaCl salinity. Touchell et al. (1992) also reported cryopreservation of *G. scapigera*, an endangered species. In this report, I discuss micropropagation of ornamental *Grevillea* cultivars 'Moonlight' and 'Superb', which were recalcitrant to nursery cutting production.

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MATERIALS AND METHODS

Actively growing shoot tips of grevillea 'Moonlight' and 'Superb' collected periodically from nursery stock were used as explants. They were surface sterilized by washing in Tween20® for 30 min at 200 rpm on a gyratory shaker at room temperature followed by treatment with dilute NaOCl solution (1% Cl_2) for 10-15 min. The chlorine-treated shoot tips were rinsed with sterile, distilled water 3-5 times before further treatment with 1% (v/v) PPM (Plant Preservative Mixture®) for 30 min before inoculating onto sterile culture medium. Initiation and shoot multiplication was achieved on MS medium (Murashige and Skoog, 1962) supplemented with 0.5-1.0 µM benzylaminopurine (BAP) and 0.01 µM naphthaleneacetic acid (NAA). Rooting was best achieved using ½ strength MS medium supplemented with 3-5 µM IBA. Acclimatization of microplants transferred to a porous potting mix was achieved in the greenhouse with control over humidity, light, and temperature.

RESULTS AND DISCUSSION

Contamination (fungal and bacterial) is a major issue with *Grevillea* species during the initiation phase. This is understandable as the explants were collected from nursery stock maintained in an open area. Additionally, the hairy nature of the *Grevillea* cultivars made them difficult to surface sterilize. About 30% success (30% clean cultures) was obtained in the initiation phase. These cultivars required 3-4 weeks for initiation to take place when cultured on MS medium supplemented with 30 g L^{-1} sucrose, 0.5-1.0 µM BAP, 0.01 µM NAA, and gelled with 7.0 g L^{-1} agar. Medium pH was adjusted to 5.8 before sterilizing by autoclaving at 121°C for 20 min. After 3-4 subcultures on a monthly interval, the shoot cultures grew well in the initiation medium. Murashige and Skoog medium supplemented with low concentrations of BAP was used to micropropagate shrubby grevilleas by various authors (Touchell et al., 1992; Watad et al., 1992; Kennedy and De Filippis, 1999; Leonardi et al., 2001). Woody plant medium modified with a high concentration of BAP was used to effect adventitious shoot formation from leaf explants of *G. scapigera* (Bunn et al., 1992). Similarly, *G. robusta* (a tree species) was propagated on woody plant medium containing 4.4 µM BAP in combination with 0.27 µM NAA (Rajasekaran, 1994). In this study, rapid growth of *Grevillea* shoot cultures to 5-6 cm per month allowed a monthly yield of 3-4 nodal cuttings from each shoot. This equated to a multiplication factor of 3-4 per month.

Rooting was not a problem with the *Grevillea* cultivars and rooting percentage varied between 70 and 100%. Reduced nutrient concentration and sucrose (50% MS medium + 20 g L^{-1} sucrose). Watad et al. (1992) rooted six cultivars of *Grevillea* on MS medium supplemented with no hormones (control), 1 mg L^{-1} IAA (indoleacetic acid), NAA, or IBA, and found that NAA was most effective (85-90% rooting) for rooting *Grevillea.* They also reported variation in rooting due to genetic differences among these cultivars. Bunn et al. (1992) could root microshoots of *G. scapigera* ex vitro in a mist chamber using commercial rooting powder (1.0 mg L^{-1} IBA). Evenor and Reuveni (2008) used 1.0 mg L^{-1} IBA in ½ MS medium to root *Grevillea*, although their shoot cultures grew well in WPM medium. Leonardi et al. (2001) also reported better rooting (rooting percentage, roots per plant, and root length) by using 1.0 mg L^{-1} IBA compared with 1 mg L^{-1} NAA.

Well rooted plants deflasked into a porous potting mix composed of peat, vermiculite, and perlite (20%:50%:30%) and maintained at ambient humidity over 90%, reduced light (80% shade), and reasonable heat (26-30°C max) during the first week after deflasking achieved greater than 90% survival. The same mix with a pinch of slow-release fertilizer (minus phosphorus) was excellent for further growth of micropropagated grevilleas. Grevilleas are known to do well in well-drained soil (Olde and Marriott, 1994).

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