HortScience 27(3):261-262. 1992.

In Vitro Propagation of the Rare and Endangered *Grevillea scapigera* (Proteaceae)

Eric Bunn and Kingsley W. Dixon
Kings Park and Botanic Garden, Kings Park, West Perth, Western Australia 6005

Additional index words. micropropagation, leaf culture, adventitious shoots, in vivo rooting

Abstract. **Micropropagation, including adventitious shoot growth from leaf sections, was achieved for *Grevillea scapigera* (Proteaceae), a rare and endangered species from Western Australia. Shoot tips were initiated on filter paper supports with liquid WPM (Woody Plant Medium) and supplemented with 20 µM zeatin riboside and 2 µM GA_3. Shoots were then incubated on WPM solidified with agar and supplemented with 5 µM kinetin and 0.5 µM BA, which produced an approximate 6-fold multiplication rate per month. Up to three adventitious shoots were induced from 0.7-cm^2 leaf sections after 6 to 7 weeks on solid 1/2 MS (Murashige and Skoog) medium supplemented with 10 µM BA and 0.5 µM IBA. Shoots, 30 to 50 mm long, were rooted in vivo in a fogged glasshouse under 70% shade using a commerical rooting powder [IBA, 0.1% (w/w)] applied to the base of the shoots. Most (67%) of the shoots treated in this way rooted after 5 weeks. Established, rooted plants have been grown on under glasshouse conditions. Chemical names used: N6-[2-isopentenyl] adenine riboside (zeatin riboside); gibberellic acid (GA_3); 6-furfurylaminopurine (kinetin); N-(phenylmethyl)-1H-purine-6-amine (BA); 1-H-indole-3-butyric acid (IBA).**

Grevillea scapigera A.S. George is an attractive, white-flowered semiprostrate shrub growing near the central wheatbelt town of Corrigin in Western Australia. A program was instigated where two grafted plants in the Royal Botanic Gardens, Sydney, were introduced to Kings Park and Botanic Garden in an attempt to propagate the species by cuttings and grafting. Since these propagation techniques met with limited success, we attempted micropropagation of the remaining grafted plants (Fig. 1).

Shoot tips and axillary nodes from side branches were taken from a whip-and-tongue-grafted plant of *G. scapigera* growing on a rootstock of *Grevillea* x 'Royal Mantle'. Shoots were washed under running tap water for 1 h, then surface-sterilized for 5 min in 0.5% sodium hypochlorite (NaOCl) plus 0.05% Tween 80, followed by immersion in distilled water. Bleached, damaged, or unwanted leaf and stem material was removed. Each shoot was then immersed in 0.5% NaOCl only for ≈5 to 10 sec, washed in sterile distilled water, then placed in 30-ml polycarbonate tubes (one explant/tube) with sterile filter paper domes for support and containing 10 ml of liquid medium. The filter paper domes were autoclaved twice at 121C for 15 min. Lloyd and McCown's (1981) Woody Plant Medium (WPM) supplemented with WPM vitamins, 500 µM *myo-inositol*, 60 mM sucrose, 20 µM zeatin riboside, and 2 µM GA,, and pH 6.0 was autoclaved at 121C for 20 min in 250-ml media bottles and poured into culture tubes. The tubes with their explants were incubated in the dark for 1 week.

Sterile shoot and bud cultures were moved into light provided by cool-white fluorescent tubes, 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 16/8 h light/dark. After another 3 weeks, explants were transferred to WPM as above, supplemented with 0.5 µM BA or 5 µM kinetin and 0.5 µM BA, and solidified with agar at 10 g-liter-l. Shoots averaged a 2- to 3-fold multiplication per 4-week incubation period with BA alone at 0.5 µM or 5- to 6-fold with 0.5 µM BA and 5 µM kinetin (Table 1). Shoots of *G. scapigera* were mostly longer than 20 mm and grew vigorously; however, some required elongation and this was readily achieved on basal WPM without growth regulators. WPM medium supplemented with 1 µM GA_3 and 0.5 µM kinetin was used to enhance shoot development from axillary buds. Shoots were incubated, then used for root induction or further shoot proliferation.

Leaves cut from plants in vitro during the subculture procedure had petioles removed and were then incubated (under the same conditions as shoot cultures), adaxial surface up, on half-strength MS (Murashige and

Received for publication 27 June 1990. Accepted for publication 20 Sept. 1991. We thank members of the Grevillea Study Group and the director and staff of the Royal Botanic Gardens, Sydney, for their efforts in preserving *Grevillea scapigera. The* cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

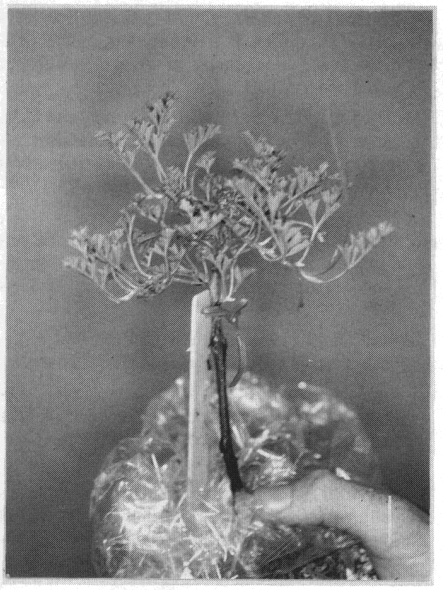

Fig. 1. Grafted plant of *Grevillea scapigera* from Sydney Botanic Gardens before removal of explants. Bar = 2 cm.

Table 1. Response of in vitro shoots and leaves of *Grevillea scapigera* to medium supplemented with plant growth regulators.[z]

Explant	Growth regulators (μM)	Mean no. shoots/ explant ± SE[y]
Shoots[y]	0.5 BA	2.4 ± 0.1
	0.5 BA + 5.0 kinetin	5.8 ± 0.2
Leaves[x]	10.0 BA + 0.5 IBA	3.0 ± 0.2

[z]Shoots incubated on WPM, leaves on 1/2 MS.
[y]Minimum of 20 replicates, repeated at least twice.
[x]Seven replicates.

Skoog, 1962) basal mineral salts, 100 μM sodium ferric EDTA, 500 μM *myo* -inositol, WPM vitamins, 90 mM sucrose, 10 g agar/liter, and supplemented with 10 μM BA and 0.5 μM IBA. In studies with leaf cultures of *Hibbertia miniata* C.A. Gardner and *Aster*-olasia species (unpublished data), contact with the medium appeared to be essential for adventitious bud development. Thus, leaves of *G. scapigera* were firmly pressed onto the medium surface, which proved difficult as the leaves are coriaceous, undulate, and hence difficult to flatten despite being grown in vitro.

After 19 to 21 days of incubation, leaves began to swell, and shoot growth commenced 9 to 14 days later (Fig. 2). Adventitious shoots appeared mainly from the cut distal petiole end and where the leaf perimeter was in contact with the medium. After 7 weeks of incubation, 15- to 20-mm-long shoots had formed on seven of nine leaf cultures. Shoots were then elongated and treated as for shoot-derived cultures. For a single leaf (0.7 cm^2), an average of three adventitious shoots were formed and ready for subculture after 6 to 7 weeks (Table 1). On average, eight to 12 larger leaves (0.5 to 0.8 cm^2) were produced per explant per culture period, of which up to half could be used for leaf cultures.

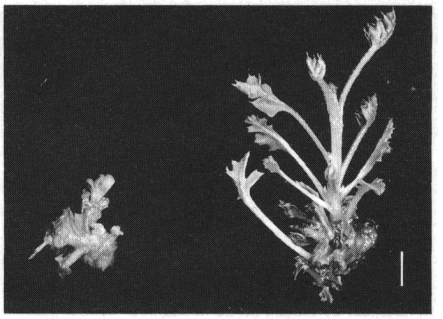

Fig. 2. Adventitious shoot production from excised leaves of in vitro-grown *Grevillea scapigera* 3 weeks (**left**) and 5 weeks (**right**) after initial culture. Bar = 0.3 cm.

Elongated shoots, ≈30 to 50 mm long, were acclimatized by placing shoots in culture vessels in a glasshouse (with 70% shade) for several days. Shoots were separated and dipped in 0.15% aqueous propyl[3-(dimethylamino)propyl]carbamate (propamocarb) antifungal solution. The stem bases were then dipped in a commercial softwood cutting powder containing 0.1% (w/w) IBA and placed in a mix of equal parts pasteurized brown peat and unpasteurized perlite in trays of pots of 40-ml capacity (64 per tray). The shoots were kept under fogged conditions for 2 weeks then moved to intermittent misting for a further 2 to 3 weeks or until roots were visible. Plants were then repotted into 70 × 70 × 100-mm tapered pots of 400-ml capacity (Fig. 3), with potting mix containing equal parts composted wood fines : coarse sand : perlite, supplemented with inorganic fertilizer (17N-1.6P-8.7K), and pasteurized at 70C for 1 h.

In vitro rooting was superseded by the ease and success of using microcuttings. Microcuttings rooted readily in potting mix, with some initiating roots in less than 2 weeks. Most (67%) *G. scapigera* shoots rooted in <5 weeks. After 6 months, shoots of *G. scapigera* produced healthy plants 10 to 12 cm tall (Fig. 3). Several plants flowered 9 to 12 months after transfer from culture.

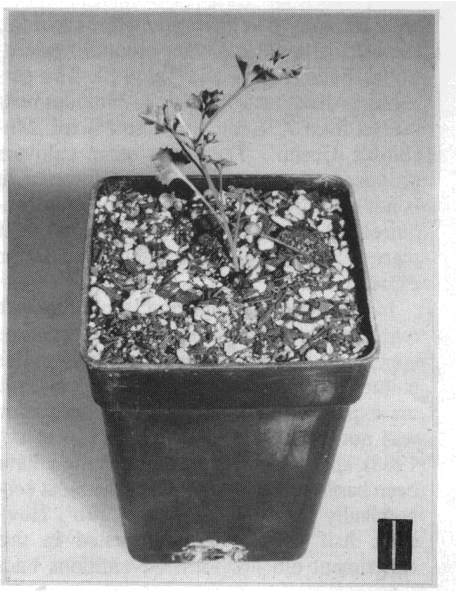

Fig. 3. *Grevillea scapigera* 6 months after removal from culture to potting mixture. Bar = 0.8 cm.

This paper outlines methods for rapid propagation of the rare and endangered species *Grevillea scapigera*, including a method for adventitious shoot production from leaf material-a new method for propagation in the Proteaceae with possible commercial applications. However, the limited genetic material available to the study does not favor successful reconstitution of the species in the natural habitat. The genetic constitution of plants derived from basal shoot clusters and leaves is unknown. Thus, the suitability of plants generated from these sources for habitat re-establishment trials requires careful assessment. The recent discovery of further plants of *G. scapigera* does hold promise for greater genetic variation than previously existed for the propagation program.

Literature Cited

Lloyd, G.B. and B.H. McCown. 1981. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Proc. Intl. Plant Prop. Soc. 30:421-437.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15:473-497.