Propagation of *Grevillea*

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ABSTRACT

Grevillea (Proteaceae) is a native Australian genus with high commercial value as landscape ornamentals, and they are known to be difficult to root. There has been only limited research into the propagation of *Grevillea*. The effect of indole-3-butyric acid (IBA) on the rooting *G.* 'Poorinda Royal Mantle' in winter, spring and summer was evaluated at UQ Gatton, southern Queensland in order to determine the rooting ability of this species in different seasons. The effect of cutting type, i.e. tip and stem cuttings, and method of auxin application, i.e. top and basal application, were also tested on *G.* 'Poorinda Royal Mantle' and *G.* 'Coastal Dawn'. *G.* 'Poorinda Royal Mantle' demonstrated a seasonal rooting and was more responsive than G. 'Coastal Dawn' to the applied IBA. Stem cuttings had a higher survival than tip cuttings, but tip cuttings had a higher capacity to root. Top application of auxin at low concentration (1 g L^{-1}) in G. 'Poorinda Royal Mantle' in spring resulted in a significantly higher rooting percentage than basal application at the same concentration. These findings could be useful for setting up a practical propagation protocol on *Grevillea*.

INTRODUCTION

*Grevillea (*Proteaceae*)* is a native Australia genus with high commercial value as landscape ornamentals and cut flowers (Joyce and Beal, 1999). *Grevillea* are generally considered a difficult-to-root species (Dupee and Clemens, 1982). *Grevillea* 'Poorinda Royal Mantle' is a vigorous prostrate shrub hybrid that is marketed for ornamental hanging baskets and as ground cover, whereas *G.* 'Coastal Dawn' is marketed as a landscape shrub. The flowers of *G.* 'Poorinda Royal Mantle' are red of a toothbrush type and are produced throughout the year, but are profuse in spring and summer. *G.* 'Coastal Dawn' only flowers during winter. Indole-3-butyric acid (IBA) powder at 16 g kg^{-1} is commercially produced and used in the nursery industry to propagate difficult-to-root, woody Australian native species such as *Banksia* and *Eucalyptus*, as well as some exotics (*Camellia)*. Exogenous IBA is generally applied at the base of the cutting. Basal application of auxin to difficult-to-root species may not lead to an increase in auxin concentration in the cells that would give rise to adventitious root formation (Ford *et al.*, 2002), depending upon how the applied auxin is taken up and transported in the cutting. Top application of auxin might enable the applied auxin to enter the natural basipetal

auxin transport in the plant so that it reaches the cells competent to form roots. The scarcity of information on propagation of *Grevillea*, particularly of the effects of applied hormones (Leonardi *et al.*, 2001), calls for more studies in this area. The objectives in this work are to determine the effectiveness of indole-3-butyric acid (IBA) at 16 g kg^{-1} (industry standard) to induce rooting of *Grevillea* 'Poorinda Royal Mantle' during different seasons. Types of cutting, i.e. tip and stem cuttings, and method of auxin application, i.e. top application to cutting stump and basal application, were also tested on *G*. 'Poorinda Royal Mantle' and *G*. 'Coastal Dawn' to determine whether they influenced rooting.

MATERIALS AND METHODS

The experiment was conducted in a mist propagation unit with the heated bench at the University of Queensland (UQ Gatton) nursery, southern Queensland. *Grevillea* cuttings were from container-grown, mature stock plants kept in a greenhouse at the UQ Gatton nursery. Tip cuttings were two nodes long with one mature, fully-developed leaf. Stem cuttings were two nodes-long, collected from the fourth to the sixth nodes below the tips with the shoot tip removed. The base of the cutting was trimmed and the leaves on the lower node were removed, leaving only one fully-developed leaf on top. The cuttings were rinsed thoroughly in tap water before being treated with IBA.

Industry standard of IBA concentration at 16 g kg^{-1} (Yates) was used to test rooting ability of G. Royal Mantle cuttings in different seasons. An IBA solution of 1 g L^{-1} was prepared for method of auxin application experiment by dissolving 1 mg of IBA powder (Sigma) in 500 µL of 50 % ethanol, which was then diluted with distilled water to make 1 g L^{-1} solution. With the basal application base of the cutting was dipped for 5 seconds in IBA powder (16 g kg^{-1}) or IBA solution (1 g L^{-1}). With the top application cuttings, the top 10-mm part of the cuttings was dipped in IBA solution (1 g L^{-1}) for 5 seconds. Cuttings were then planted in 4.5 x 4.5 x 7.5 cm tubes. The medium used was peat, perlite and vermiculite 1:1:1 with 120-g mini Osmocote per 60-L medium. Planted cuttings were placed in a mist propagation house with a

heated bench to 24 °C. The winter experiment was conducted between July-September, spring experiment between September-November, the autumn between March-June, and the summer between December-February. Light intensity in the propagation house was maintained between 1500 to 3000 lux by using shade cloth during summer. Bench temperature ranges were 21-27 °C in summer, 17-26 °C in spring, 10-25 °C in winter, and 17-26 °C in autumn.

All experiments were conducted in a randomised block design. Each of the treatments was replicated 3 times with 10 cuttings in each treatment.

Observations were done at weekly intervals beginning 3 weeks after the cuttings were planted by carefully lifting the cuttings from the medium, checking for roots and replacing the cuttings into the medium. Root numbers and root length evaluations were made two weeks after the first root initiation was observed, and roots > 2 mm in length, were recorded.

Data for each species were analysed separately. Data on percentage of rooting where appropriate were analysed using binary logistic regression (Collett, 1991) using Minitab 13.

RESULTS

Type of Cutting

Stem cuttings of both *Grevillea* cultivars resulted in a relatively higher survival compared to tip cuttings (Table 1). However, tip cuttings demonstrated a higher capacity to form roots than decapitated cuttings since tip cuttings. In *G.* Coastal Dawn only tip cuttings, and not stem cuttings, rooted without exogenous auxin, whereas in *G.* Royal Mantle tip cuttings without auxin treatment produced a significantly higher rooting percentage (67 %) than stem cuttings (25 %) (Table 1). Also, percent of cuttings rooted should be noted in relation to percent of surviving cuttings (Table 1).

Table 1. Effect of Cutting Type on Survival and Rooting of *G.* Coastal Dawn and *G.* Royal Mantle Cuttings

Treatment	*G.* Coastal Dawn [1]		*G.* Royal Mantle [1]	
	Percent of Cuttings Survived	Percent of Cuttings Rooted [2]	Percent of Cuttings Survived	Percent of Cuttings Rooted [2]
Tip Cuttings				
16 g kg^{-1} [2]	67	0 (0)	50	50 (100)
No IBA	100	12 (12)	67	67 (100)
Decapitated Cuttings				
16 g kg^{-1}	83	23 (25)	92	92 (100)
No IBA	100	0 (0)	100	25 (25)

[1] Experiment of *G.* Coastal Dawn was conducted in summer, *G.* Royal Mantle in autumn

[2] Percent of cuttings rooted out of total and out of survived (within brackets)

Response G.'Poorinda Royal Mantle' to IBA Treatment at Different Seasons

G. 'Poorinda Royal Mantle' demonstrated a seasonal response to auxin. Applied IBA significantly increased rooting in all season except for summer (Table 2). Rooting percentage of *G.* 'Poorinda Royal Mantle' stem cuttings with and without exogenous auxin was also highest in summer, and lowest in winter (Table 2). Rooting was also fastest in summer, the maximum rooting with IBA treatment being achieved by week 6 (Table 2). Rooting percentage without auxin application was also relatively high (76 %) in this season (Table 2). Except for winter, IBA at 16 g kg^{-1} resulted in a relatively high rooting percentage (more than 60 %) throughout the year.

Table 2. Influence of IBA on rooting of *Grevillea 'Poorinda Royal Mantle'* cuttings in different season

IBA Concentration	Season							
	Summer		Autumn		Winter		Spring	
	Rooting %	Rooting Period [1] (weeks)	Rooting %	Rooting Period [1] (weeks)	Rooting %	Rooting Period [1] (weeks)	Rooting %	Rooting Period [2] (weeks)
IBA 16 g kg^{-1}	90	6	74*	4	40*	16	60*	14
Control (No IBA)	76	12	39	6	0	- [2]	32	15

1) Number of weeks required to achieve maximum rooting during 16 weeks of experiment period
2) Unrooted during 16 weeks of experiment period
* Treatments significantly different from control at $p<0.05$

Methods of Auxin Application

Top application of IBA at 1 g L^{-1} to *Grevillea* cuttings resulted in a higher rooting percentage than basal application at the same concentration, particularly in *G*. Royal Mantle (P=0.039) (Table 3). The rooting percentage obtained using top application of IBA at 1 g L^{-1} in both cultivars was comparable to that of industry standard ad 16 g kg^{-1}. However, top application of IBA at 1 g L^{-1} in *G*. Royal Mantle resulted in a delayed outgrowth of axillary buds in 10 % of the rooted cuttings by about 12 weeks in spring (data not presented). Method of IBA application did not affect root number.

Table 3. Effect of Method of Auxin Application on Rooting of G. Coastal Dawn and G. Royal Mantle Stem Cuttings [1)]

Treatment	*G.* 'Coastal Dawn'		*G.*' Royal Mantle'	
	Percent of Cuttings Rooted	Root No [2)]	Percent of Cuttings Rooted	Root No [2)]
Basal IBA 1 g L^{-1}	0	- [3)]	47	1.5± 0.7
Top IBA at 1 g L^{-1}	22	1.5 ± 1.0	70*	1.2 ± 0.4
Industry Standard 16g kg^{-1}	13	1.2 ± 0.5	70*	1.2 ± 0.7
Control (No IBA)	0	-	43	1.6 ± 0.7

[1)] Experiments were conducted twice between late spring to early summer with similar results

[2)] Average of root number and standard deviation

[3)] Unrooted during the experiment period of 10 weeks

* Treatments significantly different from control at $p<0.05$

DISCUSSION

Cuttings Type

A higher rooting percentage is normally obtained using tip cuttings rather than stem (decapitated) cuttings. This has been hypothesised as due to production of endogenous auxin in the shoot tip, which is responsible for causing root induction (Marks, 1996). However, *Grevillea* tip cuttings in this experiment had a lower survival compared to decapitated cuttings,

which resulted in a low final rooting percentage. Therefore, for commercial propagation purposes the use of stem cuttings might be preferable. Without applied auxin, however, tip cuttings demonstrated higher rooting capacity than stem cuttings (Table 1).

Effect of Season

Grevillea 'Poorinda Royal Mantle' cuttings demonstrated a seasonal response to auxin application. A relatively high proportion (76 %) of the cuttings without exogenous auxin in summer rooted (Table 2), indicating that the endogenous auxin and other substances that are required for rooting were sufficient to induce rooting in this season. A seasonal response to auxin in cuttings was previously reported in *Populus nigra* (Nanda and Anand, 1970) and *Cotinus coggyria* (Blakesley *et al.*, 1991a). Plant response to applied auxin differs with season (Nanda and Anand, 1970), since seasonal changes in light, temperature, photoperiod and plant development may cause changes in endogenous hormone levels (Blakesley *et al.*, 1991a). They suggested that this might be due to different production of endogenous auxin in different seasons and in different environmental conditions under which the stock plants are grown. Difference in response to applied auxin might also be caused by differences or changes in plant tissue sensitivity to the applied hormones (Trewavas, 1986). However, the regulation of tissue sensitivity is still poorly understood.

Method of Application

The work here demonstrated that top application of auxin might be an alternative way to propagate difficult-to-root species, and has potential to reduce auxin requirement for propagation. The effectiveness of top application as opposed to basal application might be due to more efficient delivery of auxin into the competent cells to produce adventitious root (Ford *et al.*, 2002), as auxin is transported more in a basipetal direction than acropetal (Jones, 1998). Basally applied auxin might need to be transported up the cutting, and then redirected to the base through the basipetal transport system. However, more work is required before this method could be used widely in commercial propagation since top application might result in a delayed growth of axillary buds in stem cuttings. Also, more experiments need to be

conducted to determine the consistency of results in different seasons, since rooting of *Grevillea* is seasonal.

This work has demonstrated the effect of exogenous auxin on adventitious root formation in Australian native species *Grevillea*: (1) Tip cuttings of *Grevillea* had a lower survival than stem (decapitated) cuttings, however tip cuttings demonstrated a higher capacity to form roots in the absence of auxin (2) Rooting of *G.* 'Poorinda Royal Mantle' cuttings is seasonal, being highest and fastest in summer and lowest and slowest in winter (3) Top application of auxin might be an alternative method of propagation of difficult to root species. Further studies on the interaction of endogenous and exogenous auxin and its role in adventitious root formation are being undertaken.

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