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Full Length Research Paper

Rooting success using IBA auxin on endangered *Leucadendron laxum* (PROTEACEAE) in different rooting mediums

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***Leucadendron laxum* (Marsh rose Leucadendron) was tested for its rooting ability as an endangered plant species for reintroduction into the natural habitat, using IBA liquid hormone preparations and four growth media. The treatments included a control, 500, 1000, 2000 and 4000 ppm concentrations. Four growth media, namely: bark and polystyrene; peat moss and polystyrene; bark, river sand and polystyrene; and perlite and river sand were tested in an environmentally controlled greenhouse with bottom heat. The experiments used a randomised block design with 10 cuttings per treatment and 4 replicated four times. The results showed that the bark, sand and polystyrene medium had the highest (45%) significant survival rate when supplied with IBA at 500 ppm. The application of 1000 ppm of IBA in bark, sand and polystyrene medium significantly increased the number of roots per cutting (5) and produced the longest roots (4 cm). The bark and polystyrene medium supplied with zero IBA had the largest number of roots per cutting (6.5). The comparison of the four growth media showed that bark and polystyrene was the best medium that produced the highest and most significant callusing formation (30%), % of cuttings that rooted (28%), cuttings with a higher survival rate (57%), and cuttings with a higher number of roots (3.5) and the longest roots (7.35 mm). With regard to various IBA treatments tested across all treatments, the IBA supplied at 1000 ppm produced greater root numbers when compared with all other treatments.**

Key words: Hormone treatment, red data species, rooting percentage, vegetative propagation.

INTRODUCTION

Leucadendron laxum belongs to the Proteaceae family and is mostly found near the most southern point of the African continent, where the Atlantic and Indian Oceans meet. It grows on level, damp ground in the valleys between Hermanus, Bredasdorp and Agulhas (Williams, 1972). The existence of this plant in the wild is not certain, as its habitat is being drained by natural catastrophes such as wild fires and farmers (Mustard et al., 1997; Robyn and Littlejohn, 2002). As a result, *L. laxum* has been named an endangered species in South Africa (Hilton-Taylor, 1996).

Some Leucadendrons are naturally rare owing to reproductive reasons, wild fires and competition with other species, such as the spread of alien invasive species (Paterson-Jones, 2000). One report revealed that *L. laxum* has only 5 000 remaining plants in the natural habitat (Mustard et al., 1997). Improved plant propagation techniques are essential at this point to help save the remaining small plant populations.

L. laxum is an important plant that is aesthetically pleasing with its bright yellow flowers and attractive cones (Jamieson, 2001). It has a potential role in the flower and tourist industry in South Africa. As a member of the Proteaceae hybrids, these plants can be propagated by vegetative means to maintain the unique characteristics especially cut flower cultivars (Brown and Duncan, 2006) and produce uniform flowering times (Reinten et al., 2002). However, most vegetative techniques applied by Protea growers have not been published (Laubscher, 2000). To our knowledge, the vegetative

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Abbreviations: IBA, Indole-3-butyric acid; **ppm**, parts per million; and **SAFEC**, South African Flower Export Council.

propagation of *L. laxum* has not previously been described and new documentation would be seen as new research.

The appropriate vegetative propagation techniques for *L. laxum* can speed up the replacement of plants on damaged and reclaimed land and also increase production of cut flower cultivars in nurseries. Improved propagation techniques can also increase royalties and the sale of patented cultivars with an increase in quality and quantity of future export cut flowers. Therefore, faster rooting techniques are important to help save small plant populations such as *L. laxum*, especially after fires (Robyn and Littlejohn, 2002). Improved cultivation and management practices in orchards (SAFEC, 2002) need to be investigated, as threats to the natural habitat continue to escalate.

Research evidence suggests that auxins play a central role in the determination of rooting capacity, by enabling the faster production of rooted cutting material which is essential for vegetative propagation (Fogaça and Fett-Neto, 2005). Auxins are known to increase rooting percentage and rooting time together with uniformity of rooting (Hartmann et al., 2002). Under natural conditions, some Proteaceae cuttings are difficult to root, and few species have shown response to auxin treatments (Hartmann et al., 2002).

Research into various auxin concentrations and different rooting media could prove useful to the horticultural industry by enabling the faster production of rooted cutting material. IBA is one form of auxin that is effective in the rooting of a large number of plant species (Hartmann et al., 2002). In some woody species aryl esters and aryl amid of IBA are equal or more effective than acid formulations in root initiation (Hartmann et al., 2002). The use of different concentrations of IBA on *L. laxum* will identify the treatment that produces desirable rooting results. In the literature, there is no available information on the effect of auxin on rooting of *L. laxum* and further experimentation will be documented as new research.

Testing combinations of rooting components should ensure faster and better quality root formation. Components such as bark, peat, polystyrene and river sand should be tested in various combinations. Shredded milled pine bark is a medium widely used in rooting mediums to manage the pH factor, provided the quality is from a good source (Owings, 1996). Polystyrene is a coarse mineral component which provides an air-filled porosity that improves aeration, whereas river sand mostly used in combination with organic materials provides coarseness and drainage. Coarse grade peat moss similar to bark lowers the pH and enhances the water holding capacity of growing mediums (Hartmann et al., 2002).

The aim of this study is therefore to determine whether *L. laxum* responded favourably to different concentrations of IBA rooting hormones and various rooting mediums to ensure faster and more efficient rooting success in pot-plant and cut flower production.

MATERIALS AND METHODS

Experimental

The experiment was conducted in an environmentally controlled greenhouse, at the nursery of the Cape Peninsula University of Technology in Cape Town, South Africa. The clear polycarbonate greenhouse was fitted with a shade screen (40%), where temperature and humidity were monitored on a weekly basis. Midday temperatures fluctuated between 22 - 27°C and relative humidity between 39 - 86%. Bottom heat (20 - 25ºC) and mist bed conditions were supplied (Brown and Duncan, 2006). The irrigation timer was set on 15 s on and 20 min off.

The experiment started during the middle of August and continued for 8 weeks (Brown and Duncan, 2006). Cutting material of *L. laxum* was collected from selected plant populations in their natural habitat on the Agulhas Plain on the extreme south-western coast of South Africa. The material was kept dry in a sealed plastic bag and transported overnight.

Setup and design

A randomised complete block design involving four growth mediums (bark and polystyrene; peat moss and polystyrene; bark, river sand and polystyrene; and perlite and river sand and four concentrations of IBA (control, 500, 1000, 2000 and 4000 ppm) were used in this study. Altogether $4 \times 5 \times 10$ cuttings were involved in the experiment. The medium had to be light with good drainage and also had to retain some moisture to prevent quick drying; had a pH of 6.5 - 7 is preferred (SAFEC, 2002).

For each treatment, cuttings were replicated in 10 pots. The experiment was conducted in a controlled greenhouse with misting irrigation and heated beds. Readings of the greenhouse environment, that is, solar radiation, temperature, relative humidity and irrigation were monitored on a weekly basis.

Cuttings, IBA treatment and planting

Cuttings were taken from turgid stems of semi-hardwood stems after shoot elongation (Aug-Nov). Cuttings were taken in dry weather (SAFEC, 2002). For this experiment, terminal cuttings measuring approximately 150 mm long were used. All cutting material was rinsed in Benlate fungicide (10 g/l) before planting. After planting, cuttings were sprayed weekly with Captan (2 g/l) solution (Reinten et al., 2002; SAFEC, 2002). Wilted and infected cuttings were removed over the rooting period (Brown and Duncan, 2006).

Hormone treatments used IBA liquid preparations of 500, 1000, 2000, 4000 ppm. The control remained untreated. The IBA powder was dissolved in 50% ethyl alcohol and made up to the appropriate concentration by adding distilled water (Brown and Duncan, 2006). Prior to planting, the basal 5 mm of cuttings were dipped for 5 s in the rooting hormone (Reinten et al., 2002; Hartmann et al., 2002). Cuttings were then individually planted into new plug foam trays/ pots containing different growth mediums and IBA concentrations.

Data collection and statistical analyses

Samples of cuttings were drawn from the experiment on a weekly basis to monitor results of wounding, hormone treatment, rooting medium, callusing and rooting progress. The factors measured were, average shoot growth, number of cuttings callused, number of cuttings rooted, number of roots on cuttings, length of roots, and

Table 1. Effect of different concentrations of IBA on percentage survival rates of cuttings, number of roots per cutting and root length of *L. laxum* grown in bark, river sand and polystyrene medium and peat and polystyrene medium.

Conc. (ppm)	Bark, river sand and polystyrene			Peat moss and polystyrene		
	% Survival rate	Roots per cutting	Root length (cm)	% Survival rate	Roots per cutting	Root length (cm)
Control	10 ± 5.8b	0.0 ± 0.0c	0.0 ± 0.0c	60 ± 21.2	0.25 ± 0.25	0.25 ± 0.25
500	45 ± 15.0a	0.0 ± 0.0c	0.0 ± 0.0c	43 ± 23.2	0.75 ± 0.75	1.25 ± 1.25
1000	25 ± 8.7ab	5.0 ± 2.9a	4.0 ± 2.3a	58 ± 18.4	1.75 ± 0.75	0.25 ± 0.25
2000	5 ± 2.9b	0.0 ± 0.0c	0.0 ± 0.0c	65 ± 16.6	2.00 ± 1.15	3.75 ± 2.39
4000	10 ± 5.8b	1.0 ± 0.6b	0.5 ± 0.3b	60 ± 21.2	2.25 ± 0.75	1.25 ± 1.25
F Statistic	5.57*	2.71*	2.82*	0.18 ns	1.20 ns	1.14 ns

Values presented are means ± SE.
* = Significant at $P \leq 0.05$.
Means followed by the same letter(s) are not significantly different from each other at $P \leq 0.05$.

the survival rate of cuttings. Shoot growth (mm) and the length of roots (mm) were measured from cutting stage to new growth after 8 weeks. The number of cuttings callused, rooted and survival rates were counted after 8 weeks.

Data was recorded for the percentage of survival rate of cuttings, number of roots per rooted cutting and root length. Data analysis was performed in two different ways. The first consisted of one way analysis of variance for IBA treatments added to each growth medium separately. The second consisted of factorial analysis including the four growth mediums and the five IBA concentrations. Data on number of cuttings that formed callus, the number of cuttings that rooted and the cuttings that survived were transformed into percentages prior to analysis of variance. Data were presented as mean values with predicted standard errors (S.E.). These computations were done with the software program STATISTICA. The Fisher least significance difference (L.S.D.) was used to compare treatment means at $P \leq 0.05$ level of significance (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of different concentrations of IBA on the four rooting mediums

The four rooting mediums used in our study responded differently to IBA concentrations. The cuttings planted in bark, river sand and polystyrene, and bark and polystyrene rooting mediums were significantly affected by IBA concentrations in certain parameters (Tables 1 and 2). Plant cuttings inserted in peat moss and polystyrene and perlite and river sand mediums at different IBA concentrations influenced the root formation although their differences were not statistically significant (Tables 1 and 2). The IBA supplied to bark, river sand and polystyrene medium significantly ($P \leq 0.05$) affected the percentage survival rates, number of roots formed per cutting and root length (Table 1). The highest survival rate of cuttings was recorded in the 500 ppm treatment (45%), followed by the 1000 ppm treatment (25%). The higher survival rate observed in this rooting medium is probably due to the well drained conditions of the sand component which encouraged adequate oxygen circulation in the rooting zone during the rooting process (Hartmann et al., 2002). The positive fact that the cuttings survived over the 8 week period in this medium is an indication that rooting percentages could be increased if the rooting period was to be extended. According to Brown and Duncan (2006), rooting of cuttings starts between 6 and 8 weeks, and cuttings should be well rooted within 8 to 16 weeks.

The root length in bark, river sand and polystyrene medium supplied with IBA significantly ($P \leq 0.05$) increased from no reaction in the control to 4 mm in the 1000 ppm treatment (Table 1). The root length was significantly reduced to 0.5 mm with successive increases of IBA concentration to 4000 ppm. Taken together (% survival rate, number of roots per cutting and root length) our results indicated that the bark, river sand and polystyrene medium supplied with 1000 ppm IBA was the best treatment as it significantly ($P \leq 0.05$) resulted in successful survival rate of the cuttings (25%), number of roots formed (5) and root length (4 mm) of cuttings (Table 1). The successful rooting of *L. laxum* in this study was achieved at 1000 ppm and concentrations above this level inhibited root initiation and development (Table 1). The results are in agreement with those reported by Hartmann et al. (2002), who found that auxin concentrations of 500 to 1250 ppm are suitable for softwood cuttings and any levels higher than those found in the plant tissues may cause cell death.

The plant cuttings of *L. laxum* supplied with IBA and inserted in the bark and polystyrene rooting medium was significantly ($P \leq 0.05$) influenced only in the roots formed per cutting (Table 2). The control treatment formed 6.5 roots per cutting, and this was followed by supplying IBA at 500 and 1000 ppm (Table 2). Brown and Duncan, (2006), Reinten et al. (2002) and SAFEC (2002) documented the success of bark and polystyrene in rooting Proteaceae. Two other treatments (500 and 1000 ppm) in this medium were also highly significant ($P \leq 0.05$) but resulted in fewer roots (3.5 and 4.3, respectively). The IBA treatments were not as conducive to

Table 2. Effect of different concentrations of IBA on percentage survival rates of cuttings, number of roots per cutting and root length of *L. laxum* grown in bark and polystyrene medium and perlite and river sand medium.

Concentration	Bark and polystyrene			Perlite and river sand		
	% Survival rate	Roots per cutting	Root length (cm)	% Survival rate	Roots per cutting	Root length (cm)
Control	60 ± 16.3a	6.5 ± 2.1a	2.8 ± 0.6a	3 ± 2.5	0.00 ± 0.00	0.00 ± 0.00
500	58 ± 21.8a	3.5 ± 1.2ab	8.8 ± 3.8a	28 ± 14.9	0.00 ± 0.00	0.00 ± 0.00
1000	63 ± 18.9a	4.3 ± 0.8ab	6.5 ± 2.2a	15 ± 8.7	0.25 ± 0.25	0.75 ± 0.75
2000	40 ± 21.2a	1.8 ± 0.6b	12.5 ± 4.7a	5 ± 2.9	0.00 ± 0.00	0.00 ± 0.00
4000	65 ± 12.6a	1.5 ± 0.3b	6.3 ± 1.0a	3 ± 2.5	0.00 ± 0.00	0.00 ± 0.00
F Statistic	0.29 ns	3.09*	1.48 ns	1.83 ns	1.00 ns	1.00 ns

Values presented are means ± SE.
* = significant at $P \leq 0.05$; ns = not significant.
Means followed by the same letter(s) are not significantly different from each other at $P \leq 0.05$.

Table 3. Effect of different growth medium and IBA concentrations on percentage callus formed, % of cuttings that rooted, % survival rates of cuttings, number of roots per cutting and root length of *L. laxum*.

Main (Mediums)	% Callus formed	% Rooted	% Survival rate	Roots/ cutting	Root Length
Bark and polystyrene	30 ± 3.9a	28 ± 4.6a	57 ± 7.6a	3.50 ± 0.62a	7.35 ± 1.38a
Peat moss and polystyrene	19 ± 6.9ab	10 ± 3.7b	57 ± 8.2a	1.40 ± 0.36b	1.35 ± 0.61b
Bark, river sand and polystyrene	10 ± 3.3b	2 ± 0.9bc	19 ± 4.8b	1.20 ± 0.69bc	0.90 ± 0.55bc
Perlite and river Sand	7 ± 3.6b	1 ± 0.5c	11 ± 3.9b	0.05 ± 0.05c	0.15 ± 0.15c
F Statistic	4.49**	16.2***	13.6***	11.15***	18.8***
Sub (IBA- Auxins)					
Control	14 ± 4.6a	6 ± 3.3a	33 ± 9.3a	1.69 ± 0.85ab	0.75 ± 0.34
500	22 ± 7.3a	6 ± 3.0a	43 ± 9.0a	1.06 ± 0.49b	2.50 ± 1.29
1000	21 ± 7.0a	11 ± 3.1a	40 ± 8.4a	2.81 ± 0.85a	2.88 ± 0.99
2000	7 ± 2.4a	14 ± 6.8a	29 ± 8.9a	0.94 ± 0.38b	4.06 ± 1.78
4000	18 ± 5.0a	14 ± 4.2a	34 ± 9.3a	1.19 ± 0.31b	2.00 ± 0.74
F Statistic	1.22 ns	1.15 ns	0.58 ns	2.54*	2.01 ns

Values presented are means ± SE.
*, **, *** = significant at $P \leq 0.05$, 0.01 and 0.001 respectively. ns = not significant.
Means followed by the same letter(s) are not significantly different from each other at $P \leq 0.05$.

rooting however, both mediums contained polystyrene which could be linked with the availability of higher aeration in the rooting zone. Rooting numbers also progressed in this medium owing to higher moisture availability of bark in the rooting medium.

Comparisons in growth mediums and IBA concentrations

Among the four mediums tested, bark and polystyrene was significantly ($P \leq 0.01$) superior to all others in influencing all of the measured parameters (Table 3). The highest percentage of callus formed (30%), rooting (28%), survival rate (57%), number of roots per cutting (3.5) and rooting length (7.35 cm) was found in the bark and polystyrene medium (Table 3). The peat and polystyrene medium produced the second best overall results (Table 3). In comparison with all the mediums used, these two components (bark or peat) provided more moisture in the rooting zone and both had polystyrene for aeration as observed in the higher survival rate of the cuttings (Table 3). Other investigations have revealed that rooting mediums may have a profound effect on root formation of cuttings (Hartman et al., 1990). The aeration and water holding capacity of mediums are of paramount importance (SAFEC, 2002). The cuttings planted in bark, river sand and polystyrene and in polystyrene and perlite showed significantly little effect on influencing callus formation, rooting, survival rate, number of roots per cutting and rooting length (Table 3). These mediums had relatively low water-holding capacities.

With regard to various IBA treatments tested across all treatments, there was no significant influence to percentage callus formed, percentage rooting, percentage

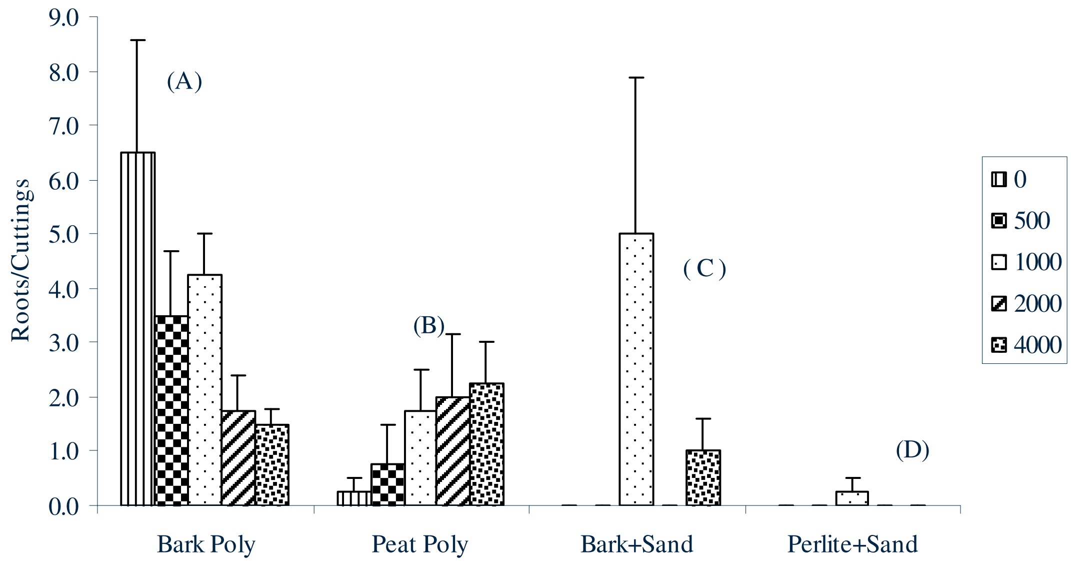

Figure 1. Interactive effects: A) Bark and polystyrene and indole butyric acid (IBA) concentration, B) peat and polystyrene and IBA concentrations, C) bark, river sand and polystyrene and IBA concentrations, D) perlite, river sand and IBA concentrations.

survival rate and rooting length. However, the number of roots per cutting was significantly ($P \leq 0.05$) affected by IBA treatments (Table 3). The IBA supplied at 1000 ppm produced greater root numbers as compared with other treatments (Table 3). The increase in root numbers after the IBA supply has also been reported in plants such as olive, *Dorycnium* spp., *Shorea leprosula*, *Leucospermum patersonii, Leucospermum patersonii* and *Protea obtusifolia* (Alegre et al., 1998; Aminah et al., 1995; Rodríguez Pérez, 1992; Wiesman and Lavee, 1995). In this study, lower concentrations of IBA were thus more conducive to root formation in *Leucadendron*. The rooting at higher IBA concentrations was significantly reduced. Too high concentrations of IBA may be toxic to cuttings of some plant species and may reduce cell differentiation in the tissues and finally affect the rooting (Hartmann and Kester, 1983; Hartmann et al., 2002).

Growth medium x IBA interaction

The growth medium x IBA interaction was significant ($P \leq 0.05$) only for number of roots per cutting (Figure 1). In general, the number of roots per cutting was increased most by IBA concentrations applied to bark and polystyrene and peat and polystyrene mediums. The use of bark or peat in rooting mediums for most Proteaceae is seen as essential as both hold added moisture in the medium (Reinten et al., 2002).

Conclusion

A hormone application of 1000 ppm IBA was successful in rooting *L. laxum.* Both bark, sand and polystyrene and the bark and polystyrene mediums contributed to successful root development of cuttings. However, the bark, sand and polystyrene medium was more successful in the survival rate and root length of cuttings. Further tests on *L. laxum* need to be conducted using different combinations of auxins and different environments to produce maximum quantity and quality rooted plants. The successful production of quality cuttings for the industry is largely defined by the grower who is able to use the correct auxins treatments and rooting mediums to produce quality Proteaceae cuttings which will succeed when planted in the field.

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