

The Effect of Indole Acetic Acid Growth Regulator and Rooting Mediums on Rooting of *Leucadendron laxum* (Proteaceae) in a Shade Tunnel Environment

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Abstract: The effects of different concentrations of IAA exogenously applied to Proteaceae plants of the genus *Leucadendron laxum* on a variety of rooting medium were studied. The aim of the study was to evaluate the rooting response of tip cuttings of nature-grown shrubs using an intermittent mist propagation system in a shaded tunnel. The IAA treatments included a control, 500, 1000, 2000 and 4000 ppm IAA auxins. The growth mediums tested were: a) bark/polystyrene (1:1); b) peat moss/polystyrene (1:1); c) bark/river sand /polystyrene (1:1:1); and d) perlite/river sand (1:1). Each treatment was replicated in ten pots. Overall, the exogenous supply of IAA had a positive effect in the advancement of rooting in *L. laxum* grown in a) peat/polystyrene and b) bark/river sand/polystyrene mediums. With regard to growth mediums, results indicated that the a) bark/polystyrene and b) peat/polystyrene mediums were significantly superior in promoting different aspects of rooting when compared with the other mediums. Evidence from this study was conclusive that the shade tunnel was an economical and successful environment in rooting *L. laxum*.

Abbreviations: IAA = Indole-3-acetic acid; SAFEC = South African Flower Export Council

Key words: Auxin % Bark % Flower industry % Peat moss % Polystyrene % River sand % Threatened species

INTRODUCTION

Leucadendron laxum is one of the world's 329 Protea species and is amongst more than 70 commercial cut flowers of Southern Africa [1]. Most *Leucadendrons* are grown from seed. However, this propagation method is mainly restricted to species grown for retail sales, while vegetative propagation techniques are more widely used in propagating higher quality cultivars and hybrids for commercial cut flower production [2]. Vegetative cuttings also remain cost effective due to the plants flowering a year earlier than seed grown plants [3]. Vegetative propagation techniques for most Proteaceae are broadly described [4] and give little reference to the propagation of individual species.

Certain plant species in the Proteaceae family remain difficult to root [4], and some such as *L. elimense* subsp. *Elimense*, do not propagate vegetatively [5]. The application of auxins such as IAA may induce economically viable results in Proteaceae by increasing rooting percentages, although the aryl esters of IAA are equal or more effective than acid formulations in root

initiation [4]. Additional experiments with auxin formulations in order to increase rooting percentages of woody species remain necessary for the development of commercial crops [4]. Further research on the use of different concentrations of IAA on *L. laxum* in shade conditions will show which treatment can provide faster and more desirable rooting results of vegetative cuttings.

The function of the rooting medium should not be excluded during rooting trials. Rooting mediums are essential in increasing rooting percentages, as the selection and combination of medium components are of importance in the rooting success of any vegetative propagation [6]. The combination of suitable rooting components is essential in providing adequate aeration and drainage to ensure faster and better quality root development. These requirements can be obtained from components such as bark, peat, polystyrene and river sand. Milled pine bark is a medium widely used and must be of good quality [7]. A coarse grade peat moss will enhance the water holding capacity of the medium [4]. Polystyrene will improve aeration and coarse grade, washed river sand will improve the water drainage [8].

The ideal rooting environment is necessary to provide the optimum conditions for successful rooting. The use of a shade tunnel will reduce electricity usage and the maintenance and construction costs compared to environmentally controlled greenhouses. Vegetative propagation techniques were chosen because they provide uniform flowering times and replicate the same characteristics and growth habit as parent stock plants [9, 3]. The results of this study will indicate new study, as the propagation of *L. laxum* and the use of IAA auxins and shaded rooting conditions in Proteaceae have never been recorded.

This study aimed at testing the rooting potential of *L. laxum* in shade tunnel conditions using various strengths of IAA auxin applications and different growth mediums.

MATERIALS AND METHODS

The experiment was conducted at the nursery of the Cape Peninsula University of Technology in Cape Town, South Africa. The experiment lasted for 8 weeks from mid August to mid October. *L. laxum* cutting material was sourced from nature grown populations on the Agulhas Plain and was kept dry and transported overnight to Cape Town.

The IAA treatments and growth mediums were randomised in a complete block design of four concentrations of IAA (control, 500, 1000, 2000 and 4000 ppm) and four growth mediums: a) bark/polystyrene (1:1); b) peat moss/polystyrene (1:1); c) bark/river sand/polystyrene (1:1:1); and d) perlite/river sand (1:1). Each treatment was replicated in ten pots. Tip cuttings were made 150 mm long from semi-hardwood stems [9, 10, 3, 11] and half of the lowest leaves were removed [12, 7]. The cuttings were rinsed in Benlate fungicide (10 g l⁻¹) before planting and sprayed with Captab fungicide (2 g l⁻¹) weekly to prevent fungal infections [3, 11]. Wilted and infected cuttings were removed over the rooting period [9]. Adequate drainage was provided to prevent *Phytophthora* fungal infection of roots.

IAA powder was dissolved at g l⁻¹ in 50% ethyl alcohol. The basal 5 mm of cuttings were dipped for 5 seconds in the rooting hormone [9, 4, 3] and then planted into foam plug trays and placed in a white shade (40%) tunnel. Midday temperatures were measured between 18-25°C and a relative humidity between 20 and 62% was recorded. The irrigation timer was set on 10 sec on and 60 min off.

Data were recorded on the percentage of callused cuttings, rooted cuttings and survival rates. Quality of growth was assessed on the numbers of roots, their length and the length of shoots obtained. These results were transformed into data percentages prior to analysis of variance. Data analysis was performed in two different ways. The first method consisted of one way analysis of variance for IAA treatments added to each growth medium separately. The second method consisted of factorial analysis including the four growth mediums and the five IAA concentrations. 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<0.05 level of significance [12].

RESULTS AND DISCUSSION

Effects of IAA Treatments in A) Peat/Polystyrene and B) Bark/River Sand/Polystyrene Mediums: Statistical analysis of data showed that the addition of different IAA treatments in *L. laxum* had evident significant rooting effect in the a) peat/polystyrene and b) the bark/river sand/polystyrene mediums (Table 1). In the bark/river sand/polystyrene medium, the IAA supplied at 500 and 4000 ppm significantly (P#0.05) produced more roots (3.3 and 5.7, respectively) per cutting than all other treatments (Table 1). Furthermore, the callusing percentage was significantly increased (P#0.05) in the IAA treatment of the peat/polystyrene medium (Table 1). The only significant callusing (30%) of the *L. laxum* cuttings was observed in the 4000 ppm IAA treatment (Table 1). The root length of *L. laxum* was also significantly increased (P#0.01) in the 500 and the 4000 ppm IAA treatments, with measurements of 2.0 and 1.7 mm, respectively (Table 1). Overall, according to the results in (Table 1), the IAA had a positive effect in advancement of rooting in *L. laxum* grown in a) peat/polystyrene and b) the bark/river sand/polystyrene mediums. The overall success of the IAA treatments on the number of roots, callusing and root length in these two mediums are in agreement with Hartmann *et al.* [4] who reported successful effect of IAA in root promotion and development. Similar success in bark/river sand/polystyrene medium indicated a suitable rooting medium for Proteaceae. These results are in agreement with Brown and Duncan [9] and Reinten *et al.* [3] where cuttings required a well drained and light rooting medium.

Table 1: Effect of different IAA concentrations on number of roots per cutting, percentage callus formed and root length of *Leucadendron laxum* grown in peat/polystyrene and bark/river sand/polystyrene medium

	Peat/Polystyrene		Bark/River sand/Poly	
	Number of roots (cutting)	% Callused	Root Length	Number of roots (cutting)
Control	1±0.58b	10±5.8b	0.7±0.33b	1.0±0.58b
500	4±2.00a	13±3.3b	2.0±0.00a	3.3±0.88a
1000	4±1.53a	3±3.3b	0.7±.33b	1.3±0.67b
2000	3±0.58a	13±6.7b	0.0±.00b	0.0±0.00b
4000	3±1.53a	30±5.8 a	1.7±0.33a	5.7±2.19a
F Statistic	0.80*	3.63*	10.0**	4.0*

Values presented are means± SE. *, ** = significant at P#= 0.05 and 0.01, respectively

Means followed by the same letter are not significantly different from each other at P#0.05

Table 2: Effect of different growth medium and IAA concentrations on percentage callus formed, % of cuttings that rooted, % survival rates of cuttings, shoot growth, root length and the number of roots per cutting of *Leucadendron laxum*

Treatment	% Callused	% Rooted	% Survival	Shoot Growth	Root Length	Number of roots/cutting
Main Treatment						
Bark Polystyrene	42±9.2a	29±4.8a	81±4.5a	0.40±0.13b	2.87±0.52a	4.40±0.96a
Peat Polystyrene	31±6.9ab	31±4.0a	79±3.2a	0.53±0.17ab	3.00±0.59a	3.73±0.50ab
Bark River Sand Poly	14±3.1bc	8±1.7b	28±4.7b	1.07±0.28a	1.00±0.22b	2.27±0.69bc
Perlite River Sand	8±2.4c	4±1.3b	29±6.4b	0.20±0.11b	0.60±0.21b	0.93±0.47c
F Statistic	5.3**	18.2***	37.4***	3.53*	9.22***	6.66***
Sub-Treatment						
Control	18±6.5a	12±3.9b	58±9.6a	0.33±0.14a	0.67±0.19b	0.92±0.29b
500	27±9.8a	23±6.9a	58±8.9a	0.75±0.25a	2.58±0.54a	4.33±0.81a
1000	20±7.7a	13±4.1b	52±10.2a	0.33±0.14a	2.00±0.75a	2.58±0.90a
2000	27±9.2a	20±5.5ab	53±10.4a	0.58±0.19a	1.75±0.52ab	3.00±1.06a
4000	28±5.5a	22±3.7ab	50±7.5a	0.75±0.33a	2.33±0.54a	3.33±0.74a
F Statistic	0.32 ns	2.2*	0.46 ns	0.90 ns	2.62*	2.48*

Values presented are means± SE. **, *** = significant at P = 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#0.05

The Effects of Growth Mediums on Callusing, Rooting, Survival, Shoot Growth, Root Length and Number of Roots:

According to the results presented in Table 2, the a) bark/polystyrene and b) peat/polystyrene mediums were significantly superior growth mediums compared with the rest used in this study. These two mediums had the best similar performances in callusing, rooting, survival, shoot growth, root length and number of roots as compared with a) bark/river sand/polystyrene and b) the perlite/river sand (Table 2). For instance, the highest significant (P#0.001) rooting percentage in *L. laxum* was observed in the a) bark/polystyrene and b) the peat/polystyrene mediums with the highest rooting percentages of 31 and 29%, respectively. The a) bark/river sand/polystyrene and b) the perlite/river sand rooting percentages were significantly much lower (8 and 4%) than the first two mediums (Table 2). The survival rate of the cuttings was significantly different (P#0.001) in all four mediums, but more so in the a) bark/polystyrene

and b) the peat/polystyrene mediums (81 and 79%). The *L. laxum* cuttings propagated in the a) bark/river sand/polystyrene and b) the perlite/river sand showed lower survival rates of 28 and 29 percent respectively (Table 2). It is therefore likely that the higher survival measurements in the a) bark/polystyrene and b) the peat/polystyrene mediums were due to the superior moisture holding capacity of the bark and peat and the drainage supplied by the polystyrene compared with the other two mediums used. The fact that a higher survival was recorded in these mediums is an indication that a higher rooting percentage was possible. The rooting period of 8 weeks used in might have been too short. Brown and Duncan [9] reported a rooting period for Proteaceae to be between 8 and 12 weeks, while Kibbler *et al.* [13] suggested that an extended rooting period increases survival and rooting percentages. Extending the rooting period could produce different results. This should be considered in future studies.

Shoot growth was significantly ($P \leq 0.05$) influenced by growth mediums. The a) bark/river sand/polystyrene and b) peat/polystyrene medium were superior with shoots growing to an average of 1.07 and 0.53 mm, respectively (Table 2). The a) bark/polystyrene and b) peat/polystyrene mediums showed less successful shoot growth of 0.40 and 0.20 mm respectively (Table 2). The success in shoot growth in *L. laxum* using bark/peat is in agreement with Matkin [8]. The addition of polystyrene and river sand in some of the mediums provided more aeration and drainage. Species which are slower to root need to stay longer in the medium while the medium must continually supply the optimum rooting requirements to ensure optimum conditions.

The measurements of root length on the cuttings of *L. laxum* showed significant differences ($P \leq 0.001$) with mediums. Values ranging from 3.0-2.87 mm were recorded in a) the peat/polystyrene and b) the bark/polystyrene mediums respectively (Table 2). This was followed by significantly lower values (1.0-0.60 mm) in a) bark/river sand/polystyrene and b) the perlite/river sand mediums, respectively. The root numbers per cutting were significantly ($P \leq 0.001$) influenced by growth mediums (Table 2). The a) bark/polystyrene and the b) peat/polystyrene medium were significantly better in developing roots per cutting (4.4-3.73) as compared with other mediums. Less significant results in the number of roots formed on the stem cuttings was recorded in the a) bark/river sand/polystyrene and b) perlite/river sand medium mediums (2.27-0.93, Table 2). These results are not in agreement with results reported by Lamb [14], where other species rooted faster in a similar rooting mediums. This lower success rate in perlite/river sand could be contributed to the possibility of faster draining capacity of the sand or the fact that perlite water retention of 23 percent [8] was too high for rooting *L. laxum* cuttings.

Results from this study clearly shows that rooting of *L. laxum* was significantly influenced by rooting medium. Overall, the a) bark/polystyrene and b) peat/polystyrene mediums were more responsive to rooting and shooting characteristics than the other two mediums tested (Table 2). The overall results in these two rooting mediums may be attributed to their good aeration characteristics and good water holding capacity [15, 16]. Taken together, these two characteristics are documented to positively favour the rooting physiology in different plant species [17, 18].

The Effect of Iaa Concentrations on Callusing, Rooting, Survival, Shoot Growth, Root Length and Number of Roots: As shown in Table 2 all IAA treatments, including

the control, did not significantly influence the percentage of cuttings that callused and survived and shoot growth in *L. laxum*. However, the IAA treatments significantly ($P \leq 0.05$) influenced the percentage rooting, root length and the number of rooted cuttings in *L. laxum*. The addition of 500 ppm of IAA was the most significant treatment in increasing the rooting percentages (23%) of cuttings (Table 2). These results were closely followed by the 4000 and 2000 treatments which obtained 22 and 20% respectively. The control and 1000 ppm IAA treatment were the lowest in influencing rooting, with 12 and 13%, respectively (Table 2). Results from this study showed that IAA was successful in rooting *L. laxum*. This is in agreement with other research findings which accredited auxins such as IAA in playing a significant role in the stimulation of rooting characteristics in cuttings of different plant species [4]. The rooting efficiency may be influenced by the exogenous supply of auxins, especially in difficult to root plants such as those belonging to Proteaceae family including *L. laxum*. Auxins have been reported to increase the number of roots formed per cutting in different plant species [19].

In the data obtained in Table 2, the root length and number of roots per cutting were significantly ($P \leq 0.05$) affected by all IAA treatments as compared with the control. Root length in these treatments measured between 0.67 in the control to 2.58 in the cuttings supplied with IAA at 500 ppm. The number of roots per cutting counted between 0.92 in the control and 4.33 in the treatment supplied with 500 ppm. Overall, for the number of roots per cutting and root length, the IAA treatment applied at any level produced positive results which were not significantly different from each other when compared with the control treatment. This suggests that the IAA treatments were equally effective in influencing these two parameters. The effects of these IAA treatments are well known to show a significant impact in rooting other plant species [20, 1]. This study also concurs with Hartmann *et al.* [4] theory that a quick dip application of auxin is an effective method of applying auxins to cuttings. Furthermore, shade tunnel conditions was proved sufficient in providing a stable rooting environment for *L. laxum*.

CONCLUSION

The preliminary results from the study indicated that the possibilities exist for the successful vegetative propagation of *L. laxum* under cheaper shaded tunnel conditions that may be affordable to many flower producers in the farming communities with limited greenhouse facilities. In such areas, the use of this

approach in combination with the appropriate doses of IAA and rooting mediums should be able to promote rooting of the difficult to root plant specie such as *L. laxum*. Additionally, this may add value to the rapid multiplication and hence the domestication of this plant species which is listed as endangered in South Africa.

The present study provides new scientific opportunities for the vegetative propagation of *L. laxum*, the rooting possibilities of which were previously undocumented and which remains threatened in South Africa. The reported technology which makes use of a shaded tunnel can be cost effective, sustainable and could be used by flower growers with little resources.

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