Effects of pretreatment of mother plants with BA and concentration of IBA on rooting of *Leucospermum* 'Succession II' stem cuttings

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INTRODUCTION

The commercial export of proteas from the Canary Islands began in 1987, when the first flowers of *Protea obtusifolia* were sent to Europe from Tenerife. Subsequently, this offer was extended with other species and cultivars of the genera *Protea*, *Leucadendron* and *Leucospermum*. Currently, the main interest is in the production of flowers of selected cultivars of *Leucospermum*, since they have a great versatility when they can be used both in a vase, similar to roses and other traditional flowers, as in flower arrangements (centers, baskets , etc.).

Among the pincushions in cultivation, *Leucospermum* 'Succession II' is one of the most interesting for its vigorous growth, its high production of long and straight stemmed flowers and a deep orange color.

This cultivar is propagated by stem cuttings, but the percentage of rooting varies throughout the year, so the scarce nurseries on the islands cannot meet the demand for plants by growers. As a protocol for the micropropagation of this cultivar has not yet been established, nor in general, of commercial-scale *Leucospermum*, the alternative proposed by Norton and Norton (1986) for pretreatment of the mother plants with benzyladenine (benzilaminopurine) (BA) to stimulate the production of lateral shoots, which would be used later as cuttings, could be applied to *L.* 'Succession II'. This procedure has been used successfully to produce basal trunk shoots in *Eucalyptus ficifolia* (Mazalewski and Wesley (1979), to produce cuttings in *Gerbera jamesonii* (Zieslin et al., 1988) and *Spiraea* (Norton and Norton, 1988), to produce lateral shoots in some species of *Tillandsia* (Bessler, 1997).

The recommended substrate for proteas propagation by South African researchers is a mixture of polystyrene and peat (Jacobs and Steenkamp, 1975), and is the one that has been used in the Canary Islands, although coconut fiber alone has also been employed as an alternative to cited mixture. As a growth regulator to stimulate root production, the IBA (indole butyric acid) is used at a concentration of 4000 ppm for semi-hardwood cuttings (Jacobs and Steenkamp, 1975; Malan, 1992 and 2012, Rodríguez-Pérez, 2007), although for less lignified material it is recommend lower concentrations (Harré, 1988).

In this work the effect of the concentration of IBA on the production of rooted cuttings of *L.* 'Succession II' was studied using cuttings prepared from lateral shoots that were developed by pretreatment of mother plants with BA.

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MATERIAL AND METHODS

The assay was carried out between July 2002 and Marzo 2003 at the Escuela T.S. de Ingeniería Agraria, (currently Higher Polytechnic School of Engineering, Agricultural Engineering Section) University of La Laguna, Tenerife, Canary Islands, Spain (28º 29´N).

The first phase of the trial consisted of the treatment of the mother plants with BA, in order to obtain the axillary shoots for the preparation of the cuttings. For this, plants of about 4 years of age established in the experimental fields of the School, were sprayed in summer with a solution of 100 and 200 ppm of BA in distilled water (2 plants per treatment, 2 sprays with intervals of 1 week). A few drops of Tween 20 surfactant was added to each solution before spraying. Leaves were sprayed to drip point. The plants responded to the treatment with the growth regulator by emitting numerous axillary shoots along the stems. The 200 ppm concentration of BA produced the highest number of shoots, but caused burns on some stems.

The second phase of the trial consisted of rooting cuttings prepared from axillary shoots of plants pretreated in summer with 100 and 200 ppm of BA.

Cuttings were prepared on October 10 of the same year. The length of the cuttings varied between 3 and 6 cm, in the case of plants pretreated with 100 ppm of BA, and between 3 and 7 cm in those pretreated with 200 ppm of BA. A randomized block design with 2 x 4 treatments (10 cuttings per treatment) and four replications was used in this experiment. The treatments resulted of the combination of two types of pretreatment (100 and 200 ppm of BA) and four concentrations of IBA (0, 500, 1000 and 1500 ppm of IBA). The total number of cuttings was 320.

Cuttings were removed of leaves on their basal half, and the bases were treated with IBA in a solution of 50% ethanol to give treatments of 0 (control), 500, 1000 and 1500 ppm using the quick-dip method (5 sec), followed by a dip in talc containing benomyl and captan, both at 5% of a.m. concentration. Then they were planted in a mixture of polystyrene foam pellets and peat moss (6:4 v/v ratio) in plastic propagating trays, which were placed on a bed with bottom heat (22 ± 2ºC) in a well-ventilated greenhouse. Irrigation was supplied by microjets from 15-40 s, according to the weather, every 30 min from 9:00 to 17:00 h. Supplementary irrigation was provided when necessary. Cuttings were sprayed weekly with a mixture of benomyl, captan, chlortalonil or ipropdione to control diseases. Every week from the fourth week from planting cuttings were scored according to the following scale: a = alive cuttings; b = cuttings with roots, but not transplantable; c = transplantable cuttings.

Analysis of variance was applied to the data on survival, rooted and transplantable cuttings to test significant differences between treatments. Significant differences in means were separated using Duncan´s multiple comparison test. The data were transformed using the arcsine transformation before analysis of variance was performed. However, the values shown in the tables are original untransformed values.

RESULTS AND DISCUSSION

At 10 weeks from the planting of the cuttings, the first transplantable cuttings were obtained in treatments 2, 3, 4, 6 and 8, in percentages not exceeding 7.5%.

At 16 weeks, 70% of transplantable cuttings were obtained in treatment 4 (cuttings treated with 1,500 ppm of IBA obtained from plants treated with 100 ppm of BA), followed by treatments 2 (cuttings treated with 500 ppm of IBA obtained from plants treated with 100 ppm of BA) with 52.5%, and 8 (cuttings treated with 1,500 ppm of IBA obtained from plants treated with 200 ml of BA) with 45%. There were no significant differences between these treatments (Table 1). The average percentage of transplantable cuttings from plants treated with 100 ppm of BA was 49.4%, 15.8% higher than the cuttings from plants treated with 200 ppm of BA.

Table 1. Percentages of transplantable plants of *Leucospermum* 'Succession II' at 16 and 22 weeks from planting.

Treatments	% transplantables cuttings	
	At 16 weeks	At 22 weeks
1. 100 BA + 0 IBA	32.5 ^{ab}	40.0 ^{bc}
2. 100 BA + 500 IBA	57.5 ^{ab}	62.5 ^{ab}
3. 100 BA + 1000 IBA	42.5 ^{ab}	50.0 ^{bc}
4. 100 BA + 1500 IBA	70.0 ^{a}	82.5 ^{a}
5. 200 BA + 0 IBA	17.5 ^{b}	17.5 ^{c}
6. 200 BA + 500 IBA	37.5 ^{ab}	45.0 ^{bc}
7. 200 BA + 1000 IBA	37.5 ^{ab}	42.5 ^{bc}
8. 200 BA + 1500 IBA	45.0 ^{ab}	50.0 ^{bc}

Within the same column, treatments that show the same letter are not significantly different (P>0.05) to 5%. Treatment notation: BA = benzyladenine; IBA = indole butyric acid.

At the end of the trial, at 22 weeks treatment 4 continued to show the highest percentage of transplantable cuttings (82.5%), followed by treatments 2 (62.5%), 3 (50%) and 8 (50%). There were also no significant differences between these treatments (Table 1). As at 16 weeks, the average percentage of transplantable cuttings from plants treated with 100 ppm of BA (58.8%), was higher than that of cuttings from plants treated with 200 ppm of BA (by 20%). Pretreatment with BA had a significant effect on percentages of rooted cuttings (rooted but not transplantable + transplantable) and transplantable cuttings. Treatment with IBA affected significantly the yield of transplantable cuttings, but the interaction BA × IBA was not significant. IBA treatment significantly influenced cutting survival (Table 2).

Table 2. Effects of pretreatment of mother plants with BA and concentration of IBA on percentages of survival, rooted but not transplantable and transplantable stem cuttings of *Leucospermum* 'Succession II' at 22 weeks from planting.

Treatments	Survival (%)	Rooted (%)	Transplantable (%)
BA (ppm)			
100	87.8	81.0	61.6
200	80.7	49.6	38.1
IBA (ppm)			
0	98.1	49.9	27.4
500	63.6	73.7	54.9
1000	91.8	63.9	45.8
1500	72.9	76.0	70.6
Significance			
BA	ns[1]	**	**
IBA	*	ns	*
BA x IBA	ns	ns	ns

[1]= significant (P<0.05); **= highly significant (P<0.01); [1]ns = not significant. Treatment notation: BA = benzyladenine; IBA = indole butyric acid.

The rooting process is much longer and more expensive than when apical cuttings normally used in commercial nurseries are employed, since it took 22 weeks to obtain rooting percentages greater than 80% when 1500 ppm of IBA were applied to cutttings prepared from mother plants pretreated with 100 ppm of BA, while Rodríguez-Pérez et al. (2003) obtained 95% of rooting in 8 weeks using terminal cuttings of 'Succession II', prepared from mother plants without pretreatment with BA. In any case, pretreatment con BA can be used by nurseries that have a small number of mother plants since at 16 weeks they could obtain 70% of rooted plants, which is commercially acceptable.

CONCLUSIONS

In summary, it seems that cuttings prepared from axillary shoots from plants treated with 100 ppm of BA tend to take root faster and in greater proportion than those prepared from shoots of plants treated with 200 ppm of BA, and within the first, the use of 1500 ppm of IBA seems the most advisable to obtain adequate percentages of transplantable cuttings, from the commercial point of view.

Leucospermum 'Succession II'

Cutting of *Leucospermum* 'Succession II' showing callus at its base

Rooted cutting of *Leucospermum* 'Succession II'

Plants of *Leucospermum* 'Succession II'

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