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Vegetative Cutting Propagation of *Protea* Hybrid 'Susara' (*Proteaceae*)

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Summary

The rooting of stem cuttings of *Protea* 'Susara' (*P. magnifica* x *P. susannae*) was examined with regard to auxin application, basal wounding and position on the shoot. Three levels of indole-3-butyric acid (IBA) (0, 2000 and 4000 ppm) and four types of cuttings (terminal and basal cuttings from the last flush of growth and terminal and subterminal cuttings prepared with complete growth flush from terminal and subterminal flush of growth, respectively) were used. Terminal and basal cuttings from the last flush of growth treated with 4000 ppm of IBA can be employed for the propagation of this protea. The type of cutting did not have an overall effect on rooting ($P > 0.05$). Terminal stem cuttings prepared with complete growth flush can also be used for the propagation of *Protea* 'Susara'. The presence of auxin, or basal wounding, or a combination of both did not improve rooting significantly. Wounding negatively affected rooting. However, in similarly prepared subterminal cuttings, including the complete subterminal flush of growth, the presence of auxin (2000 or 4000 ppm of IBA) was required in order to achieve analogous results.

Key words. wounding – auxin concentration – rooting – cutting position

Introduction

One of the most noteworthy cultivars of the genus *Protea* is *P.* 'Susara' (*P. magnifica* x *P. susannae*), which adapts well to clayey soils, displays vigorous and fast growth and produces an attractive salmon-pink flower. This cultivar has been introduced successfully in Portugal (mainland, Madeira and Azores) and Spain (Canary Islands). Proteas cultivation has also been introduced in the South and East of Spain, Greece and Cyprus.

Protea plant propagation is carried out by stem cuttings. Several *Protea* species such as *P. cynaroides* root easily, while others such as *P. magnifica* present great difficulty. In general, propagation from cuttings of the species and cultivars of genus *Protea* is more difficult and slower than from those of genera *Leucadendron* and *Leucospermum* (Coetzee and Littlejohn 2001). Therefore, several of these commercially highly interesting cultivars require a specific study in order to determine the most suitable techniques and environmental conditions to achieve their efficient propagation, thus ensuring rapid diffusion of this crop.

In many woody species it has been observed that rooting is influenced by the position of the stem on the mother plant (Hartman and Kester 1983; Brits 1986; Montarone et al. 1997). In several species, cuttings from basal and medial positions root best, whereas in other species apical cuttings may root best (Al-Saqri and Alderson 1996). Terminal cuttings of *Leucospermum* cv. 'Succession II' (*L. cordifolium* x *L. lineare*) rooted significantly better than basal cuttings (Rodríguez-Pérez et al. 2003). Peach cuttings from the middle of the shoots rooted better than basal and terminal ones (Tsipouridis et al. 2003).

In *Protea*, terminal cuttings prepared from the last growth flush are normally employed, although subterminal or basal cuttings from that same flush have also been used. The cuttings are treated with IBA (1000–4000 ppm) to enhance rooting. A standard treatment consists in applying 4000 ppm of IBA (Malan 1992). Other growth regulators have also been employed (Criley and Parvin 1979; Gouws et al. 1990). A minimum rooting percentage of 80 %, regarded as ideal for effective nursery propagation, is however seldom achieved (Malan 1992). Specific requirements have to be adapted for each cultivar for optimum results (Harré 1995). Faruchi et al. (1997) reported that the use of semi hardwood cuttings and treating them with IBA 2000 ppm gave the highest rooting percentage in *P. obtusifolia*. Rooting of leaf bud cuttings is also possible in *P. obtusifolia* (Rodríguez-Pérez 1992).

Nurseries using only terminal cuttings often lack sufficient plant material to produce the plants to fulfil the demand. Therefore providing them with the most suitable techniques for use with other types of cuttings would largely improve the quantity and quality of rooted cuttings.

In several plant species, the use of basal stem wounding, either alone or in combination with auxin treatment (IBA), was found to stimulate root formation (Howard et al. 1984; Majumder and Prasad 1988; Al-Salem and Karma 2001). The wounded stem cuttings probably absorb more water and growth regulators from the medium than unwounded cuttings (Howard 1979; Hartman and Kester 1983). The wounded tissues can be stimulated to produce

ethylene that can promote the formation of adventitious roots (HOWARD 1979). The use of this technique in proteas improved rooting of *P. obtusifolia* (RODRÍGUEZ-PÉREZ 1990), *Leucadendron* 'Safari Sunset' propagated in spring, when rooting is more difficult, *Leucospermum cordifolium* 'California Sunshine' and *Leucospermum* 'Succession II' (RODRÍGUEZ-PÉREZ et al. 1993, 2001 and 2003, respectively).

Since an exhaustive search of the literature afforded no information on the plant propagation of *P.* 'Susara', the objective of this study was to determine the effects of the stem cutting position (on the terminal and subterminal flush of growth), of auxin concentration (IBA) and of basal stem wounding on the velocity and rooting percentage of *P.* 'Susara'.

Materials and Methods

General aspects

The assays were carried out between February 1998 and April 1999 at the Escuela T. S. de Ingeniería Agraria, University of La Laguna, Tenerife, Canary Islands, Spain (28° 29' N).

The cuttings were taken from mother plants 6 years old cultivated in the experimental plots of the Escuela. Cuttings preparation began half an hour after harvesting. Cuttings were clipped of leaves on their basal half, the basal 2 mm dipped into a 50 % ethanol solution of IBA for 5 s, followed by a dip in talc containing benomyl and captan, both at 5 % of active materia concentration. Fresh cuts were made at the base of the cuttings shortly before they were dipped in the auxin solution. In wounded cuttings, prior to auxin treatment, two shallow, opposite incisions were made with a sharp blade in the basal bark, penetrating as far as the outer cortex and extending upwards for about 2 cm. Then they were planted in a mixture of polysterene foam pellets and peat moss (6:4 v/v ratio) in plastic propagating trays, which were placed on a bed with bottom heat (24 ± 2 °C) 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 second week from planting cuttings were scored according to the following scale: 0 = dead cuttings; 1 = cuttings without callus; 2 = cuttings with callus; 3 = cuttings with roots, but not transplantable; 4 = transplantable cuttings. Cutting scores were averaged within a treatment to obtain the rooting index. This was calculated every 4 weeks from planting to reflect the quality of the root system produced (CRILEY and PARVIN 1979).

Effect of cutting position on rooting using the standard technique (Expt. 1)

Semihardwood terminal and basal cuttings, 10–12 cm long, were prepared on 11 February, 1998, from the newest flush of growth. Cuttings were treated with 4000 ppm of IBA. A randomized block design was employed with 2 treatments, terminal (Control) and basal cuttings, and 4 replications. The experimental unit consisted of 10 cuttings. The total number of cuttings was 80.

A t-test was applied to the data to detect significant differences between treatments. The data on percentage rooting were transformed using the arcsin transformation to obtain normality of the distribution before t-test was performed. However, the values shown in the text and tables are original untransformed values. The data for rooting index were averaged within a replicate and subjected to analysis of variance, previous testing for normality of the distribution (Komogorov-Smirnov test) and homogeneity of variances (Levene's test).

Effect of cutting position, wounding and IBA concentration (Expt. 2)

Cuttings were prepared on 14 October, 1998, from the terminal and subterminal flush of growth of stems collected from 5 plants. Stems had only two flushes of growth. Each cutting consisted of one flush of growth (terminal or subterminal). The base of the cuttings included the transition zone of one flush to the other, marked by scars left by the budscales of the previous flush of growth. The terminal cuttings were of semihardwood, while the wood of the subterminal cuttings was more lignified. 10 cuttings per treatment were used. The total number of cuttings was 120. The experimental design was a completely randomized block with 2 x 3 x 2 treatments. The treatments resulted from the combination of 2 types of cuttings: terminal and subterminal; 3 concentrations of IBA: 0 (control), 2000 and 4000 ppm; and with or without wounding of cuttings. After cutting preparation, weight, length, number of leaves and basal diameter of cuttings were recorded. Chi square tests for independence were performed at 24 weeks from planting on data of transplantable cuttings. The correlation among weight, number of leaves, diameter and length of transplantable cuttings was studied. A t-test was applied to the rooting index data, previous testing for normality of the distribution (Komogorov-Smirnov test) and homogeneity of variances (Levene's test). Each cutting was considered as a replicate within a treatment.

Results

Effect of cutting position on rooting using the standard technique (Expt.1)

The roots formed from the basal callus in both terminal and basal cuttings. They appeared at 3 weeks from planting. The first transplantable cuttings of both treatments appeared 9 weeks after planting. At the end of the experiment (24 weeks) both treatments showed similar percentages of transplantable cuttings (terminal cuttings = 75 % and basal cuttings = 77.5 %) ($t = -0.100$; $P > 0.05$). The rooting index scores for terminal and basal cuttings were 3.65 and 3.77, respectively, showing also that the type of cutting did not have an overall effect on rooting ($P > 0.05$) (data not shown).

Effect of cutting position, wounding and IBA concentration (Expt.2)

In the unwounded cuttings, roots formed at the base of the cutting or from a wound caused in the cutting during preparation. Calluses appeared in the wounded cuttings

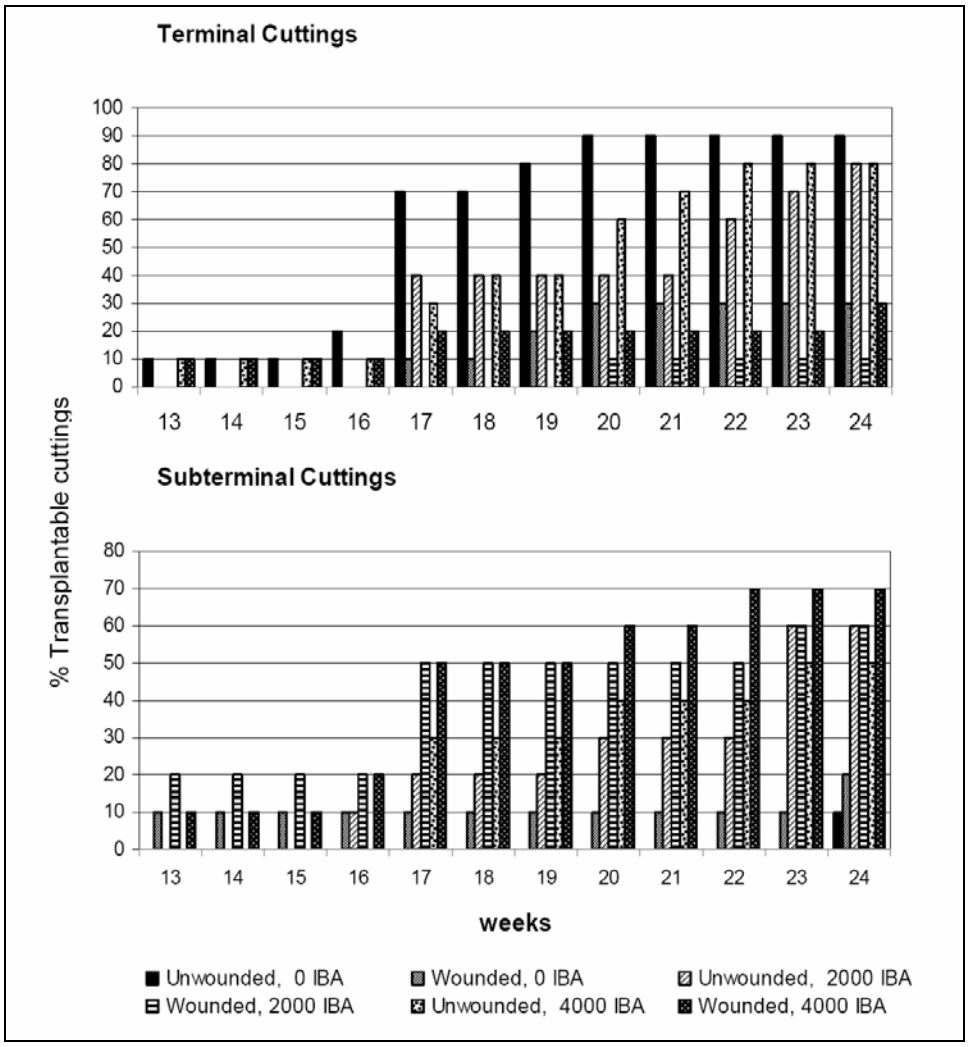


Fig. 1. Influence of cutting position, wounding and IBA on the production of transplantable cuttings of *Protea* 'Susara'

covering the entire incision. Roots appeared from the base of the incisions or along same. The first roots appeared after 4–7 weeks from planting in terminal cuttings and after 4–6 weeks in subterminal cuttings, except in those unwounded and without auxin treatment which started rooting at 11 weeks.

At 13 weeks from planting, the first transplantable cuttings were observed in terminal and subterminal cuttings (Fig. 1). At 20 weeks, unwounded terminal cuttings without IBA treatment showed the greatest percentage of transplantable cuttings (90 %), followed by unwounded terminal cuttings treated with 4000 ppm of IBA and wounded subterminal cuttings with the same IBA treatment (60 % each) and wounded subterminal cuttings treated whith 2000 ppm of IBA and unwounded subterminal cuttings treated with 4000 ppm of IBA (both 50 %).

At the end of the experiment (24 weeks), in terminal cuttings, unwounded cuttings without hormonal treatment showed 90 % of transplantable cuttings, followed by unwounded cuttings treated with 2000 or 4000 ppm of IBA, both giving 80 %. No statistical differences were observed among them (Table 1). Terminal cuttings with wounding presented lower rooting percentages (30 % maximum) and were significantly different from unwounded cuttings. Wounding had a significantly negative effect on rooting. It should be pointed out that the presence of IBA was not necessary to achieve a satisfactory rooting percentage (90 %).

In subterminal cuttings the best results were obtained with wounded cuttings treated with 4000 ppm of IBA showing 70 % of transplantable cuttings, followed by unwounded or wounded cuttings treated with 2000 ppm of IBA, both giving 60 %. No significant differences were observed among them (Table 1). The presence of auxin was necessary to obtain significant rooting percentages. In wounded cuttings, increasing IBA concentration favoured root formation (Table 1)

A comparison of the treatments of the terminal cuttings that gave the highest rooting percentages with those of the most successful subterminal cuttings revealed no significant differences.

In terminal cuttings, the analysis of the rooting index data showed that wounding had a negative effect on rooting, since rooting was significantly affected by wounding ($P < 0.05$) (Table 1). In subterminal cuttings, the analysis of the rooting index data showed that IBA concentration, in general, had a significantly positive effect on rooting ($P < 0.05$) (Table 1).

Significant correlations were established among the weight, number of leaves, diameter and length in the transplantable terminal and subterminal cuttings, taken

Table 1. Effects of cutting position, wounding and IBA concentration on rooting percentage and index of *Protea* 'Susara' at 24 weeks from planting.

Treatments	IBA	Rooting of transplantable cuttings (%)		Rooting index	
Wounding	(ppm)	Terminal[a]	Subterminal[a]	Terminal[b]	Subterminal[b]
Unwounded	0	90 a	10 b	3.8 a	2.0 c
Wounded	0	30 bc	20 b	2.2 b	2.4 b
Unwounded	2000	80 a	60 a	3.7 a	3.1 b
Wounded	2000	10 c	60 a	2.3 b	3.3 a
Unwounded	4000	80 a	50 ab	3.7 a	3.3 a
Wounded	4000	30 bc	70 a	2.6 b	3.3 a

[a]Within the same column, treatments that show the same letter are not significantly different (P > 0.02) to 2 %.
[b]Within the same column, treatments that show the same letter are not significantly different (P > 0.05) to 5 %.

Table 2. Relationship between the length, diameter, weight and number of leaves of cuttings in transplantable terminal and subterminal cuttings of *P.*'Susara'

	Terminal		Subterminal	
Correlation	ρ	Signif.	ρ	Signif.
L-D	0.404	5 %	0.360	n.s.
L-W	0.683	1 %	0.656	1 %
L-NL	0.608	1 %	0.375	n.s.
W-D	0.854	1 %	0.745	1 %
W-NL	0.738	1 %	0.710	1 %
D-NL	0.544	1 %	0.596	1 %

Parameter notation: L = Length; D = Diameter; W = Weight; NL = No. of Leaves.
n.s.: not significant.

together, except in subterminal cuttings which showed a lack of correlatioin between the length and diameter and number of leaves (Table 2).

Discussion

Protea 'Susara' can be propagated by using terminal and basal cuttings prepared from the last growth flush. The position of the cutting above the shoot had no significant effect on the rooting percentage (P > 0.05). These results contrast with those obtained in *Leucadendron discolor* (Rodríguez-Pérez et al. 1997) and *Leucospermum* 'Succession II' (Rodríguez-Pérez et al. 2003) whereby rooting of the terminal cuttings was significantly better than that of the basal cuttings. Brits (1986) also observed significant differences with regard to the position of the cutting, whether terminal or subterminal, in the rooting of several cultivars of *Leucospermum*.

Protea 'Susara' can also be propagated by cuttings that include all the last flush of growth and cuttings including the entire preceding growth flush. The position of the cuttings on the mother plant had also no significant effect on the rooting percentage. The best treatments of each type of cuttings presented satisfactory results (Table 1). However, Montarone et al. (1997), while studying rooting of *Protea eximia*, *P.* 'Cardinal' (*P.eximia* x *susannae*) and *P.* 'Sylvia' (*P. eximia* x *susannae*) cuttings, taken from plants cultivated under soilless conditions, observed a positive acropetal rooting gradient in vegetative stems to the subterminal flush which afforded the best rooting. The terminal flush was incapable of rooting, due to the immaturity of its tissues. It would be worthwhile in the future to continue this research in order to determine the rooting possibilities of flushes preceding the subterminal cuttings, or cuttings prepared from those flushes, in plants cultivated in the field.

While the presence of auxin in terminal cuttings prepared from the last growth flush was not necessary to obtain a satisfactory rooting percentage, treatment with IBA application was essential in subterminal cuttings in order to obtain analogous results (Table 1). Subterminal cuttings were more mature than terminal cuttings, and according to Al-Saqri and Alderson (1996), the woodier the cutting, the higher the IBA concentration required.

Wounding has improved rooting of cuttings of several woody species (Howard 1979). Wounding-related compounds play an important role in rooting (Van der Krieken et al. 1997). Wounding in *Protea* 'Susara' improved absorption of the auxin solution through the incisions, which was observed as capillary action in incised cuttings during the dipping process. However, wounding negatively affected rooting of terminal prepared cuttings including all the last growth flush (Table 1). Jacobs (1981) reported that wounding in proteas at times favoured rooting, while occasionally it had a negative effect. In subter-

minal cuttings, the combined effect of auxin and wounding was not synergistic. The same result was reported by Teklehaimanot et al. (1996) in the propagation of the derived savanna provenance of *Parkia biglobosa*.

Callus formation is important in adventitious root production, as it is often a prerequisite to root initiation (Lovell and White 1986). In wounded cuttings of *Leucadendron discolor* and *L.* cv. 'Safari Sunset' (*Proteaceae*) callus formation was considered a prerequisite to initiation of root primordia (Pérez-Francés et al. 2001). In wounded cuttings of *P.* 'Susara', the formation of two rows of callus nodules was not clearly observed along the edges of each incision, as reported in wounded cuttings of *Leucospermum cordifolium* 'California Sunshine' and *Leucospermum* 'Succession II' (Rodríguez Pérez et al. 2001, 2003). *Protea* 'Susara' is relatively slow rooting (3 or more weeks to start rooting), showing a prolonged phase of dedifferentiation, which precedes the induction phase, i.e. the phase most sensitive to auxin (De Klerk et al. 1999).

Finally, this is the first extensive study of the different types of cuttings for vegetative cutting propagation of a species or cultivar of genus *Protea*, having as plant material plants cultivated in the field. This study provides important information on the most suitable techniques to make optimum use of *P.* 'Susara' plant material. The shoots from which the terminal cuttings are prepared are collected in a similar manner to flower stems, normally including several growth flushes, of which only the apical stem is employed. Under conditions similar to those of this study, it is recommendable, when using terminal or basal cuttings prepared from the last flush of growth, a treatment with 4000 ppm of IBA to get satisfactory rooting percentages. If terminal stem cuttings prepared with complete growth flush are used, it is not necessary the presence of auxin to obtain excellent results. However, in similarly prepared subterminal cuttings, including the complete subterminal flush of growth, the presence of auxin (2000 or 4000 ppm of IBA) is required in order to achieve analogous results.

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