

Short communication. Use of proleptic shoots in the cutting propagation of *Protea* 'Susara' (*Proteaceae*)

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Abstract

This study analyses the influence of auxin and wounding on the rooting of stem cuttings of *Protea* 'Susara' (*P. magnifica* × *P. susannae*) prepared from proleptic shoots. Two assays were carried out. Terminal cuttings, 10-15 cm long, treated with 4000 mg L⁻¹ of indole-3-butyric acid (IBA) and wounded or not were used in the first experiment. The combination of wounding + IBA did not significantly improved rooting compared with the use of IBA alone (70% and 53.3% of transplantable cuttings, respectively). Three levels of IBA (0, 2,000 and 4,000 mg L⁻¹), with or without wounding, and cuttings including the total length of the shoot were used in the second assay. At the end of the trial (24 weeks), wounded cuttings treated with 2,000 mg L⁻¹ or 4,000 mg L⁻¹ of IBA yielded 90% of transplantable cuttings. The combination of wounding + IBA significantly favoured rooting in comparison with the use of IBA or wounding alone.

Additional key words: auxin concentration; proteas; terminal cuttings; wounding.

Resumen

Comunicación corta. Uso de brotes prolépticos en la propagación por estaca de *Protea* 'Susara' (*Proteaceae*)

El objetivo de este estudio fue analizar la influencia de la aplicación de auxina y lesionado sobre el enraizamiento de estacas de *Protea* 'Susara' (*P. magnifica* × *P. susannae*) preparadas a partir de brotes prolépticos. Se realizaron dos ensayos. En el primero se emplearon estacas terminales, de 10-15 cm de longitud, tratadas con 4.000 mg L⁻¹ de ácido indol-3-butírico (IBA) y lesionadas o no. La combinación lesionado + IBA no mejoró significativamente el enraizamiento, en comparación con el uso solo de IBA (70% y 53,3% de estacas trasplantables, respectivamente). En el segundo experimento se utilizaron tres niveles de IBA (0, 2.000 y 4.000 mg L⁻¹) y lesionado o no de las estacas que incluían toda la longitud del brote. Al final del ensayo (24 semanas), las estacas lesionadas tratadas con 2.000 ó 4.000 mg L⁻¹ de IBA produjeron un 90% de estacas trasplantables. La combinación lesionado + IBA mejoró significativamente el enraizamiento, en comparación con el uso solo de IBA o de lesionado.

Palabras clave adicionales: concentración de auxinas; estacas terminales; lesionado; proteas.

Protea 'Susara' (*P. magnifica* × *P. susannae*) has been introduced in the Canaries in the nineties of the last century due to its vigorous and fast growth, its attractive salmon-pink flowers and its tolerance to the clayey soils frequently found in potential cultivation areas.

Protea plant propagation is carried out by stem cuttings. Terminal cuttings prepared from the last growth flush are normally employed, although subterminal or

basal cuttings from that same flush have also been used (Malan, 1992). A standard treatment consists in applying 4.000 mg L⁻¹ of IBA (indole-3-butyric acid) (Malan, 1992). Other growth regulators have also been employed (Criley and Parvin, 1979; Gouws *et al.*, 1990).

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

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Abbreviations used: D (diameter), IBA (indole-3-butyric acid), L (length), NL (number of leaves), W (weight).

and Karam, 2001). The wounded stem cuttings probably absorb more water and growth regulators from the medium than unwounded cuttings (Howard, 1979; Hartmann and Kester, 1983). The use of this technique in proteas improved rooting of *P. obtusifolia* (Rodríguez-Pérez, 1990), *Leucadendron* 'Safari Sunset' and some *Leucospermum* cultivars (Rodríguez-Pérez et al., 1993, 2001, 2003, respectively).

The aim of this work was to study the propagation of *P.* 'Susara' utilizing cuttings prepared from the proleptic shoots bunched below the apex of the flower stems, which are usually discarded when flower stems are collected.

Two assays were carried out between February 1998 and April 1999. The cuttings were taken from mother plants 6 years old. Cuttings preparation began 1/2 h 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 ($24 \pm 2^\circ\text{C}$) in a well-ventilated greenhouse. Irrigation was supplied by microjets (40 L h^{-1}) 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 recommended pesticides 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 (most of roots longer than 3.5 cm). 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).

In the first trial, semihardwood terminal cuttings, 10-15 cm long, were prepared on 20 February, 1998. Cuttings were treated with 4,000 mg L^{-1} of IBA. A randomized block design was employed with two treatments, unwounded (control) and wounded cuttings, and 3 replicates. The experimental unit consisted of 10

cuttings and the total number of cuttings was 60. A *t* test, using SPSS 14.0 for Windows, 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 analysed in the same way.

In the second trial, cuttings were prepared on 21 October, 1998. Cuttings included the total length of the shoot. Ten cuttings per treatment were used. The total number of cuttings was 60. The experimental design was a randomized complete block with 3×2 treatments, without replicates. The treatments resulted from the combination of 3 concentrations of IBA: 0 (control), 2,000 and 4,000 mg L^{-1} , with or without wounding of cuttings. After preparation of cuttings, 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. The data for rooting index were transformed using the arcsin transformation and subjected to analysis of variance. Each cutting was considered a replicate within a treatment.

At the end of the first experiment (24 weeks) treatment using wounded cuttings showed 70% of transplantable cuttings, versus 53.3% in treatment control. There were no significant differences between both treatments ($t = -0.405$; $p > 0.05$). The combination of wounding + IBA did not significantly improve rooting compared with the use of IBA alone. The analysis of the rooting index data showed that wounding had no significant effect on rooting ($t = 0.789$; $p > 0.05$), the rooting scores for unwounded and wounded cuttings being 2.93 and 3.50, respectively (data not shown).

In the unwounded cuttings, a callus formed at the base of the cutting, from which the roots then grew. In the wounded cuttings, the callus appeared on both sides of the incision, although a continuous mass was occasionally observed to fill the incision. The roots fundamentally originated at the base of the incision, and roots were observed along the entire incision only in a few instances.

In the second trial, the first transplantable cuttings could be seen at 12 weeks in wounded cuttings treated with 4,000 mg L^{-1} of IBA (10%) (Fig. 1). At 20 weeks,

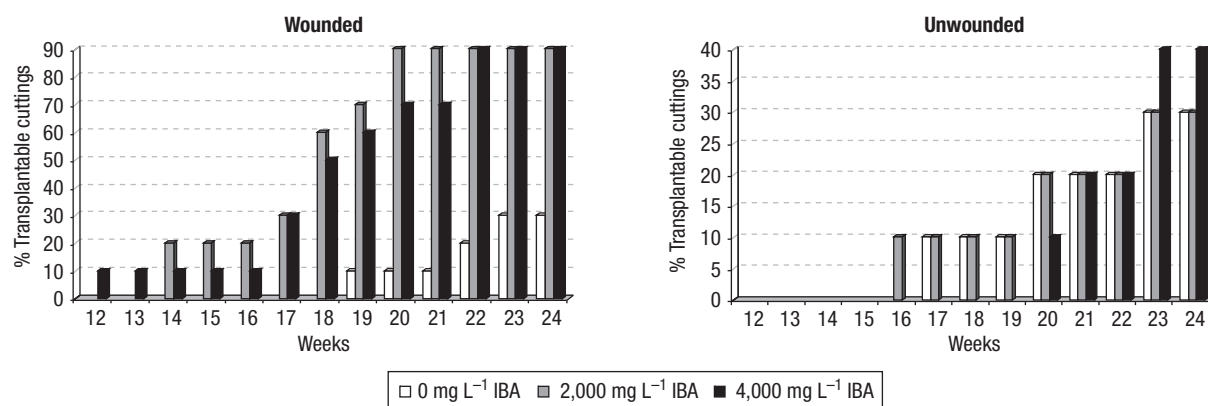


Figure 1. Effect of wounding and IBA concentration on rooting percentage of cuttings prepared including the entire length of proleptic shoots of *Protea* 'Susara'.

wounded cuttings treated with 2,000 mg L⁻¹ or 4,000 mg L⁻¹ of IBA gave 90% and 70% of transplantable cuttings, respectively, while unwounded cuttings without hormonal treatment yielded only 20%. No significant differences were observed between wounded cuttings treated with 2,000 mg L⁻¹ or 4,000 mg L⁻¹ of IBA, but both treatments were significantly different from the remainder of treatments. At the end of the trial (24 weeks), wounded cuttings treated with 2000 mg L⁻¹ or 4000 mg L⁻¹ of IBA yielded 90% of transplantable cuttings. These treatments continued to be significantly different from the remainder of the treatments (Table 1). The analysis of variance of the rooting index data showed that wounding had a significantly positive effect on rooting ($p = 0.035$) at 24 weeks from planting (Table 2). No significant effects of auxin were observed on rooting ($p > 0.05$). The interaction wounding \times IBA was not significant ($p > 0.05$).

Table 1. Effects of wounding and IBA concentration on rooting percentage of cuttings prepared including the entire length of proleptic shoots of *Protea* 'Susara' at 24 weeks from planting

Wounding	IBA (mg L ⁻¹)	Rooting of transplantable cuttings (%)
Unwounded	0	30 ^b
Wounded	0	30 ^b
Unwounded	2,000	30 ^b
Wounded	2,000	90 ^a
Unwounded	4,000	40 ^b
Wounded	4,000	90 ^a

Within the same column, treatments that show the same letter are not significantly different ($p > 0.02$).

Significant correlations were determined among weight (W), number of leaves (NL), diameter (D) and length (L) of transplantable cuttings [L-D: ρ (Pearson's correlation coefficient) = 0.731, signif. 1%; L-W: $\rho = 0.752$, signif. 1%; L-NL: $\rho = 0.732$, signif. 1%; W-D: $\rho = 0.912$, signif. 1%; W-NL: $\rho = 0.747$, signif. 1%; D-NL: $\rho = 0.613$, signif. 1%]. Callus and roots formation was as in the first experiment.

This study has shown that *Protea* 'Susara' can be propagated by cuttings prepared from proleptic shoots. Wounding of terminal cuttings, treated with 4,000 mg L⁻¹ of IBA, improved rooting in comparison with unwounded cuttings, although not significantly. 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*. Subterminal cuttings

Table 2. Index rooting of *Protea* 'Susara' proleptic cuttings prepared including the entire length of shoots after wounding and IBA treatment at 24 weeks from planting

Treatments	Rooting index
<i>IBA (mg L⁻¹)</i>	
0	2.47
2,000	3.35
4,000	2.76
<i>Wounding</i>	
Unwounded	2.66
Wounded	3.50
<i>Significance</i>	
IBA	$p = 0.138$
Wounding	$p = 0.035$
IBA \times Wounding	$p = 0.096$

of *P. 'Susara'* prepared with complete flush of growth from subterminal flush of growth showed the same behaviour (Rodríguez-Pérez *et al.*, 2009). In prepared cuttings including the entire length of the proleptic shoot, wounding significantly improved rooting, as observed for cuttings of other woody species (Howard, 1979). It is possible that as the base of these cuttings were more lignified, the incisions at the end of the stem increased the area for uptake of water and auxin, thus enhancing rooting. The wounded tissues can be stimulated to produce ethylene that can promote the formation of adventitious roots (Howard, 1979). Wounding might have also helped in the stimulation of cell division and promotion of root primordia by better utilising the auxins and other root promoting substances (Malla Reddy, 1991). Wounding-related compounds play an important role in rooting (Van der Krieken *et al.*, 1997).

The use of auxin alone did not improve rooting significantly in prepared cuttings including the entire length of the proleptic shoot. However, the combination of auxin and wounding significantly favoured rooting (Table 1), although it was not confirmed by the rooting index data (Table 2). The combined effect of both factors was synergistic. This result is concordant with that obtained by Howard *et al.* (1984) in M.26 apple rootstocks, Majumder and Prasad (1988) in mango, Rodríguez-Pérez (1990) in *Protea obtusifolia*, and Rodríguez-Pérez *et al.* (1993 and 2003) in *Leucadendron 'Safari Sunset'* and *Leucospermum 'Succession II'*, respectively.

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 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). Rooting of both types of cuttings showed a prolonged phase of dedifferentiation (3 or more weeks to start rooting), which precedes the induction phase, *i.e.* the phase most sensitive to auxin (De Klerk *et al.*, 1999). These results agree with those of Rodríguez-Pérez *et al.* (2009), confirming that *Protea 'Susara'* is relatively slow rooting.

The significant relation among weight, number of leaves, diameter and length of transplantable cuttings

including the total length of the proleptic shoots agree with the results reported by Rodríguez-Pérez *et al.*, (2009), using terminal cuttings prepared from the last flush of growth of *Protea 'Susara'*.

In conclusion, the use of the types of cuttings studied in this work will allow the nursery worker to increase the number of cuttings prepared from mother plants, and therefore the production of plants of this cultivar. Under conditions similar to those of this study, when employing terminal cuttings, the use of 4,000 mg L⁻¹ of IBA would be enough to get satisfactory results. If the cuttings used include the entire length of the shoot, it would be recommendable to employ a combination of wounding + IBA (2,000 or 4,000 mg L⁻¹ of IBA).

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