Micropropagation of *Eucalyptus maidenii* elite trees

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Summary

A method to propagate *in vitro* elite genotypes of *Eucalyptus globulus* Labill. ssp. *maidenii* (F. Muell.) Kirkp. was successfully established. The most favourable period for plant material collection was November to April. In that period the time needed for an epicormic bud to sprout was smaller, and the ratio of sprouted branches and the average number of epicormic buds sprouted were higher than in the other collection time (March to July). To establish axenic cultures the best response was obtained when micropropagation was started from shoot tips. The shoots developed on the Quoirin and Lepoivre (1977) basal medium had the best shape, size and colour. In relation to the growth regulators the best response for multiplication was obtained with 6-benzilaminopurine (BA) and Indolebutyric acid (IBA) and the best treatment was 0.2 mg.L^{-1}/0.02 mg.L^{-1} respectively. Five of 58 clones evaluated were considered to have a good response to multiplication. The best elongation treatment was BA 0.1 mg.L^{-1}/IBA 0.5 mg.L^{-1}. This treatment produced 4 shoots elongated per explant, sizing more than 2 cm. The rooting response was variable depending on the clone and the treatment, and it did not surpass 25%. The rooted shoots were successfully transferred into potting soil (1peat:$1^{1/2}$ coconut fibre:1sand) in the greenhouse. After 30 months of outplanted, the survival percentage observed was around 80%, a percentage comparable to that obtained on plantations established from seeds, according to the company registers.

Key words: *in vitro* plant culture, rooting, 6-benzylaminopurine, indolebutyric acid, *Eucalyptus maidenii*

Resumen

Micropropagación de árboles elite de *Eucalyptus maidenii*

Se estableció un método para propagar *in vitro* genotipos elite de *Eucalyptus globulus* Labill. ssp. *maidenii* (F. Muell.) Kirkp. El período más favorable para la colecta del material vegetal fue de noviembre a abril. En este período el tiempo necesario para la brotación de una yema epicórmica fue menor, la proporción de ramas brotadas y los valores medios de yemas epicórmicas brotadas fueron mayores que en el período de marzo a julio. El método más adecuado para establecer cultivos axénicos fue la introducción a partir de ápices meristemáticos. Los brotes desarrollados en el medio basal de cultivo Quoirin and Lepoivre (1977) tenían la mejor forma, tamaño y color. En relación a los reguladores de crecimiento los mejores resultados de multiplicación fueron obtenidos con la combinación de 6-benzilaminopurina (BA) y ácido indolbutírico (IBA) 0.2 mg.L^{-1}/0.02 mg.L^{-1} respectivamente. Cinco de 58 clones evaluados tuvieron una buena respuesta a la multiplicación. El mejor tratamiento de elongación fue BA 0.1 mg.L^{-1}/IBA 0.5 mg.L^{-1}, produciéndose 4 brotes elongados por explanto de más de 2 cm. El enraizamiento fue variable dependiendo del clon y del tratamiento, no sobrepasando el 25%. Los explantos enraizados fueron transplantados exitosamente en sustrato (1 turba:1 ½ fibra de coco: 1 arena) y aclimatados en invernáculo. Después de 30 meses de crecimiento a campo, la tasa de sobrevivencia fue de 80%, resultados comparables a los obtenidos por la empresa en plantaciones a partir de semillas de la misma especie.

Palabras clave: cultivo de plantas *in vitro*, enraizamiento, ácido indol butírico, 6-benzylaminopurina, *Eucalyptus maidenii*

Introduction

The well-known advantages of plantations of vegetatively propagated eucalyptus, namely uniformity, adaptation, cost and wood production make it attractive to develop a breeding program for *Eucalyptus globulus* Labill. ssp. *maidenii* (F. Muell.) Kirkp. This specie showed higher volume growth, good shape, higher wood density compared with *E. globulus* ssp. *globulus* in studies developed in north of Uruguay (Resquin and Balmelli, 1999).

In horticulture amenable clones have long been propagated by stem cuttings, but with the exception of a few easy-to-propagate species like poplars and willows, clonal forestry is recent. Much of the pioneering development work has been done by trial and error, which has given good initial progress, for example in some pines and eucalyptus. However, at present, only the easiest-to-propagate clones are acceptable for large scale production, often a small minority of the total within a species, and the basis of propagation ability is poorly understood (Wilson, 1998).

Micropropagation has the potential to provide very high multiplication rates of selected tree genotypes, with resulting short-term silvicultural gains. Methods for tissue culture propagation of some *Eucalyptus* species were described previously (Bonga and Von Aderkas, 1992; Bandyopadhyay, 1999; Calderon, 1994; Debergh and Read, 1991; Guimaraes *et al.*, 1997; Mac Rae and Cotterill, 1997; Texier and Faucher, 1985; Watt *et al.*, 2003; Bennett *et al.*, 2004). Aseptic cultures have been established from seeds, seedlings, shoots, flowers and lignotubers. Callus cultures have been established from a wide range of tissue sources for at least 30 species of *Eucalyptus*. Plant regeneration from callus was successful for 12 of these species. Micropropagation through axillary proliferation, or adventitious shoot proliferation on nodal explants, or both, has been successful (Le Roux and Van Staden, 1991). However, to date, there have been no reports concerning *in vitro* plant regeneration of *Eucalyptus globulus maidenii*.

The main objective of this work was to develop a method to propagate *in vitro* elite genotypes of *Eucalyptus maidenii* selected from plantations designated for paper pulp production.

Materials and methods

Plant material

Seventy three "elite" trees were selected from 3 - 8 year old *Eucalyptus globulus* Labill. ssp. *maidenii* (F. Muell.) Kirkp. (*E. maidenii*) plantations originated from Australian's seeds (Bolaro, latitude 35º 40', longitude 150º 02', source: CSIRO, Australia) growing in Uruguay (Tacuarembó, latitude 31º 42', longitude 55º 59'). The trees were selected based on growth, form, wood density, frost tolerance and sanitary conditions. Five to ten branches of 50 x 3 cm were collected from each 'elite' tree during two periods of time (November to April and March to July). Branches were brought to the laboratory in plastic bags with cotton soaked in citric acid solution 100 $mg.l^{-1}$. The bags were sealed and kept in temperatures around 4º C for its transport. Once in the laboratory the branches were sealed with paraffin and kept with their bases in tap water with Benlate® 2 $g.L^{-1}$ and Captan® 2 $g.L^{-1}$ in a greenhouse. 6-benzylaminopurine (BA) 5 $mg.l^{-1}$ was used to induce the production of epicormic shoots.

The following parameters were evaluated for each "elite" tree: 1) time needed for an epicormic bud to sprout, 2) ratio of sprouted branches, 3) ratio of sprouted "elite" trees, 4) average number of epicormic buds sprouted, 5) number of times the branches sprout during a given period. A generalized linear model (McCullagh and Nelder, 1989) assuming binomial distribution and link function logit was used to analyse the statistical differences of the ratio of sprouted "elite" trees and sprouted branches. Poisson distribution and link function logarithm was used to analyse the statistical differences of the average number of epicormic buds sprouted and the time needed for an epicormic bud to sprout.

Surface sterilization

Cultures were initiated from shoot tips and nodal pieces of young epicormic shoots obtained from lateral buds of branches. The explants were washed with water and soap and surface sterilized with Benlate® 2 $g.L^{-1}$ and Captan® 2 $g.L^{-1}$ for 60 min and sodium hypochlorite 0.1% (v/v) for 5 min. Then washed 3 times with sterilized water and immersed in Polyvinil pirrilidona (PVP) 1.5 $g.L^{-1}$ for 60 min at 4º C.

For the experiments initiated from shoot tips, the shoot tips with four leaf primordia were dissected under microscope and cultured on haemolysis tubes. For the experiments initiated from nodal pieces, pieces with one or two buds were cut and cultured in tubes (20 mm x 25 cm).

Culture media

1. Basal Media

For the basal media, the mineral salts, full strength and three dilutions (1/2, 1/3, 1/4) of Murashige and Skoog (1962) And Quoirin and Lepoivre (1977) (QL) and the vitamins of de Fossard (1974) were tested. All the media contained sucrose at 30 $g.L^{-1}$ and were solidified with 10 $g.L^{-1}$ of agar, unless stated otherwise. The pH was adjusted at 5.8 and autoclaved (1.1 $kg.cm^{-2}$ at 121º C for 20 min) after addition of growth regulators.

2. Initiation media

For the initiation phase in experiments from nodal pieces, a cytokinin, 6-benzylaminopurine (BA) ranging in concentration from 0.01 $mg.L^{-1}$ to 0.1 $mg.L^{-1}$ and two auxins, indolebutyric acid (IBA), naphthalenacetic acid (NAA) ranging in concentration from 0.01 $mg.L^{-1}$ to 0.1 $mg.L^{-1}$ were tested in combinations. The nodal pieces were subcultured in the initiation media and after 20 days were transferred to multiplication media for rapid multiplication. When the explants were shoot tips, giberelin (GA_3) at 0.035 $mg.L^{-1}$ was tested for the initiation phase. After 10 days the shoot tips were transferred to multiplication media and subcultured in intervals of 20 days until they reached a good size for rapid multiplication. Shoot health was assessed by counting the number of explants contaminated and the number of oxidized explants. Each treatment contained 5 replications with at least 10 explants per replication. The rate of contamination was assessed and data analysed with ANOVA and Tukey's Test ($p = 0.10$).

3. Multiplication media

For shoot tip culture a combination of BA ranging in concentration from 0.1 $mg.L^{-1}$ to 0.01 mg L^{-1} and IBA 0.01 $mg.L^{-1}$ was tested. For rapid multiplication two cytokinins BA/Kinetin (KIN) (1 $mg.L^{-1}$ to 0.2 $mg.L^{-1}$) in several combinations with two followings auxins IBA (0.01 $mg.L^{-1}$ to 0.05 $mg.L^{-1}$) or NAA (0.01 $mg.L^{-1}$ to 0.05 $mg.L^{-1}$) were tested. Each treatment contained 5 replications with at least 10 explants per replication. Shoot multiplication was assessed by counting the number of shoots obtained from a single initial shoot after 5 subcultures and data analysed with t Student Test ($p = 0.001$).

4. Elongation media

Six different treatments for shoot elongation were tested:

1) BA 0.1 $mg.L^{-1}$/IBA 0.5 $mg.L^{-1}$; 2) 6-furfurylaminopurine (kinetin) 0.1 $mg.L^{-1}$/IBA 0.5 $mg.l^{-1}$; 3) BA 0.1 $mg.L^{-1}$/IBA 0.5 $mg.L^{-1}$/activated charcoal 5 $g.L^{-1}$; 4) GA_3 0.1 $mg.L^{-1}$; 5) IBA 0.5 $mg.L^{-1}$/GA_3 0.1 $mg.L^{-1}$; 6) activated charcoal 5 $g.L^{-1}$.

Each treatment contained 5 replications with at least 10 explants per replication. Elongation was assessed counting the number of shoots elongated per explant and their length and visual appearance and data analysed with ANOVA and Tukey's Test ($p = 0.10$).

5. Rooting induction

Individual shoots were cut and exposed to 6 different induction treatments:

1) ¼ Basal media mineral salts + thiamine 10 $mg.L^{-1}$ + sucrose 20 $g.L^{-1}$ + IBA 1 $mg.L^{-1}$, placed in the dark during 7 days.

2) ¼ Basal media mineral salts + thiamine 10 $mg.L^{-1}$ + sucrose 20 $g.L^{-1}$ + IBA 2 $mg.L^{-1}$, placed in the dark during 7 days.

3) and 4) the same media were tested in light.

5) Immersion during 30 s in IBA 5 $mg.L^{-1}$.

6) Immersion during 30 s in IBA 10 $mg.L^{-1}$.

6. Root growth.

¼ Basal media mineral salts + thiamine 10 $mg.L^{-1}$ + sucrose 20 $g.L^{-1}$.

After rooting induction treatment, the explants were transferred to root growth media.

Each treatment contained 5 replications with at least 10 explants per replication. The number of explants rooted were counted for establish rooting ability and data analysed with ANOVA.

Culture condition

Cultures were incubated in a 16/8 h light/dark photoperiod at temperatures of 24 ± 2º C. Light intensity was 12 $\mu mol.m^{-2}.s^{-1}$ for culture initiation, 18 $\mu mol.m^{-2}.s^{-1}$ for multiplication and elongation and 36 $\mu mol.m^{-2}.s^{-1}$ for root growth and acclimatisation.

Transfer into soil

After the adventitious shoots established a root system on the agar growth root media, they were taken out, washed free of all agar and planted into potted soil mix of 1 peat:1½ coconut fibre:1 sand and maintained in the greenhouse for 6 weeks until the plants were well established. For the first two weeks, the pots were kept in a bag to maintain high humidity and then the bags were gradually opened to acclimatise the plants. Some of these were then outplanted in the field to establish a clonal trial to evaluate their performance in the field. After 30 months of the trial set up, plant survival, height and diameter were evaluated.

Results and discussion

Sprouting ability

The epicormic sprouting success (Fig. 1A) of 73 'elite' trees and the number of days required to sprout in greenhouse conditions were assessed. The material evaluated came from three sites: El Puente, Agua Clara y Cacique Cepê and two collection time were evaluated.

With regard to the ratio of sprouted 'elite' trees no significant differences were observed between the collection time of branches (Table 1). From the 73 trees evaluated, 15 did not sprout. This fact may have been due to suboptimum conditions of the branches such as diameter and health, and/or a sprouting disability of the tree.

On the other hand, it was observed that the sprouting ability after stimulation with citokinins (assessed as the time needed for an epicormic bud to sprout, the ratio of sprouted branches and the average number of epicormic buds sprouted) differed with the collection time (Table 1). The time needed for an epicormic bud to sprout was smaller, the ratio of sprouted branches and the average number of epicormic buds sprouted were higher for branches collected between November and April than for those collected between March and July (Table 1). From these results we conclude that the sprouting induction of epicormic buds varies with branch collection time, with a probability of 0.0001 that it was a hazardous event. We propose that the November to April collection time is the most favourable because the mother plants are in full activity. In this period the environmental conditions correspond to the annual growth cycle in which some tissues and particularly the meristems increase their morphogenetic plasticity (Bonga and Von Aderkas, 1992).

Other factors that can affect the epicormic sprouting induction response are the age of the mother plant (Table 2). It has been found that the ratio of sprouted 'elite' trees for mother plants of 8 years old was significantly lower than for mother plants of 3 and 5 years old (Table 2). Furthermore, the time needed for an epicormic bud to sprout was significantly lower for the branches collected from the mother plants of 3 years old than from those collected from mother plants of 5 and 8 years old (Table 2). In this sense, the existence of a juvenility gradient has been recognized for forest trees (Eldridge *et al*., 1997). An hypothesis proposing a cycle of senescence and rejuvenation in plants would explain why juvenile zones could be found in the crowns of mature trees (Waering, 1959). The duration of the juvenile stage becomes shorter the greater the distance from the roots that each cyclical flush occurred. Thus in the very mature stages of some species the juvenile stage might be absent. In this way, the juvenility stage of trees can explain the sprouting response.

Table 1. Influence of collection time on the ratio of sprouted 'elite' trees, the time needed for an epicormic bud to sprout, the ratio of sprouted branches and the average number of epicormic buds sprouted per branch.

Branches collection time	Ratio of sprouted 'elite' trees	Time needed for an epicormic bud to sprout (days)[1]	Ratio of sprouted branches	Average number of epicormic buds sprouted/branch
November-April	87.8 ± 14.6 a	14.3 ± 0.5 a	86.3 ± 4.5 a	5.3 ± 0.4 a
March-July	68.8 ± 20.7 a	23.8 ± 0.8 b	51.8 ± 5.6 b	2.6 ± 0.4 b

[1]Days required from BA application to sprout initiation. The values correspond to the mean and standard deviation.
Different letters indicate significant differences from other treatments.
p = 0.0001

Some of the trees of Agua Clara were in bloom at the moment of branch extraction. This means that the mature state has begun and may be the reason why the ratio of sprouted 'elite' trees was smallest or the time needed for an epicormic bud to sprout was longer for branches from this site (Table 2).

Initiation

With respect to the kind of explants used to initiate the culture, with shoot tips axenic cultures were established and maintained during the process of micropropagation. Nodal pieces (Fig. 1B) were a good explants source for micropropagation in regard to the generation rate but had the disadvantage of a higher rate of contamination (80% compared with 30% for shoot tips). This method is not profitable because of the high cost on labour and culture media used, due to the high subculture frequency needed to avoid bacteria proliferation. The use of cultures obtained from shoot tips was adopted to all the experiments including basal media evaluation and hormonal treatments.

The percentage of clones showing shoot induction on the QL basal medium (60%) differed significantly ($p = 0.05$) from that obtained in MS medium and its dilutions (15%). Furthermore, QL developed the best quality of shoots (size and colour), in contrast these clones developed vitrification on MS medium. Many hypotheses have been proposed to explain vitrification, such as: use of liquid medium, high Cl^- and NH_4^+ concentrations, high citokinine concentrations and low agar concentrations (Böttcher *et al.*, 1988). Therefore, the QL medium was used in all our hormonal experiments reported here.

Multiplication

The response of the clones to the different multiplication treatments was evaluated with qualitative indicators like shoot vigour (colour and consistency) and basal callus formation or callus formation on leaves; and quantitative indicators such as multiplication rate (number of shoots obtained from a single initial shoot after 5 subcultures).

In relation to the growth regulators, the combination of BA and IBA gives an optimal hormonal stimulation for rapid multiplication and the best treatment was BA 0.2 $mg.L^{-1}$/IBA 0.02 $mg.L^{-1}$, the differences between this treatment and the rest was highly significant ($p < 0,001$) (Fig. 2). In general, this treatment promoted higher rates of shoot multiplication and they appeared healthier.

When NAA was used in multiplication media no desirable results were found due to the induction of callus formation in the base of the explants and on leaves that lead to poor bud developing. Kinetin was also ineffective because induced red leaves of small size and retarded growth.

BA has been the growth regulator more used in culture media for eucalyptus species to induce the proliferation of axilary buds (Del Ponte *et al.*, 2001; Alves *et al.*, 2004). It was found that BA produced more vigorous shoots, while kinetin induces small leaves, some of which had modified shapes (Trindade, 1990).

For *E. globulus* it has been reported that IBA promoted the best multiplication rates whereas IAA and NAA retarded growth, and in some cases produced explants death (Trindade, 1990). This author found that IBA plus BA was the best combination for rapid

Table 2. Influence of age and site on the ratio of sprouted 'elite' trees, the time needed for an epicormic bud to sprout, the ratio of sprouted branches and the average number of epicormic buds sprouted per branch.

Age, Site	Ratio of sprouted 'elite' trees	Time needed for an epicormic bud to sprout (days)[1]	Ratio of sprouted branches	Average number of epicormic buds sprouted/branch
3, Cacique Cepê	87.8 ± 5.2 a	14.3 ± 0.5 a	86.3 ± 4.3 a	5.3 ± 0.4 a
5, El Puente	82.6 ± 7.9 a	23.4 ± 0.9 b	46.6 ± 6.0 b	2.4 ± 0.4 b
8, Agua Clara	33.3 ± 15.3 b	26.0 ± 2.4 b	75.0 ± 11.1 ba	4.0 ± 1.2 ab

[1]Days required from BA application to sprout initiation. The values correspond to the mean and standard deviation.
Different letters indicate significant differences from other treatments.
$p = 0.0001$

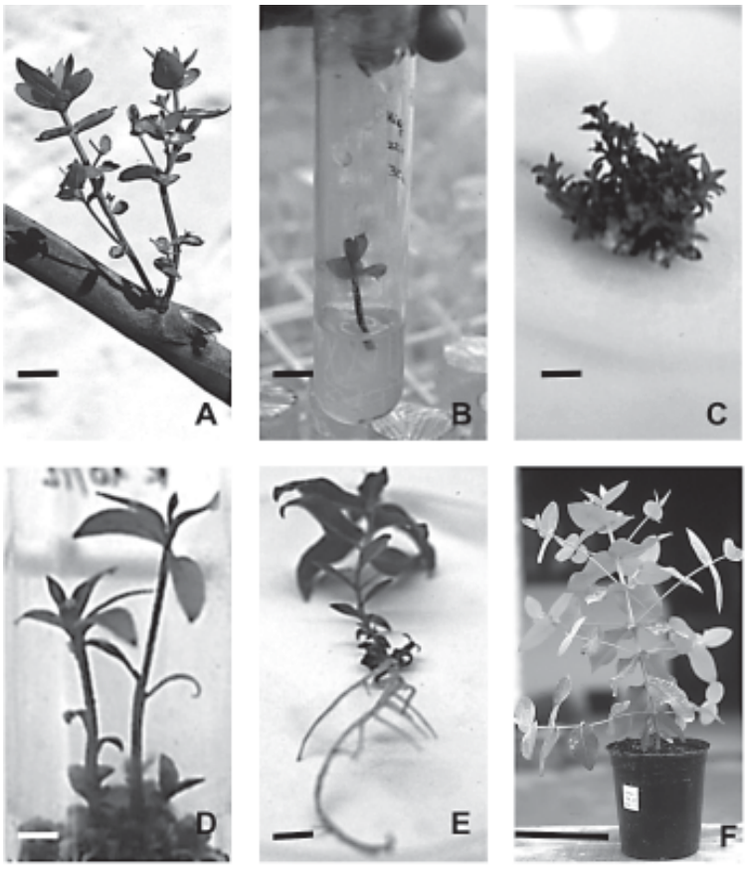

Figure 1. Micropropagation steps of *E. maidenii* (A) Epicormic shoot > 3 cm (bar 1 cm) (B) Culture initiation from nodal pieces (bar 1cm) (C) Explant in rapid multiplication (bar 1 cm) (D) Elongation phase (bar 0.5 cm) (E) *In vitro* rooted shoot in IBA 2 mg.L^{-1} (induction in the dark), showing a good root-shoot connection (bar 1 cm) (F) Micropropagation derived plant acclimatised in greenhouse conditions (bar 10 cm).

multiplication during large subculture periods. For other authors the best combination for *E. globulus* was BA and NAA (Serrano *et al.*, 1996; Calderon, 1994; Salinero, 1985) or BA and KIN. In accordance with the results obtained in this work for *E. maidenii* shoot multiplication with BA, IBA or NAA in different combinations, the best growth regulator combination (BA/IBA) (Fig. 1C) was similar to that used by Trindade, 1990 for *E. globulus*. As far as we know is the first time that data on *E. maidenii* micropropagation are reported.

Five of 58 clones evaluated were considered to have a good response to multiplication. The clones named 1, 9, 77, 79, 82 developed an average multiplication rate of 4. The multiplication rate on eucalyptus depends on the species, the clone and whether the shoots were juvenile or mature. In *E. margirata* multiplication rates from 3 to 7 in 4 weeks were registered in materials proceeding from seeds, while in materials proceeding from adult trees lower rates (between 2 and 5) were reached in the same period of time (Castro and González, 2002). In this way the results obtained in this work with *E. maidenii* were similar to those obtained with other species of eucalyptus.

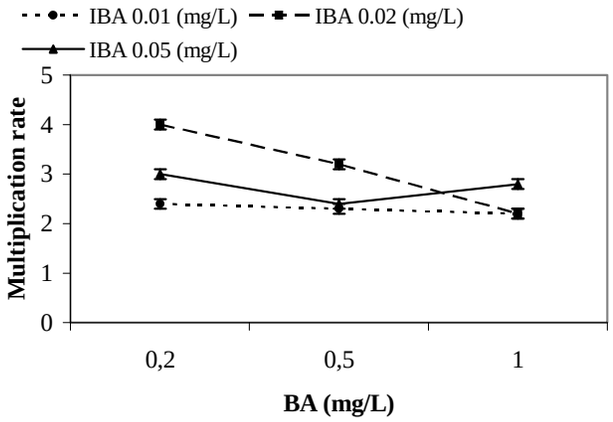

Figure 2. Effect of benzylaminopurine (BA) in combination with various indolebutyric acid (IBA) concentrations on the multiplication rate of shoots of *E. maidenii* after 5 subcultures. Values are means of 5 replications with at least 10 explants per replication. The error bar represents the standard deviation. The best treatment was BA 0.2 mg.L^{-1}/IBA 0.02 mg.L^{-1}, the differences between this treatment and the rest was highly significant ($p < 0.001$).

Elongation

To favour elongation different strategies were used, such as transfer into liquid medium, reduction of citokinin concentration, addition of giberelic acid and activated charcoal with different success depending on the specie (Calderón, 1994).

For elongation, as for rooting phase, the work was done with 5 clones that had a good response to multiplication.

We have found that only treatment 1(BA 0.1 mg. L^{-1}/IBA 0.5 mg.L^{-1}) and 3 (BA 0.1 mg.L^{-1}/IBA 0.5 mg.L^{-1} and activated charcoal 5 g.L^{-1}) differed significantly from the rest at p = 0.10. They produced 4 shoots elongated per explants, sizing more than 2 cm. Activated charcoal in the medium produced good elongation but also produced callus in stem and leaves which made the subsequent micropropagation steps difficult.

The use of GA_3 or kinetin produced weak elongation (no more than 3 mm) and deformed explants.

We have found that the best elongation treatment was BA 0.1 mg.L^{-1}/IBA 0.5 mg.L^{-1}, because it produced 4 shoots elongated per explants, sizing more than 2 cm and promoted the explants hardening (Fig. 1D).

Rooting

The rooting response to different treatments was assessed after four and ten months of multiplication.

The induction treatments consisting in auxin immersion were discarded because they produced sporadic rooting unlike the best treatment for rooting *E. globulus*, which was a rapid immersion in IBA (Salinero, 1985).

Rooting percentage per clone and the treatment used for rooting induction are presented in Table 3. There were no significant differences between these treatments. The rooting response was variable depending on the clone and the treatment, and it did not surpass 25%, but it is very important to note that the explants rooted had a good root-shoot connection (Fig. 1E) and rarely formed intermediate callus. In this study a better result was obtained to rooting if the explants were placed in the dark during the induction phase.

Adventitious rooting is essential for vegetative propagation of woody species. The effects of light and auxins in the root formation phase are well known. *E. globulus* has been found to be recalcitrant to rooting if the explants are illuminated during the formation phase of rooting. It was found that rooting was effective when the explants were exposed to IBA 10 mg.l^{-1} during 4 days and the root formation phase was developed in the dark. It is also known that rooting ability decreases with the age of the mother plant, and this fact is more important in some species than in others, for example it is more important for *E. globulus* than in *E. saligna* (Fett-Neto, 2001).The IBA requirement is variable and inherent to genotypes and to the culture conditions previous to rooting, such as number of subcultures, kind and concentration of growth regulators, juvenility and elongation (Del Ponte *et al.*, 2001).

Table 3. Average rooting percentage in different clones and treatments.

Clone	Treatment IBA	% rooting
1	1 mg.l^{-1}	8.1
1	2 mg.l^{-1}	12.9
9	1 mg.l^{-1}	23.1
9	2 mg.l^{-1}	16.3
77	1 mg.l^{-1}	13.0
77	2 mg.l^{-1}	25.9
79	1 mg.l^{-1}	11.1
79	2 mg.l^{-1}	16.7
82	1 mg.l^{-1}	2.6
82	2 mg.l^{-1}	5.0

Data were collected from 50 explants per treatment (5 replications with at least 10 explants per replication). Analysis of variance did not detect any significant difference between treatments. The explants were placed in the dark during 7 days of the induction treatment.

The results obtained for *E. maidenii* were in accordance with the data found for other *Eucalyptus* species in which the variation in response of different clones highlights the importance of examining a range of genotypes before concluding that a species responds in a particular way to plant growth regulators in *in vitro* culture media (Bennett *et al.*, 1994).

The rooted shoots were successfully transferred into potting soil (1 peat:$1^{1/2}$ coconut fibre:1 sand) in the greenhouse (Fig. 1F). The survival percentage (Table 4) observed was in the range of that obtained for plantations established from seeds according with the company registers.

It is not possible to make statistical comparisons between the volumes of plantations from seeds and the

Table 4. Clonal performance in the field.

Sowing date	Average diameter (cm)	Average height (m)	Average volume/tree (m^3)	% Survival
Spring	7.6	6.7	0.0232	89.4
Fall	2.4	3.0	0.0014	73.9

clonal trial since the clones did not reach half rotation age which is the age for final assessement of eucalyptus (Eldridge *et al*., 1997). However, an approximation can be determined from the comparison of the volume of 30 months plantation from seeds with a commercial volume of 17.1 m^3/ha, considering 1250 plants/ha, and from clones, with a commercial volume of 29 m^3/ha, which represents 11.9 m^3/ha more.

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