

TOWARDS AN EFFICIENT REGENERATION PROTOCOL FOR *EUCALYPTUS UROPHYLLA*

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The aim of the present study was to establish an efficient regeneration system for *Eucalyptus urophylla* by means of organogenesis. The hypocotyls from seedlings of *E. urophylla* clone U6 were used as explants and cultured in a modified Murashige and Skoog (MS) medium, supplemented with 13.2 $\mu\text{M L}^{-1}$ N-phenyl-N'-[6-(2-chlorobenzothiazol-yl)] urea (PBU) and 0.285 $\mu\text{M L}^{-1}$ indole-3-acetic acid (IAA). After culture for 5 days, 98.8% explants formed callus. After 30 days, the calli obtained were transferred to Schwarz differential medium (SDM) containing different combinations of 6-benzyladenine (6-BA) and naphthalene acetic acid (NAA). Compared with other growth regulator combinations, PBU stimulated more vigorous calli and restrained browning. In addition, a large percentage (58.6%) of the calli induced by PBU showed adventitious bud formation. Proliferation of adventitious buds was obtained on SDM medium supplemented with 2.2 $\mu\text{M L}^{-1}$ 6-BA and 0.25 $\mu\text{M L}^{-1}$ NAA and shoot elongation was then stimulated on SDM medium supplemented with 5.28 $\mu\text{M L}^{-1}$ PBU and 0.25 $\mu\text{M L}^{-1}$ NAA for 20 days. For rooting, the elongated shoots were cultivated on root induction medium containing 2.46 $\mu\text{M L}^{-1}$ indole-3-butyric acid (IBA). Plantlets were then successfully transplanted to a greenhouse. This paper presents an efficient procedure for regeneration of *E. urophylla*.

Keywords: In-vitro organogenesis, adventitious bud induction, adventitious bud propagation, substituted urea

INTRODUCTION

The genus *Eucalyptus*, belonging to the family Myrtaceae, is the most widely planted hardwood crop in tropical and subtropical regions because many eucalypt species show superior growth to other trees and adapt well to the environment, plus the wood is useful for multiple purposes. *Eucalyptus* represents the most important plantation tree in China, covering more than 3 mil ha of commercial plantations (Qi 2006). Plantation forestry of eucalypts supplies high quality raw materials for pulp, paper, plywood and energy and in doing so, reduces pressure on native forests. However, the expansion of these eucalypt plantations may become limited due to agricultural and biofuel land needs. From a strategic standpoint, increasing the plantation productivity and wood quality by transgenic technology has become increasingly important for forest industry (Grattapaglia & Kirst 2008). This species can be transformed

by *Agrobacterium tumefaciens*, but the major difficulty lies in shoot regeneration from transformed tissues (Gonzales et al. 2002). Though significant progress has been made in the regeneration of some *Eucalyptus* species via indirect organogenesis, few studies have reported successful regeneration of *E. urophylla* via callus. Synthetic phenyl urea derivatives are potent plant growth regulators and exhibit cytokinin-like activity in various culture systems (Turker et al. 2009). Among them, N-phenyl-N'-[6-(2-chlorobenzothiazol-yl)] urea (PBU) has been successfully synthesised and purified in the laboratory (Li & Luo 2001). The aim of this work was to establish an efficient regeneration system for the *E. urophylla* by organogenesis from stem segment. We used PBU for efficient callus and bud induction as a preliminary step towards regeneration of explants derived from axenic seedlings.

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MATERIALS AND METHODS

Plant material

Viable seeds of *E. urophylla* clone U6 were provided by the China Eucalypt Research Center. Seeds were dipped in distilled water for 3 hours and surface sterilised with 70% (v/v) ethanol for 1 min, followed by 20% (v/v) sodium hypochlorite for 10 min twice. The seeds were then rinsed six times in sterile distilled water and sown on 1/2 Murashige and Skoog (MS) medium (half strength salts and full organic compounds) for germination at 25 °C in darkness. Hypocotyls (5–8 mm) of 8-day-old seedlings were used as explants for regeneration procedure.

Regeneration procedure

The regeneration procedure included the continuous stages described below. For each stage, a number of treatments using plant growth regulators (hormones) were designed with five replicates per treatment and 16 explants/shoots per replicate.

Callus induction

Explants were inoculated on MS medium supplemented with 100 mg L⁻¹ vitamin C, 30 g L⁻¹ sucrose, 7 g L⁻¹ agar and different plant growth regulator combinations for callus induction. MS medium was sterilised at 120 °C for 20 min after adjusting the pH between 5.8 and 6.0. Vitamin C was sterilised by filtration using 0.22-µm pore diameter membrane before combining with other components. The explants were incubated at 25 ± 2 °C in darkness for 2 weeks and then for another 2 weeks under a 16-hour photoperiod with light irradiance of approximately 50 µmol m⁻² s⁻¹ emitted by cool fluorescent tubes (standard culture conditions).

As browning occurred during the callus induction stage, we assessed the influence of anti-browning agents on phenol accumulation. Calli derived from the explants were cultivated on MS medium containing PBU 13.2 µM L⁻¹ and indole-3-acetic acid (IAA) 0.285 µM as well as different concentrations of sodium dithionite (Na₂S₂O₄), vitamin C and polyvinylpyrrolidone (PVP). The calli were maintained at standard

culture conditions. After 20 days, rate of callus browning was evaluated.

To detect the effects of basic medium on callus induction, explants were inoculated on MS medium, Schwarz differential medium (SDM) and sweet potato medium (SPM) (He 2009) supplemented with 100 mg L⁻¹ vitamin C, 30 g L⁻¹ sucrose, 7 g L⁻¹ agar, 13.2 µM L⁻¹ PBU and 0.285 µM L⁻¹ IAA for callus induction. After 20 days, callus formation was evaluated.

Adventitious bud induction

To induce formation of adventitious buds, calli derived from the explants were cultivated on SDM (He 2009) containing different plant growth regulator combinations. The calli were maintained at standard culture conditions. After 30 days, rates of total adventitious bud formation and of shoot formation (shoots > 5 mm) were evaluated.

To detect the effects of basic medium on adventitious bud induction, calli derived from the explants were cultivated on half-strength MS medium, SDM and SPM supplemented with 100 mg L⁻¹ vitamin C, 30 g L⁻¹ sucrose, 7 g L⁻¹ agar, 500 µmol L⁻¹ putrescine, 100 µmol L⁻¹ spermidine, 4.4 µM L⁻¹ 6-benzyladenine (6-BA) and 0.25 µM L⁻¹ naphthalene acetic acid (NAA) for adventitious bud induction. After 30 days, adventitious bud formation and rate of shoot formation were evaluated.

Adventitious bud propagation

Calli with short adventitious buds were first transferred to SDM supplemented with 100 mg L⁻¹ vitamin C, 30 g L⁻¹ sucrose, 7 g L⁻¹ agar and different concentrations of 6-BA and NAA for 30 days. After this period, the adventitious bud propagation coefficients were evaluated.

Shoot elongation, rooting and acclimatisation

To stimulate shoot elongation, calli with short adventitious buds were transferred to half-strength MS medium supplemented with different plant growth regulator combinations for 20 days. The elongated shoots (approximately 35 mm long) were cultivated in modified half-strength MS medium containing different concentrations of indole-3-butyric acid (IBA)

or other plant growth regulator for rooting. Subsequently, rooted plantlets in conical flasks covered with ventilate pellicle were moved to a greenhouse (25 ± 2 °C and 80% relative humidity) for 7 days, followed by transplanting to a mixture of soil and fine sand (2:1 v/v) in the greenhouse to allow normal growth.

Statistical analysis

Values used for statistical analysis were means obtained for each replicate. Effects of treatment were analysed by ANOVA and means were compared using Duncan's multiple range test ($p = 0.05$). All percentage data were subjected to arc-sine transformation prior to ANOVA with SAS/STAT® software (1997).

RESULTS AND DISCUSSION

Callus formation

Explants were found to be important for callus induction. The length of explants should not be more than 8 mm. If the original explants contained meristems of axillary buds, shoots would grow out directly from those buds. Callus colours were related to the presence of anthocyanins and buds could sprout from greenish-yellow calli (Huang et al. 2010, Ouyang et al. 2012; Figure 1a). These calli maintained the ability to grow until approximately 30 days on MS medium. After this period, the calli gradually became brown while no adventitious buds could be observed. The differences between treatments were significant (Table 1). About 98.8% of the cut surfaces had grown calli on the sixth day when the explants were cultured on MS medium containing $13.2 \mu\text{M L}^{-1}$ PBU and $0.285 \mu\text{M L}^{-1}$ IAA (Table 1). Compared with the combination of 6-BA and 2, 4-dichlorophenoxy (2, 4-D), PBU stimulated more vigorous calli and efficiently prevented them from browning. In addition, calli induced by PBU showed higher frequency of adventitious buds after transferring to adventitious bud inducing medium. In this study, PBU was used to induce the calli formation for 30 days. This is the first study which used PBU to induce *E. urophylla* callus. Similarly, PBU could induce higher percentage of callus formation than other plant growth regulators in *E. urophylla* × *E. grandis* hybrids (Ouyang

et al. 2012). Synthetic cytokinins act through activating the cytokinin response pathway (Yamada et al. 2001).

The rate of callus browning was significant among additives. Vitamin C (100 mg L^{-1}) yielded the best results, with only 18.3% of the explants grew browning on the sixth day of culture (Table 2), while those of PVP and $\text{Na}_2\text{S}_2\text{O}_4$ resulted in higher browning rates.

Between the three basic media, MS medium yielded mean callus formation of 98.8%, being significantly higher than the rest of the two media, SDM and SPM (Figure 2a).

Adventitious bud induction

The adventitious buds were observed 40 days after the calli had been cultured on MS medium supplemented with the combinations of NAA with 6-BA, PBU or thidiazuron (TDZ) (Table 3). Regarding the percentage of explants regenerating buds and number of buds per explant, the interaction between NAA with 6-BA, PBU or TDZ concentrations was significant. NAA in combination with TDZ induced organogenesis and just 28% of the calli induced by 6-BA showed adventitious bud formation (Tibok et al. 1995).

Combination of $0.25 \mu\text{mol L}^{-1}$ NAA and $6.6 \mu\text{mol L}^{-1}$ BA yielded the highest induction rate, 58.6% of adventitious buds formed. This differed from those observed in *E. camaldulensis* by Ho et al. (1998) and Diallo and Duhoux (1984), who reported bud regeneration on cotyledonary leaf explants cultured in the presence of $16.2 \mu\text{M}$ NAA and $4.44 \mu\text{M}$ BA and $5.4 \mu\text{M}$ NAA and $2.22 \mu\text{M}$ BA respectively. These authors used auxin at concentrations higher than that of BA. In the present study, however, the best results were obtained when concentration of BA was higher than NAA. The present results and those reported in other studies together indicated that the efficiency of *Eucalyptus* micropropagation was highly dependent on adventitious bud induction. The percentage of adventitious bud formation was 58.6%, which was higher than that reported in other studies (Cid et al. 1999, Hervé et al. 2001, Alves et al. 2004). The effect of basic media on adventitious bud induction was significant. SDM yielded the highest percentages in both adventitious buds regenerated and good buds

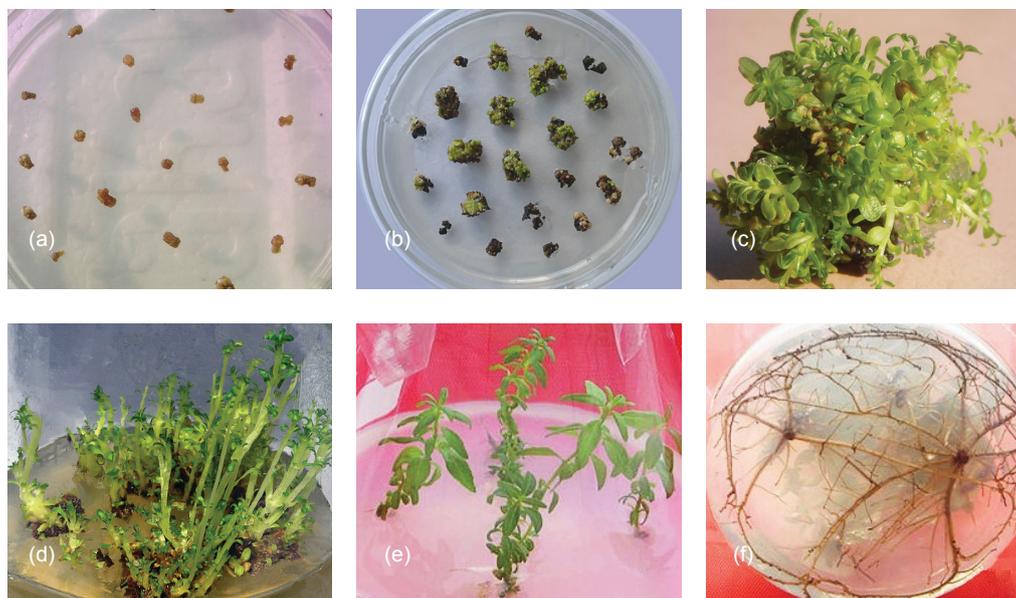


Figure 1 Regeneration of *Eucalyptus urophylla* from hypocotyl explants: (a) callus inoculated on MS medium with $13.2 \mu\text{M L}^{-1}$ PBU and $0.285 \mu\text{M L}^{-1}$ IAA, (b) differentiation of adventitious buds on SDM with $0.25 \mu\text{M L}^{-1}$ NAA and $2.2 \mu\text{M L}^{-1}$ 6-BA, (c) propagation of adventitious buds inoculated on SDM supplemented with $1.1 \mu\text{M L}^{-1}$ 6-BA and $0.25 \mu\text{M L}^{-1}$ NAA, (d) elongation of shoots inoculated on half-strength MS medium supplemented with $5.28 \mu\text{M L}^{-1}$ PBU and $0.25 \mu\text{M L}^{-1}$ NAA and (e) and (f) rooting of elongated shoots cultured in modified half-strength MS medium supplemented with $2.46 \mu\text{M L}^{-1}$ IBA; PBU = N-phenyl-N'-[6-(2-chlorobenzothiazol-yl)] urea, IAA = indole-3-acetic acid, NAA = naphthalene acetic acid, 6-BA - 6-benzyladenine, IBA = indole-3-butyric acid, MS = Murashige and Skoog, SDM = Schwarz differential medium

formed (> 5 mm in length), being 57.8 and 48.9% respectively (Figure 2b).

Adventitious bud propagation

The propagation coefficient of adventitious buds varied with 6-BA and NAA concentrations from 1.4 to 4.5 (Table 4). The combination of $1.1 \mu\text{mol L}^{-1}$ 6-BA and $0.25 \mu\text{mol L}^{-1}$ NAA yielded the greatest propagation coefficient in which the adventitious buds were of high quality (Figure 1c).

Shoot elongation

Percentage of shoot-induced adventitious buds varied with combination (treatment) of plant growth regulators and mean length of shoots induced per combination ranged from 2.2 to 5.5 cm (Table 5). Combination of $5.28 \mu\text{mol L}^{-1}$ PBU and $0.25 \mu\text{mol L}^{-1}$ NAA yielded maximum

shoot induction, showing 98% of elongating shoots and 5.5 cm in shoot length (Figure 1d).

Shoot rooting and acclimatisation

The hormone IBA generally resulted in markedly higher rooting ability of shoots, ranging between 69.0 and 97.4%, whereas that of control was only 19.3% when cultured on the half-strength MS medium without any hormones added (Table 6). In treatments with IBA, 6–10 adventitious roots were usually visible in 12-day culture and the roots could reach 30–80 mm in length in 10-day culture (Figure 1e, f), indicating the importance of IBA pre-treatment for efficient rooting of shoots in regeneration of *E. urophylla*.

Finally, all rooted plantlets survived in greenhouse conditions and developed into normal plants without any obvious variant phenotypes. However, regeneration was via callus

Table 1 Callus induction and growth from hypocotyls of *Eucalyptus urophylla* incubated on MS medium

2,4-D ($\mu\text{M L}^{-1}$)	IAA ($\mu\text{M L}^{-1}$)	6-BA ($\mu\text{M L}^{-1}$)	PBU ($\mu\text{M L}^{-1}$)	Explant number	Number of explants forming callus	Mean of induction rate (% \pm SD)	Character of callus
4.5	0	0.44	0	80	54	67.5 \pm 1.4 f	Dead white and loose
9.0	0	0.44	0	80	45	56.3 \pm 1.2 g	Dead white and loose
13.5	0	0.44	0	80	30	37.5 \pm 1.1 h	Dark red and close
4.5	0	0.88	0	80	58	72.5 \pm 1.6 ef	Dark red and close
9.0	0	0.88	0	80	60	75.0 \pm 0.9 de	Dead white and loose
13.5	0	0.88	0	80	45	56.3 \pm 2.3 n	Thin green and close
4.5	0	1.32	0	80	65	81.3 \pm 2.7 cd	Thin green and close
9.0	0	1.32	0	80	58	72.5 \pm 1.9 ef	Dark red and close
13.5	0	1.32	0	80	52	65.0 \pm 0.8 f	Thin green and close
0	0.171	0	6.6	80	67	83.8 \pm 2.4 c	Dead white and loose
0	0.285	0	6.6	80	69	86.3 \pm 1.5 bc	Dead white and loose
0	0.570	0	6.6	80	74	92.5 \pm 1.1 a	Thin green and close
0	0.171	0	13.2	80	72	90.0 \pm 2.2 ab	Thin green and close
0	0.285	0	13.2	80	79	98.8 \pm 2.3 a	Thin green and close
0	0.570	0	13.2	80	74	92.5 \pm 0.9 a	Thin green and close
0	0.171	0	19.8	80	73	91.3 \pm 2.8 a	Dark red and loose
0	0.285	0	19.8	80	65	81.3 \pm 2.1 c	Dark red and close
0	0.570	0	19.8	80	60	75.0 \pm 0 de	Dark red and close

MS = Murashige and Skoog medium, 2,4-D = 2,4-dichlorophenoxy, IAA = indole-3-acetic acid, 6-BA = 6-benzyladenine, PBU = N-phenyl-N'-(6-(2-chlorobenzothiazol)-yl) urea; SD = standard deviation, means followed by the same letter do not differ significantly ($p > 0.05$); close means that the callus is fairly solid and difficult to differentiate while loose means it is light and crisp

Table 2 The effects of different additive on callus tissue antibrowning

$\text{Na}_2\text{S}_2\text{O}_4$ (mg L^{-1})	Additive		Mean of browning rate (% \pm SD)
	PVP (g L^{-1})	Vitamin C (mg L^{-1})	
0	0	50	24.6 \pm 1.3 cd
0	0	100	18.3 \pm 2.1 e
0	0	150	21.2 \pm 0.9 de
0	1	0	33.7 \pm 2.3 ab
0	5	0	26.7 \pm 1.6 c
0	10	0	23.7 \pm 3.3 cde
10	0	0	34.1 \pm 1.7 a
15	0	0	29.8 \pm 3.3 bc
20	0	0	26.4 \pm 1.5 c

$\text{Na}_2\text{S}_2\text{O}_4$ = sodium dithionite, PVP = polyvinylpyrrolidone; SD = standard deviation, means followed by the same letter do not differ significantly ($p > 0.05$)

Table 3 Average adventitious bud induction rates of different combinations of plant growth regulators supplemented with MS medium

Plant growth regulator				Mean induction rate (% ± SD)	Mean of good induction rate (% ± SD)
NAA (µM L ⁻¹)	6-BA (µM L ⁻¹)	PBU (µM L ⁻¹)	TDZ (µM L ⁻¹)		
0.1	0.55	–	–	0 ± 0 q	0 ± 0 l
0.25	0.55	–	–	13.4 ± 0.8 k	0 ± 0 l
0.5	0.55	–	–	21.2 ± 0.8 i	7.6 ± 0.3 i
0.1	1.1	–	–	30.2 ± 0.6 h	13.9 ± 1.4 gh
0.25	1.1	–	–	32.2 ± 0.6 h	14.5 ± 2.4 gh
0.5	1.1	–	–	38.9 ± 0.3 de	31.3 ± 0.5 cd
0.1	2.2	–	–	35.1 ± 0.2 fg	29.2 ± 0.5 de
0.25	2.2	–	–	58.6 ± 0.5 a	48.9 ± 1.4 a
0.5	2.2	–	–	51.3 ± 0.6 b	39.8 ± 0.9 b
0.1	–	3.3	–	17.5 ± 0.6 j	6.2 ± 1.1 i
0.1	–	5.28	–	32.5 ± 0.4 gh	16.9 ± 1.0 g
0.1	–	6.6	–	44.4 ± 0.2 c	40.6 ± 1.0 b
0.25	–	3.3	–	41.5 ± 0.3 cd	32.3 ± 0.8 cd
0.25	–	5.28	–	37.1 ± 0.5 ef	24.6 ± 1.1 f
0.25	–	6.6	–	32.2 ± 0.6 h	13.1 ± 0.9 h
0.5	–	3.3	–	43.1 ± 0.1 c	35.3 ± 0.2 c
0.5	–	5.28	–	38.5 ± 1.0 de	26.6 ± 0.8 ef
0.5	–	6.6	–	21.3 ± 0.9 i	7.7 ± 0.6 i
2.5	–	–	2.275	6.3 ± 0.6 m	0 ± 0 l
5	–	–	2.275	4.3 ± 0.4 n	0 ± 0 l
10	–	–	2.275	0.0 ± 0 q	0 ± 0 l
2.5	–	–	4.55	13.6 ± 0.3 k	3.0 ± 0.3 j
5	–	–	4.55	6.2 ± 0.1 m	0 ± 0 l
10	–	–	4.55	1.6 ± 0.7 p	0 ± 0 l
2.5	–	–	9.1	3.1 ± 0.5 o	0 ± 0 l
5	–	–	9.1	10.8 ± 0.1 l	1.4 ± 0.5 k
10	–	–	9.1	0.0 ± 0 q	0 ± 0 l

MS = Murashige and Skoog medium, 6-BA = 6-benzyladenine, NAA = naphthalene acetic acid, PBU = N-phenyl-N'-[6-(2-chlorobenzothiazol-yl)] urea, TDZ = thidiazuron; SD = standard deviation, means followed by the same letter in the same column do not differ significantly (p > 0.05)

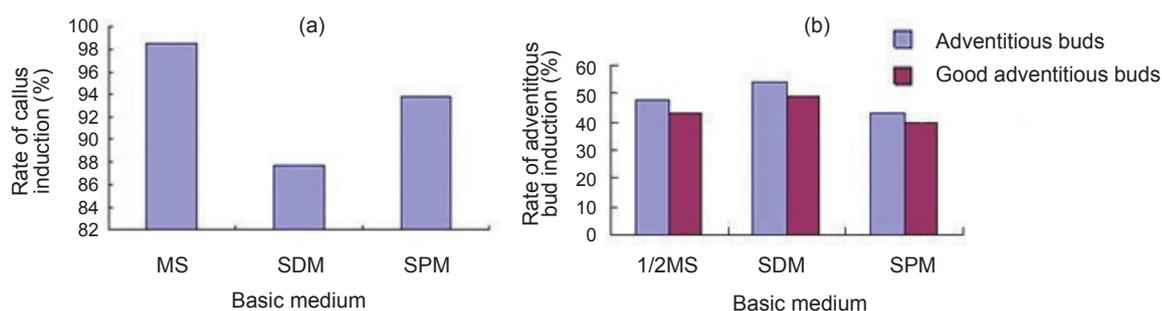


Figure 2 Effect of basic media on (a) callus and (b) adventitious bud induction; MS = Murashige and Skoog medium, SDM = Schwarz differential medium and SPM = sweet potato medium

Table 4 Number of propagation times of adventitious buds for different 6-BA and NAA concentrations

Plant growth regulator		Proliferation times (\pm SD)
6-BA ($\mu\text{mol L}^{-1}$)	NAA ($\mu\text{mol L}^{-1}$)	
0.22	0.1	2.6 \pm 0.1 def
1.1	0.1	3.2 \pm 0.2 c
0.22	0.25	2.5 \pm 0.1 efg
1.1	0.25	4.5 \pm 0.2 a
0.22	0.5	2.2 \pm 0.1 g
1.1	0.5	2.4 \pm 0.2 fg
0.22	7.5	1.9 \pm 0.1 h
1.1	7.5	2.3 \pm 0.1 fg
0.22	–	1.4 \pm 0.1 fg
0.44	–	1.7 \pm 0.2 h
1.1	–	2.4 \pm 0.1 efg
2.2	–	2.7 \pm 0.2 de
4.4	–	2.8 \pm 0.1 cd
6.6	–	3.6 \pm 0.1 b
8.8	–	3.4 \pm 0.1 b

6-BA = 6-benzyladenine, NAA = naphthalene acetic acid; SD = standard deviation, means followed by the same letter do not differ significantly ($p > 0.05$)

and took 3 months, so there was a chance of somaclonal variation during tissues culture. The regeneration procedure in this study has proven to be in genetic transformation of elite *Eucalyptus* species (Herve et al. 2001, Alves et al. 2004).

CONCLUSIONS

This simple, reliable and efficient procedure to regenerate *E. urophylla* plants comprised five culture stages: callus induction on PBU-containing medium, adventitious bud formation on medium containing NAA and 6-BA, adventitious bud proliferation on medium containing NAA and 6-BA, elongation on medium with PBU and NAA and finally rooting with IBA pre-treatment. The procedure took about 3 months to produce regenerated plants from hypocotyls. PBU was more efficient in inducing callus than 6-BA and 2, 4-D. In addition, callus induced by PBU showed higher frequency of induction of adventitious buds. In this study, there was nearly 100% callus production, from which only 50% produce buds

with about 27 shoots per bud (proliferation times was 4.5) and 92% of the shoots root. Thus, with 80 explants, a total of 994 shoots could be produced, proving that this was an efficient protocol.

Regeneration from clonal material has the advantage of retaining all traits of the original elite tree which will not happen if using seedling materials. Explants used in this study were from germinated seeds, which were of unknown genotype. However, the regeneration protocol described regenerated primary leaf explants and in-vitro shoots. Finally, this regeneration procedure is also an important step in genetic transformation of elite *Eucalyptus* genotypes.

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Table 5 Effect of plant growth regulators on shoot elongation

Treatment	Plant growth regulator			Mean of shoot length (cm ± SD)
	NAA ($\mu\text{mol L}^{-1}$)	6-BA ($\mu\text{mol L}^{-1}$)	PBU ($\mu\text{mol L}^{-1}$)	
1	0.25	0.22	–	3.4 ± 0.3 hi
2	0.5	0.22	–	3.8 ± 0.1 gh
3	0.75	0.22	–	4.5 ± 0.2 d
4	0.25	0.44	–	3.7 ± 0.4 gh
5	0.5	0.44	–	4.2 ± 0.2 e
6	0.75	0.44	–	4.8 ± 0.3 bcd
7	0.25	0.66	–	3.9 ± 0.2 fg
8	0.5	0.66	–	4.4 ± 0.1 de
9	0.75	0.66	–	4.9 ± 0.6 bc
10	0.1	–	3.3	2.2 ± 0.1 j
11	0.1	–	5.28	3.1 ± 0.2 i
12	0.1	–	6.6	2.4 ± 0.4 j
13	0.25	–	3.3	5.0 ± 0.5 ab
14	0.25	–	5.28	5.5 ± 0.1 a
15	0.25	–	6.6	4.8 ± 0.2 bcd
16	0.4	–	3.3	4.7 ± 0.2 cde
17	0.4	–	5.28	4.3 ± 0.1 ef
18	0.4	–	6.6	4.1 ± 0.4 f

NAA = naphthalene acetic acid, 6-BA = 6-benzyladenine, PBU = N-phenyl-N'-[6-(2-chlorobenzothiazol-yl) urea; SD = standard deviation, means followed by the same letter do not differ significantly ($p > 0.05$)

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Table 6 Effect of plant growth regulators on shoot rooting

Plant growth regulator			Percentage of rooting shoots (% ± SD)
IAA ($\mu\text{mol L}^{-1}$)	NAA ($\mu\text{mol L}^{-1}$)	IBA ($\mu\text{mol L}^{-1}$)	
–	–	–	19.2 ± 1.0 hi
1.14	–	–	43.1 ± 1.0 g
2.85	–	–	48.5 ± 0.8 g
4.56	–	–	51.2 ± 0.5 fg
5.7	–	–	52.3 ± 0.7 efg
8.55	–	–	58.1 ± 0.6 ef
11.4	–	–	61.4 ± 0.9 de
–	1	–	31.2 ± 1.4 h
–	2.5	–	32.5 ± 0.3 h
–	4	–	30.6 ± 0.6 h
–	5	–	27.4 ± 0.4 hi
–	7.5	–	27.5 ± 0.7 hi
–	10	–	22.7 ± 0.6 hi
–	–	0.984	69.0 ± 1.1 d
–	–	2.46	97.4 ± 6.7 a
–	–	3.936	91.8 ± 0.6 b
–	–	4.92	78.5 ± 1.4 c
–	–	7.38	82.2 ± 0.1 c
–	–	9.84	81.2 ± 0.7 c

IAA = indole-3-acetic acid, NAA = naphthalene acetic acid, IBA = indole-3-butyric acid, SD = standard deviation, means followed by the same letter do not differ significantly ($p > 0.05$)

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