ALTERNATIVE CLONAL PROPAGATION OF DRYOBALANOPS BECCARII AND D. RAPPA

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JONG LK & SANI HB. 2012. Alternative clonal propagation of *Dryobalanops beccarii* and *D. rappa*. Application of rooted cuttings derived from epicormic shoots from mature trees of *Dryobalanops beccarii* and *D. rappa* in current tree propagation and improvement programmes is suggested and discussed. Development of epicormic shoot indicated partial rejuvenation which was exhibited by plagiotropic growth habit, adult leaf morphology and delayed bud flushing of rooted cuttings. The juvenility of epicormic shoots showed that the 'ageing' component of cyclophysis had been reversed by the 'maturation' component which remained unaltered. The incomplete rejuvenation was further demonstrated by poor rooting of cuttings derived from epicormic shoots. The potential of using these characteristics as possible markers of juvenility and maturity is also discussed. Cuttings derived from epicormic shoots from the stump of *D. beccarii* and *D. rappa* rooted more readily than those from other parts of the tree bole. It was suggested that epicormic shoots obtained from stumps were more juvenile than those from upper part of the stem. This phenomenon, described as topophysis, supported the theory that maturation occurred in different phases.

Keywords: Epicormic shoot, partial rejuvenation, ageing, juvenility, maturation, plagiotropic, topophysis

JONG LK & SANI HB. 2012. Kaedah alternatif bagi pembiakan klon *Dryobalanops beccarii* dan *D. rappa*. Artikel ini mencadang serta membincangkan aplikasi keratan berakar yang dihasilkan daripada pucuk epikormik pokok *Dryobalanops beccarii* dan *D. rappa* matang dalam program pembiakan dan penambahbaikan pokok. Pembentukan pucuk epikormik menunjukkan rejuvenasi separa yang dipamerkan melalui tabiat pertumbuhan plagiotropik, morfologi daun dewasa serta pengeluaran tunas daun yang lewat pada keratan berakar. Kejuvenilan pucuk epikormik menunjukkan bahawa komponen penuaan siklofisis telah diterbalikkan oleh komponen kematangan yang kekal tidak berubah. Rejuvenasi yang tidak lengkap ini turut ditunjukkan oleh pengakaran yang kurang pada keratan yang dihasilkan daripada pucuk epikormik. Potensi menggunakan ciriciri ini sebagai penanda kejuvenilan serta kematangan turut dibincangkan. Keratan yang dihasilkan daripada pucuk epikormik tunggul *D. beccarii* and *D. rappa* berakar lebih cepat berbanding bahagian lain batang pokok. Kami mencadangkan bahawa pucuk epikormik yang diperoleh daripada tunggul menunjukkan lebih banyak sifat juvenil berbanding bahagian batang pokok yang lebih tinggi. Fenomena ini yang dinamakan sebagai topofisis menyokong teori yang menyatakan bahawa kematangan berlaku dalam fasa-fasa yang berbeza.

INTRODUCTION

The presence of epicormic shoot is generally undesirable as it can increase the presence of knots and blemishes and reduce the quality and value of harvested wood. It can also congest the canopy, thus, blocking light and preventing fruit from gaining colour, and contribute little carbohydrate to fruit development (Tymoszuk 1984). In commercial uses, however, epicormic shoots are utilised as propagation material for certain species, e.g. *Arbutus* sp., *Quercus* sp., *Tilia* sp. and *Eucalyptus* sp. due to their superior rooting ability (Chalupa 2002) and as coppice shoots in some forestry management system (Smith et al. 1997). If a species can be induced for production of epicormic shoots, then there is a possibility that a system of controlled production of epicormic shoots can be developed. Hence, it would allow more available material for vegetative propagation from mature trees. This will be great advantage because 'juvenile' material arising from adult ortet has superior genetic stability in phenotypes (Libby 1974).

Juvenile root suckers, epicormic shoots and stump sprouts are superior and easier

materials for propagation compared with adult plant materials found at the top of tree (Suzuki 2002). Physiological condition determines the adventitious root formation, not the chronological age of the plant. When roots produce shoots, they are physiological juvenile and are a good source for production of cuttings. However, stem cuttings from the same plant may not root (Davies 1983).

This study was thus aimed at evaluating the rooting capacity and growth performance of stem and branch cuttings derived from epicormic shoot induced by isolated stem segment, stump of trees and bending of trees with different ages. In this study, the effects of ortet age, position of branch (i.e. first and second order) and position of stem segments from where the epicormic shoots sprouted were investigated with respect to (1) length of the longest root and rooting success of cuttings, and (2) growth of rooted cuttings in terms of height and plagiotropism.

MATERIALS AND METHODS

Cuttings of *Dryobalanops beccarii* and *D. rappa* were collected from epicormic shoots growing on stumps and isolated stem segments. Cuttings were also derived from epicormic shoots which were induced by bending of standing mature trees of *D. beccarii* at different age groups. These cuttings were collected from the field at Sampadi Forest Reserve, Kuching, Sarawak, Malaysia.

Leafy single-node cuttings for stem and branch were collected from the field in the morning. The base of each cutting was cut to expose fresh tissue shortly before it was placed in the rooting bed. The base of each cutting was cut at right angle to the stem and without treatment of rooting hormone. All cuttings were cut 4 to 7 cm in length. Half of a whole leaf was retained on each cutting. The cuttings were inserted 1 to 2 cm deep into rooting media of cleaned river sand (sand particles $\leq 2 \text{ mm}$) at $5 \times 5 \text{ cm}$ spacing. Rooting was carried out in a plastic-covered greenhouse. The plastic enclosures were then shaded with 50% black plastic netting. High humidity was maintained in the rooting beds using a mist system three times per day (i.e. 8.00 a.m., 11.30 a.m. and 5.00 p.m.) with 15 min misting. The temperature, humidity and light intensity during the experiment were 25 ± 2 °C, $83 \pm 5\%$ and 17.5 ± 0.1 Lux respectively.

Assessment of the cuttings

Monthly assessments of cuttings were carried out for five months, i.e. until no more new roots were formed. At the end of each month, the number of rooted cuttings, number of roots and length of the longest root were recorded. The cuttings were then replanted into a rooting bed and monthly assessment was carried out for another five months. A cutting was recorded as rooted when it produced root length of 10 mm or more.

After five months, rooted cuttings were potted in black polythene bags $(17 \times 8 \text{ cm})$ containing potting mixture of forest top soil and sand at a ratio of 3:1. After potting for six months at the nursery, shoot height and plagiotropism were recorded. The degree of plagiotropism was visually quantified by classifying each rooted cutting that had new shoot growth as orthotropic (0), slightly plagiotropic, i.e. $< 45^{\circ}$ (1), and very plagiotropic, i.e. > 45° (2). Cuttings that did not produce bud in the rooting bed could not be evaluated for plagiotropism. First-year shoot growth was measured from the base of the terminal bud scar to the tip. Shoot height was measured to the nearest millimeter. For each experiment, five potted plants per treatment were randomly chosen due to limitation of potted cuttings. These plants were randomly arranged at the nursery. Throughout the experiment, plants were shaded with one layer of plastic netting, allowing 50% full sunlight intensity. Fertiliser (NPK green, $15N: 15P_2O_5: 15K_2O$) was applied at a rate of 1 g plant⁻¹ month⁻¹ to maintain healthy growth of the plants.

Experimental design and statistical analysis

Experiment 1

A total of 180 cuttings were derived from epicormic shoots induced by bending of standing mature trees (clear bole height, 8-year-old: 0.7–2.1 m, 10-year-old: 1.5–2.5 m and 15-year-old: 1.0–2.0 m) of *D. beccarii*. A two-factor experiment on rooting of cuttings with age (8, 10 and 15 years old) as one factor and the two types of cuttings (stem and branch cuttings) as the other was laid out in randomised complete block design with five replicates. All data were subjected to analysis of variance (ANOVA) using Minitab Statistical Program (1985) and the means obtained, except for rooting percentage, were arcsine transformed. Results in all tests were considered significant if $p \le 0.05$.

Experiment 2

A total of 120 branch cuttings were collected from the epicormic shoot induced by bending of standing mature trees (10 years old) of *D. beccarii* (clear bole height 5.8–6.1 m) and *D. rappa* (clear bole height 3.6–4.4 m). A randomised complete block design with treatment in 2×2 factorial, five replications and six cuttings per plot was employed. The two-factor experiment involved two species (*D. beccarii* and *D. rappa*) and two branch orders (1° and 2° branch cuttings). The data were analysed as described earlier (Experiment 1).

Experiment 3

This experiment was focused on *D. beccarii*, which involved the factorial combination of two methods induction of epicormic shoot (i.e. isolated stem segment and stump) and two types of cuttings, namely, stem and branch cuttings. These four treatments were in randomised complete block design with five replicates and six cuttings per plot. All data were subjected to statistical analyses as described earlier (Experiment 1).

Experiment 4

This experiment using *D. rappa* involved factorial combination of two methods of induction of

epicormic shoot, namely, bending (clear bole height 2.8–3.9 m) and stumping, with two types of cuttings (stem and branch cuttings). These four treatments were in randomised complete block design with five replicates and six cuttings per plot. A total of 120 cuttings were used. All data were subjected to statistical analyses as described earlier (Experiment 1).

RESULTS

Experiment 1

In the branch cutting, rooting success of D. beccarii after five months in the propagation bed increased with ortet age from 23.3 (8 years old) to 33.3% (15 years old) (Table 1). However, in stem cuttings, rooting rate declined from 26.7 (10 years old) to 20.0% (15 years old). By comparison, the lowest (3.3%) rooting success of stem cuttings was from 8-year-old plant. However, the differences between ages and types of cuttings were not significant (Tables 2). There was also no significant interaction between age and type of cutting in the number of roots produced (Tables 1 and 2). Conversely, the length of the longest root was found to be significantly influenced by ortet age. There was significant difference in interaction between ortet age and type of cutting. However, there was no significant difference in the length of the longest root between stem and branch cuttings in this experiment (Table 2).

After six months of potting at nursery stage, the highest shoot growth of *D. beccarii*

Table 1Comparison of parameters of rooted cuttings derived from epicormic shoots induced by
bending of standing mature trees of *Dryobalanops beccarii* with respect to different age groups
and types of cuttings

Parameter	Type of		Ortet age (years)	
	cutting	8	10	15
Rooting success (%)	Stem	3.3 (30)	26.7 (30)	20.0 (30)
	Branch	23.3 (30)	30.0 (30)	33.3 (30)
Number of roots	Stem	3.4(5)	1.2 (5)	2.2 (5)
	Branch	2.8 (5)	2.0 (5)	2.2 (5)
Length of the longest root (mm)	Stem	21.3 (5)	29.0 (5)	55.3 (5)
	Branch	15.0 (5)	49.9 (5)	31.2 (5)
Shoot growth (mm)	Stem	90.9 (5)	123.3 (5)	17.3 (5)
	Branch	48.3 (5)	63.3(5)	6.0 (5)
Plagiotropism	Stem	0.8(5)	0.0 (5)	0.0 (5)
	Branch	1.0(5)	1.4(5)	1.0 (5)

Number in parenthesis is the number of cuttings for each treatment

Table 2Analysis of variance of growth parameters of rooted cuttings derived from
epicormic shoots induced by bending of standing mature trees of *D. beccarii*
with respect to different age groups and types of cuttings

Source of variation	F value	Source of variation	F value
Rooting success		Shoot growth	
Ortet age (A)	2.89 ns	Ortet age (A)	7.71**
Type of cutting (B)	3.92 ns	Type of cutting (B)	4.73*
$\mathbf{A} \times \mathbf{B}$	0.53 ns	$\mathbf{A} \times \mathbf{B}$	0.66 ns
Number of roots		Plagiotropism	
Ortet age (A)	2.83 ns	Ortet age (A)	1.20 ns
Type of cutting (B)	0.02 ns	Type of cutting (B)	16.90**
$\mathbf{A} \times \mathbf{B}$	0.61 ns	$\mathbf{A} \times \mathbf{B}$	2.80 ns
Length of the longest root			
Ortet age (A)	12.08**		
Type of cutting (B)	0.49 ns		
$\mathbf{A} \times \mathbf{B}$	8.50**		

*, ** = significant at $p \le 0.05$ and $p \le 0.01$ respectively, ns = non-significant

was found in stem (123.3 mm) and branch (63.3 mm) cuttings of epicormic shoot derived from 10-year-old ortet (Table 1). Shoot growth was significantly influenced by ortet age and type of cutting. However, there was no significant interaction between ortet age and type of cutting. In comparison with branch cuttings, shoot height produced by stem cuttings was significantly greater than that of branch cuttings (Table 2).

Stem cuttings from three ages of ortet grew plagiotropically at the end of the first year, but the differences were not significant. Branch cuttings from 10- and 15-year-old trees were more orthotropic than 8-year-old tree and, thus, resulted in significant differences between stem and branch cuttings.

Experiment 2

After five months in the propagation bed, rooting success of *D. beccarii* and *D. rappa* was found to be significantly different (Table 3 and 4). For *D. beccarii*, the average rooting rates of the first and second order branches were 40.0 and 50.0% respectively (Table 3). The rooting success for *D. rappa* was significantly much lower than that of *D. beccarii* (10.0 and 16.7% respectively).

Length of the longest root and plagiotropism were significant between both species (Table 4). It can also be seen that the branch orders have significant effect on the number of roots, shoot growth and plagiotropism. After six months of potting at the nursery stage, there was no significant interaction between species and branch orders in rooting success, number of roots, length of the longest root and shoot growth. However, there was significant interaction between species and branch orders in plagiotropism (Table 4). Shoot grew more orthotropic in rooted cuttings of the second order branch (0.4) for *D. rappa* than the first order branch (2.0) (Table 3). In contrast, the shoot was more plagiotropic in the second order branch (2.0) of rooted cuttings for *D. beccarii*.

Experiment 3

From the results in Tables 5 and 6, rooting success of *D. beccarii* was significantly higher in epicormic shoots derived from stump than isolated stem segment. Average rooting values of stem and branch cuttings by induction of epicormic shoot through isolated stem segment were 30 and 10% respectively. Average rooting values of stem and branch cuttings by induction of epicormic shoot through stump were 56.7 and 66.7% respectively.

The induction of epicormic shoot was not the only factor in determining rooting success of cuttings. It was also significantly affected by length of the longest root, plagiotropism and shoot growth of the rooted cuttings (Table 6). However, no significant effect was found in

Parameter	Branch	Spe	cies
	order	D. beccarii	D. rappa
Rooting success (%)	1°	40.0 (30)	10.0 (30)
	2°	50.0 (30)	16.7 (30)
Number of roots	1°	1.2 (5)	1.4 (5)
	2°	2.4 (5)	2.0 (5)
Length of the longest root (mm)	1°	44.3 (5)	80.6 (5)
	2°	37.6 (5)	102.2 (5)
Shoot growth (mm)	1°	34.2 (5)	52.1 (5)
	2°	11.4 (5)	16.5 (5)
Plagiotropism	1°	1.6 (5)	2.0 (5)
	2°	2.0 (5)	0.4(5)

Table 3Comparison of parameters of rooted cuttings with respect to different species
and branch orders

Number in parenthesis is the number of cuttings for each treatment

 Table 4
 Analysis of variance of growth parameters of rooted cuttings with respect to species and branch orders

Source of variation	F value	Source of variation	F value
Rooting success		Length of the longest root	
Species (A)	12.86**	Species (A)	53.61**
Branch order (B)	1.07 ns	Branch order (B)	1.16 ns
$\mathbf{A} \times \mathbf{B}$	0.03 ns	$\mathbf{A} \times \mathbf{B}$	4.22 ns
Number of roots		Shoot growth	
Species (A)	0.07 ns	Species (A)	3.00 ns
Branch order (B)	5.79*	Branch order (B)	19.32**
$\mathbf{A} \times \mathbf{B}$	0.64 ns	$\mathbf{A} \times \mathbf{B}$	0.94 ns
Plagiotropism			
Species (A)	6.55*		
Branch order (B)	6.55*		
$\mathbf{A} \times \mathbf{B}$	18.18**		

*, ** = significant at $p \le 0.05$ and $p \le 0.01$ respectively, ns = non-significant

Table 5Comparison of parameters of rooted cuttings of *D. beccarii* with respect to different methods of
induction of epicormic shoot and types of cuttings

Parameter	Isolated stem segment		Stump		
	Stem cutting	Branch cutting	Stem cutting	Branch cutting	
Rooting success (%)	30.0 (30)	10.0 (30)	56.7 (30)	66.7 (30)	
Number of roots	1.4 (5)	1.4 (5)	2.2 (5)	4.4 (5)	
Length of the longest root (mm)	20.4 (5)	57.9 (5)	49.1 (5)	33.0 (5)	
Shoot growth (mm)	56.2 (5)	57.8 (5)	106.7 (5)	89.7 (5)	
Plagiotropism	0.0 (5)	0.4 (5)	0.0(5)	2.0 (5)	

Number in parenthesis is the number of cuttings for each treatment

number of roots. The average numbers of roots by branch and stem cuttings were 4.4 and 2.2 respectively. The average number of roots for stem and branch cuttings from isolated stem segment was 1.4. It was also observed that rooted cuttings from isolated stem segment and stump had more orthotropic growth in stem than branch cuttings.

Plagiotropism is the only parameter that was significantly influenced by types of cuttings (Table 6). The rest of the parameters did not show any significant difference between stem and branch cuttings.

Interaction between methods of induction of epicormic shoot and types of cuttings was significant with respect to rooting success, number of roots and also plagiotropism, but no significant interaction was obtained with type of cutting with respect to length of longest root and shoot growth.

Experiment 4

Rooting success of *D. rappa* stem (53.3%) and branch (46.7%) cuttings from stump was higher than those of stem (23.3%) and branch (40.0%) cuttings obtained by bending of standing mature trees (Tables 7 and 8). Unfortunately, both methods of induction of epicormic shoot did not influence rooting success, length of longest root, number of roots, shoot growth and plagiotropism. There was significant difference between branch and stem cuttings with respect to shoot growth and plagiotropism.

Shoot height produced by rooted cuttings from stem (191.7 mm) and branch (109.2 mm) obtained from epicormic shoot of the stump was significantly higher than those (127.9 and 100.3 mm respectively) of bending standing mature trees (Tables 7 and 8). There was no significant interaction between methods of

Table 6	Analysis of variance of rooting success, length of the longest root, number of roots, shoot growth
	and plagiotropism of rooted cuttings of D. beccarii with respect to different methods of induction
	of epicormic shoot and types of cuttings

Source of variation	F value	Source of variation	F value	
Rooting success		Number of roots		
Method of induction (A)	31.48**	Method of induction (A)	0.03 ns	
Type of cutting (B)	0.89 ns	Type of cutting (B)	1.05 ns	
$\mathbf{A} \times \mathbf{B}$	4.56^{*}	$\mathbf{A} \times \mathbf{B}$	6.58*	
Length of the longest root		Shoot growth		
Method of induction (A)	26.89**	Method of induction (A)	3.07*	
Type of cutting (B)	3.56 ns	Type of cutting (B)	0.11*	
$\mathbf{A} \times \mathbf{B}$	3.56 ns	$\mathbf{A} \times \mathbf{B}$	0.15 ns	
Plagiotropism				
Method of induction (A)	12.50**			
Type of cutting (B)	12.50**			
$\mathbf{A} \times \mathbf{B}$	4.50*			

*, ** = significant at $p \le 0.05$ and $p \le 0.01$ respectively, ns = non-significant

Table 7Comparison of parameters of rooted cuttings of *Dryobalanops rappa* with respect to different
methods of induction of epicormic shoot and types of cuttings

Parameter	Bending		Stump	
	Stem cutting	Branch cutting	Stem cutting	Branch cutting
Rooting success (%)	23.3 (30)	40.0 (30)	53.3 (30)	46.7 (30)
Number of roots	3.8 (5)	3.0 (5)	3.6 (5)	2.4 (5)
Length of the longest root (mm)	43.5 (5)	39.9 (5)	29.7 (5)	28.9 (5)
Shoot growth (mm)	127.9 (5)	100.3 (5)	191.7 (5)	109.2 (5)
Plagiotropism	0.2 (5)	2.0 (5)	0.0 (5)	1.4 (5)

Number in parenthesis is the number of cuttings for each treatment

Table 8Analysis of variance of rooting success, length of the longest root, number of roots, shoot
growth and plagiotropism of rooted cuttings of *D. rappa* with respect to different methods
of induction of epicormic shoot and types of cuttings

Source of variation	F value	Source of variation	F value	
Rooting success		Number of roots		
Method of induction (A)	2.93 ns	Method of induction (A)	4.15 ns	
Type of cutting (B)	0.25 ns	Type of cutting (B)	0.13 ns	
$\mathbf{A} \times \mathbf{B}$	1.33 ns	$\mathbf{A} \times \mathbf{B}$	0.05 ns	
Length of the longest root		Shoot growth		
Method of induction (A)	0.51 ns	Method of induction (A)	2.91 ns	
Type of cutting (B)	3.17 ns	Type of cutting (B)	6.68*	
$\mathbf{A} \times \mathbf{B}$	0.13 ns	$\mathbf{A} \times \mathbf{B}$	1.66 ns	
Plagiotropism				
Method of induction (A)	3.20 ns			
Type of cutting (B)	51.20**			
$\mathbf{A} \times \mathbf{B}$	0.80 ns			

*, ** = significant at $p \le 0.05$ and $p \le 0.01$ respectively, ns = non-significant

induction of epicormic shoot and types of cuttings in all parameters for rooted cuttings of *D. rappa.*

Effects of branch order and methods of induction of epicormic shoot on leaf characteristic

Rooted cuttings by branch order had carried-over juvenile markers from epicormic shoots which were rejuvenated by bending of standing mature trees. Such juvenile markers were demonstrated by acute tip and narrow base for D. beccarii, and acute tip but round base for *D. rappa* (Figure 1). Leaf characteristics of rooted cuttings derived from epicormic shoots varied with ortet age and position of stem segments. Leaves of rooted cuttings derived from epicormic shoots by bending of standing 8-year-old tree had more rounded base compared with the older ortets, i.e. 10- and 15-year-old trees (Figure 2). Leaves of stem and branch cuttings had acute leaf tip, narrow leaf base and no hair. Leaves of rooted cuttings derived from epicormic shoots from stump and isolated segments had acute tips and narrow bases. However, leaf of rooted stem cuttings had shorter length than those of rooted branch cuttings (Figure 2). In D. rappa, however, leaf of rooted cuttings derived from epicormic shoots varied with position of stem segments. Leaves of rooted cuttings obtained from bending of standing mature tree, stump and isolated stem segments had acute tips and more rounded base (Figure 3).

DISCUSSION AND CONCLUSIONS

There was non-significant difference between three levels of ortet ages in rooting success, number of roots and plagiotropism. However, significant difference was obtained between types of cuttings in shoot growth and plagiotropism. This indicated that after the ontogenetical rejuvenation by bending of standing mature trees, rooting success, number of roots and plagiotropism were not influenced by chronological age of the mature tree. However, ortet age influenced length of the longest roots and shoot growth of rooted cuttings of *D. beccarii*. Types of cuttings influenced shoot growth and plagiotropism of rooted cuttings.

All cuttings from first and second order branches of *D. beccarii* and *D. rappa* revealed significant differences in number of roots, shoot growth and plagiotropism. However, branch order influenced rooting success and length of the longest roots. First order branch cuttings of *D. beccarii* grew more upright than higher order branch cuttings and this agreed with findings by Power et al. (1986). Conversely, rooted cuttings of *D. rappa* grew less upright than second order branch. It has been reported

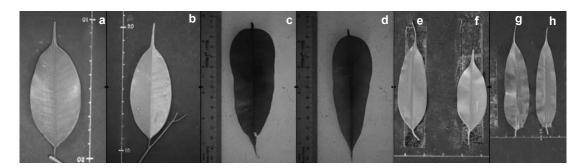


Figure 1Leaf characteristics by branch orders: (a & b) first and second branch order of Dryobalanops beccarii,
(c & d) first and second order branch of D. rappa; and leaf characteristic of rooted cuttings derived
from: (e & f) first and second order branch of D. beccarii and (g & h) first and second order branch
of D. rappa

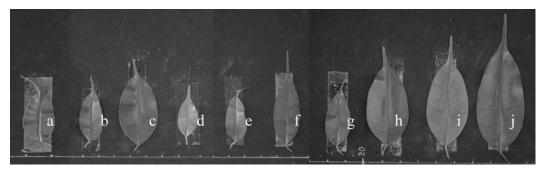


Figure 2 Leaf characteristics of rooted cuttings derived from epicormic shoots from bending of standing mature tree, stump and isolated stem segments of different ortets; leaf was taken from the third node from the shoot tip; rooted cuttings of epicormic shoots derived from bending of standing mature tree of *D. beccarii* of (a–c) 8-, 10- and 15-year-old (stem cuttings), (d–f) 8-, 10- and 15-year old (branch cuttings); and rooted cuttings of epicormic shoots of *D. beccarii* derived from: (g & h) stem and branch cuttings (tree stump) and (i & j) stem and branch cuttings (isolated stem segment)

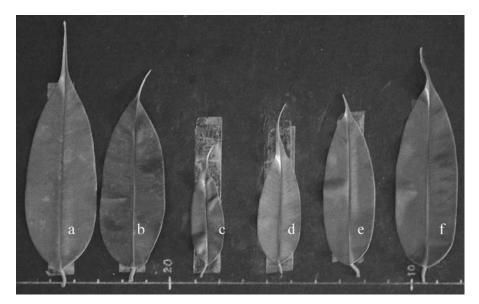


Figure 3 Leaf characteristics of rooted cuttings of epicormic shoots from bending of standing mature tree, stump and isolated stem segments; leaf was taken from the third node from the shoot tip; rooted cuttings of epicormic shoots of *D. rappa* derived from: (a–c) tree stump, bending of standing tree and isolated stem segment (stem cuttings), and (d–f) tree stump, bending of standing tree and isolated stem segment (branch cuttings)

that micropropagated Douglas-fir plantlets from cotyledonary tissue were plagiotropic (Ritchie & Long 1986). This contradicts the theory by Power et al. (1986) whereby plagiotropism is a function of maturation since cotyledonary material is presumably very juvenile.

The effect of position of stem segments strongly influenced rooting parameters and subsequent growth of newly-formed plants. Significant differences were found between using isolated stem segment and stump in rooting success, length of longest root and plagiotropism. This proves that the stage of ontogency is different in different parts of the plant (Fortanier & Jonkers 1976).

Cuttings derived from epicormic shoots from stump rooted more readily than those from other parts of *D. beccarii* and *D. rappa*. The results suggested that epicormic shoots derived from stumps are more juvenile than those derived from the upper part of the stem. This phenomenon, topophysis, supports the theory that maturation occurs in different phases (Olesen 1978). In transition from juvenile to mature phase, specific genes are turned on or off and this can negatively influence rooting since certain enzymes may or may not be produced (Davies 1993). Nevertheless, the primary control of rooting is still unclear (Heuser & Witham 1993, Hartmann et al. 2002).

Based on results obtained from the experiment, development of epicormic shoot has partially rejuvenated. The epicormic shoot bared some juvenile morphological characteristics while still inheriting certain mature characteristics. The 'ageing' component of cyclophysis has been reversed but the 'maturation' component remains unaltered (Medford 1992). Thus, true rejuvenation did not occur in epicormic shoots.

On the basis of leaf characteristics, rooted cuttings derived from epicormic shoots revealed that physiological rejuvenation occurred with morphologically more juvenile leaf than those derived from older ortets (Lanner 2002, Husen & Pal 2006). Moreover, physiological rejuvenation also varied with position of stem segment and this was supported by Fortanier and Jonkers (1976). This shows that the stage of ontogeny is different in different parts of the plant. However, complete rejuvenation does not happen. Therefore, rejuvenation through epicormic shoot development has only achieved partial rejuvenation (Husen & Pal 2003).

Procedure for clonal propagation of *D. beccarii* and *D. rappa* from epicormic shoots

Epicormic shoots from tree stumps have great potential as an alternative source of propagation material, at least if the objective is to initially propagate clones from mature material. Cuttings derived from epicormic shoots, particularly those from tree stumps proved to be the most successful in this study. These cuttings had many juvenile morphological characteristics in both stem and branch cuttings and had demonstrated promising level of rooting success and shoot growth compared with the rest of the sources of propagation materials. Interestingly, all rooted stem cuttings of D. beccarii and D. rappa had demonstrated equal potential to attain orthotropic growth for six months growing in containers.

From a practical point of view, epicormic shoots derived from isolated stem segments and bending of standing mature trees could provide abundant cutting materials for the initial stage of propagating adult genotypes. Furthermore, shoot growth of rooted cuttings from these sources was equal or even better compared with those derived from stumps. For initiation of clonal propagation from mature material, with the objective of obtaining true-to-type propagation material, production of epicormic shoots using isolated stem segment and bending of standing mature trees may contribute some promising cutting materials. This study also provides some preliminary information towards development of proper management schedule such as timing of collection of cuttings. This may help in future plant breeding programmes for both D. beccarii and D. rappa.

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