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Feasibility study of quality plantation pulpwood breeding on fibre length, vessel element length and their ratio sought by within-tree variations in *Eucalyptus* trees

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Abstract. The relationship between fibre length and vessel element length was examined by their within-tree variations for breeding quality plantation pulpwood from the view points of pulp properties, that is, shorter vessel element and longer fibre are preferable. Because the genetic difference between trees may express the different ratio of fibre length to vessel element length. Within-tree variations in the trunk of fibre length and vessel element length were studied in *Eucalyptus camaldulensis* and *E. globulus* trees grown at the same site. Within-tree variations of both cell length properties in both species were generally high in the upper and outer parts of the trunk in both fibre and vessel. No large difference of tendency was observed between individuals and between species except in the axial variation between individuals of *E. globulus* for vessel element length by statistical analysis. The relationships between fibre length and vessel element length were significant in all trees although the rate of fibre length to vessel element length was significantly different both between individuals in each species and between species. From these, the breeding program is expected to select a tree possessing genetically longer fibre length (more elongation of cambial initial) indicating higher breaking length with shorter vessel element length (shorter cambial initial) indicating higher printability, regardless the difference in the within-tree variations.

Key words: quality breeding, *Eucalyptus*, within-tree variation, fibre length, vessel element length.

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Introduction

Eucalyptus is the most popular plantation species for pulpwood and is cultivated extensively in many countries. Breeding trees possessing high pulp yield and strength are important for the management of pulpwood forests. Elite tree selection based on quality is also needed to reduce atmospheric CO_2 and the cost of pulp, especially for plantations with short rotations (Ona *et al*., 1996).

Fibre morphology is an important determinant of pulp properties in *Eucalyptus* (Higgins, 1978; Bamber, 1985; DuPlooy, 1988; Malan *et al*., 1994; O'Neill *et al*., 1996; Downes *et al*., 1997; Ona *et al*., 2001; Wimmer *et al*., 2002; Kibblewhite *et al*., 2004). In particular, fibre length is recognized as an important property for paper strength (Bamber

1985; Ona *et al.*, 2001). Similarly, vessel elements have a major effect on paper properties (Colley, 1973; Colley & Word, 1976; Ogata, 1978; Malan *et al.*, 1994; Ona *et al.*, 2001) and the penetration of pulping liquors (Hillis, 1969). These suggest that cell length properties, namely fibre length and vessel element length can be used as selection indices for quality breeding.

Within-tree variation of cell length property in *Eucalyptus* has been previously examined (Rudman, 1970; Valente *et al.*, 1992; Ridoutt & Sands, 1993; Jorge *et al.*, 2000; Hudson *et al.*, 2001; Muneri & Raymond, 2001; Rao *et al.*, 2002; Quilhó *et al.*, 2006). However, while a few papers have been published on the radial variation of vessel element length (Dadswell, 1958; Ohbabayashi & Shiokura, 1990; Sharma *et al.* 2005), there is little information on the variation in the axial direction. In addition to this, the tendency of within-tree variation of cell length property either between individuals or between species has been examined to a small extent using statistical data analysis with a quantitative approach.

From the view points of pulp properties, shorter vessel element and longer fibre are preferable (Ona *et al.*, 2001; Colley, 1975). The genetic difference between trees may express the different ratio of fibre length to vessel element length. Therefore, the examination of relationship between fibre length and vessel element length is important by their within-tree variations. This brings to improve the ratio of fibre length to vessel element length for breeding quality plantation pulpwood although little is investigated.

In this paper, we first examined the within-tree variations of fibre length and vessel element length in *E. camaldulensis* and *E. globulus* trees. We investigated the pattern in within-tree variations of the cell length properties between individuals and between species, respectively, in both radial and axial directions. To compare the trend of within-tree variation, it often occurs that one individual or species is normally distributed and the other polarized at some extremes (Ohshima *et al.*, 2005a), even though the same central tendency of within-tree variation was observed. Consequently, the Moses test was hired for this study. The Moses test is classified as one of the nonparametric analyses for two independent sample sets and is utilized in experimental studies, which assume that the treatment variable will influence subjects in either a positive or a negative way, creating a polarizing effect (Moses 1952; SPSS 11.0J). The Moses test concentrates on the findings in the distribution tails. If the possibility associated with the Moses test is lower than the desired significance level, then the two sample sets are not different. Second, we discussed the relationship between fibre length and vessel element length using within-tree values. Since total two trees in each species were limitedly used, this paper is a feasibility study.

Material and Methods

Materials

The sample trees were 14-year-old *E. camaldulensis* and *E. globulus* cultivated in a seed orchard at Manjimup, Western Australia. The plantation site has an annual average temperature of 15.5°C and rainfall of 1,000 mm (Ohshima *et al.*, 2005b). The two sample trees of each species, No.1 and No.2 trees, possessed average and superior growth characteristics, respectively in the seed orchard under the same silvicultural conditions. The height (h) and diameter (d) at breast height of the two sample trees were as follows. *E. camaldulensis* No.1: h = 15.2 m, d = 18.8 cm; No.2: h = 18.1 m, d = 23.5 cm; *E. globulus* No.1: h = 19.9 m, d = 24.4 cm; No.2: h = 30.0 m, d = 23.8 cm.

Discs of 6 cm thickness were cut from the sample trees at 1 m intervals, 0.3 m above the ground up to the small end diameter of 8 cm. Two strips, 2 cm wide were cut through the centre of each disc (Figure 1). One strip was divided into 2 cm (radial)

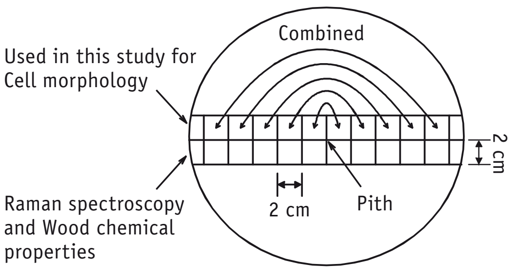

Figure 1. Sampling procedure.

× 2 cm (tangential) × 6 cm (axial) wood blocks from pith to bark. These blocks were stored in ethanol and utilized for the measurement of cell length properties. Another strip was used for Raman spectroscopy analysis and wet chemical analysis (Ona *et al.*, 1999, 2001).

Measurement of cell length properties

Fibre length and vessel element length were measured using small sticks (c.a. 1 × 1 × 10 mm) randomly taken from the whole block samples randomly and macerated with Schultze's solution (Saiki, 1985; Baba *et al.*, 1996). Fifty fibres and thirty vessel elements were randomly selected and measured using a digitizer (Oscon, Gradimate SQ-3000, Tokyo, Japan) equipped with a light microscope (Olympus CH, Tokyo, Japan).

Statistical analysis

The trend of within-tree variation of fiber length and vessel element length was examined between individuals and between species for both radial and axial directions using statistical data analysis (Ohshima *et al.*, 2005a). The linear regression line was created for radial variation at a given distance from the pith and for axial variation at a given height above the ground by the SigmaPlot 8.0 software (Systat Software, Chicago, IL, USA). The slopes for one individual and for one species were grouped as one sample data set and utilized for the examination of tendency difference of within-tree variations of the cell length properties between individuals and between species, respectively, in both radial and axial directions by the Moses test of extreme reactions (Moses, 1952) using a software of SPSS 11.0J (IBM, Armonk, NY, USA). All sample data sets were used without data trimming. The Moses test was performed with the significance level set to 0.05. Regression analysis between fibre length and vessel element length was performed logarithmically and the correlation coefficient was calculated by two-way ANOVA using software of SigmaPlot 8.0. The tolerance, stepwise and iterations were set at 0.0001, 100 and 100, respectively.

Results

Measured data of cell length properties are summarized in Table 1. The linear regression lines at given heights above the ground are plotted for fibre length against a distance from the pith for *E. camaldulensis* tree No. 1 (Figure 2).

The slopes of regression lines in both radial and axial directions are summarized in Tables 2 and 3, respectively. The slopes for one individual and for one species were grouped as one sample data set and utilized for the examination of tendency difference in within-tree variations

Table 1. Data of cell length properties (average ± standard deviation).

Cell length	*E. camaldulensis*		*E. globulus*	
	No. 1 (n=33)	No. 2 (n=44)	No. 1 (n=56)	No. 2 (n=93)
Fibre length / mm	0.671 ± 0.050	0.612 ± 0.066	0.846 ± 0.124	0.804 ± 0.146
Vessel element length / mm	0.214 ± 0.032	0.189 ± 0.027	0.237 ± 0.032	0.248 ± 0.060

Table 2. Slopes of linear regression lines from pith to bark at given heights above the ground in radial variation.

Sample	Height above the ground (m)	Fibre length (x 10^{-2} / m^2)	Vessel element length (x 10^{-2} / m^2)
E. camaldulensis No. 1			
	0.3	13.4	2.9
	1.3	1.4	1.1
	2.3	2.0	6.5
	3.3	13.0	7.3
	4.3	17.0	0.0
	5.3	15.7	18.7
	6.3	27.2	21.3
	7.3	26.5	13.0
	8.3	107.5	20.5
	9.3	27.0	26.0
E. camaldulensis No. 2			
	0.3	15.3	4.2
	1.3	11.6	4.2
	2.3	16.1	12.6
	3.3	33.3	7.4
	4.3	31.4	15.8
	5.3	25.3	5.7
	6.3	34.2	8.0
	7.3	43.0	12.0
	8.3	21.0	8.4
	9.3	29.3	-1.8
	10.3	24.5	-12.0
	11.3	24.5	-12.0
E. globulus No. 1			
	0.3	20.3	6.9
	1.3	31.6	2.7
	2.3	37.5	12.0
	3.3	39.2	12.2
	4.3	54.1	4.5
	5.3	40.5	8.0
	6.3	59.8	12.9
	7.3	15.6	9.8
	8.3	36.7	7.6
	9.3	29.5	4.0
	10.3	33.2	18.5
	11.3	97.7	16.0
	12.3	86.5	26.0
	13.3	46.5	31.0
E. globulus No. 2			
	0.3	35.9	8.5
	1.3	34.5	13.7
	2.3	48.2	22.5
	3.3	51.2	18.3
	4.3	42.6	16.5
	5.3	51.6	21.8
	6.3	54.3	14.0
	7.3	42.8	23.4
	8.3	56.4	23.4
	9.3	36.8	15.3
	10.3	62.9	30.2
	11.3	58.1	20.3
	12.3	61.7	27.5
	13.3	66.8	20.3
	14.3	46.7	22.4
	15.3	54.4	20.5
	16.3	35.8	11.4
	17.3	95.2	20.0
	18.3	68.8	31.0
	19.3	79.0	27.2
	20.3	83.0	24.2
	21.3	102.0	40.5
	22.3	114.5	-3.0

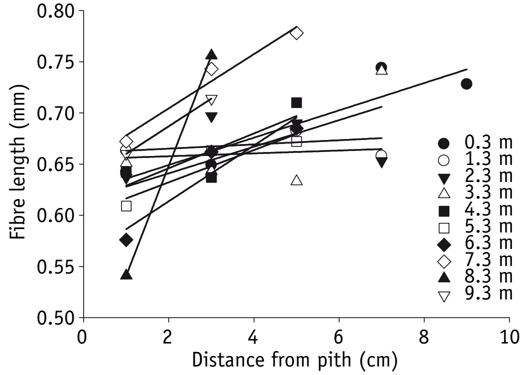

Figure 2. Plots of linear regression lines at given heights above the ground for fibre length against distances from pith in *E. camaldulensis* tree No. 1.

Table 3. Slopes of linear regression lines from bottom to top at given distances from pith in axial variation.

Sample	Distance from pith (cm)	Fibre length (x 10^{-2} / m^2)	Vessel element length (x 10^{-2} / m^2)
E. camaldulensis No. 1			
	1	-4.7	1.4
	3	10.4	7.3
	5	8.4	11.4
	7	-1.4	12.3
E. camaldulensis No. 2			
	1	8.9	7.1
	3	7.8	4.1
	5	13.6	5.3
	7	20.0	12.6
	9	9.0	38.5
	11	35.0	-2.0
E. globulus No. 1			
	1	4.1	1.0
	3	5.7	1.3
	5	2.4	1.1
	7	2.6	0.5
	9	27.3	-5.2
	11	2.0	-22.0
E. globulus No. 2			
	1	2.0	2.6
	3	7.9	4.0
	5	9.0	6.0
	7	5.0	5.7
	9	10.4	8.9
	11	-112.0	41.0

Table 4. Statistical differences of tendency in within-tree variations of cell length properties between trees and between species by the Moses test of extreme reactions ($P<0.05$) and its probability. P values are shown in parentheses. NS means not significant.

Trait	Fibre length	Vessel element length
Radial difference between individuals of *E. camaldulensis*	NS (1.000)	NS (0.368)
Radial difference between individuals of *E. globulus*	NS (0.684)	NS (0.684)
Radial diffrence between species	NS (0.865)	NS (0.120)
Axial difference between individuals of *E. camaldulensis*	NS (0.452)	NS (0.452)
Axial difference between individuals of *E. globulus*	NS (0.773)	Significant (0.000)
Axial diffrence between species	NS (0.805)	NS (0.368)

of cell length properties between individuals and between species, respectively, in both radial and axial directions by the Moses test of extreme reactions. The results are summarized in Table 4.

Within-tree variation of fibre length

Within-tree variation of fibre length in *E. camaldulensis* and *E. globulus* are shown in Figures 3 and 4, respectively.

In *E. camaldulensis*, both trees had long fibres in the upper and outer parts of the trunk. In particular, the upper parts in No.1 showed long fibres compared to the other parts. There was a pattern of increasing fibre length from pith to bark in the

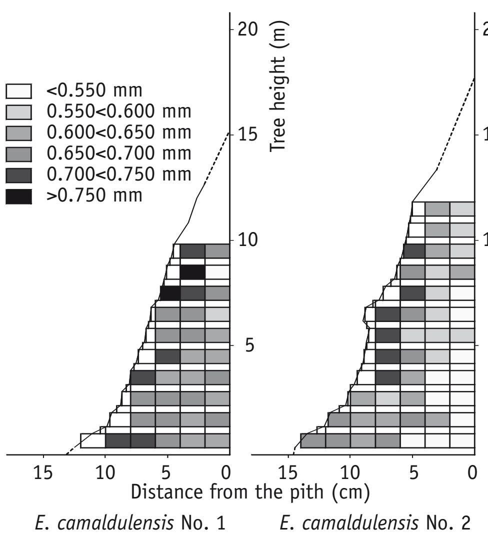

Figure 3. Within-tree variation of fibre length in *E. camaldulensis*.

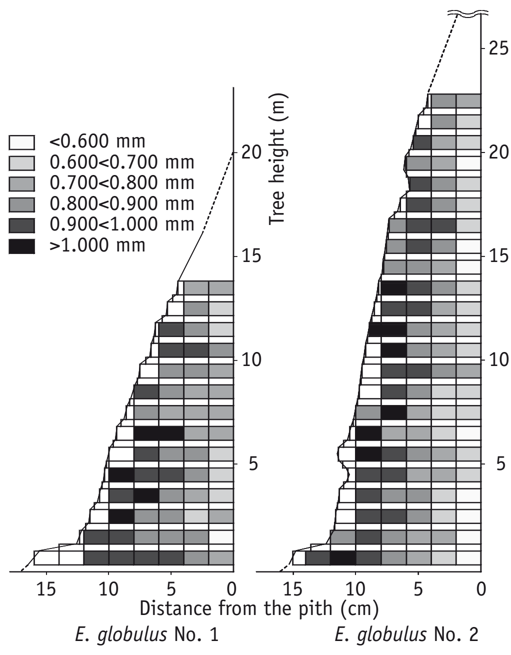

Figure 4. Within-tree variation of fibre length in *E. globulus*.

radial direction, and from bottom to top in the axial direction. Tree-to-tree variation was very small in fibre length regardless tree growth rate. Similarly, *E. globulus* trees had long fibres in the outer parts of the trunk. The outer parts at about one-third of the tree height above the ground showed particularly long fibres compared to the other parts. There was a pattern of increasing fibre length from pith to bark in the radial direction and from bottom to top in the axial direction. A relatively weak pattern was observed in the axial direction in the inner parts. Tree-to-tree variation was very small in *E. globulus* regardless tree growth rate.

Within-tree variation of vessel element length

Within-tree variations of vessel element length in *E. camaldulensis* and *E. globulus* are shown in Figures 5 and 6, respectively.

Both *E. camaldulensis* trees had long vessel elements in the upper and outer parts of the trunk. A pattern of increasing vessel element length was observed from pith to bark in the radial direction, and from bottom to the top in the axial direction. Tree-to-tree variation was very small regardless tree growth rate. Compared to this, *E. globulus* trees had long vessel elements in the outer parts of the trunk only. In particular, the outer parts in tree No. 2 showed long vessel elements compared to the inner parts. A pattern of increasing vessel element length was observed from pith to bark in the radial direction; however, different patterns between individuals in the axial direction were observed. A relatively uniform pattern in the axial direction was observed for tree No. 1, but a weak pattern of increasing vessel element length occurred from bottom to the top for tree No. 2. Tree-to-tree variation was large in the axial direction of *E. globulus*.

Relationship between fibre length and vessel element length

The relationships between fibre length and vessel element length in *E. camaldulensis*

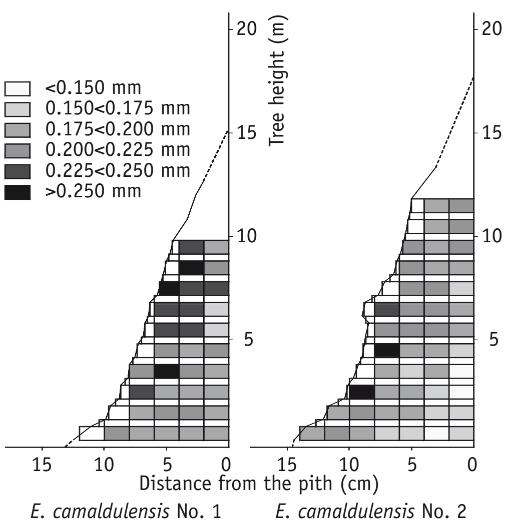

Figure 5. Within-tree variation of vessel element length in *E. camaldulensis*.

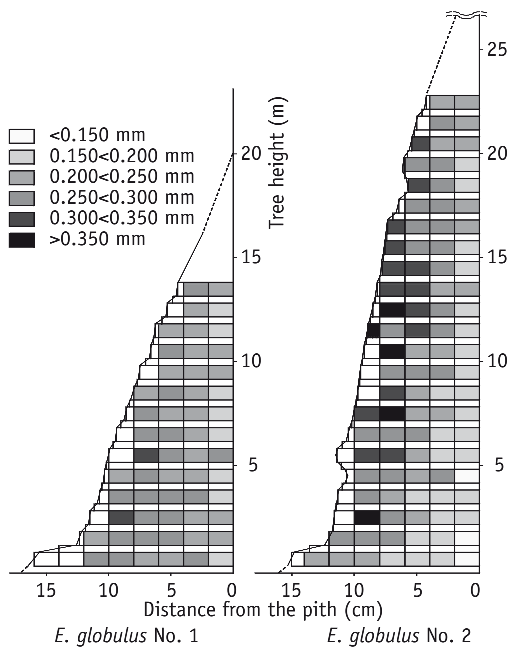

Figure 6. Within-tree variation of vessel element length in *E. globulus*.

and *E. globulus* are shown in Figure 7A and B, respectively. Statistical data are summarized in Table 5.

The relationships between fibre length and vessel element length were significant in all trees. However, the rate of fibre length to vessel element length was different both between individuals in each species and between species, respectively.

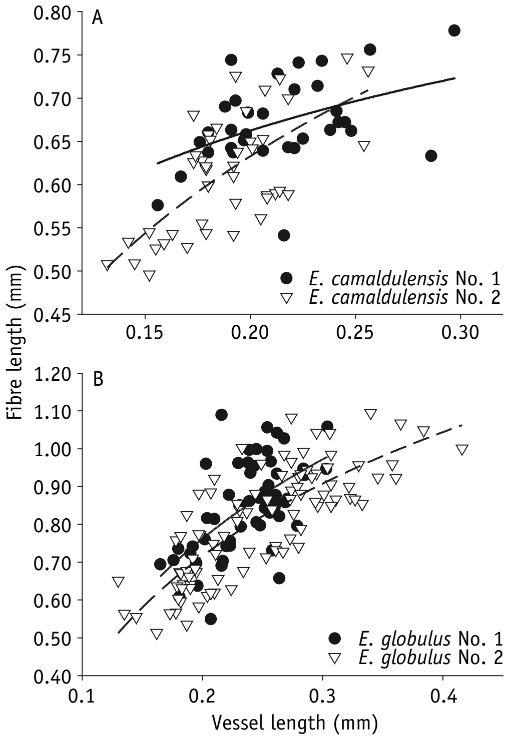

Figure 7. The relationship between vessel element length and fibre length using their within-tree variations. (A) *E. camaldulensis*, (B) *E. globulus*. The regression line is solid for tree No. 1 and dash for tree No. 2, respectively.

Discussion

Within-tree variation of fibre length

We previously examined within-tree variation of fibre morphology, including radial, tangential, and average diameters, wall thickness, and lumen diameter in *Eucalyptus camaldulensis* and *E. globulus* using the same trees in this study (Ohshima *et al*., 2003). In *E. camaldulensis*, within-tree variation was generally high in the lower and inner parts of the trunk for radial diameter, the outer parts for tangential diameter, the lower parts for average and lumen diameters, and the upper and outer parts for wall thickness. In *E. globulus*, within-tree variation was high in the outer parts for radial, tangential, average diameters, and the upper and outer parts for wall thickness. It was high in the lower parts for lumen diameter.

The pattern of within-tree variation of fibre length is almost the same for both species in fibre wall thickness previously reported using the same trees (Ohshima *et al*., 2003). This implies longer fibres have thicker walls and agrees with previous reports in *E. tereticornis* (Sharma *et al*., 2005).

Many papers have been published on the consistent radial variation of fibre length (increasing fibre length from pith to bark) reported for *E. grandis* (Bamber *et al*., 1969; Hans *et al*., 1972; Brasil & Ferreira, 1972; Taylor, 1973; Malan & Gerischer, 1987; Bhat *et al*., 1990; Malan, 1991), *E. grandis x urophylla* (Quilhó *et al*. 2006), *E. nitens* (Nicholls & Pederick, 1979; Malan, 1991; Muneri & Raymond, 2001), *E. pilularis* (McKimm &

Table 5. Statistical data of regression analysis between fibre length and vessel element length.

	E. camaldulensis		*E. globulus*	
	No. 1 (n=33)	No. 2 (n=44)	No. 1 (n=56)	No. 2 (n=93)
Correlation coefficient	0.443*	0.678***	0.583***	0.798***
Regression equation	$y = 0.908 + 0.153 \ln x$	$y = 1.1314 + 0.310 \ln x$	$y = 1.5951 + 0.518 \ln x$	$y = 1.4773 + 0.473 \ln x$

*Significant at 1%; **Significant at 0.1%; ***Significant at 0.01%; *y* – Fibre length; *x* – Vessel element length

Ilic, 1987), E. *saligna* (Bamber & Curtin, 1974; Ohbayashi & Shiokura, 1990), *E. camaldulensis* (Rudman, 1970), and *E. globulus* (Jorge *et al*., 2000; Hudson *et al*., 2001; Miranda *et al*., 2001). The pattern observed in our samples was similar to common eucalypts, which is not affected by different growth rates. This suggests that the increase in fibre length is based on an increase in the length of the cambial initials with increasing cambial age as reported in *E. globulus* (Ridoutt *et al*., 1993) since the trees utilized in this study were grown in one plot under the same silvicultural conditions to minimize environmental influence.

The maximum fibre length in the outer parts of *E. camaldulensis* and *E. globulus* was 0.778 and 1.095 mm, respectively, shorter than the reported mature wood fibre lengths of 0.840 and 1.210 mm (Dadswell, 1972). Hillis (1990) reported that, in many fast growing *Eucalyptus* species, the first 10–15 years of growth constitutes juvenile wood. This suggests the sample trees used in this study consisted of juvenile wood with the fibre length still increasing towards the bark.

In the axial direction, Wilkes (1988) described the fibre length as often increasing to a point well up the trunk and then declining at higher levels to the top in Eucalyptus. A similar pattern was reported in *E. grandis* (Bhat *et al*., 1990), *E. nitens* (Muneri & Raymond, 2001), *E. saligna* (Sardinha & Hughes, 1979), *E. regnans* (Raymond *et al*., 1998) and *E. globulus* (Muneri & Raymond, 2001). On the other hand, a weak pattern of decreasing fibre length from bottom to top was reported in *E. nitens* (Hudson *et al*., 2001) *E. grandis x urophylla* (Quilhó *et al*. 2006) and *E. globulus* (Valente *et al*., 1992; Ridoutt & Sands, 1993; Jorge *et al*., 2000; Hudson *et al*., 2001). In addition, a mixed pattern was reported in *E. tereticornis* (Rao *et al*., 2002). The axial variation of fibre length is also considered to be concomitant with the variation of cambial initial length in the vascular cambium as reported in *E. globulus* (Ridoutt & Sands, 1993).

Our results showed increasing fibre length from bottom to top and might be affected by the young age of our sample.

Ona *et al*. (2001) reported that fibre length significantly is related to breaking length. The results obtained suggest the outer parts of the trunk will have higher pulp strength than the inner. Similarly, *E. globulus* will be superior to *E. camaldulensis*.

Within-tree variation of vessel element length

For vessel morphology (radial, tangential and average diameters, and wall thickness) using the same trees, vessel morphology value was observed to increase in the outer parts of the trunk in both species (Ohshima *et al*., 2004).

The pattern of within-tree variation of vessel element length is almost the same for both species in vessel wall thickness. This implies longer fibres own thickened wall and agrees with previous reports in *E. tereticornis* (Sharma *et al*., 2005).

Vessel element length increased slightly from pith to bark in the radial direction in *E. saligna* (Ohbayashi & Shiokura, 1990) and in *E. tereticornis* (Sharma *et al*., 2005). Our results are in good agreement with these for both species in the radial direction. In the axial direction, it is difficult to compare our results to others because of few reports on this topic. Since vessel element length is reported as same as the length of the cambial initial (Bailey, 1920; Chalk, 1983; Kitin *et al*., 1999), within-tree variation of vessel element length is considered to be dependent on that of the cambial initial. In addition to this, one species type shows increase in vessel element length with age and another shows relatively constant pattern (Bailey, 1920; Furukawa *et al*., 1983). In the axial direction, the former type was clearly observed in *E. camaldulensis*, but both types were observed in *E. globulus*. Further studies are encouraged.

Longer vessel elements cause higher frequency of vessel picking (Colley, 1975). The results suggest *E. camaldulensis* may have higher printability than *E. globulus* due to less vessel picking.

Relationship between fibre length and vessel element length

Fibre length and vessel element length can have either significant or not significant correlations due to the various increase pattern of vessel element length with cambial age described elsewhere (Bailey, 1920; Furukawa *et al*., 1983). Even though slightly increased pattern of vessel element length from the pith to bark was observed, no significant correlation between the cell length properties was obtained in *E. tereticornis* (Sharma *et al*., 2005). Our results showed significant relationship between the cell length properties for both species. Since fibre length is derived from the length of cambial initial and its elongation (Honjo *et al*., 2005), the pattern of within-tree variation of fibre length depends on both internal factor of cambial age and external factor of climate. In this study, the utilized trees were grown under the same silvicultural conditions at the same site. The external factor is removed. From these, our results show genetic difference of cambial initial elongation from the same length of cambial initial.

In both species, tree-to-tree difference was observed and the breeding program is expected in the comparison of fibre length (elongation of cambial initial) to vessel element length (cambial initial) within the stem.

Among utilized four trees, *E. globulus* No. 1 may be a preferable quality plantation pulpwood because of higher rate of fibre length to vessel element length indicating higher breaking length with higher printability.

Conclusions

The within-tree variations were examined for fibre length and vessel element length in *E. camaldulensis* and *E. globulus* trees. No large difference of tendency was observed between individuals and between species in both radial and axial directions, excluding in the axial variation between individuals of *E. globulus* for vessel element length.

Regardless the difference in the within-tree variations, the relationships between fibre length and vessel element length were significant in all trees. However, in the rate of fibre length to vessel element length, the different magnitude was observed both between individuals in each species and between species. This is regardless the difference in the within-tree variations.

Consequently, the tree breeding program as quality plantation pulpwood is encouraged to select a tree owing longer fibre (≈ higher breaking length) with shorter vessel element (≈ higher printability).

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