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Original article

Cellulose content as a selection trait in breeding for kraft pulp yield in *Eucalyptus urophylla*

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Keywords:
cellulose content /
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pulp yield

Abstract

• The aim of this study was to investigate the effectiveness of using cellulose content, measured by the diglyme-HCl method, as a selection trait in breeding programs for kraft pulp yield in *Eucalyptus urophylla*.

• A total of 275 trees from sixty-two families were sampled from a thinned progeny trial of *E. urophylla* in northern Vietnam to evaluate cellulose content from breast-height increment cores. Among those, twenty unrelated trees were felled to evaluate cellulose content and pulp yield from breast-height disk samples.

• The regression of pulp yield of disk samples on cellulose content was strong either from disks (R^2 = 0.83) or increment cores (R^2 = 0.69). There was no significant difference in cellulose content between the provenances. The narrow-sense within-provenance heritability of cellulose content was 0.50 and the coefficient of additive genetic variation was 3.9%. Genetic correlations between cellulose content and growth (0.28–0.45) or wood basic density (–0.02) were not significantly different from zero.

• Breast-height increment core cellulose content measured by diglyme-HCl method is under strong genetic control and can be used to rank trees for pulp yield in *E. urophylla* plantations. Selection for increased cellulose content would have only minor effects on growth and wood basic density.

Mots-clés :
teneur en cellulose /
***Eucalyptus urophylla* /**
corrélation génétique /
héritabilité /
rendement en pâte

Résumé – La teneur en cellulose comme un trait de sélection pour l'amélioration du rendement en pâte kraft d'*Eucalyptus urophylla*.

• L'objectif de cette étude était d'étudier l'efficacité de l'utilisation de la teneur en cellulose, mesuré par la méthode diglyme-HCl, comme un trait de sélection dans les programmes d'amélioration du rendement en pâte kraft chez *Eucalyptus urophylla*.

• Un total de 275 arbres issus de soixante-deux familles ont été échantillonnés, à partir d'un essai de descendance d' *E. urophylla* dans le nord du Vietnam, pour évaluer la teneur en cellulose de carottes d'accroissement prélevées à hauteur de poitrine. Parmi ces arbres, vingt ont été abattus afin d'évaluer la teneur cellulose et le rendement en pâte de disques échantillons, prélevées à hauteur de poitrine.

• La régression de la production de pâte du disques échantillons a été forte sur la teneur en cellulose, soit à partir des disques (R^2 = 0.83) ou des carottes d'accroissement (R^2 = 0.69). Il n'y a pas de différence significative pour la teneur en cellulose entre les provenances. Le sens restreint dans l'héritabilité intra-provenance de la teneur de la cellulose était 0,50 et le coefficient de variation génétique additive a été de 3,9 %. Les corrélations génétiques entre la teneur en cellulose et la croissance (0,28–0,45) ou l'infra densité du bois (–0,02) ne sont pas significativement différentes de zéro.

• La teneur en cellulose des carottes d'accroissement mesurée par la méthode diglyme-HCl est sous contrôle génétique fort et peut être utilisée pour classer les arbres pour le rendement en pâte dans les plantations d'*E. urophylla*. La sélection pour l'augmentation de la teneur en cellulose aurait seulement des effets mineurs sur la croissance et l'infra-densité du bois.

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1. INTRODUCTION

Eucalyptus is the most widely planted hardwood tree genus in the world. Eucalypt plantations are the largest short fiber pulp resources, occupying globally more than 18 million hectares, mainly in South America, South Africa, India, China and South East Asia (FAO, 2001). Breeding objectives for pulp production systems have been developed for eucalypts (Borallho et al., 1993; Greaves et al., 1997). These studies have concluded that the traits with greatest impact on improving profitability or reducing cost of pulp production are wood volume, wood basic density and pulp yield. Pulp yield is defined as oven dry weight of pulp per unit oven dry weight of wood (Smook, 1992). A higher pulp yield will allow a reduction in the wood requirement per ton of pulp produced, and hence wood costs. Kraft pulp is the most important, accounting for about 73% of world total pulp production in 2007 (RISI, 2008).

Kraft pulp yield is traditionally assessed by cooking wood chips in an alkaline solution at elevated temperature and pressure to dissolve lignin and leave fibres composed of cellulose and hemicellulose intact (Smook, 1992). This method is time-consuming and expensive, restricting the number of samples that may be processed. The minimum sample size for laboratory pulping is usually about 500 g. This makes it destructive in that the tree must be felled to obtain such a large sample. Therefore, the method has limitations when screening many genetic entries in a breeding program.

Cellulose contributes approximately 40–50% of the dry weight of wood, occurring predominantly in the secondary cell wall and is the main component (74–86% by weight) of kraft pulp (Sjöström, 1992). Cellulose content is strongly correlated with kraft pulp yield (Kube and Raymond, 2002; Wallis et al., 1996) and can therefore be used as an indirect measure of it. The methods to determine cellulose content are simpler and less expensive than the direct measurement of pulp yield, require a much smaller wood sample of about 1 g dry weight, and therefore allow samples to be taken without trees having to be felled (Kube and Raymond, 2002; Raymond and Schimleck, 2002; Wallis et al., 1996). There are several methods to determine cellulose content in wood. Among these methods, the diethylene glycol dimethyl ether-hydrochloric acid (diglyme-HCl) method developed by Wallis et al. (1997) uses less toxic chemicals, can be undertaken in sealed bottles and allows multiple samples to be processed at the same time. Cellulose content determined by this method is strongly correlated with whole tree kraft pulp yield estimated for *E. globulus* and *E. nitens*, with correlation coefficients ranging from 0.82 to 0.91 (Apiolaza et al., 2005; Kube and Raymond, 2002; Wallis et al., 1996).

To date, there are few published studies on the genetic control of cellulose content in wood. These focus mainly on two temperate eucalypt species, *E. globulus* and *E. nitens* (Apiolaza et al., 2005; Kube et al., 2001; Raymond and Schimleck, 2002; Schimleck et al., 2004), and *Populus deltoides* (Olson et al., 1985). Narrow-sense heritability of cellulose content is reported to be high, ranging from 0.32 to 1 (Apiolaza et al., 2005; Kube et al., 2001; Raymond and Schimleck, 2002; Schimleck et al., 2004), but the coefficient of additive genetic variation in one study was low (3%) (Apiolaza et al., 2005). Genetic gain in cellulose content from selecting the top 5% of the population, in studies by Kube et al. (2001) and Schimleck et al. (2004), was about 3–4% of the original population mean, with little effect on growth or wood density. Such levels of genetic improvement could reduce total pulping cost by 2–3% (Greaves and Borallho, 1996).

Eucalyptus urophylla S. T. Blake and its interspecific hybrids, particularly with *E. grandis*, are extensively used by the pulp and paper industry in tropical regions of Brazil, South Africa, Congo, coastal southern China and South East Asia because of their acceptable wood quality, rapid growth, canker disease resistance and high volumetric yield (Eldridge et al., 1994). Attempts at genetic improvement of these taxa have mainly focused on increasing volume and wood basic density (Kien et al., 2009; Kien et al., 2008; Santos, 1990; Wei and Borralho, 1997). Better understanding of genetic control of cellulose content would assist breeding for improved profitability of pulpwood plantations of this species.

The overall objective of this study was to evaluate the use of cellulose content as a selection trait in breeding programs for kraft pulp yield in plantations of *E. urophylla*. In order to confirm the effectiveness of the diglyme-HCl method for predicting pulp yield in *E. urophylla*, we first determined the relationship between cellulose content, estimated by the diglyme-HCl method, and kraft pulp yield of wood chip samples taken from a breast-height disk. The following questions were then addressed:

(i) How strong is the genetic control of cellulose content in *E. urophylla*?
(ii) What are the genetic relationships between cellulose content, growth and wood density?

2. MATERIALS AND METHODS

Trees included in this study were sampled from a thinned progeny trial of *E. urophylla* at Ha Tay province in northern Vietnam (latitude 21° 07′ N; longitude 105° 26′ E; altitude 44 m above sea level), at age ten years. The soil is degraded ferralitic clay-loam with general loss of topsoil through erosion and low levels of nitrogen, phosphorus and potassium and soil depth is about 40–70 cm. At planting, the progeny test included 144 open-pollinated families from nine natural provenances in Indonesia (Tab. I), set out in a row-column design (12 rows × 12 columns) (Williams and Matheson, 1994) with eight replicates and four-tree row plots. The initial spacing was 4 m between rows and 1.5 m within rows. The progeny test had a first thinning at age two years by leaving the single tree with the best vigour and stem form in each plot, and a second thinning at age five years in which the 17 slowest-growing families, and inferior trees of some other families, were removed, leaving 127 families with four to eight trees per family.

Sixty-two families from nine provenances, in total 275 trees, were included in this study for evaluating genetic parameters (Tab. I). To determine the relationship between cellulose content and pulp yield, among these selected individuals, 20 unrelated trees were analysed.

Table I. Provenances and number of families per provenance sampled in the study.

CSIRO Seedlot	Provenance and island	Lat.	Long.	Alt. (m)	No. of families
17 564	Mandiri, Flores	08° 15′ S	122° 58′ E	410	4
17 565	Lewotobi, Flores	08° 32′ S	122° 48′ E	375	16
17 567	Egon, Flores	08° 38′ S	122° 27′ E	450	14
17 831	N Ilwaki, Wetar	07° 52′ S	126° 27′ E	515	8
17 836	SW Uhak, Wetar	07° 39′ S	126° 29′ E	350	6
17 840	Wai Kui, Alor	08° 14′ S	124° 44′ E	540	3
17 841	Piritumas, Alor	08° 19′ S	124° 31′ E	355	4
17 842	Dalaki Mt, Pantar	08° 31′ S	124° 05′ E	440	3
17 843	Baubilatung, Pantar	08° 20′ S	124° 02′ E	285	4

2.1. Pulp yield evaluation

A total of twenty trees from twenty different families were randomly selected and felled to evaluate the relationship between cellulose content and pulp yield. Before felling the selected trees, we collected three 5-mm increment cores just below the disk: one pith to bark core to determine wood basic density using the water displacement method (Olesen, 1971); and two bark to bark cores were combined to provide sample for cellulose content determined by diglyme-HCl method (Wallis et al., 1997). From each felled tree, a 10 cm disk was cut at breast height (1.3 m). Each disk was divided by two diametrical cuts, 10° apart, yielding two narrow sectors which together provided the sample for cellulose analysis and two large sectors for pulp yield analysis. Duplicated analysis of cellulose content of cores, cellulose content of disk and pulp yield were carried out for each tree.

Pulp yield was analysed by cooking 500 g of wood chips sized approximately 25 × 25 × 2 mm at average moisture content of 12.5% in alkali at 170 °C to Kappa number 18 ± 1 and determined as the ratio between oven dry weight of residue after cooking and oven dry weight of wood chips, expressed as a percentage. The Kappa number is an indication of the residual lignin in the pulp and measures the amount of bleach required during digestion of a wood pulp in order to obtain a pulp with a given degree of whiteness (Smook, 1992).

2.2. Determination of cellulose content using the diglyme-HCl method

Wood samples were ground to a particle size less than 1 mm. Air-dried wood meal (1.0 g oven dry) was added to a 50 mL reaction bottle together with 10 mL of diglyme and 2 mL of 10 M hydrochloric acid. The bottle was sealed with a teflon-coated cap and then shaken at low speed in a water bath at 90 °C for one hour. The residue was then collected by vacuum filtration through an oven-dry weighed filtering funnel and washed with 50 mL methanol and then 250 mL distilled boiling water. The funnel was dried at 105 °C overnight and then cooled to room temperature in a desiccator. Cellulose content was determined as the ratio of oven dry weight of residue to oven dry weight of original material, expressed as a percentage.

2.3. Genetic evaluation

To study heritability and additive genetic variation of cellulose content, we randomly selected 62 families from all nine provenances from all four islands Flores, Wetar, Pantar and Alor, choosing three to sixteen families per provenance, representing about half of the families of each provenance planted in the trial (Tab. I). All trees in each selected family (four to six trees per family) remaining in the trial were sampled, yielding a total of 275 trees. On each tree, we measured diameter over bark at breast height (hereafter referred to as diameter) and height. Three 5-mm increment cores were collected at breast height: one pith to bark core was used to determine wood basic density using the water displacement method (Olesen, 1971); and two other cores (bark to bark) were combined to provide a sample for cellulose content using the diglyme-HCl method (Wallis et al., 1997).

Wood basic density (*DEN*) was determined as:

$$DEN = \frac{w_2}{w_1} (\text{g cm}^{-3})$$

where w_1 = weight of water displaced by immersion of core, which indicates fresh volume of sample; and w_2 = oven dry weight of sample. Conical stem volume (*VOL*) of each sampled tree was calculated as:

$$VOL = \frac{1}{3}\ \pi\ \frac{DBH^2 \times HT}{40} (\text{dm}^3)$$

where *HT* = total tree height in m and *DBH* = diameter over bark in cm at breast height assessed at age 10 years.

2.4. Data analysis

We first calculated linear regressions between (i) pulp yield and cellulose content of increment cores and (ii) pulp yield and cellulose content of disks at breast height using the means of the duplicate pulp yield and cellulose samples of the 20 trees which were felled. The linear regressions were estimated using the model:

$$y = a + bx \quad (1)$$

using PROC REG in SAS software (SAS Institute, 2004), where y is pulp yield as percentage by weight of oven dry wood chips and x is cellulose content as percentage by weight of oven dry wood meal, a is the intercept and b is the slope of the line.

Repeatabilities of measurements of pulp yield and cellulose content of increment cores and disks were estimated as:

$$r = \frac{\sigma_S^2}{\sigma_S^2 + \sigma_W^2}$$

where r is the repeatability of measurements; σ_S^2 is the variance of measurements between trees and σ_W^2 is the variance between different measurements on the same tree. Mean within-tree standard deviations for the three variates were also calculated.

Estimation of variances and covariances in the genetic study using ASReml (Gilmour et al., 2006) was based on a multivariate individual tree mixed-effects model:

$$\mathbf{y} = \mathbf{X_B b} + \mathbf{X_M m} + \mathbf{X_P p} + \mathbf{Z u} + \mathbf{e} \quad (2)$$

where **y** is the vector of observations of diameter, height, volume, wood density and cellulose content, **b** is the vector of fixed replicate effects, **m** is the vector of fixed island effects, **p** is the vector of fixed provenance effects, **u** is the vector of random individual effects, and **e** is the vector of random residuals, $\mathbf{X_B}$, $\mathbf{X_M}$, $\mathbf{X_P}$ and **Z** are incidence matrices relating **b, m, p** and **u** to **y**. The random effects were assumed

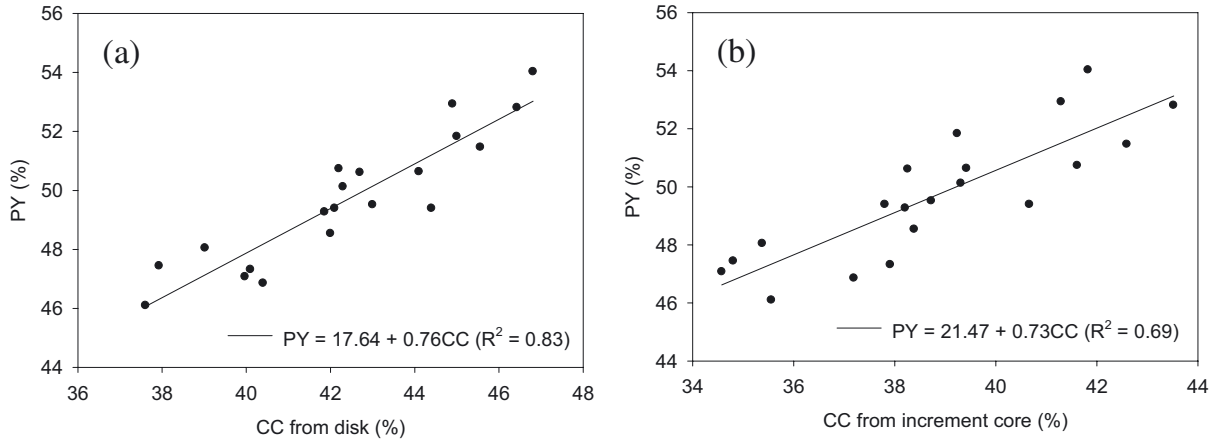

Figure 1. Linear relationships between pulp yield (PY) from disks and cellulose content (CC) from (a) discs and (b) increment cores.

to follow an independent multivariate normal distribution with zero means and (co)variances

$$V\begin{bmatrix}\mathbf{u}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{G}\otimes\mathbf{A} & \mathbf{0}\\ \mathbf{0} & \mathbf{R}\otimes\mathbf{I}\end{bmatrix}$$

where **0** is a null matrix, **I** is an identity matrix of order equal to the total number of genetic entries, and residuals, respectively; and ⊗ is the direct (Kronecker) product operation. $\mathbf{G} = \{\sigma_{u_i u j}\}$, and $\mathbf{R} = \{\sigma_{e_i e j}\}$ are the additive genetic and residual variance-covariance matrices between trait i and j, denoting variance when $i = j$. **A** is the numerator relationship matrix. The significance of fixed effects was tested using F-tests. The outcrossing rate in natural stands of *E. urophylla* has been estimated to be about 90% (House and Bell, 1994; Gaioto et al., 1997). This information was incorporated in the calculations of genetic parameters in ASReml by declaring a selfing rate of 10% in the population in the model (Gilmour et al., 2006).

Phenotypic variance (σ_P^2), narrow-sense individual tree heritability (h^2), coefficient of additive genetic variation (CV_A), and genetic (r_A) and environmental correlation (r_E) between traits, were estimated as:

$$\sigma_P^2 = \sigma_A^2 + \sigma_E^2$$

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2}$$

$$CV_A = \frac{100\sigma_A}{\bar{X}},\ \text{where } \bar{X} \text{ is mean value of the trait}$$

$$r_A = \frac{\sigma_{A_1A_2}}{\sigma_{A_1}\sigma_{A_2}}$$

$$r_E = \frac{\sigma_{E_1E_2}}{\sigma_{E_1}\sigma_{E_2}}$$

where σ_A^2 is the additive variance, σ_E^2 is the environmental variance, σ_A is the square root of additive genetic variance, σ_E is the square root of environmental variance, $\sigma_{A_1A_2}$ and $\sigma_{E_1E_2}$ are the additive genetic and environmental covariance between trait 1 and trait 2, respectively. Standard errors of the estimates of heritabilities and genetic correlations were calculated using a standard Taylor series approximation in the ASReml software (Gilmour et al., 2006). Log likelihood tests were used to test whether environmental and genetic correlations differed significantly from zero.

Table II. Mean cellulose content (CC), pulp yield (PY), and mean standard deviation (SD) for duplicates and its repeatability (r) based on a sample of 20 unrelated trees.

	Mean (%)	Min (%)	Max (%)	Mean SD for duplicates (%)	r
CC from cores	38.8	34.6	43.5	0.44	0.96
CC from disk	42.4	37.6	46.8	0.37	0.98
PY from disk	49.7	46.1	54.0	0.68	0.90

3. RESULTS

3.1. Relationship between pulp yield and cellulose content

To validate selection based on small samples, we determined the correlation between the cellulose content of 1 g samples, from increment cores or disks that can be taken without serious damage to the tree, and pulp yield estimated from 500 g samples from felled trees.

For the 20 unrelated trees in the progeny test, the mean pulp yield was 49.7%, the mean cellulose content of the disks was 42.4% and mean cellulose content of the increment cores was 38.8%. Repeatability was 0.90 for pulp yield and 0.96 and 0.98 for the cellulose cores and disks (Tab. II). The mean standard deviation of duplicates was 0.68% for pulp yield (1.4% of the mean), and from 0.37% to 0.44% for cellulose (0.9–1.0% of the means). The regression of pulp yield of disk samples on cellulose content of disk samples was strong ($R^2 = 0.83$, $p < 0.001$) (Fig. 1a). The regression for pulp yield of disk samples on cellulose content of core samples was somewhat weaker ($R^2 = 0.69$, $p < 0.001$) (Fig. 1b), but still explained 69% of the variance in pulp yield.

3.2. Genetic parameters

To assess the consequences of selection for high cellulose content, we estimated the degree of genetic control of cellulose content and genetic correlations between cellulose with

Table III. Mean values, *p*-values for difference between provenances and islands, heritabilities, and coefficients of additive variation (CV_A) for traits in the study (sample of 275 trees).

Trait	Mean	*p*-value		h^2 ± s.e.	CV_A (%)
		Provenances	Islands		
Diameter (cm)	20.1	0.62	0.10	0.32 ± 0.18	10.4
Height (m)	20.1	0.55	0.14	0.22 ± 0.17	7.8
Volume (dm^3)	251.6	0.88	0.13	0.38 ± 0.19	30.8
Cellulose content (%)	39.6	0.55	0.83	0.50 ± 0.20	3.9
Wood density (g cm^{-3})	0.5	0.32	0.65	0.48 ± 0.20	5.6

Table IV. Environmental (below diagonal) and genetic correlations (above diagonal) between diameter (DBH), height (HT), volume (VOL), wood basic density (DEN) and cellulose content (CC).

	DBH	HT	VOL	DEN	CC
DBH		0.98 ± 0.05	0.99 ± 0.02	0.30 ± 0.38	0.45 ± 0.29
HT	0.92 ± 0.02		0.97 ± 0.05	0.35 ± 0.44	0.28 ± 0.36
VOL	0.93 ± 0.02	0.90 ± 0.02		0.31 ± 0.35	0.45 ± 0.27
DEN	–0.44 ± 0.23	–0.42 ± 0.23	–0.47 ± 0.25		–0.02 ± 0.31
CC	0.44 ± 0.19	0.53 ± 0.17	0.35 ± 0.21	-0.25 ± 0.27	

growth and wood basic density. Cellulose content and wood density had heritabilities of 0.50 and 0.48, respectively, substantially higher than the heritabilities of the growth traits, which ranged from 0.22 to 0.38 (Tab. III). Heritabilities of all traits had large standard errors which can be explained by the small sample size. Coefficients of additive genetic variation of cellulose content and wood density were 3.9 and 5.6%, respectively, lower than those estimated for growth traits (7.8–30.8%). There were no significant differences either between provenances, or between islands, for any of the traits examined in this study (Tab. III). Genetic correlations between cellulose content and growth; and between cellulose content and wood density had high standard errors and were not significantly different from zero (Tab. IV). Environmental correlations between cellulose content and growth were moderately positive (0.25 to 0.53); and weak to moderate but negative between wood density and other traits (–0.02 to –0.47) (Tab. IV).

4. DISCUSSION

4.1. Relationship between pulp yield and cellulose content

Cellulose content of increment cores was shown to be a reasonably reliable indirect measure of pulp yield. The reasonably good correlations between pulp yield and cellulose content either from disks or increment cores indicated that cellulose content determined by the diglyme-HCl method is a good predictor of pulp yield as previously has been shown in *E. globulus* and *E. nitens* (Wallis et al., 1997; Raymond and Schimleck, 2002; Schimleck et al., 2004). Cellulose content determined by this method had high repeatability (r = 0.96–0.98) with small standard deviation of duplicate measurements (about 1% of the mean) indicating that cellulose content measured by this method can give reliable results. The somewhat higher standard deviation obtained for the estimate of pulp yield duplicates (0.68%, or about 1.4% of the mean) contributes to imprecision of the relationships between cellulose and pulp yield. Several recent studies evaluating cellulose content have used NIR (near infrared reflectance) prediction of cellulose, using calibrations developed from a sub-set of the samples that have been chemically analysed for cellulose (Raymond and Schimleck, 2002; Schimleck et al., 2004; Apiolaza et al., 2005). Such calibrations enable accurate prediction of cellulose content from NIR information in *E. globulus* and *E. nitens* (R^2 = 0.83–0.92) (Raymond and Schimleck, 2002; Schimleck et al., 2004). NIR prediction is faster than the direct measure using diglyme-HCl method (Schimleck et al., 2004) and its use would increase the number of samples that can be analysed. As discussed above, cellulose content measured by the diglyme-HCl method was determined with high repeatability, and could be used to develop NIR-predicted cellulose content in this species.

The higher cellulose content of the disk samples relative to the cores probably arose because cores over-sample the inner wood, whereas disks give a representative sample at the sampling height (Downes et al., 1997). Breast-height pulp yield reported in this study may not be truly representative of the whole tree pulp yield. However the average pulp yield (49.7%) is similar to that of other reports on *E. urophylla* (Schimleck et al., 2006). The correlation between cellulose content of 12 mm increment cores and whole tree kraft pulp yield was reported to be relatively high (R^2 = 0.58) in hybrid poplar (Schimleck et al., 2005). Correlation between pulp yield measured from disk sample at breast height and whole tree values have been reported to be good (R^2 = 0.54–0.75) in *E. globulus* and *E. nitens* (Raymond et al., 2001b; Schimleck and Mitchell, 1998). In our study, increment core cellulose content was less strongly correlated with pulp yield (R^2 = 0.69) compared to cellulose content of disk samples (R^2 = 0.83), which might indicate that the prediction of pulp yield will not

be highly accurate if it is based on increment core cellulose. However, the two regression equations had very similar slopes (b_{disk} = 0.76 and b_{core} = 0.73), and the difference between the intercepts was 3.6%, which accounted for slightly different mean cellulose content of cores and disks (3.8%) (Fig. 1). The regression of cellulose content of core samples on cellulose content of disk samples was also strong (R^2 = 0.81, $p < 0.001$), and both gave high repeatability. The results suggest that cellulose content of cores determined using the diglyme-HCl method should be useful for ranking trees for breeding for kraft pulp yield in *E. urophylla*.

4.2. Effect of thinning and sampling method on heritabilities and genetic correlations

The trial used in this study was selectively thinned; and only 62 families with 4–5 trees per family were sampled in this study. Heritabilities and genetic correlations estimates could be biased because of removing the slowest-growing trees and the poorest families at earlier ages. Effects of thinning on heritability have been reported for growth traits, resulting either in increased (Gapare et al., 2003; Matheson and Raymond, 1984) or decreased heritability (Wei and Borallho, 1998b). Use of longitudinal multivariate REML analysis has been reported to reduce the effects of thinning and selection on the estimation of genetic parameters (Apiolaza et al., 2000; Wei and Borallho, 1998b). Kien et al. (2009) conducted a simulated selective thinning at age 2 years in the same trial used in this study and found stronger relationship between family breeding values using thinned data predicted by longitudinal multivariate and unthinned data (R^2 = 0.68) than those observed from univariate analysis between thinned and unthinned data (R^2 = 0.54). We have also tested heritability of growth traits using data from the entire population using the longitudinal mixed model described in Kien et al. (2009). Resulting heritabilities were 0.33 and 0.24 for DBH and HT, respectively, similar to the heritabilities estimated from sample population in this study but with lower standard error (0.08). Barnes et al. (1992) reported that heritability estimates are often inflated by selective thinning if there are dominance effects present. Wood quality traits are usually under strong additive genetic control (Cornelius, 1994; Raymond, 2002) while dominance effects can be significant in the genetic control of growth traits in eucalypts (Bouvet et al., 2009). Therefore, selective thinning for growth should have had little effect on heritability of wood density and cellulose content. Wood density assessed from the same population at age 8 years using all trees in the population gave a heritability estimate of 0.61 ± 0.13 (Kien et al., 2008), similar to that from the current study (0.48 ± 0.20). Coefficients of additive genetic variation were similar in both studies. The coefficient of additive genetic variation estimated for cellulose content in our study was also similar to the value reported by Apiolaza et al. (2005) from an unthinned progeny test of *E. globulus* in Australia.

To our knowledge, there are no published studies reporting on the effect of selective thinning on genetic correlations among wood properties and growth traits. However, estimated genetic correlations of cellulose content and wood density with growth traits in our study are in general agreement with previous studies in *Eucalyptus* species; these report a wide range of estimated genetic correlations, many of them non-significant (Apiolaza et al., 2005; Kien et al., 2008; Kube et al., 2001; Raymond and Schimleck, 2002; Wei and Borralho, 1997). The genetic correlations between wood density and growth in our study agreed well with those of Kien et al. (2008) who found genetic correlations between wood density and growth traits to be in the range from 0.10 to 0.28 and non-significant.

4.3. Genetic parameters and implications for breeding

Heritability for cellulose content was moderate (0.50), while the coefficient of additive genetic variation for cellulose content was relatively low at 3.9%. The heritability of cellulose content was comparable to results reported in *E. globulus* and *E. nitens* which are in the range from 0.32 to 0.76 (Kube et al., 2001; Raymond and Schimleck, 2002; Schimleck et al., 2004). Heritability estimates for wood density were similar to those reported by Kien et al. (2008), which were based on all the families represented in the progeny trial. The heritabilities reported in this study should be interpreted with caution as they were estimated from limited material and from a single site which may be biased by the presence of additive genetic-by-environment interaction variance in the estimates of the additive genetic variance. Kien et al. (2009) reported high genetic correlation in growth traits between this trial and a parallel trial in northern Vietnam, probably owing to high similarity in soil and climatic conditions between the two sites, which were separated by less than 60 km. The genetic correlation for basic density of wood cores between the two sites was also strong (r_A = 0.89) (Kien et al., 2008). Significant genotype-by-environment interactions in growth traits for this species have been reported (Mori et al., 1988; Tripiana et al., 2007). In other *Eucalyptus* species, genotype-by-environment interaction has commonly been found to be moderate for growth traits, but small and of no practical importance for wood density, cellulose content and pulp yield (Kube et al., 2001; Raymond et al., 2001a). Therefore, across-site heritabilities for growth traits in *E. urophylla* across contrasting environments might be reduced owing to the presence of genotype-by-environment interaction while those for wood density and cellulose content would probably tend to remain stable.

Genetic correlations between cellulose content and growth or wood density were weak and non-significant in the present study. This information might suggest that independent selection for any of these traits would have minor effects on the other traits. However, the conclusion should be interpreted with caution as it was based on a small sample size. Nonetheless, a selection index combining growth, wood density and cellulose content could be used when breeding for kraft pulp yield in *E. urophylla*, which would increase these traits simultaneously to maximise enterprise profit. Published estimates of genetic correlations between cellulose content and growth

or wood density for eucalypts are highly variable, both positive and negative, either weak or strong in different studies (Apiolaza et al., 2005; Kube et al., 2001; Raymond and Schimleck, 2002; Tibbits and Hodge, 1998). Genetic correlations between cellulose content and diameter are usually positive (0.24 to 0.86) in *E. nitens* (Kube et al., 2001; Tibbits and Hodge, 1998), but variable (–0.43 to 0.61) in *E. globulus* (Apiolaza et al., 2005; Raymond and Schimleck, 2002). Generally, genetic correlations between cellulose content and growth or wood density have been estimated with large standard errors. There are several possible reasons for the variability in estimates of genetic correlations. Firstly, as in the current study, genetic correlations involving wood property traits have typically been estimated using relatively small sample sizes that reduce the precision of estimation. Secondly, there may be differences between species. Thirdly, there may be variation arising from differences in genetic expression at different sites. Raymond and Schimleck (2002) reported genetic correlations between cellulose content and wood density to be different among sites, ranging from –0.33 to 0.74.

The genetic and environmental correlations between cellulose content and growth were both positive, while the genetic correlations between wood density and growth were positive but the environmental correlations were negative. The difference in sign of these two correlations may be caused by different physiological mechanisms that affect the genetic and environmental sources of variation of the traits (Falconer and Mackay, 1996).

In this study, we did not find significant difference either between provenances or islands in cellulose content in *E. urophylla*. However, this result should be interpreted with caution because selective thinning at earlier ages and the limited number of families per provenance could have affected the ranking between provenances. Significant differences between provenances for cellulose content and pulp yield have been reported in several eucalypt species (Clarke et al., 1997; Miranda and Perreira, 2002). Previous studies reported significant difference in growth between provenances in this species (Hodge et al., 2001; Kien et al., 2009; Tripiana et al., 2007; Wei and Borallho, 1998a). Tripiana et al. (2007) found a strong and negative relationship between altitude of seed source and its performance in Congo, showed strong clinal variation due to altitude in this species. In a study on the entire population of trees in this trial and a parallel progeny trial located in the same region, Kien et al. (2009) found that Lewotobi provenance was significantly superior in growth and form to the other provenances tested, while wood density did not differ significantly between provenances (Kien et al., 2008). This result agreed well with those of Wei and Borralho (Wei and Borralho, 1997; 1998a). However, Darrow and Roeder (1983) detected differences in wood basic density between *E. urophylla* seed sources in provenance trials in South Africa.

5. CONCLUSIONS

The reasonably good correlation between cellulose content determined by the diglyme-HCl method and pulp yield demonstrated the practicality of using this method in the evaluation of pulp yield in *E. urophylla* plantations. Cellulose content of increment cores taken at breast height is a reasonably good predictor of pulp yield at the same height and can be used to rank trees for pulp yield in *E. urophylla* plantations. Heritability for cellulose content is moderately high in *E. urophylla* and substantial gains can be achieved through selection. Genetic correlations between cellulose content and growth or wood density appeared to be weak, suggesting that independent selection for cellulose can be made with minor effect on the other traits.

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