Genetic improvement of pulp yield in *Eucalyptus nitens* using cellulose content determined by NIR

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Introduction

Eucalyptus nitens (Deane and Maiden) Maiden (Shining gum) is widely grown for pulp production in South Eastern Australia on cold sites subject to frequent frosts.[1,2] Improving the yields of pulp obtained from this species is an important part of increasing plantation profitability but assessment using traditional methods is destructive (sample trees need to be felled), time-consuming and expensive. The traditional methods are therefore not well suited to screening large numbers of samples. Cellulose content has been used as an alternative in tree breeding programs. It is strongly correlated with pulp yield, is lower cost, allows larger numbers of samples to be taken, and samples can be taken without falling the tree.[3–5]

A limitation of a direct measure of cellulose content is that it relies on wet chemistry, which requires specialised laboratories and skilled laboratory staff. The number of samples that can be processed has been found to be limited by these practical constraints and this, in turn, limits the gains that can be made in a tree-breeding program. An indirect method such as near infrared (NIR) spectroscopy provides a large increase in the numbers of samples that can be processed[6] however the relative benefits of more samples compared to less accuracy needs to be determined. In this study the genetic gains in cellulose content of *E. nitens* were compared using wet chemistry cellulose content and NIR predicted cellulose content based on calibrations developed using selected samples.

Materials and methods

Sample origin

The genetic material used was open pollinated progeny from 40 native forest families from Toorongo Plateau in the central highlands of Victoria. Progeny trials were established on three sites in northern Tasmania, Australia, in 1984 and site details are given in Table 1. The trial design was a randomised complete block with 16 replications per site and single tree plots spaced at 3 m by 3 m. Wood samples were taken at age 13 years by taking a 12 mm bark-to-bark core at a height of 0.9m. Approximately 5 trees were randomly sampled from each family. More details about this study are given elsewhere.[4,5]

Table 1. Description of field sites.

	Dial Range	Gog Range	Kamona
Number of samples	168	182	188
Latitude (South)	41º 10´	41º 29	41º 08
Longitude (East)	146º 04´	146º 23´	147º 40´
Altitude (m)	100	300	160
Rainfall (mm/yr)	1060	1200	1150

Determination of cellulose content

Wood cores were reduced to small fragments using a disc pulveriser and then reduced to wood meal in a Wiley mill fitted with a 1 mm screen. Crude cellulose content (g cellulose per g o.d. wood) was measured using the method of Wallis *et al*.[7] Non-cellulosic compounds were solubilised by digestion in diglyme and hydrochloric acid for one hour on a shaker table in a water bath at 90°C. The residue was collected by filtration, washed, dried and weighed to determine the mass of crude cellulose.

Near infrared spectroscopy

The wood meal was placed in a large NIRSystems sample cup (NR-7070). The NIR spectra were measured in diffuse reflectance mode from samples held in a spinning sample holder in a NIRSystems Inc. Model 5000 scanning spectrophotometer. The spectra were collected at 2 nm intervals over the wavelength range 1100–2500 nm. The instrument reference was a ceramic standard. Fifty scans were accumulated for each sample and the results averaged. After the spectrum had been obtained, the sample cup was emptied, repacked and a duplicate spectrum obtained.

The duplicate spectra were averaged and converted to the second derivative using the instrument's NSAS software. A segment width of 10 nm and a gap width of 20 nm were used for the conversion.

Calibration development

Samples were selected from each site for calibration development using an existing northern Tasmania pulp yield calibration to predict the pulp yield of all samples. It is known that the relationship between pulp yield and cellulose is strong and it was assumed that the variation in predicted pulp yields would represent the range of cellulose contents. Based on the predicted yields 40 samples were selected from each site to cover the range of predicted pulp yields.

Cellulose calibrations were developed using NSAS software (version 3.52) and second derivative spectra. Partial least squares (PLS) regression was used to develop the calibrations with four cross validation segments and a maximum of ten factors. The NSAS software recommended the final number of factors to use for each calibration.

Calibration statistics

The measure of how well a calibration fits the data is the standard error of calibration (*SEC*). The measure of how well the calibration predicts the constituent of interest in a separate test set is given by the standard error of prediction (*SEP*). Note: generally a calibration is applied to a test set of unknown samples that are different from the calibration set. Owing to the nature of this study the calibration samples were included in the prediction set ensuring that the calibrations were applied to test sets of the same size and composition. The co-efficient of determination (R^2) was also used to assess calibration performance.

Estimating genetic gains

Genetic gains were estimated after calculating individual tree breeding values for cellulose content. This was done separately for each of two variables, which were wet chemistry cellulose content and NIR predictions based on 40 calibration samples per site. Breeding values were calculated by fitting the following individual tree model using ASREML:

$$Y = \mu + SITE + REP(SITE) + TREE + FAM.SITE + \varepsilon$$

where Y is a vector of the data for each trait; μ is a vector of means for each trait; SITE are site effects for each trait fitted as a fixed factor; REP(SITE) are within site replicate effects for each trait fitted as a fixed factor; TREE are individual tree breeding values (additive genetic effects); FAM.SITE is the interaction between family and site; and $\boldsymbol{\varepsilon}$ is a vector of residuals for each trait.

For each of the variables, the top (highest breeding value) 30 trees were selected. This represents the top 5% of the population. Genetic gains were calculated by averaging breeding values of the selected population and were expressed relative to gains that could be obtained by assessing all trees using wet chemistry methods.

Results and discussion

Relationship between predicted pulp yield and cellulose

The relationship between NIR predicted pulp yield and laboratory determined cellulose content was investigated. R^2 ranged from 0.68 (Dial) to 0.52 (Gog), the R^2 for Kamona was 0.65. The strong relationships suggest that selection of samples based on predicted pulp yield should provide adequate representation of the range of cellulose contents.

Cellulose calibrations for each site

Cellulose calibrations were developed for each site and then used to predict the cellulose contents of all samples from the site. Summary statistics for each calibration are provided in Table 2.

The calibration developed for Dial had a strong R^2 (0.69) and it performed well when used to predict the cellulose content of all Dial samples. The Gog calibration was developed using two factors and had a stronger R^2 (0.77) than the Dial calibration but it did not perform as well in prediction. The Kamona calibration provided the strongest calibration statistics of the three sites (R^2 = 0.93, *SEC* = 0.46) and also provided the best predictions of cellulose content [Figure 1(a) and (b)].

Table 2. Summary of cellulose calibrations developed for each site.

Calibration set					Prediction set	
Site	# factors	R^2	*SEC*		R^2	*SEP*
Dial	3	0.69	0.67		0.71	0.65
Gog	2	0.77	0.77		0.55	1.08
Kamona	4	0.93	0.46		0.83	0.57

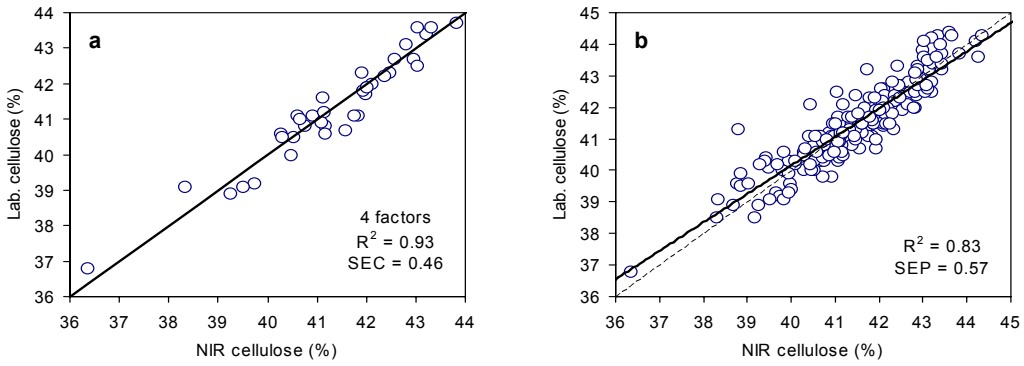

Figure 1. Results for Kamona, (a) 40 sample calibration and (b) prediction on all 188 samples.

Genetic gains

The genetic gains obtained using direct cellulose content and cellulose as predicted by the 40 sample calibrations are given in Table 3. The NIR calibrations delivered a high proportion of the potential genetic gain and it appears that NIR spectroscopy is a reliable method for selecting improved genotypes.

Table 3. Gain in cellulose content (%) using 40 calibration samples.

Calibration method	Genetic gain[a]	Proportion of maximum possible gain	Heritability (± se)
Cellulose direct	1.51	100%	0.61 ± 0.15
NIR 40 samples/site	1.27	84%	0.71 ± 0.16

[a]Genetic gain is the gain in cellulose content after selecting the top 30 trees from the 538 trees sampled (top 5%). For example, a gain of 1.24 means the cellulose content has improved from 41.51% (the average value) to 42.75%.

Future work

The work described here is preliminary and future work is planned to:

a. compare genetic gains when using NIR calibrations based on different sampling intensities;
b. compare genetic gains when using NIR calibrations based on samples selected by WinISI software, rather than selecting samples using predicted pulp yield; and
c. to compare genetic gains when specific site calibrations are applied across sites.

Conclusions

Strong calibrations for cellulose content were obtained using selected samples. The strongest relationships were obtained for Kamona.

Genetic gains based on NIR predicted cellulose content were a large proportion of the maximum possible gain.

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