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Research Article

Identification of putative trait based markers for Genetic Improvement of *Eucalyptus tereticornis*

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Abstract

Tree breeding is basically aimed at producing quality products like seeds and clones and the most valuable contribution of molecular markers in breeding programs would be to reduce the time of selection or to reduce the number of breeding cycles. This can be achieved either by the early identification of superior progeny or by the identification of parents that will yield superior progeny. The present study highlights the use of microsatellite markers towards development of putative markers tagging the adventitious rooting trait in *Eucalyptus tereticornis*. Further, putative cellulose synthase specific markers were identified and correlated with pulping character of wood tissues. The validation of these putative markers in larger populations can lead to more efficient and broadly applicable early selection procedure for key traits in eucalypt breeding programs.

Key Words: Adventitious rooting; Cellulose; DNA marker; Lignin; Wood property traits

Introduction

Eucalypts occupy 19.61 M hectares globally and India ranks first in area under eucalypts plantation (3.943 M ha) which act as an important source of carbon neutral renewable energy and raw material for paper and solid wood (Iglesias Trabado and Wilstermann 2008). The average productivity in India is 20 $m^3ha^{-1}year^{-1}$, followed by Brazil with 3.0 million hectares of intensively cultivated clonal plantations with average productivity of 45–60 $m^3ha^{-1}year^{-1}$ (Mora and Garcia, 2000). Lack of sufficient genetic variability is one of the important reasons for low productivity of *Eucalyptus* plantations in India, since it restricts the intensity of selection in breeding populations. Further, the populations are highly inbred and the existing variability has been over exploited through intensive selection of promising trees and their multiplication for commercial plantations.

The two main areas to accelerate domestication in tree species include exploitation of genetic diversity in breeding programs and genetic modification, by introducing new genes into already existing elite genotypes (Boerjan, 2005).

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Most phenotypic traits of interest for tree breeding are characterized by continuous variation. Such traits are usually influenced by a number of genes with a small effect interacting with other genes and the environment. The main traits targeted in tree species are wood properties and traits related to adaptation and growth (Sewell *et al.,* 2000). In addition, QTLs have been identified for disease resistance, growth, flowering, vegetative propagation, frost tolerance and leaf oil composition (Butcher *et al.,* 2004; Kaya *et al.,* 1999; Yoshimaru *et al.,* 1998; Hurme *et al.,* 2000; Cervera *et al.,* 2004). Genetic linkage maps have been constructed for most of the commercially important forest tree genera, and updated information on genetic linkage maps for forest trees is available at http://dendrome.ucdavis.edu/ index.php.

In eucalypts, QTLs have been successfully identified for wood properties (Grattapaglia *et al.,* 1996; Verhaegen *et al.,* 1997; Myburg *et al.,* 2001); juvenile traits such as seedling height and leaf area

(Byrne *et al.,* 1997); insect and pathogen resistance and essential oil traits (Shepherd *et al.,* 1999; Junghans *et al.,* 2003) and vegetative propagation traits (Grattapaglia *et al.* 1995, Marques *et al.,* 1999; 2005). The first published association study in forest trees found an association between two SNP markers from the *CCR* gene and microfibril angle in *Eucalyptus nitens,* explaining approximately 5% of the total phenotypic variation. (Thumma *et al.,* 2005).

The present paper highlights the development of putative DNA markers for two economically important traits of *E. tereticornis* including adventitious rooting and pulping traits. These leads on further validation can provide resources for identification of early selection markers for trait identification in eucalypt breeding program.

Materials and Methods

Identification of adventitious rooting marker

Germplasm used for phenotyping: Eight clones with variable adventitious rooting percent were selected for marker analysis. The selected clones (Table 1) were vegetatively propagated and parameters including percent rooting, number of roots and root length of each ramet were recorded. Further, free and endogenous IAA and ABA content in root and leaf samples were estimated from three ramets of each clone according to the methods described by Unyayar *et al.* (1996). The validation population for the rooting marker included five families from Orobay provenance which showed maximum variation in rooting percent.

Genotyping with adventitious root specific SSR primers

DNA isolation

Genomic DNA was extracted from young leaves of eucalypt clones as described earlier by Doyle and Doyle (1987) with minor modifications. Five Microsatellite EMBRA primers targeting the QTL region for vegetative propagation in *Eucalyptus* species with synteny across the species belonging to symphomyrtus subgenus including *E. globulus, E. grandis, E. urophylla* and *E. tereticornis* (Marques *et al*., 2002) were synthesized at Sigma Aldrich USA (Table 2). Template DNA (50 ng) was amplified in a reaction volume of 10µL containing 1.0µL 10X PCR buffer (Bangalore Genei Ltd., India), 40nM of each primer (forward and reverse primer), 0.4mM dNTPs mix, and 0.3 U of Taq DNA polymerase (Bangalore Genei Ltd., India). The PCR conditions for amplifications were as follows: Initial denaturation (5 min, 94 °C), followed by 30 cycles consisting of denaturation (1 min, 94 °C), annealing (1 min; see table 2 for temperature conditions), extension (2 min, 72 °C) and a final extension (10 min, 72 °C). The amplified products were separated in a 6% denaturing polyacrylamide gels at 50 Watts at 42 ^{0}C for two hours and stained with silver nitrate (Bassam *et al* 1991). The gel profiles were documented with Camedia digital camera.

Identification of pulping trait marker

Selection of suitable population and determination of population structure

Seven provenances from the International provenance cum seed orchard trial at Karunya Research Plot, Coimbatore were selected for this study (Table 3). DNA was isolated from young leaves of ten randomly selected individuals belonging to seven provenances using Doyle and Doyle (1987) protocol and amplified with seven ISSR primers (Table 4). The putative markers were further validated in individuals selected from five families of Orobay provenance.

Phenotyping for pulping trait in wood samples

The wood tissues from three randomly selected individuals from seven provenances were subjected to proximate analysis at Tamil Nadu Newsprint Ltd., Karur using the TAPPI procedures (Anonymous, 1978).

Genotyping using cellulose synthase primer pairs

Genomic DNA was extracted from young leaves of three individuals belonging to seven provenances as earlier described by Doyle and Doyle (1987). The primers used for amplification were developed based on the gene sequences available in the public domain database (www.ncbi.nlm.nih..gov). Three primer pairs for cellulose synthase (*CesA*) were custom synthesized from sequences of *Populus tremuloides*, *Gossypium hirsutum* and *Hordeum vulgare* (Table 3.8). SSR amplifications were performed for seven *E. tereticornis* provenances using three *CesA* primers (Table 5).

Template DNA (50 ng) was amplified in a total volume of 10-µL containing 1.0µL 10X PCR buffer (Bangalore Genei Ltd., India), 40nM of each primer (forward and reverse primer), 0.4mM dNTPs mix, and 0.3 U of Taq DNA polymerase (Bangalore Genei Ltd., India). PCR was performed in MJ Research DNA engine thermal cycler (PTC-200). The conditions for amplifications were as follows: initial denaturation (5 min, 94 °C), followed by 30 cycles consisting of denaturation (1 min, 94 °C), annealing (1 min; see table for temperature conditions), extension (2 min, 72 °C) and a final extension (10 min, 72 °C). The amplified fragments were separated in a 6% denaturing polyacrylamide gels and stained with silver nitrate (Bassam *et al* 1991). The gel profiles were documented with Camedia digital camera.

Results

Identification of adventitious rooting marker

The rooting percentage in the studied clones ranged from 0 to 100 per cent with an average of 74 per cent. In Et clone, no root elongation was observed in any of the replications, resulting in failure of vegetative propagation of this clone (Figure 1). The root length ranged from 0.3 cm to 21.3 cm in all clones with mean of 13.96 ± 4.93 cm and CV of 47 per cent (Table 6). The individuals in the provenance Orobay was selected for validation of markers since it revealed the maximum variation for rooting percentage. Family 18 and 20 showed only root initiation but no root elongation while the other families showed varying percent of adventitious rooting ranging between 30 % to 80 %.

The endogenous levels of free and bound IAA and ABA were estimated in leaves and root samples of the eight above mentioned clones. The leaf IAA content varied from 17.42 µg in Et clones showing zero percent rooting to 30.0 µg in Et-17-01, with no significant correlation with rooting percentage. The root free IAA was high in Et clone (10.49 µg) which showed no root elongation. The leaf ABA content varied from 5.83 µg in Et clone to 9.37 µg in ITC 3. The root ABA content varied from 2.84 µg in Et-10-06 to 7.43 µg in Et clone. The root free ABA was high in Et clone (7.43 µg) which showed no root elongation, while all other clones registered low root free ABA content ranging from 2.84 µg to 4.47 µg. (Table 6).

The five EMBRA primer pairs amplified a total of 73 alleles across the eight clones. The total number of alleles per primer pair ranged from 9 (amplified by EMBRA 18) to 24 (amplified by EMBRA 6). The allele size ranged from 76 bp (amplified by EMBRA 18) to 164 bp (amplified by EMBRA 6). The per cent polymorphism for each primer pair ranged from 88.9 to 100 per cent. The primer EMBRA 10 amplified a specific allele at 110 bp in non rooted clone (Et clone). Similarly, the primer pair EMBRA 13 amplified three specific alleles at 139 bp, 138 bp and 135 bp in Et clone (Figure 2). The SSR marker profiles clearly showed that there was no specific allele for hundred per cent rooting clones. The putative non adventitious rooting marker generated by EMBRA 13 was validated in the five families of Orobay provenance. The primer amplified specific alleles at 150 bp and 152 bp in the families 18 and 20 which showed no root elongation.

Correlation analysis

The data generated from the SSR marker profiles were correlated with rooting percentage, root free IAA and root free ABA using SPSS. The four non-rooting specific alleles (EMBRA 10_{110}, EMBRA 13_{139}, EMBRA 13_{138} and EMBRA 13_{135}) significantly correlated with rooting per cent (-0.777^{*}), root free IAA (0.713^{*}) and root free ABA (0.840^{**}) (Table 7).

Identification of pulping trait marker

Population Structure analysis in *E. tereticornis* provenances

Analysis of genetic relationship within and between seven *E. tereticornis* provenances showed significant polymorphism with seven ISSR primers. A total of 540 markers amplified from 70 individuals and the PCR products varied in length from 255 bp to 2711 bp. Among the seven provenances, high genetic diversity in terms of per cent polymorphic loci (30.9) was observed in SW Mt. Garnet provenance and minimum was recorded in Cardwell provenance (14.8). Similar trend was observed in mean gene diversity, where in minimum was registered in Cardwell provenance (0.043) and maximum in SW Mt. Garnet (0.084). Shannon's information index was maximum in SW Mt. Garnet provenance (0.132) and minimum in Cardwell provenance (0.067) (Table 8). The results of AMOVA indicated that 40 per cent of genetic variability was attributable to the differences among population and 60 per cent within populations (Table 9). A dendrogram based on UPGMA cluster analysis of genetic distance values showed that clusters did not accurately reflect the geographic position of populations (Figure 3).

Proximate analysis of wood samples

The estimated results showed that the holocellulose content in the seven provenances ranged from 62.2% in Norman by River to 69.3% in Orobay with an average of 65.4%. The Orobay provenance recorded the highest holocellulose content. Similarly, the pentosan content was maximum in Norman by River provenance (22.7%) and minimum in Orobay provenance (18.5%). The lignin content was highest in Norman by River (33.1) and lowest in Orobay provenance (27.6 %) respectively. The pentosans and lignin content were negatively correlated with holocellulose content (Table 10).

Genotyping using *CesA* specific primer pairs

CesA1 primer pair developed from Cellulose synthase sequence of *Gossypium hirsutum* amplified 8 alleles ranging from 150 to 300 bp in all individuals of seven provenances while *CesA2* and *CesA*3 amplified 2 and 5 alleles respectively in the expected range of 153 and 180 bp respectively. The presence of specific amplicons in genotypes with high cellulose content was not documented using the primers *CesA1* and *CesA3*. However, specific bands for *CesA2* at 174 bp and 176 bp were observed in all the three individuals of Orobay provenance, one individual of S.W. of Mt. Garnet provenance and two individuals of Cardwell and North Kennedy River provenance (Figure 4). The results showed that these two alleles positively correlated with holocellulose (0.945^{**}) and negatively correlated with lignin (-0.932^{**}) and Pentosans (-0.857^{**}) (Table 11). The results were further corroborated in all the five families of Orobay provenance consisting of four individuals each and the 174 bp and 176 bp products amplified in all the individuals except few (Figure 5). Allelic diversity within and between the families of Orobay was also observed.

Discussion

Pulp and paper industries have a continuous demand for raw material with uniform physical and chemical properties. Therefore, investigation on genetic control of industrially important traits like wood property and vegetative propagation traits involving identification of genes that govern these complex traits, is of key importance.

Vegetative propagation is the technique used to transform genetic gains into industrial benefits. Root initiation and elongation are internally regulated by phytohormones (Sundberg and Uggla, 1998; Wang and Cui, 1999), including Indole-3-Acetic Acid (IAA) and Gibberellic Acid (GA) and inhibitors like Abscisic Acid (ABA) and ethylene. The cross-talk of IAA with other phytohormones has been convincingly demonstrated in the last decade (Swarup *et al.,* 2002). In the present investigation, an attempt was made to correlate the endogenous hormone levels (IAA and ABA) in the root and leaf tissues with adventitious rooting. This is in consonance with the report by Saugy and Pilet (1987), where high concentration of endogenous IAA inhibited the root elongation in maize. Similarly, Noda *et al.* (2000) also reported that the high concentration root IAA and ABA inhibited the root elongation in citrus root stocks.

DNA markers linked to vegetative propagation trait has been earlier reported in eucalypt species and QTLs tagging the trait was reported in *E. grandis* and *E. urophylla* using RAPD (Grattapaglia *et al.* 1995), AFLP (Marques *et al.* 1999) and SSR markers (Marques *et al.* 2002). Microsatellite markers developed based on the report by Marques *et al.* (2002) produced four non-rooting putative specific alleles (EMBRA 10 $_{110}$, EMBRA 13_{139}, EMBRA 13_{138} and EMBRA 13_{135}) in the non –rooted Et clone in the present study. This supports the earlier report of Marques *et al.* (2002) which described the transferability of SSR markers tagged to the vegetative propagation trait in *E. tereticornis* and *E. globulus.* However, in the present study, the alleles 110, 139, 138 and 135 bp generated by EMBRA 10 and EMBRA13 were found to significantly associate (-0.777*, 0.713*, 0.840**) with the non root elongation trait of Et clone, which were not linked with this trait in the earlier study by Marques *et al.* (2002).

Population genetic structure analysis reveals distribution of alleles and change in allele frequencies under the influence of the evolutionary forces and it helps to assess the distribution of diversity within and between populations. It is affected by a number of factors like mating system, gene flow, seed dispersal, mode of reproduction and natural selection (Hamrick and Godt, 1990). In Association mapping, presence of population structure may lead to spurious associations (Buckler and Thornsberry, 2002). Inbreeding species usually have less variation within populations but greater genetic differentiation between populations (Hamrick and Godt, 1996) while in out breeding species, with in population diversity is predominantly high revealing a low genetic population structure when compared to inbreeding species. The confounding effect of population structure on Association mapping is not a serious problem in out crossing species (Thumma *et al.,* 2005). The present investigation too revealed a high genetic differentiation with in *E. tereticornis* provenances revealing its suitability for association analysis.

The first published association study in forest trees found an association between two SNP markers from the *CCR* gene and microfibril angle in *Eucalyptus nitens,* explaining approximately 5% of the total phenotypic variation. (Thumma *et al.,* 2005). In a powerful demonstration of the resolution of association genetics they detected an alternatively-spliced variant of the *CCR* gene from the region of the significant haplotype, thereby revealing the probable molecular basis of the trait variation. In the present study, the allelic diversity of cellulose synthase gene was assessed in the natural population and a significant correlation of specific alleles with lignin/cellulose content was observed, suggesting that future in depth study in *CesA* genes could lead to identification of wood property trait markers using candidate gene based association analysis.

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Table 1: List *E. tereticornis* clones used for adventitious rooting studies

Clone No	Height (cm)	DBH (cm)	Source
Et-04-05	34.66	0.22	Odandurai, Reserve forest, Mettupalayam
Et-10-06	26.82	0.14	Odandurai, Reserve forest, Mettupalayam
Et-01-07	36.82	0.28	Odandurai, Reserve forest, Mettupalayam
Et-17-01	37.00	0.24	Odandurai, Reserve forest, Mettupalayam
Et-12-11	40.50	0.30	Odandurai, Reserve forest, Mettupalayam
SMD-7(Et clone)	15.62	0.06	Sethumadai, Coimbatore
ITC-10	41.50	0.32	ITC, Badrachalam, Andhrapradesh
ITC-3	42.50	0.31	ITC, Badrachalam, Andhrapradesh

Table 2: List of SSR primers used for developing markers for vegetative propagation trait

Locus	Repeat Motifs	Primer Sequences (5'-3')	Expected Length (bp)	Annealing Temperature°C
EMBRA 6	$(AG)_{19}$	F AGAGAATTGCTCTTCATGGA R GAAAAGTCTGCAAAGTCTGC	121-165	58
EMBRA 10	$(CCT)_3(AG)_{14}$	F GTAAAGACATAGTGAAGACATTCC R AGACAGTACGTTCTCTAGCTC(A)	115-149	60
EMBRA 11	$(AG)_4GG(AG)_{13}$	F GCTTAGAATTTGCCTAAACC R GTAAAATCCATGGGCAAG	124-158	56
EMBRA 13	$(AG)_{27}$	F ATTTCCCTAGGTTTGACATG R TCCAACATCTTACTCAACCA	73-111	60
EMBRA 18	$(AG)_3GG(AG)_{19}$	F CAGCTAGGATGTTAGACTTGG R GCACACCTAGAATTTTCAAACTA	70-110	61

Table 3: Details of *E. tereticornis* provenances used for pulping trait studies

No	Provenance	Provenance/ seedlot.No	No.of Families	Locality	Latitude (N)	Longitude (E)	Altitude (m)
1	Cardwell (CW)	13277	4	QLD	18° 14'	145° 58'	30
2	N Kennedy River (NK)	17864	5	QLD	15° 17'	144° 00'	70
3	Normanby River (NR)	16547	1	QLD	15° 46'	144° 58'	140
4	Orobay (OR)	13399	5	PNG	8° 57'	148° 28'	200
5	Palmer river (PR)	13847	1	QLD	16° 07'	144° 47'	365
6	Sogeri Plateau (SP)	13418	5	PNG	9° 30'	147° 26'	580
7	SW of Mt. Garnet (SWG)	16554	4	QLD	18° 24'	144° 45'	890

Table 4: List of ISSR primers used for population structure analysis

Primer code	Nucleotide sequence
5' anchored	
$R(CA)_7$	5' GRTRCYGRTRCACACACACACACA 3'
$T(GT)_9$	5' CRTAYGTGTGTGTGTGTGTGTGT 3'
$TA(CAG)_4$	5' ARRTYCAGCAGCAGCAG 3'
$RA(GCT)_6$	5' AYARAGCTGCTGCTGCTGCTGCT 3'
3' anchored	
$(GA)_8R$	5' GAGAGAGAGAGAGAGARGY 3'
UBC810	5' GAGAGAGAGAGAGAGAT 3'
UBC842	5' GAGAGAGAGAGAGAGAYG 3'

Table 5: List of gene specific primers used for development of pulping trait marker

Gene	Primer Sequences (5'-3')	Expected Length(bp)	Annealing Temperature°C	Accession number
CesA1	F GAAGGTGTTTTTGTGCCCAT R ACTTGCTGGCTGGCTGTATT	241	45	AY483152
CesA2	F GACATGCAACTGCTTGCCTA R TACCCTCCAAAGTCCCACAG	139	54	AF417485
CesA3	F GGTTTATTGGTGTGTTGGGG	153	45	AF254895

Table 6: Rooting parameters, IAA and ABA content of eight *E. tereticornis*

Clones	Rooting per cent	Root length (cm)	No.of roots (No)	Leaf IAA (µg/mg)	Root IAA (µg/mg)	Leaf ABA (µg/mg)	Root ABA (µg/mg)
Et-04-05	40	21.3	6.0	22.80	3.10	6.33	3.73
Et-10-06	70	18.0	7.0	26.17	4.00	7.28	2.84
Et-01-07	100	9.6	14.0	24.17	3.48	8.47	3.48
Et-17-01	80	15.8	9.0	30.00	4.00	8.80	2.87
Et-12-11	100	11.8	12.0	23.17	2.94	7.50	3.13
Et clone	0	0.3	0.0	17.42*	10.49*	5.83*	7.43*
ITC 10	100	14.7	20.0	26.42	4.50	8.17	4.30
ITC 3	100	19.9	23.0	24.83	4.30	9.37	4.47
Mean ± SE	74 ± 11.95	13.96 ± 4.93	11.4 ± 2.86	23.4 ± 1.76	5.5 ± 1.02	7.7 ± 0.43	4.0 ± 0.53
CV (%)	40.00	47.00	55.83	21.28	53.7	15.84	37.50

clones

Table 7: SSR markers associated with rooting traits

	Rooting %	Leaf IAA total	Root free IAA	Root ABA total
EMBRA 10	110* (-0.777)	110 (-0.486)	110* (0.713)	110*(0.840)
EMBRA 13	139* (-0.777)	139 (-0.486)	139 *(0.713)	139* (0.840)
EMBRA 13	138* (-0.777)	138 (-0.486)	138* (0.713)	138* (0.840)
EMBRA 13	135* (-0.777)	135 (-0.486)	135* (0.713)	135 *(0.840)

* Indicates that marker corresponds with QTL for the trait.

The values in Parenthesis denotes correlation with trait

Table 8: Genetic variability within populations of *E. tereticornis* provenances

Provenances	Observed number of alleles	Effective number of alleles	Gene diversity	Shanon information index	Percent polymorphic loci	Sample size
CW	1.147 (0.355)	1.069 (0.205)	0.043 (0.117)	0.067 (0.174)	14.8	10
NK	1.263 (0.441)	1.127 (0.262)	0.078 (0.149)	0.122 (0.221)	26.4	10
NR	1.256 (0.437)	1.118 (0.257)	0.073 (0.145)	0.113 (0.214)	25.6	10
OR	1.173 (0.379)	1.089 (0.237)	0.054 (0.133)	0.083 (0.195)	17.3	10
PR	1.234 (0.424)	1.105 (0.238)	0.066 (0.137)	0.104 (0.204)	23.5	10
SP	1.172 (0.377)	1.086 (0.227)	0.053 90.129)	0.081 (0.192)	17.2	10
SWG	1.309 (0.462)	1.134 (0.263)	0.084 (0.149)	0.132 (0.221)	30.9	10

Table 9: Analysis of molecular variance (AMOVA) in *E. tereticornis* provenances

Source	df	Sum of Squares	Mean Sum Squares	Varience components	Percentage of variation	P Value
QLD and PNG provenances						
Among all Provenances	6	673.429	112.238	9.755	40	< 0.001
Within all Provenances	63	925.200	14.686	14.686	60	< 0.001

Table 10: Proximate analysis of wood samples of *E. tereticornis* provenances

Provenances	Sample No	Moisture per cent	Ash per cent	Hot Water Solubility per cent	NaOH Solubility per cent	AB Extractives per cent	Acid Insoluble Lignin per cent	Pentosans per cent	Holocellulose per cent
SW Mt Garnet	1	8.40	0.47	3.80	16.30	3.65	31.91	23.07	63.50
	2	8.20	0.61	3.30	18.80	3.45	31.65	22.24	63.95
	3	8.60	0.42	3.00	18.20	3.34	28.27	19.36	68.10
Cardwell	4	8.40	0.45	3.84	19.92	3.93	28.17	20.12	67.20
	5	8.70	0.48	4.45	16.23	3.89	28.84	19.76	66.85
	6	8.90	0.45	4.56	19.79	3.19	33.41	22.40	62.51
Orobay	7	8.60	0.24	4.09	18.98	3.08	27.37	18.14	69.12
	8	8.24	0.27	4.55	19.88	3.04	28.16	18.44	69.33
	9	8.10	0.33	4.68	20.12	2.63	27.78	18.86	69.26
Sogri Plateau	10	8.11	0.43	3.87	19.89	3.76	33.77	21.90	62.23
	11	7.88	0.29	3.24	22.85	3.06	34.06	21.84	62.18
Palmer River	12	7.35	0.59	3.29	16.44	2.84	31.31	21.05	64.35
	13	7.66	0.51	3.18	17.83	3.61	31.95	20.60	63.46
	14	8.10	0.41	4.39	19.72	3.37	31.16	20.29	64.02
Norman By River	15	8.53	0.36	5.11	18.50	2.72	33.12	22.89	62.12
	16	8.56	0.25	4.76	17.98	2.70	33.37	22.43	62.19
	17	8.51	0.34	4.82	17.33	3.15	32.68	22.75	62.27
North Kenndy River	18	8.30	0.36	4.12	18.53	3.25	28.56	19.60	67.67
	19	8.26	0.35	3.41	18.56	2.84	28.23	18.56	69.28
	20	8.40	0.35	4.96	20.50	3.12	30.68	20.10	63.84
Mean ± SE		8.29 ± 0.08	0.40 ± 0.02	4.07 ± 0.15	18.82 ± 0.36	3.23 ± 0.09	30.72 ± 0.52	20.72 ± 0.36	65.17 ± 0.63
CV (%)		4.4	15.9	16.4	8.6	12.1	7.5	7.8	4.3

Table 11: Correlation analysis of wood property traits with *CesA* alleles

	174	176	Moisture per cent	Ash per cent	Hot Water Solubility	1% NaOH Solubility	AB Extractives	Lignin	Pentason	Holocellulose
174	**1.000**	1.000**	0.346	-0.331	-0.145	0.102	0.246	-0.932**	-0.857**	0.945**
176	1.000**	**1.000**	0.346	-0.331	-0.145	0.102	0.246	-0.932**	-0.857**	0.945**

Figure 1: Comparison of rooting behaviour in *E. tereticornis* clones

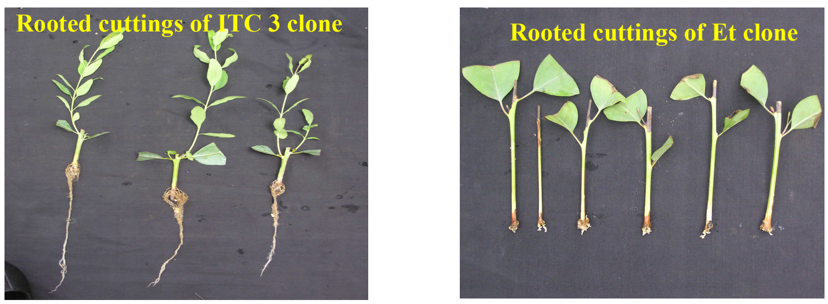
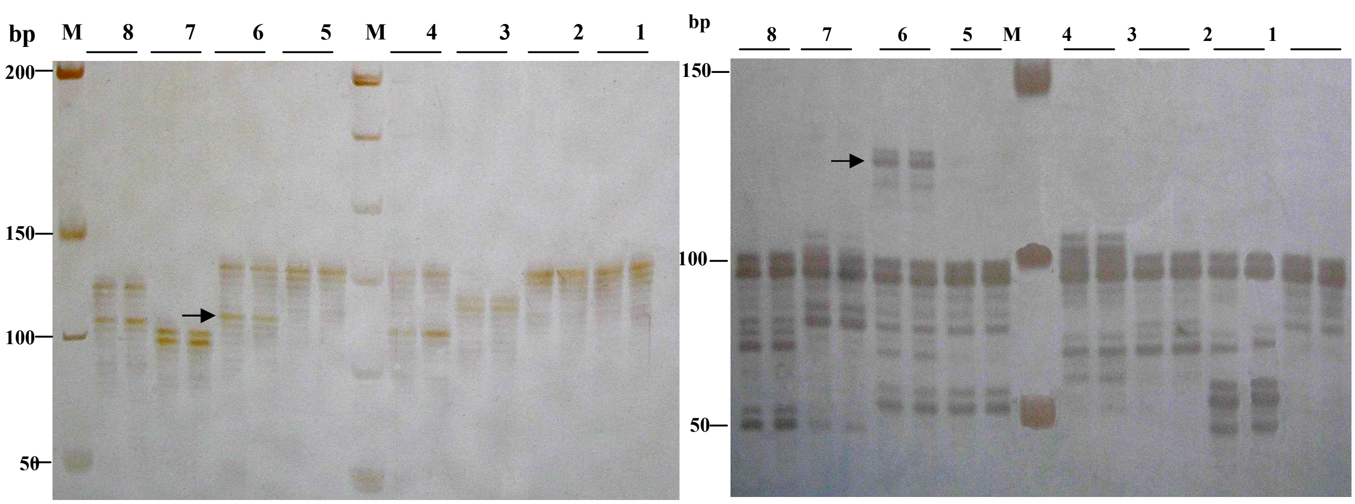


Figure 2: SSR Profile of *E. tereticornis* clones with primer EMBRA 10 & EMBRA 13



1 - 8 : Et-04-05; Et-10-06 ; Et-01-07; Et-17-01; Et-12-11 ; SMD-7(Et clone); ITC10 ; ITC3
M: 50 bp ladder (Fermentas, Ltd. USA)

1 - 8: Et-04-05; Et-10-06 ; Et-01-07; Et-17-01; Et-12-11 ; SMD-7(Et clone); ITC10 ; ITC3
M: 50 bp ladder (Fermentas, Ltd. USA)

Arrow indicates putative markers for non root elongation trait

Figure 3: Genetic similarity among seven *E. tereticornis* provenances

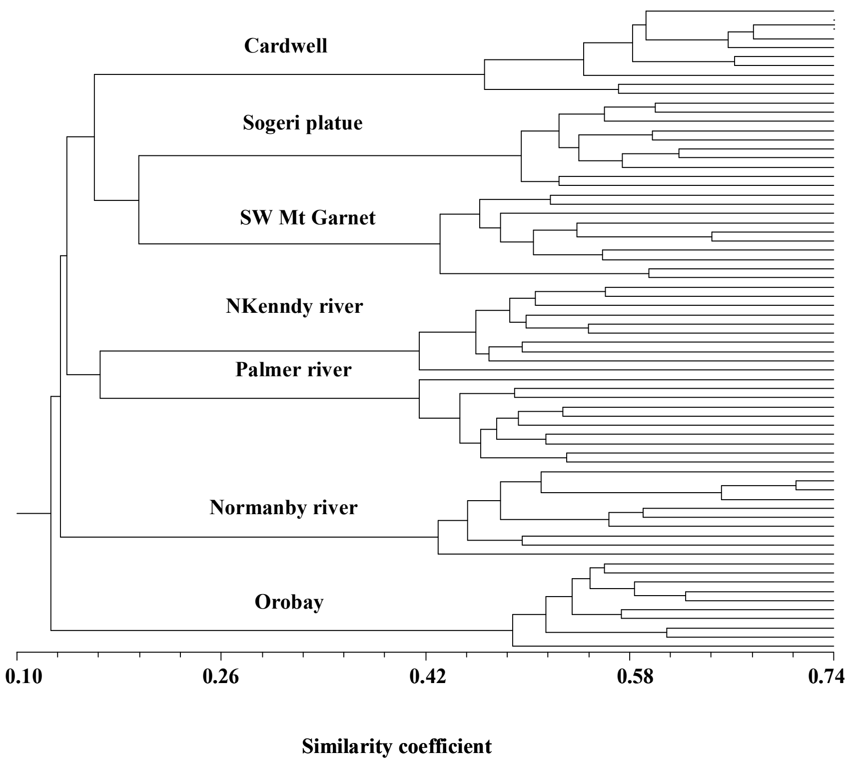
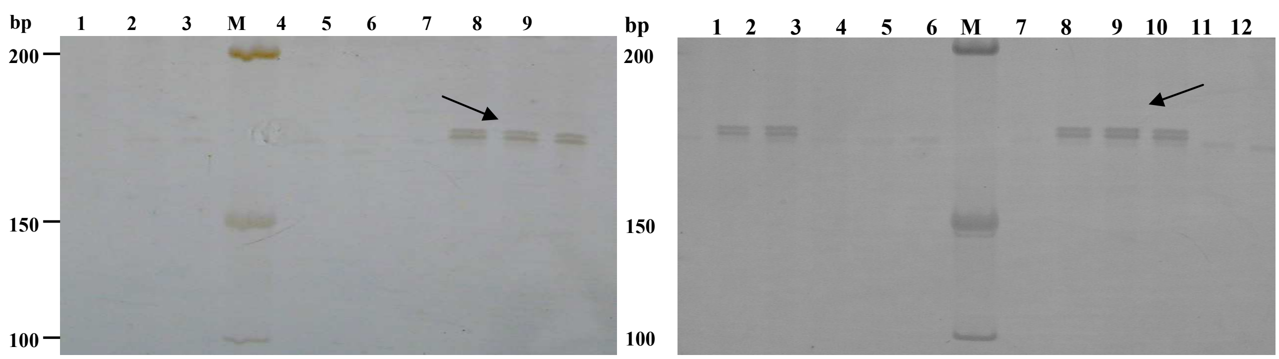


Figure 4: Marker profile of *E. tereticornis* provenances with *CesA2*

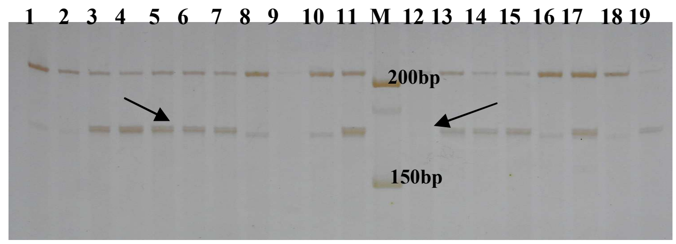


Lanes 1 to 3 Palmer River; Lanes 4 to 6 Sogeri Plateau; Lanes 7 to 9 Orobay
M : 50 bp ladder (Fermentas, Ltd. USA)

Lanes 1 to 3 North Kennedy River ; Lanes 4 to 6 Norman By River; Lanes 7 to 9 Cardwell; Lanes 10 to 12 SW Mt Garnet
M : 50 bp ladder (Fermentas, Ltd. USA)

Arrow indicates putative markers for pulping trait

Figure 5: Marker profile of five Orobay families with *CesA2*



Lanes 1 to 4 family 20; Lanes 5 to 7 family 19; Lanes 8 to 11 family 18; Lanes 12 to 15 family 17; Lanes 16 to 19 family 16

Arrow indicates putative markers for pulping trait