

Quantitative trait locus (QTL) analysis of growth and vegetative propagation traits in *Eucalyptus nitens* full-sib families

Bala R. Thumma · Brian S. Baltunis · John C. Bell ·
Livinus C. Emebiri · Gavin F. Moran ·
Simon G. Southerton

Received: 11 January 2010 / Revised: 21 March 2010 / Accepted: 2 April 2010 / Published online: 22 May 2010
© Springer-Verlag 2010

Abstract Tree growth and vegetative propagation are complex but important traits under selection in many tree improvement programmes. To understand the genetic control of these traits, we conducted a quantitative trait locus (QTL) study in three full-sib families of *Eucalyptus nitens* growing at two different sites. One family growing at Ridgley, Tasmania had 300 progeny and two clonally replicated families growing at Mt. Gambier, South Australia had 327 and 210 progeny. Tree growth was measured over several years at both sites and percentages of roots produced by either stem cuttings or tissue culture were assessed in the two Mt. Gambier families. Linkage analysis of growth traits revealed several QTLs for later year traits but few for early year traits, reflecting temporal differences in the heritabilities of these traits. Two growth QTL positions, one on LG8 and another on LG11 were common

between the Ridgley and Mt. Gambier families. Four QTLs were observed for each of the two vegetative propagation methods. Two QTLs for vegetative propagation on LG7 and LG11 were validated in the second family at Mt. Gambier. These results suggest that growth and vegetative propagation traits are controlled by several small effect loci. The QTLs identified in this study are useful starting points for identifying candidate genes using the *Eucalyptus grandis* genome sequence.

Keywords Candidate genes · Single nucleotide polymorphism · Stem cuttings · Tissue culture

Introduction

Improving tree growth is an important breeding objective in many forest tree-breeding programmes. The heritability of tree growth is generally moderate to low (Hamilton and Potts 2008; Whiteman et al. 1992); however, growth QTLs have been identified in a range of tree species (reviewed in Butcher and Southerton (2007)). Several studies have shown a significant genotype by environment interaction for growth traits (e.g. Hamilton and Potts (2008)). In spite of these limitations, substantial progress has been made in improving tree growth through breeding programmes. This has been mainly achieved due to the large variation observed for these traits. However, molecular markers associated with growth traits may be useful for increasing selection efficiency for these low heritability traits.

Several studies have been conducted in eucalypts to identify QTLs associated with growth traits. The number of QTLs detected for growth traits is generally low compared with other traits such as wood quality. For example, in a

Communicated by: D. Grattapaglia

Electronic supplementary material The online version of this article (doi:10.1007/s11295-010-0298-6) contains supplementary material, which is available to authorized users.

B. R. Thumma (✉) · B. S. Baltunis · J. C. Bell · S. G. Southerton
CSIRO Plant Industry, GPO Box 1600,
Canberra, ACT 2601, Australia
e-mail: Reddy.thumma@csiro.au

L. C. Emebiri
EH Graham Centre for Agricultural Innovation,
Wagga Wagga Agricultural Institute,
Wagga Wagga, NSW 2650, Australia

G. F. Moran
School of Biology, Australian National University,
Canberra, ACT 2601, Australia

recent study we detected 36 QTLs associated with several wood quality traits in *Eucalyptus nitens* (Thumma et al. 2010). In contrast, only three QTLs were observed for stem volume in *Eucalyptus grandis* (Grattapaglia et al. 1996). Similarly, three QTLs were identified for tree diameter in a hybrid cross between *E. grandis* and *Eucalyptus urophylla* (Verhaegen et al. 1997). In *E. globulus*, Bundock et al. (2008) identified one QTL for cumulative growth while Freeman et al. (2009) identified two QTLs for tree diameter. The low number of QTLs detected in these studies may reflect the low heritability generally associated with growth traits. QTL studies are useful approaches for exploring the genetic control of low heritability traits and may shed light on the molecular control of poorly understood phenotypes such as tree growth. Co-location of candidate genes to the QTL intervals may shed light on the potential genes controlling growth traits and further candidate genes underlying QTL positions can be identified using the eucalypt genome (<http://eucalyptusdb.bi.up.ac.za/>). However, to exploit the full potential of the genome sequence there is a need to conduct more QTL studies in different species using large populations.

Vegetative propagation through macro-propagation (stem cuttings) and/or micro-propagation (tissue culture) is an important feature of many tree-breeding programmes. Clonal propagation will help in the rapid deployment of selected or improved germplasm into commercial plantations. The ability to produce roots from stem cuttings has been shown to vary among different species. Conifer species such as *Pinus* (e.g. *Pinus taeda*, *Pinus radiata*) and *Picea* have been shown to be propagated easily through stem cuttings. In eucalypts, tropical species such as *Eucalyptus camaldulensis*, *E. grandis*, *E. urophylla* are easy to vegetatively propagate through stem cuttings. However, temperate species such as *E. globulus* and *E. nitens* are difficult to propagate clonally either through macropropagation by stem cuttings or micropropagation by tissue culture because of low root initiation (de Little et al. 1992; England and Borralho 1995; Hetherington and Orme 1989).

One approach to improve the vegetative propagation in *E. nitens* is to identify markers linked to propagation traits using QTL analysis. There are a few studies in eucalypts where progeny from eucalypt hybrids have been used for QTL analysis (Grattapaglia et al. 1995; Marques et al. 1999). Some of the large effect QTLs identified by Marques et al. (1999) were validated in different progeny from the same family (Marques et al. 2005). However, as these studies were mainly based on dominant marker systems, it is not possible to compare the QTLs identified in these studies with other studies. While direct comparison of QTLs between different studies is difficult when dominant markers are used in linkage map construction, it should be possible to establish linkage group level homology of QTL

effects if transferable markers such as microsatellites or single-nucleotide polymorphisms (SNPs) are mapped.

There are no studies in eucalypts where tissue culture data was used for QTL analysis, and there are no studies comparing QTLs detected for vegetative propagation traits from stem cuttings with tissue culture. In this study, we analysed the QTLs associated with growth and vegetative propagation traits in three full-sib families of *E. nitens* growing at two sites. The main objectives of this study are (a) to identify and compare the growth QTLs in different families growing at different sites, and (b) to detect QTLs associated with vegetative propagation traits based on stem cuttings and tissue culture methods.

Materials and methods

Full-sib family at Ridgley

The full-sib family at Ridgley was part of a three-generation out-crossed pedigree (Byrne et al. 1998). In 1993, seedlings of the third generation were planted in a field experiment in Northwest Tasmania, 10 km southwest of Ridgley. The design of the field experiment was a row/column layout consisting of 20 rows and 11 columns, with five-tree line plots from the same family in each row/column grid position. The growth traits, diameter at breast height (DBH-cm) and height (Ht-m) were measured at 2 years of age, (DBH-2 and Ht-2); at 3 years (DBH-3 and Ht-3); at 4 years (DBH-4 and Ht-4); and at 7 years (DBH-7).

Full-sib families at Mt. Gambier

Two full-sib families with a common parent were selected from the Gunns Ltd. breeding programme. Family 1 (TO163 X SN382) had the largest number of progeny (327 individuals) and was used for mapping and discovering QTL for vegetative propagation and growth traits. Family 2 (SN382 X TO15) had a progeny size of 210 individuals and was used for validating QTLs discovered in family 1. The two families had a common parent SN382 which was used as a male parent in family 1 and as a female parent in family 2.

Seed from both families were germinated using standard medium. For each seedling, the stem was severed into two sections between the cotyledons and first pair of leaves. The apical tip was removed and used as a source for DNA extraction. The remainder of the upper portion comprising the leaf pairs was placed onto standard micropropagation medium (de Little et al. 1992). The lower segment including the root and cotyledons (ortet) was relocated to the glasshouse and new growth was used as the source of material for cutting assays.

Tissue culture—rooting assays

Cloned plants were transferred (i.e. subcultured) to fresh multiplication medium using standard techniques (de Little et al. 1992). The number of clones in family 1 was reduced from 327 to 300 due to ortet mortality. After about 12 weeks of multiplication, cultures were transferred from small containers to larger containers. The first rooting assay was conducted 24 weeks after germination when cultures had multiplied sufficiently. A maximum of ten shoots per clone per assay were tested for root induction. Shoots of good health and a minimum size of 1 cm were selected. Dominant shoots in a clump were selected where possible. Four rooting assays were conducted in family 1 and three rooting assays were conducted in family 2. Each shoot was scored for presence or absence of roots and the percentage of rooted shoots was obtained for each clone.

Stem cuttings—rooting assays

The ortets were transferred from root trainers into 125 mm pots and remained in the glasshouse over winter to promote shoot growth. Pruning was carried out periodically to remove apical dominance and to promote the maximum number of shoots. Shoots were harvested, dipped in auxin rooting hormone and planted in pots. Five rooting trials were conducted for family 1 and two trials were performed for family 2. Setting 2 in family 1 had some burning of cuttings due to lack of misting.

Field trial of ortets and stem cuttings from two families

The ortets and stem cuttings from the vegetative experiments were used to establish a field trial. The field trial was planted at Mt. Gambier, South Australia in August 1999. Two to five replications of rooted cuttings were established for each clone. The ortets were planted as a further replicate. Diameter at breast height was measured in 2002 (DBH-2) and 2008 (DBH-8), while a height measurement was taken in 2003 (Ht-3).

Molecular methods

In the Ridgley full-sib family, more than 290 markers were used for constructing a linkage map. Details of the different markers mapped on the linkage map were presented in Thumma et al. (2010).

In the Mt. Gambier full-sib families, the three parents were screened for polymorphic loci using RFLP and microsatellite markers. These markers were chosen to give full genome coverage based on the genetic linkage maps of *E. nitens* (Byrne et al. 1995) and *E. globulus* (Thamarus et al. 2002). In family 1, 106 markers comprising 96 RFLPs

and 12 microsatellites were assayed in 327 seedlings. Thirty-eight markers were assayed in the 210 progeny of the validation family 2. As the number of markers genotyped in family 1 was low, a consensus linkage map was developed based on linkage information from previous maps of *E. nitens* (Byrne et al. 1995; Thumma et al. 2010) and *E. globulus* (Thamarus et al. 2002).

Statistical analyses of vegetative propagation and growth traits in Mt. Gambier full-sib families

Analyses were conducted in ASReml (Gilmour et al. 2006) using an individual-tree mixed effects model for vegetative propagation and growth traits in the Mt. Gambier families.

$$y_{ijklmn} = \mu + R_i + F_j + RF_{ij} + \text{row}_{k(i)} + \text{col}_{l(i)} + \text{clone}_{m(j)} + e_{ijklmn}, \quad (1)$$

where y_{ijklmn} is the individual tree measurement, μ is the overall mean, R_i is the fixed effect of replicate ($i=1, 2, 3, 4, 5$), F_j is the fixed effect of family ($j=1, 2$), $\text{row}_{k(i)}$ is the random effect of row within replicate $\sim N(0, \hat{\sigma}_{\text{row}}^2)$, $\text{col}_{l(i)}$ is the random effect of column within replicate $\sim N(0, \hat{\sigma}_{\text{col}}^2)$, $\text{clone}_{m(j)}$ is the random effect of clone within family $\sim N(0, \hat{\sigma}_{\text{clone}}^2)$, and e_{ijklmn} is the random residual effect $\sim N(0, \hat{\sigma}_{\text{error}}^2)$. Broad-sense within-family heritability for full-sib families (\hat{H}_{WF}^2) was estimated for each trait and theoretically contains one half the additive genetic variance, three fourths of the dominance genetic variance, and most of the epistatic genetic variance (Falconer and Mackay 1996).

$$\hat{H}_{WF}^2 = \frac{\hat{\sigma}_{\text{clone}}^2}{\hat{\sigma}_{\text{clone}}^2 + \hat{\sigma}_{\text{error}}^2}, \quad (2)$$

where $\hat{\sigma}_{\text{clone}}^2$ is the estimated variance among clones within full-sib family and the summation of $\hat{\sigma}_{\text{clone}}^2 + \hat{\sigma}_{\text{error}}^2$ is the phenotypic variance.

Additionally, repeatability of clonal means was estimated as

$$\hat{H}_C^2 = \frac{\hat{\sigma}_{\text{clone}}^2}{\hat{\sigma}_{\text{clone}}^2 + \hat{\sigma}_{\text{error}}^2/t}, \quad (3)$$

where t is the mean number of ramets per clone.

Approximate standard errors for heritability and clonal repeatability estimates were calculated using a Taylor series expansion method using ASReml (Gilmour et al. 2006). Clonal values were predicted in ASReml using BLUP (best linear unbiased predictions) procedures.

Additionally, the coefficient of genetic variation (CGV) was estimated in order to compare the genetic variances of the traits. The CGV expresses the genetic variance relative to the mean of the trait of interest and gives a standardised

measure of the genetic variance relative to the mean of the trait. The higher the CGV for a trait, the higher is its relative variation.

$$\text{CGV} = \frac{\hat{\sigma}_{\text{clone}}}{\bar{x}} \times 100\%, \quad (4)$$

where $\hat{\sigma}_{\text{clone}}$ is the square root of the variance among clones and \bar{x} is the population mean for the trait.

QTL analysis

Both single marker analysis using analysis of variance and interval mapping were used to detect QTL in the Ridgley family and in family 1 of Mt. Gambier while only single-marker analysis was used in family 2 of Mt. Gambier. In the Ridgley family, interval mapping was done using QTL express (Seaton et al. 2002). The QTLs that were significantly associated with each trait were used as cofactors for searching for additional QTL with composite interval mapping (Zeng 1993). Significance thresholds were determined from permutation tests. Confidence intervals (95%) for QTL were estimated using the method of Darvasi and Soller (1997).

The clonal values representing the genetic effects adjusted for the fixed effects (replicate) were used for QTL analyses of Mt. Gambier families. As grand parental genotype information was not available, we used MapQTL 6.0 (Van Ooijen 2009) for interval mapping of the full-sib family 1 at Mt. Gambier. Genotype phase information required for QTL analysis was determined by using Join-Map (Van Ooijen 2006). Chromosome-wide and genome-wide significance levels of QTL positions were determined by permutation tests. The 95% confidence intervals were determined by the two-LOD support interval method. Multiple QTL model (MQM) mapping was used to detect additional QTLs by using the significant markers from interval mapping as co-factors. Regression approximation to the maximum likelihood algorithm implemented in MapQTL 6.0 was used in interval and MQM mapping. QTL positions were mapped using MapChart (Voorrips 2002).

Results

Trait variation in full-sib family at Ridgley

DBH on average ranged from 2.4 cm in 2-year-old trees to 16.5 cm in 7-year-old trees, while height ranged from 2.2 m in 2-year-old trees to 7.6 m in 4-year-old trees (Table 1). The correlation between early and later year growth traits was weaker compared with the correlation within early and later year growth traits (Table 2). Correlation between year 2 height and DBH was the highest, followed by the correlation between year 4 height and year 3 height and year 3 height and year 2 height. In general, correlations among height traits were higher compared with diameter traits (Table 2).

Trait variation in full-sib families at Mt. Gambier

Variation and heritability of growth traits

Significant clonal variation was detected ($p < 0.05$) for all of the growth traits measured in the two full-sib families at Mt. Gambier with greatest genetic variation detected for DBH-8 compared with growth measurements at younger ages. Additionally, highly significant differences ($p < 0.001$) were detected between the two-full sib families for DBH-2 and Ht-3, but this source of variation disappeared for DBH-8 ($p = 0.523$). The coefficient of genetic variation ranged from 5.1% for Ht-2 to greater than 10% for the two diameter traits (Table 3). Broad-sense heritability estimates for growth traits were small and ranged from 0.06 for DBH-2 to 0.16 for DBH-8 (Table 3). Similarly, clonal repeatability estimates ranged from 0.17 for DBH-2 to 0.36 for DBH-8. Within different age groups, heritability and clonal repeatability estimates for early ages, i.e. DBH-2 and Ht-3 were lower than that of later years, i.e. DBH-8.

Variation and heritability of vegetative propagation traits

In both families, the percentage rooting was consistently higher in stem cuttings (40.8%) compared with rooting in

Table 1 Variation in growth traits in *Eucalyptus nitens* full-sib family at Ridgley

	DBH-2 (cm)	Ht-2 (m)	DBH-3 (cm)	Ht-3 (m)	DBH-4 (cm)	Ht-4 (m)	DBH-7 (cm)
Min	0.5	0.2	0.8	1.0	2.3	2.9	3.7
Max	4.1	5.0	10.4	7.1	14.4	10.2	24.6
Range	3.6	4.8	9.6	6.1	12.1	7.3	20.9
Average	2.4	2.2	6.4	5.1	9.7	7.6	16.5

DBH-2 diameter at breast height at 2 years of age; Ht-2 height at 2 years of age; DBH-3 diameter at breast height at 3 years of age; Ht-3 height at 3 years of age; DBH-4 diameter at breast height at 4 years of age; Ht-4 height at 4 years of age; DBH-7 diameter at breast height at 7 years of age

Table 2 Phenotypic trait correlations among growth traits in *Eucalyptus nitens* full-sib family at Ridgley

	DBH-2 ^a	Ht-2	DBH-3	Ht-3	DBH-4	Ht-4
DBH-7	0.60 ^b	0.71	0.83	0.76	0.84	0.69
Ht-4	0.67	0.79	0.78	0.90	0.77	
DBH-4	0.68	0.76	0.89	0.79		
Ht-3	0.78	0.90	0.88			
DBH-3	0.87	0.89				
Ht-2	0.91					

^a Trait abbreviations as in Table 1

^b All trait correlations were significant at $p < 0.01$

tissue culture (25.5%) (Table 4). The mean percentage of rooting in family 1 for tissue culture was 24.6, and for stem cuttings it was 34.5. In family 2, the mean percentage of rooting in tissue culture was 22.5 and 47.2 for rooting from stem cuttings. There was a decline in rooting percentage over time particularly in tissue culture. The second setting of stem cuttings for the family 1 pedigree was burned from water stress, which probably explains the lower percentage of rooting. The increase in rooting percentage in setting 4 was unexpected. It appears that generally the decline in rooting over time is slower in stem cuttings compared with tissue culture with the percentage of rooting dropping by 50% after three rounds of setting in tissue culture in family 2.

Clonal variation for vegetative propagation (% rooting) for both tissue culture and stem cuttings was highly significant ($p < 0.0001$). Significant family differences were detected for % rooting in stem cuttings ($p < 0.001$) but not in tissue culture ($p = 0.27$). Broad-sense heritability for percentage of roots produced with tissue culture was higher (0.43) compared with stem cuttings (0.33) (Table 3). In general, heritability was higher for vegetative propagation traits compared with growth traits reflecting the higher percentage of genetic variation associated with vegetative propagation traits compared to growth traits (Table 3).

Table 4 Percentage of rooting in tissue culture and in stem cuttings at each setting for both *Eucalyptus nitens* families at Mt. Gambier

Temporal rooting tests	Tissue culture		Stem cutting	
	Family 1	Family 2	Family 1	Family 2
1	35.4	31.1	44.0	56.1
2	25.6	20.5	23.0	38.3
3	14.4	16.0	34.0	–
4	23.1	–	37.0	–

QTL analysis of growth traits in Ridgley full-sib family

QTL analysis of growth at different ages was performed using interval mapping. The number of QTLs detected for growth traits was lower (Table 5; Fig. 1) than those detected for wood quality traits in an earlier study using the same family (Thumma et al. 2010). The number of QTLs detected ranged from one for DBH-2 to three for DBH-7 and Ht-3. No significant QTLs were detected for Ht-2. Composite interval mapping using the significant markers as co-factors did not reveal any further QTLs. The number of QTLs detected for later year traits was higher than early year traits. This may reflect the higher heritability of later year traits compared with earlier years as seen in the Mt. Gambier families. A cluster of QTLs for year 3 and year 4 height and DBH was observed on LG5, possibly reflecting a high correlation among these traits. Similarly, QTLs for DBH-3 and DBH-4 and QTLs for Ht-3 and DBH-7 were clustered on LG6 and LG12, respectively. The percentage of variance explained by each QTL was small and varied between 2.7% and 6.4%. Most of the QTL effects were paternal in origin while the QTL effects for DBH-7 on LG1 and Ht-3 on LG12 were maternal in origin. The QTL effects for Ht-3 on LG4, DBH-2 on LG8 and Ht-4 on LG11 were due to interaction between maternal and paternal alleles. At the two common QTLs, the QTL effects for height and DBH traits on LG5 and the QTL effects for

Table 3 Mean, percentage coefficient of genetic variation (%CGV) within family broad-sense heritability (\hat{H}_{WF}^2) and clonal repeatability (\hat{H}_C^2) for traits in *Eucalyptus nitens* full-sib families at Mt. Gambier

Trait	Mean±SD	% CGV	$\hat{H}_{WF}^2 \pm SE$	$\hat{H}_C^2 \pm SE$
DBH-2 (mm)	35.62±23.06	10.9%	0.07±0.03 ($p < 0.05$)	0.17±0.07 ($p < 0.05$)
Ht-3 (m)	4.89±1.65	5.1%	0.06±0.03 ($p < 0.05$)	0.16±0.07 ($p < 0.05$)
DBH-8 (mm)	242.32±66.93	10.6%	0.16±0.03 ($p < 0.0001$)	0.36±0.06 ($p < 0.0001$)
SC (%)	36.9±34.5	52.3%	0.33±0.03 ($p < 0.0001$)	0.59±0.03 ($p < 0.0001$)
TC (%)	24.1±26.4	69.0%	0.43±0.03 ($p < 0.0001$)	0.70±0.02 ($p < 0.0001$)

DBH-2 diameter at breast height in 2002; Ht-3 height in 2003; DBH-8 diameter at breast height in 2008; SC percentage of rooting of stem cuttings; percentage of rooting of TC tissue cultured plants

Table 5 Interval mapping of growth QTL in *Eucalyptus nitens* full-sib family at Ridgley

LG	Trait	Position (cM)	F^a	Paternal effect ^b	Maternal effect ^b	Interaction effect ^b	$R^2(\%)^c$
1	DBH-7	58	6.68 ^d	0.5902	0.7556	0.1158	5.64
4	Ht-3	110	4.37 ^e	0.0264	-0.1215	0.2032	3.42
5	Ht-3	67	7.46 ^e	0.2935	0.0474	0.0152	6.36
5	DBH-3	70	4.78 ^e	0.3624	0.0505	-0.0895	3.84
5	Ht-4	67	5.35 ^e	0.2941	-0.0012	0.0082	4.42
5	DBH-4	69	5.97 ^d	0.5244	-0.0972	-0.0178	5.02
6	DBH-3	14	6.47 ^e	-0.4443	0.0877	-0.0364	5.47
6	DBH-4	15	5.1 ^e	-0.5110	0.1125	-0.0328	4.18
8	DBH-2	246	5.75 ^d	0.0998	0.0117	-0.2747	5.06
8	DBH-7	172	4.98 ^e	0.6392	-0.1827	-0.6324	4.03
11	Ht-4	119	5.35 ^e	0.1883	-0.0052	0.2587	4.42
12	DBH-7	0	4.05 ^e	-0.7502	-0.2291	-0.0634	3.11
12	Ht-3	0	3.63 ^e	-0.1381	-0.1825	0.0596	2.69

Trait abbreviations as in Table 1.

^a Significance of the F -statistic is based on 1,000 permutations

^b Paternal effect is paternal and maternal effect is maternal contribution to additive effect. Interaction effect is interaction between the two parental alleles

^c Percentage of phenotypic variance explained by the QTL

^d Significant at <0.01

^e Significant at <0.05

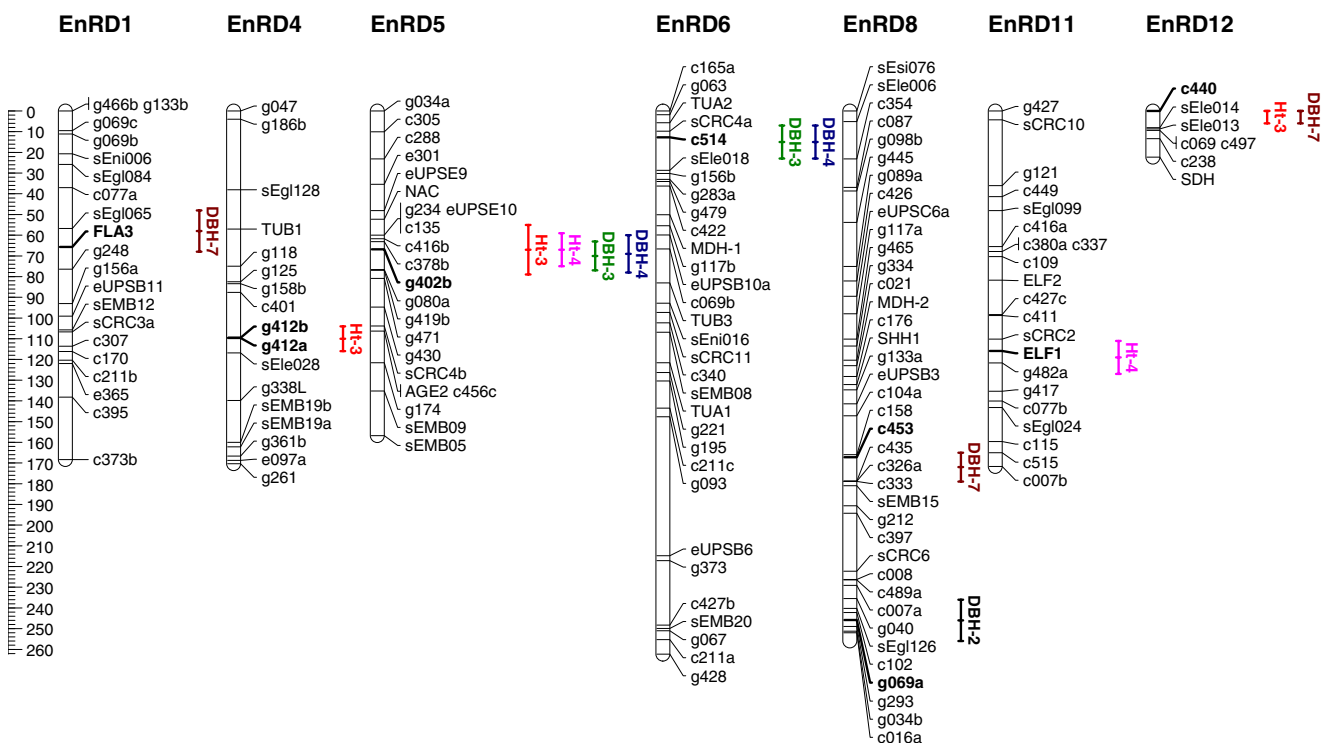


Fig. 1 QTL positions for growth traits in *Eucalyptus nitens* full-sib family at Ridgley (RD). Bars indicate 95% confidence intervals. Scale on left indicates distance in centiMorgans (cM). Markers mapping

nearest to the centre of the QTL interval are shown in **bold**. Trait abbreviations as in Table 1. Numbering of linkage groups is based on the *Eucalyptus* consensus map (Brondani et al. 2006)

DBH-3 and DBH-4 on LG6 were paternal in origin. However, for the common QTL on LG12, the QTL effect for Ht-3 was maternal while the QTL effect for DBH-7 was paternal in origin. Three candidate genes co-located to QTL intervals for different growth traits. The flowering gene *ELF1* co-located to a QTL for Ht-4 on LG11, a fasciclin-like AGP gene (*FLA3*) co-located to a QTL for DBH-7 on LG1 and an EST (*eUPSE10*) co-located to a common height QTL on LG5 (Fig. 1).

QTL analysis of growth traits in Mt. Gambier full-sib families

Interval mapping of growth QTLs in family 1

A consensus map developed by comparison with the linkage map from the full-sib family at Ridgley was used for QTL analysis in family 1 at Mt. Gambier. Interval mapping in family 1 using the predicted genetic values revealed two QTLs each for DBH-8 on LG3 and LG11, and for Ht-3 on LG8 and LG9 (Table 6; Fig. 2). However, no QTLs were identified for DBH-2. Further analysis with MQM mapping did not reveal any additional QTL. The percentage of genotypic variance explained by the QTL is only marginally higher than the percentage of phenotypic variance explained by the QTL in the full-sib family at Ridgley (8.5% vs 6.4%). The percentage of genotypic variance explained varied between 6 and 8.5% in family 1 at Mt. Gambier.

Single-marker analysis of growth QTLs in family 2

As the number of markers genotyped in family 2 was small, QTL analysis was performed using single marker analysis only. Family 2 was primarily used to validate the results from family 1. Two markers on LG11 (g482 and g417) which map to the 95% confidence interval of QTL for DBH-8 observed in family 1 (Fig. 2) were significantly

associated with DBH-8 (Table 7) in family 2. Similarly, g243 at 225.4 cM on LG3 was significantly associated with DBH-8. However, this marker is 182 cM away from the QTL for DBH-8 observed on LG3 (Fig. 2). One marker on LG8 at 118 cM (c104) was significantly associated with Ht-3 which was close to the QTL for Ht-3 observed in family 1. Similarly, two markers (c378 and g010) on LG9 near the QTL for Ht-3 in family 1 were significantly associated with Ht-3 in family 2. Analysis of the parental contribution to the QTL effect at marker g482 on LG11, which showed a significant effect on DBH-8 by single marker analysis in both families, revealed that the QTL effect was derived from the common parent (SN382) in both families.

Comparison of QTLs of growth traits between Ridgley and Mt. Gambier families

The QTL for DBH-7 on LG8 in the Ridgley family overlaps with the QTL for Ht-3 in the Mt. Gambier family. Similarly, the QTL for Ht-4 on LG11 in the Ridgley family overlaps with the QTL for DBH-8 in Mt. Gambier family (Fig. 3). The QTL for DBH-8 on LG11 observed in family 1 of Mt. Gambier was validated in family 2 using single marker analysis (Table 7). The flowering gene, *ELF1* mapped to the QTL on LG11.

QTL analysis of vegetative propagation traits

Interval mapping of vegetative propagation traits in family 1

QTL analysis was performed using the predicted genetic values of mean percentage of roots produced from all settings. Four QTLs were observed for percentage of roots produced by both stem cutting and tissue culture methods (Table 8; Fig. 2). The percentage of genotypic variation explained by the QTL for percentage of roots produced ranged from 4.9% to 15.4%. On LG1 there was a common QTL for percentage of roots produced by both methods. The QTL on LG3 explained the highest percentage of genetic variation (15.4%) in roots produced by tissue culture while the QTL on LG7 explained the highest percentage of genetic variation (11.1%) in roots produced by the stem cutting method. The percentage of genotypic variation explained by vegetative propagation traits was generally higher than growth traits. Similarly, the LOD scores associated with vegetative propagation QTLs were higher than those of the growth traits. Analysis of the QTL effect at the common QTL on LG1 using the flanking markers revealed that the effect at this position was mainly derived from the paternal parent (common parent, SN382).

Table 6 QTL analysis of growth traits in *Eucalyptus nitens* full-sib families at Mt. Gambier

LG	Trait	Position (cM)	Locus	LOD	R^2 (%)
3	DBH-8	102	c116	3.4 ^a	6.0
8	Ht-3	180.7	c397	3.59 ^a	6.3
9	Ht-3	101.2	g435	3.35 ^a	5.9
11	DBH-8	135.3	c251	4.84 ^b	8.5

Interval mapping in family 1. Trait abbreviations as in Table 3

^a Significant with chromosome-wide permutation tests

^b Significant with genome-wide permutation tests

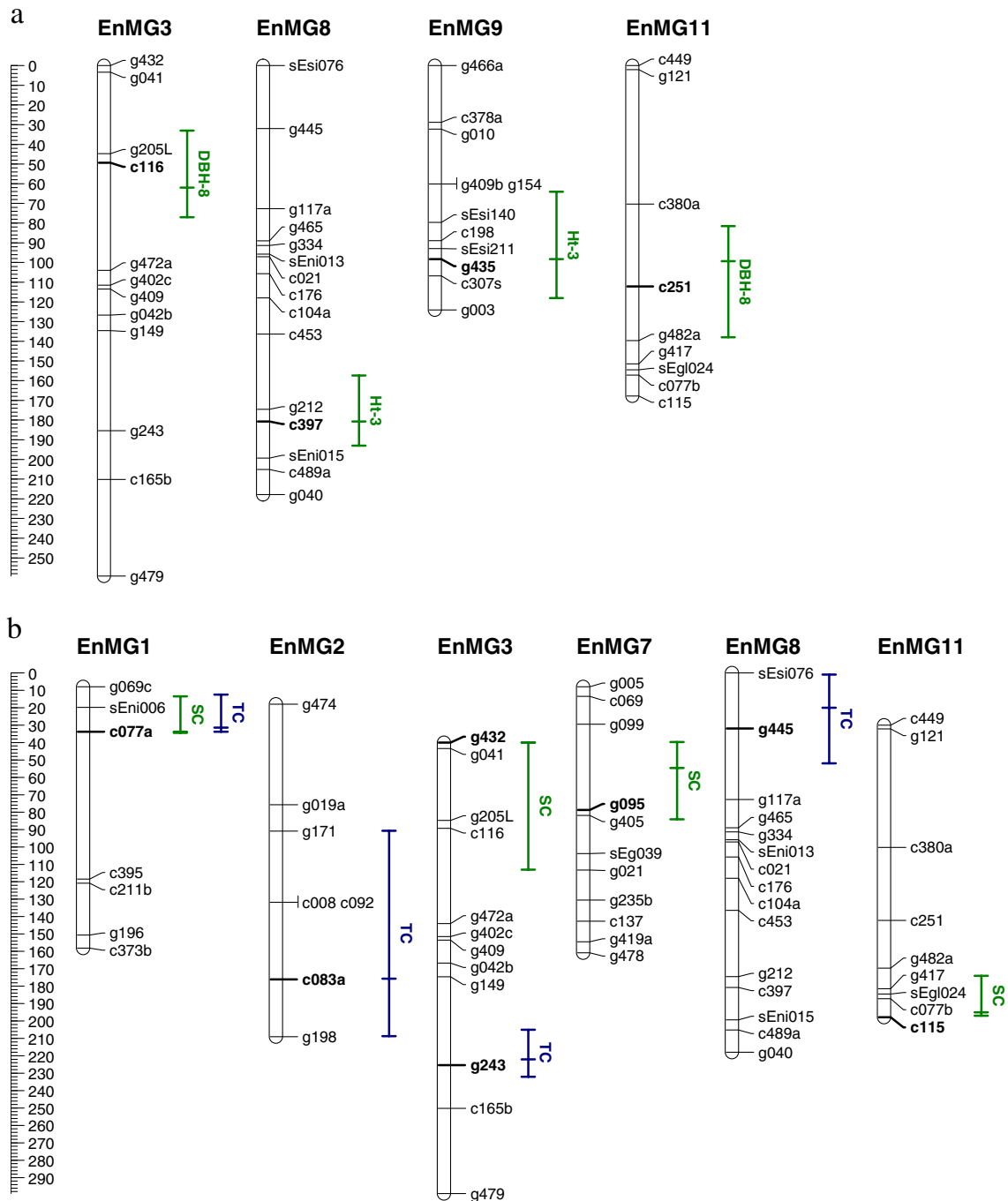


Fig. 2 QTL positions for growth and vegetative propagation traits in *Eucalyptus nitens* full-sib family 1 at Mt. Gambier (MG). Bars indicate 95% confidence intervals. Scale on left indicates distance in

cM. Markers mapping nearest to the centre of the QTL interval are shown in **bold**. **a** QTL positions of growth traits; **b** QTL positions of vegetative propagation traits. Trait abbreviations are as in Table 3

Single-marker analysis of vegetative propagation traits in family 2

Single-marker analysis of vegetative propagation traits in family 2 revealed that two markers (g095 on LG7, and c077B on LG11) were significantly associated with percentage of roots produced by tissue culture (Table 9).

Similarly, two markers (g417 and c115) on LG11, one marker (Es140) on LG9 and one marker (c288) on LG5 were significantly associated with percentage of roots produced by stem cuttings. None of the QTLs for percentage of roots produced by tissue culture in family 1 could be validated in family 2. However, two markers (g095 on LG7 and c077B on LG11) which were signifi-

Table 7 Single-marker analysis of growth traits in family 2

LG	Trait	Position (cM)	Locus	<i>P</i>	<i>R</i> ² (%)
3	DBH-8	225.4	g243	0.0281	3.3
8	Ht-3	118	c104	0.0413	4.36
9	Ht-3	31.7	c378	0.0176	3.88
9	Ht-3	35.2	g010	0.0165	6.95
11	DBH-8	175.6	g482	0.046	5.43
11	DBH-8	187.5	g417	0.0132	7.26

Trait abbreviations as in Table 3

cantly associated with percentage of roots produced by tissue culture, co-located to QTLs for percentage of roots produced by stem cuttings on LG7 and LG11, respectively in family 1. Of the four QTLs for percentage of roots produced by stem cuttings in family 1, at least one QTL was validated in family 2. Two markers (g417 and c115) on LG11, which were significantly associated with roots produced by stem cuttings, co-located with QTL for the same trait on LG11 in family 1. The QTL effect for percentage of roots produced by stem cuttings on LG11 in family 1 was derived mainly from the common parent (SN382), while in family 2, the QTL effect was from the paternal parent (TO15).

Table 8 QTL analysis of vegetative propagation traits in *Eucalyptus nitens* full-sib families at Mt. Gambier

LG	Trait	Position (cM)	Locus	LOD	<i>R</i> ² (%)
1	SC	35.3	c077a	3.53 ^a	4.9
1	TC	32.3	c077a	4.94 ^b	7.5
2	TC	176.4	c083	4.43 ^b	6.8
3	SC	40	g432	4.68 ^b	6.4
3	TC	221.6	g243	10.54 ^b	15.4
7	SC	64.3	g095	8.35 ^b	11.1
8	TC	20	sEsi076	3.24 ^a	5.0
11	SC	203.8	c115	3.53 ^a	4.9

Interval mapping in family 1

SC stem cutting, TC tissue culture

^a Significant with chromosome-wide permutation tests

^b Significant with genome-wide permutation tests

Discussion

Heritability and the number of growth QTLs

The number of QTLs detected for growth traits in the Ridgley full-sib family was lower than the number of QTLs

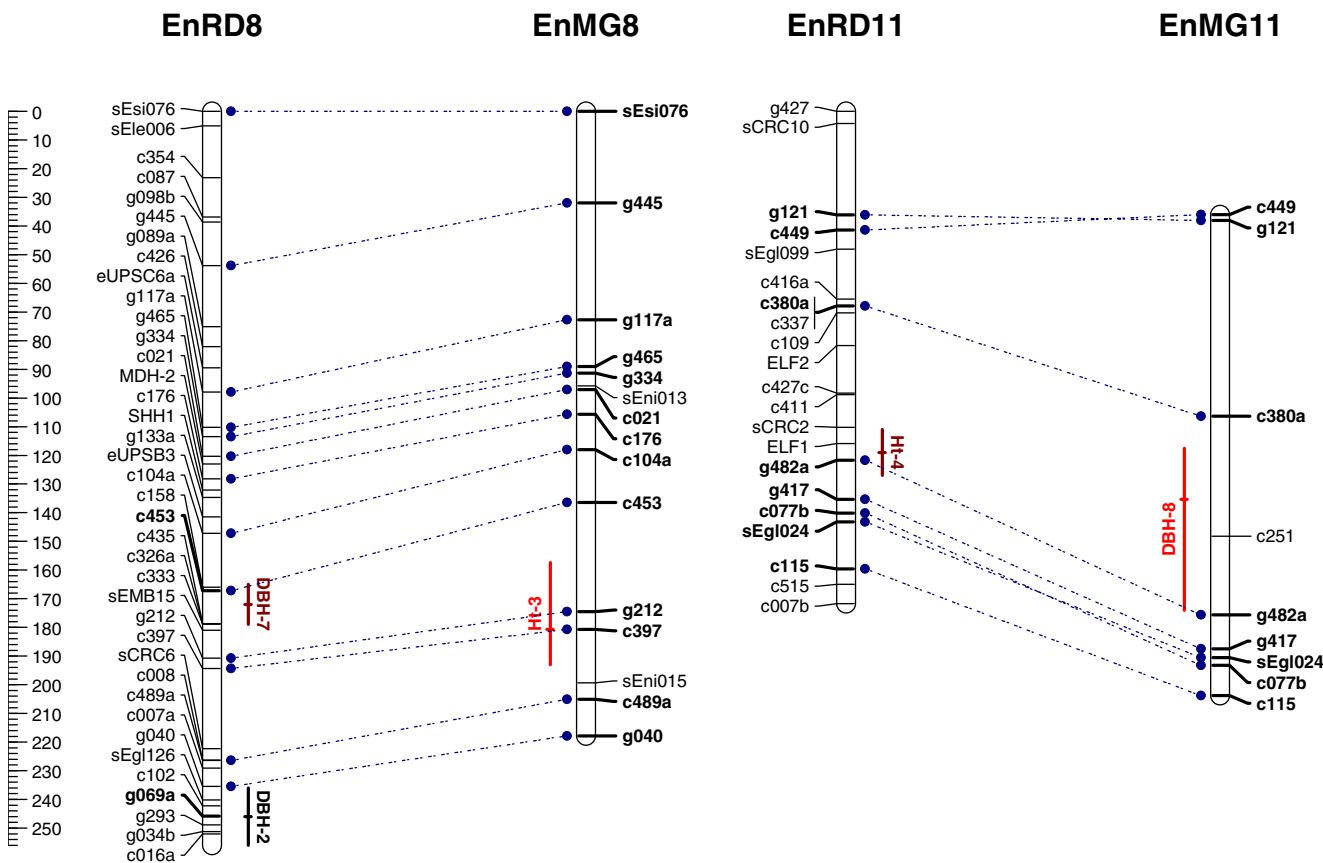


Fig. 3 Comparison of growth QTL between *Eucalyptus nitens* full-sib families at Ridgley (RD) and Mt. Gambier (MG)

Table 9 Single-marker analysis of vegetative propagation traits in family 2

LG	Trait	Position (cM)	Locus	<i>P</i>	<i>R</i> ² (%)
5	SC	43.4	c288	4.23E-04	6.69
7	TC	79.6	g095	0.002	5.00
9	SC	82.5	sEsi140	0.0097	7.08
11	TC	148.2	c251	0.0238	5.05
11	SC	187.5	g417	0.0589	4.09
11	TC	193.2	c077b	0.0199	3.04
11	SC	203.8	c115	0.0344	4.76

SC-stem cutting TC Tissue culture.

detected for wood quality traits using the same family in an earlier study (Thumma et al. 2010). However, the number of QTLs detected for growth traits in this study is higher than in comparable studies (Bundock et al. 2008; Freeman et al. 2009). The number of QTLs detected for later year traits was higher than early year growth traits (Table 5). For some early year growth traits (Ht-2) no QTLs were detected. The number of QTLs detected, therefore, appears to be directly related to the heritability of the traits. Heritability of the early year growth traits was lower than later year growth traits in both Mt. Gambier families (Table 3). Similarly, the higher number of wood quality QTLs detected in several studies may reflect the higher heritability of these traits compared with growth traits. However, heritability of a trait and the percentage of phenotypic variation explained by the QTL appear to be poorly correlated. The estimated percentage of phenotypic variation explained for growth traits reported in a number of studies is moderate (<15%; (Grattapaglia et al. 2009). Some of these values may have been overestimated as the size of the populations used in these studies was small. In a recent study Buckler et al. (2009) identified more than 30 QTLs for flowering time in maize using several large families. The percentage of phenotypic variation explained by each of these QTLs is generally less than 3% even though flowering time is highly heritable. This is in contrast to inbred species such as *Arabidopsis* and rice where a few QTLs explained a large percentage of variation in flowering time (El-Lithy et al. 2006; Izawa et al. 2003). Similarly, several SNPs each explaining less than 0.3% of phenotypic variation were associated with human height, although the heritability of height is very high (Weedon et al. 2007). Even though the individual QTL effect was small, flowering time in the maize parental lines was accurately predicted ($R^2 > 0.87$) by considering all the significant QTLs together. Buckler et al. (2009) suggested that mating system and demographics influence the genetic architecture of adaptive traits. Similar small effect QTL may underlie

many economically important traits in forest trees irrespective of their heritability. This is supported by results from recent association studies which show that the percentage of variation explained by SNP-trait associations is less than 5% for wood quality and adaptive traits such as cold tolerance (Eckert et al. 2009; Gonzalez-Martinez et al. 2007; Thumma et al. 2009; Thumma et al. 2005). However, together the significant SNPs may explain a substantial proportion of trait variation.

Comparison of growth QTLs

The QTL for DBH-7 on LG8 and for Ht-4 on LG11 found in Ridgley overlap with the QTL intervals for Ht-3 on LG8 and DBH-8 on LG11 in the Mt. Gambier family. Single marker analysis results from family 2 of Mt. Gambier also suggest a QTL for DBH-8 on LG11. These QTLs, therefore, may represent the effects of common genes underlying the growth traits.

There are few studies analysing QTLs associated with growth traits in eucalypts and even fewer studies with common markers mapped between different studies making it difficult to compare the results from this study with others. Grattapaglia et al. (1996) observed several QTLs for growth traits including a QTL for DBH on LG5 in *E. grandis*. Freeman et al. (2009) also identified a QTL for DBH on LG5 in *E. globulus*. Similarly, in the present study a number of QTLs was observed on LG5. Even though all these studies suggest a growth QTL on LG5, within the linkage group the position of the QTL may not be the same. Fine mapping with several markers may determine the position of the QTL and candidate genes underlying the QTL positions.

The homolog of the *Arabidopsis* LFY flowering gene *ELF1* maps to the common growth QTL found on LG11 (Fig. 1). The co-location of the *ELF1* gene with a growth QTL may reflect the correlation between flowering time and growth observed in several *Eucalyptus* species (Mora et al. 2009; Wiltshire et al. 1998). Southerton et al. (1998) found that the *ELF* gene was expressed in floral primordia and leaf primordia in vegetative meristems of *E. globulus*. Similarly, *Populus* flowering locus (FT2) initiates the onset of flowering thus changing from the vegetative phase to the reproductive phase, and regulates seasonal growth and flowering (Hsu et al. 2006). The co-location of *ELF* gene with growth QTL observed in this study and the correlation between flowering time and growth seen in several *Eucalyptus* species may be clues to the potential molecular mechanism underlying the QTL. However, identification of candidate genes based on co-location to the QTL regions needs to be treated cautiously. Within a QTL position, there could be several linked genes. Further studies are needed to confirm the role of a gene controlling the trait.

Establishing the identity of *ELF* genes

We mapped *ELF* gene on *E. nitens* linkage map using an RFLP probe. This probe detected two loci segregating in the Ridgley family. These two loci mapped to LG11: one at 82 cM, and one at 116 cM, close to the QTL interval for Ht-4 (Fig. 1). Southerton et al. (1998) identified two copies of *ELF* in *E. globulus*. One of the *ELF* genes, *ELF2*, was found to be a pseudo gene. In order to find out if the *ELF* gene mapping to the QTL interval is the functional gene, we sequenced the nearby genomic marker (g482). We then BLAST searched the *E. grandis* genome sequence (<http://eucalyptusdb.bi.up.ac.za/>) with the two *ELF* sequences of *E. globulus* and the g482 sequence. This revealed a scaffold which contained the homologs for both *ELF* and the g482 sequences. We were able to determine that the *ELF* locus near g482 is the functional copy of the gene based on a 37 bp insertion in the 3' region of the gene which is absent in the pseudo gene. The physical distance between the *ELF* gene and g482 on the scaffold is 633 kb. This translates into a genetic distance of about 1.60 cM between these two markers by considering 1 cM as equivalent to a mean physical distance of 395 kb (Grattapaglia and Bradshaw 1994). The genetic distance observed between these two markers on the *E. nitens* linkage map is 5.7 cM (Fig. 1). Increasing the marker density should improve the discrepancy observed between these two estimates. This analysis represents one of the first studies to utilise the public *Eucalyptus* genome resource (<http://eucalyptusdb.bi.up.ac.za/>) to establish the identity of a gene underlying a QTL interval.

Comparison of growth and wood quality QTLs in Ridgley family

Comparison of the growth QTLs detected in the Ridgley family with wood quality QTLs detected in an earlier study (Thumma et al. 2010) revealed several common QTLs between the two traits (Electronic supplementary material, Figure 1). For some shared QTL positions, the QTL effects are in a favourable direction. For example a QTL for cellulose content in the earlier study was observed near the QTL for DBH-7 on LG1. At this common interval, the QTL effect for both traits was derived from the maternal parent and the QTL effect was positive for both traits. Similarly near the common QTL for height and DBH traits on LG5, a QTL for microfibril angle (MFA) was observed. The QTL effect at this position was negative between growth traits and MFA indicating higher growth is associated with lower MFA, a favourable condition from a breeding perspective. However, there appears to be a negative or unfavourable correlation between growth and density QTL. On LG6, 11 and 12 a QTL for density was

observed near the QTL for growth traits. At all these positions the QTL effects for growth and density were negative indicating higher growth was associated with lower density. All these correlations between QTL effects may reflect the phenotypic correlations observed between the growth and wood quality traits in the Ridgley family. For example, a significant negative relationship ($r=-0.22$) was observed between DBH-7 and wood density in the Ridgley family. The negative QTL effects between growth and density traits observed in this study reinforce similar findings from earlier studies in eucalypts (Bundock et al. 2008; Grattapaglia et al. 1996; Verhaegen et al. 1997). These results suggest that selection for growth may have some adverse impact on wood density. However, in a recent study Stackpole et al. (2009) observed that the adverse correlation found between density and growth traits at selection age (4–5 years) in *E. globulus* breaks down at harvest age (16–17 years). QTL analysis of density and growth traits collected at harvest age may therefore reveal QTLs independent of each other.

Vegetative propagation QTLs in Mt. Gambier families

The higher heritability of vegetative propagation traits compared with growth traits resulted in the detection of more QTLs for vegetative propagation traits. The percentages of variation explained by the vegetative propagation QTLs are also higher than for growth traits. Co-location of QTLs for percentage of roots produced by both tissue culture and stem cutting methods on LG1 suggests common genes underlying these traits. The QTL with the strong effect on LG7 was validated by single marker analysis in family 2. However, in family 1 this QTL was associated with roots produced by the stem cutting method, while in family 2 it was associated with roots produced by tissue culture. Similarly, the QTL on LG11 was validated in family 2 for percentage of roots produced by both methods. Previous studies have reported a number of putative QTL for the ability to produce roots from stump sprout cuttings (Grattapaglia et al. 1995; Marques et al. 1999). Marques et al. (2002) by comparing different studies identified a homologous linkage group (LG1) containing a putative QTL for vegetative propagation. The QTL for roots produced by stem cutting and tissue culture observed on LG1 in the present may be the same as the common QTL observed in other studies.

Grattapaglia et al. (1995) suggested that a small number of QTLs of large effect may underlie vegetative propagation traits in eucalypts (Grattapaglia et al. 1995). This is in contrast to the results from the present study. It seems likely that the large effects are in part due to the small sample size employed in the study (Beavis 1998). In an *Eucalyptus tereticornis* x *E. globulus* pedigree the QTL effects for the

same traits tended to be smaller and the sample size used was much larger (Marques et al. 1999). Comparison of QTLs between different studies is difficult as QTL detection is influenced by a number of factors such as population size, experimental design and the analytical techniques employed.

Conclusions

We have identified several QTLs for growth and vegetative propagation traits using three families growing at two sites. QTL analysis in Mt. Gambier families had more power as genetic data predicted from clonal replications was used. At two positions, QTLs for growth are conserved in Ridgley and Mt. Gambier families. Two vegetative propagation QTLs on LG7 and LG11 were validated in a separate family. However, these results need to be treated cautiously. While clonal replicates were used to obtain the phenotype data, fewer markers were genotyped in the Mt. Gambier families compared with the Ridgley family. In Ridgley family 1, the other hand the trait data was obtained from single trees.

Currently, there are few candidate genes for complex traits such as growth in eucalypts. The flowering gene *ELF2* on LG11 could be considered a candidate gene controlling growth. It is difficult to compare QTL positions from this study with other *Eucalyptus* studies. There are few QTL reports examining growth traits and even fewer on vegetative propagation traits in eucalypts and the number of common markers between the different studies is very low. There is a clear need for the use of common gene-based markers to better understand the genetic control of complex traits such as growth and to compare results from different studies. At present, the candidate gene markers on the *E. nitens* linkage map are primarily related to cell wall biosynthesis. Currently, we are mapping several candidate genes for adaptive traits on the *E. nitens* linkage map. The public *Eucalyptus* genome resource (<http://eucalyptusdb.bi.up.ac.za/>) will be very useful for revealing candidate genes underlying QTL intervals identified in this study. QTL mapping represents the first step in identifying candidate genes controlling complex but important traits such as growth and vegetative propagation traits. Marker-assisted selection is expected to be very useful for growth traits as phenotypic correlations between early and later year growth traits is low and markers linked to growth traits may be used for increasing selection efficiency at an early age.

Acknowledgements We thank Jan Murrell and Kylie Groom for their help in generating molecular marker data used in this study and John Owen for planting the stem cuttings and ortets at the Mt. Gambier field site. We are grateful to Tim Hingston, Helen Jones and

Gillian Rasmussen (Gunn's Ltd.) for providing the vegetative propagation trait data used in this study. For analysing the ELF genes, we have used Eucalyptus DB provided by DOE Joint Genome Institute and *Eucalyptus* Genome Network, EUCAGEN.

References

- Beavis WD (1998) QTL analysis: power, precision, and accuracy. In: Patterson HA (ed) Molecular dissection of complex traits. CRC, Boca Raton, pp 145–162
- Brondani R, Williams E, Brondani C, Grattapaglia D (2006) A microsatellite-based consensus linkage map for species of *Eucalyptus* and a novel set of 230 microsatellite markers for the genus. *BMC Plant Biol* 6:20
- Buckler ES, Holland JB, Bradbury PJ, Acharya CB, Brown PJ, Browne C, Ersoz E, Flint-Garcia S, Garcia A, Glaubitz JC, Goodman MM, Harjes C, Guill K, Kroon DE, Larsson S, Lepak NK, Li H, Mitchell SE, Pressoir G, Peiffer JA, Rosas MO, Rocheford TR, Romay MC, Romero S, Salvo S, Villeda HS, Sofia da Silva H, Sun Q, Tian Q, Upadyayula N, Ware D, Yates H, Yu J, Zhang Z, Kresovich S, McMullen MD (2009) The Genetic Architecture of Maize Flowering Time. *Science* 325: 714–718
- Bundock P, Potts B, Vaillancourt R (2008) Detection and stability of quantitative trait loci (QTL) in *Eucalyptus globulus*. *Tree Genet Genom* 4:85–95
- Butcher PA, Southerton SG (2007) MAS in forestry species. Marker-assisted selection (MAS) in crops, livestock, forestry and fish: current status and the way forward. FAO, Rome, pp 283–305
- Byrne M, Murrell JC, Allen B, Moran GF (1995) An integrated genetic linkage map for eucalypts using RFLP, RAPD and isozyme markers. *Theor Appl Genet* 91:869–875
- Byrne M, Parrish TL, Moran GF (1998) Nuclear RFLP diversity in *Eucalyptus nitens*. *Heredity* 81:225–233
- Darvasi A, Soller M (1997) A simple method to calculate resolving power and confidence interval of QTL map location. *Behav Genet* 27:125–132
- de Little DW, Tibbits WN, Rasmussen GF, Ravenwood I (1992) Genetic improvement strategy for APPM Eucalypt tree farms in Tasmania. Mass Production Technology for Genetically Improved Fast Growing Forest Tree species ACTES proceedings. AFOCEL, France, Bordeaux, pp 177–188
- Eckert AJ, Bower AD, Wegrzyn JL, Pande B, Jermstad KD, Krutovsky KV, St. Clair JB, Neale DB (2009) Association genetics of coastal Douglas fir (*Pseudotsuga menziesii* var. *menziesii*, Pinaceae). I. Cold-hardiness related traits. *Genetics* 182:1289–1302
- El-Lithy ME, Bentsink L, Hanhart CJ, Ruys GJ, Rovito D, Broekhof JLM, van der Poel HJA, van Eijk MJT, Vreugdenhil D, Koormneef M (2006) New Arabidopsis Recombinant Inbred Line Populations Genotyped Using SNPWave and Their Use for Mapping Flowering-Time Quantitative Trait Loci. *Genetics* 172:1867–1876
- England N, Borrallho N (1995) Heritability of rooting success in *Eucalyptus globulus* stem cuttings. In: Eds BM, Potts NMGB, Reid JB, Cromer RN, Tibbits WN, Raymond CA (ed) *Eucalypt plantations: improving fibre yield and quality*. Proceedings CRC-IUFRO Conference, Hobart, Tasmania, Australia, 19–24 Feb 1995 (CRC for Temperate Hardwood Forestry). pp 237–238
- Falconer DS, Mackay TFC (eds) (1996) *Introduction to Quantitative Genetics*, 4th edn. Longman Scientific and Technical, New York
- Freeman J, Whittcock S, Potts B, Vaillancourt R (2009) QTL influencing growth and wood properties in *Eucalyptus globulus*. *Tree Genet Genom* 5:713–722

- Gilmour AR, Gogel BJ, Cullis BR, Thompson R (2006) ASReml User Guide Release 2.0. VSN International Ltd, Hemel Hempstead, HP1 1ES, UK
- Gonzalez-Martinez SC, Wheeler NC, Ersoz E, Nelson CD, Neale DB (2007) Association genetics in *Pinus taeda* L. I. Wood property traits. *Genetics* 175:399–409
- Grattapaglia D, Bradshaw HD Jr (1994) Nuclear DNA content of commercially important *Eucalyptus* species and hybrids. *Can J For Res/Rev Can Rech For* 24:1074–1078
- Grattapaglia D, Bertolucci FL, Sederoff RR (1995) Genetic mapping of QTLs controlling vegetative propagation in *Eucalyptus grandis* and *E. urophylla* using a pseudo-testcross strategy and RAPD markers. *Theor Appl Genet* 90:933–947
- Grattapaglia D, Bertolucci FLG, Penchel R, Sederoff RR (1996) Genetic mapping of quantitative trait loci controlling growth and wood quality traits in *Eucalyptus grandis* using a maternal half-sib family and RAPD markers. *Genetics* 144:1205–1214
- Grattapaglia D, Plomion C, Kirst M, Sederoff RR (2009) Genomics of growth traits in forest trees. *Curr Opin Plant Biol* 12:148–156
- Hamilton MG, Potts BM (2008) Review of *Eucalyptus nitens* genetic parameters. *NZ J For Sci* 38:102–119
- Hetherington S, Orme RK (1989) Seedling variation in root formation of *Eucalyptus globulus* cuttings. *Comb Proc Int Plant Propag Soc* 39:56–60
- Hsu C-Y, Liu Y, Luthe DS, Yuceer C (2006) Poplar FT2 shortens the juvenile phase and promotes seasonal flowering. *Plant Cell* 18(8):1846–1861
- Izawa T, Takahashi Y, Yano M (2003) Comparative biology comes into bloom: genomic and genetic comparison of flowering pathways in rice and Arabidopsis. *Curr Opin Plant Biol* 6:113–120
- Marques CM, Vasquez-Kool J, Carocha VJ, Ferreira JG, O'Malley DM, Liu BH, Sederoff R (1999) Genetic dissection of vegetative propagation traits in *Eucalyptus tereticornis* and *E. globulus*. *Theor Appl Genet* 99:936–946
- Marques MC, Brondani BR, Grattapaglia GD, Sederoff SR (2002) Conservation and synteny of SSR loci and QTLs for vegetative propagation in four *Eucalyptus* species. *TAG Theor Appl Genet* 105:474–478
- Marques C, Carocha V, Pereira de Sá A, Oliveira M, Pires A, Sederoff R, Borralho N (2005) Verification of QTL linked markers for propagation traits in *Eucalyptus*. *Tree Genet Genom* 1:103–108
- Mora F, Gleadow R, Perret S, Scapim C (2009) Genetic variation for early flowering, survival and growth in sugar gum (*Eucalyptus cladocalyx* F. Muell) in southern Atacama Desert. *Euphytica* 169:335–344
- Seaton G, Haley CS, Knott SA, Kearsley M, Visscher PM (2002) QTL Express: mapping quantitative trait loci in simple and complex pedigrees. *Bioinformatics* 18:339–340
- Southerton SG, Strauss SH, Olive MR, Harcourt RL, Decroocq V, Zhu XM, Llewellyn DJ, Peacock WJ, Dennis ES (1998) *Eucalyptus* has a functional equivalent of the Arabidopsis floral meristem identity gene LEAFY. *Plant Mol Biol* 37:897–910
- Stackpole D, Vaillancourt R, de Aguilar M, Potts B (2009) Age trends in genetic parameters for growth and wood density in *Eucalyptus globulus*. *Tree Genet Genom*. doi:10.1007/s11295-009-0239-4
- Thamarus KA, Groom K, Murrell J, Byrne M, Moran GF (2002) A genetic linkage map for *Eucalyptus globulus* with candidate loci for wood, fibre, and floral traits. *Theor Appl Genet* 104:379–387
- Thumma BR, Nolan MR, Evans R, Moran GF (2005) Polymorphisms in *cinnamoyl CoA reductase (CCR)* are associated with variation in microfibril angle in *Eucalyptus* spp. *Genetics* 171:1257–1265
- Thumma BR, Matheson BA, Zhang D, Meeske C, Meder R, Downes GM, Southerton SG (2009) Identification of a Cis-acting regulatory polymorphism in a Eucalypt COBRA-like gene affecting cellulose content. *Genetics* 183:1153–1164
- Thumma B, Southerton S, Bell J, Owen J, Henery M, Moran G (2010) Quantitative trait locus (QTL) analysis of wood quality traits in *Eucalyptus nitens*. *Tree Genet Genom* 6:305–317. doi:10.1007/s11295-11009-10250-11299
- Van Ooijen JW (2006) JoinMap® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen
- Van Ooijen JW (2009) MapQTL® 6, Software for the mapping of quantitative trait loci in experimental populations of diploid species. Kyazma BV, Wageningen
- Verhaegen D, Plomion C, Gion JM, Poitel M, Costa P, Kremer A (1997) Quantitative trait dissection analysis in *Eucalyptus* using RAPD markers: 1. Detection of QTL in interspecific hybrid progeny, stability of QTL expression across different ages. *Theor Appl Genet* 95:597–608
- Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. *J Hered* 93:77–78
- Weedon MN, Lettre G, Freathy RM, Lindgren CM, Voight BF, Perry JRB, Elliott KS, Hackett R, Guiducci C, Shields B, Zeggini E, Lango H, Lyssenko V, Timpson NJ, Burt NP, Rayner NW, Saxena R, Ardlie K, Tobias JH, Ness AR, Ring SM, Palmer CNA, Morris AD, Peltonen L, Salomaa V, Smith GD, Groop LC, Hattersley AT, McCarthy MI, Hirschhorn JN, Frayling TM (2007) A common variant of HMGA2 is associated with adult and childhood height in the general population. *Nat Genet* 39:1245–1250
- Whiteman PH, Dean CA, Doran JC, Cameron JN (1992) Genetic parameters and selection strategies for *Eucalyptus nitens* (Dean and Maiden) in Victoria. *Silvae Genet* 41:77–81
- Wiltshire RJE, Reid JB, Potts BM (1998) Genetic Control of reproductive and vegetative phase change in the *Eucalyptus risdonii-E. tenuiramis* complex. *Aust J Bot* 46:45–63
- Zeng ZB (1993) Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. *Proc Natl Acad Sci USA* 90:10972–10976