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Genetic dissection of vegetative propagation traits in *Eucalyptus tereticornis* and *E. globulus*

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Abstract We have detected quantitative trait loci (QTLs) affecting vegetative propagation traits in *Eucalyptus tereticornis* and *Eucalyptus globulus*. Using amplified fragment length polymorphism (AFLP) genetic linkage maps, the inheritance of 199 markers was assessed in 94 F_1 individuals with extreme adventitious rooting response, and in 221 randomly chosen F_1 individuals. Phenotypes were scored in 1995 and 1996. QTL analyses were performed using chi-square tests (χ^2), single-marker analysis (SMA), interval mapping (IM) and composite interval mapping (CIM). All approaches yielded similar QTL detection results. Three QTLs are hypothesized for mortality (MORT=% dead cuttings), nine for adventitious rooting (ROOT, RCT=% rooted cuttings relative to the surviving or total cuttings, respectively), four for petrification (PETR=% surviving unrooted cuttings), one for sprouting ability (SPR=number of stump sprout cuttings harvested in 1995) and four for the stability of adventitious rooting (STAB=absolute value of the difference ROOT95-ROOT96). All putative QTLs for MORT and PETR were located on the *E. tereticornis* map, and for SPR and STAB on the *E. globulus* map. We found different QTLs for MORT, ROOT, RCT, SPR and STAB. Putative QTLs accounted for 2.6–17.0% of the phenotypic variance of a trait (R^2). Estimated standardized gene substitution effects varied between 0.13 and 0.49 phenotypic standard deviations (σ_p). These results indicate that the phenotypic variation in these traits has a meaningful genetic component and that stable QTLs can be found in a family of reasonable size where no previous knowledge of the trait was available.

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Introduction

Tree improvement is hampered by long generation intervals, inbreeding depression and weak juvenile-adult correlations. In addition, most traits of commercial importance are quantitatively inherited and are expressed late in tree development. Genomic mapping applied to tree breeding offers promise for the identification of genes that contribute to the variation of quantitative traits. It can also provide new insights into genome structure and evolution. The AFLP technique allows a considerable decrease in the cost and time required to construct genetic linkage maps, relative to other PCR-based marker systems (Zabeau and Vos 1993; Vos et al. 1995). This facilitates the construction of single-tree parental maps of elite breeding families. Recent reports on the genetic architecture of quantitatively inherited traits in interspecific hybrids of forest trees support the existence of a few QTLs controlling large proportions of the total genetic variation (Bradshaw and Stettler 1995; Grattapaglia et al. 1996; Verhaegen et al. 1997). These QTLs could potentially be manipulated through marker-assisted selection (MAS) strategies. The implementation of MAS would be advantageous for the identification of superior clones for propagation and for the selection of superior parents with complementary genotypes, for the succeeding generation (O'Malley and McKeand 1994).

Vegetative-propagation traits are appropriate for QTL dissection in *Eucalyptus* as there is abundant intra- and inter-specific variation (Reuveni et al. 1990), genetic control is reasonable ($h^2 \approx 0.4$) (Borralho and Wilson 1994) and clonal propagation allows an increased accuracy in trait measurement (Bradshaw and Foster 1992). Vegetative propagation can capture both additive and non-additive genetic variation in tree breeding (Zobel and Talbert 1984). Major pulp and paper companies propagate selected *Eucalyptus* genotypes resulting from

recurrent selection and/or interspecific hybridization. *Eucalyptus tereticornis* has good potential for rooting (60–90% from cuttings) but modest pulping qualities (Chaperon and Quillet 1977). It is frequently employed in hybridization programs to improve adventitious rooting of other eucalypt species (Eldridge et al. 1994). *Eucalyptus globulus* is a major plantation species, with excellent wood properties for cellulose pulp production, yet with a very irregular (5–64%) adventitious rooting behavior (Hetherington and Orme 1989). This constitutes a bottleneck in the production of elite genotypes (England and Borralho 1995). While physiological and environmental factors can be important in the success of adventitious rooting, there is still ample room for genetic improvement (Reuveni et al. 1990; Borralho and Wilson 1994).

Elucidation of the genetic basis of vegetative propagation in *Eucalyptus* would help target breeding efforts. The purpose of the present work was to investigate the genetic basis of stump sprouting and adventitious rooting ability. We relied on linkage disequilibrium within an interspecific *E. tereticornis*×*E. globulus* full-sib family to identify major genetic factors. In any pseudo-testcross experiment the only detectable QTLs are those for which one or both parents are heterozygous for alleles of strong alternative effect which are not masked either by dominance (Groover et al. 1994) or the environment in which phenotyping is conducted (Bradshaw 1996). We expected that outbreeding and mostly undomesticated eucalypts would be heterozygous in factors affecting vegetative propagation. The availability of data from 2 years allowed the study of QTL stability in different environments. We wanted to know the number and location of genetic loci affecting trait expression, the parental source of beneficial QTL alleles, the magnitude of their effect on the phenotype and their stability across time.

Materials and methods

Plant material

The QTL mapping pedigree was initiated in 1993 by hybridization of an *E. tereticornis* seed parent (clone TT Esc 87/90) and an *E. globulus* pollen parent (clone GB MJ 6/90). Both species belong to the subgenus *Symphyomyrtus* (Eldridge et al. 1994). No information about the parent's adventitious rooting ability was available. An F_1 full-sib progeny set (895 individuals) was established in the field in 1994 as mother stock plants for vegetative propagation. In July of 1995 and 1996 all plants were cut back to stimulate axillary sprouting. Two-months later, stump sprout cuttings were harvested. The basal tip of each cutting was dipped in a 5,000-ppm IBA solution in talc and placed into a 6:4 rooting potting mix (turf and styrofoam beads). The plant material was kept for 30 days in tunnels (70–90% humidity) under mist irrigation (combined with 1.5% of Previcur fungicide) before transfer to a shaded acclimation area.

Linkage-map construction

The AFLP-based genetic linkage maps of the parent trees have been reported elsewhere, together with procedures for DNA extraction and AFLP assays (Marques et al. 1998). The maps were constructed using 73 F_1 progeny. A subset of 199 evenly spaced (1 per 10 cM on average) 1:1 segregating markers were selected for QTL analysis (108 for *E. tereticornis* and 91 for *E. globulus*).

Phenotypic measurements

Vegetative propagation traits were measured during operational cloning, on individually potted plants 90 days after root induction. Data for all 895 progeny were collected in 1995 and 1996 for: (1) mortality (MORT=dead/total cuttings), (2) adventitious rooting (ROOT=rooted/surviving cuttings, RCT=rooted/total cuttings), and (3) petrification (PETR=surviving unrooted/total cuttings). We have further assessed the mother stock plants sprouting ability (SPR=number of cuttings harvested in 1995) and dwarfism (number of dwarfs). The stability of the adventitious rooting response (STAB) in both years was also appraised (absolute value of the difference ROOT95-ROOT96). In 1995 it was assumed that the conditions in the rooting tunnels were homogeneous and data from all available cuttings per clone were averaged (generally 20 cuttings per clone). In 1996 we grouped seven cuttings (when available) per plot, in up to four blocks, to assess possible variation across and along the tunnels. Data were averaged per plot and weighted means were computed for each clone. Correlations between pairs of traits were calculated using Pearson's correlation coefficient (SAS 1988).

Data analysis

Selective genotyping

Among the F_1 individuals with the most reproducible adventitious rooting response (STAB <40), we selected 50 with 0–4% ROOT and 44 with 53–100% ROOT. The parents were also genotyped. Chi-square tests (1 df) for goodness of fit to the expected 1:1 allele frequency were performed. Significant differences ($P \leq 0.05$) were interpreted as an indication of putative marker-trait associations (SAS 1988).

Random genotyping

A random sample of 221 F_1 individuals and the parents were also genotyped. This group included the individuals used for the construction of the genetic linkage maps. QTL detection was performed by single-marker analysis (SMA) (analogous to linear regression), interval mapping (IM) (Lander and Botstein 1989), and composite interval mapping (CIM) (Zeng 1994). QTL searches were done separately for each trait and year. For SMA *F*-tests were considered significant at a genome-wide $P \leq 0.005$ (SAS 1988). For IM and CIM analyses (number of background parameters set to 5; window size set to 10 cM), implemented by QTL-Cartographer v1.13a (Basten et al. 1998), a numerical method was used to estimate the critical level for type-I error rates (Churchill and Doerge 1994). One-thousand permutations of the phenotypic data were executed and the threshold value of the likelihood-ratio (LR) test statistic determined to give genome-wide Type-I error rates of $P \leq 0.1$, 0.05, 0.025 and 0.01. When more than one marker from a single linkage group was associated with a QTL, the LR test-statistics values generally peaked at one position and decreased in value with distance from this peak. The utility Eqtl (QTL-Cartographer v1.13a) was used to find the peaks above the specified significant threshold values. When enumerating markers we refer to that closest to the LR test-statistics peak.

Separate QTL analyses were carried out for each parent, under a backcross model (Grattapaglia and Sederoff 1994). Consensus-significant marker-trait associations (across different analytical tests) were hypothesized to be caused by the presence of a nearby QTL. The proportion of phenotypic variation explained by each significant marker was estimated as the coefficient of determina-

tion (R^2) for the single-locus model from the least-squares analysis of variance (Type-III Sum of Squares/Total Sum of Squares) (Stuber et al. 1992). Simultaneous multilocus estimates of the total proportion of phenotypic trait-variation explained by the joint action of the putative QTLs were obtained by multiple linear regression (PROC GLM, SAS 1988). Assessment of the effect of each significant marker on the phenotypic mean-trait value was done using PROC GLM and the Duncan test (SAS 1988). The effects of the putative QTLs were expressed in phenotypic standard deviations (σ_p). The marker with the highest level of significance was selected to test for digenic epistasis. A stepwise regression procedure was applied to exclude multi-collinearity (SAS 1988). Two-way interactions between unlinked putative QTLs were investigated by stepwise regression on all significant markers and pairwise regression on the significant unlinked markers associated with each trait (SAS 1988).

Results

Quantitative traits

There were 22% dwarfs in the 895 *E. tereticornis*×*E. globulus* F_1 progeny. In 1995, it was possible to harvest cuttings from 646 individuals, 302 of which never rooted. In 1996, we collected cuttings from 427 individuals, 106 of which never produced roots. Approximately 76% of the F_1 progeny gave consistent adventitious rooting results in both years (STAB <40). The frequency plots of the vegetative-propagation traits studied showed a continuous distribution (Fig. 1) differing from normality (except SPR). Log-transformation of the data did not significantly improve normality. A large degree of phenotypic variation was observed for all traits. A rough estimate of the adventitious rooting ability (ROOT) of the parents used in this experiment was obtained from their progeny when crossed with other individuals of the same species: 12% for *E. tereticornis* and 14% for *E. globulus*. As expected, the phenotypic correlation between the same traits measured in successive years and between ROOT-RCT was high, and likewise between PETR-MORT in the same year (Table 1). Unexpected low values were found for MORT and PETR in successive years (Table 1).

QTL discovery

For *E. tereticornis* (Table 2) 26 markers (in nine linkage groups) displayed a significant ($P \leq 0.05$) deviation from the expected 1:1 allele frequency ratio in the selective genotyping experiment. From the random sample and SMA, 21 *F*-tests (corresponding to markers in seven linkage groups and one unlinked marker) were significant, indicating markers associated with trait expression ($P \leq 0.005$). One significant marker with SMA (A145) was not considered in the QTL-Cartographer analysis as

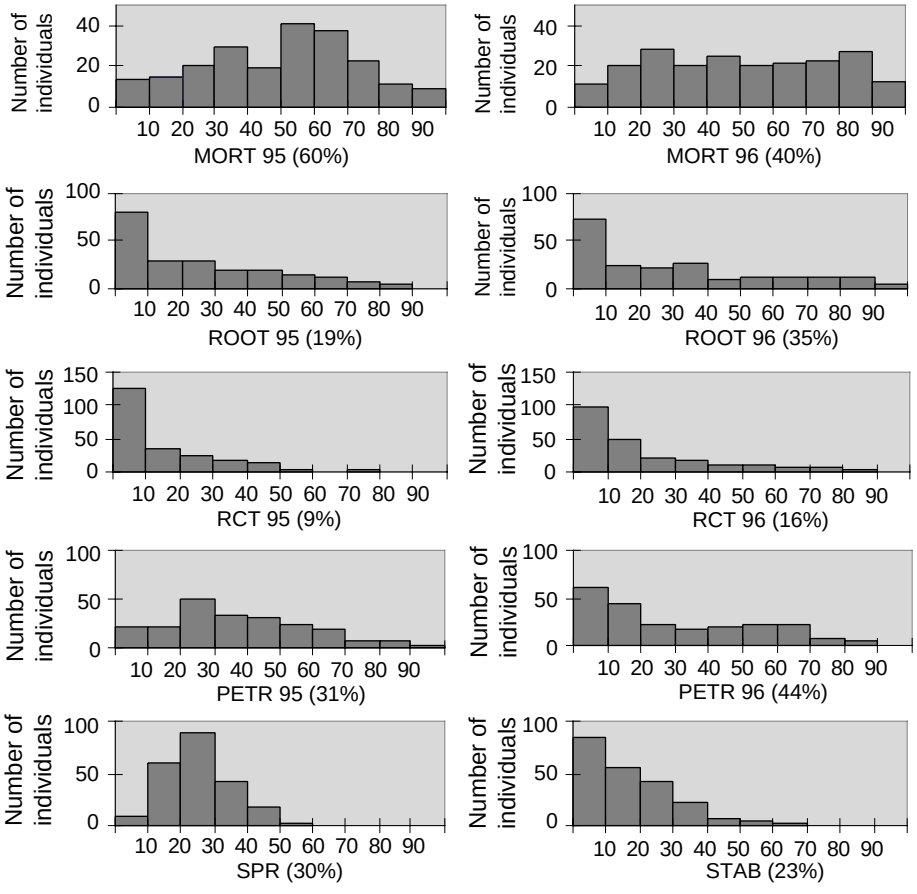

Fig. 1 Frequency distributions of the vegetative propagation traits measured (in 1995 and 1996) in the full-sib *E. tereticornis*×*E. globulus* hybrid family: MORT (dead/total cuttings), ROOT (rooted/surviving cuttings), RCT (rooted/total cuttings), PETR (surviving unrooted/total cuttings), SPR (number of stump sprout cuttings harvested in 1995), STAB (absolute value of the difference ROOT95–ROOT96). F_1 mean values are presented in parenthesis

Table 1 Pearson's correlation coefficients (SAS 1988) and significance level for relationships between characters in the full-sib *E. tereticornis*×*E. globulus* hybrid family: MORT (dead/total cuttings), ROOT (rooted/surviving cuttings), RCT (rooted/total cuttings), PETR (surviving unrooted/total cuttings), SPR (number of stump sprout cuttings harvested in 1995), STAB (absolute value of the difference ROOT95-ROOT96), SPR (number of stump sprout cuttings harvested in 1995). Significant at **P*=0.01, ***P*=0.005, ns=non significant

Item	MORT96	ROOT95	RCT95	ROOT96	RCT96	PETR95	PETR96	STAB	SPR
MORT95	0.46**	ns	–0.45**	ns	–0.26**	–0.76**	–0.31**	ns	ns
MORT96	–	ns	–0.26**	–0.24**	–0.53**	–0.31**	–0.71**	ns	ns
ROOT95		–	0.84**	0.74**	0.62**	–0.54**	–0.41**	0.30**	ns
RCT95			–	0.63**	0.65**	–0.24**	–0.25**	0.26**	ns
ROOT96				–	0.85**	–0.35**	–0.44**	0.54**	ns
RCT96					–	–0.18*	–0.22**	0.47**	ns
PETR95						–	0.52**	–0.14*	ns
PETR96							–	0.35**	ns
STAB								–	ns
SPR									–

Table 2 Significant marker-trait associations in *E. tereticornis* detected by selective genotyping ($\chi 2$, $P \leq 0.05$, *0.005) and/or random genotyping (SMA, genome-wide $P \leq 0.005$, *0.0002; IM and CIM, genome-wide $P \leq 0.1$, *0.05, **0.025, ***0.01). In the table, the "/" means "and". The position of the nth marker in the linkage group (starting from the top) is indicated as LG(n), as in Marques et al. 1998

Marker	LG(n)	$\chi 2$	Single marker analysis	Interval mapping	Composite interval mapping
A206	1(8)	0.012	–	–	–
A88r	2(10)	–	–	–	MORT95*
A48	2(11)	–	MORT95/96	–	MORT95*
A618	4(5)	0.046	–	–	–
A118	4(10)	0.029	–	PETR95	–
B506	4(11)	0.013	ROOT95, PETR95/96	PETR95	–
A333r	5(7)	0.050	–	–	–
A227	6(1)	0.003*	ROOT/RCT95, ROOT/RCT96, PETR96	ROOT*/RCT95, ROOT**RCT***96	ROOT**/RCT95, ROOT***/RCT**96, PETR96
A218	6(2)	0.001*	ROOT/RCT95, ROOT**/RCT**96, PETR96	ROOT*/RCT95, ROOT***/RCT***96	ROOT/RCT95, ROOT***/RCT**96, PETR96
A256r	6(3)	–	–	RCT96	–
A426r	7(2)	0.017	RCT95	–	–
A24	7(4)	0.050	–	–	–
B406r	7(10)	0.027	ROOT/RCT95, RCT96	ROOT*/RCT95, RCT96	RCT95
A152r	7(11)	0.001*	ROOT*/RCT95, RCT96	ROOT*/RCT95, RCT96	RCT95
B50	7(12)	–	–	ROOT*/RCT95	ROOT*/RCT95
A63	7(13)	0.019	ROOT/RCT95	ROOT***/RCT*95, RCT*96	ROOT***95, RCT**96
A43r	7(14)	0.001*	ROOT*/RCT95, RCT96	ROOT**/RCT95, RCT*96	ROOT***95, RCT**96
A23r	7(15)	0.005*	ROOT/RCT95	ROOT*/RCT95	ROOT95
A531r	7(17)	0.029	–	–	–
B293	8(1)	–	MORT95/96*, PETR95*/96*	MORT95/96***, PETR95***/96***	ROOT95*, MORT95*/96***, PETR95***/96***
B366r	8(2)	0.024	ROOT95, MORT96*, PETR95/96*	MORT96***, PETR95*/96***	ROOT95*
B90	8(3)	–	RCT96, MORT96	MORT96**	–
A590r	10(3)	–	MORT96	MORT96*	–
A592	10(4)	–	MORT96	MORT96	–
B255r	10(5)	–	–	MORT96**, PETR96	–
B453	10(6)	–	MORT96, PETR96	–	–
A196	11(1)	0.012	–	–	–
A253	11(2)	–	–	ROOT95**/96	ROOT95**/96, PETR95
B511	11(3)	0.000*	ROOT95*/96, PETR95	ROOT95***/96**, PETR95	ROOT95***/96**, PETR95**
B336r	11(4)	0.002*	ROOT95*/96, PETR95	ROOT95***/96**	ROOT95**/96**, PETR95*
B316	11(5)	0.000*	ROOT95/96	–	–
B425	12(1)	0.016	–	–	–
A71	12(2)	0.048	–	–	–
A160	12(3)	0.013	–	–	–
B352	12(5)	0.001*	–	–	–
B306	13(2)	0.022	–	–	–
A610	13(4)	0.020	–	–	–
A430	14(3)	–	STAB	–	–
A145	Unlinked	–	MORT95/96*, PETR95/96*	Not considered	Not considered

Table 3 *E. tereticornis*: summary of the most stable marker-trait associations across the different analyses, assumed to be putatively associated with QTLs. The position of the nth marker in the linkage group (starting from the top) is indicated as LG(n), as in Marques et al. 1998. The first number in the table refers to the percentage of phenotypic variation explained by the marker (%R^2). **The second number is the effect of the putative QTLs expressed in phenotypic standard deviations (σ_p).** A positive σ_p value indicates that the favourable effect is associated with the band-present phenotype. A negative σ_p value indicates that the favourable effect is associated with the band-absent phenotype. The "xxxx" indicate alternative locations of the putative QTLs. Trait mean and variance refer to the 221 individuals genotyped

Marker	LG (n)	MORT95	MORT96	ROOT95	RCT95	ROOT96	RCT96	PETR95	PETR96
A48	2(11)	3.19 **0.17**	3.50 **0.15**	–	–	–	–	–	–
B506	4(11)	–	–	2.95 **–0.19**	–	–	–	4.80 **0.20**	3.15 **0.17**
A227	6(1)	–	–	xxxx	xxxx	xxxx	xxxx	–	3.61 –0.20
A218	6(2)	–	–	5.27 **0.24**	5.05 **0.24**	5.57 **0.28**	5.40 **0.28**	–	xxxx
A426r	7(2)	–	–	–	3.78 **–0.18**	–	–	–	–
B406r	7(10)	–	–	xxxx	xxxx	–	xxxx	–	–
A152r	7(11)	–	–	xxxx	xxxx	–	xxxx	–	–
B50	7(12)	–	–	xxxx	xxxx	–	–	–	–
A63	7(13)	–	–	3.59 **0.27**	4.36 **0.26**	–	xxxx	–	–
A43r	7(14)	–	–	xxxx	xxxx	–	4.82 **–0.18**	–	–
A23r	7(15)	–	–	xxxx	xxxx	–	–	–	–
B293	8(1)	4.76 **0.24**	14.45 **0.49**	xxxx	–	–	–	7.28 **–0.32**	17.03 **–0.49**
B366r	8(2)	–	xxxx	5.37 **–0.20**	–	–	–	xxxx	xxxx
B90	8(3)	–	xxxx	–	–	–	2.79 **–0.15**	–	–
A590r	10(3)	–	3.51 **0.17**	–	–	–	–	–	–
A592	10(4)	–	xxxx	–	–	–	–	–	–
B511	11(3)	–	–	5.52 **–0.26**	–	xxxx	–	3.26 **0.20**	–
B336r	11(4)	–	–	xxxx	–	3.85 **0.23**	–	–	–
Overall %R^2	–	8.29	24.35	24.21	13.89	12.37	14.69	16.21	24.27
Trait mean	–	49.60	53.03	25.87	13.56	33.19	18.59	37.14	28.45
Trait variance	–	22.74	27.29	24.93	15.27	30.77	20.07	20.93	23.66
No. putative QTLs	–	2	3	5	3	2	3	3	3

it was an unlinked marker. Twenty significant markers (in five linkage groups) were disclosed by IM ($P \leq 0.1$). All but 1 of 15 markers (in five linkage groups) detected by CIM ($P \leq 0.1$) were identified in at least one previous analysis. Overlapping significant markers for MORT, ROOT, RCT and/or PETR were found in linkage groups 4, 6, 8 and 11. A total of 18 markers were significant in at least two analyses and were assumed to be putatively associated with QTLs for MORT, ROOT, RCT and PETR in linkage groups 2, 4, 6, 7, 8, 10 and 11 (Table 3). Most of these markers were detected by SMA (94%) and IM (89%). Some were also located by the χ^2 (67%) and by CIM (72%). All linkage groups with significant markers related to ROOT (4, 6, 7, 8 and 11) were identified by the selective genotyping strategy. The other two linkage groups (2 and 10) hosted significant markers related to MORT. No putative QTLs for SPR and STAB were detected in the *E. tereticornis* map.

For *E. globulus* (Table 4) 18 markers (in five linkage groups and one unlinked marker) displayed a significant ($P \leq 0.05$) deviation from the expected 1:1 allele frequency ratio in the selective genotyping experiment. From a random sample and SMA, ten *F*-tests (corresponding to markers in five linkage groups and one unlinked marker) were significant, indicating markers associated with trait expression ($P \leq 0.005$). Two significant markers in the χ^2 and SMA were not considered in the QTL-Cartographer analysis, as one (B324) was not a framework marker and the other (B296) was an unlinked marker. Seven significant markers were disclosed by IM (in two linkage groups, $P \leq 0.1$) and by CIM (in five linkage groups, $P \leq 0.1$). Overlapping significant markers (for ROOT96 and STAB) were found on linkage group 3. A total of nine markers were significant in at least two analyses and were assumed to be putatively associated with QTLs for ROOT, RCT, SPR and STAB in linkage groups 2, 3,

Table 4 Significant marker-trait associations in *E. globulus* detected by selective genotyping ($\chi 2$, $P \leq 0.05$, *0.005) and/or random genotyping (SMA, genome-wide $P \leq 0.005$, *0.0002; IM and CIM, genome-wide $P \leq 0.1$, *0.05, **0.025, ***0.01). In the table, the "/" means "and". The position of the nth marker in the linkage group (starting from the top) is indicated as LG(n), as in Marques et al. 1998

Marker	LG (n)	$\chi 2$	Single-marker analysis	Interval mapping	Composite interval mapping
A86r	2(2)	0.014	–	–	–
B77r	2(3)	0.007	–	–	–
B624	2(4)	0.003*	STAB	–	STAB
A95r	3(3)	0.047	–	–	–
B323	3(4)	0.005*	–	–	–
A205	3(5)	0.006	–	–	–
A535r	3(6)	0.031	–	–	–
B324	3(7)	0.005*	STAB, ROOT96*	Not considered	Not considered
A552	5(4)	–	–	ROOT96*	–
B583	5(5)	–	–	RCT95*, ROOT96**	ROOT96*
B355r	5(6)	0.002*	ROOT/RCT95, ROOT*/RCT96	RCT95*, ROOT96**	RCT95*, ROOT96*
A243r	5(7)	0.003*	ROOT/RCT95, ROOT*/RCT96	ROOT*/RCT***95, ROOT96**	ROOT*/RCT***95, ROOT96
B526	5(8)	0.002*	ROOT/RCT*95, ROOT/RCT96	ROOT**/RCT***95, ROOT*/RCT96	ROOT**/RCT***95
B9	5(9)	0.019	ROOT/RCT96	–	–
B587	6(1)	–	–	STAB*	STAB*
B100r	6(2)	–	STAB	STAB**	STAB**
A351	7(9)	0.037	–	–	–
B62	8(3)	0.037	ROOT/RCT95	–	–
B42'r	8(10)	0.036	–	–	–
A141	8(11)	0.015	–	–	–
A358	8(12)	0.006	–	–	SPR
B405cr	8(13)	–	SPR	–	–
B296	Unlinked	0.001*	STAB*	Not considered	Not considered

5, 6 and 8, and close to one unlinked marker (Table 5). Most of these markers were detected by SMA (90%) and χ^2 (80%). Some were also located by CIM (63%) and IM (50%). All linkage groups with significant markers related to ROOT (3, 5 and 8) were identified by the selective genotyping strategy. The other two linkage groups (2 and 6) and the unlinked marker hosted significant markers related to STAB and SPR. No putative QTLs for MORT and PETR were detected in the *E. globulus* map.

QTL stability

In *E. tereticornis* (Table 3) we detected three putative QTLs influencing MORT: two were found in both years and one solely in 1996. A total of six putative QTLs were detected for ROOT and/or RCT: one for ROOT/RCT in both years; one for ROOT/RCT95 and RCT96; one for ROOT95 and RCT96; one for ROOT95/96; one exclusively for ROOT95 and another only for RCT95. Four chromosomal regions influenced PETR: two were detected in both years and two were exclusive to either 1995 or 1996. In *E. globulus* (Table 5) three putative QTL influenced ROOT and/or RCT: one for ROOT/RCT in both years; one for ROOT/RCT95 and another for ROOT96 alone.

QTL effects

In *E. tereticornis* (Table 3) for all traits, individual putative QTLs explained on average 5.3% of the phenotypic variation ($2.8\% \leq R^2 \leq 17.0\%$) having an average effect of 0.23 σ_p ($0.15\ \sigma_p \leq$ effect $\leq 0.49\ \sigma_p$). On average, putative QTLs explained 16.3% of the phenotypic variation in MORT and ROOT/RCT, and 20.2% of the phenotypic variation in PETR. One putative QTL in linkage group 8 was alone responsible for 0.49 σ_p in MORT and PETR in 1996.

In *E. globulus* (Table) for all traits, individual putative QTLs explained on average 5.3% of the phenotypic variation ($2.6\% \leq R^2 \leq 8.1\%$) having an average effect of 0.22 σ_p ($0.13\ \sigma_p \leq$ effect $\leq 0.31\ \sigma_p$). On average, putative QTLs explained 9.6% of the phenotypic variation in ROOT/RCT, 3.9% of the phenotypic variation in SPR and 21.1% of the phenotypic variation in STAB.

QTL interactions

The presence of interactions between putative QTLs was investigated in two ways. Pairwise regression detected one significant interaction in *E. globulus* (markers B624-B100r for STAB) and two in *E. tereticornis* (markers B506-A63 for ROOT95 and A227-B293 for PETR96). Taking these interactions into account increased R^2 values by 6.3% (on average). Stepwise regression on all putative QTLs revealed an average of seven significant interaction terms per trait (at $P \leq 0.02$). Most of these occurred between markers (in the two species) that were not significant (for that trait) when considered individually. Taking these interactions into account increased R^2 values by 17.8% (on average).

Table 5 *E. globulus*: summary of the most stable marker-trait associations across the different analyses, assumed to be putatively associated with QTLs. The position of the nth marker in the linkage group (starting from the top) is indicated as LG(n), as in Marques et al. 1998. The first number in the table refers to the percentage of phenotypic variation explained by the marker (%R^2). **The second number is the effect of the putative QTLs expressed in phenotypic standard deviations (σ_p).** A positive σ_p value indicates that the favourable effect is associated with the band-present phenotype. A negative σ_p value indicates that the favourable effect is associated with the band-absent phenotype. The "xxxx" indicate alternative locations of the putative QTLs. Trait mean and variance refer to the 221 individuals genotyped

Marker	LG (n)	ROOT95	RCT95	ROOT96	RCT96	SPR	STAB
B624	2(4)	–	–	–	–	–	2.55 **0.24**
B324	3(7)	–	–	4.74 **–0.23**	–	–	3.00 **–0.24**
B355r	5(6)	xxxx	xxxx	7.70 **–0.31**	5.53 **–0.26**	–	–
A243r	5(7)	xxxx	xxxx	xxxx	xxxx	–	–
B526	5(8)	6.88 **0.22**	8.11 **0.23**	xxxx	xxxx	–	–
B100r	6(2)	–	–	–	–	–	6.25 **–0.26**
B62	8(3)	2.62 **–0.13**	4.19 **–0.17**	–	–	–	–
B405cr	8(13)	–	–	–	–	3.88 **0.26**	–
B296	Unlinked	–	–	–	–	–	7.90 **–0.27**
Overall %R^2	–	9.13	11.80	12.29	5.33	3.88	21.12
Trait mean	–	26.07	13.66	32.78	18.60	13.67	16.70
Trait variance	–	25.27	15.34	30.83	20.16	15.30	14.65
No. putative QTLs	–	2	2	2	1	1	4

Discussion

Genetic analysis of traits

The strong phenotypic correlation observed between ROOT-RCT and PETR-MORT within each year was not related to a majority of shared QTLs, unlike what Yadav et al. (1997) reported for root architecture QTLs in rice. For both species and years only 6 of 13 putative QTLs were shared for ROOT and RCT. The same trend occurred for PETR and MORT. Notwithstanding this, as reported in Verhaegen et al. (1997), correlated traits had QTLs in the same chromosomal locations. One notable example is marker B293 (*E. tereticornis*), significant for both MORT and PETR. The weak phenotypic correlation found for MORT95-MORT96 and PETR95-PETR96 was not related to a minority of shared QTLs. Four of seven putative QTLs (for both traits) were common between years. Weakly correlated traits had QTLs in the same chromosomal locations, with similar allelic effects. This was also reported by Grattapaglia et al. (1995). There was no straightforward relationship between the number of shared QTLs and the presence/absence of phenotypic correlations. Possible explanations for this are that a large proportion of the phenotypic variance is explained by QTLs that were not detected or else by the presence of other genetic effects (Wu et al. 1997).

Selective and random genotyping

We have used a selective and a random genotyping strategy in marker-QTL linkage determination for ROOT. Selective genotyping was very effective in targeting linkage groups with QTLs. This confirms theoretical predictions (Lander and Botstein 1989; Darvasi and Soller 1992; Muranty and Goffinet 1997) on the efficiency of selective genotyping, provided the size of the population phenotyped is adequate. In this work, the whole F_1 progeny set was phenotypically evaluated and approximately 10% of the individuals were selected at each extreme (Lander and Botstein 1989; Darvasi and Soller 1992). The major limitation of this approach is the inability to analyze different traits simultaneously. Lin and Ritland (1996) found that selective genotyping may decrease the power of mapping multiple linked QTLs. Yet, our ability to distinguish between effects caused by a single major QTL or by clusters of multiple QTLs with smaller effects is still restricted (Liu and Dekkers 1998).

Statistical analyses

Most statistical methods for QTL mapping were developed for inbred lines (Muranty 1996). Some strategies for mapping QTLs in outbred populations are available (reviewed in Hoeschele et al. 1997), but none is unbi-

ased. The precise location of QTLs is limited by the number of meioses studied, marker coverage, environmental effects, measurement error, segregation of other QTLs, interaction effects, experimental design, the magnitude of the type-I error allowed and the method of statistical analysis (Liu 1997). QTL analysis in our work was predicated on consensus-significant marker-trait associations with the χ^2, SMA, IM and CIM. The rationale was that results yielded by different approaches are more likely to be real and reproducible, although the statistical methodologies are not independent and equally powerful (Rebai et al. 1995).

Results were similar across different analytical tests for QTL detection. The chi-square test and regression analysis are useful for initial data exploration and the verification of results obtained with other methodologies (Kearsey and Farquhar 1998). Despite being more robust to violations of normality, these methods cannot extract all the information in the data (Liu 1997). Interval mapping allows asymptotically unbiased estimates of QTL location and effect, if the assumption that there is only one QTL on a chromosome is true (Lander and Botstein 1989). Composite interval mapping improves the precision of mapping multiple QTLs (Jansen 1993; Zeng 1994). It has been shown that least-squares and maximum likelihood are very similar in terms of power and the estimation of QTL effects (Haley and Knott 1992) despite some bias in the estimation of residual variance (Xu 1995). A total of 84% significant marker-trait associations in this work were detected simultaneously by SMA and maximum likelihood methods. A similarity between regression and IM results in QTL mapping has also been previously reported (Grattapaglia et al. 1995).

Genetic architecture of vegetative propagation traits in *Eucalyptus*

QTL discovery

Putative QTLs were found in a family of reasonable size where the adventitious rooting response of the parents was unknown, and was later found to be modestly expressed. This indicates that the observed phenotypic variation has a meaningful genetic component. Different QTL loci were detected for MORT, ROOT, RCT and STAB. This does not exclude the possible existence of clusters of QTLs or QTLs with pleiotropic effects in common loci. For example, all QTLs influencing PETR were also involved with MORT, ROOT and/or RCT. Borralho and Wilson (1994) reported that petrification and rooting of *E. globulus* stem cuttings should be assessed separately. We discovered both common and independent loci influencing these traits. The existence of three unique QTLs influencing STAB is also very interesting. It hints at the influence of other factors (hypothetically physiological) in the stable expression of the adventitious rooting response. Spurious linkages between markers in both maps associated to QTLs are unlikely, as the putative QTLs reported involve several nearby markers. We were not able to determine whether one or more QTLs were present in some chromosomal areas. The QTL positions reported here should be considered tentative (within each linkage group).

QTL stability

In both maps, some putative QTLs were detected in successive years for MORT, ROOT, RCT and PETR. Others were year or index (ROOT/RCT) specific. Few studies address the issue of QTL stability in different years or environments. Studies done in a particular environment are likely to underestimate the number of QTLs that influence a trait (Paterson et al. 1991). Our results are in agreement with evidence presented by Tibbits et al. (1997) suggesting that *Eucalyptus* spp. can be screened for adventitious rooting at any time of the year. A majority of stable putative QTLs influenced MORT and ROOT/RCT. Half of the putative QTLs for PETR were detected in both years.

QTL effects

The quantitative value of alternative marker genotypes was measured as the effect of one allelic substitution averaged over potentially two alternative alleles inherited from the other parent. Intralocus interactions (e.g. dominance) may be present and cannot be accounted for (Gratappaglia et al. 1995). Therefore, estimates of individual QTL effects should be considered indicative (Yadav et al. 1997). QTLs explaining small portions of the phenotypic variance far out-number those explaining larger portions, in this experiment. The smallest individual effect detected was 2.6%. The largest was 17.0%. Knapp et al. (1992) observed that estimates of R^2 obtained from non-simultaneous single-locus models can be significantly inflated by sampling bias. In our data the arithmetic sum of the individual R^2 effects was generally not larger than the multilocus estimates. Furthermore, estimates of R^2 (explained by the joint action of all putative QTLs mapped) obtained by linear regression are generally smaller than those obtained by interval mapping (Grattapaglia et al. 1995; Plomion et al. 1996). The simultaneous multilocus estimates of the total proportion of phenotypic variation detected here (3.9–24.4%) are similar to those reported in Grattapaglia et al. 1995 (4.2–32.2%).

Epistasis

Only minor evidence of epistasis was found between unlinked QTLs influencing the same trait. This observation is common in the literature (Grattapaglia et al. 1996; Plomion et al. 1996; Byrne et al. 1997). The power to detect epistatic effects is limited in view of the large

number of potential pairwise interactions and the relatively small sampling of any particular combination of genotypes at a pair of loci (Paterson et al. 1991). In contrast to this expectation, a lot of interactions were revealed between significant markers for different traits, comparable in magnitude to the main effects. These results suggest the presence of significant epistasis between regions of different main effects. It is also possible that regions without significant main effects could interact with other regions (Groover et al. 1994). Epistasis should be expected, given that phenotypes are the result of interactive and interrelated metabolic and ontogenic pathways (Lee 1995).

Vegetative propagation traits

The mechanisms by which adventitious roots are formed are not well understood but have been associated with rooting inhibitors (Paton et al. 1970), peroxidases (Phytoud and Buchala 1989), rooting co-factors (Wilson and Staden 1990), phenolic substances (Curir et al. 1990), growth regulators (Liu and Reid 1992), polyamines (Tepfer et al. 1994) and thiamine (Chee 1995), among others. The large array of physiological and biochemical processes involved suggests an underlying complexity in adventitious root formation. Grattapaglia et al. (1995) identified six QTLs for SPR and four for RCT in a full-sib cross between *E. grandis* and *E. urophylla*. Most of the inherited phenotypic variation in SPR (13.5%) was attributed to *E. grandis*, and in RCT (28.5%) to *E. urophylla*. In our experiment, *E. tereticornis* was responsible for the explained average phenotypic variance in MORT (16.3%) and PETR (20.2%), and *E. globulus* for the variance in SPR (3.88%) and STAB (21.12%). On average, more adventitious rooting phenotypic variation was contributed by *E. tereticornis* (16.3%) relative to *E. globulus* (9.7%). The success of cloning depends on high adventitious rooting and low mortality and petrification. The ability to dissect the genetic components of vegetative propagation traits is important to clarify the way they should be incorporated in selection strategies.

Marker assisted selection (MAS) in *Eucalyptus*

There are many opportunities for the integration of MAS in forestry. As in *Populus*, QTL maps could help the choosing of long-term strategies in *Eucalyptus* breeding (Bradshaw 1996). Also, marker-based estimates of genetic affinity could conduct the program for improvement at the within-provenance level versus wide crossing and interspecific hybridization (Williams 1995). Marker information could also facilitate the development of selection indexes (Fernando and Grossman 1989). Moreover, complex trait dissection could be carried out for parents with high breeding values or in pedigrees where biometrical analysis detected the segregation of major genes. Markers could also minimize linkage drag during the introgression of QTLs by backcrossing (Kearsey and Farquhar 1998) and help break unfavorable correlations between quantitative characters of interest (Verhaegen et al. 1997). Lack of precise knowledge on the location of a QTL, the magnitude of its effects and their biological significance for tree growth and development is one of the major limiting factors to the application of MAS in tree breeding (O'Malley and McKeand 1994; Hospital et al. 1997; Moreau et al. 1998).

It is likely that some QTLs are important across environments while others vary in different environments (Groover et al. 1994, Verhaegen et al. 1997). The assessment of QTLs across genetic backgrounds is also important for elucidation of the distribution of genetic variation at the population level. The consistent QTLs would be most useful in MAS and very interesting candidates for positional cloning. We are currently validating the QTLs detected, in an independent set of the same F_1 progeny. We will investigate synteny in eucalypts using AFLP loci (Marques et al. 1998) and microsatellite markers (Brondani et al. 1998) for which both parents are heterozygous. There is growing evidence of corresponding chromosome regions carrying similar QTLs in different species (Verhaegen and Plomion 1996; Kearsey and Farquhar 1998). The progressive accumulation of individual linkage maps with subsets of common markers among them will help to clarify the relationships of QTLs in different species. The existence of generalized genomic regions associated with trait expression could be verified, and the hypothesis of heterogeneity of QTLs across populations tested. This information would be very useful for the application of MAS.

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