C. Weng · T. L. Kubisiak · C. D. Nelson · M. Stine

Mapping quantitative trait loci controlling early growth in a [longleaf pine x slash pine) x slash pine BC₁ family

Abstract Random amplified polymorphic DNA (RAPD) markers were employed to map the genome and quantitative trait loci controlling the early growth of a pine hybrid F, tree (*Pinus palustris* Mill. *x I? elliottii* Engl.) and a recurrent slash pine tree (*P. elliottii* Engl.) in a (longleaf pine x slash pine) x slash pine BC, family consisting of 258 progeny. Of the 150 hybrid F_1 parent-specific RAPD markers, 133 were mapped into 17 linkage groups covering a genetic distance of 1,338.2 cM. Of the 116 slash pine parent-specific RAPD markers, 83 were mapped into 19 linkage groups covering a genetic distance of 994.6 cM. A total of 11 different marker intervals were found to be significantly associated with 13 of the 20 traits on height and diameter growth using MAPMAKER/QTL. Nine of the eleven marker intervals were unique to the hybrid parent 488 genome, and two were unique to the recurrent parent 18-27 genome. The amount of phenotypic variance explained by the putative QTLs ranged from 3.6% to 11.0%. Different QTLs were detected at different ages. Two marker intervals from the hybrid parent 488 were found to have QTL by environment interactions.

Keywords *Pinus palustris* . *Pinus elliottii* . QTLs . Random amplified polymorphic DNAs . Early height growth

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Introduction

Longleaf pine (*Pinus palustris* Mill.) has many desirable characters, such as good wood quality, fusiform rust resistance and southern pine bark beetle resistance. However, it has a delay in early height growth (EHG) known as the "grass stage." The grass stage has been an important factor limiting the artificial regeneration of longleaf pine (Schmidtling and White 1989). Efforts to genetically improve the EHG of longleaf pine through the introgression of genes controlling EHG from either loblolly pine or slash pine began in the 1960s. Brown (1964) and Derr (1966, 1969) made crosses between longleaf pine and loblolly pine or slash pine. C.D. Nelson (unpublished data) made crosses between longleaf pine and slash pine in 1990 and backcrosses in 1995. No "grass stage" was observed in progeny from both crosses, and great variation of height growth was observed among the progeny. Previous studies also revealed that EHG is a quantitative trait controlled by a small number of major effect genes (Brown 1964, Nelson unpublished data) and has a heritability ranging from 0.47 to 0.68 (Snyder and Namkoong 1978; Layton and Goddard 1982). On the basis of these studies, a more efficient approach to genetically improve EHG may be to map these gene loci with molecular markers and then use the markers that are tightly linked to these loci for marker-assisted selection.

While mapping quantitative trait loci (QTLs) controlling the EHG in a (longleaf pine x slash pine) × longleaf pine BC, family is the more direct way to address the issue of introgression of EHG genes into longleaf pine, mapping them in a (longleaf pine × slash pine) × slash pine BC, family may be more efficient for identifying QTLs originating from the longleaf pine grandparent. We expected to find that the EHG trait of BC_1 individuals is regulated by positive-effect QTLs mainly from slash pine and/or negative-effect genes mainly from longleaf pine and that the homology level between longleaf pine and slash pine is lower than within slash pine species. The negative-effect genes in the F_1 parent that come from the longleaf pine grandparent are less likely to be

shared by the slash pine parent. Consequently, we expected more genes would be detected in the F_1 parent than in the recurrent parent and that these identified genes would be more likely to be from the longleaf pine grandparent than from the slash pine grandparent. In contrast, if a (longleaf pine × slash pine) x longleaf pine BC, family is used, the genes identified in the F_1 would be more likely to be from the slash pine grandparent. The negative-effect genes identified in a (longleaf pine × slash pine) x slash pine BC, family would provide some extra information that cannot be obtained using a (longleaf pine x slash pine) × longleaf pine BC, family. With this information, we may be able to avoid the EHG QTLs with negative effect while introgressing the ones with positive effect by means of marker-assisted selection in breeding longleaf pine with improved EHG.

Random amplified polymorphic DNAs (RAPDs) markers are attractive markers for genome mapping, QTL mapping, map-based cloning, and analysis of genetic variation for several reasons. RAPDs are polymerase chain reaction (PCR)-based DNA markers. PCR-based markers can be used to map repetitive regions of a genome as efficiently as they can map the gene-rich regions (Monna et al. 1994). This property is one of the advantages that PCR-based markers have over cDNA-based restriction fragment length polymorphism (RFLP) markers (Gill et al. 1996a, b). This is particularly significant in mapping genomes of organisms that have large genomes where repetitive DNAs comprise a large proportion of the genome (Miksche and Hotta 1973; Rake et al. 1980; Kriebel 1985). The genome size of *Pinus* species has been estimated to be between 33 and 57 pg (equivalent to about 3×10^{10} bp perihaploid genome) (Ohri and Khoshoo 1986; Walkamiya et al. 1993; Plomion et al. 1995), which is relatively large compared to most other organisms. Besides the properties that are shared with other PCR-based DNA markers, RAPD analysis is fast and simple and uses trace amounts of DNA template (Welsch and McClelland 1990; Williams et al. 1990). RAPDs have been the most frequently used molecular markers for mapping pines, including maritime pine (Plomion et al. 1995), sugar pine (Devey et al. 1995), longleaf pine (Nelson et al. 1993; Kubisiak et al. 1995), slash pine (Nelson et al. 1994; Kubisiak et al. 1995) and radiata pine (Emebiri et al. 1998).

In this paper, we present the RAPD genetic linkage maps of a longleaf pine x slash pine F_1 tree and slash pine tree 18-27 using a BC, mapping population and detect QTLs using a single marker-based SAS procedure, analysis of variance (ANOVA), and the marker interval-based computer program, MAPMAKER/QTL.

Materials and methods

Mapping population

Progeny from a (longleaf pine x slash pine) x slash pine BC_1 family were used for genome mapping and QTL mapping. The hybrid parent (tree 488, F,, ♀) was developed by Derr (1966) by crossing a longleaf pine (tree 8R, ♀) with a slash pine (tree 5 1, Or), and the recurrent parent 18-27 (d) is a superior selection originally chosen for growth rate, form, and disease tolerance. Seeds of this backcross were germinated in June of 1996 and grown in containers (10 cubic inches of rooting volume) in a greenhouse. During this period all seedlings were protected from diseases (primarily damping-off and brown spot needle blight) and given uniform growing conditions. In January of 1997, the seedlings were planted at three different field locations [southwest Georgia (GA; 92 seedlings), east-central Louisiana (LA; 92 seedlings) and southeast Mississippi (MS; 101 seedlings)] using a completely randomized design with a between-tree spacing of 10 feet × 10 feet. The overall survival rate was 90.5%, leaving a total of 258 seedlings for linkage mapping and QTL searching (82 at GA site, 83 at LA site and 93 at MS site)

Field data

As of November 1999, a total of eight measurements had been taken on the BC, family. These measurements included total height (h1, h2, h3 'and h4) from ground level to bud tip and stem diameter (d1, d2, d3 and d4) at 3-5 cm above the ground on the 7th (in the greenhouse), 16th, 29th, and 41st month (in the field). Measurements were made in January 1997 (prior to out-planting from the greenhouse), October 1997, November 1998, and November 1999. Changes in height and diameter between these measurement dates were also calculated. In total, there were 20 traits: height at the 7th month (h1), 16th month (h2), 29th month (h3), 41st month (h4); height increments from the 7th to the 16th month (Ah 12), 7th to the 29th month (Ah 13), 7th to the 4 1 st month (Δh14), 16th to the 29th month (Δh23), 16th to the 41st month (Δh24), 29th to the 41st month (Ah34); collar diameter at the 7th month (d1), 16th month (d2), 29th month (d3), 41st month (d4); diameter increments from the 7th to the 16th month (Δd12), 7th to the 29th month (Δd13), 7th to the 41st month (Δd14), 16th to the 29th month (Δd23), 16th to the 41st month (Δd24), 29th to the 41st month (Ad34). For the marker-QTL analysis all traits except h1 and d1 were standardized for location effects as follows: $Y\_hat_{ij} = (Y_{ij} - Y_i)/S_{Yi}$, where Y_{ij} is the actual trait measurement taken on the jth individual planted at site i; Y_i is the trait mean for site i; S_{Yi} is the standard deviation of the trait for planting site i. Each of the 20 traits was tested for normality using the Wilk-Shapiro test. These tests suggested that four-of the traits (d2, Ad24, Ad34 and Ah34) were not normally distributed. As transformations using log, square root, square and inverse could not obtain normality for any of the measures, these measures were analysed based on a non-normal distribution.
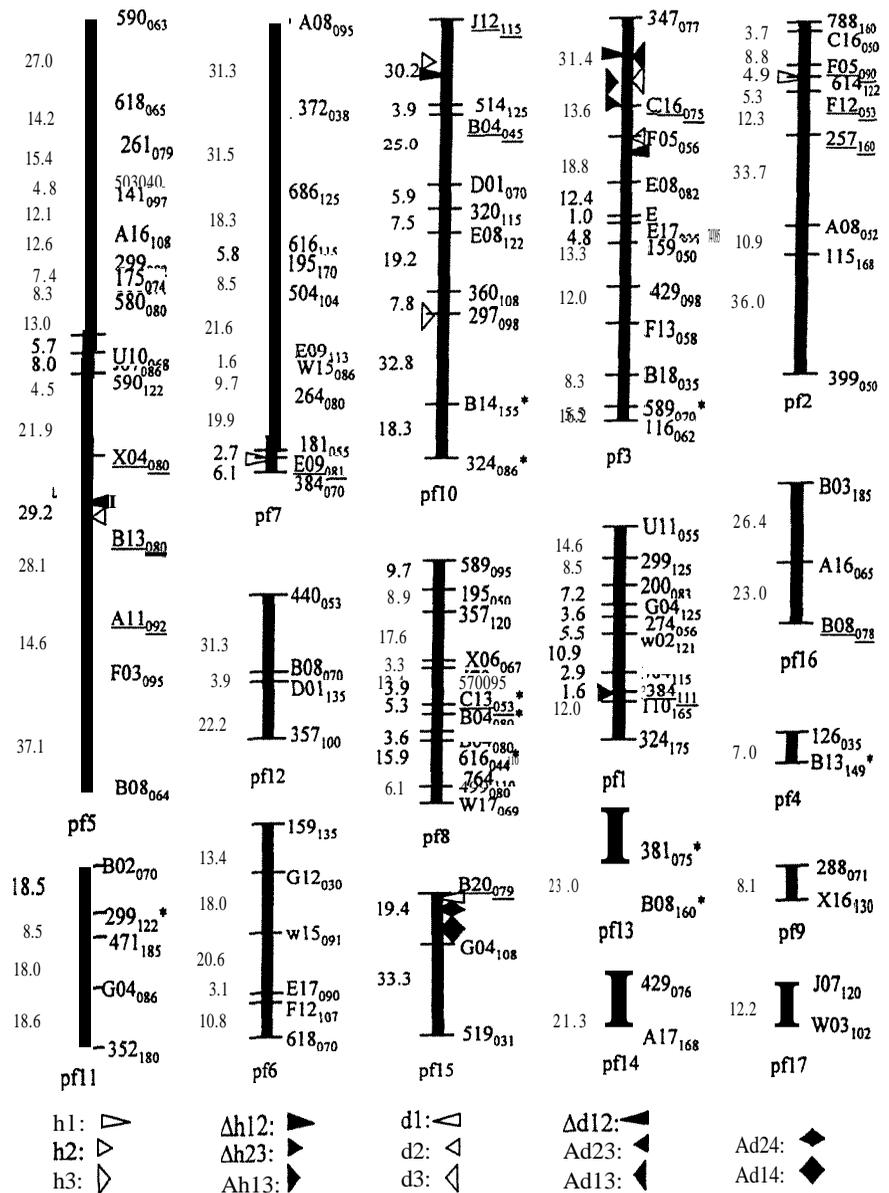
DNA isolation and purification

Total DNA was extracted from 1.0 g leaf samples of individual trees using a CTAB procedure (Murray and Thompson 1980). The DNA samples were purified further using Prep-A-Gene (Bio-Rad, Richmond, Calf.). The DNA samples were diluted to a working concentration of approximately 20 ng/μl with low TE (10 mM Tris:0.1 mM EDTA).

RAPD analysis

Decamer primers were purchased from either Operon Technologies (Alameda, Calif.) or J. Hobbs (University of British Columbia, Vancouver, B.C., Canada). Primers were selected either randomly or because they had previously identified polymorphic RAPD markers in longleaf pine or slash pine. To identify useful polymorphisms, we screened primers against the F_1 parent, recurrent parent and six BC, progeny. Primers that amplified testcross loci (segregating in 1:1) were further characterised on the entire mapping population ($n=258$). Presence of a band was scored as an 'H' (heterozygous), while absence of a band was scored as 'A' (homozygous band absent). Those cases in which a reaction

Fig. 1 Linkage maps for the hybrid parent 488. The *last digit* of all markers was truncated. Markers with *an asterisk* (*) were distorted from a 1: 1 segregation ratio ($P<0.01$). *Underfined* markers were associated with QTLs detected using ANOVA. Loci marked with *arrows* represent the most likely locations of QTLs using MAPMAKER/QTL

Legend:
h1: ▷ (open right arrow, h1)
h2: ▷
h3: ▷
Δh12: ▶ Δh23: ▶ Ah13: ▶
d1: ◁ d2: ◁ d3: ◁
Δd12: ◀ Ad23: ◀ Ad13: ◀
Ad24: ◆ Ad14: ◆

Linkage groups: pf5, pf11, pf7, pf12, pf6, pf10, pf8, pf15, pf3, pf1, pf13, pf14, pf2, pf16, pf4, pf9, pf17

completely failed or when the presence or absence of bands was unclear, were recorded as missing data. RAPDs were named by the manufacturer primer code corresponding to the primer responsible for their amplification, followed by a three-digit number indicating the approximate fragment size in base pairs, with the last digit being the tens position digit of fragment size in base pairs (the ones position digit was truncated).

Linkage analysis

The RAPD data were divided into two subsets; one consisting of all the markers heterozygous in the hybrid parent 488, and a second consisting of all the markers heterozygous in the recurrent parent 18-27; each subset was analysed separately. The marker data were entered into the computer package MAPMAKER/EXP 3.0 (Lander et al. 1987) and analysed using a modified backcross format (Nelson et al. 1993). Each marker was tested for goodness of fit to its expected Mendelian inheritance ratio using chi-square (χ^2) analysis ($P<0.01$). Linkage groups were established using a minimum log of odds (LOD) threshold of 4.0 and maximum dis-

tance of 40 cM. Those loci that appeared to be experiencing segregation distortion were first excluded from linkage analyses and were placed in their most likely positions after the non-distorted markers were ordered. Linkage groups were assigned a two-letter name followed by a number. The letter designation 'pf' indicates a hybrid parent-specific linkage group, and 'pe' indicates a recurrent parent-specific linkage group.

QTL analysis

Two different methods were employed to investigate the degree of association between the marker loci and each of the 20 traits. The first method was single marker-based SAS ANOVA in which the individual marker genotypes were used as class variables. An association between a marker and trait was considered significant if the Type-I error rate was lessthan 0.005. The interaction between marker and planting site was considered significant if the Type-I error rate was lessthan 0.005. The proportion of the phenotypic variance explained by segregation of the marker was determined by the R-square (R^2) value. The second method utilised the

Fig. 2 Linkage maps for the slash pine parent 18-27. The *last* digit of all markers was truncated. Markers with an *asterisk* (*) were distorted from 1: 1 segregation ratio (*P<0.0* I). *Underlined* markers were associated with QTLs detected using ANOVA. Loci marked with arrows represent the most likely locations of QTLs using MAPMAKER/QTL
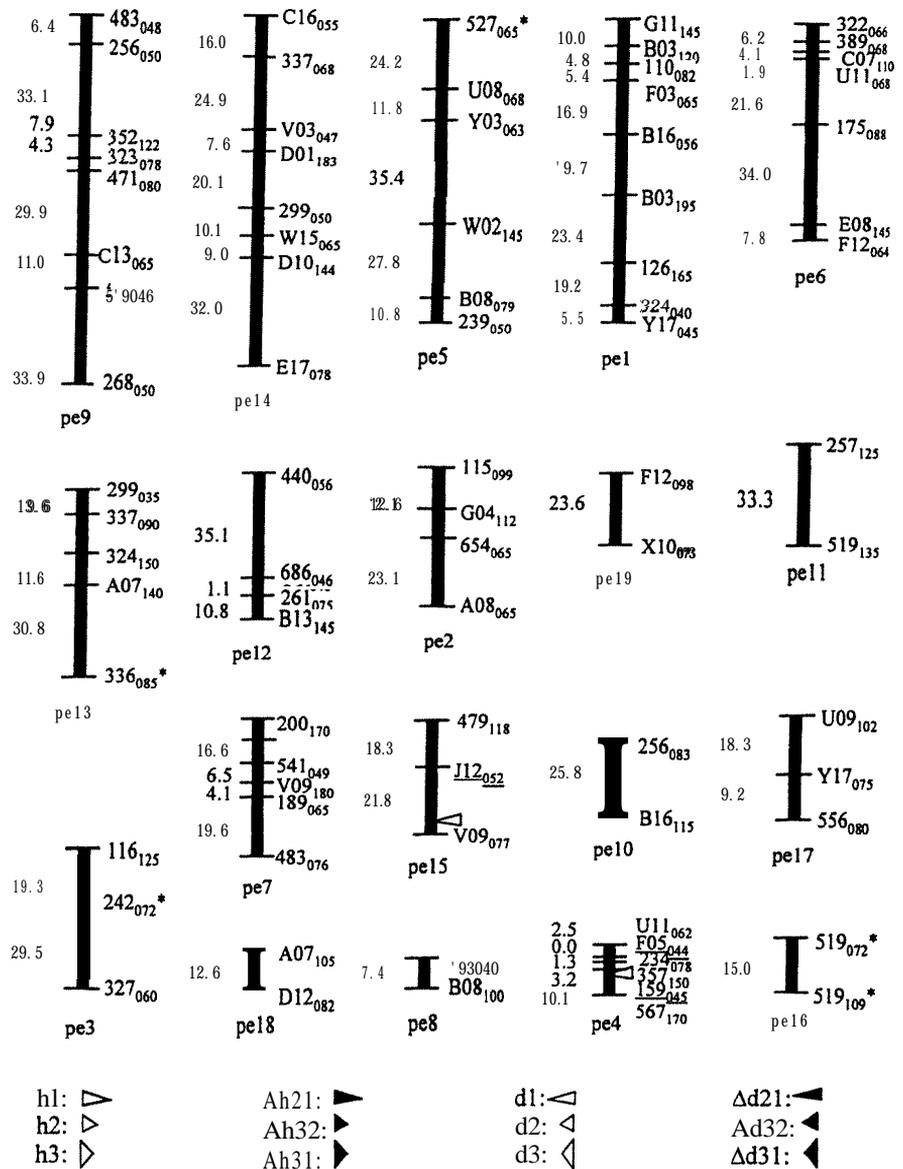
interval mapping approach available in the software package MAPMAKER/QTL 1.0 (Lander et al. 1987). Although there are no statistical inferences available to directly test for marker interval by planting site interaction in MAPMAKER/QTL 1 .O, the data for each planting site were analysed separately and the difference in LOD scores between sites used as an indicator of interaction. An association between a marker interval and trait was considered significant if the LOD score observed was greater than 2.0. The interaction between a marker interval and planting site was considered significant if the LOD score difference between any two sites was greater than 2.0.

Results

Linkage mapping

A total of 266 RAPD markers (150 heterozygous in the hybrid parent 488 and 116 heterozygous in recurrent parent 18-27) were scored on the BC, population. Chisquare tests suggested that 215 (80.8%) of these markers (119 heterozygous in the hybrid parent 488 and 96 heterozygous in recurrent parent 18-27) segregate at a ratio of 1: 1, while the remaining 51 (19.2%) (31 heterozygous in the hybrid parent 488 and 20 heterozygous in recurrent parent 18-27) are distorted from the 1: 1 ratio.

Based on two-point analyses, 113 of the 150 hybrid parent 488-specific marker loci were grouped into 17 groups (5 with two loci, 12 with three or more loci). These markers covered a genetic distance of 1,338.2 cM (Fig. 1). Genome size in pine has been estimated to be approximately 2,300–2,400 cM (Plomion et al. 1995; Echt and Nelson 1997) using the method described by Hulbert et al. (1988). Assuming that each of the 23 unlinked markers accounts for 20 cM and that each of the 34 ends of our 17 groups cover 10 cM, the total map coverage is estimated at 2,138.2 cM or 9 1 .0% of the pine genome.

Table 1 RAPD markers significantly associated with the inheritance of various growth measurements in a (longleaf pine x slash pine) x slash pine BC_1 family based on single-marker ANOVA models (Pr>F=0.005). (LG linkage group . UL unlinked marker)

Marker[a]	Parent[b]	LG	Trait[c]	df	F value	Pr>F[d]	R2[e]	Δ[df]
159045	1827	pe4	d1	252	10.36	0.0015	0.0403	-0.4056
257160	488	pf2	h1	250	9.11	0.0028	0.0352	0.3818
384080	488	UL	h3	252	8.45	0.0040	0.0328	0.3662
384111	488	Pfl	Ah23	239	8.28	0.0044	0.0340	-0.372 l
503037	18_27	UL	d2	158	9.12	0.0030	0.0552	0.4805
618060	1827	UL	d1	83	12.18	0.0008	0.1423	0.7661
A11092	488	Pf5	d2	160	10.40	0.0015	0.0618	-0.5100
B04045	488	pf10	h2	234	11.39	0.0009	0.0472	-0.4411
			Δh12	234	10.04	0.0017	0.0418	-0.4143
B08078	488	pf16	d4	166	8.40	0.0043	0.0482	0.4498
			Ad14	166	8.46	0.004 1	0.0485	0.4516
			Ad24	1.54	8.73	0.0036	0.0537	0.4765
B13080	488	Pf5	d2	158	11.68	0.0008	0.0697	0.5438
			Δd12	157	10.15	0.0017	0.056 1	0.5086
B20079	488	pf15	d1	250	8.78	0.0033	0.0386	-0.3749
			Δd14	244	8.83	0.0033	0.0349	-0.3805
			Ad24	152	9.99	0.0019	0.0617	-0.5127
			Ad34	244	8.27	0.0044	0.0328	-0.3682
C13053	488	pf8	d4	246	8.20	0.0046	0.0322	0.3652
C16075	488	pf3	h3	251	9.91	0.0018	0.0384	0.3974
			Δh13	2.51	8.98	0.0030	0.0352	0.3782
			Ah23	238	8.71	0.0035	0.0359	0.3826
			d3	2.51	9.89	0.0019	0.0386	-0.3970
			Δd13	251	9.32	0.0025	0.0365	-0.3855
E09081	488	pf7	h1	2.50	16.54	0.000 1	0.0620	0.5144
F05044	18_27	pe4	d1	253	9.04	0.0029	0.0333	-0.378 1
F05090	488	pf2	h1	2.51	12.30	0.0005	0.0467	-0.4428
F12053	488	pf2	hl	252	10.86	0.0011	0.0413	0.4152
G04108	488	pf15	Ad34	239	8.02	0.0050	0.0325	-0.3664
J12052	18_27	pe15	d1	253	8.32	0.0043	0.0297	-0.3626
J12115	488	pf10	h2	237	14.22	0.0002	0.0568	0.4897
			Δh12	240	10.08	0.0017	0.0410	0.4098
			Ad14	247	8.14	0.0047	0.0319	0.3630
W03102	488	pf17	Ad34	80	9.17	0.0033	0.1028	-0.6773
X04050	488	UL	Δh14	80	8.74	0.0041	0.0985	-0.6611
			Ah34	80	10.80	0.0015	0.1189	-0.7348
X04080	488	pf5	h1	251	10.85	0.0011	0.0415	0.4158

[a] Marker locus
[b] Informative parent
[c] Growth metric
[d] Probability of a larger F value
[e] R-square or the proportion of the phenotypic data explained by the marker locus
[f] Difference between the QTL allele and the population mean expressed in phenotypic standard deviations

Of the 116 recurrent parent 18–27-specific markers 87 were grouped into 19 groups, covering a total genetic distance of 994.6 cM (Fig. 2). Adjusting for the 29 unlinked loci and the 38 ends of the 19 linkage groups, the total map coverage is estimated at 1,954.6 cM or 8 1.1% of the pine genome.

Detecting QTLs using the single marker method

A total of 23 different markers were found to be significantly associated with QTLs for 17 of the 20 growth traits using single marker regression (Table 1). No markers were found to be significantly associated with diameter increments from the 16th to the 29th month (Ad23) and from 4 Ith to 16th month (Δd24), and total height at 4 lth month (h4). Of the 23 significant associations, 18 were unique to the hybrid parent 488 genome, and five were unique to the recurrent parent 18-27 genome. Of the 18 hybrid parent 488-specific markers, nine were associated only with height growth, seven were associated only with diameter growth, with two being associated with both height and diameter growth. Two markers were significantly associated with growth metrics at more than

one age (Table 1). Of these 18 markers, 16 were located on ten different hybrid parent 488-specific linkage groups (Fig. 1) and two were unlinked with any other markers. The amount of variation explained by the various QTLs ranged from 3.19% (0.36300) to 11.89% (0.7348 σ) of the total phenotypic variance. All five markers unique to recurrent parent 18-27 were associated with diameter growth measurements. Three of these were located on two different linkage groups (Fig. 2), while two markers were unlinked. The famount of variation explained by these five QTLs ranged from 2.97% (0.36260) to 14.23% (0.76610) of the total phenotypic variance.

Detecting QTLs using MAPMAKER/QTL

A total of 11 different marker intervals were found to be significantly associated with 13 of the 20 growth traits using the interval mapping method of MAPMAKER/QTL (Table 2). Nine of the eleven marker intervals were unique to the hybrid parent 488 genome, and two were unique to the recurrent parent 18-27 genome. Of the nine hybrid parent 488 marker intervals, five were

Table 2 RAPD marker intervals significantly associated with the inheritance of various growth measurements in a (longleaf pine x slash pine) x slash pine BC_1 family based on the interval mapping method (LOD>2.0) (*LG* linkage group)

Interval[a]	Parent[b]	LG	Trait	LOD	Variance explained[c] (%)	Substitution effect
159_{045}–567_{170}	18_27	pe4	h3	2.37	4.1	Positive
297_{098}–$B14_{155}$	488	pf10		2.01	3.6	Negative
347_{077}–$C16_{075}$	488	pf3		2.05	5.0	Negative
			$\Delta h12$	2.18	4.3	Negative
			$\Delta h23$	2.02	4.5	Negative
			$\Delta h13$	2.54	6.1	Negative
			d3	2.54	6.1	Negative
			Ad13	2.76	5.1	Negative
384_{111}–110_{165}	488	Pfl	$\Delta h23$	2.76	5.1	Positive
$B04_{045}$–$J12_{115}$	488	pf10	$\Delta h12$	3.29	8.5	Negative
$B20_{079}$–$G04_{108}$	488	pf15	d1	4.34	14.0	Negative Negative
			Ad14	2.07	4.2	Negative
			Ad24	2.38	5.9	Negative
$E09_{081}$–264_{080}	488	pf7	h1	3.40	6.3	Negative
$F05_{056}$–$E08_{082}$	488	pf3	d2	2.20	...	Negative
			Ad12	2.40	6.9	Negative
$F05_{090}$–$F12_{053}$	488	pf2	h1	2.80	5.4	Positive
$J12_{052}$–$V09_{077}$	18_27	pe15	h1	2.01	5.7	Negative
$X04_{080}$–$B13_{080}$	488	pf5	d2	2.35	4.8	Positive
				2.71	10.0	Negative
			Ad12	2.74	11.0	Negative

[a] Marker interval
[b] Informative parent
[c] Percentage of the phenotypic data explained by the marker interval

Table 3 Markers that were associated with QTL by environmental interaction using simple regression and a Type-I error rate of 0.005. See Table 1 for definitions

Marker	Parent	LG	Trait	F value	Pr>F	R^2
590_{063}*site	488	pf5	d2	10.67	0.0013	0.0640
618_{065}*site	488	pf5	d2	8.15	0.0049	0.0497
590_{063}*site	488	Pf5	Ad12	9.63	0.0023	0.058 1

Table 4 Intervals that were associated with QTLs by environmental interactions

Interval	LG	Trait	LOD			Combined LOD
			GA site	LA site	MS site	
$X04_{080}$–$B13_{080}$	pf5	d2	–	0.49	2.57	3.18
347_{077}–$C16_{075}$	pf3	Ad12	–	2.70	0.24	1.74
$X04_{080}$–$B13_{080}$	pf5	Ad12	–	0.47	2.77	3.18

associated only with height growth, two were associated only with diameter growth, and two were associated with both height and diameter growth. Two of the marker intervals were significantly associated with growth metrics at more than one age (Table 2). These nine marker intervals were located on seven different hybrid parent-specific linkage groups (Fig. 1). The amount of variation explained by the various QTLs ranged from 3.60% to 11.0% of the total phenotypic variance. The two marker intervals in the recurrent parent 18-27 genome were only associated with diameter growth at one age (Table 2). These two intervals were located on two different linkage groups (Fig. 2). These QTLs explained 4.1% and 5.7% of the total phenotypic variance, respectively.

QTL by environment interactions

Only two QTL by planting site interactions were found to be significant – marker 590_{1175} associated with height growth at the 7th month (hl) and marker 347_{0780} associated with the change in diameter growth from the 7th to the 19th month. Both markers were from the hybrid parent 488 (Table 3) and were located in linkage group pf.5. Two intervals, 347_{077}–$C16_{075}$ and $X04_{080}$–$B13_{080}$, both from the hybrid parent 488, showed interaction effects. Interval 347_{077}–$C16_{075}$ was from linkage group pf3 and interval $X04_{080}$–$B13_{080}$ was from linkage group pf5 (Table 4).

Discussion

Twenty-three markers were found to be associated with 17 of the 20 traits using ANOVA, with variance explained by associated QTLs ranging from 2.97% (0.36260) to 14.23% (0.76610) of the total phenotypic variance. Twenty-one QTLs influencing 13 of the 20 traits were located in 11 different marker intervals using MAPMAKER/QTL, with the variance, explained by associated QTLs, ranging from 3.60% to 11 .0% of the total phenotypic variance. The results showed a trend: the amount of variance explained jointly by all major-effect QTLs influencing the same measurement became smaller when trees became older. At the 7th month, the amount of variance explained jointly by all QTLs was 14.5% and 12.1% for hl and dl , respectively, when analysed using multiple regression, while no major QTLs were detected for Ah34 and Ad34. Two markers were found to associate with interactions between three QTLs and their environments using ANOVA. Two intervals showed

QTL by enviromnent interaction using MAPMAKER/QTL. No marker was found to associate with QTLs that have significant effects on all the four ages.

Markers detected to be associated with the traits using MAPMAKER/QTL were similar to those detected using ANOVA, and the two methods could be used complementarily. Of the 19 linked markers associated with QTLs detected using ANOVA, 13 were found in the intervals associated with QTLs detected using MAPMAKER/QTL, and three markers, 257_{160}, Al 1_{092} and F05$_{044}$, were found to link (at genetic distance of 12.3, 28.1, and 4.5 cM, respectively) with intervals associated with QTLs detected using MAPMAKER/QTL, which suggested that both methods are feasible for QTL detection. ANOVA and MAPMAKER/QTL could be used complementarily. First, except for the case of marker $E09_{081}$ versus interval $E09_{081}$–264_{080}, the QTL effects estimated by MAPMAKER/QTL were always greater than those estimated by ANOVA. On average, the percentage variance explained by each interval using MAPMAKER/QTL was 0.02 l higher than the highest R-square value partitioned by the corresponding marker in a linkage group using ANOVA. One explanation could be that misclassification of QTL genotypes occurs whenever there is a crossover between the QTL and the marker (Weng et al. 1999) and that this misclassification will result in smaller mean difference and lead to a decrease in R-square. This may imply that MAPMAKER/QTL is more powerful than ANOVA. Second, unlinked markers that cannot be used by MAPMAKER/QTL can be analysed using ANOVA because no marker linkage information is needed for ANOVA to detect marker-QTL association. Four QTLs were found to be associated with four markers unlinked to any other markers. The marker 618_{060}, one of these four, explained 14.23% of the total phenotypic variance of trait dl .

In terms of amount of variance explained by the QTLs, the results were comparable to results published previously for some other pine species. The three QTLs associated with hl in MAPMAKER/QTL analysis explained 14.5% of the total phenotypic variance of hl, and the three QTLs associated with dl explained 12.1% of the total phenotypic variance of dl. Compared to results from some well-studied plant species, these numbers were low. In soybean, a QTL for plant height was found to explain 67.7% of the total phenotypic variance, a number almost rive times as much as the one we obtained for our populations. In the outcrossing species *Eucalyptus nitens*, three QTLs, each with an effect between 10% and 15%, have been detected for the total height of seedlings at 55 days after planting out (Byrne et al. 1997). However, our results were not unusual when we compared them to published results for maritime pine (two QTLs explaining 17% of total phenotypic variance of height at 15 weeks, three QTLs explaining 21% of height at 38 weeks and one QTL explaining 10% of height at 92 weeks) (Plomion et al. 1996), loblolly pine (one QTL explaining 20% of total phenotypic variance of height increment at age 2 years and one QTL explaining 12.6% of height increment at age 4 years) (Kaya et al.

1999) and cocoa (two QTLs explaining 16.5% of total variance of height and two QTLs explaining 11.2% of total variance of diameter of two-year-old cocoa) (Crouzillat et al. 1996).

The information about from which grandparent the QTLs detected in the F_1 parent were derived is not a must for marker-assisted selection. Due to the lack of linkage information for the grandparents, we could not determine the derivation of the QTLs detected in the F_1 parent. Thus, we could not tell whether a band-present-associated negative effect was due to a band-present-associated negative effect allele, which would be expected to be more likely from the longleaf pine grandparent, or a band-absent-associated positive effect allele, which would be expected to be more likely from the slash pine grandparent. However, this situation would not be a problem in marker-assisted selection. When selecting a QTL from the F_1 parent using marker-assisted selection, selecting individuals that contain a band-present-associated positive effect allele or avoiding individuals that contain a band-present-associated negative effect allele would be adequate for selection on this QTL. Of course, with information on the derivation of these QTLs, we would be able to increase the certainty of the existence of these QTLs.

The results from this research may suggest some recommendations for future longleaf pine breeding programs for early height and diameter growth. First, more markers will be needed to cover the genome of the population. Without taking the unlinked markers into account, only about 70% and 58% of the genome was covered. With more markers, more QTLs located in the unmapped-regions may be detected. Second, the interactions across planting sites suggest that different lines should be developed for different planting sites. Developing a set of lines for a series of environments may facilitate the breeding program. Third, more loci will need to be taken into account than we had expected. The number of loci involved in regulating height and diameter growth was estimated to be five. However, our results have shown that different QTLs were activated at different growth ages and, consequently, the total number we need to work on may be more than ten, with about three for each age.

There were some limitations in this experiment. First, the results of this research can only provide some extra information about QTLs, mainly the negative-effect ones from the longleaf pine grandparents. None of the BC_1 individuals were designed to make any further crosses. Second, many putative QTLs had a low LOD score. Those loci were subjected to further tests to confirm their existence of EHG effects. The LOD scores for 12 of the 21 QTLs were smaller than 2.5, and 18 of the 21 QTLs were smaller than 3.0. The reasons for the low LOD scores may be small sample size for each environment, small effects of the corresponding QTLs compared to the environment effects or spurious QTLs detected. By increasing the sample size, LOD scores could be increased, and the probability of detecting spurious QTLs could be decreased.

References

Brown CL (1964) The seedling habit of longleaf pine. Ga For Res Counc Rep No. 10

Byrne M, Murrel JC, Owen JV, Kriedemann P, Williams ER, Moran GF (1997) Identification and mode of action of Quantitative trait loci affecting seedling height and leaf area in *Eucalyptus nitens*. Theor Appl Genet 94:674–68 1

Crouzillat D, Lerceteau E, Petiard V, Morera J, Rodriguez H, Walker D, Philips W, Ronning C, Schnell R, Osei J, Fritz P (1996) *Theobroma cacao* L.: a genetic linkage map and quantitative trait loci analysis. Threor Appl Genet 93:205–2 14

Derr HJ (1966) Longleaf xslash pine hybrids at age 7: survival, growth, and disease susceptibility. J For 64:236–239

Derr HJ (1969) Intraspecific and interspecific crosses for testing brown-spot resistance of longleaf pine. Final Report on Study FS-SO-1102-5.2. USDA For Serv S For Exp Stn, New Orleans, La.

Devey ME, Delfino-Mix A, Kinloch BB, Neale DB (1995) Random amplified polymorphic DNA markers tightly linked to a gene for resistance-to white pine blister rust in sugar pine. Proc Natl Acad Sci USA 92:2066–2070

Echt CS, Nelson CD (1997) Linkage mapping and genome length in eastern white pine (*Pinus strobus* L.). Theor Appl Genet 94:1031–1037

Emebiri LC, Devey ME, Matheson AC, Slee MU (1998) Interval mapping of quantitative trait loci affecting NESTUR, a stem growth efficiency index of radiate pine seedlings. Theor Appl Genet 97: 1062-1 068

Gill KS, Gill BS, Endo TR, Boyko EV (1 996a) Identification and high density mapping of gene rich regions in chromosome group 5 of wheat. Genetics 143: 1001–1012

Gill KS, Gill BS, Endo TR, Taylor T (1996b) Identification and high density mapping of gene rich regions in chromosome group 1 of wheat. Genetics 144: 1883-1891

Hulbert TW, Legg EJ, Lincoln SE, Lander ES, Michehnore RW (1988) Genetic analysis of the fungus, *Bremia lactucae*, using restriction fragment length polymorphisms. Genetics 120: 947-958

Kaya Z, Sewell MM, Neale DB (1999) Identification of quantitative trait loci influencing annual height- and diameter-increment in loblolly pine. Theor Appl Genet 98:586–592

Kriebel HB (1985) DNA sequence components of the *Pinus strobus* nuclear genome. Can J For Res 15: 1-4

Kubisiak TL, Nelson CD, Nance WL, Stine M (1995) RAPD linkage mapping in a longleaf pine xslash pine F_1 family. Theor Appl Genet 90: 1119-1 127

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174– 18 1

Layton PA, Goddard RE (1982) Environmental and genetic effects on duration of the grass stage of longleaf pine. In: Proc 7th N Am For Bio-Workshop. University of Kentucky, Lexington, pp131–136

Miksche JP, Hotta Y (1973) DNA base composition and repetitive DNA in several conifers. Chromosoma 4 1:29–36

Monna L, Miyao A, Inoue T, Fukuoka S, Yamazaki M, Sun ZH, Sasaki T, Monobe Y (1994) Determination of RAPD markers in rice and their conversion into sequence-targeted sites (STSs) and STS-specific primers. DNA Res 1: 139-148

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:432 1-4325

Nelson CD, Nance WL, Doudrick RL (1993) A partial genetic linkage map of slash pine (*Pinus elliottii* Englem. var. *elliottii*) based on random amplified DNAs. Theor Appl Genet 87: 145–151

Nelson CD, Kubisiak TL, Stine M, Nance WL (1994) A genetic linkage map of longleaf pine (*Pinus palustris* Mill.) based on random amplified polymorphic DNAs. J Hered 85:433–439

Ohri D, Khoshoo TN (1986) Genome size in gymnosperms. Plant Syst Evol 153:119–131

Plomion C, O'Malley DM, Durel CE (1995) Genome analysis in maritime pine (*Pinus pinaster*): comparison of two RAPD maps using selfed and open-pollinated seeds of the same individual. Theor Appl Genet 90: 1028-I 034

Plomion C, Durel CE, O'Malley DM (1996) Genetic dissection of height in maritime pine seedlings raised under accelerated growth conditions. Theor Appl Genet 93:849–858

Rake AV, Mikshe JP, Hall RB, Hansen KM (1980) DNA reassociation kinetics of four conifers. Can J Genet Cytol 22:69–79

Schmidtling RC, White TL (1989) Genetics and tree improvement of longleaf pine. In: Proc Symp Manage Longleaf Pine. Southern Forest Experimental Station. New Orleans. pp1.14– 126

Snyder EB, Namkoong G (I 978) Inheritance in a diallel crossing experiment with longleaf pine. USDA For Ser Res Pap SO- 140

Walkamiya I, Newton RJ, Johnston JS, Price HJ (1993) Genome size and environmental factors in the genus *Pinus*. Am J Bot 80:1235–1241

Welsch J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:72 13-72 18

Weng C, Kubisiak TL, Nelson CD, Geaghan JP, Stine M (1999) Comparison of Maximum Likelihood Estimation Approach and Simple Regression Approach in Detecting Quantitative Trait Loci Using RAPD Markers. In: Proc 25th S For Tree Improv Conf. LSU Agriculture Centre, New Orleans, La. pp177–185

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 653 1-6535