A Genetic Linkage Map of Longleaf Pine (*Pinus palustris* Mill.) Based on Random Amplified Polymorphic DNAs

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Eight megagametophyte DNA samples from a single longleaf pine (*Pinus palustris* Mill.) tree were used to screen 576 oligonucleotide primers for random amplified polymorphic DNA (RAPD) fragments. Primers amplifying repeatable polymorphic fragments were further characterized within a sample of 72 megagametophytes from the same tree. Fragments segregating in a 1:1, present-to-absent, ratio were classified as Mendelian markers and mapped using multipoint linkage analysis. The analysis revealed 16 linkage groups of at least three markers, ranging in size from 21.1 to 185.6 cM, and six linked pairs (5.5 to 23.0 cM) of markers. The 22 groups and pairs included 133 RAPD markers and covered approximately 1,635 cM of genetic map distance. Genome size estimates, based on the linkage data, ranged from 2,612 to 2,656 cM. Using a 30-cM map scale and including the 11 unlinked markers and the ends of the 16 linkage groups and six linked pairs, the set of RAPD markers accounts for approximately 2,265 cM, or 85% of the genome.

Genetic studies of long-lived, outcrossing species such as those of the *Pinaceae* have been hampered by a lack of genetic markers. For the most part, available genetic markers have been inadequate in providing detailed genetic information due to their low level of polymorphism and limited number (Beckmann and Soller 1983; Conkle 1981; Tanksley 1983). Recent introductions of several DNA-based genetic marker systems have allowed the construction of genetic linkage maps in a wide range of plant species (Al-Janabi et al. 1993; Bernatzky and Tanksley 1986; Gebhardt et al. 1989; Helentjiaris et al. 1986; Landry et al. 1987; McCouch et al. 1988; Nam et al. 1989; Reiter et al. 1992), including forest trees (Grattapaglia and Sederoff 1994; Liu and Furnier 1993; Nelson et al. 1993; Tulsieram et al. 1992). These markers, namely, restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNAs (RAPDs), are generally useful for genetic studies because they contain relatively high levels of polymorphism and are obtainable in large numbers (Botstein et al. 1980; Welsh and McClelland 1990; Williams et al. 1990). Genetic linkage maps composed primarily of RFLP and RAPD markers are now being used to search for quantitative trait loci (QTLs) in several plant species (Keim et al. 1990; Lander and Botstein 1989; Martin et al. 1989; Paterson et al. 1988).

Longleaf pine is native to the southeast United States, but is known worldwide as an important timber- and resin-producing species. Breeding objectives in the United States have emphasized rapid early height growth and resistance to brown spot needle blight (caused by *Scirrhia acicola* [Dearn.] Siggers) (Bey 1979; Schmidtling and White 1989). Variation in early height growth among and within families is large (Synder 1969, 1973; Synder and Derr 1972; Synder and Namkoong 1978; Wells and Synder 1976). Most of this variation can be attributed to the grass-stage habit, in which seedlings do not initiate height growth until their second or third year at the earliest and indefinitely (>15 years) at the latest (Wahlenberg 1946). In field tests in which seedlings are grown under brown spot disease pressure, the least infected seedlings tend to initiate height growth earlier, effecting a positive correlation between resistance and height growth (Kais and Griggs 1986; Synder and Derr 1972). However, in Synder and Derr's (1972) study, the correlation between family mean heights in fungicide-treated (brown spot controlled) and nontreated plots was not significant, implying a nongenetic basis for the positive correlation between disease resistance and height growth. In general, the lack of height growth in the first years after planting, even under optimal field conditions, has caused tree

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breeders to limit their efforts to improve longleaf pine for planting.

Brown (1964) and Derr (1966, 1969) have observed intermediate height growth in interspecies hybrid families of longleaf × loblolly (*Pinus taeda* L.) pines and longleaf × slash (*P. elliottii* Engelm. var. *elliottii*) pines. Using data from parental, F_1, BC_1, and F_2 generations, Brown (1964) calculated that the minimum number of factors controlling first-year height growth was approximately 10. Recent calculations using Lande's (1981) methods have suggested fewer than 10 factors as the minimum number (Nelson CD, unpublished data). The availability of genetic markers greatly enhances the proposition of implementing a backcross breeding program designed to introgress genes for first-year height growth from slash or loblolly pine into longleaf pine. For example, RAPD markers monomorphically present within the donor species and absent within longleaf pine could be used to monitor the relative amounts of each species' DNA contained within backcross individuals. Individuals expressing excellent first-year height growth and being homozygous band absent for a large proportion of RAPD markers would be candidates for selection. In this way, the recovery of longleaf pine DNA could be greatly enhanced in the first and second backcross generations (Hillel et al. 1990; Hospital et al. 1992; Tanksley et al. 1981; Tanksley and Rick 1980), especially in regions unlinked to the genes for first-year height growth. Given the long generation length in these species, this marker-aided selection (MAS) approach should prove extremely beneficial.

In the study reported here, we used RAPD markers to develop a genetic linkage map of longleaf pine. The mapping population consisted of 80 megagametophytes of a single tree (clone 3-356). The large size of pine megagametophytes makes them especially useful for genetic linkage studies with various marker systems (Bahrman and Damerval 1989; Conkle 1981; Guries et al. 1978). RAPD markers segregate 1:1 (band present to band absent) in megagametophytes of single trees, allowing the phase of most linked markers to be unambiguously determined (Hulbert et al. 1988; Raeder and Broda 1986) and single-tree genetic linkage maps to be constructed (Nelson et al. 1993; Tulsieram et al. 1992). Currently, clone 3-356 is being used as an elite longleaf parent in several F_1 crosses with elite slash and loblolly pines. Our long-term research goals are to develop a MAS method for introgressing genes for first-year height growth into longleaf pine and to map early height growth QTLs and brown spot resistance genes segregating in the backcross and F_2 generations of these F_1 interspecies crosses.

Materials and Methods

DNA Extraction

We dissected megagametophytes of longleaf pine clone 3-356 from wind-pollinated seeds. Total DNA was prepared from individual megagametophytes with a modified SDS extraction, as previously described by Nelson et al. (1993). DNA concentrations were determined spectrophotometrically by averaging several (4–6) absorbance readings taken at 260 nm. The extraction protocol typically yielded 20 μg of DNA per megagametophyte (range 9 to 34 μg). We standardized working DNA preparations to 12.5 ng/μl in low TE (10 mM Tris, 0.1 mM EDTA pH 8.0) and stored them until used at −20°C.

RAPD Procedure

We based RAPD reactions on the protocol reported by Williams et al. (1990), with modifications suggested by R. R. Sederoff (North Carolina State University) and tested by Nelson et al. (1993) on slash pine. The reaction consisted of the following in 16 μl total volume: 3.125 ng template DNA, 5 pmoles primer DNA (0.3125 μM), 3.2 nmoles each dNTP (200 μM each) (Promega), 1.6 μl 10× *Taq* DNA polymerase reaction buffer (Boehringer-Mannheim) (100 mM Tris-HCl pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin), and 1.0 U *Taq* DNA polymerase (Boehringer-Mannheim). Reactions were loaded in flexible microtiter plates (Becton-Dickinson) and overlaid with 50 μl of mineral oil. We placed the microtiter plates in preheated (85°C) programmable temperature cyclers (MJ Research PTC-100) and covered them with sheets of mylar film. The RAPD reactions were driven to completion using the following thermal profile: 5 s at 95°C; 1 min 55 s at 92°C; followed by 45 cycles of 5 s at 95°C, 55 s at 92°C, 1 min at 35°C, and 2 min at 72°C; followed by 7 min at 72°C. The reactions ended with an indefinite hold at 4°C.

We electrophoresed the completed RAPD reactions in 2% agarose gels and TAE buffer (40 mM Tris base, 20 mM sodium acetate, 1 mM EDTA, glacial acetic acid to pH 7.2) for approximately 3.5 h at 3 V/cm (150 V). Prior to gel electrophoresis, 3 μl of loading buffer (10× TAE, 50% glycerol, 0.25% bromophenol blue) were added to each reaction. After electrophoresis, the gels were stained with ethidium bromide (0.4 μg/ml) for 20 min, washed in water (7× gel vol) for 45 min, and photographed over UV light using a Polaroid MP-4 camera and Polaroid 667 instant film.

All primers used in this study were arbitrary sequence, 10-base-oligonucleotide primers with C+G contents ranging from 50% to 80%. We obtained 220 primers from Operon Technologies (Oligo Sets A, B, C, D, E, F, G, J, W, X, and Y) and an additional 356 primers from J. E. Carlson of the University of British Columbia (numbers 101–360 and 620–635, and 80 primers within the range of numbers 361–590). The group of 80 primers was deliberately selected based on positive screening and mapping results with at least one slash pine tree (Nelson et al. 1993; van Buijtenen JP, unpublished manuscript). All 576 primers were initially screened for polymorphism against a sample of eight megagametophytes of clone 3-356. We re-screened primers revealing amplification and potential polymorphism with the same set of eight megagametophytes to check for repeatability. Primers producing one or more repeatable, polymorphic fragment(s) were chosen for the mapping phase, which involved assaying each selected primer against an additional sample of 72 megagametophytes of clone 3-356. The 72 template DNAs were arranged into three sets of 24 megagametophytes. Each set was amplified separately with the selected primers. Only RAPD fragments that were readily scored for each template set were included in the linkage analysis.

Linkage Analysis

We scored polymorphic fragments as + for present, − for absent, and 0 for missing (i.e., failed or unscorable reaction) in the screening and mapping phases. The RAPD fragments were tested for 1:1 segregation ratio using chi-square (χ^2) tests. All fragments with an α-value greater than 0.05 were assumed to represent Mendelian genetic loci and were used in the linkage analysis. Multipoint linkage analysis was performed with MAPMAKER II version 1.9 (Lander et al. 1987) using a modified backcross data file (Nelson et al. 1993). The modified file permitted the identification of both coupling (++/−−) and repulsion (+−/−+) phase linkages.

We calculated genome size estimates using a method-of-moments estimator, $G(Z) = M(M-1)X(Z)/K(Z)$ (method 3 in Chakravarti et al. 1991; Hulbert et al. 1988),

Table 1. Linkage group assignment and chi-square (χ^2) test information for 174 RAPD markers in longleaf pine 3-356

Group	Order[a]	Primer[b]	Band[c]	Size[d]	$\chi^2_{(1:1)}$	Probability
A	1	Y17	A	0350	3.66	056
A	2	G06	A	0775	1 85	174
A	3	186	B	0790	0.21	651
A	4	533	A	0675	0.45	.502
A	5	503	A	0400	0 65	.419
A	6	299	C	0780	0.82	.365
A	7	G17	A	0460	0.2	655
A	8	D12	A	1000	0.01	904
A	9	B13	A	0550	0 51	.473
A	10	B13	C	0800	0 06	.811
A	—[e]	210	A	1100	0 64	.423
A	—	258	A	0500	0 02	893
B	1	429	A	0400	0.34	.558
B	2	F07	B	0825	1 03	311
B	3	B13	B	0775	0 23	.633
B	4	119	B	0750	0 46	.497
B	5	499	B	1250	1 03	.311
B	6	269	C	0750	1.25	264
B	7	F07	C	1000	1.53	216
B	8	122	A	0400	0 46	497
B	8	306	A	0450	0 82	365
B	9	A07	A	0275	0 62	.431
B	10	B04	B	0800	1 03	.311
B	11	362	B	1300	0 12	732
B	12	C01	A	0500	0.21	651
B	13	G04	A	1090	0 33	564
B	—	213	A	0400	0 62	431
B	—	256	A	0520	1.8	.18
B	—	493	A	0700	1 8	.18
BR[f]	14	B09	A	0440	0.64	425
BR	15	327	B	1090	0 21	.651
BR	15	B04	A	0500	0 21	651
BR	16	173	A	0550	1.03	.311
BR	—	Y17	B	0750	2.85	.091
C	1	271	A	0890	2.85	091
C	2	G09	A	0750	2.92	087
C	3	203	A	1050	0	1.000
C	4	135	B	0650	0 01	91
C	5	570	A	1100	0 12	729
C	6	171	B	0850	0 2	.655
C	7	225	A	0900	1 8	18
C	8	186	A	0700	0.82	.365
C	9	C05	B	1600	0 07	789
C	10	248	A	0500	0 29	593
C	11	299	D	1600	1.03	311
C	12	119	A	0525	1 57	.21
C	13	254	B	0600	0.8	371
C	—	195	C	1725	2.14	144
C	—	A12	A	1700	3.76	052
D	1	319	A	1150	0 05	.816
D	2	243	A	0500	1 03	.311
D	3	J08	A	0670	2 45	118
D	4	A06	A	1200	1.25	264
D	5	184	B	0575	1 25	264
D	6	297	B	0520	0 8	371
D	7	348	A	0600	0 05	.821
D	8	102	B	1450	0.32	.574
D	9	297	A	0400	1.25	264
D	—	159	B	0525	0.29	593
E	1	257	A	1050	1 53	.216
E	2	370	A	0850	1.28	.258
E	3	B20	B	0775	0.32	574
E	4	504	A	0650	0 33	564
E	5	169	A	0875	1 03	311
E	6	256	B	0700	0	1.000
E	7	C05	A	0900	0.07	.789
E	—	381	A	0760	0.12	732
E	—	J06	B	1000	0.62	431
F	1	B05	C	0650	1 25	264
F	2	299	A	0300	0 01	91
F	3	153	B	0525	0.01	.91
F	4	E12	B	0575	0 01	91
F	5	B05	A	0475	0	1 000

Table 1. Continued

Group	Order[a]	Primer[b]	Band[c]	Size[d]	$\chi^2_{(1:1)}$	Probability
F	6	509	A	0775	1.85	.174
F	7	499	A	0850	2.45	118
F	8	D12	B	1350	0 01	.904
F	—	195	B	0780	1 53	216
F	—	258	B	1500	0 89	.345
F	—	B05	B	0500	0 05	823
FQ[f]	9	460	A	0800	0.01	909
FQ	10	254	C	0800	0	1.000
FQ	11	360	A	1100	0.22	642
FQ	—	327	A	1000	0 05	821
G	1	533	B	1100	0 05	823
G	2	G12	A	0900	0 2	.655
G	3	171	A	0600	0.45	502
G	4	168	A	0600	0.62	431
G	5	517	A	0650	0.45	502
G	6	185	A	0850	1 81	179
G	7	402	A	0900	3 75	053
H	1	184	C	0900	1 25	264
H	2	181	A	0350	0 8	.371
H	3	123	A1	1000	2.51	113
H	3	123	A2	1100	2.51	113
H	4	268	B	0875	2.14	144
H	5	248	B	0900	0 8	371
H	6	299	B	0450	3 28	07
H	—	264	B	0500	0 89	345
H	—	269	B	0400	0	1 000
H	—	348	B	1000	1.53	.216
I	1	254	D	0900	0	1 000
I	2	E08	A	1200	0 8	371
I	3	E12	A	0450	0.8	371
I	4	297	C	0900	0.05	823
I	5	F07	A	0625	0.05	821
I	6	621	A	0500	0.12	729
I	7	180	A	0875	0 2	.655
I	—	G12	B	1100	1 8	18
I	—	Y20	A	0875	0 32	574
J	1	J01	B	0700	0	1 000
J	2	B20	A	0330	1.85	174
J	3	184	A	0325	0.8	.371
J	4	J01	A	0500	0.82	365
J	5	264	A	0440	0 16	.686
J	6	E17	A	0440	0 2	655
J	—	550	C	1800	0 22	637
K	1	362	A	0600	0.64	425
K	2	116	A	1450	1.08	.299
K	3	242	A	0750	1.8	.18
K	4	295	A	0500	0 8	371
K	5	256	C	0800	1 25	264
L	1	195	A	0300	0	1.000
L	2	132	B	0900	2.14	144
L	3	X18	A	0650	0.12	729
L	4	187	A	1050	0.32	574
L	5	403	A	1500	0	1 000
L	—	268	C	1050	0 01	91
L	—	269	A	0350	0.05	823
L	—	G06	B	0975	0 11	736
L	—	G17	B	1100	0	1 000
M	1	550	B	0800	1 53	216
M	2	269	D	0825	1 8	18
M	3	F06	A	0625	0 45	.502
M	4	550	A	0675	0.45	.502
M	5	628	A	0630	0 12	.732
N	1	427	B	0825	1 25	264
N	2	J06	A	0600	1 53	216
N	3	122	B	1000	0 46	497
N	4	318	A	0790	2.45	118
N	5	C01	B	0675	0 46	.497
N	6	159	A	0350	3 5	061
N	—	504	B	0725	0 33	.564
O	1	J08	B	0900	0.8	371
O	2	135	A	0400	0.01	91
O	3	327	C	1650	0 05	.821
O	4	168	B	0650	0 01	91
O	5	254	A	0440	0 45	502
O	6	267	A	0600	0 29	593

Table 1. Continued

Group	Order[a]	Primer[b]	Band[c]	Size[d]	$\chi^2_{(1:1)}$	Probability
P	1	153	A	0275	0.24	.628
P	2	Y19	A	0525	0 2	655
P	3	W19	C	1600	0 46	497
Lp1[g]	1	132	A	0520	0 82	365
Lp1	2	181	B	1700	0.32	.574
Lp2	1	216	A	0875	0 05	.821
Lp2	2	268	A	0700	0.62	.431
Lp3	1	244	A	0800	0.46	497
Lp3	2	386	A	0550	0 64	425
Lp4	1	370	B	1475	0.82	.365
Lp4	2	E19	A	0550	0 2	655
Lp5	1	D12	C	1700	0 13	718
Lp5	2	W19	B	1000	0.12	.732
Lp6	1	G10	A	0450	0 62	.431
Lp6	2	W19	A	0600	3 28	.07
UL[h]	—	102	A	1050	1 03	311
UL	—	111	A	1550	0.62	.431
UL	—	146	A	0400	0	1.000
UL	—	153	C	0800	1 53	216
UL	—	357	A	0400	2 51	.113
UL	—	357	B	1400	0 21	.651
UL	—	381	B	1450	1 32	251
UL	—	427	A	0520	0.8	371
UL	—	429	B	0650	0 84	359
UL	—	479	A	0750	1 21	272
UL	—	J01	C	0975	1 57	.21

[a] Map order within the linkage group

[b] Primer ID

[c] Fragment ID.

[d] Approximate size (bp) of fragment

[e] Dash indicates map order is uncertain (LOD < 2 0)

[f] Groups BR and FQ are linked to B and F, respectively (see text)

[g] Lp = linked pair, identifies the members of the pair.

[h] UL = unlinked marker

where for a given LOD score, Z, $G(Z)$ is genome size in cM, M is the number of markers analyzed, $X(Z)$ is the maximum cM distance between linked markers, and $K(Z)$ is the number of linkages. Two-point linkages at LOD values of 2.0, 3.0, and 4.0 between the 170 distinct loci were determined and used to provide three different estimates. The maximum of these estimates was used in further calculation and consideration of genome coverage.

Results

For the 576 primers screened, 248 initially appeared to reveal polymorphism between megagametophytes of clone 3-356. These primers were run again, with the same megagametophytes, to test for repeatability of the apparent RAPD fragments. A large proportion (ca. 40%) of these primers failed to produce repeatable polymorphisms. Of those primers producing repeatable RAPD fragments, 128 were selected for mapping. With these primers, 200 fragments were scored on the three template sets of the mapping phase. Due to faint or inconsistent amplification, 12 of

these fragments were classified as unreliable and omitted from further analysis. Chi-square analysis indicated that 14 of the remaining 188 fragments were not segregating 1:1 for presence-to-absence. The 174 RAPD fragments that were apparently segregating 1:1 and their assigned linkage map positions are listed in Table 1. These fragments were produced by 102 primers. Fifty primers produced one polymorphic fragment, 35 produced two, 14 produced three, and 3 produced four. Fragment sizes ranged from 275 to 1,725 base pairs (bp).

Using two-point data, 139 RAPD markers were classified into 18 linkage groups of three or more loci with a linkage criteria of LOD 5.0 and distance 25 cM. Three-point analyses were then performed for each group. Orders of markers that were consistent for all three-point tests with LOD 3.0, distance 25 cM, and an exclusion threshold LOD of 3.0 were taken as framework orders. This resulted in the positioning of 92 markers within the 18 groups. Potential positions for the remaining 47 markers within the groups were then tested. Positions that were 100 times more likely than the next best position were accepted. Twenty-one markers were positioned, while 26 markers were found to have two or more similarly likely (LOD < 2.0) positions (Table 1).

The 35 unlinked markers were then tested for linkage to the 18 groups with a linkage criteria of LOD 4.0 and distance 30 cM. Nine markers met this criteria the first time, three markers the second, and zero the third. Three- and multipoint analyses were used to order the enlarged groups after each reiteration. As expected, the map positions determined for the newly linked markers were distal to the end markers of the initial groups. Linkages between all possible pairs of these 18 groups were then tested. Two LOD scores for linkage between groups were significant (LOD > 3.0) and mapped less than 30 cM apart when the respective groups were combined. Two-point analysis of the remaining 23 loci resulted in six pairs of linked markers, with LOD 4.0 and distance 25 cM, and 11 unlinked markers (Table 1).

Figure 1 presents our genetic linkage map of longleaf pine clone 3-356. Sixteen linkage groups consist of three or more RAPD markers, and six groups contain linked pairs of markers only (Table 1 and Figure 1). Groups B and F each contain two of the initial 18 groups (R and Q, not shown separately). The LOD scores for linkage of groups R (B09-A, 327_B, 173-A) and B and groups Q (460-A, 254-C, 360-A) and F, respectively, were 6.67 and 5.63, with between-group map distances of 25.2 cM and 27.2 cM. LOD scores of 2.50 and 2.14 were found for linkage between groups J and G and groups K and H, respectively. These were not considered significant, however, as map distances between these groups would be approximately 50 cM. The 22 linkage groups and pairs contain 133 mapped loci (LOD ≥ 2.0) and cover an estimated 1,634.7 cM of map distance. The weighted-average distance between markers within the 22 linkage groups is 14.7 cM (14.9 cM in the groups and 12.2 in the pairs).

Genome size estimates were 2,612, 2,656, and 2,631 cM for LOD scores of 2.0, 3.0, and 4.0, respectively. For this calculation we used data for 170 markers, because four pairs of the 174 markers were linked (0% recombination) in the repulsion phase and may represent two alleles

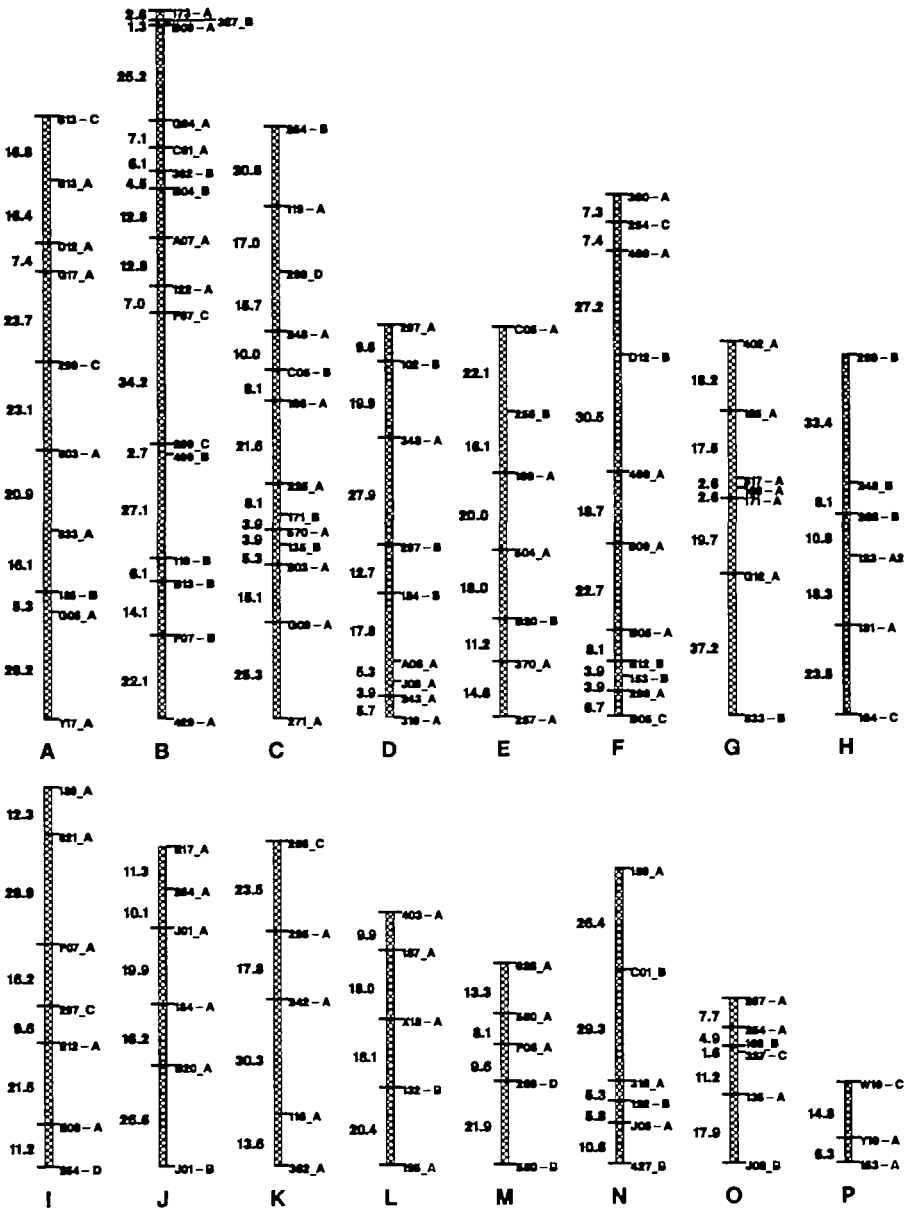

Figure 1. Genetic linkage map of longleaf pine clone 3-356 Linkage groups with three or more RAPD markers are indicated by letters A through P Marker names are given on the right-hand side of the linkage groups, and Haldane centimorgan (cM) distances are given on the left-hand side. The marker names contain the primer ID and a letter code for the specific fragment (Table 1) The symbols (– or _) separating the primer and fragment in the marker names indicate phase relationships between linked markers, i e., – – and _ markers are in coupling (– –/ ++ or ++/– –). Framework markers (LOD > 3 0, distance < 25 cM) are marked with crossbars that completely pass through the rectangle Markers ordered at LOD scores between 2 0 and 3.0 are marked with crossbars that touch the right-hand side of the rectangle. Markers added to the framework groups at distances between 25 cM and 30 cM (LOD > 4 0) or used to link framework groups together are marked with crossbars that touch the left-hand side of the rectangle

at the same locus. Utilizing the formulation of Bishop et al. (1983), we expect a random sample of 170 markers (each covering 30 cM) drawn from a uniform distribution over 12 linkage groups totaling 2,656 cM to cover 84.2% of the genome. Using a 30-cM map scale and assuming that 24 of the 32 ends of our 16 linkage groups are located within 15 cM (30 cM/2) of the 24 telomeres ($x = n = 12$ in longleaf pine), we can account for 2,265 cM (85.3% of the genome) with the 170 markers. Without the telomere assumption, the 170 markers would cover nearly 98% of the longleaf pine genome. In light of Bishop's et al. (1983) theoretical work, this level of coverage would seem highly unlikely.

Discussion

In the study reported here, we used megagametophytic DNA of a single longleaf pine tree and RAPD markers to produce a medium-density genetic linkage map (14.7 cM average spacing). The map includes 16 linkage groups, containing at least three loci, and six linked pairs of markers. The groups and pairs cover a contiguous distance of approximately 1,635 cM. Using the two-point linkage data, we estimate the genome size to be 2,656 cM. Including the 11 unlinked markers, we calculated our genome coverage to be 85.3%, which closely agrees to a theoretical estimate (84.2%) assuming 170 markers, 30 cM coverage per marker, 2,656 total cM, and 12 chromosomes (haploid number). Based on a similar mapping study, we previously estimated the genome size of slash pine to be in the range of 2,880 to 3,360 cM (Nelson et al. 1993). More recent estimates made with larger data sets on the same and additional trees suggest that the genome sizes of longleaf and slash pines are in the range of 2,300 to 2,900 cM (Nelson CD, Kubisiak TL, van Buijtenen JP, Stine M, and Nance WL, unpublished data).

Estimates of the physical size of several pine species vary from 33 pg to 57 pg per diploid (2C) nucleus (Ohri and Khoshoo 1986). An estimate for longleaf pine was not given, but the average for the most closely related species (Subgenera *Pinus*, Section *Pinus*, Subsection *Australes*) was 38.2 pg (or 1.84×10^{10} bp per haploid nucleus). This suggests that the average physical size of a centimorgan in longleaf pine is approximately 6.9×10^6 bp. Because the bp/cM relationship is known to vary widely within various genomes, this average value is useful for comparative purposes only. Estimates of the average bp/cM ratio in plants range from 2.9×10^5 for *Arabidopsis thaliana* to 1.4×10^6 for *Zea mays* (Arumuganathan and Earle 1991, Nam et al. 1989; Whitkus et al. 1992). The large bp/cM ratio in longleaf pine will require very high resolution mapping (<0.1 cM average spacing) for applications involving map-based cloning techniques. This level of resolution may be afforded for specific genomic regions only, such as those adjacent to disease-resistance loci. In addition, the large bp/cM ratio could also cause problems in QTL mapping experiments, because a 10-cM interval, containing 6.9×10^7 bp, could contain many loci—potentially with both positive and negative effect alleles.

The efficiency of RAPD mapping is largely a function of the information content per primer screened and mapped. For this map, we screened 576 primers with eight megagametophytes for polymorphism within a single longleaf pine tree. Of these 576 primers, 80 were previously identified by their ability to produce mappable polymorphisms in two unrelated slash pine trees. In the preselected group, 24.8% of the primers produced mappable polymorphisms, while only 16.7% did in the nonselected group. However, in terms of the number of mappable RAPD markers per primer used in mapping, the preselected group provided no advantage. For the two groups, the preselected primers produced 0.36 mapped RAPD markers per primer screened, while the nonselected group produced 0.29. We have obtained similar results with other species in *Pinus* and *Picea* (Nelson CD, van Buijtenen JP, Echt C, Binelli G, and Nance WL, unpublished data). Tingey et al. (1992) cited similar (0.3 markers per primer screened) and much higher estimates (up to 2.5) for various plant species. Selecting the tree or pedigree for mapping efficiency may also prove beneficial as we have observed large variation between trees within several *Pinus* and *Picea* species (Nelson CD, Nance WL, van Buijtenen JP, and Binelli G, unpublished data). However, it appears that mapping trees and pedigrees selected for trait variation will prove most valuable, as most trees apparently have sufficient DNA polymorphisms to identify and map genetic marker loci.

One advantage of RAPD markers compared to RFLPs and allozymes is the potential to automate the laboratory process. In this experiment, we automated several steps of the RAPD process, allowing a single person to completely process an average of 768 reactions per day (Nance and Shumate 1992). In addition to the increased throughput afforded by automation, we also observed a reduction in failed reactions (no or recognizable problem amplifications). In an earlier RAPD mapping experiment (Nelson et al. 1993), we found 10% failed reactions (missing data) and approximated a 5% error rate due mostly to nonrecognizable problem amplifications (i.e., partially failed reactions). Partially failed reactions are especially problematic in that they usually result in misscored data. Kubisiak et al. (1993) found that misscored mapping data at and above the 4% level resulted in statistically detectable differences in several linkage map construction parameters. Most notable was the reduced numbers of mapped framework (LOD > 5 0 for group inclusion and LOD > 3.0 for placement within groups) markers. In the study reported here, with the help of automation, we found 5% failed reactions and estimated a 2% error rate (data not shown). Weeden et al. (1992) and Hemmat et al. (1994) found similar error rates in RAPD markers assayed in pea, lentil, and apple mapping experiments, owing the reduction in error rates to using high-quality template DNA, scoring only clear polymorphisms, and anchoring linkage groups with allozyme or RFLP markers. Clearly these measures, combined with automation, would further contribute to reducing the error rates associated with RAPD markers.

The level of RAPD data throughput currently achievable with automation should allow for the design and rapid execution of large MAS and QTL mapping experiments. However, the nature of RAPD markers—dominance, low polymorphism information content (PIC), and low marker homology between crosses (Hemmat et al. 1994)—requires that individual- or pedigree-specific maps be constructed for each project, adding to the need for increased automation (Grattapaglia et al. 1992). Attempts to overcome these problems by converting RAPD markers to sequence characterized amplified region (SCAR) markers (Paran and Michelmore 1993) are in progress. Other PCR-based markers, such as microsatellites, may also prove beneficial, as they have in mammalian species (Dietrich et al. 1992; Serikawa et al 1992). Clearly much work remains to be done, but for applications within backcross or testcross families, such as those produced in backcross breeding programs, RAPD markers should be useful for monitoring introgression and mapping

QTLs and disease-resistance genes in longleaf pine.

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