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Utility of Random Amplified Polymorphic DNA (RAPD) Markers for Linkage Mapping in Turkish Red Pine (*Pinus brutia* Ten.)

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Summary

We have applied the random amplified polymorphic DNA (RAPD) marker system to estimate linkage relationships in *Pinus brutia* TEN.. We used DNA samples from 30 haploid seed megagametophytes from each of 4 mother trees. Ninety-five 10-base oligonucleotide primers were evaluated and 34 revealed at least one polymorphic RAPD locus. The number of segregating RAPD loci per polymorphic primer varied from 2.32 to 3.25, but when segregating loci per tested primer was considered it was low, ranging on the average, from 0.28 in tree 4 to 0.59 in tree 1. Based on the RAPD loci segregating in 1:1 ratio, genetic linkage groups formed from 6 for genotype-4 (total map distance=163.91 cM) to 13 for genotype-3 (total map distance=511.2 cM). It was also found that a number of segregating loci in all 4 genotypes (ranging from 14 to 21) could not be assigned into any of the constructed linkage groups. It was difficult to compare linkage groups among the genotypes since most of RAPD loci segregating in one genotype were not found in others. Thus, the linkage map provided very little information on the genomic organization of RAPD markers at the species level. The utility of RAPD markers in forest genetics is also discussed in the paper.

Key words: Pinus brutia, RAPD markers, genetic linkage mapping, polymerase chain reaction.

FDC: 165.3; 174.7 *Pinus brutia.*

Introduction

Turkish red pine (*Pinus brutia* TEN.) is found throughout the eastern Mediterranean and is a commercially important timber species in Turkey. Frequent forest fires, excessive utilization of stands and conversions of forest to agricultural have likely impacted the genetic diversity of Turkish populations, however, the magnitude of the impact is unknown. Red pine tree improvement programs have been established (ISIK, 1989; ISIK and KAYA, 1993), but there is an urgent need to assess the genetic diversity in Turkish populations. Inexpensive and easy to apply genetic markers would greatly facilitate this effort.

Since the early 1970's, allozymes have been the most widely used genetic markers in forestry since they are inexpensive, rapid to apply and require only modest technical skills. Examples of such studies include; geographic variation (GURIES and LEDIG, 1982), systematic relationships (CONKLE *et al.,* 1988), within stand variation (NEALE, 1985), mating systems (SHAW and ALLARD, 1982), gene flow (MILLAR, 1983) and estimation of pollen contamination in seed orchards (FRIEDMAN and ADAMS, 1985).

The main limitation of allozyme techniques is the small number of markers available. Linkage studies in conifers based on allozyme markers have mapped no more than about 10% of the genome (226.4 cM) (CONKLE, 1981). DNA markers, however, have numerous advantages over isozymes and have gradually replaced isozymes for many applications in forestry (NEALE and WILLIAMS, 1991; NEALE *et al.,* 1992). However, genetic markers such as restriction fragment length polymorphisms (RFLP) are technically demanding and expensive and would be difficult to apply in Turkey given the current laboratory standards.

Random amplified polymorphic DNA (RAPD) markers are new types of genetic markers which are based on the polymerase chain reaction (PCR) (WELSH and MCCLELLAND, 1990; WILLIAMS *et al.,* 1990). RAPD markers are inherited in a Mendelian manner and can be generated for any species without prior DNA sequence information (WELSH and MCCLELLAND, 1990). One advantage of RAPD markers is the increased speed of analysis and dramatic reduction in the amount of DNA required for analysis. Furthermore, when haploid megagametophyte tissues from conifers are used as the source of template DNA, there is no need to have specific crosses to carry out linkage mapping (CARLSON *et al.,* 1991; TULSIERAM *et al.,* 1992).

In a preliminary study, KAYA and NEALE (1993) assessed the utility of RAPD markers for studying genetic polymorphism in *Pinus brutia* (Turkish red pine) and *Pinus nigra* var *pallasiana* (Anatolian black pine). This study showed that variation could be detected with RAPD markers and it should be possible to construct genetic maps. The objectives of the current study

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Table 1. – Geographical information on *Pinus brutia* populations from which genotypes were selected as a seed source.

Populations	Seed trees ID.	Latitude	Longitude	Aspect	Altitude	Distance from Mediterranean Sea
Düzlerçami	#1	36 26	30 31	flat	300 m	30 km
Bük	#2	36 26	30 25	SW	500 m	50 km
Pamucak	#3	37 23	30 33	W	800 m	90 km
Köroglu beli	#4	37 23	30 25	W	950 m	130 km

Table 2. – The numbers of primers yielding segregating loci, total number of segregating loci per tree, average number of segregating loci per primer for each tree.

	Seed Trees			
	Tree-1	Tree-2	Tree-3	Tree-4
-Total number of primers tested	95	95	95	95
-Number of primers revealing segregating loci	19(20%)	19(20%)	16(16.8%)	11(11.6%)
-Number of segregating loci	56	44	52	27
-Average number of segregating loci per primer	0.59	0.46	0.55	0.28
-Average number of segregating loci per polymorphic primer	2.95	2.32	3.25	2.45

were (1) to identify a large number of RAPD loci in Turkish red pine and to determine their Mendelian inheritance and (2) to construct genetic linkage maps for 4 trees.

Materials and Methods

Plant materials

Open-pollinated seeds were collected from a large number of seed trees at 4 locations in Turkey (*Table 1*). These collections were made in 1990 for studying genetic structure of natural populations of Turkish red pine. For RAPD analysis, we selected 30 seeds from 1 tree at each location.

DNA isolation procedures

Seeds were soaked in distilled water at 4 °C for 24 hours and then megagametophytes were excised from the seed coat and embryo. DNA extractions from seed megagametophytes were performed according the method of KREIKE (1990). Megagametophytes were homogenized with a glass pestle in 60 µl of the extraction buffer (0.1 M Tris.HCl pH 8.0, 0.1 M EDTA, 0.25 M NaCl, 1% SDS) in 1.5 ml Eppendorf centrifugation tubes. After complete homogenization (30 s to 40 s), extraction buffer was added to a final volume of 400 µl. Homogenized tissues were placed in a 60 °C water bath for 30 minutes to 40 minutes and DNA was extracted twice with 500 µl chloroform-octanol (24:1). The DNA was precipitated with 500 µl ethanol/0.3 M sodium acetate, and washed twice with 70% ethanol kept at –20 °C in a refrigerator. DNA was dissolved in 200 µl to 300 µl TE buffer (10 mM Tris/1 mM EDTA, pH 8.0). The yields per megagametophyte varied from 1 µg to 1.2 µg.

RAPD primers and PCR conditions

Random 10-base oligonucleotide primers were obtained from Operon technologies (Alameda, California, USA). The sequence of each primer was arbitrary and generated on a random basis with the requirement that their (G+C) content be 60% to 70% and that ends were not self-complimentary. In this study, primers from kits A(opa), B(opb), D(opd), E(ope), F(opf), H(oph), J(opj), G(opg), and K(opk) were used.

PCR reactions contained 1 µl of the DNA sample (5 ng/µl); 2.5 µl of buffer (100 mM Tris-HCl pH 8.3, 500 mM KCl; Perkin-Elmer, USA); 0.2 µl (1 unit) of *Taq* DNA polymerase (Perkin-Elmer, USA); 4 µl of deoxynucleoside triphosphate mix (200 µM of each nucleotide); 2.5 µl of 25 mM $MgCl_2$; 1 µl of 1 *pmol* primers (Operon Technologies, USA), 0.13 µl Tween (Sigma, USA) and 13.66 µl of doubled distilled sterile water. DNA amplifications were conducted for 40 cycles of a denaturation step at 94 °C for 1 minute, an annealing step at 37 °C for 1 minute, and an extension step at 72 °C for 2 minutes. The last cycle was followed by a holding step for 10 minutes at 72 °C. Amplification products were visualized on 2% agarose gel with ethidium bromide (0.5 mg/ml) staining for 30 minutes.

Strategy for identification of polymorphic RAPD loci

All primers were initially screened against a set of four megagametophyte DNAs from each of 4 trees to identify segregating loci. Primers revealing 1 or more polymorphic loci were then screened against the remaining 26 megagametophyte DNAs from each tree to obtain single locus segregation data. The size of the segregating RAPD bands were estimated by using the 100 bp DNA ladder (Gibco BRL, USA) as a reference on gels.

Segregation analysis and linkage mapping

A Chi-square tests of goodness of fit to the 1:1 ratio were performed to confirm inheritance of RAPD loci. Due to problems associated with reproducibility of RAPD analysis, we excluded markers which did not segregate according to the expected 1:1 ratio. RAPD markers conforming to the 1:1 segregation ratio were analyzed for linkage using the Mapmaker

program (LANDER *et al.*, 1987). The linkage data was analyzed as an F2 intercross. Linkage groups were obtained by setting the maximum allowable recombination value to 4.0 and minimum log likelihood (LOD) to 3.0. Map orders of markers within linkage groups were determined by the multi-point analysis. Distance between markers were expressed in cM as derived by the Kosambi function.

Results and Discussion

This study demonstrates that a genetic map based on RAPD markers can be constructed for Turkish red pine and this map might ultimately be used to map loci for important traits. These markers mapping those Quantitative trait Loci (QTL) might subsequently be used in marker breeding.

More than 80% of the primers tested produced RAPD bands, but the number of primers revealing polymorphic loci was significantly less, ranging from 11.6% in tree 4 to 20% in trees 4 and 5. In total, 56, 44, 52, and 27 segregating loci were obtained in seed trees 1, 2, 3, and 4, respectively. The average number of segregating loci per primer varied from 0.28 in tree 4 to 0.59 in tree 1. When only the primers revealing polymorphic loci are considered, the number of polymorphic loci per polymorphic primer ranged from 2.3 (tree 2) to 3.2 (tree 3) (*Table 2*).

Although few of RAPD bands per primer were initially identified in each tree as polymorphic (e.g., ranging from 0.77 polymorphic RAPD bands per studied primer in tree 4 to 1.18 in tree 3), a great portion of these RAPD bands showed signifi-

Table 3. – Segregation data and Chi-square ($\chi 2$) test of 1:1 segregation for 112 RAPD loci based on assays of 30 megagametophyte DNAs from each of 4 Turkish red pine trees. P = present, and A = absent.

Locus and its length as base pairs	Tree-1 P	A	$\chi 2$ test	Tree-2 P	A	$\chi 2$ test	Tree-3 P	A	$\chi 2$ test	Tree-4 P	A	$\chi 2$ test
opa-1a$_{1000}$	14	12	0.15(.30)[a]	-[b]	-	-	-	-	-	-	-	-
opa-1b$_{800}$	16	10	1.39(.76)	-	-	-	14	13	0.04(.15)	-	-	-
opa-2a$_{>1500}$	16	12	0.57(.55)	14	15	0.03(.15)	12	14	0.15(.30)	-	-	-
opa-2b$_{1100}$	17	11	1.29(.74)	17	12	0.87(.65)	14	12	0.15(.30)	-	-	-
opa-2c$_{700}$	11	17	1.29(.74)	-	-	-	-	-	-	-	-	-
opa-3a$_{>1500}$	-	-	-	12	11	0.04(.15)	-	-	-	-	-	-
opa-3b$_{200}$	-	-	-	12	11	0.04(.15)	-	-	-	-	-	-
opa-7a$_{1500}$	9	8	0.59(.19)	-	-	-	-	-	-	-	-	-
opa-7b$_{1100}$	7	11	0.89(.65)	-	-	-	-	-	-	14	11	0.36(.45)
opa-7c$_{600}$	-	-	-	10	10	0.00(.00)	-	-	-	-	-	-
opa-7d$_{400}$	-	-	-	11	9	0.20(.35)	-	-	-	-	-	-
opa-8a$_{700}$	-	-	-	14	8	1.66(.80)	-	-	-	-	-	-
opa-8b$_{300}$	-	-	-	10	12	0.18(.33)	-	-	-	-	-	-
opa-9a$_{>1500}$	-	-	-	9	14	1.09(.70)	9	15	1.52(.78)	16	9	1.98(.84)
opa-9b$_{650}$	11	13	0.17(.32)	-	-	-	-	-	-	-	-	-
opa-9c$_{550}$	13	11	0.17(.32)	13	10	0.39(.47)	-	-	-	13	12	0.04(.16)
opa-9d$_{450}$	-	-	-	-	-	-	14	10	0.67(.59)	-	-	-
opa-11a$_{700}$	11	7	0.90(.66)	-	-	-	11	8	0.48(.51)	-	-	-
opa-11b$_{600}$	9	9	0.00(.00)	11	8	0.48(.51)	8	11	0.48(.51)	-	-	-
opa-12a$_{600}$	-	-	-	-	-	-	-	-	-	16	9	1.98(.84)
opa-14a$_{>2072}$	11	14	0.36(.45)	10	13	0.39(.47)	-	-	-	-	-	-
opa-14b$_{800}$	15	10	1.01(.68)	14	9	1.09(.70)	-	-	-	-	-	-
opa-14c$_{1100}$	-	-	-	-	-	-	15	9	1.52(.78)	-	-	-
opa-14d$_{550}$	-	-	-	-	-	-	15	9	1.52(.78)	-	-	-
Opa-14e$_{1500}$	-	-	-	-	-	-	-	-	-	12	11	0.04(.16)
opa-16a$_{1300}$	12	12	0.00(.00)	-	-	-	13	13	0.00(.00)	12	12	0.00(.00)
opa-16b$_{550}$	-	-	-	-	-	-	15	11	0.62(.57)	-	-	-
opa-16c$_{600}$	14	10	0.67(.59)	11	13	0.17(.32)	-	-	-	11	13	0.17(.32)
opa-16d$_{2072}$	-	-	-	13	10	0.39(.47)	-	-	-	-	-	-
opa-16e$_{1100}$	-	-	-	12	12	0.00(.00)	-	-	-	-	-	-
opa-17a$_{2072}$	11	13	0.17(.32)	11	13	0.17(.32)	-	-	-	-	-	-
opa-17b$_{1100}$	14	10	0.67(.59)	14	10	0.67(.59)	11	10	0.05(.17)	-	-	-
opa-17c$_{300}$	-	-	-	11	13	0.17(.32)	-	-	-	-	-	-
opa-17d$_{700}$	11	13	0.17(.32)	-	-	-	-	-	-	11	14	0.36(.45)
opa-17e$_{800}$	-	-	-	-	-	-	15	7	2.98(.91)	-	-	-
opa-18a$_{800}$	-	-	-	16	11	0.93(.66)	15	10	1.01(.68)	-	-	-
opa-18b$_{300}$	-	-	-	-	-	-	13	12	0.04(.16)	-	-	-
opa-20a$_{1300}$	-	-	-	-	-	-	-	-	-	15	10	1.01(.68)
opa-20b$_{500}$	-	-	-	-	-	-	-	-	-	13	12	0.04(.60)
opa-20c$_{800}$	-	-	-	-	-	-	17	9	2.50(.87)	14	11	0.36(.45)
opa-20d$_{1100}$	-	-	-	-	-	-	15	11	0.62(.57)	-	-	-
opb-13a$_{950}$	-	-	-	-	-	-	-	-	-	12	7	1.33(.75)
opb-13b$_{800}$	-	-	-	-	-	-	-	-	-	12	7	1.33(.75)
opb-14a$_{700}$	-	-	-	-	-	-	13	15	0.14(.29)	-	-	-
opb-14b$_{300}$	-	-	-	-	-	-	14	14	0.00(.00)	-	-	-
opb-16a$_{1400}$	9	10	0.05(.18)	-	-	-	-	-	-	-	-	-
opb-19a$_{1300}$	13	9	0.73(.61)	13	9	0.73(.61)	-	-	-	-	-	-
opb-20a$_{800}$	-	-	-	-	-	-	11	16	0.93(.66)	-	-	-
opb-20b$_{1100}$	-	-	-	-	-	-	14	13	0.04(.15)	-	-	-
opd-1a$_{1500}$	15	10	1.01(.68)	-	-	-	-	-	-	-	-	-
opd-1b$_{1300}$	-	-	-	-	-	-	13	9	0.73(.61)	-	-	-
opd-1c$_{850}$	17	8	3.31(.93)	-	-	-	-	-	-	13	9	0.73(.61)
opd-1d$_{650}$	-	-	-	-	-	-	12	10	0.18(.33)	14	8	1.66(.80)

Locus and its length as base pairs	Tree-1 P	A	χ2 test	Tree-2 P	A	χ2 test	Tree-3 P	A	χ2 test	Tree-4 P	A	χ2 test
opd-3a$_{1100}$	-	-	-	-	-	-	14	12	0.15(.30)[a]	-[b]	-	-
opd-3b$_{900}$	12	13	0.04(.16)	-	-	-	16	10	1.40(.76)	-	-	-
opd-3c$_{800}$	10	15	1.01(.68)	15	13	0.14(.29)	16	10	1.40(.76)	-	-	-
opd-3d$_{350}$	-	-	-	-	-	-	12	14	0.15(.30)	-	-	-
opd-11a$_{700}$	15	12	0.33(.44)	14	12	0.15(.30)	15	11	0.62(.57)	-	-	-
opd-11b$_{600}$	13	14	0.04(.15)	-	-	-	-	-	-	10	13	0.39(.47)
opd-11c$_{550}$	-	-	-	10	16	1.40(.76)	-	-	-	-	-	-
opd-15a$_{>1500}$	-	-	-	-	-	-	14	12	0.15(.30)	-	-	-
opd-15b$_{1250}$	17	10	1.83(.82)	17	10	1.83(.82)	-	-	-	-	-	-
opd-15c$_{850}$	15	12	0.33(.44)	-	-	-	11	15	0.62(.57)	-	-	-
opd-15d$_{600}$	-	-	-	-	-	-	17	9	2.50(.88)	-	-	-
ope-1a$_{>1500}$	14	14	0.00(.00)	-	-	-	-	-	-	-	-	-
ope-1b$_{1500}$	-	-	-	14	14	0.00(.00)	-	-	-	-	-	-
ope-1c$_{800}$	17	11	1.29(.74)	17	10	1.83(.82)	-	-	-	-	-	-
ope-1d$_{700}$	-	-	-	-	-	-	15	10	1.01(.68)	9	14	1.09(.70)
ope-9a$_{1400}$	14	9	1.09(.70)	11	12	0.04(.16)	10	13	0.39(.47)	-	-	-
ope-9b$_{1100}$	-	-	-	-	-	-	14	8	1.66(.80)	9	12	0.43(.49)
ope-9c$_{900}$	-	-	-	-	-	-	14	8	1.66(.80)	-	-	-
ope-12a$_{1500}$	14	13	0.04(.15)	-	-	-	-	-	-	-	-	-
ope-12b$_{1100}$	15	12	0.33(.44)	13	12	0.04(.16)	-	-	-	-	-	-
ope-12c$_{800}$	16	11	0.93(.66)	15	10	1.01(.68)	-	-	-	9	13	0.73(.61)
ope-12d$_{650}$	12	15	0.33(.44)	-	-	-	-	-	-	-	-	-
ope-12e$_{950}$	-	-	-	-	-	-	13	8	1.20(.73)	-	-	-
ope-12f$_{>1400}$	-	-	-	-	-	-	12	9	0.43(.49)	-	-	-
opf-3a$_{1400}$	16	10	1.40(.76)	-	-	-	13	11	0.17(.32)	-	-	-
opf-3b$_{1100}$	16	10	1.40(.76)	14	10	0.67(.59)	16	8	2.72(.90)	-	-	-
opf-5a$_{>1500}$	-	-	-	-	-	-	10	14	0.67(.59)	-	-	-
opf-5b$_{1000}$	-	-	-	-	-	-	12	12	0.00(.00)	-	-	-
opf-5c$_{850}$	14	13	0.04(.15)	-	-	-	-	-	-	-	-	-
opf-5d$_{700}$	14	13	0.04(.15)	-	-	-	-	-	-	-	-	-
opf-5e$_{600}$	19	8	4.61(.96)	-	-	-	13	11	0.17(.32)	-	-	-
opf-8a$_{1200}$	19	8	4.61(.96)	-	-	-	11	11	0.00(.00)	-	-	-
opf-8b$_{1100}$	11	16	0.93(.66)	12	14	0.15(.30)	-	-	-	9	13	0.73(.61)
opf-8c$_{800}$	14	13	0.04(.15)	11	15	0.62(.57)	-	-	-			
opf-8d$_{750}$	-	-	-	-	-	-	14	8	1.66(.80)	13	9	0.73(.61)
opf-8e$_{650}$	-	-	-	10	16	1.40(.76)	9	13	0.73(.61)	-	-	-
opf-8f$_{250}$	-	-	-	-	-	-	-	-	-	10	12	0.18(.33)
opf-16a$_{1100}$	16	12	0.57(.55)	-	-	-	-	-	-	-	-	-
opf-16b$_{900}$	16	12	0.57(.55)	12	14	0.15(.30)	12	10	0.18(.33)	9	15	1.52(.78)
opf-16c$_{800}$	19	9	3.65(.94)	-	-	-	-	-	-	-	-	-
opf-16d$_{250}$	12	16	0.57(.55)	12	14	0.15(.30)	-	-	-	-	-	-
opf-16e$_{700}$	-	-	-	9	17	2.50(.88)	-	-	-	-	-	-
opf-16f$_{>1200}$	-	-	-	-	-	-	14	8	1.66(.80)	-	-	-
opf-16g$_{500}$	-	-	-	-	-	-	-	-	-	11	13	0.17(.32)
oph-11a$_{1200}$	13	15	0.14(.29)	-	-	-	11	12	0.04(.16)	-	-	-
oph-11b$_{1100}$	-	-	-	-	-	-	-	-	-	14	11	0.36(.45)
oph-11c$_{750}$	16	12	0.57(.55)	-	-	-	13	10	0.39(.47)	-	-	-
oph-11d$_{650}$	-	-	-	-	-	-	12	11	0.04(.16)	-	-	-
oph-11e$_{450}$	-	-	-	-	-	-	-	-	-	-	-	-
oph-11f$_{400}$	-	-	-	12	12	0.00(.00)	-	-	-	-	-	-
oph-14a$_{>1500}$	-	-	-	10	14	0.67(.59)	-	-	-	-	-	-
oph-14b$_{1150}$	-	-	-	14	10	0.67(.59)	-	-	-	-	-	-
oph-14c$_{700}$	-	-	-	-	-	-	15	9	1.52(.78)	-	-	-
oph-14d$_{550}$	-	-	-	13	11	0.17(.32)	-	-	-	-	-	-
oph-14e$_{450}$	14	12	0.15(.30)	-	-	-	-	-	-	-	-	-
oph-17a$_{1100}$	19	9	3.65(.94)	-	-	-	-	-	-	-	-	-
opg-12a$_{>1500}$	16	10	1.40(.76)	16	8	2.72(.90)	-	-	-	-	-	-
opg-12b$_{650}$	16	10	1.40(.76)	11	13	0.17(.32)	12	13	0.04(.16)	15	10	1.01(.68)
opg-12c$_{450}$	15	11	0.62(.57)	-	-	-	-	-	-	13	12	0.04(.16)
opk-8a$_{600}$	16	12	0.57(.55)	9	15	1.52(.78)	-	-	-	-	-	-
opk-8b$_{500}$	16	12	0.57(.55)	-	-	-	-	-	-	-	-	-

[a]) The numbers in parenthesis are Chi-square prohabilities.
[b]) Data was not available either locus not being segregated 1:1 or locus was not present in the tree.

cant deviation from 1:1 segregation ratios after conducting Chi-square tests for them. This may be due to problems associated with reproducibility of RAPD analysis since PCR reactions were so sensitive to changes in the reaction conditions. As a result, on the average, the excluded RAPD bands varied from 0.38 per primer in tree 4 (38 RAPD bands were excluded) to 0.73 in tree 2 (71 such RAPD bands were excluded). The remaining polymorphic markers listed in *table 3* which did not show any significant deviation from 1:1 segregation ratio were used in linkage analysis.

Separate linkage analyses were conducted for each tree for 2 reasons; (1) there were very few loci common to 2 or more trees (*Table 3*), and (2) a combined analysis would have required determining close relationships between linked markers common to 2 or more trees prior to estimating linkages. The number of linkage groups with 2 or more markers ranged from

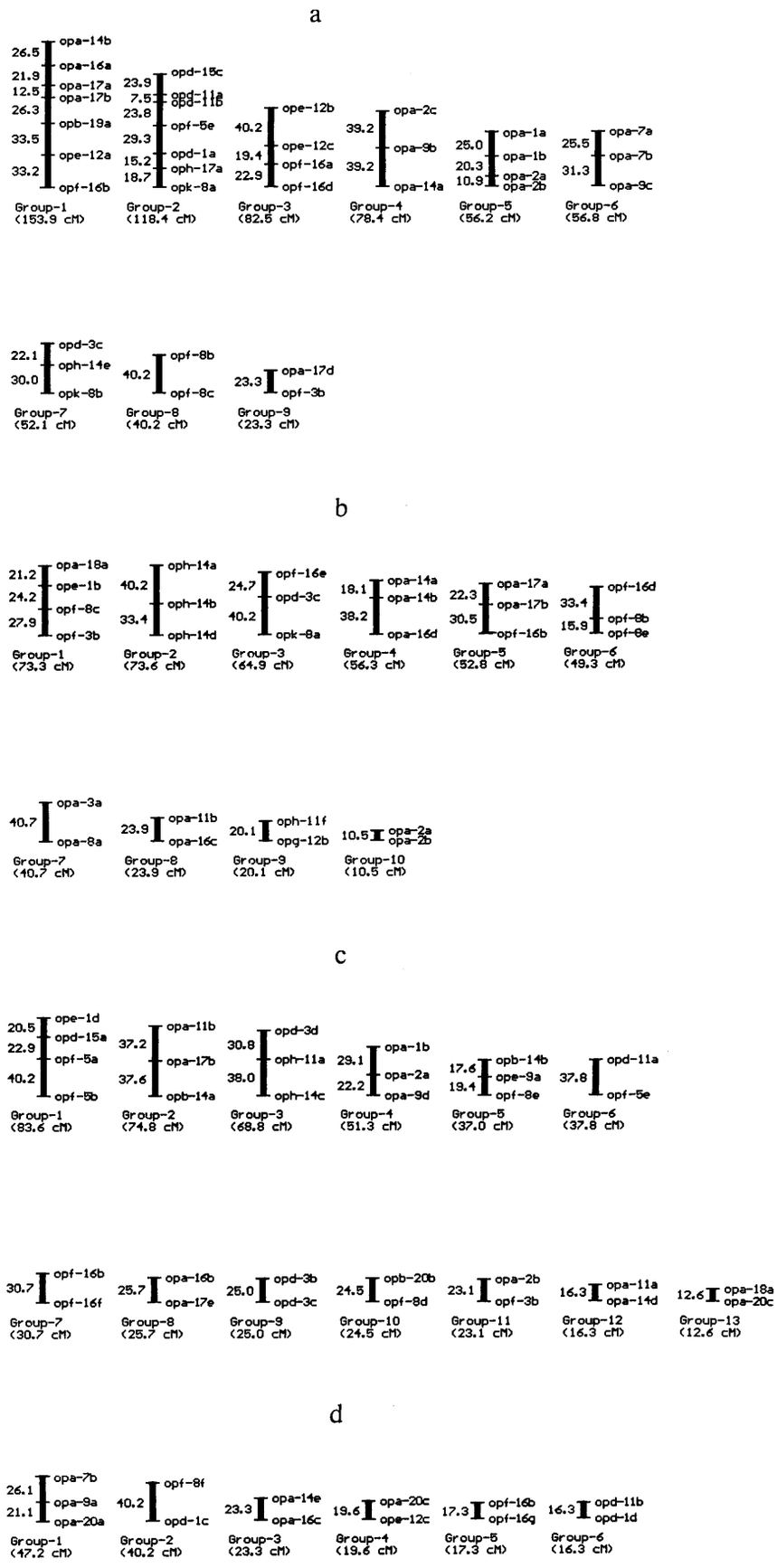

Figure 1. – RAPD linkage maps in Turkish red pine. The loci are listed on the right and map distance in centiMorgans on the left. a) Showing the linkage groups constructed by Mapmaker in tree 1. b) Showing the linkage groups constructed by Mapmaker in tree 2. c) Showing the linkage groups constructed by Mapmaker in tree 3. d) Showing the linkage groups constructed by Mapmaker in tree 4.

Table 4. – Number of segregating loci, number of unlinked loci, number of mapped loci, number of linkage groups and total map distance for each tree.

	Seed Trees			
	Tree-1	Tree-2	Tree-3	Tree-4
-Number of segregating loci	56	44	52	27
-Number of unlinked loci	21	17	20	14
-Number of segregating loci mapped	35	27	32	13
-Number of linkage groups	9	10	13	6
-Total map distance	661.8cM	465.4cM	511.2cM	163.9cM

6 (tree 4) to 13 (tree 3). In trees 1 and 2, the number of linkage groups were 9 and 10 respectively (*Table 4*). The total map distance covered by the linkage groups was variable. It ranged from 163.9 cM (6 linkage groups in tree 4) to 661.8 cM (9 linkage groups in tree 1) (*Table 4, Figure 1*). The number of segregating loci mapped to linkage groups (2 or more loci in each linkage groups) varied from 13 in tree 4 to 35 in tree 1. Many loci were unlinked in each tree (e.g., ranging from 14 loci in tree 4 to 21 loci in tree 1) (*Table 4*).

Considering the basic chromosome number for the Turkish red pine (n=12) and the number of constructed linkage groups as well as the total map distance covered by the linkage groups, it is obvious that we were unable to identify all linkage groups corresponding to all chromosomes. With the addition of new markers, the smaller linkage groups may join to other linkage groups and most of the unlinked RAPD loci will become parts of existing linkage groups. Furthermore, it was not possible to make valid comparisons among trees about linkage relationships due to the small number of markers common to the 4 trees.

Despite frequent use of RAPD markers in crop species for various purposes (e.g., determining genetic distances between cultivars of papaya (STILES *et al.,* 1993), constructing genetic linkage maps in blueberry (ROWLAND and LEVI, 1994), determining genetic polymorphisms in domesticated onion species (WILKE *et al.,* 1993)), there are few published reports of RAPD maps for forest trees (CARLSON *et al.,* 1991; GRATTAPAGLIA *et al.,* 1992; DALE *et al.,* 1992; TULSIERAM et al., 1992; NELSON *et al.,* 1993; GRATTAPAGLIA and SEDEROFF, 1994; BINELLI and BUCCI, 1994). It has not been demonstrated whether RAPD markers will be useful for breeding or gene resource conservation in forest species.

The subject of genetic mapping and the choice of molecular markers for mapping in forestry have been discussed thoroughly in NEALE and HARRY (1994). They pointed out that the most useful genetic markers for forest trees should have some desirable attributes such as (1) abundant polymorphic loci with multiple alleles, (2) having quick and inexpensive assays, (3) shared among related species, (4) detectable in different tissues, in different developmental stages, and (5) showing codominance.

This study shows that RAPD markers have met with some of the above requirements such as being simple, quick and inexpensive to apply and detectable in different tissues. Lack of codominance restricts the use of RAPD markers to single tree genetic mappings using haploid material. Since most forest tree species have not been fully domesticated, RAPD markers for studying genetic diversity in both natural and plantation populations will not be used in a great deal in forestry for a while. However, as specific genetic materials from inbreeding and backcross breeding programs become available for many tree species, these makers will be preferred over other markers. At the moment, RAPD markers do not offer sufficient advantages over isozymes to replace them as the markers of choice for studies of genetic diversity. RAPD markers also lack reproducibility among labs, experiments, and also among individual DNA preparations. In the present study, it was found that PCR reactions were very sensitive to many factors, e.g., DNA preps, $MgCl_2$ concentrations, cycling conditions etc. Also, comparisons of markers in genetically different samples only on molecular weight basis were very difficult. Thus, the results of the present and previous studies indicate that forest geneticists have to develop new type of DNA markers since RFLP is technically demanding and RAPD markers do not have a sufficiently wide range of application in forestry. The new type of marker will certainly be based on PCR because of its technical simplicity and inherent power. In fact, in some laboratories, investigations toward the development of new DNA markers quick and inexpensive to apply (NEALE and HARRY, 1994) have already started and they will be available soon.

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Survival, Growth Trends and Genetic Gains in 17-year Old *Picea abies* Clones at Seven Test Sites

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Abstract

Rooted cuttings (stecklings) from 40 different clones, and seedlings from one seed source of Norway spruce (*Picea abies* L. KARST.) were planted on 7 contrasting test sites in northern Germany. Survival rates and total heights (Ht) were observed at ages 3, 5, 8, 10, 13 and 17 years. Diameters at breast height (dbh) were also measured at age 17. Test site means for survival rate ranged from 81% to 95%, except site Kattenbühl (73%). About 70% of all deaths on the test sites occured within the first four growing seasons after outplanting. Clones taller in nursery tended to show higher death rates in the early years in the field than shorter clones. There were significant differences among the test sites in survival rates, but no rank interactions over the years. Seedlings and stecklings from the same origin (i.e. Westerhof) showed similar survival rates at all the test sites.

Stecklings planted on low elevation test sites showed better Ht performance than those at high elevation test sites (at age 17 years avg Ht at Syke 826 cm, at Lautenthal 492 cm). Overall means for Ht, dbh and volume index (VI) were 648 cm, 88 mm and 48.9 dm^3, respectively. Both the test sites and clones showed statistically significant differences in Ht, dbh and VI values. There were also significant clone x site interactions. Overall steckling Ht and VI values were larger than those of seedlings, relative difference being 11% and 37%, respectively, at age 17 years. Steckling heights at nursery were not reliable enough to predict future field performances. Broad sense heritabilities for Ht was 0.14, and for dbh was 0.13 at age 17 years. When 20% of the clones (8 clones out of 40) were selected, expected genetic gain in Ht is about 10.0%, in VI it is 33.0%.

Key words: Norway spruce, survival, height growth, clonal forestry, genetic gain, heritability.

FDC: 165.441; 232.11; 174.7 *Picea abies.*

Introduction

"Clonal Forestry" came into the scientific scene more intensively in the late 1960s. Its theoretical grounds and promising potentials in practical forestry have been discussed by several authors (e.g. KLEINSCHMIT et al., 1973; LEPISTO, 1974; TODA, 1974; SHELBOURNE and THULIN, 1974; KLEINSCHMIT and SCHMIDT, 1977; ROULUND, 1981; LIBBY, 1983). Along with these developments, many clonal experiments have been established at that period to realize the results of these potentials. The recent 2 books edited by AHUJA and LIBBY (1993) give an excellent overall review of the topic.

This study presents the results of 1 of those early clonal experiments which started in the late 1960s at the Lower Saxony Forest Research Institute (LSFRI), Dept. of Forest Tree Breeding. The objectives of this study are to examine the survival and growth trends over years in 17-year old trees, and estimate heritabilities for height and diameter growths.

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