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Segregating random amplified polymorphic DNAs (RAPDs) in *Betula alleghaniensis*

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Summary. Molecular markers are currently being developed for *Betula alleghaniensis* Britton using random amplified polymorphic DNA (RAPD). Arbitrarily designed 11-mer primers were tested on three intraspecific controlled crosses for which more than 15 full-sibs were available. Using two of these primers, we were able to genetically characterize a total of nine polymorphic RAPD markers. Segregation of these markers was consistent with a biparental diploid mode of inheritance, and all appeared dominant. RAPDs were valuable in detecting contaminants and, therefore, in assessing the validity of controlled crosses. Limitations of the technique are discussed in relation to the determination of parental genotypes and construction of linkage maps for hardwood species.

Key words: RAPD – *Betula alleghaniensis* – Segregation analysis – Genetic markers – Controlled crosses

Introduction

Isozymes have proven to be useful markers for estimating genetic diversity and population structure in hardwood species (Li et al. 1992). However, isozyme loci are in limited number because they are restricted to genes encoding soluble proteins. In contrast, DNA markers allow direct access to the coding and non-coding regions of the genome, making their number potentially unlimited. Several methods have been developed to detect DNA polymorphisms in hardwoods. The most common approach relies on restriction fragment length polymorphisms (RFLP) using conserved or variable regions of the genome as DNA probes. Probes consisting of conserved ribosomal genes (rDNA) or chloroplast DNA (cpDNA) have been used to study interspecific variability, to monitor gene flow between species or to study evolutionary relationships, while hypervariable DNA probes such as M13 have been valuable in distinguishing individuals within species (Strauss et al. 1992; Li et al. 1992). However, the main disadvantages of RFLPs are that the technique involves DNA transfer and hybridizations, and requires quite large amounts of DNA.

Based on the polymerase chain reaction (PCR), a new class of DNA markers called random amplified polymorphic DNA (RAPD), arbitrarily primed PCR (AP-PCR), or DNA amplification fingerprinting (DAF) have been recently developed to detect genetic variation among individuals within plant species (Williams et al. 1990; Welsh et al. 1991 a, b; Caetano-Anollés et al. 1991; Goodwin and Annis 1991; Hu and Quiros 1991). RAPDs have also been used to construct genetic maps (Welsh et al. 1991 a; Carlson et al. 1991; Klein-Lankhorst et al. 1991; Michelmore et al. 1991; Giovannoni et al. 1991) and to evaluate levels of gene flow between species (Arnold et al. 1991). Moreover, RAPD markers have proved to be useful for parentage determination (Welsh et al. 1991 b). The technique used to generate RAPDs consists of amplifying unspecified regions of genomic DNA with single or combined short primers of arbitrary nucleotide sequence and thus, does not require a specific sequence information for the design of amplification primers. It uses very small amounts of template DNA and can rapidly generate fingerprints of DNA fragments that appear to be inherited in a Mendelian fashion (Williams et al. 1990; Hu and Quiros 1991). One limitation of RAPDs is that in most cases, heterozygotes cannot

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be directly identified because of the dominant nature of these markers, as compared to isozyme and RFLP markers that are usually co-dominant and consequently facilitate the identification of heterozygotes (Neale and Williams 1991).

Recently, the Ministère des Forêts du Québec has initiated a tree-improvement program for *Betula alleghaniensis* Britton (yellow birch), a species of high economic value in eastern Canada. As part of this program, the objectives of the study presented here were: (1) to develop RAPD markers and (2) to determine the mode of inheritance of these markers by using controlled crosses. In this paper, we show that RAPDs are valuable for assessing the validity of controlled crosses, and also that the observed segregation of the markers among the full-sibs is consistent with a biparental mode of inheritance and supports the dominant nature of RAPDs.

Materials and methods

Plant material

Branches from 36 individuals of *Betula alleghaniensis* Britton were collected in natural stands located in different regions of the province of Québec (Canada). The scions were carefully emasculated and grafted in mid-February (1990). Controlled pollinations were performed in the greenhouse on grafted branches bearing flowers using the bottle graft method as described by Clausen (1973) and Wright (1976). Pollen was obtained by forcing maturation on branches placed in water in the greenhouse (Johnson 1945) and was stored as described by Clausen (1973). Female flowers emerged 3–5 weeks after grafting. These were bagged and pollinated with approximately 0.1 cc of pollen per bag using a syringe and a needle to pierce the bag. The flowers were pollinated at least twice at 2-day intervals. The bags were removed after the receptive stage of the female flowers, and the seeds were allowed to mature in the greenhouse. The seeds were collected in September and October, cleaned and stored at 3 °C until sowing; they were germinated in early March (1991) on seed beds of peatmoss, vermiculite, perlite and sand (6:2:1:1), under mist irrigation, and transferred to seedling containers using the same substrate. Seedlings were irrigated and fertilized following standard nursery methods. The controlled crosses that were studied involved 11 parents (clones 304, 305, 308, 401, 404, 501, 502, 601, 603, 607, and 704).

Total genomic DNA isolation

DNA extractions from leaves of grafted parents and young seedlings were performed according to the method of Bousquet et al. (1990) with a few modifications. Approximately 100 mg of fresh or frozen (−80 °C) tissue was ground under liquid nitrogen and homogenized in an extraction buffer (100 m*M* TRIS, 1.4 *M* NaCl, 20 m*M* EDTA, 2% CTAB, 0.2% β-mercaptoethanol, pH 9.5). The homogenate was then incubated at 65 °C for 30 min, emulsified with an equal volume of chloroform-isoamyl alcohol (24:1), and centrifuged at 13,000 *g* for 15 min. The aqueous phase was transferred to a new 1.5-ml Eppendorf tube and emulsified again until a clear aqueous phase was obtained. DNA was precipitated with the addition of 1/10 volume of 3 *M* sodium acetate and an equal volume of isopropanol. After 15 min of centrifugation at 13,000 *g*, the DNA pellet was washed with 70% ethanol, vacuum dried, and resuspended in about 75 µl of TE buffer (10 m*M* TRIS, pH 8.0, 0.1 m*M* EDTA, pH 8.0). DNA concentration was measured using a fluorometric assay (Cesarone et al. 1979).

DNA amplification (RAPD)

Reaction mixtures (25 µl) contained 10 m*M* TRIS-HCl (pH 8.3), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.001% gelatin, 100 µ*M* of each dNTP (Pharmacia), 0.25 µ*M* of primer, 20–75 ng of genomic DNA and 0.2 units of *Taq* DNA polymerase (Perkin Elmer Cetus) overlayed with mineral oil. Amplification was conducted in a thermal cycler model N801-0150 (Perkin Elmer Cetus). To prevent DNA synthesis that could result from the non-specific pairing of primers below 37 °C, a "hot start" was performed at 72 °C for every amplification experiment (D'Aquila et al. 1991; Mullis 1991). The amplification conditions, similar to those of Williams et al. (1990), were as follows: 45 cycles, each consisting of a denaturation step of 1 min at 94 °C, followed by an annealing step of 1 min at 37 °C, and an extension step of 2 min at 72 °C. The last 25 extension steps were progressively extended by 5 s/cycle. The last cycle was followed by 10 min at 72 °C to ensure that primer extension reactions proceeded to completion. Heteroduplex formation was verified on RAPD products using the following program: a 2-min denaturation step at 94 °C followed by a 1-h renaturation step where the temperature of the heating bloc gradually decreased until it reached 65 °C, thus allowing slow reannealing of the DNA molecules. The amplification products were electrophoresed in 2% agarose gels and visualized by staining with ethidium bromide. The reproducibility of the major amplification products was tested at least twice for each experiment. The molecular weight marker used was ϕX174 digested with *Hae*III (Pharmacia).

Six 11-mer primers of arbitrary sequence (except for T at the last position) were tested (see Table 1). Before they were synthesized, primers were checked for internal secondary structures using the UWGCG (University of Wisconsin Genetic Computer Group) system. Primer synthesis was performed with an Applied Biosystem 391 DNA synthesizer. Protecting groups were removed in 30% ammonium hydroxide at 55 °C for 5 h, and the primers were purified on Sep Pak C18 cartridges (Waters).

Genetic analysis of RAPD variants

In the genetic analysis, only those major fragments whose presence or absence was unambiguous were retained. On the basis of the assumption that there is a diploid single locus mode of inheritance and that RAPDs are dominant markers, a parent not harboring a fragment was attributed a recessive genotype (*aa*) whereas the presence of the fragment was interpreted either as a dominant-heterozygote (*Aa*) or a dominant-homozygote (*AA*) genotype. Observed frequencies of presence versus absence of a fragment in the full-sibs were confronted with the expected

Table 1. Eleven-mer primers tested in this study

Name	Sequence
MMUL111	5′-TCGATCTACGT-3′
MMUL112	5′-GTCAATCCGAT-3′
MMUL113	5′-ATCCTATGGGT-3′
MMUL114	5′-GGCATCGTAAT-3′
MMUL115	5′-AACTGAGCGCT-3′
MMUL116	5′-GTATCGCAGAT-3′

Table 2. Results of segregation analysis of RAPD markers for cross 603 (♀) × 404 (♂)

Primer	Fragment (bp)	Parents	Full-sibs				
		Putative genotypes 603 × 404	Expected		Observed		Probability
			aa	*Aa*+*AA*	*aa*	*Aa*+*AA*[a]	
MMUL114	1,280	*aa* × *Aa*	9.5	9.5	7	12	0.096
	440	*aa* × *Aa*	9.5	9.5	9	10	0.176
	410	*Aa* × *Aa*	4.8	14.2	3	16	0.152
MMUL112	1,010	*aa* × *Aa*	9.5	9.5	3	16	0.002*
	450	*aa* × *Aa*	9.5	9.5	12	7	0.096

* Significant at $P<0.05$; that is, the model is rejected

[a] Heterozygote (*Aa*) and dominant homozygote (*AA*) classes were pooled since they are undistinguishable

1:1 (from crossing *aa* × *Aa*) or 3:1 (from crossing *Aa* × *Aa*) ratios. Lack of fit between observed and expected frequencies was assessed using the binomial law (Sokal and Rohlf 1981), and the level of significance was fixed at $P<0.05$. Since the number of full-sibs was small, these tests are to be interpreted cautiously.

Southern hybridization

RAPDs were electrophoresed as described previously and transferred overnight onto GenescreenPlus nylon membranes (DuPont) using 0.4 *M* NaOH and 1.0 *M* NaCl as transfer solution. The membranes were neutralized in 0.5 *M* TRIS-HCl and 2.5 *M* NaCl for 10 min. The selected RAPD fragment to be used as a probe was excised from a 2% low-melting-point agarose gel and labelled with α-[^{32}P]dCTP using the ^{T7}QuickPrime kit (Pharmacia) following the manufacturer's recommendations. Hybridization and washes were performed at 65 °C in a rotary hybridization oven (Robbins Scientific) according to the manufacturer's instructions (GenescreenPlus, DuPont). The membranes were then exposed with a X-Omat AR film (Kodak) at room temperature for one hour.

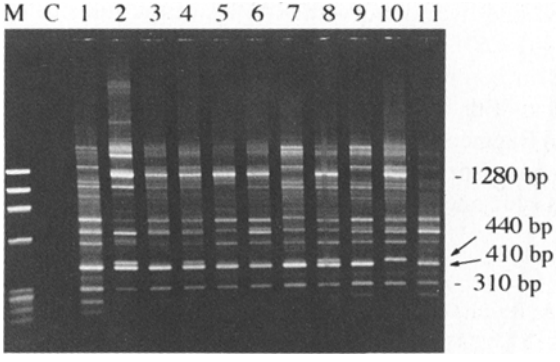

Fig. 1. Gel electrophoresis of RAPD fragments obtained with primer MMUL114 for cross 603 (♀) × 404 (♂). *Lane M* is molecular marker ϕX174 digested with *Hae*III (1,353, 1,078, 872, 603, 310, 281, 271, 234, 194, 119 bp). *Lane C* is the negative control without DNA. *Lanes 1* and *2* are parents 603 and 404, respectively. *Lanes 3–11* show RAPD products for 9 out of 19 full-sibs of the cross. The sizes of the fragments considered in the segregation analysis are indicated on the *right*

Results

In order to examine the extent of variability of RAPD markers in the parental generation, DNA was extracted from the 11 parents involved in the intraspecific controlled crosses and was amplified using each of six 11-mer primers (see Table 1). The use of four primers resulted in the production of faint bands or in the complete absence of fragments (MMUL111, 113, 115, and 116). However, primers MMUL112 and MMUL114 revealed simple and intense band patterns that could be inferred genetically.

Following this initial screening of variation, three intraspecific controlled crosses were selected for segregation studies based on differences in fragment patterns of the parents and on the availability of a minimum number of 15 full-sibs: 603 (♀) × 404 (♂), 704 (♀) × 601 (♂), and 401 (♀) × 601 (♂). DNA was extracted from their progeny, and primers MMUL112 and MMUL114 were used for amplification. Each experiment was replicated and resolved the same products. No heteroduplex formation was observed. For a given cross and primer combination, the number of full-sibs that had or did not have a specific fragment transmitted from one or both parents was scored, and the binomial law was used to test if the observed segregation was consistent with a biparental diploid mode of inheritance.

Primer MMUL114

RAPDs obtained with primer MMUL114 for the intraspecific cross 603 (♀) × 404 (♂) and for some of its 19 full-sibs are shown in Fig. 1. Four fragments (1,280, 440, 410, and 310 bp) were considered for analysis, of which three (1,280, 440, and 410 bp) segregated in a diploid dominant-recessive fashion (Table 2). For example, the 440-bp fragment was absent in parent 603 but present in parent 404 and in 10 out of 19 full-sibs. A putative genotype (*aa*) (absence of the fragment) could be deduced for parent 603 and an *Aa* genotype (presence of the frag-

ment) for parent 404. The observed segregation ratio among the full-sibs fitted the expected 1:1 ratio (from crossing $aa \times Aa$) (Table 2). The 310-bp fragment was present in the whole family so that parental genotypes could not be determined.

For cross 401 (♀) × 601 (♂) and its 17 full-sibs, five fragments (1,280, 950, 850, 500 and 410 bp) were considered for analysis, of which four segregated among the F_1 progeny (Fig. 2a). A 410-bp fragment unexpectedly appeared in 3 out of 17 full-sibs, whereas neither parent 401 nor 601 harbored it (Fig. 2a). However, this 410-bp fragment was observed in other parents such as 404, 603, and 704 (Figs. 1, 2c, and 3). This indicated a possible case of cross contamination that could have occurred during the elaboration of controlled cross 401 × 601. To ascertain this hypothesis, the 410-bp fragment of parent 704 was labelled and hybridized with RAPDs of the full-sibs of cross 401 × 601 and with RAPDs of the 11 parents involved in controlled crosses. The hybridization signals shown in Fig. 2b and d clearly demonstrate that the 410-bp fragment was detected only in the 3 putative contaminant offsprings and in 8 out of 11 parents, but not in parents 401 and 601. Consequently, there were 3 illegitimate full-sibs within this particular family. The number of legitimate offsprings was thus reduced to 14 for this cross. Two fragments (1,280 bp and 500 bp) segregated in a diploid dominant-recessive fashion (Table 3). For the 950-bp fragment, the 1:1 ratio that was expected from crossing $aa \times Aa$ was not observed in this cross, but a diploid model was accepted in the next cross (see below). The 850-bp fragment was found to be present in both parents and all F_1 progeny. Putative parental genotypes are indicated in Table 3.

Finally, the third cross 704 (♀) × 601 (♂) and its 24 full-sibs (partly shown in Fig. 3) were analyzed. In this cross, six fragments were considered for analysis (Fig. 3, Table 4). Because this cross involved parent 601, it shares three segregating fragments that were analyzed previously for cross 401 × 601 (1,280, 950, and 410 bp) The distribution of some fragments among the parents and full-sibs of cross 704 × 601 indicated other potential contaminants. For example, the 850-bp fragment which was present in both parents was absent in 1 out of 24 full-sibs (not shown). Moreover, the 690-bp fragment of parent 704 was absent in 2 full-sibs (Fig. 3), which is unexpected if parent 704 is to be considered homozygous for the

Table 3. Results of segregation analysis of RAPD markers for cross 401 (♀) × 601 (♂)

Primer	Fragment (bp)	Parents	Full-sibs				
		Putative genotypes 401 × 601	Expected		Observed		Probability
			aa	*Aa*+*AA*	*aa*	*Aa*+*AA*[a]	
MMUL114	1,280	*aa* × *Aa*	7	7	7	7	0.210
	950	*aa* × *Aa*	7	7	12	2	0.006*
	500	*Aa* × *Aa*	3.5	10.5	5	9	0.147
MMUL112	1,010	*Aa* × *Aa*	3.5	10.5	4	10	0.220

* Significant at $P < 0.05$; that is, the model is rejected
[a] Heterozygote (*Aa*) and dominant homozygote (*AA*) classes were pooled since they are undistinguishable

Table 4. Results of segregation analysis of RAPD markers for cross 704 (♀) × 601 (♂)

Primer	Fragment (bp)	Parents	Full-sibs				
		Putative genotypes 704 × 601	Expected		Observed		Probability
			aa	*Aa*+*AA*	*aa*	*Aa*+*AA*[a]	
MMUL114	1,280	*aa* × *Aa*	10.5	10.5	12	9	0.140
	950	*Aa* × *Aa*	5.2	15.8	5	16	0.199
	690	*AA* × *aa*	0	21	0	21	–
	500	*aa* × *Aa*	10.5	10.5	10	11	0.168
	410	*Aa* × *aa*	10.5	10.5	6	15	0.026*
MMUL112	1,010	*Aa* × *Aa*	5.2	15.8	5	16	0.199

* Significant at $P < 0.05$; that is, the model is rejected
[a] Heterozygote (*Aa*) and dominant homozygote (*AA*) classes were pooled since they are undistinguishable

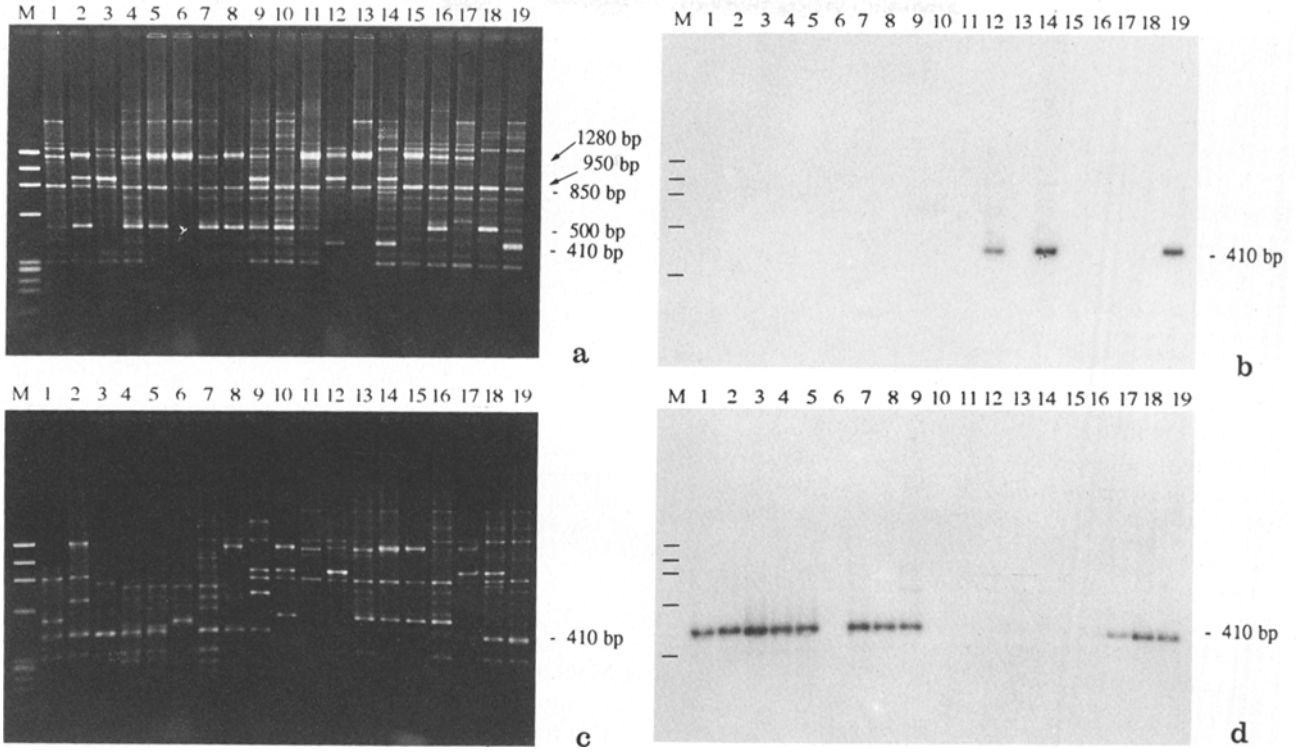

Fig. 2a–d. Gel electrophoresis and Southern hybridizations of RAPD fragments used for the detection of contaminants among the full-sibs of cross 401 (♀) × 601 (♂). **a** RAPD fragments obtained with primer MMUL114 for cross 401 × 601 and its 17 full-sib progeny. **b** Southern hybridization of the 410-bp fragment from parent 704 to the RAPDs shown in **a**; *lanes 12*, *14* and *19* correspond to full-sibs 10, 12 and 17, respectively, determined to be contaminants. For **a** and **b**, *lanes 1* and *2* are parents 401 and 601, respectively; *lanes 3–19* are the 17 full-sibs of the cross. **c** RAPD fragments obtained with primer MMUL114 for several *B. alleghaniensis* parents and for some full-sibs of cross 401 × 601. **d** Southern hybridization of the 410-bp fragment of parent 704 to the RAPDs shown in **c**. For **c** and **d**, *lanes 1–11* are parents 304, 305, 308, 501, 502, 607, 603, 404, 704, 601 and 401, respectively; *lanes 12–19* are full-sibs 1, 3, 6, 7, 8, 10, 12 and 17, respectively, of cross 401 × 601. *Lane M* is molecular marker ϕX174 digested with *Hae*III. In Southern hybridizations, only the 1,353-, 1,078-, 872-, 603-, and 281-bp fragments of ϕX174 are indicated in *lanes M*

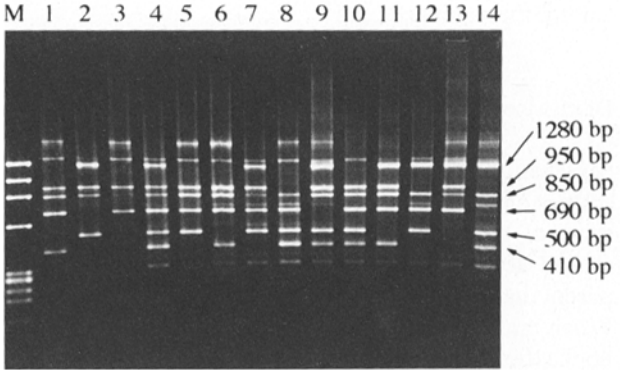

Fig. 3. Gel electrophoresis of RAPD fragments obtained with primer MMUL114 for cross 704 (♀) × 601 (♂). *Lane M* is molecular marker ϕX174 digested with *Hae*III. *Lanes 1* and *2* are parents 704 and 601, respectively. *Lanes 3–14* show RAPD products for 12 out of 24 full-sibs of the cross. The sizes of the fragments considered in the segregation analysis are indicated on the *right*

dominant allele. Therefore, a total of 3 full-sibs might be illegitimate and were thus excluded from the analysis (Table 4). Fragments of 850 and 690 bp appeared in all full-sibs while three other fragments (1,280, 950, and 500 bp) segregated in a diploid dominant-recessive fashion. Note that segregation of the 1,280-bp fragment in crosses 603 × 404 and 401 × 601, and the 500-bp fragment in cross 401 × 601 (Tables 2, 3, and 4) also fitted the expected ratios. In cross 704 × 601, the diploid dominant-recessive model was rejected for the 410-bp fragment. However it was accepted for the cross 603 × 404 (see Table 2).

Primer MMUL112

RAPD patterns of some of the full-sibs of crosses 603 (♀) × 404 (♂) and 704 (♀) × 601 (♂) are illustrated in Figs. 4 and 5, respectively. Only two segregating fragments, 1,010 and 450 bp, were considered for analysis

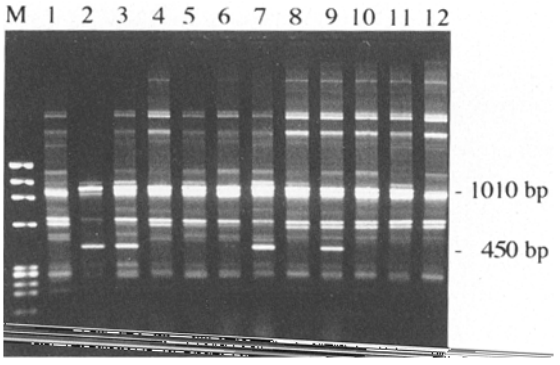

Fig. 4. Gel electrophoresis of RAPD fragments obtained with primer MMUL112 for cross 603 (♀) × 404 (♂). *Lane M* is molecular marker ϕX174 digested with *Hae*III. *Lane 1* and *2* are parents 603 and 404, respectively. *Lanes 3–12* show RAPD products for 12 out of 24 full-sibs of the cross. The sizes of the fragments considered in the segregation analysis are indicated on the *right*

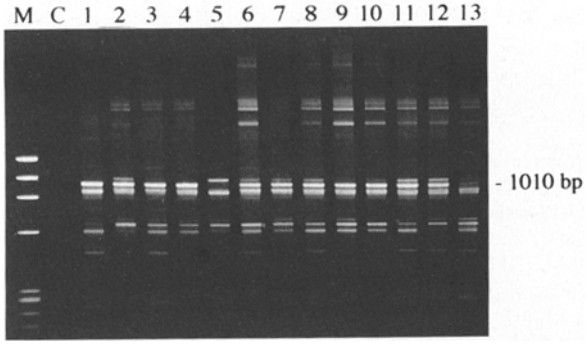

Fig. 5. Gel electrophoresis of RAPD fragments obtained with primer MMUL112 for cross 704 (♀) × 601 (♂). *Lane M* is molecular marker ϕX174 digested with *Hae*III. *Lane C* is the negative control without DNA. *Lanes 1* and *2* are parents 704 and 601, respectively. *Lanes 3–13* show RAPD products for 11 out of 24 full-sibs of the cross. The size of the fragment considered in the segregation analysis is indicated on the *right*

because these were intense and easy to score. The 930-bp fragment just below the 1,010-bp fragment was discarded because it appeared to be composed of two fragments (see Fig. 5). Putative parental genotypes and segregation ratios are shown in Tables 2, 3, and 4. The 450-bp fragment, present in parent 404 but not in other parents, was detected in 7 out of the 19 offsprings of cross 603 × 404. Thus, a homozygote recessive genotype (*aa*) and a dominant heterozygote genotype (*Aa*) were deduced for parents 603 and 404, respectively. The observed frequencies of presence versus absence of the fragment fitted the expected 1:1 ratio (Table 2). The 1,010-bp fragment was present in all the parents involved in the three crosses analyzed. The observed segregation of this fragment in the progenies fitted the expectations for crosses 401 × 601 and 704 × 601, but not for cross 603 × 404 (Tables 2, 3, and 4). As for the 950- and 410-bp fragments of primer MMUL114, this discrepancy between crosses could be accounted for by the small number of F_1 progeny available for the segregation analysis.

Table 5. Multilocus genotypes deduced from RAPDs

Primer	Fragment (bp)	Parent				
		401	404	601	603	704
MMUL114	1,280	*aa*	*Aa*	*Aa*	*aa*	*aa*
	950	*aa*	*aa*	*Aa*	*aa*	*Aa*
	850	*A–*	*aa*	*A–*	*aa*	*A–*
	690	*aa*	*aa*	*aa*	*aa*	*AA*
	500	*Aa*	*aa*	*Aa*	*aa*	*aa*
	440	*aa*	*Aa*	*aa*	*aa*	*aa*
	410	*aa*	*Aa*	*aa*	*Aa*	*Aa*
	310	nd	*A–*	nd	*A–*	nd
MMUL112	1,010	*Aa*	*Aa*	*Aa*	*aa*	*Aa*
	450	*aa*	*Aa*	*aa*	*aa*	*aa*
Heterozygosity[a]		0.25	0.56	0.50	0.11	0.38

nd, not determined
[a] Average observed heterozygosity is 0.36

Multilocus parental genotypes deduced from the segregation analysis of RAPD markers are summarized in Table 5. In addition to the parental genotypes, parents of a given cross were genotyped *aa* when they did not harbor a fragment that was present in another cross. For instance, controlled cross 603 × 404 did not exhibit the 690-bp fragment that was present in cross 704 × 601. Thus, parents 603 and 404 were attributed a recessive genotype (*aa*) for this particular locus. Therefore, if the MMUL114-310-bp fragment is excluded, a total of nine polymorphic RAPD markers among the parents could be obtained. As expected from such a small number of loci, heterozygosity was found to be quite variable among the parents.

Discussion

Segregation analysis of RAPD markers among the offsprings has led to the detection of illegitimate full-sibs in some of our controlled crosses. In one case, we have clearly demonstrated that pollen from more than one parent had contributed to the controlled pollination. When multiple crosses are performed, the unintentional application of pollen from more than one parent is likely to occur. Furthermore, accidental mixing of pollen due to the mislabelling of ramets or an inadequate pollen-isolation technique could also account for pollen contaminations. In conifers, variable error rates in controlled crosses have also been detected (Adams et al. 1988). The importance of making available powerful molecular or

biochemical markers to monitor the identity of pedigree material within the framework of a tree-improvement program is thus obvious. RAPDs have been used to determine parentage in maize hybrids (Welsh et al. 1991 b). Our results show that RAPD markers could be useful, in addition to isozymes and RFLP markers, in assessing the validity of controlled crosses or in conducting paternity analysis.

The segregation of all of the variable markers in the F_1 progenies was generally consistent with a biparental diploid mode of inheritance, and all of the markers appeared to be dominant, as also reported in Douglas-fir and white spruce (Carlson et al. 1991), in broccoli and cauliflower (Hu and Quiros 1991), in lettuce (Michelmore et al. 1991), in tomato (Giovannoni et al. 1991) and in canola (Deragon and Landry 1992). RAPDs were mostly found to be dominant and rarely co-dominant in a study on soybean (Williams et al. 1990). These authors also reported that in a genetic map of *Neurospora crassa*, of the 88 RAPD markers tested, 84 were dominant and only 4 were co-dominant. In our study, homozygote and heterozygote genotypes could be determined unambiguously in most cases using progeny arrays. However, when a fragment was detected in both parents and in all of the full-sib family, it was impossible to clearly distinguish between a dominant homozygote (*AA*) and a dominant heterozygote genotype (*Aa*). To discriminate between these genotypes, one would need to test progenies from more crosses involving one of the parents with other parents that do not exhibit these specific fragments. Alternatively, if haploid tissues were readily available, the allelic composition of any individual for a given locus could be determined more rapidly. In gymnosperms, megagametophytes are haploid and can be used to determine parental genotypes, whereas in angiosperms, the endosperm contains a genetic contribution of both parents. This advantage of gymnosperms over angiosperms may enable the construction of single tree linkage maps using RAPDs (Carlson et al. 1991). In angiosperms, pollen could represent a valuable source of haploid cells. However, additional anther culture manipulations render the technique less manageable. Because of their dominant nature, RAPD markers are therefore less informative than co-dominant isozyme and RFLP markers.

Nevertheless, we feel that RAPDs are potent molecular markers, not only for fingerprinting purposes and the construction of linkage maps, but also for assessing the validity of controlled crosses. They can be easily and rapidly obtained, and are particularly useful when vegetative material is available in minute amounts, and when tissues contain high levels of secondary metabolites that inhibit enzyme activity, such as in *B. alleghaniensis*. Under these conditions, isozymes and RFLPs are of limited value. Also, new strategies are being developed aimed at expanding the informative value of RAPD markers in angiosperms. The use of inbred isogenic lines to construct RAPD linkage maps is one of these strategies (Williams et al. 1990), but these lines can hardly be obtained with hardwoods. The recently reported 'bulked segregant analysis' strategy (Michelmore et al. 1991; Giovannoni et al. 1991) seems to be one of the most promising approaches to detect quantitative trait loci (QTLs) in woody angiosperms using RAPDs. This technique consists of forming two groups of bulked DNA samples from a segregating population of a single cross. Each bulk contains individuals that are identical for a particular trait but arbitrary at all unlinked regions. The two bulks are then screened for polymorphisms using RAPD markers. Segregation analysis of the markers confirm that they are effectively linked to the trait of interest. This illustrates the promising potential of RAPDs in tree-improvement programs of hardwoods.

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