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RAPDs as an aid to evaluate the genetic integrity of somatic embryogenesis-derived populations of *Picea mariana* (Mill.) B.S.P.

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Summary. The usefulness of random amplified polymorphic DNA (RAPD) in assessing the genetic stability of somatic embryogenesis-derived populations of black spruce [*Picea mariana* (Mill.) B.S.P.] was evaluated. Three arbitrary 11-mer primers were successfully used to amplify DNA from both in-vivo and in-vitro material. Twenty-five embryogenic cell lines, additional zygotic embryos and megagametophytes from three controlled crosses involving four selected genotypes of black spruce were used for the segregation analysis of RAPD variants. Ten markers were genetically characterized and used to evaluate the genetic stability of somatic embryos derived from three embryogenic cell lines (one cell line per cross, 30 somatic embryos per cell line). No variation was detected within clones. The utilization of RAPD markers both for the assessment of genetic stability of clonal materials and to certify genetic stability throughout the process of somatic embryogenesis is discussed.

Key words: RAPD – *Picea mariana* – Genetic markers – Genetic stability – Somatic embryogenesis

Introduction

Since somatic embryogenesis was first demonstrated in spruce by Hakman et al. (1985), thousands of somatic embryo-derived plants have been produced and are currently being evaluated in the field (von Arnold and Hakman 1988; Becwar et al. 1989; Attree et al. 1990; Roberts et al. 1990a, b; Webster et al. 1990). Before somatic embryogenesis can be exploited for clonal propagation in reforestation programmes, the genetic integrity of the somatic embryo-derived plants, or emblings, must be evaluated.

Plantlets derived from in-vitro culture might exhibit somaclonal variation (Larkin and Scowcroft 1981) which is often heritable (Larkin et al. 1984; Breiman et al. 1987) and therefore, results from genetic change (Karp and Bright 1985; Karp 1991). Several types of genetic changes associated with somaclonal variation have been reported, notably variation in chromosome number (Karp et al. 1984, 1989; Karp 1991) or gene copy number (Landsmann and Uhrig 1985; Brettel et al. 1986; Zheng et al. 1987), DNA mutations (Brown 1989; Müller et al. 1990), transpositional changes (Peschke et al. 1987; Peschke and Phillips 1991), rearrangements and amplification of minor forms of the mitochondrial genome (Rode et al. 1988; Hartmann et al. 1989; Shirzadegan et al. 1991), and rearrangements of the chloroplast DNA (Zong-Xui et al. 1983; Day and Ellis 1985; Dunford and Walden 1991). In conifers, no somaclonal variation was observed in Norway spruce [*Picea abies* (L.) Karst] embryogenic tissue and emblings using a flow cytometric analysis (Mo et al. 1989), nor in Interior spruce (*Picea glauca-engelmannii* complex) using an abscisic acid (ABA) profile for somatic embryo maturation and 15 different isozyme assays representing a minimum of 25 loci (Eastman et al. 1991). However, variation in chromosome number was reported for two immature somatic embryos of Norway spruce (Lelu 1987).

Several strategies can be used to assess the genetic integrity of in-vitro-derived clones, but most of them have limitations. Karyological analysis cannot reveal alterations in specific genes or small chromosomal

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rearrangements. Isozyme markers provide a convenient method for the detection of genetic changes but they are subject to ontogenic variation, are limited in number, and only DNA regions coding for soluble proteins can be sampled. Restriction fragment length polymorphism (RFLP) markers are useful for sampling various regions of the genome and are potentially unlimited in number. However, they are time-consuming, costly, and they require large amounts of plant tissue. Using the polymerase chain reaction (PCR) in conjunction with short primers of arbitrary sequence (Williams et al. 1990), randomly amplified polymorphic DNA (RAPD) markers were recently shown to be sensitive for detecting variation among individuals within tree species (Carlson et al. 1991; Roy et al. 1992). The advantages of this technique over others are that large numbers of samples can be analyzed economically and quickly, that only micro-quantities of material are needed, that the specific DNA fingerprints obtained are independent of ontogenic expression, and that most of the genome can be sampled with a potentially unlimited number of markers.

The purpose of the present study was to inquire about the applicability, and to identify the limitations, of RAPDs as an alternative method for assessing the genetic integrity of embryogenic tissues and somatic embryos of black spruce [*Picea mariana* (Mill.) B.S.P.] produced through somatic embryogenesis.

Materials and methods

Plant material

Seeds (seedlots 11977, 11996, 11990) were obtained from three controlled crosses between four selected genotypes [416(♂), 422(♀), 430(♀) and 433(♀)] of black spruce [*P. mariana* (Mill.) B.S.P.]. These parents are part of a large breeding population maintained by the Ministère des Forêts du Québec. The crosses were performed with one male parent and three female parents using a nested design (Zobel and Talbert 1984). Therefore, within each cross, seeds represent a full-sib family while among crosses seeds represent half-sibs families having one parent in common. Seeds were collected in 1988 and stored at 3–5 °C in sealed jars at an average seed moisture content of 5.6% for 3 years before utilization.

Embryogenic cultures

For each cross, embryogenic cell lines were obtained from excised mature zygotic embryos and maintained by subculturing every 2 weeks on HLM-1 medium, according to previously published procedures (Tremblay 1990). The medium consisted of Litvay's salts (Litvay et al. 1985) used at half-strength, supplemented with iron given as Sequestrene 330 Fe at 28 mg/l, 500 mg/l L-glutamine, 1g/l casein hydrolysate, 1% (w/v) sucrose, 10 µM 2,4-dichlorophenoxyacetic acid (2,4-D), 5 µM benzylaminopurine (BAP), and 0.9% (w/v) Difco Bacto-agar. The pH of the medium was adjusted to 5.7 before autoclaving at 121 °C. Glutamine was filter-sterilized and added to the cooled medium.

Maturation of somatic embryos

Seven days after subculture on maintenance medium, portions of embryogenic tissue were transferred onto maturation medium under a 16-h/day photoperiod provided by Vita-Lite (Duro-Test Electric Ltd., Ontario, Canada) (Tremblay and Tremblay 1991). After 2 weeks on maturation medium, the tissues were transferred intact onto fresh maturation medium. The maturation medium was HLM basal medium supplemented with 1g/l filter-sterilized glutamine, 1g/l casein hydrolysate, 6% (w/v) filter-sterilized sucrose, 22.5 µM ABA and 0.5% (w/v) Gelrite gellan gum (Kelco, Calif., USA.).

Sampling scheme

For genotyping the parental generation, DNA was isolated from needles of each of the four parents used in the three crosses. For the segregation analysis of RAPD variants, DNA was extracted from megagametophytes and zygotic embryos obtained from the controlled crosses. DNA was also isolated from embryogenic tissue of cell lines which originated from the same controlled crosses. The genetic stability of somatic embryos was assessed on one embryogenic cell line from each of the three crosses: for each cell line, DNA was isolated from over 30 mature somatic embryos [stage 3 according to Hakman and von Arnold (1988)] produced on three different pieces of tissue. The time between the induction of the embryogenic cultures and the maturation of the somatic embryos was approximately 2 years.

DNA isolation

DNA was extracted from needles, embryogenic tissues, and somatic embryos using the procedure of Bousquet et al. (1990) modified. For needles, approximately 100 mg of tissue were ground to a powder in liquid nitrogen using a mortar and pestle. The powdered tissue was transferred into a 1.5 ml sterile microcentrifuge tube and resuspended in 800 µl of CTAB buffer. Megagametophytes were excised from sterilized seeds under aseptic conditions. Each megagametophyte was ground in a micromortar with 100 µl of CTAB buffer. The homogenate was transferred into a 1.5 ml sterile microcentrifuge tube. The micromortar and pestle were rinsed twice with successively 300 and 400 µl of CTAB buffer and the rinses were pooled with the homogenate. For each embryogenic cell line, DNA was isolated from 100 mg of embryogenic tissue, 2 weeks after subculture, and placed in a 1.5 µl sterile microcentrifuge tube with 800 µl of CTAB buffer. The tissue was squashed directly with a plastic pestle. Mature somatic embryos were taken aseptically from the embryogenic tissue and their DNA extracted as described for the megagametophytes.

The extraction buffer consisted of 2% (w/v) CTAB (cetyltrimethylammonium bromide, Sigma), 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 9.5, and 0.2% (v/v) β-mercaptoethanol. The homogenate was incubated at 65 °C for 30 min, extracted with an equal volume of chloroform, and then centrifuged at 13,000 g for 15 min. DNA was precipitated from the aqueous phase by mixing with 1/10 volume of 3 M sodium acetate and an equal volume of isopropanol. After centrifugation at 13,000 g for 30 min, the DNA pellet was rinsed with 70% ethanol, vacuum dried, and resuspended in 10 mM Tris pH 8.0, 0.1 mM EDTA.

DNA amplification

Eleven-mer primers were used for PCR amplification following a protocol previously described by Roy et al. (1992). Amplification reactions were performed in volumes of 25 µl containing 1 × Cetus reaction buffer, 200 µM of each dNTP (Pharmacia),

0.25 µM of primer, 20–75 ng of genomic DNA, and 0.2 unit of *Taq* DNA polymerase (Perkin Elmer Cetus). The mixture was covered with 25 µl of mineral oil. For the DNA amplification, a Perkin Elmer Cetus 480 DNA Thermal Cycler was programmed for 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. After amplification was completed, 5 µl of the samples were loaded and electrophoresed on 2% agarose gel, followed by staining with ethidium bromide. In all cases ϕx174 DNA digested with *Hae*III (Pharmacia) was used as size marker.

Identification and selection of RAPD variants

DNA samples isolated from the four parents were amplified with a set of seven arbitrary 11-mer primers. The amplification was repeated at least twice and only bands reproducible on several runs were considered for analysis. Following this preliminary screening, three 11-mer primers (MMUL111: 5′-TCGATCTACGT-3′, MMUL118: 5′-CGTGGTAAACT-3′, and MMUL112: 5′-GTCAATCCGAT-3′) were retained for the segregation studies. These primers resulted in repeatable and variable fragment patterns among the parents and also displayed simple band patterns.

In all tree species analyzed so far, RAPDs are usually expressed in a dominant fashion (Carlson et al. 1991; Roy et al. 1992). In gymnosperms, the availability of haploid megagametophytes makes it possible to discriminate between dominant homozygous (AA) and dominant heterozygous (Aa) genotypes. For this reason, marker segregation was verified with zygotic embryos and embryogenic cell lines as well as with megagametophytes. After the inheritance of the markers was certified, they were used to assess the genetic stability of embryogenic tissues and somatic embryos.

Results

Embryogenic cell lines

Ten embryogenic cell lines were obtained from the cross involving parents 416(♂) and 433(♀), six from the cross 416(♂) × 430(♀), and nine from the cross 416(♂) × 422(♀).

Parental generation and their progenies

Primer MMUL111. For this primer, three fragments (1.25 kb, 1.10 kb, and 900 bp) were considered for segregation analysis. The 1.25-kb fragment was present only for parents 422 and 433, the 1.10-kb fragment was present for parents 422, 430, and 433, and the 900-bp fragment was observed for all four parents (Fig. 1). The 1.25-kb fragment appeared in five out of nine cell lines of the cross 416(♂) × 422(♀) (Fig. 2). For the same cross, the 1.10-kb fragment appeared in three out of nine cell lines and the 900-bp fragment in six out of nine. This suggested that parent 422 was heterozygous for each of the three segregating fragments, while parent 416 was heterozygous only for the 900-bp fragment. To

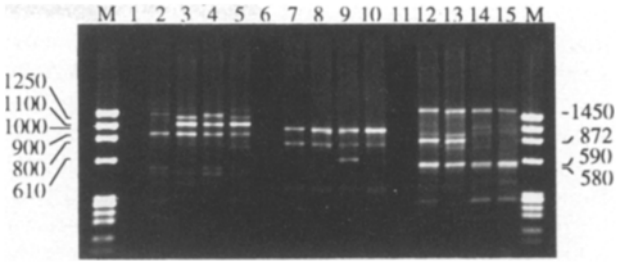

Fig. 1. Gel electrophoresis of RAPD fragments obtained with primers MMUL111 (*lanes 2 to 5*), MMUL118 (*lanes 7 to 10*), and MMUL112 (*lanes 12 to 15*) for the four black spruce parents 416(♂), 422(♀), 430(♀), and 433(♀), respectively. *Lanes M* are the size marker ϕx174 digested with *Hae*III (1353, 1078, 872, 603, 310, 281, 271, 234, 194, and 119 bp). *Lanes 1, 6, and 11* are the negative controls (PCR amplification without DNA). The size of the fragments considered is indicated on the left side for primers MMUL111 and MMUL118, and on the right side for primer MMUL112

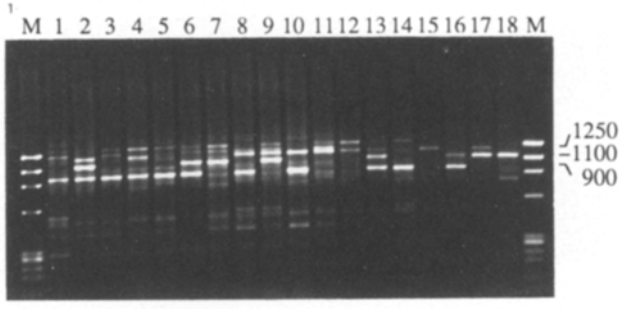

Fig. 2. Gel electrophoresis of RAPD fragments obtained with primer MMUL111. *Lanes M* are the size marker ϕx174 digested with *Hae*III. *Lanes 1 and 2* are parents 416 and 422, respectively. *Lanes 3 to 11, 12 to 15, and 16 to 18* show RAPD products for embryogenic cell lines, zygotic embryos, and megagametophytes, respectively, obtained from the controlled cross 416(♂) × 422(♀). The size of the frgments considered in the segregation analysis is indicated on the right

test the first hypothesis, ten zygotic embryos (Fig. 2, only four are shown) and ten megagametophytes (Fig. 2, only three are shown) derived from the cross 416(♂) × 422(♀) were analyzed. Among the zygotic embryos, the 1.25-kb and 1.10-kb fragments were detected in five out of ten and the 900-bp fragment in six out of ten. For the megagametophytes, the 1.25-kb and 1.10-kb fragments were detected in four out of ten and the 900-bp fragment in six out of ten. It was concluded that each of these three fragments is inherited in a simple Mendelian fashion and that parent 422 was heterozygous for the three fragments.

The 1.10-kb and 900-bp fragments were considered for the cross 416(♂) × 430(♀) (data not shown). The 1.10-kb fragment appeared in four out of six cell lines and the 900-bp fragment in five out of six. As in the

case of parent 422, parent 430 could be considered heterozygous for the 1.10-kb and 900-bp fragments. For the third cross, 416(♂) × 433(♀) (data not shown), the 1.25-kb fragment appeared in all cell lines. The 1.10-kb fragment was detected in six out of ten cell lines and the 900-bp fragment in eight out of ten. Hence, parent 433 could be considered homozygous for the 1.25-kb fragment and heterozygous for the two other fragments. From these various segregation analyses, the male parent 416 appeared heterozygous for the 900-bp fragment.

Primer MMUL118. RAPD patterns obtained with primer MMUL118 from the cell lines of the cross 416(♂) × 430(♀) are illustrated in Fig. 3. Three fragments (1 kb, 800 bp, and 610 bp) were considered for analysis. The 610-bp fragment, only present for parent 430, was detected in all the cell lines derived from the

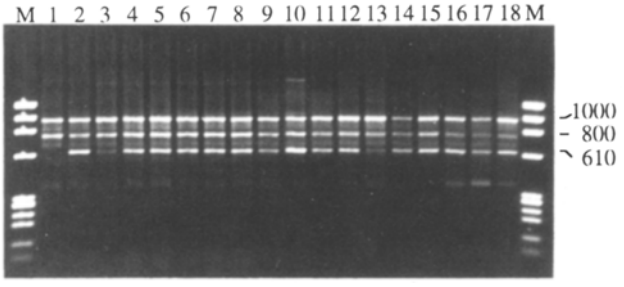

Fig. 3. Gel electrophoresis of RAPD fragments obtained with primer MMUL118. *Lanes M* are the size marker ϕx174 digested with *Hae*III. *Lanes 1 and 2* are parents 416 and 430, respectively. *Lanes 3 to 8, 9 to 15, and 16 to 18* show RAPD products for embryogenic cell lines, zygotic embryos, and megagametophytes, respectively, obtained from the controlled cross 416(♂) × 430(♀). The size of the fragments considered in the segregation analysis is indicated on the right

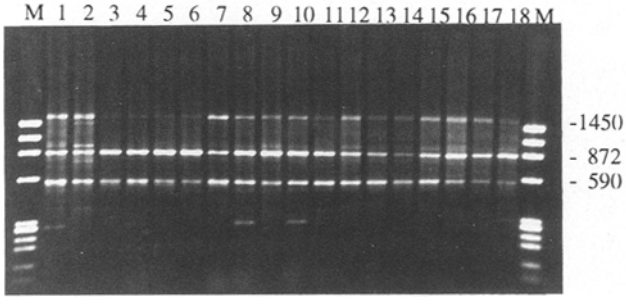

Fig. 4. Gel electrophoresis of RAPD fragments obtained with primer MMUL112. *Lanes M* are the size marker ϕx174 digested with *Hae*III. *Lanes 1 and 2* are parents 416 and 422, respectively. *Lanes 3 to 11, 12 to 15, and 16 to 18* show RAPD products for embryogenic cell lines, zygotic embryos, and megagametophytes, respectively, obtained from the controlled cross 416(♂) × 422(♀). The size of the fragments considered in the segregation analysis is indicated on the right

cross 416(♂) × 430(♀). RAPDs obtained with ten zygotic embryos (Fig. 3, only seven are shown) and ten megagametophytes (Fig. 3, only three are shown) derived from this cross showed all three fragments. This suggested that parent 430 was homozygous for these three fragments. The 1-kb and 800-bp fragments appeared monomorphic since they were detected with all three parents (Fig. 1) as well as in diverse megagametophytes, zygotic embryos, and cell lines derived from the two other crosses analyzed with MMUL118 (data not shown).

Primer MMUL112. For this primer, four fragments were considered (1.45 kb, 872 bp, 590 bp, and 580 bp). The 1.45-kb and 590-bp fragments were detected for all four parents whereas the 872-bp fragment was present only for parents 416 and 422, and the 580-bp fragment only for parents 430 and 433 (Fig. 1). The 1.45-kb, 872-bp, and 590-bp fragments were observed in all cell lines involving parents 416 and 422 (Fig. 4). RAPDs obtained with the zygotic embryos (Fig. 4, only four are shown) and the ten megagametophytes (Fig. 4, only three are shown) derived from this cross, all had the three fragments, suggesting that parent 422 was homozygous for these fragments.

For the other crosses, 416(♂) × 430(♀) and 416(♂) × 433(♀), the 1.45-kb, 590-bp, and 580-bp fragments appeared in all cell lines, zygotic embryos, and megagametophytes (data not shown). In all cases, maternal parents appeared homozygous for these three fragments. From all the segregation analyses, the male parent 416 could be considered heterozygous for the remaining 872-bp fragment.

The preceding segregation analyses allowed parents to be genotyped for the ten RAPD markers characterized (Table 1). Heterozygosity ranged from 0.20 to 0.33. With a high degree of confidence, six out

Table 1. Multilocus genotypes deduced from RAPDs

Primer	Fragment (bp)	Parent			
		416	422	430	433
MMUL111	1250	aa	Aa	aa	AA
	1100	aa	Aa	Aa	Aa
	900	Aa	Aa	Aa	Aa
MMUL118	1000	A-	AA	AA	AA
	800	A-	AA	AA	AA
	610	aa	aa	AA	aa
MMUL112	1450	A-	AA	AA	AA
	872	Aa	AA	aa	aa
	590	A-	AA	AA	AA
	580	aa	aa	AA	AA
Heterozygosity		0.33	0.33	0.20	0.20

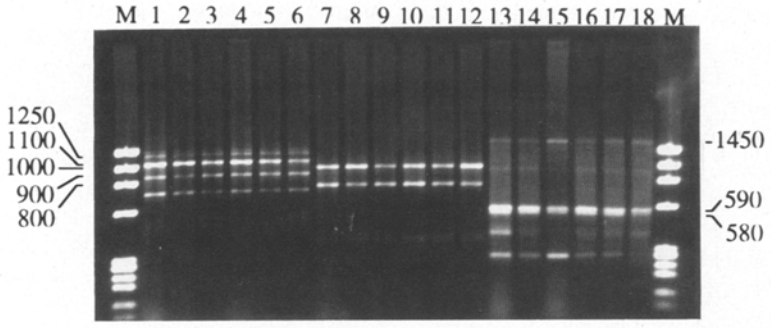

Fig. 5. Gel electrophoresis of RAPD fragments obtained with primers MMUL111 (*lanes 1 to 6*), MMUL118 (*lanes 7 to 12*), and MMUL112 (*lanes 13 to 18*). *Lanes M* are the size marker ϕx174 digested with *Hae*III. *Lanes 1, 7, and 13* are embryogenic tissue from the cell line MA-13 [cross 416(♂) × 433(♀)]. *Lanes 2 to 6, 8 to 12, and 14 to 18* show RAPD products of MA-13 somatic embryos. The size of the fragments used to assess genetic stability is indicated on the left side for primers MMUL111 and MMUL118 and on the right side for primer MMUL112

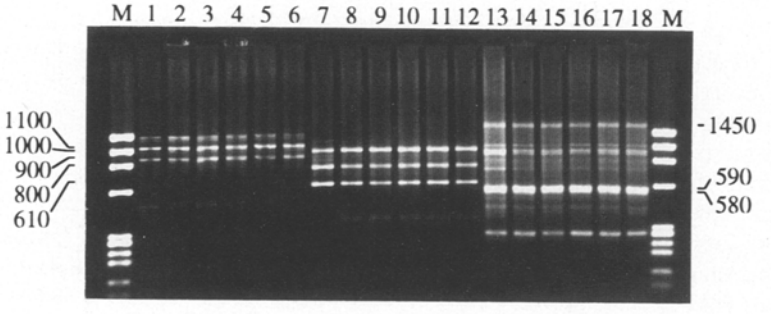

Fig. 6. Gel electrophoresis of RAPD fragments obtained with primers MMUL111 (*lanes 1 to 6*), MMUL118 (*lanes 7 to 12*), and MMUL112 (*lanes 13 to 18*). *Lanes M* are the size marker ϕx174 digested with *Hae*III. *Lanes 1, 7, and 13* are embryogenic tissue from the cell line MB-2 [cross 416(♂) × 430(♀)]. *Lanes 2 to 6, 8 to 12, and 14 to 18* show RAPD products of MB-2 somatic embryos. The size of the fragments used to assess genetic stability is indicated on the left side for primers MMUL111 and MMUL118 and on the right side for primer MMUL112

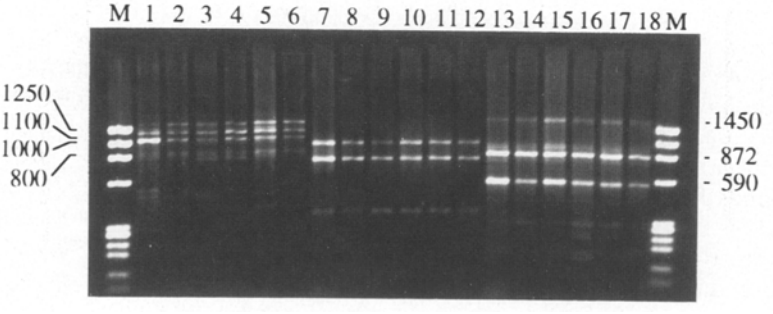

Fig. 7. Gel electrophoresis of RAPD fragments obtained with primers MMUL111 (*lanes 1 to 6*), MMUL118 (*lanes 7 to 12*), and MMUL112 (*lanes 13 to 18*). *Lanes M* are the size marker ϕx174 digested with *Hae*III. *Lanes 1, 7, and 13* are embryogenic tissue from the cell line MC-12 [cross 416(♂) × 422(♀)]. *Lanes 2 to 6, 8 to 12, and 14 to 18* show RAPD products of MC-12 somatic embryos. The size of the fragments used to assess genetic stability is indicated on the left side for primers MMUL111 and MMUL118 and on the right side for primer MMUL112

of ten markers were shown to be polymorphic, each with two putative alleles, in the sample of parents analyzed.

Genetic stability of somatic embryos

Three embryogenic cell lines, MA-13, MB-2, and MC-12, derived from the crosses 416(♂) × 433(♀), 416(♂) × 430(♀), and 416(♂) × 422(♀), respectively, and their somatic embryos (Figs. 5, 6 and 7) were tested with the ten RAPD markers previously characterized. In this preliminary study, and for the three cell lines used, the fragments selected as markers were easily detected among the somatic embryos. With primer MMUL111, the 1.10-kb fragment was present in all the somatic embryos derived from the three cell lines MA-13, MB-2, and MC-12. The 1.25-kb fragment was also observed in all the somatic embryos derived from MA-13 and MC-12 whereas the 900-bp fragment was detected in all the somatic embryos derived from MA-13 and MB-2. With primer MMUL118, the 1-kb and 800-bp fragments were found in all the somatic embryos derived from MA-13, MB-2, and MC-12. The 610-bp fragment was also detected in all somatic embryos derived from MB-2. Using primer MMUL112, the 1.45-kb, 590-bp, and 580-bp fragments were identified in all the somatic embryos derived from MA-13 and MB-2. The 1.45-kb, 872-bp, and 590-bp fragments were also present in all the somatic embryos derived from MC-12. No genetic instability among somatic embryos within a particular cell line could be detected with the ten markers used.

Discussion

All recent studies reporting segregation of RAPD variants, notably for soybean (Williams et al. 1990), Douglas-fir and white spruce (Carlson et al. 1991),

broccoli and cauliflower (Hu and Quiros 1991), lettuce (Michelmore et al. 1991), tomato (Giovannoni et al. 1991), canola (Deragon and Landry 1992), and birch (Roy et al. 1992), showed that the segregation in consistent with a biparental diploid mode of inheritance and that RAPDs are most often expressed in a dominant fashion. Similar results were obtained in this study for the segregation of RAPD markers in the megagametophytes and the F_1 progenies. Furthermore, because of the haploid nature of the megagametophytes in gymnosperms, it was possible to distinguish unambiguously between dominant homozygotes (AA) and dominant heterozygotes (Aa) for the female parents.

Although the aim of this study was not to assess the overall stability of in-vitro-derived clonal materials, no somaclonal variation could be detected among the somatic embryos and the embryogenic cell lines from which they were produced, using ten markers (four monomorphic and six polymorphic). The small sample size used in this study, in terms of the number of somatic embryos, cell lines, and markers, was designed to evaluate the potential and applicability of RAPD markers for assessing the genetic stability of somatic embryogenesis-derived materials. Our results show that this strategy could be useful in assessing the genetic integrity of such materials.

In our hands, the method appeared simple and the results reproducible. Because only micro-amounts of material are necessary, this approach can be used to assess each stage of in-vitro culture. Large sample sizes can be treated rapidly and the technique lends itself to automation (Williams et al. 1990; Welsh et al. 1991; Hedrick 1992). Furthermore, the genome is most probably randomly sampled without any variation due to ontogenic expression. However, only major fragments genetically characterized through segregation analysis should be used as markers. Minor fragments, which tend to be unstable in staining intensity and therefore not reliable, should not be considered.

The dominant nature of RAPDs does not allow one to distinguish the dominant homozygote from the heterozygote solely from the banding patterns in somatic embryos or young emblings. Therefore, for a dominant homozygote at a particular locus, mutations affecting only one of the two alleles would remain undetected. Selection of RAPD markers for which male and female parents of cell lines are of different homozygote classes (AA vs aa) might alleviate this inconvenience. These markers would only sample for mutations at heterozygous loci in progeny.

Ideally, to test the genetic integrity of somatic embryogenesis-derived clonal materials for a particular species with RAPDs, a number of markers equal to or larger than the one required to obtain a saturated linkage map would be desirable. For conifers, this number has been estimated to be larger than 200–300 (Carlson et al. 1991; Neale and Williams 1991). Once a diagnostic of stability has been obtained with such a number of markers for every targetted species and set of production procedures, somatic embryogenesis could proceed with higher confidence at an industrial scale as part of current tree improvement programmes.

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References

Attree SM, Tautorus TE, Dunstan DI, Fowke LC (1990) Somatic embryo maturation, germination, and soil establishment of plants of black and white spruce (*Picea mariana* and *Picea glauca*). Can J Bot 68:2583–2589

Becwar MR, Noland TL, Wyckoff JL (1989) Maturation, germination, and conversion of Norway spruce (*Picea abies* L.) somatic embryos to plants. In Vitro Cell Dev Biol 25(6):575–580

Bousquet J, Simon L, Lalonde M (1990) DNA amplification from vegetative and sexual tissues of trees using polymerase chain reaction. Can J For Res 20:254–257

Breiman A, Rotem-Abarbanell D, Karp A, Shaskin H (1987) Heritable somaclonal variation in wild barley (*Hordeum spontaneum*). Theor Appl Genet 74:104–112

Brettel RIS, Pallotta MA, Gustafson JP, Appels R (1986) Variation at the *nor* loci in Triticales derived from tissue culture. Theor Appl Genet 71:637–643

Brown PTH (1989) DNA methylation in plants and its role in tissue culture. Genome 31:717–729

Carlson JE, Tulsieram LK, Glaubitz JC, Luk VWK, Kauffeldt C, Rutledge R (1991) Segregation of random amplified DNA markers in F_1 progeny of conifers. Theor Appl Genet 83:194–200

Day A, Ellis THN (1985) Deleted forms of plastid DNA in albino plants from cereal anther culture. Curr Genet 9:671–678

Deragon J-M, Landry BS (1992) RAPD and other PCR-based analyses of plant genomes using DNA extracted from small leaf disks. PCR Methods Appl 1:175–180

Dunford R, Walden RM (1991) Plastid genome structure and plastid-related transcript levels in albino barley plants derived from anther culture. Curr Genet 20:339–347

Eastman PAK, Webster FB, Pitel JA, Roberts DR (1991) Evaluation of somaclonal variation during somatic embryogenesis of interior spruce (*Picea glauca engelmannii* complex) using culture morphology and isozyme analysis. Plant Cell Rep 10:425–430

Giovannoni JJ, Wing RA, Ganal MW, Tanksley S (1991) Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acids Res 19:6553–6558

Hakman I, Fowke LC, von Arnold S, Eriksson T (1985) The development of somatic embryos in tissue cultures initiated

from immature embryos of *Picea abies* (Norway spruce). Plant Sci 38:53–60

Hakman I, von Arnold S (1988) Somatic embryogenesis and plant regeneration from suspension cultures of *Picea glauca* (white spruce). Physiol Plant 72:579–587

Hartmann C, Henry Y, Aubry C, Rode A (1989) Identification of new mitochondrial genome organizations in wheat plants regeneration from somatic tissue cultures. Theor Appl Genet 77:169–175

Hedrick P (1992) Shooting the RAPDs. Nature 355:679–680

Hu J, Quiros CF (1991) Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep 10:505–511

Karp A (1991) On the current understanding of somaclonal variation. In: Miflin BJ (ed) Oxford Surveys of Plant Molecular and Cell Biology, vol 7, pp 1–58

Karp A, Bright SWJ (1985) On the causes and origins of somaclonal variation. In: Miflin BJ (ed) Oxford Surveys of Plant Molecular and Cellular Biology, vol 2, pp 199–234

Karp A, Rissiott R, Jones MGK, Bright SWJ (1984) Chromosome doubling in monohaploid and dihaploid potatoes by regeneration from cultured leaf explants. Plant Cell Tissue Org Cult 3:363–373

Karp A, Jones MGK, Foulger D, Fish N, Bright SWJ (1989) Variability in potato tissue culture. Am Potato J 66:669–684

Landsmann J, Uhrig H (1985) Somaclonal variation in *Solanum tuberosum* detected at the molecular level. Theor Appl Genet 71:500–506

Larkin PJ, Scowcroft WR (1981) Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor Appl Genet 60:197–214

Larkin PJ, Ryan SA, Brettell RIS, Scowcroft WR (1984) Heritable somaclonal variation in wheat. Theor Appl Genet 67:443–455

Lelu MA (1987) Etude préliminaire des variations morphologiques et génétiques obtenues après embryogenèse somatique chez *Picea abies*. In: Annales de Recherches Sylvicoles, AFOCEL, pp 35–47

Litvay JD, Verma DC, Johnson MA (1985) Influence of loblolly pine (*Pinus taeda* L.) culture medium and its components on growth and somatic embryogenesis of the wild carrot (*Daucus carota* L.) Plant Cell Rep 4:325–328

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Mo LH, von Arnold S, Lagercrantz U (1989) Morphogenic and genetic stability in longterm embryogenic cultures and somatic embryos of Norway spruce [*Picea abies* (L.) Karst]. Plant Cell Rep 8:375–378

Müller E, Brown PTH, S. H, Lörz H (1990) DNA variation in tissue-culture-derived rice plants. Theor Appl Genet 80:673–679

Neale DB, Williams CG (1991) Restriction fragment length polymorphism mapping in conifers and applications to forest genetics and tree improvement. Can J For Res 21:545–554

Peschke VM, Phillips RL (1991) Activation of the maize transposable element *Suppressor-mutator* (*Spm*) in tissue culture. Theor Appl Genet 81:90–97

Peschke VM, Phillips RL, Gegenbach BG (1987) Discovery of transposable element activity among progeny of tissue-culture-derived maize plants. Science 238:804–807

Roberts DR, Flinn BS, Webb DT, Webster FB, Sutton BCS (1990a) Abscisic acid and indole-3-butyric acid regulation of maturation and accumulation of storage proteins in somatic embryos of interior spruce. Physiol Plant 78: 355–360

Roberts DR, Sutton BCS, Flinn BS (1990b) Synchronous and high-frequency germination of interior spruce somatic embryos following partial drying at high relative humidity. Can J Bot 68:1086–1090

Rode A, Hartmann C, De Buyser J , Henry Y (1988) Evidence for a direct relationship between mitochondrial genome organization and regeneration ability in hexaploid wheat somatic tissue cultures. Curr Genet 14:387–394

Roy A, Frascaria N, MacKay J, Bousquet J (1992) Segregating random amplified polymorphic DNAs (RAPD) in *Betula alleghaniensis*. Theor Appl Genet 85:173–180

Shirzadegan M, Palmer JD, Christey M, Earle ED (1991) Patterns of mitochondrial DNA instability in *Brassica campestris* cultured cells. Plant Mol Biol 16:21–37

Tremblay FM (1990) Somatic embryogenesis and plantlet regeneration from embryos isolated from store seeds of *Picea glauca*. Can J Bot 68:236–242

Tremblay L, Tremblay FM (1991) Effects of gelling agents, ammonium nitrate, and light, on the development of *Picea mariana* (Mill) B.S.P. (black spruce) and *Picea rubens* Sarg. (red spruce) somatic embryos. Plant Sci 77:233–242

von Arnold S, Hakman I (1988) Regulation of somatic embryo development in *Picea abies* by abscisic acid (ABA). J Plant Physiol 132:164–169

Webster FB, Roberts DR, McInnis SM, Sutton BCS (1990) Propagation of interior spruce by somatic embryogenesis. Can J For Res 20:1759–1765

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: 6531–6535

Welsh J, Petersen C, McClelland M (1991) Polymorphisms generated by arbitrarily primed PCR in the mouse: application to strain identification and genetic mapping. Nucleic Acids Res 19:303–306

Zheng KL, Castiglione S, Biasini MG, Morandi C, Sala F (1987) Nuclear DNA amplification in cultured cells of *Oryza sativa* L. Theor Appl Genet 74:65–70

Zobel B, Talbert J (1984) Applied Forest Tree Improvement. John Wiley and Sons. New York

Zong-Xui S, Cheng-Zhang Z, Kang-Le Z, Xui-Fang Q, Yaping F (1983) Somaclonal genetics of rice, *Oryza sativa* L. Theor Appl Genet 67:67–72