cankers on stressed red and jack pines in Minnesota and Wisconsin. Plant Dis. **74**: 54–56.
Palmer, M.A., and Nicholls, T.H. 1985. Shoot blight and collar rot of *Pinus resinosa* caused by *Sphaeropsis sapinea* in forest tree nurseries. Plant Dis. **69**: 739–740.
Palmer, M.A., Nicholls, T.H., and Croghan, C.F. 1986. Fungicidal control of shoot blight caused by *Sphaeropsis sapinea* on red pine nursery seedlings. Plant Dis. **70**: 194–196.
Palmer, M.A., McRoberts, R.E., and Nicholls, T.H. 1988. Sources of inoculum of *Sphaeropsis sapinea* in forest tree nurseries. Phytopathology, **78**: 831–835.
Peterson, G.W., and Wysong, D.S. 1968. Diplodia tip blight of pines in the central great plains: damage and control. Plant Dis. Rep. **52**: 359–360.
Punithalingham, E., and Waterston, J.M. 1970. *Diplodia pinea*. *In* Descriptions of pathogenic fungi and bacteria. Commonw. Mycol. Inst. Phytopathol. Pap. 273.
Slagg, C.M., and Wright, E. 1943. Diplodia blight in coniferous seedbeds. Phytopathology, **33**: 390–393.
Soeda, Y., Shogo, K., and Noguchi, T. 1972. Identification of alkyl 2-benzimidizole-carbamates as a major metabolite of thiophanates fungicide in/on the bean plant. Agric. Biol. Chem. **36**: 817–823.
Swart, W.J., and Wingfield, M.J. 1991. Biology and control of *Sphaeropsis sapinea* on *Pinus* species in South Africa. Plant Dis. **75**: 761–765.
Warren, C.G., Sanders, P., and Cole, H. 1974. *Sclerotinia homeocarpa* tolerance to benzimidazole configuration fungicides. Phytopathology, **64**: 1139–1142.

Development of random amplified polymorphic DNA markers for genetic mapping in Pacific yew (*Taxus brevifolia*)

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Abstract: A genetic linkage map was constructed for Pacific yew (*Taxus brevifolia* Nutt.) based on random amplified polymorphic DNA (RAPD) markers. A series of optimization experiments were conducted to develop a highly repeatable protocol for Pacific yew. In these experiments, a high $MgCl_2$ concentration (5.5 mM) together with a low primer concentration (0.2 μm) in the polymerase chain reaction (PCR) mixture yielded the best amplification products. PCR amplification products were further improved by treating the template DNAs with RNase. Experiments showed that bovine serum albumine had the same effect as RNase on PCR amplification. The segregating mapping population consisted of 39 haploid megagametophytes from a single mother tree. DNA extracted from a subset of 6 megagametophytes was screened with 345 ten-base oligonucleotide primers of arbitrary sequence. Of the screened primers, 28% revealed at least one polymorphic locus. Eighty-six of these primers revealed at least one polymorphic locus and were used with the entire set of megagametophyte DNAs. One-hundred-two loci were scored and segregated in the expected 1:1 ratio (1.19 locus per primer). Linkage analysis was conducted using MAPMAKER. Forty-one of 102 markers were distributed into 17 linkage groups and covered 305.8 centimorgans. The remaining 61 unlinked markers should be assigned to linkage groups as more markers are added to the map.

Résumé : Les auteurs ont construit une carte de linkage génétique pour l'if occidental (*Taxus brevifolia* Nutt.) à partir de marqueurs d'ADN polymorphe résultant de l'amplification de régions anonymes du génome (random amplified polymorphic DNA, RAPD). Une série d'expériences d'optimisation fut réalisée afin d'établir un protocole fiable pouvant facilement être répété chez l'if occidental. Ces expériences ont permis de découvrir qu'une forte concentration en $MgCl_2$ (5,5 mM) et une faible concentration en amorce (0,2 μm) permettaient d'obtenir les meilleurs produits d'amplification par PCR. Ces produits étaient encore meilleurs lorsque l'ADN génomique cible était traité à la RNase. De plus, les expériences d'optimisation ont démontré que lors de l'amplification par PCR, l'albumine du sérum de bovin engendrait le même effet que la RNase. Afin d'établir

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la carte de linkage, une population de ségrégation constitutée de 39 mégagamétophytes haploïdes ayant le même parent maternel fut utilisée. Les échantillons d'ADN extraits pour un sous-ensemble de 6 mégagamétophytes furent utilisés afin de cribler 345 amorces oligonucléotidiques décamères de composition arbitraire. De ces 345 amorces, 28% ont démontré au moins un locus polymorphe. Les échantillons d'ADN de l'ensemble des mégagamétophytes furent par la suite amplifiés par PCR à l'aide de 86 de ces amorces démontrant au moins un polymorphisme. Un total de 102 loci polymorphes furent détectés (1,19 locus par amorce en moyenne), chacun démontrant une ségrégation des deux allèles selon la proportion théorique de 1:1. L'analyse de linkage fut réalisée à l'aide du logiciel MAPMAKER, permettant de regrouper 41 de ces 102 marqueurs en 17 groupes de linkage couvrant 305,8 centimorgans. Les 61 autres marqueurs non liés devraient pouvoir être assignés aux groupes de linkage existants à mesure que des marqueurs supplémentaires s'ajouteront à la carte.

[Traduit par la Rédaction]

Introduction

Pacific yew (*Taxus brevifolia* Nutt.) is one of seven species in the *Taxus* genus, family Taxaceae. It has a limited value as a commercial timber species, though its secondary metabolites are very valuable. For example, Taxol® (paclitaxel),[2] a diterpene product of secondary metabolism extracted from yew bark, has been known to be a promising anticancer agent since the late 1960s (Wani et al. 1971; Fuchs and Johnson 1978). Recently, it has been approved by the Food and Drug Administration for use in the United States. Bark from mature Pacific yew plants will not provide an abundant supply of taxol. Alternatives to bark for taxol production, such as needle tissue, tissue cultures, semisynthesis of taxol from naturally occurring taxol analogs, and total synthesis are being investigated. In recent years, interest in the research of its biology and genetics has become more evident because of the importance of taxol as an anticancer agent (Wheeler et al. 1992).

Detailed genetic linkage maps are fundamental tools for studying the organization of plant genomes (Tanksley 1983; Neale and Williams 1991). A detailed genetic map of Pacific yew could be used in a variety of ways, such as determining the level and organization of genetic variation in the species, which will be useful for the gene conservation, gene resource management, and improvement of the species (Cheliak et al. 1988). A detailed genetic map might also be used to identify chromosomal locations of important genes such as the genes in the pathways leading to the production of taxol.

Several types of genetic markers could be used in Pacific yew, including isozymes, restriction fragment length polymorphisms (RFLPs), and random amplified polymorphic DNA (RAPDs). Recently, El-Kassaby and Yanchuk (1994) used allozyme markers to study genetic diversity and were able to determine the inheritance of only 21 allozyme loci in Pacific yew. Allozyme markers will be useful for diversity studies in Pacific yew, but do not provide enough markers for the construction of detailed linkage maps. DNA-based genetic markers such as RFLPs and RAPDs can be used to construct detailed maps (Neale and Williams 1991; Neale et al. 1992). An RFLP map for loblolly pine (*Pinus taeda* L.) has been constructed (Devey et al. 1994), and it has been shown that the loblolly pine probes can be used for other species (Ahuja et al. 1994). However, most loblolly pine probes would not cross-hybridize in *Taxus* (Ahuja et al. 1994). Furthermore, there are no large F_1 families currently available for establishing the inheritance of RFLP markers. Because of the long stratification period of yew seeds (1 year) and the slow growth rate of seedlings, it would be several years before large amounts of DNA necessary for Southern blot hybridization from F_1 families could be isolated. For these reasons, RAPD markers are currently the best alternative for Pacific yew genetic mapping.

RAPD markers are genetic markers that are based on the polymerase chain reaction (Williams et al. 1990; Welsh and McClelland 1990). RAPD markers are dominant genetic markers, e.g., the homozygote for "visible band" cannot be distinguished from the heterozygote. This limitation of RAPDs can be overcome in conifers if assays are performed on DNA from the haploid megagametophytes (Tulsieram et al. 1992). The zygosity of an individual tree is determined by a small sample (6–10) of segregating megagametophytes from that tree. However, an important problem with the RAPD technique encountered in the previous studies is the lack of reproducibility of RAPD markers among experiments and also among individual DNA preparations. Thus, RAPD technique should be optimized for a particular species before RAPD data can be obtained.

The objectives of this study were (1) to optimize the RAPD protocol for Pacific yew, (2) to identify RAPD markers using DNA from megagametophytes of a single Pacific yew mother tree, and (3) to establish a genetic map of these markers by classical linkage analysis.

Materials and methods

Plant material

Seeds were collected from a single tree growing near Cherokee Creek on the Tahoe National Forest, California (39°34'N, 102°59'W). Seeds were stratified for 6 months at alternating temperatures of 10°C and 20°C for 12 h each. This was followed by an incubation at 5°C for another 6 months. To make the dissection of seed and separation of megagmetophytes easy, seed germination was performed at 25°C for 10–40 days until the embryo had emerged from the seed coat.

DNA isolation

DNA was extracted from megagametophyte tissues of 39 individual seeds following the modified extraction protocol of Kreike (1990), in which octanol:chloroform extraction was performed instead of phenol:chloroform extraction because of the known inhibitory effects of phenol on polymerase chain reactions (PCR). DNA yield per megagametophyte ranged from 648 to 5220 ng. Each of the 39 DNA samples was diluted to 10 ng/μL with TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) prior to use.

[2] Taxol® is the registered trade name for the drug derived from paclitaxel. Hereafter paclitaxel will be referred to as taxol.

Table 1. Testing the effects of RAPD reaction components on PCR amplification.

Reaction mixture for PCR*	Tested reaction component	Component concentration	Result
5.5 mM $MgCl_2$, 0.20 μM primer, 200 μM dNTPs, 1.0 U*Taq* polymerase, 0.13 μL Tween-20, 2.5 μL PCR buffer	DNA	4 ng/25 μL	No amplification
		8 ng/25 μL	Best amplification
		15 ng/25 μL	Very faint bands
		20 ng/25 μL	Very faint bands
8 ng DNA, 0.20 μM primer, 200 μM dNTPs, 1.0 U*Taq* polymerase, 0.13 μL Tween-20, 2.5 μL PCR buffer	$MgCl_2$	2.0 mM	No amplification
		2.5 mM	No amplification
		3.0 mM	No amplification
		3.5 mM	No amplification
		4.0 mM	No amplification
		4.5 mM	No amplification
		5.0 mM	Very faint bands
		5.5 mM	Best amplification
		6.0 mM	Very faint bands
8 ng DNA, 5.5 mM $MgCl_2$, 200 μM dNTPs, 1.0 U *Taq* polymerase, 0.13 μL Tween-20, 2.5 μL PCR buffer	Primer	0.16 μM	Clear bands, template
		0.20 μM	Clear bands, template
		0.28 μM	Clear bands, template
		0.32 μM	Clear bands, template
		0.40 μM	No amplification
8 ng DNA, 5.5 mM $MgCl_2$, 0.20 μM primer, 1.0 U *Taq* polymerase, 0.13 μL Tween-20, 2.5 μL PCR buffer	dNTPs	20 μM	No amplification
		50 μM	Very faint bands
		100 μM	Good amplification
		150 μM	Good amplification
		200 μM	Best amplification
		250 μM	Very faint bands
8 ng DNA, 5.5 mM $MgCl_2$, 0.20 μM primer, 200 μM dNTPs, 0.13 μL Tween 20, 2.5 μL PCR buffer	*Taq* DNA polymerase	0.5 U	No amplification
		1.0 U	Good amplification
		1.5 U	Good amplification
		2.0 U	Blurred backgrounds
		2.5 U	Blurred backgrounds
8 ng DNA, 5.5 mM $MgCl_2$, 0.20 μM primer, 200 μM dNTPs, 1.0 U *Taq* polymerase, 2.5 μL PCR buffer	Tween-20	0.00 μL/25 μL	Very faint bands
		0.13 μL/25 μL	Best amplification
		0.25 μL/25 μL	No amplification

Note: Each test was carried out at a standard cycling condition of 40 cycles of 1 min at 94°C (denaturing), 1 min at 37°C (annealing), and 2 min at 72°C (extension) with postholding at 72°C for 5 min.

*See the text for the definitions of reaction components.

RAPD optimization experiments

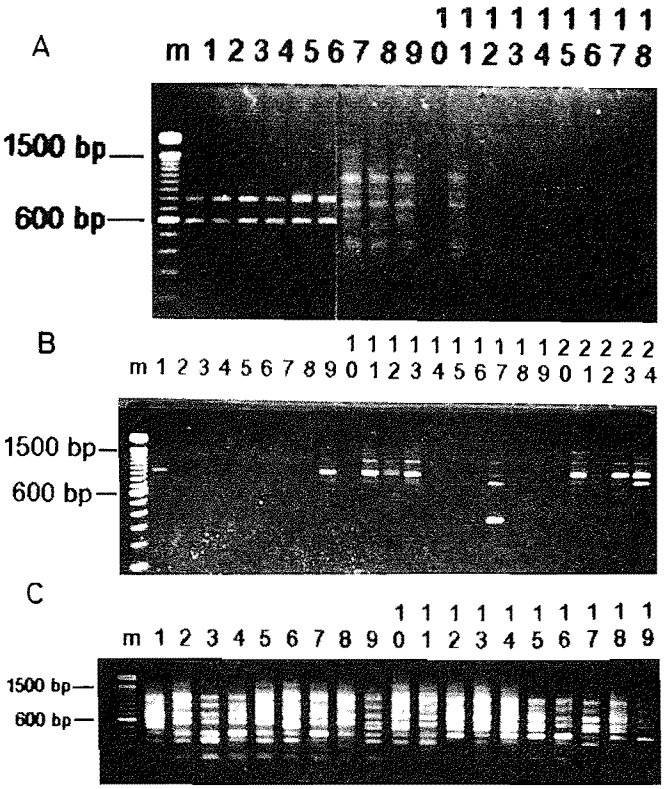
The conditions reported by Williams et al. (1990) for generating RAPD markers by PCR were modified to optimize RAPD assays for DNA from Pacific yew megagametophytes. Optimization reactions were performed in 25-μL volumes. Gels (2% agarose) were run in 1× TBE (Trisma base – boric acid – EDTA) buffer at 70 V for 3.5 h and stained with ethidium bromide.

RAPD reaction components

Tests to optimize RAPD components included varying DNA, $MgCl_2$, primer, dNTP mixture, and *Taq* DNA polymerase concentrations. DNA amounts of 4, 8, 15, 20 ng per reaction volume of 25 μL, $MgCl_2$ concentrations within the range of 2.0 to 6.0 mM, primer concentrations of 0.16, 0.20,0.28, 0.32, and 0.40 μM, deoxynucleoside triphosphate concentrations between 20 and 250 μM (the four dNTPs were at equivalent concentrations in the mixture), and 0.5, 1.0, 1.5, 2.0, and 2.5 units (U) of Perkin-Elmer Cetus Ampli*Taq* DNA polymerase were tested. The effect of Tween-20 (Sigma, U.S.A.) as a nonionic detergent was also tested; experiments without Tween-20 and with 0.13 and 0.25 μL Tween-20 were performed (Table 1).

Additionally, the effects of RNase and bovine serum albumine (BSA) on RAPD reactions were tested. In these tests, 0.02, 0.04, 0.06, 0.08, 0.10, and 0.12 μg RNase/ng DNA and the same amounts of BSA per nanogram DNA were added to the reaction mixture. Furthermore, small aliquots from the template DNAs were treated with 0.02 μg RNase/ng DNA at 37°C for 3 h. All

Fig. 1. (A) RAPD amplification with primer OPE-18 showing the effect of Tween-20. *m*, size of markers, 100 base pair DNA ladder (Gibco, BRL). Lanes 1–6 are the results of 0.13 μL Tween-20 per 25 μL reaction volume, lanes 7–12 are the results of 0.0 μL Tween-20 per 25 μL reaction volume, and lanes 13–18 are the results of 0.25 μL Tween-20 per 25 μL reaction volume. (B) RAPD amplifications with primer OPQ-18 without RNase in the reaction mixture. (C) RAPD amplifications with primer OPQ-18 with 0.2 μL RNase per 25 μL reaction volume.



these tests were carried out with standard amplification conditions of 40 cycles of 1 min at 94°C (denaturing), 1 min at 37°C (annealing), and 2 min at 72°C (extension) with postholding at 72°C for 5 min in a thermal cycler (Perkin-Elmer Cetus DNA 9600).

Cycling parameters

A range of cycling conditions were tested to determine an optimum: (1) 35 cycles of 1 min at 94°C, 1 min at 37°C, 2 min at 72°C with and without preholding at 94°C for 1.5 min, and with postholding at 72°C for 5 min; (2) 40 cycles of 1 min at 94°C, 1 min at 37°C, 2 min at 72°C with postholding at 72°C for 10 min; (3) 45 cycles of 1 min at 94°C, 1 min at 37°C, 2 min at 72°C with postholding periods of 5, 10, and 15 min at 72°C; (4) 45 cycles of 1 min at 94°C, 1 min at 37°C, 2 min at 72°C with preholding at 94°C for 1.5 min and postholding at 72°C for 5 and 10 min. In all cycling experiments, the optimum reaction mixture of 8 ng DNA, 5.5 mM $MgCl_2$, 0.2 μM primer, 200 M dNTPs, 1 U *Taq* DNA polymerase, 2.5 L PCR buffer, and 0.13 L Tween-20 was used.

Primer screening

Ten-base oligonucleotide primers from Operon Technologies (Alameda, California) (kits OPC-OPK and OPP-OPZ) were used. Initially, 345 RAPD primers were screened against a subset of six megagametophyte DNAs. Two hundred forty-nine primers did not reveal at least one polymorphic RAPD band and were eliminated from the study. From the remaining 96 primers, only 86 with polymorphic RAPD band(s) were screened with the remaining 33 template DNAs to obtain single-locus segregation data. Because of template DNA limitation, 10 primers yielding polymorphic bands were eliminated, since a considerable amount of template DNA was used up during the optimization trials.

Single-locus segregation and linkage analysis

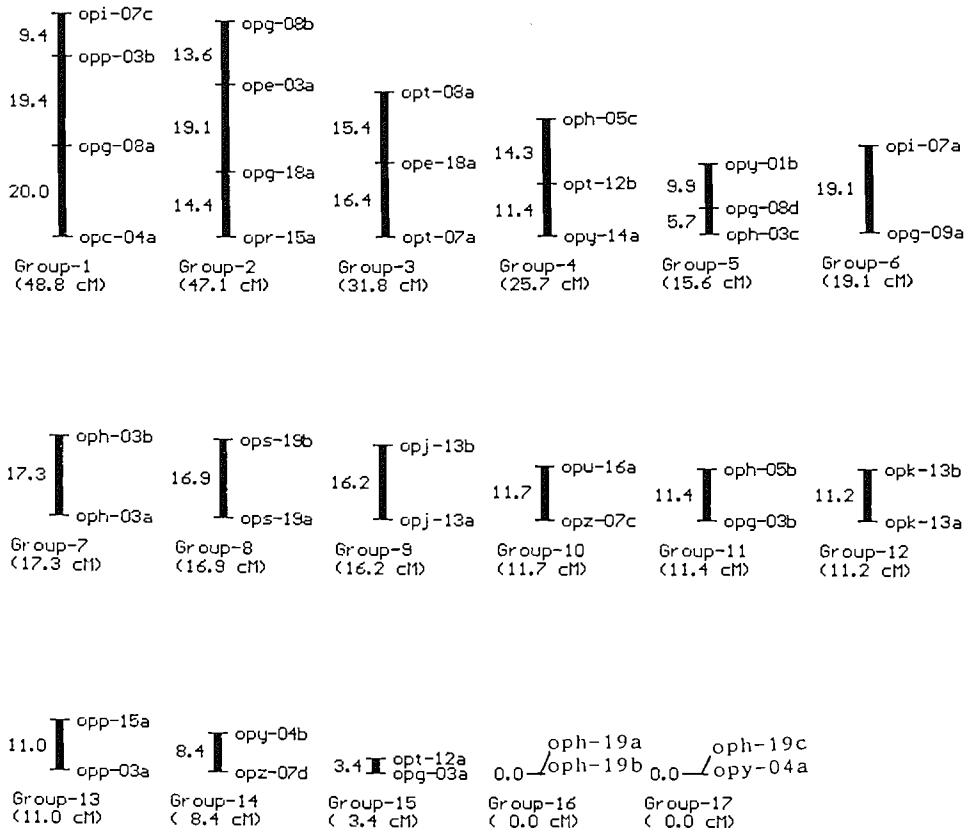
A χ^2 goodness of fit test to a 1:1 Mendelian ratio was performed for all segregating loci at the 0.05 significance level. Since megagametophytes derived from seeds of single tree show 1:1 segregation and are equivalent to a backcross mating, linkage analysis was performed as a backcross using the MAPMAKER/EXP (version 3.0) program (Lander et al. 1987.). Linkage criteria of 0.40 recombination value and log likelihood (LOD) of 3.00 were used in the analysis. Markers that did not segregate according to the expected ratio were excluded from this analysis, since in both crop and forest tree species, the occurrence of distorted ratios in segregating RFLP and isozyme alleles is not uncommon (Carlson et al. 1991; Tulsieram et al. 1992; Binelli and Bucci 1994; Mukai et al. 1995). The actual mechanism underlying this phenomenon is not well understood. There appears to be a lack of consensus among researchers about whether markers expressing segregation distortion should be used in linkage mapping studies.

Results and discussion

RAPD optimization experiments

The DNA template concentration of 8 ng per reaction volume of 25 μL yielded the best amplification products, while 4 ng produced no bands at all and 15 ng and 20 ng produced very faint bands (Table 1). $MgCl_2$ concentrations within the range of 2.0–4.5 mM resulted in no amplification products. When the concentration of $MgCl_2$ was increased to 5.0 mM, very faint bands occurred. The optimum $MgCl_2$ concentration was in the range of 5.5–6.0 mM (Table 1). There was no appreciable difference between the amplification products using 0.16, 0.20, and 0.28 μM primer concentrations; however, it appeared that 0.20 μM primer produced slightly better RAPD bands. Primer concentrations exceeding 0.32 μM resulted in a reduced number of bands or no bands at all. Deoxynucleotide triphosphate (dNTP) concentration of 200 μM resulted in the best RAPD bands. Concentrations lower than 100 μM and higher than 200 μM produced either very faint bands or no bands at all. Both 1.0 and 1.5 U of *Taq* DNA polymerase produced very clear bands. Higher concentrations (2.0 and 2.5 U) of *Taq* DNA polymerase produced very blurred backgrounds, and lower concentrations (0.5 U) produced no RAPD bands. Reactions without Tween-20 yielded very faint bands, whereas the addition of 0.13 μL Tween-20 per reaction volume of 25 μL resulted in bright RAPD bands (Table 1, Fig. 1A). The addition of 0.06 μg RNase/ng DNA directly to the reaction mixture universally improved the RAPD reaction (Figs. 1B and 1C). However, RNAse concentrations higher than 0.06 μg yielded faint bands with blurred backgrounds or no bands at all. RNase concentrations below 0.02 μg/ng DNA did not yield strong bands with many DNA samples. Substitution of 0.02 μg

Fig. 2. RAPD linkage map in Pacific yew. The loci are listed on the right and map distances in centimorgans (cM) on the left. Forty-one of the 102 markers were mapped to 17 linkage groups, while the remaining 61 were unlinked, using a recombination value of 0.40 and log likelihood ratio (LOD) score of 3.00. The 17 linkage groups covered a total of 305.8 cM.



BSA/ng DNA for 0.06 μg RNase/ng DNA produced identical results. Like RNase, increased amounts of BSA resulted in faint bands with blurred backgrounds or no bands at all.

The optimum set of cycling conditions was determined to be 45 cycles of 1 min at 94°C, 1 min at 37°C, and 2 min at 72°C with postholding period of 10 min at 72°C. The other cycling procedures tested did not produce satisfactory PCR amplification products. Following the optimization experiments, a standard protocol was applied for the subsequent mapping study. Megagametophyte DNAs were first treated with 0.02 μg RNase/ng DNA at 37°C for 3 h. The standard RAPD reaction mixture included 8 ng DNA, 5.5 mM $MgCl_2$, 0.2 μM primer, 200 μM dNTPs (for each), 1 U of *Taq* DNA polymerase, 2.5 μL PCR buffer, and 0.13 μL Tween-20 per reaction volume of 25 μL. Reactions were run for 45 cycles of 1 min at 94°C, 1 min at 37°C, and 2 min at 72°C with a postholding period of 10 min at 72°C.

Through a series of optimization experiments, we attempted to develop a highly repeatable RAPD protocol for Pacific yew megagametophyte DNAs. Specific primer–template interactions were the main difficulties during these optimization experiments. Although firstly we assumed the presence of significant RNA contamination in the genomic DNA preparations suppressing the PCR amplification (Pikaart and Villeponteau 1993) and treated the template DNAs with RNase to eliminate these contaminations, subsequent experiments showed that there was actually no detectable amount of RNA in these preparations (Göçmen 1994). Since the addition of certain amounts of RNase and BSA directly to the reaction mixture also improved the PCR amplification (Figs. 1B and 1C), it could be concluded that the RNase treatment of the template DNAs somehow helped to stabilize the enzyme rather than digesting the RNA. For the same purpose, Tween-20 as a nonionic detergent had already been added to the reaction mixture, but it was not enough for the desired PCR products alone. Because of the lack of information about the exact action mechanisms of such components in the PCR procedure, it is difficult to explain why the PCR amplification of Pacific yew megagametophyte DNAs required both of them for the best PCR products. However, depending on the known inhibitory effects of phenol in PCR, it could only be said that some phenolics or other secondary metabolites of the species were the inhibitory substances in the template DNA preparations.

Primer screening, single-locus segregation, and linkage analysis

Ninety-six of the 345 RAPD primers that were screened against a subset of six megagametophyte DNAs revealed at least one polymorphic locus. Subsequently, 86 of these primers revealed that polymorphic loci were applied to the remaining 33 megagametophyte DNAs to obtain single-locus

segregation data. Forty-seven of those 86 primers revealed that the total number of loci was 102, which conforms to the expected segregation ratio of 1:1 (segregation data could be accessed from the Dendrome Data Base, IFG, USDA Forest Service, Albany, California, U.S.A.). The size of the markers ranged from 370 to 2070 base pairs. Sixteen of the 47 primers detected 1 segregating locus, 15 detected 2 loci, 10 detected 3 loci, 5 detected 4 loci, and 1 detected 6 loci. The estimated average number of segregating loci per primer was 0.30. When only the polymorphic primers showing 1:1 segregation for at least 1 locus were considered, the average number of segregating loci was determined as 2.17. Also, the average number of bands (including monomorphic and polymorphic) produced per polymorphic primer that revealed at least one locus segregating to 1:1 ratio was calculated as 3.87.

A significant portion (30%) of the RAPD loci showed siginificant deviation from 1:1 segregation ratios based on χ^2 tests ($p = 0.05$). After elimination of those RAPD loci showing significant deviation from the expected 1:1 segregation ratio, 102 RAPD loci were found to be segregating according to the expected ratio. Forty-one of the 102 markers were mapped to 17 linkage groups, while the remaining 61 were unlinked, using a recombination value of 0.40 and log likelihood ratio (LOD) score of 3.00. The 17 linkage groups covered a total of 305.8 centimorgan (cM) (Fig. 2). The minimum and maximum map distances covered by the linkage groups are 0.00 cM (group 16 and group 17 with 2 markers in each) and 48.80 cM (group 2 with 4 markers), respectively (Fig. 2). Although there are published reports of RAPD maps in other forest trees (Carlson et al. 1991; Grattapaglia et al. 1992; Dale et al. 1992; Tulsieram et al. 1992; Nelson et al. 1993; Binelli and Bucci 1994; Grattapaglia and Sederoff 1994; Grattapaglia et al. 1995; Plomion et al. 1995; Mukai et al. 1995), this is the first genetic map for Pacific yew based on RAPD markers and this is an important step towards the application of these markers in forest trees. With more RAPD markers, a detailed genetic map will be constructed in the future, since the total map distance covered in this study is very small when compared with an average genome size in conifers.

This study and most of the previous studies indicate that none of the genetic markers currently available have all of the desirable attributes of the most useful genetic markers for forest trees. Furthermore, it has not been demonstrated whether RAPD markers will be useful for marker breeding or gene resource conservation in forest species (Kaya and Neale 1995). Therefore, forest geneticists must develop new DNA markers that will overcome most of the limitations currently associated with RAPDs and RFLPs. In fact, Neale and Harry (1994) report that in some laboratories, investigations toward the development of such new DNA markers have already started and they will be available soon. Because of the inherent power and technical simplicity of PCR, newly developed molecular markers will almost certainly be PCR based.

References

Ahuja, M.R., Devey, M.E., Groover, A.T., Jermstad, K.D., and Neale, D.B. 1994. Mapped DNA probes from loblolly pine can be used for restriction fragment length polymorphism mapping in other conifers. Theor. Appl. Genet. **88**: 279–282.

Binelli, G., and Bucci, G. 1994. A genetic linkage map of *Picea abies* Karst., based on RAPD markers, as a tool in population genetics. Theor. Appl. Genet. **88**: 283–288.

Carlson, J.E., Tulsieram, L.K., Glabubita, J.C., Luk, V.W.K., Kauffeldt, C., and Rutledge, R. 1991. Segregation of random amplified DNA markers in F1 progeny of conifers. Theor. Appl. Genet. **83**: 194–200.

Cheliak, W.M., Murray, G., and Pitel, J.A. 1988. Genetic effects of phenotypic selection in white spruce. For. Ecol. Manage. **24**: 139–149.

Dale, G., Gates, C., Vogel, H., Daggard, G., and Teasdale, B. 1992. Mapping of the interspecific hybrid between *Pinus caribaea* and *Pinus elliottii* using RAPD markers. *In* 6th International Conifer Biotechnology Working Group, 23–28 Apr. 1992, Radisson Governors Inn, Research Triangle Park, N.C.

Devey, M.E., Fiddler, T.A., Liu, B.H., Knapp, S.J., and Neale, D.B. 1994. An RFLP linkage map for loblolly pine based on a three-generation outbred pedigree. Theor. Appl. Genet. **88**: 273–278.

El-Kassaby, Y.A., and Yanchuk, A.D. 1994. Genetic diversity, differentiation and inbreeding in Pacific yew from British Columbia. J. Hered. **85**: 112–117.

Fuchs, D.A., and Johnson, R.K. 1978. Cytological evidence that taxol, an antineoplastic agent from *Taxus brevifolia*, acts as a mitotic spindle poison. Cancer Treat. Rep. **62**: 1219–1222.

Göçmen, B. 1994. Single tree genetic linkage mapping of *Taxus brevifolia* Nutt. based on random amplified polymorphic DNA (RAPD) markers using DNA from haploid megagametophytes. Master's thesis, Middle East Technical University, Ankara, Turkey.

Grattapaglia, D., and Sederoff, R. 1994. Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus uraphylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics, **137**: 1121–1137.

Grattapaglia, D., Chapparo, J., Wilcox, P., McCord, S., Werner, D., Amerson, H., McKeand, S., Bridgwater, F., Whetten, R., O'Malley, D., and Sederoff, R. 1992. Mapping in woody plants with RAPD markers: application to breeding in forestry and horticulture. *In* Joint Plant Breeding Symposia Series on Applications of RAPD Technology to Plant Breeding, 1 Nov. 1992, Minneapolis, Minn.

Grattapaglia, D., Bertolucci, F.L., and Sederoff, R.R. 1995. Genetic mapping of QTLs controlling vegetative propagation in *Eucalyptus grandis* and *E. urophylla* using a pseudo-testcross strategy and RAPD markers. Theor. Appl. Genet. **90**: 933–947.

Kaya, Z., and Neale, D.B. 1995. Linkage mapping in Turkish red pine (*Pinus brutia* Ten.) using random amplified polymorphic DNA (RAPD) genetic markers. Silvae Genet. **44**: 110–116.

Kreike, J. 1990. Genetic analysis of forest tree populations: isolation of DNA from spruce and fir apices. Plant Mol. Biol. **14**: 877–879.

Lander, E.E., Green, P., Abrahamson, J., Barlow, A., Daly, H.J., Lincoln, S.E., and Newberg, I. 1987. Mapmaker and interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics, **1**: 174–181.

Mukai, Y., Suyuma, Y., Tsumura, Y., Kawahara, T., Yoshimaru, H., Kondo, T., Tomaru, N., Kuramto, N., and Murai, M. 1995. A linkage map for sugi (*Cryptomeria japonica*) based on RFLP, RAPD, and isozyme loci. Theor. Appl. Genet. **90**: 835–840.

Neale, D.B., and Harry, D.E. 1994. Genetic mapping in forest trees: RFLPs, RAPDs, and beyond. Agric. Biotechnol. News Inf. **6**: 107N–114N.

Neale, D.B., and Williams, C.G. 1991. Restriction fragment length polymorphism mapping on conifers and applications to forest tree genetics and tree improvement. Can. J. For. Res. **21**: 545–554.

Neale, D.B., Devey, M.E., Jermstad, K.D., Ahuja, M.R., Alosi, M.C., and Marshall, K.A. 1992. Use of DNA markers in forest tree improvement research. New For. **6**: 391–407.

Nelson, C.D., Nance, W.L., and Doudrick, R.L. 1993. A partial genetic linkage map of slash pine (*Pinus elliottii* Engelm var. *elliottii*) based on randomly amplified polymorphic DNAs (RAPD). Theor. Appl. Genet. **87**: 145–151.

Pikaart, M.J., and Villeponteau, B. 1993. Suppression of PCR amplification by high levels of RNA. Biotechniques, **14**: 24–25.

Plomion, C., O'Malley, D.M., and Durel, C.E. 1995. Genomic analysis in maritime pine (*Pinus pinaster*). Comparison of two RAPD maps using selfed and open-pollinated seeds of the same individual. Theor. Appl. Genet. **90**: 1028–1034.

Tanksley, S.D. 1983. Molecular markers in plant breeding. Plant Mol. Biol. Rep. **1**: 3–8.

Tulsieram, K.L., Glaubitz, J.C., Kiss, G., and Carlson, J.E. 1992. Single tree genetic linkage mapping in conifers using haploid DNA from megagametophytes. Bio/Technology, **10**: 686–690.

Wani, M.C., Taylor, H.L., Wall, M.E., Coggon, P., and McPhial, A.T. 1971. Plant anti-tumor agents. Part 6. The isolation and structure of taxol, a noval anti-leukemic and anti-tumor agent from *Taxus brevifolia*. J. Am. Chem. Soc. **93**: 2325–2327.

Welsh, J., and McClelland, M. 1990. Finger-printing genomes using PCR with arbitrary primers. Nucleic Acids Res. **18**: 7213–7218.

Wheeler, N.C., Jech, K., Masters, S., Brobst, S.W., Alvarado, A.B., and Hoover, A.J. 1992. Effect of genetic, epigenetic, and environmental factors on taxol content in *Taxus brevifolia* and related species. J. Nat. Prod. **55**: 432–440.

Williams, J.G.K., Kubelic, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**: 6531–6535.

pH distribution in radial sections of the stem and root of *Cryptomeria japonica*

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Abstract: The pH distribution in the outer bark, inner bark, cambium, and xylem layer of a conifer, *Cryptomeria japonica*, was measured using an iridium oxide electrode and a flat-type glass electrode. The outer bark of *C. japonica* was strongly acidic, and there was a difference in pH between the outer and inner bark. The outer bark had a pH of about 3 from the surface to the inside. The inner bark pH was about 4 at the outer bark side and about 6 near the cambium side. The pH was maximal (about 6.3) in the cambium, and slightly lower in the outer xylem. pH values determined in subsections of the outer bark after chemical washing with pure water, hydrochloric acid, barium chloride, and formic acid suggested that water-insoluble carboxyl (—COOH) groups bound to organic polymers contribute to the strong acidity of the outer bark.

Résumé : La répartition du pH dans l'écorce externe et interne, le cambium et le xylème d'un conifère, *Cryptomeria japonica*, a été mesurée avec une électrode à l'oxide d'iridium et une électrode de verre de type plat. L'écorce externe de *C. japonica* était très acide et il y avait une différence de pH entre l'écorce externe et l'écorce interne. Le pH de l'écorce externe mesurait environ 3 en allant de la surface vers l'intérieur. Le pH de l'écorce interne mesurait environ 4 du côté de l'écorce externe et environ 6 du côté du cambium. Le pH était le plus élevé, environ 6,3, dans le cambium et légèrement plus faible dans la partie externe du xylème. Les valeurs de pH mesurées dans des sous-sections de l'écorce externe après un lavage à l'eau pure, à l'acide hydrochlorique, au chlorure de barium et à l'acide formique suggèrent que des groupements carboxyliques (—COOH) liés à des polymères organiques contribuent à l'acidité élevée de l'écorce externe.

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