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Identification of a RAPD marker linked to the pendula gene in Norway spruce (*Picea abies* (L.) Karst. f. *pendula*)

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Abstract The pendula phenotype of Norway spruce [*Picea abies* (L.) Karst f. *pendula*] is characterized by narrow crowns and strong apical dominance and is controlled by a single dominant gene (*P*). This defined genetic control presents one of the few opportunities to map a single gene controlling a morphological trait in a forest tree. We used random amplified polymorphic DNA (RAPD) markers and bulked segregant analysis to identify one locus OPH10_720, linked to the pendula gene. The estimated recombination frequency (*r*) between OPH10_720 and *P* was 0.046 (SE_r = 0.032). Mapping of the pendula gene is an important first step towards the ultimate identification and cloning of this gene.

Key words Norway spruce · Pendula phenotype · Bulked segregant analysis · RAPD markers

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Introduction

Most traits of interest for forest-tree improvement are quantitatively inherited (e.g. growth and yield); there are very few examples of traits controlled by single genes. The pendula phenotype in Norway spruce [*Picea abies* (L.) Karst. f. *pendula*] is a naturally occurring mutant with narrow crowns and strong apical dominance (Pöykkö and Pukkinen 1990). Crosses between pendula and wild-type trees have demonstrated that this phenotype is controlled by a single dominant gene (Pukkinen and Pöykkö 1990; Lepistö 1985). This simple Mendelian inheritance presents a rare opportunity to genetically map a gene for a morphological trait of major economic importance in a forest tree. Spruces expressing this gene could be grown at higher stand densities and might have improved wood quality due to a decrease in the size of lateral branches (Kärki 1985). The isolation and transfer of this gene to other species might also lead to new varieties for reforestation.

RAPD markers and bulked segregant analysis have successfully been used to map genes affecting disease resistance in lettuce (Michelmore et al. 1991; Kesseli et al. 1993) and barley (Barua et al. 1993). Hormaza et al. (1994) used this approach to identify a RAPD marker linked to sex determination in *Pistacia vera*.

Forest trees are considered to be difficult subjects for genetic analysis due their large genome size and lack of genetic-linkage information. However, Devey et al. (1995) recently mapped a gene for resistance to white-pine blister rust (*Cronartium ribicola* Fisch.) in sugar pine (*Pinus lambertiana* Dougl.) using bulked segregant analysis and RAPD markers.

In the present paper we report the identification of a RAPD locus linked to the pendula gene using bulked segregant analysis. The recombination frequency between the RAPD marker OPH10_720 and the pendula gene was estimated to be 0.046.

Materials and methods

Plant material

Forty-three full-sib progeny of the cross P289 × E477 were selected for genetic analyses. The female parent (P289) was normal (*pp*) and the male parent (E477) was pendula (*Pp*). The 43 progeny trees were 5 years old and segregated for both phenotypes. Eighteen trees exhibited the pendula phenotype (42%) and twenty-five had the wild-type (58%) phenotype.

DNA extraction

Genomic DNA from the two parent trees and the 43 progeny was isolated from needle tissue using standard procedures (Murray and Thompson 1980).

Bulked segregant analysis

Two bulked DNA samples from 15 pendula and 15 normal progeny were constructed (Michelmore et al. 1991) Four-hundred decamer primers (Operon Technologies, Alameda, Calif.) were used to screen the two bulked DNA samples based on small modifications of the method of Williams et al. (1990). The presence of a RAPD fragment in one bulk and absence in the other provided evidence for a putatively linked marker. To exclude false positives, assays were repeated on the parental DNAs and ten individual DNAs from each bulk (pendula and normal) using the appropriate primer.

RAPD assays

PCR templates for both bulked and individual progeny reactions consisted of 20 ng of genomic DNA. Amplification reactions were done in a buffer containing 10 mM TrisHCl (pH 8.3 at 20 °C), 50 mM KC1, 1.5 mM $MgCl_2$, 0.33 μM primer, and 1 U of *Taq* DNA Polymerase (Boehringer Mannheim) in a total volume of 25 μl. Forty-five cycles of amplification were performed on a Biometra TrioBlock Thermocy-cler: 2 min 95 °C; 45 cycles of: 1 min 95 °C; 1 min 36 °C; 2 min 72 °C and an additional 5 min 72 °C step. Amplification products were electrophoresed on 1.5% agarose gels in 0.5 × TBE buffer. DNA fragments were visualized by ethidium bromide staining and fluorescence.

Southern hybridization

Southern hybridization was conducted to verify the segregation of RAPD bands at the OPH10_720 locus. Approximately 100 ng of the 720 bp fragment, which had been cut out of an agarose gel and re-amplified, was labelled with 50 μCi of 32 P-dCTP. Amplification products were transferred to Zetaprobe GT (Biorad) nylon membrane and blots were hybridized and washed at 65 °C in 0.1 × SSPE/0.1% SDS.

Linkage analysis

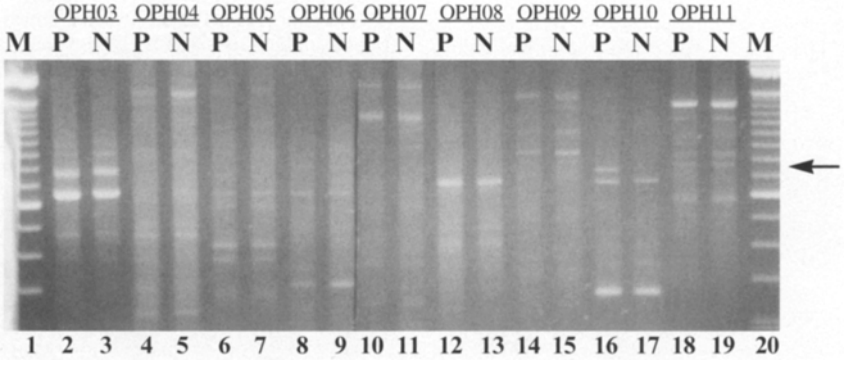
Maximum-likelihood estimation was used to calculate recombination frequency ($r = R/N$), where R equals the number of recombinants and N equals the total number of progeny. The maximum-likelihood estimate of the standard error of r was $SE_r = \sqrt{r(1-r)/N}$ (Adams and Joly 1980).

Results and discussion

RAPD markers and bulked segregant analysis were used to quickly identify one genetic marker linked to the pendula gene in Norway spruce. An example of the RAPD patterns from the pendula and normal bulks for 9 of the 400 primers is shown in Fig.1. The presence of a band in one bulk and absence in the other is putative evidence for a linked locus, as shown for pirmer OPH10. Twelve putatively linked loci were identified during this first screening. Assays were then performed on DNA isolated from the parents and ten individual progeny DNAs from each of the pools to exclude false positives based on BSA. Seven primers were excluded based on these analyses. The remaining five primers were applied to all 43 individuals. Only one RAPD locus (OPH10_720) was linked to the pendula gene. RAPD patterns of the parental and 20 progeny DNAs for primer OPH10 are shown in Fig. 2. Two recombinant individuals were found at the OPH10_720 locus, indicating tight linkage between the marker and the *P* locus. Southern hybridization was conducted to verify the RAPD-band segregations (data not shown). A single band of approximately the same size (720 bp) was detected in every individual exhibiting a pendula phenotype and in the recombinant individual. The estimated recombination frequency (r) between OPH10_720 and *P* was 0.0465 ($SE_r = 0.032$).

The power of bulked segregant analysis is not nearly as great under the conditions presented here as was

Fig. 1 Bulk samples of DNA from pendula (*P*) and wild-type (*N*) individuals were used to screen 400 RAPD primers. Shown here are amplification products from nine primers, *OPH03* through *OPH11*, on the *P* and *N* bulks. *M* = 100-bp molecular-weight ladder, BRL. Any difference between the two samples identifies a putatively linked marker, such as with *OPH10*, where one fragment (720 bp, solid arrow-head) is present in the pendula bulk sample (*lane 16*) but absent in the normal sample (*lane 17*)



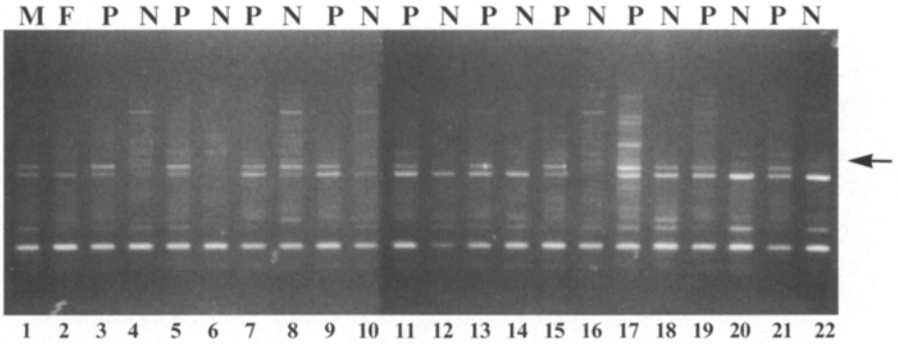


Fig. 2 Segregation analysis with primer OPH10 and DNA from male (*lane 1*) and female (*lane 2*) parent and 20 individuals of both pendula (*P*) and wild-type (*N*) progeny, (*lanes 3–22*). The *solid arrowhead* indicates the pendula-linked RAPD fragment (OPH10_720). Recombinant individuals are shown in *lanes 8* and *18*.

originally reported for backcross or F_2 populations (Michelmore et al. 1991). The mating used in this study was between two non-inbred and highly heterozygous parent trees. Thus, there is likely to be differences in the linkage phase of a marker gene and the target gene between parents of the cross. The power to detect linked dominant marker loci using bulked segregant analysis with this type of cross is significantly reduced. An alternative and more powerful approach for using bulked segregant analysis in conifers is to take advantage of the haploid genetic system in seed megagametophytes. Each megagametophyte represents a single meiotic product, thus linkage analyses can be conducted on segregations from individual mother trees.

The next stage of this project is to identify markers more closely linked to *P* by using the haploid genetics approach and a large number of progeny. Direct analysis of the linkage relationship of markers to the pendula gene would then be possible without the use of controlled crosses. The use of significantly larger sample sizes will also identify markers more closely linked to the pendula gene. Ultimately, a genetic marker which tags the pendula gene must be identified to proceed with positional cloning.

Norway spruce might be a good model system for the positional cloning of conifer genes because a regenerable tissue-culture system, using somatic embryogenesis, has been reported (Hakman and von Arnold 1985) and stable transformation has recently been successful using biolistic transfer into embryogenic suspensions (Ellis et al. 1993). Transformation and regeneration would enable complementation assays to be performed for verification of the cloning of the pendula gene.

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