THE USE OF BULKED SEGREGANT ANALYSIS TO IDENTIFY AFLP™
MOLECULAR MARKERS CLOSELY LINKED TO *MELAMPSORA
LARICI-POPULINA* RESISTANCE IN *POPULUS*

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1. Abstract

We have identified three AFLP markers tightly linked to the locus conferring resistance
to the leaf rust *Melampsora larici-populina* in *Populus* by Bulked Segregant Analysis.
The study was carried out using a hybrid progeny derived from an inter-specific,
controlled cross between a resistant *Populus deltoides* female and a susceptible *P. nigra*
male. The segregation ratio of resistant to susceptible plants suggested that a single,
dominant locus (*Mer*) defined the resistance to *Melampsora*.

2. Introduction

*Melampsora larici-populina* is one of the most damaging fungi for poplar in Central-
and North-Europe. Infection by *M. larici-populina* causes premature defoliation and can
reduce growth by more than 20%. Trees defoliated early in the growing season are
more susceptible to other pathogens and to environmental stress. Infections over
successive years can result in a complete loss of the plantation. Therefore, tree breeders
have generated and selected inter-specific hybrids that are resistant to different races of
*M. larici-populina*.

Since association between genetic markers and traits of interest was first reported
by Sax (1923), much attention has been given to the potential applications of markers
to improve plant breeding. This approach opens a multitude of new ways in tree
improvement. It provides the basis for accelerated breeding through increased intensity
of selection of larger progenies, allows early selection of traits, and permits more
efficient selection of parents for subsequent breeding programs. Different molecular
techniques, such as Restriction Fragment Length Polymorphism (RFLP) or polymerase
chain reaction (PCR)-based techniques, have been used with the aim of detecting
molecular markers associated with simply inherited or complex traits (quantitative trait
loci, QTLs) of poplar, especially those related to disease resistance and commercially
important traits (Bradshaw and Stettler, 1995a, 1995b). The efficiency of the indirect
selection will depend on the easiness of marker detection and on the degree of linkage to the locus of interest. Several high-density marker technologies have recently been developed. The AFLP™ technique developed by Vos et al. (1995) can be considered the most powerful and is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. The AFLP technique combines the reliability of the RFLP technique, avoiding Southern blotting, with the specific detection of restriction fragments by PCR amplification (Vos et al., 1995). The AFLP technique detects polymorphisms such as the presence or absence of restriction enzyme sites, sequence polymorphisms adjacent to these sites, insertions, deletions, and rearrangements. Michelmore et al. (1991) described a screening method, the Bulked Segregant Analysis (BSA) based on bulking DNAs from individual plants to rapidly screen for molecular markers, tightly linked to a locus of interest. We report the identification of three molecular markers tightly linked to the locus conferring resistance to *M. larici-populina* in *Populus* using AFLP and BSA. This strategy combines one of the most important advantages of the AFLP technology, the high number of loci that can be analyzed per experiment, with the rapid screening approach of BSA.

3. Materials and Methods

3.1. PLANT MATERIAL

The three-generation *Populus* pedigree was founded in 1948 by crossing a female *P. deltoides* V5, resistant to races E1, E2, and E3 of *M. larici-populina* and a susceptible male *P. deltoides* V1. The obtained F1 hybrid family S 9 consisted of 500 clones. A controlled cross between one clone of the family S 9, the female S 9-2 resistant to races E1, E2, and E3 of *M. larici-populina* and the susceptible male *P. nigra* Ghy was carried out in 1987, to produce the hybrid family 87001 which contained 262 clones.

3.2. *MELAMPSORA LARICI-POPULINA* RESISTANCE TEST

Resistance tests were carried out in the experimental nursery and by artificial infections in the laboratory. For the nursery tests, 10 cuttings of all 262 seedlings were planted in the nursery in June. Plants were observed for their susceptibility to *M. larici-populina* in October. The score system used comprised 11 values ranging between 0 and 5 including half-values: 0 (tree is entirely free of rust), 0.5 (rust spores on 1-10% of the leaves, no leaves are shed), 1 (rust spores on 11-20% of the leaves, no leaves are shed), 1.5 (rust spores on 21-30% of the leaves, no leaves are shed), 2 (rust spores on 31-40% of the leaves, no leaves are shed), 2.5 (rust spores on 41-50% of the leaves, no leaves are shed), 3 (rust spores on 51-60% of the leaves, 1-20% of the leaves are shed in the lower crown-half), 3.5 (rust spores on 61-70% of the leaves, 21-40% of the leaves are shed in the lower crown-half), 4 (rust spores on 71-80% of the leaves, 41-60% of the leaves are shed), 4.5 (rust spores on 81-90% of the leaves, 61-80% of the leaves are shed), and 5 (rust spores on 91-100% of the leaves, 81-100% of the leaves are shed). The score reflects an average for the 10 cuttings per seedling.