

Molecular Dissection of White Pine Genetic Resistance to *Cronartium ribicola*

Jun-Jun Liu, Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria, British Columbia, Canada; and **Richard Sniezko**, USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR

Abstract—*Pinus monticola* (Dougl. ex D. Don.) maintains a complex defence system that detects white pine blister rust pathogen (*Cronartium ribicola* J.C.Fisch.) and activates resistance responses. A thorough understanding of how it functions at the molecular level would provide us new strategies for creating forest trees with durable disease resistance. Our research focuses on molecular dissection of *P. monticola* major gene (Cr2) resistance and quantitative partial resistance. To characterize the Cr2 gene, resistance gene family encoding proteins with nucleotide-binding-site and leucine-rich-repeat (NBS-LRR) was identified and used to search for DNA polymorphisms by genotyping with a modified approach of amplified fragment length polymorphism (AFLP). Multiple DNA markers for NBS-AFLPs and LRR-AFLPs were mapped on the Cr2 linkage, potentially useful for marker-assisted selection (MAS) in breeding programs. A fine Cr2 genetic mapping is in progress for a final positional cloning of this R gene. The Cr2-triggered defence response was investigated by both genomic and proteomic strategies. A cDNA library was constructed from needles of a Cr2 family at early stages (at time 0-control to 96 hrs) post *C. ribicola* infection. From 5,000 clones randomly sequenced, 3,034 unique expressed sequence tags (ESTs) were identified, including 31 signal transduction genes, 47 transcription factor genes, and 326 down-stream stress-responsive genes. A case study of the thaumatin-like protein (PmTLP) family using quantitative reverse transcription-polymerase chain reaction (RT-qPCR) revealed that a coordinated up-regulation of multiple PmTLP genes was involved in *P. monticola* Cr2 resistance. A further analysis of changes in global gene expression patterns will allow us to identify candidate genes of other types that are involved in compatible and incompatible white pine-*Cronartium* interactions. In the *P. monticola* proteome, we sequenced 105 proteins that were differentially

expressed between early stages post infection on resistant and susceptible seedlings. Among those identified proteins, the 14-3-3 proteins are significant in that they bind a number of functionally diverse signalling proteins such as kinases, phosphatases and transmembrane receptors. Multiple families of pathogenesis-related (PR) proteins are believed to contribute to plant quantitative resistance to various pathogens. Along with other host PR proteins, PR3 chitinase (PmCh4) and anti-microbial peptide (PmAMP1) are protein components participating in western white pine partial resistance to *Cronartium*. These researches on gene expression profiling have resulted in identifying different resistance mechanisms in western white pine. We also investigated single nucleotide polymorphisms (SNPs) of the PmCh4 gene family and protein accumulation of PmAMP1 in seed families, their partial resistance phenotypes were quantitatively assessed in USDA-FS trials using a series of parameters, including presence of aecia, canker damage and severity, number of stem symptoms by type (normal canker, partial bark reaction, complete bark reaction), or 'clean'/stem symptom free. A low level of PmCh4 intragenic linkage disequilibrium (LD), but most of them with statistic significance, was found within a distance of ~800 bp. The PmCh4 SNP data also revealed moderate to high levels of genetic structure among these seven seed families (average $F_{st} = 0.163$, $p < 0.001$). Our findings suggest that LD-based association analysis is as effective strategy to dissect genetic mechanisms underlying partial resistance phenotypes in conifers. Our studies using molecular genetic, genomic, and proteomic approaches are yielding a detailed understanding of how *P. monticola* confers resistance to *Cronartium*. We believe that similar approaches are feasible to investigate rust resistance in *P. albicaulis* and other white pines.

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