ENHANCING *Septoria Musiva* RESISTANCE IN POPLAR WITH MOLECULAR GENETIC APPROACHES

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Populus species and their hybrids are among the fastest-growing temperate trees. The primary commercial uses of poplar trees include pulpwood, engineered lumber products, and bioenergy. In anticipation of shrinking petroleum reserves and a reduced land base to produce forest products, interest in short-rotation intensive poplar culture as an alternative fuel and fiber resource has increased. However, widespread adoption of short-rotation intensive poplar production is hindered by the occurrence of several important foliar and stem infecting pathogens. Among them, leaf spot and stem canker caused by the necrotrophic fungus *Septoria musiva* Peck is the most serious disease affecting hybrid poplar production in North America (1). Severe leaf spot outbreaks can reduce the photosynthetic area and cause premature defoliation; thereby decreasing annual growth. Stem cankers reduce growth, predispose the tree to colonization by secondary organisms, and cause severe girdling and breakage of the main stem. Biomass losses due to this pathogen vary among clones and have been reported to be as high as 63% of total yield (2). Furthermore, Ostry et al. (3) found that 86% of the clones in a plot located near infected plantations in Michigan had cankers five years after plot establishment. Chemical and biological control of Septoria leaf spot and canker has been attempted. For example, spraying disease-suppressive *Streptomyces* strains can also significantly reduce leaf disease caused by *S. musiva* (4). However, all these approaches are expensive and problematic. In the case of *Streptomyces*, spore application needed to be done at least monthly but preferably weekly during periods of *S. musiva* spore release. As a result, planting resistant clones appears to be the best means of managing Septoria diseases. In this report, we evaluated three candidate resistance genes by genetic engineering. We also conducted the first global analysis of the *Populus* defense transcriptome dynamics in response to *S. musiva* leaf spot using the RNA-Seq method. Differential expression analyses were performed between resistant and susceptible clones and infected and uninfected leaf tissues.

MATERIALS AND METHODS

Poplar transformation: *Agrobacterium tumefaciens* strain EHA105 carrying candidate gene(s) that are listed in Table 1 were used to transform a *S. musiva* susceptible hybrid clone OGY (*P. deltoides* × *nigra*). Transformation and selection were conducted according to Liang et al. (5).

Host Propagation, pathogen propagation, and inoculations: Dormant branch cuttings from 4 clones of hybrid poplar, DN34, NM6, NC11505, and DN164, representing 2 resistant and 2 susceptible poplar clones, were cut into 10-cm-long sections and planted into 21-cm-deep Ray Leach cone-tainers TM with only the top-most bud exposed above the growing medium surface. Following the initial 90 days of growth trees were transplanted from the cone- tainers into 1 gallon plastic pots. Three isolates of *S. musiva*, isolated from Septoria cankers, were used in the experiment. Inoculations were conducted according to LeBoldus et al. (6).

Table 1. Candidate genes for poplar transformation (provided by Dr. William A. Powell at SUNY-ESF)

Gene(s) in construct	Promoter	Number of transgenic events	Notes
ESF39	A vascular promoter from American chestnut stem tissue (ACS2)	3	• ESF39: an antimicrobial peptide gene having a similar amphipathic α-helix found in magainins • Lac: a laccase gene from Chinese chestnut • Kanamycin as selection agent for all constructs
Laccase	A vascular promoter from American chestnut stem tissue (ACS9A)	2	
ESF39+Laccase	Same as individual gene	3	
OxO+Laccase	Same as individual gene	4	
OxO+ESF39+Laccase	Same as individual gene	14	

Illumina sequencing and data processing: Four days after inoculation, leaves were collected and immediately frozen in liquid nitrogen. Total RNA was extracted with a CTAB method. The integrity and quality of RNA were verified with an Agilent Technologies 2100 Bioanalyzer before subjecting the RNA to cDNA synthesis and library construction. A total of 16 cDNA samples were individually bar-coded and run in two lanes of HiSeq 2000 v3. The *Populus trichocarpa* genome and gff annotation file were downloaded from Phytozome, while the *Septoria musiva* genome and gff annotation file were from JGI. The RNA-Seq reads were aligned to both reference genomes using the software package TopHat (7). The resulting alignments were analyzed by Cufflinks (8) to assemble the transcripts and estimate their abundance in each indexed sample.

Identification of differentially expressed (DE) genes: A statistical package, DESeq, was used to analyze the transcript abundances (9) with default parameters. For the comparisons with combined genotypes, a multifactor design was used to account for the genotype differences as well as the sample condition ("Septoria challenged" vs "control"). An adjusted p-value of less than .05 was considered significant across all comparisons.

RESULTS AND DISCUSSION

As shown in Table 1, several transgenic lines have been obtained for the candidate genes as evidenced by PCR with gene-specific primers. The expression levels of transgenes have also been determined. We are currently propagating the plants for whole-plant *S. musiva* assays. Individual expression of the wheat oxalate oxidase gene and several antimicrobial peptide genes having a similar structure as ESF39A was able to enhance resistance in OGY to *S. musiva*, although the plants ultimately succumbed to the disease (5). By combining 2 or 3 resistance genes with different action mechanisms, we expect to achieve a higher level of and more durable resistance.

In total, 511 million reads were generated, constituting 22.8 Gb of sequences. An average of 78% of the reads was successfully aligned to the *P. trichocarpa* genome, 28% of which was

uniquely aligned. Functional annotation of differentially expressed genes based on comparisons between resistant and susceptible clones revealed that there were significant differences in the expression of genes involved in disease/stress resistance and oxidation reduction in mock treated leaves. Four days post inoculation of *S. musiva*, differentially expressed genes were most enriched with GO terms of leucine-rich repeats and disease defense. Among them were 23 loci involved in plant-type hypersensitive responses (GO:0009626), corresponding to 8 kinds of proteins: NB-ARC domain-containing disease resistance protein (11 loci), flavin-dependent monooxygenase 1, phospholipase A 2A, MAC/Perforin domain-containing protein, MutT/nudix family protein, EF-TU receptor, mitogen-activated protein kinase 3, and elicitor-activated gene 3-1. In particular, Potri.001G426500 locus, encoding a homolog of NB-ARC domain-containing disease resistance protein, was up regulated in the relative resistant clones of all compressions, suggesting a strong candidate R gene in response to *S. musiva* perception. The results from this study indicate that strong defense mechanisms involved in the pathogen perception, oxidation reduction, and accumulation of defense-related gene products may contribute to Septoria resistance in DN34 and NM6. We will use qRT-PCR to validate the expression data from RNA-Seq.

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