Transcriptome Analysis of Rpp4 Silenced Plants via virus-induced gene silencing (VIGS).

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Five Asian Soybean Rust (ASR) resistance genes have been identified in soybean: *Rpp1*, *Rpp2*, *Rpp3*, *Rpp4* and *Rpp5*. Of particular interest is *Rpp4*, which has remained stable and confers resistance against *Phakopsora pachyrhizi* isolates from around the world. *Rpp4* was mapped to soybean linkage group G (chromosome 18), 1.9cM from simple sequence repeat (SSR) marker Satt288. Sequencing of this region in the susceptible genotype Williams 82 (Wm82) identified a cluster of three CC-NBS-LRR resistance genes. We developed Virus Induced Gene Silencing (VIGS) constructs from the NBD and LRR regions of the Wm82 Rpp4 candidate genes to test whether paralogous genes were responsible for resistance in the resistant parent (PI459025B). To perform the VIGS experiments, 14-day-old PI459025B plants were subjected to one of five pre treatments: no treatment, mock inoculation with buffer and carborundum, inoculation with a Bean pod mottle virus (BPMV) vector lacking an insert, or inoculation with LRR- BPMV VIGS vectors. At 21 days after BPMV inoculation, all plants were inoculated with a spore suspension from *P. pachyrhizi* isolate LA04-1. RNA was extracted from the *Rpp4* LRR silenced plants and transcriptome analyses of three independent biological replicates was performed using the GeneChip® Soybean Genome Array (Affymetrix®). Since the plant samples used in this study differed only in the expression of *Rpp4,* comparisons of these samples by microarray would identify genes downstream of *Rpp4* in the signaling pathway. A total of 383 genes were found to be significantly differentially expressed between *Rpp4* silenced and mock silenced plants infected with ASR. Out of 383 genes differentially expressed genes, 22 were induced, and 361 were suppressed. Most of the up-regulated genes shared homology to known genes such as Pectin acetylesterase, Aspartyl protease, GDP mannose pyrophosphorylase, phosphatidylinositol transfer protein PDR16. Of the down-regulated genes identified, many had functions related to defense, disease resistance and metabolism. Statistical analyses of overrepresented gene ontology functional categories highlighted the importance of genes involved in lignin biosynthesis, flavonoid biosynthesis, response to oxidative stress and phenylpropanoid biosynthesis for defense. In addition, we used Clover (cis-element over representation) software and TRANSFAC, a transcription factor database, to identify transcription factor binding sites over-represented in the promoters of our differentially expressed genes. From this analysis we identified 39 transcription factor binding sites significantly over represented in our differentially expressed genes when compared to all genes in the soybean genome. Most of the cis-elements we identified were related to defense, such as MYB80, MYBBAS1, MYB.PH3, CRF-2. This study revealed part of metabolic pathways potentially activated by Rpp4 gene.

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