Different sets of wheat genes are used in *Dn7*-mediated resistance to feeding by two biotypes of Russian wheat aphid

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ABSTRACT

Diuraphis noxia (Kurdjumov) (Russian wheat aphid) is an economically significant pest in wheat-growing areas, particularly in the U.S. and South Africa. The interaction between wheat and the Russian wheat aphid is poorly understood. When new biotypes appeared in the U.S. between 2003 and 2006, it became evident that specific interactions exist between resistance loci and aphid biotypes. *Dn7* provides a high level of resistance to eight currently existing biotypes. This study was conducted to gain a greater understanding of *Dn7*-mediated resistance to two different biotypes (RWA1 and RWA2). Using wheat Genechip® arrays, we compared the transcript profiles of resistant and susceptible lines (94M370 and Gamtoos) infested with either RWA1 or RWA2. The number of differentially expressed genes was higher in both resistant and susceptible plants fed upon by RWA1 compared to those fed upon by RWA2. Common sets of genes in response to both biotypes were involved in basic functions such as carbohydrate metabolism and energy generation. Common genes also included cell wall synthesis enzyme genes, and defense-response or stress-related genes. Many genes that were unique to RWA1 or RWA2 response were transcription factors. The results suggest that while common pathways are involved in *Dn7*-mediated resistance to RWA1 or RWA2 attack, divergent pathways appear to be involved as well. Silencing of candidate genes identified from microarray experiments using virus-induced gene silencing (VIGS) enabled us to identify genes that play important roles in wheat's defense response to the Russian wheat aphid.

INTRODUCTION

The Russian wheat aphid (RWA), is a significant insect pest of bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare*) in many areas of the world. Estimates of direct and indirect costs of RWA to small grain producers in the US totaled $893.1 million from 1987-1993, averaging out to about $127 million per year ((Morrison and Peairs 1998). The damage caused by the RWA has been effectively controlled by the use of resistant cultivars. In 2003, however, a new biotype was discovered in Colorado that was virulent against all known resistance genes with the exception of a gene called *Dn7* (Haley et al. 2004). The new population was designated RWA2, while the original population was designated as RWA1. In the following three years, at least five more biotypes were identified in the US central great plains (Burd et al. 2006; Weiland et al. 2008). The rapid emergence of new biotypes has created a dilemma for breeding programs because development of a single cultivar could take as long as 10-12 years. Innovative strategies for protecting cereal crops from this pest are needed. Understanding the mechanism of RWA-host interaction may provide the basis for new strategies of plant protection.

Dn7 is a rye gene that was transferred to wheat via a 1R.1B translocation (Marais et al. 1994) and it confers a high level of resistance to all currently existing biotypes in the U.S. as well as South African biotypes (Lapitan et al. 2007a; Weiland et al. 2008; Zaayman et al. 2008). This study was conducted to elucidate the defense response mechanisms of *Dn7*-mediated resistance during feeding by two different aphid biotypes (RWA1 and RWA2).

MATERIALS AND METHODS

Plant materials and aphid infestation

Hexaploid wheat (*Triticum aestivum* L.) 9M370 containing *Dn7* and Gamtoos (*Dn7*$^-$) were infested with RWA1 or RWA2 at the 4- leaf stage. Non-infested plants of both cultivars served as controls. Three replications of each treatment were conducted. Leaf tissues were collected 5 h post infestation (5 hpi).

RNA preparation and hybridization to wheat GeneChip® arrays

Frozen leaf tissue was ground and incubated with RNA purification reagent from Invitrogen (Carlsbad, CA) at room temperature for 10 minutes. Total RNA was extracted using Qiagen RNeasy Plant Mini Kit with RNase Free/DNase (Qiagen, Valencia, CA) following the manufacturer's instructions. Integrity and quantity of the RNA was tested using Bio-Rad Experion RNA StdSen Chips (Bio-Rad, Hercules, CA). The RNA samples were sent to the Virginia Bioinformatics Institute Core facility or the Microarray Core Lab (Aurora, CO), where additional quality control was performed. These facilities then performed RNA labeling, processing, and data gathering according to Affymetrix protocols.

Microarray data quality control and analysis

A total of 18 samples were hybridized to arrays. Different quality control checks were performed

including inspection of hybridized images, boxplots and histograms of $\log_2$(PM) values, examination of hybridization and PolyA controls. Data analysis was carried out using Bioconductor in R (Gentleman et al. 2004). Data preprocessing and summarization were performed using Robust Multichip Average (RMA) (Irizarry et al. 2003). Statistical tests of differential expression were conducted using the moderated t-test through the limma (Linear Models for Microarrays) package in Bioconductor. The Benjamini-Hochberg multiple testing adjustment was applied in order to control the comparison-wise false discovery rate (Benjamini and Hochberg 1995). Genes corresponding to probe sets with an average absolute value of less than or equal to 0.05 were considered differentially expressed. The target sequence corresponding to genes identified as differentially expressed were obtained from Affymetrix. Target sequences were then searched against the KEGG (http://www.genome.jp/kegg/), BRENDA (http://www.brenda-enzymes.info/), and Gene Ontology (GO) (http://www.geneontology.org/) databases using BLASTX via the program PLAN (He). Annotationwas obtained for the top significant hit (using an e-value cutoff of 1e-10) for each target sequence.

RESULTS AND DISCUSSION

More genes were differentially expressed after infestation with RWA1 (1138 and 1147, up- and down-regulated with RWA1, respectively; 965 and 165, up- and down-regulated with RWA2, respectively). Of these genes, only 486 were in common during RWA1 and RWA2 infestation. More genes appeared to be unique in response to RWA1 or RWA2 at this time point (Fig.1).

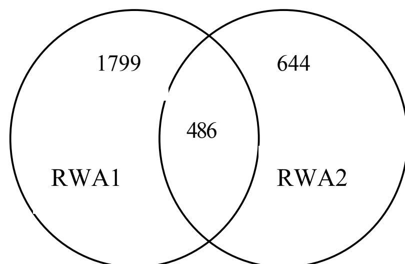

Figure 1. Venn diagram showing the number of differentially expressed genes 5hpi with RWA1 or RWA2.

Genes that were in common in response to RWA1 and RWA2 included those coding for cell wall synthesis enzymes, carbohydrate metabolism enzymes, protein processing enzymes and genes in the jasmonic acid pathway. This group also contains heat shock proteins, zinc finger transcription factors, and calmodulin. However, a majority of the genes in this group are of unknown function.

Tables 1 and 2 show examples of genes that were uniquely differentially expressed during RWA1 or RWA2 infestation, respectively. Many of the genes in this group consist of transcription factors.

Table 1. Examples of genes differentially expressed in response to RWA1

Probe Set ID	Annotation
Ta.12219.1.A1_at	cysteine protease
Ta.1321.1.S1_at	chloroplast chaperone protein
Ta.13256.1.A1_a_at	pathogenesis-related group 5 protein
Ta.1739.2.S1_at	senescence-associated protein-like
Ta.22828.2.S1_at	wkm2c.pk005.n2:fis, amino acid metabolism
Ta.23807.4.S1_s_at	Heat shock protein 70

Table 2. Examples of genes differentially expressed in response to RWA2

Probe Set ID	Annotation
Ta.5367.1.S1_s_at	NAC domain protein
Ta.20429.1.S1_at	ammonia-lyase (wali4)
Ta.2882.1.S1_at	EF-hand Ca2+-binding protein CCD1
Ta.4678.1.S1_at	(A.thaliana) transcription factor
Ta.320.1.S1_at	calcium-binding protein - like (Arabidopsis thaliana)
Ta.11671.1.S1_at	Heat shock factor protein 4

Similar results were previously observed using cDNA AFLP transcript profiling of the same genotypes infested with RWA1, RWA2 and two South African biotypes. Zaayman et al. (2008) showed that the response of *Dn7* to the South African biotypes infestation noticeably differed from the response to the US biotypes RWA1 and RWA2. Similar genes were identified in response to RWA1 and RWA2 as those identified in the present study.

The results suggest that while there are common pathways in *Dn7*-mediated resistance to different biotypes, there appears to be diverging pathways as well. It appears that *Dn7* recognises and interacts in a highly specific manner with different aphid's putative eliciting agents, consistent with previous findings (Lapitan et al. 2007b; van Zyl and Botha 2008). This in turn activates specific defense pathways unique to that interaction. On the other hand, it is possible that the observed differences are due to temporal rather than qualitative differences. To test this, we are currently investigating the expression of selected genes at different time points following aphid infestation using quantitative RT-PCR.

Differentially expressed genes identified in this study are being validated for their function in resistance using virus-induced gene silencing (VIGS). Silencing of an unknown gene (*r20c*) in 94M370 resulted in the development of susceptible symptoms following aphid infestation. Aphid fecundity more than doubled in the *r20c*-silenced plant compared to 94M370 inoculated with an empty viral vector (Table 3). The result suggests that this gene is important in resistance.

Table 3. Average number of nymphs per aphid on wheat plants inoculated with BSMV

Genotype	VIGS Construct	Ave	St Dev
94M370	BSMV:00	5.8	6.5
	BSMV:r20C	14.5	5.9
Gamtoos	No BSMV	15.25	5.7

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