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Transcriptomics of induced defense responses to greenbug aphid feeding in near isogenic wheat lines

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ABSTRACT

The greenbug aphid, *Schizaphis graminum* (Rondani) is an important cereal pest, periodically threatening wheat yields in the United States and around the world. The single dominant gene, *Gb3*-based resistance is highly durable against prevailing greenbug biotypes under field conditions; however, the molecular mechanisms of *Gb3*-mediated defense responses remain unknown. We used Affymetrix GeneChip Wheat Genome Arrays to investigate the transcriptomics of host defense responses upon greenbug feeding on resistant and susceptible bulks (RB and SB, respectively) derived from two near-isogenic lines. The study identified 692 differentially expressed transcripts and further functional classification recognized 122 transcripts that are putatively associated to mediate biotic stress responses. In RB, *Gb3*-mediated resistance resulted in activation of transmembrane receptor kinases and signaling-related transcripts involved in early signal transduction cascades. While in SB, transcripts mediating final steps in jasmonic acid biosynthesis, redox homeostasis, peroxidases, glutathione S-transferases, and notable defense-related secondary metabolites were induced. Also transcripts involved in callose and cell wall decomposition were elevated SB, plausibly to facilitate uninterrupted feeding operations. These results suggest that *Gb3*-mediated resistance is less vulnerable to cell wall modification and the data provides ample tools for further investigations concerning *R* gene based model of resistance.

1. Introduction

Bread wheat (*Triticum aestivum*) is the third largest cereal crop in the world (http://faostat.fao.org/) and second most important cereal in the United States with a production of 60 million tons during 2010. Most of the hard-red winter wheat in the US is cultivated in the Southern Great Plains where the yields are hampered by several phloem feeding insect pests primarily, the greenbug aphid, *Schizaphis graminum* (Rondani). Economic losses due to greenbug vary each year but are estimated to reach $ 405 million annually (http://www.wheatworld.org/wp-content/uploads/Wheat-Pest-Initiative-FY11-Final.pdf). Given the number of alternative hosts, biotypes, modes of reproduction, and frequency of outbreaks, the greenbug poses a threat to farming and continues to vex the scientific community. Deployment of resistance cultivars is an important component of integrated pest management for control of greenbug aphids, but host resistance can potentially be defeated by new virulent biotypes [1]. Hence, a thorough understanding of the physiological and molecular basis of resistance mechanisms serves as a key to the development of cultivars with durable resistance and better tactics for insect control.

During the course of evolution, plants have developed sophisticated sensory mechanisms enabling them to perceive the nature of herbivore feeding habits and to elicit appropriate defense responses. In the context of crop production, ecology, and host plant resistance, induced defense signaling plays a very important role by allowing plants to make necessary adaptation to herbivore attack [2]. The induced defense responses of greenbug aphids with piercing/sucking feeding behavior (preferentially feeding on phloem sap) contrast to those of chewing insects. The phloem-feeders pierce through the physical barrier and consume photosynthates to alter photosynthate composition and resource allocation that is primarily driven for defense [3,4]. For successful feeding operations, aphids navigate their stylets between intercellular spaces to reach phloem sieve elements [5]. Once the connection is established with phloem sieve elements, the aphids may feed continuously for hours to days and even weeks. To facilitate an uninterrupted feeding the

Abbreviations: JA, jasmonic acid; ET, ethylene; ABA, abscisic acid; SA, salicylic acid; GA, gibberellic acid; BR, brassinosteroid; R, resistant; S, susceptible; RB, resistant bulk; SB, susceptible bulk; hai, hours after inoculation; FC, fold change.

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aphids secrete salivary substances which may not only assist in easy stylet penetration but also serve as an elicitor to trigger downstream pathways and suppress the plant induced defense cues [6,7].

Perception of herbivore induced elicitors and effectors by the membrane bound receptors activate the putative herbivore or damage-associated molecular patterns (HAMP or DAMP), some that are parallel to pathogen or microbe-associated molecular patterns (PAMP or MAMP), critical in basal or induced immune system [8–10]. Since most phloem-feeders cause minimal physical damage to the plant tissue, the resulting defense responses are similar to those associated with PAMP or MAMP and are recognized by pattern recognition receptors (PRRs) present on the surface of plant cells [3,8]. Perception of phloem-feeding herbivores by *R* genes similar to disease resistance R proteins encodes a coiled coil nucleotide-binding leucine rich repeat (CC-NBS-LRR) protein [11–14]. Perception of feeding behavior by plants with *R* gene was shown to activate phytohormone, salicylic acid (SA)-dependent signaling cascade and callose deposition [14]. The detailed signaling cascades involving NB-LRR motif has been thoroughly summarized in the context of disease resistance [15,16] and plant-aphid interactions [2] suggesting that the induced defense responses involve multiple signal transduction pathways.

Following perception of piercing/sucking feeding patterns, plants activate early signal networks those trigger massive transcriptional reprogramming and downstream responses to defend against phloem-feeders [17]. The early signal transduction events induced by phloem-feeding insects are largely mediated by calcium flux, reactive oxygen species (ROS), phytohormones, volatiles organic compounds, and nonvolatile secondary metabolites that can serve as repellants, toxins, and even attract natural enemies [9,18–20]. Calcium ions (Ca^{2+}) in the plants serve as secondary messengers mediating developmental responses, stress signaling, and herbivore attack [18]. After sensing aphid feeding, Ca^{2+} sensors activate downstream defense signaling cascades by increasing expression of calmodulin, calmodulin binding proteins, and calcium-dependent protein kinases (CDPKs) [21]. The role of ROS in mediating herbivore attack by either chewing or piercing/sucking insects is unquestionable; however, the nature of response depends on type of herbivory and duration and intensity of feeding [4,22–24].

The phytohormones SA, jasmonic acid (JA), and ethylene (ET) activate herbivore induced signals via independent, antagonistic, and synergistic pathways and interface with other hormones auxin, abscisic acid (ABA), brassinosteroid (BR), gibberellins (GA), and cytokinin (CK) [25–28]. Additionally, as part of the defense mechanisms against phloem-feeding insects and other herbivores, plants are known to alter secondary metabolites, glutathione S-transferases (GSTs), peroxidases, and redox homeostasis [19,29–31]. As a defensive mechanism, plants resistant to phloem feeding herbivores increase callose deposition in sieve tubes; while susceptible plants promote callose-decomposing enzymes such as β-1,3-glucanase (also present in aphid saliva), resulting in unplugging of phloem occlusion [32].

From a plant breeding perspective, *R* gene-mediated host defenses play critical roles against herbivore damage; however, the detailed physiological and molecular basis of gene-for-gene interactions in the grass genomes like wheat remains unclear [33,34]. Host resistance to piercing/sucking insects is usually controlled by single or major genes [35]. Our previous studies indicated that host plant resistance to greenbug infestation in the wheat cultivar TAM 110 is due to a single dominant gene *Gb3*, which has been mapped in the distal end of wheat chromosome arm 7DL and tagged with molecular markers [36–38]. Previous behavioral and phenotypic studies on greenbug biotype E infestation in the resistant (TXGBE273) and susceptible (TXGBE281) NILs of *Gb3* suggested that antixenosis, antibiosis, and tolerance were responsible for host plant resistance against the greenbug aphid [39–43]. Using these preconditioned R and S NILs we found that *Gb3*-mediated induced defense responses were systemic, rendering uninfected young leaves more protected [43]. Systemic induced resistance was also noticed in S NIL but at a much lower level compared to *Gb3*-induced resistance in R NIL. When feeding on resistant TXGBE273 plants, the greenbugs spent more time wandering on the leaf surface compared to susceptible plants (TAM 105) where feeding begins soon after infestation [41]. However, the molecular basis of *Gb3*-mediated early defense responses and associated signal transduction pathways triggered by greenbug feeding remain unknown.

The current study was conducted to explore the global transcriptomic responses of greenbug feeding to elucidate the molecular mechanisms underlying *Gb3*-mediated as well as basal defense responses. We used Affymetrix GeneChip Wheat Genome Arrays to assess the transcriptomic changes in the resistant and susceptible bulks (RB and SB, respectively) within 24 h and 48 h after greenbug infestation. A functional classification was performed based on pairwise biologically meaningful comparisons constructed between R and S genotypes at 0 h, 24 h, and 48 h after greenbug infestation and the results were validated using qRT-PCR.

2. Materials and methods

2.1. Plant materials, growth conditions, and greenbug infestation

F_8 recombinant inbred lines (RILs) derived from two near isogenic lines of the greenbug resistance gene *Gb3*, TXGBE273 (*Gb3Gb3*) and susceptible TXGBE281 (*gb3gb3*) [37,44] were developed. Sixteen RILs, eight homozygous resistant (*Gb3Gb3*) and eight homozygous recessive, were chosen to construct two bulks, the resistant bulk (RB) and susceptible bulk (SB), as the starting materials for transcriptome profiling in the present study. Homozygosity of the 16 RILs at the *Gb3* locus was verified in three consecutive generations (F_6, F_7, and F_8) in greenbug biotype E infestation tests with at least 100 plants for each RIL. Nine plants from each of the 16 RILs were grown in three replicates in plastic trays using LC1 growth medium (three plants per replication per RIL). The plants were grown under controlled environmental conditions in a growth chamber with mixed fluorescent and incandescent lights providing ~300 μmol m^{-2} s^{-1} PPFD for a 12 h photoperiod. Healthy plant growth conditions were maintained throughout the experiment with periodic watering. When the plants reached three-leaf stage, each plant was infested with 25 biotype E greenbugs, as previously described [42,43].

2.2. Sample collection and RNA preparation

Leaf tissues from three plants of each RIL were collected at 0 h, 24 h, and 48 h after infestation (hai). Prior to leaf sample collection, all greenbug aphids were carefully removed from the seedlings with a fine hair brush. The leaf tissues were flash frozen in liquid nitrogen and stored at −80° C until further processing. For RNA extraction, the leaf samples were ground into fine powder in liquid nitrogen using mortar and pestle. The total RNA was extracted using the TRIzol reagent according to manufacturer's instructions (Invitrogen, Carlsbad, CA, USA) and quantified. Then the R and S bulks were constructed in such a way that for each replication at each time point, equal amounts of total RNA from each of the eight resistant and susceptible RILs was pooled to make the RB and SB respectively. Thus 18 total RNA samples (2 bulks; 3 time points; and 3 replications) were prepared for subsequent expression profiling using Affymetrix GeneChip Arrays. RNA quality and concentration was determined using NanoDrop