Phloem Feeding Regulates the Plant Defense Pathways Responding to Both Aphid Infestation and Pathogen Infection

Yinghua Huang

USDA-ARS Plant Science Research Laboratory and Oklahoma State University, 1301 N. Western Street, Stillwater, OK 74075, USA.
Email: yinghua.huang@ars.usda.gov

Abstract

Molecular basis of plant defense response to diseases has already been explored in great detail, but little is known about the molecular mechanisms controlling host defense against insect attack. Our research aims to improve understanding of plant defense against aphid, a major pest on many cereal crops. In this study, transcriptome of sorghum in response to greenbug feeding was investigated with microarrays carrying thousands of cDNA clones from the SSH libraries of plants treated by greenbug aphid. Analysis of genome-wide transcriptional regulation allowed the discovery of all defense-related genes and these resultant expression profiles helped in elucidating their function. These data showed that phloem-feeding induced defense response pathways associated with both aphid attack and pathogen infection and indicated signaling pathways were partially overlapping among plant responses to pathogens, insects, and wounding.
Introduction

Plants have evolved diverse defense mechanisms to combat various enemies, including phytopathogens and herbivorous insects. Activation of host defense responses is controlled by a complex signal transduction network. Plant responses to phloem-feeding insects, such as aphids which make little wounding while feeding, are not as well understood as those to chewing insects or plant pathogens. It is believed that plant response to aphids is complicated, involving several defense strategies (Thompson and Goggin, 2006). Thus, it is important to explore the genetic mechanisms underlying plant defense against aphids and to identify the factors that regulate resistance or susceptibility of the host plants. In our current studies, we have used sorghum/aphid as the model system to examine aphid-induced responses in crop plants through genome-wide transcript analysis. After infestation with a virulent biotype of greenbug, substantial changes in gene expression (up- and down-regulation) in the host plants were observed based on the parallel analyses between various treatments (Park et al., 2006). This report focuses on the recent research progress in understanding of sorghum plant defense responses to greenbug aphid and other stresses at the molecular level.

Materials and methods

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analysis. After infestation with a virulent biotype of greenbug, substantial changes in gene expression (up- and down-regulation) in the host plants were observed based on the parallel analyses between various treatments (Park et al., 2006). This report focuses on the recent research progress in understanding of sorghum plant defense responses to greenbug aphid and other stresses at the molecular level.

Sorghum (*Sorghum bicolor*) seedlings were used to study plant defense response to attack by greenbug aphid (*Schizaphis graminum*), a phloem-feeding insect. The induction of genes associated with host defense responses to greenbug was investigated using cDNA microarrays and gene expression profiles of the greenbug challenged plants were obtained. The differentially expressed genes were validated by Northern analysis and quantitative real-time PCR (qRT-PCR), and their function was analyzed by sequence annotation against Genbank databases. The transcript profiles and their expression patterns were also compared to those induced by *Pseudomonas andropogonis*, a causing bacterial leaf stripe, and wounding.

**Results and discussion**

Similar to plant responses to other attackers, plants respond to phloem-feeding aphids with a complicated arsenal of defensive responses. Analysis of gene expression profiles generated from our studies indicated that the aphid induced a surprisingly complex set of transcriptional products, of which are often known as anti-aphid proteins, insecticidal metabolites, and other stress-related compounds. Gene annotation for over 100 differentially expressed cDNA clones were categorized into ten major function groups, including a large portion of genes with unknown function. The two large groups of candidate genes were for defense-related functions and signal transduction pathways.

There were some specific defense responses to greenbug challenge in sorghum plant. A cDNA coding for cyanogenic β-glucosidase was identified in the microarray experiment. This enzyme catalyzes the process of generation of hydrogen cyanide (HCN) from a cyanogenic glucoside precursor. The toxic HCN is usually associated with
plant defense against herbivores. Another sorghum β-glucosidase gene (glucan endo-1,3-β-glucosidase) also responded to greenbug infestation. The gene encoding glucan endo-1,3-β-glucosidase expressed at a relatively higher level in resistant sorghum seedling tissues after exposed to greenbug. Other cDNAs coding for insect-specific defense compounds such as mannose binding lectin precursor and polyphenol oxidase (PPO) were also detected as up-regulated in the expression profile of greenbug-challenged resistant sorghum tissues. Lectins are anti-nutritive proteins that bind reversibly to specific mono- or oligosaccharides, becoming toxic plant proteins which are very effective in combating herbivores. The anti-nutritive effect of PPO has been well documented for defense against herbivores. RT-qPCR analysis showed that PPO transcripts were induced to a significant level (982-fold). This strong up-regulation of PPO in the resistant sorghum plants further supports the defensive role of this protein against greenbug aphid.

In addition, in response to attack by greenbug aphids, sorghum plants expressed a subset of plant defensive compounds which are typically induced by pathogen attack. For example, the expression level of β-1,3-glucanase (BGL) increased 3.22 folds, and the class III chitinase had 3.44-fold increase in sorghum seedling tissues 72 h after treatment with greenbug. Another cDNA coding for a pathogenesis-related protein (PR-5) was also identified as an up-regulated gene in greenbug-infested seedlings. Detection of disease-responsive genes in greenbug-challenged tissues suggests their functions in defense response against greenbug infestation as well.

Comparison of plant transcriptional responses to the greenbug and a bacterial pathogen, *Pseudomonas andropongonis*, provided further evidence for a coordinated regulation of plant defense pathways in the host. Several signal molecules and regulatory proteins were identified in the transcript profiles, including lipoxygenase (LOX) and mitogen-activated protein kinases (MAPK). These data suggest that the regulation of crop plant’s response to greenbug aphid involved multiple signaling systems, including salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) signal transduction pathways, and the crop plants responded to the two types of attackers, bacterial
pathogen and greenbug aphid, with distinctive and partial overlapping defense products involving antimicrobial or insecticidal activities.

References
