Deep and Comparative Transcriptome Analysis of Rice Plants Infested by the Beet Armyworm (Spodoptera exigua) and Water Weevil (Lissorhoptrus oryzophilus)

R. C. Venu · M. Sheshu Madhav · M. V. Sreerekha · Kan Nobuta · Yuan Zhang · Peter Carswell · Michael J. Boehm · Blake C. Meyers · Kenneth L. Korth · Guo-Liang Wang

Received: 14 December 2009 / Accepted: 11 January 2010 / Published online: 10 February 2010
© Springer Science+Business Media, LLC 2010

Abstract The beet armyworm (Spodoptera exigua) and the rice water weevil (Lissorhoptrus oryzophilus) are two important insect pests in rice production. To identify insect-responsive genes in rice, we performed a deep transcriptome analysis of Nipponbare rice leaves infested with both beet armyworm and water weevil using massively parallel signature sequencing (MPSS). Many antisense, alternative, and novel transcripts were commonly and specifically induced and suppressed in the infested tissue. Key genes involved in the defense metabolic pathways such as salicylic acid and jasmonic acid biosynthesis pathways were up-regulated in the infested leaves. To validate the MPSS results, we analyzed the transcriptome of the rice leaves infested with water weevils using Solexa’s sequencing-by-synthesis (SBS) method. The MPSS and SBS data were highly correlated (Pearson’s correlation coefficient=0.85), and 83% of genes had similar gene expression in both libraries. Our comprehensive and in-depth survey of the insect-infested libraries provides a rich genomic resource for further analyzing the function of key regulatory genes involved in insect resistance in rice.

Keywords Beet armyworm · Water weevil · MPSS · SBS · Transcriptome analysis

Introduction

Herbivorous insects are responsible for destroying one fifth of the world’s total annual crop production. Plants have evolved several layers of defense mechanisms against herbivorous insects (Mello and Silva-Filho 2002; Korth 2003). Understanding the molecular basis of these host mechanisms to insect attack is essential for effective control of insect damage in crop production. In the last decade, extensive research has revealed the expression pattern of defense-related genes in the infested plants by using different gene expression profiling technologies such as microarrays. Microarray-based genome-wide transcriptomic analyses have been performed in several plant species, including Arabidopsis thaliana (De Vos et al. 2005; Reymond et al. 2000, 2004; Stotz et al. 2000), bean (Arimura et al. 2000), Nicotiana attenuata (Voelkel et al. 2005), and rice (De Vos et al. 2006; Stotz et al. 2006).
Results

Library characteristics and sequence matching analysis

About 1.0 to 1.2 million individual 17-base signatures were obtained in the four MPSS libraries (PLA, PLW, PLC, and NLD, Table 1). These signatures were processed with reliability and significance filters as described by Meyers et al. (2004a). A total of 46,904 distinct 17-base signatures were obtained from the MPSS libraries (Table 1 and Fig. 1). To compare the expression levels across the libraries, we normalized the frequency of signatures in each library to one million (transcripts per million or TPM). Figure 1 shows the number of pooled distinct signatures from the four MPSS libraries separated by reliability and significance filters and further grouped based on their match in the Nipponbare genomic sequence. When all the unique, reliable, and significant signatures (≥4 TPM) from the four libraries were clustered, a total of 37,532 unique signatures were obtained. Clustering of reliable significant signatures led to identification of 26,282 unique signatures that had only one hit in the genome (hits=1). A total of 5,358 reliable significant signatures matched the genome more than once (hits>1). About 5% of the signatures were significant unreliable, and 30% of them had genome
matches (Fig. 1). Distinct reliable significant signatures in PLA (14,480), PLW (15,912), PLC (13,779), and NLD (14,395) were identified. Thirteen transcripts with expression level >10,000 TPM were expressed in the PLC library (Table 1).

All the reliable experimental signatures were matched to the rice genomic sequence to determine the precise location of expressed sense and antisense transcripts in the rice genome. About 87–90% of the signatures matched to the japonica (Nipponbare) genomic sequence (Table S2 of the Electronic Supplementary Material). Also, 80–83% of the signatures matched to rice annotated genes, of which nearly 73% and 15% signatures belonged to sense and antisense transcripts, respectively. Among them, about 5% represented both sense and antisense signatures. About 86% of the signatures matched to the existing ESTs at the TIGR database. In addition, based on the precise location/matching of the experimental signatures on the annotated genes, the number of sense (classes 1, 2, 5, and 7) and antisense (classes 3 and 6) signatures were identified in PLA (11,182 and 2,025), PLW (11,858 and 2,008), PLC (10,779 and 1,925), NLD (8,935 and 1,432), and SPLW (13,813 and 3,378; Table 2).

Expression pattern of antisense, alternative, and novel transcripts, and transcripts of transcription factors (TFs) after insect infestation

About 63–68% of the reliable signatures in the two libraries generated from infested plants matched the Knowledge-Based Oryza Molecular Biological Encyclopedia (KOME) full-length (FL) cDNAs. Among the matched signatures, about 57–60% were sense signatures and 10% were antisense (Table 2, Table S2 of the Electronic Supplementary Material), and about 2% matched both sense and antisense.

### Table 1 Statistics of Insect-Infested and Control Rice MPSS and SBS Libraries

| Technology | MPSS | SBS |
|------------|------|-----|
| Signature category | Beetle armyworm-infested plants (PLA) | Water weevil-infested plants (PLW) | Mechanical wounded plants (PLC) | Unwounded control plants (NLD) | Water weevil-infested plants (SPLW) |
| Total sequenced | 1,150,869 | 1,012,170 | 1,213,577 | 1,254,824 | 3,051,005 |
| Distinct | 21,365 | 20,282 | 18,202 | 20,791 | 99,837 |
| Reliable | 18,311 | 18,593 | 17,048 | 18,659 | 64,332 |
| Unreliable | 3,054 | 1,689 | 1,154 | 2,132 | 35,505 |
| Significant | 15,326 | 16,673 | 14,259 | 14,901 | 33,123 |
| Nonsignificant | 6,039 | 3,609 | 3,943 | 5,890 | 66,714 |
| Reliable significant | 14,480 | 15,912 | 13,779 | 14,395 | 31,955 |
| Reliable nonsignificant | 3,831 | 2,681 | 3,269 | 4,264 | 32,377 |
| Unreliable significant | 846 | 761 | 480 | 506 | 1,168 |
| Unreliable nonsignificant | 2,208 | 928 | 674 | 1,626 | 34,337 |
| Distinct genes expressed | 7,941 | 8,871 | 7,738 | 8,146 | 13,964 |
| 1–100 TPM | 19,370 | 17,865 | 16,147 | 18,132 | 96,570 |
| 101–1,000 TPM | 1,826 | 2,251 | 1,874 | 2,497 | 3,002 |
| 1,001–10,000 TPM | 160 | 160 | 168 | 153 | 254 |
| >10,000 TPM | 9 | 6 | 13 | 9 | 11 |

*a Genome-matched reliable significant signatures
strands of the same full-length cDNA. Expression of the antisense transcripts was confirmed by matching the significant reliable signatures from each library with the rice antisense full-length cDNAs in the KOME database. The total number of genes with antisense transcripts was 1,769 in PLA, 1,773 in PLW, 1,691 in PLC, and 1,432 in NLD (Table 2), suggesting a significant induction of antisense gene expression in the insect-infested and wounded leaves. The specifically induced or suppressed genes with antisense in the PLA and PLW libraries were identified by comparison with the two control libraries (PLC and NLD). Forty antisense genes in PLAwere ≥5-fold induced, and none was suppressed relative to PLC and NLD, respectively (Table 3). Similarly, 44 and 3 genes were ≥5-fold induced and suppressed in PLW, respectively, when compared to the two controls (Table 3). The identities of the genes encoding antisense transcripts with ≥5-fold induction or suppression are listed in Table S3 of the Electronic Supplementary Material.

About 10–12% of the expressed genes showed alternative splicing when compared with the TIGR alternative-splice-form clusters. Among them, the genes with alternative transcripts that were induced or suppressed specifically in PLA and PLW relative to the two controls were identified (Table 3). A total of 223 and 40 specifically induced genes (≥5-fold induction) produced alternative transcripts in PLA and PLW, respectively. Some of the pathogen defense-related genes, such as those encoding metallothionein-like protein type 2 (TC334871, TC323823, TC320546, TC311902, TC319184), nonspecific lipid transfer protein (TC327106), aspartic proteinase precursor (TC300646), BTH-induced protein phosphatase 1 (TC300268), thioredoxin (TC304211), calmodulin (TC338165), catalase (TC342436), Rho-GTPase-activating protein (TC355505), cysteine proteinase inhibitor 2 (TC315144), small GTP-binding protein (TC335268), and stress-related protein (TC341761), generated alternative transcripts (Table S3 of the Electronic Supplementary Material).

### Table 2 Classification of the Reliable MPSS Signatures from PLA, PLW, and PLC Libraries Based on their Location on the Annotated Genes

| Signature category b | PLA | PLW | PLC | NLD | SPLW |
|---------------------|-----|-----|-----|-----|------|
|                     | Total signatures | Grouped by gene a | Total signatures | Grouped by gene a | Total signatures | Grouped by gene a | Total signatures | Grouped by gene a | Total signatures | Grouped by gene a |
| Class 1 (exon, sense strand) | 5,335 | 4,707 | 5,861 | 5,161 | 5,206 | 4,534 | 5,758 | 5,090 | 5,868 | 5,250 |
| Class 2 (500 bp 3′-UTR) | 5,263 | 4,673 | 5,314 | 4,746 | 5,020 | 4,483 | 4,825 | 4,368 | 11,490 | 9,053 |
| Class 3 (exon, antisense strand) | 1,862 | 1,635 | 1,836 | 1,628 | 1,771 | 1,559 | 1,471 | 1,336 | 3,634 | 3,044 |
| Class 4 (un-annotated region) | 825 | 0 | 872 | 0 | 819 | 0 | 857 | 0 | 2,858 | 0 |
| Class 5 (intron, sense strand) | 404 | 383 | 498 | 473 | 412 | 390 | 485 | 452 | 2,095 | 1,781 |
| Class 6 (Intron, antisense strand) | 163 | 160 | 172 | 165 | 154 | 149 | 113 | 110 | 425 | 398 |
| Class 7 (span splice site, sense strand) | 180 | 178 | 185 | 183 | 141 | 139 | 173 | 171 | 337 | 328 |
| Classes 1, 2, 5, 7 (Sense signatures) | 11,182 | 8,786 | 11,858 | 9,284 | 10,779 | 8,407 | 11,241 | 8,935 | 19,790 | 13,813 |
| Classes 3, 6 (antisense signatures) | 2,025 | 1,769 | 2,008 | 1,773 | 1,925 | 1,691 | 1,584 | 1,432 | 4,059 | 3,378 |
| Total | 21,365 | 9,469 | 20,282 | 10,013 | 18,202 | 9,097 | 20,791 | 9,558 | 26,707 | 14,301 |

a Grouped by gene including transposons
b See Meyers et al. 2004a for class definitions

### Table 3 Specifically Induced or Suppressed (Fivefold or More) Antisense Genes (with KOME Antisense Transcripts Support), Alternative Transcripts (with TIGR Alternative Transcripts Support) and Genes Encoding Transcription Factors in Insect-Infested Plants Compared to Wound and Unwound Plants

| Library | Antisense genes | Alternative transcripts | Transcription factor genes |
|---------|-----------------|------------------------|---------------------------|
| PLA | 40 | 223 | 137 |
| PLW | 44 | 40 | 166 |
The novel transcripts that matched the genome sequence but were not present in the KOME FL-cDNAs and TIGR-EST databases, and the novel genes that matched the genome sequence but were not present in the TIGR ESTs, KOME FL-cDNAs, and TIGR annotated rice genes, were searched in both PLA and PLW. About 1,000 novel transcripts and 1,200–1,300 novel genes were identified (Table S3 of the Electronic Supplementary Material).

The TF genes that were induced or suppressed in PLA or PLW compared to PLC and NLD are given in the Table S4 of the Electronic Supplementary Material. Some of the important stress-related transcription factor genes encoding LIM domain-containing protein (Os06g13030), heat shock protein (Os03g63750), zinc finger domain protein (Os03g55540), homeobox-leucine zipper protein (Os10g39030), and Myb-related transcription factor (Os01g09280) were highly induced in both PLA and PLW relative to PLC and NLD. However, some of the NAC domain-containing transcription factor genes (Os11g08210 and Os02g36880) were suppressed in both PLA and PLW libraries compared to PLC and NLD.

Promoter analysis of the genes responsive to insect infestation

Promoter analysis revealed the presence of many conserved cis motifs in the upstream regions of the up-regulated genes. In the 12 highly induced genes (≥50-fold) in both PLA or PLW, 17 types of cis motifs were identified (Table 4 and Table S4 of the Electronic Supplementary Material). These motifs were highly represented in the promoters of the plus or minus strand of the 12 defense-related genes. The precise locations of the known cis elements in the promoter regions of all 12 genes are listed in the Table S4 of the Electronic Supplementary Material.

Identification of genes in the defense-related metabolic pathways

A network map of defense-related metabolic pathways was generated based on the biochemical pathways reported at the Gramene website (http://www.gramene.org; Fig. 2). The important metabolic pathways responsible for the production of secondary metabolites including SA, JA, ET, and other hormones were integrated based on the genes identified in the four MPSS libraries. Genes that were at least 5-fold up- or down-regulated in PLA and PLW (relative to PLC and NLD) and that were involved in the production of these defense molecules are presented. Many genes involved in the biosynthesis of JA, like lipoxygenases (Os12g37290, Os08g39850, Os04g37430) and 12-oxophytodienolate reductase (Os06g11240), were up-regulated in both PLA and PLW libraries (Fig. 2). The key gene encoding phenylalanine ammonia-lyase (Os04g43760), which catalyzes the biosynthesis of SA through L-phenylalanine, was up-regulated in both PLA (36-fold) and PLW (44-fold). In contrast, the gene encoding isochorismate synthase 1 (Os09g19734), which produces SA through chorismate, was down-regulated in both PLA (81-fold) and PLW (72-fold). However, many of the genes belonging to ET biosynthesis were down-regulated in both PLA and PLW, such as those encoding 1-aminocyclopropane-1-carboxylate synthase (Os01g55540, 58-fold), centromere/kinetochore protein zw10 (Os11g34310, 5-fold), tyrosine aminotransferase (Os01g42510, 14-fold), tyrosine transaminase (Os10g25140, 14-fold, Os09g28050, 6-fold), and 1-aminocyclopropane-1-carboxylate oxidase (Os09g27820, 25-fold). A large group of genes involved in brassinosteroid production, cytokinin production 7-N-glucoside biosynthesis, and phenylpropanoid biosynthesis were also highly expressed.

Genes commonly expressed in both beet armyworm- and water weevil-infested plants but not in wounded or untreated control plants

A total of 878 transcripts (653 genes) were 5-fold or more up-regulated and 371 transcripts (340 genes) were 5-fold or more down-regulated in both PLA and PLW, relative to those in the two control libraries (Fig. 3; Table S5 of the Electronic Supplementary Material). Among them, the known defense genes with 5-fold induction and commonly or specifically present in the two libraries from insect-infested rice are listed in Table 5. Among the defense genes, we observed the up-regulation of the genes encoding Bowman–Birk protease inhibitors (Os01g60730, Os01g04050), lipoxygenase (Os12g37260), nucleic acid binding protein (Os03g07370), terpene synthase 8 (Os04g27790), OsWRKY78—superfamily of rice TFs having WRKY and zinc finger domains (Os07g39480), metallothionein-like protein type 2 (Os01g65650), RING-H2 finger protein (Os01g60730), cysteine-rich receptor-like protein kinase (Os07g43560), and 4-coumarate–CoA ligase (Os01g67530; Table 5). Other genes belonging to secondary metabolite production were also up-regulated, including those encoding squalene monoxygenase (Os03g12910), tyrosine decarboxylase gene (Os07g25590), phenylalanine ammonia-lyase gene (Os04g4376), and N-acylethanolamine amido hydrolase (Os11g06900; Table 5; Table S5 of the Electronic Supplementary Material). Some of the genes involved in the protein degradation pathway were up-regulated in the plants infested with either pest but not in the wounded and untreated plants (Table 5; Table S3 of the Electronic Supplementary Material); these genes included 26S protease regulatory subunit 7 (Os06g09290), brix domain-containing proteins (Os01g33030), hecox carrier protein HEX6 (Os10g41190), tab2 protein (Os02g39740), F-box domain-containing proteins (Os09g32870, Os08g09760,
### Table 4  Conserved cis Motifs in the Promoters of Beet Armyworm and Water Weevil-Induced Defense-Related Genes

| GeneID          | Os01g03320 | Os01g04050 | Os01g05650 | Os01g09280 | Os01g53810 | Os01g72420 | Os01g73450 | Os02g24190 | Os02g32200 | Os03g07370 | Os06g13030 | Os07g33110 |
|-----------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| PLA             | 60         | 246        | 65         | 99         | 52         | 82         | 67         | 79         | 50         | 176        | 325        | 124        |
| PLW             | 172        | 181        | 145        | 60         | 79         | 53         | 53         | 52         | 64         | 89         | 114        | 83         |
| PLC             | 0          | 0          | 2          | 0          | 2          | 0          | 0          | 0          | 0          | 0          | 0          | 0          |
| NLA             | 0          | 0          | 0          | 0          | 0          | 0          | 0          | 0          | 0          | 0          | 0          | 0          |
| ARR1AT          | (+)        | (+)        | (+)        | (+)        | (+)(−)     | (+)        | (+)        | (+)        | (+)        | (+)        | (+)(−)     | (+)(−)     |
| BIHD1OS         | (−)        | (+)        | (−)(−)     | (+)        | (+)        | (−)        | (+)        | (+)        | (−)        | (+)        | (+)        | (+)        |
| CAATBOX1        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (−)        | (+)        | (+)        | (+)        | (−)        |
| CACTFTPPCA1     | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (−)        | (+)        | (+)        | (+)        | (−)        |
| DOFCOREZM       | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (−)        | (+)        | (−)        | (+)        | (−)        |
| EBOXBNAPA       | (+)        | (+)        | (+)        | (+)(−)     | (+)(−)     | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        |
| GATABOX         | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        |
| GT1CONSENSUS    | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)(−)     | (+)(−)     |
| GT1GMSCAM4      | (+)(−)     | (+)        | (+)        | (+)        | (+)(−)     | (+)        | (+)(−)     | (+)        | (+)(−)     | (+)(−)     | (+)        | (+)(−)     |
| GTGANTG10       | (+)(−)     | (+)        | (+)        | (+)        | (+)(−)     | (+)        | (+)        | (+)        | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     |
| IBOXCORE        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        |
| MYCCONSENSUSAT  | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     |
| POLLENLELATS2   | (−)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        | (+)        |
| ROOTMOTIPTAPOX1 | (+)        | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     |
| TATABOX         | (+)(−)     | (+)        | (+)        | (+)        | (+)(−)     | (+)        | (+)        | (+)        | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     |
| WBOXNTERF3      | (+)(−)     | (+)        | (+)        | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     |
| WRKY71OS        | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     | (+)(−)     |

Plus strand (+); Minus strand (−); Conserved cis elements present in all the 12 defense-related gene promoters are shown; Os01g03320 (Bowman-Birk-type bran trypsin inhibitor precursor; GATCTATTCGTCTATCG); Os01g04050 (Bowman-Birk-type wound-induced proteinase inhibitor WIP1 precursor; GATCTGTGTGATATACA); Os01g05650 (metallothionein-like protein type 2; GATCCAGTTACAAGTGA); Os01g09280 (myb-related transcription activator; GATCACTACACGATTCC); Os01g53810 (transferrin receptor-like dimerization domain-containing protein, GATCCTGATTTAAGGCA); Os01g72420 (C2 domain-containing protein; GATCTCTCTTTGACTATT); Os01g73450 (urodolate kinase; GATCTCTAGGTTTTTA); Os02g24190 (cyclin-dependent protein kinase; GATCATTTTGTGTTGGA); Os02g32200 (thioesterase family protein; GATCCAAATGCTTCTA); Os03g07370 (endonuclease/nucleic acid binding protein; GATCTGATTTAAGGCA); Os06g13030 (LIM domain-containing protein; GATCCAGCAGACCTCA); Os07g33110 (calcium-dependent protein kinase, isoform 2; GATCCATCGAC TATGTT)
Sugar derivatives indicate up-

Network of defense-related pathways showing the expression of insect-infested plants (Fig. S1 and Table S). Up-regulation was identified for metabolism of cofactors and vitamins, carbohydrate metabolism, and energy metabolism were up-regulated in both kinds of insect-infested plants (Fig. S1 and Table S of the Electronic Supplementary Material).

We also observed the induction of the NAD(P)H-dependent oxidoreductase gene (Os04g08550 and its six isoforms), which encodes a key enzyme involved in radical scavenging and the accumulation of reactive oxygen species. The induction seems to be specific to both insect infestations because expression of these genes did not increase in the mechanically damaged plants. The transcripts encoding several key JA biosynthetic enzymes like allene oxide synthase, allene oxide cyclase, and phospholipase D were up-regulated in rice after infestation by either insect (Fig. 2; Table 5; Table S of the Electronic Supplementary Material). Up-regulation was identified for several isoforms of the phenylalanine ammonia-lyase genes (Os04g43760, Os02g41650, Os05g35290, Os02g41630, and Os02g41680) involved in the SA biosynthesis pathway.

Os11g32810, Os08g35960, Os11g07970), ubiquitin-conjugating enzyme E2N (Os01g48280), ubiquitin-conjugating enzyme E2S (Os06g45000), and ubiquitin ligase SINAT4 (Os03g24040). In addition, many genes involved in the metabolism of cofactors and vitamins, carbohydrate metabolism, and energy metabolism were up-regulated in both kinds of insect-infested plants (Fig. S1 and Table S of the Electronic Supplementary Material).

We also observed the induction of the NAD(P)H-dependent oxidoreductase gene (Os04g08550 and its six isoforms), which encodes a key enzyme involved in radical scavenging and the accumulation of reactive oxygen species. The induction seems to be specific to both insect infestations because expression of these genes did not increase in the mechanically damaged plants. The transcripts encoding several key JA biosynthetic enzymes like allene oxide synthase, allene oxide cyclase, and phospholipase D were up-regulated in rice after infestation by either insect (Fig. 2; Table 5; Table S of the Electronic Supplementary Material). Up-regulation was identified for several isoforms of the phenylalanine ammonia-lyase genes (Os04g43760, Os02g41650, Os05g35290, Os02g41630, and Os02g41680) involved in the SA biosynthesis pathway.

beet armyworm water weevil

Fig. 3 Commonly and specifically induced and suppressed genes (5-fold relative to the controls) after beet armyworm and water weevil infestations. Commonly induced/suppressed genes were those induced/suppressed in both kinds of insect-infested plants while specifically induced/suppressed genes were those induced/suppressed in only one kind of insect-infested plant.

Fig. 2 Network of defense-related pathways showing the expression of key genes belonging to metabolism of secondary metabolites including salicylic acid, jasmonic acid, ethylene, and hormones. The genes that were up- or down-regulated 5-fold in PLA or PLW are shown in parenthesis (positive numbers indicate up-regulated genes and negative numbers indicate down-regulated genes). The number next to the library code (PLA or PLW) shows the signature class as described by Meyers et al. (2004a).
In addition, calmodulin (Os06g06160) and a calcium-binding protein were also induced in plants infested with either insect (Table S5 of the Electronic Supplementary Material).

Genes differentially expressed in beet armyworm- and water weevil-infested plants

A total of 1,666 transcripts (1,570 genes) were specifically up-regulated (i.e., up-regulated in one kind of insect-infested plant but not the other), and 587 transcripts (580 genes) were specifically down-regulated in PLA compared with the two controls (Fig. 3 and Table S5 of the Electronic Supplementary Material). Similarly 2,033 transcripts (1,863) were specifically up-regulated, and 444 transcripts (432 genes) were specifically down-regulated in the PLW library (Fig. 3 and Table S5 of the Electronic Supplementary Material). The genes encoding transcription factors containing known domains such as MADS, PLATZ, RWP-RK, SET, and ZIM were highly up-regulated in the beet armyworm-infested plants (Table 5 and Tables S4 and S5 of the Electronic Supplementary Material), whereas the genes encoding transcription factors with ABI3VP1, ARF, ARID, AUX/IAA, SNF2, SBP, TCP, TUB, and WRKY domains were highly up-regulated in water weevil-infested plants (Table 5 and Table S5 of the Electronic Supplementary Material).

### Table 5 List of Defense-Related Genes Specifically and Commonly Induced in the Host After Beet Armyworm and Water Weevil Infestations

| ID | Signature | PLA | PLW | PLC | NLD | Gene ID | Gene description |
|----|-----------|-----|-----|-----|-----|---------|------------------|
| Genes commonly induced in both PLA and PLW |
| 1 | GATCTGTGATGATACAG | 246 | 181 | 0 | 0 | Os01g04050 | Bowman–Birk-type wound-induced proteinase inhibitor precursor |
| 2 | GATCGATTTCCATTTGGG | 206 | 119 | 11 | 0 | Os05g31750 | Annexin-like protein RJ4 |
| 3 | GATCACTGTAGCAGTGG | 178 | 526 | 2 | 0 | Os12g37260 | Lipoxygenase 2.1, chloroplast precursor |
| 4 | GATCTGTATTTAAGGCCA | 170 | 11 | 0 | 8 | Os03g07370 | Endonuclease/nucleic acid binding protein |
| 5 | GATCTGTTAGCTAGTGG | 146 | 218 | 0 | 0 | Os07g43560 | CRK10 |
| 6 | GATCGTGGAGGTGGAGTT | 101 | 130 | 0 | 0 | Os02g50770 | Peroxidase 65 precursor |
| 7 | GATCTGTGGTGGAGAGAG | 82 | 132 | 2 | 0 | Os04g27790 | Terpene synthase 8 |
| 8 | GATCATCAGAATTGCTGTG | 70 | 102 | 18 | 3 | Os07g39480 | OsWRKY78—superfamily of rice TFs having WRKY and zinc finger domains |
| 9 | GATCTGTCCTTGCCACCT | 69 | 127 | 0 | 17 | Os08g09860 | FMN-dependent dehydrogenase family protein |
| 10 | GATCCAGTTACAAAGTTGTA | 65 | 145 | 2 | 0 | Os01g05650 | Metallothionein-like protein type 2 |
| 11 | GATCATCCTCGCGGCGG | 60 | 141 | 4 | 0 | Os01g06750 | RING-H2 finger protein ATL5A |
| 12 | GATCTATTTCGTTATCG | 60 | 172 | 0 | 0 | Os01g03320 | Bowman–Birk-type bran trypsin inhibitor precursor |
| Genes specifically induced in PLA |
| 13 | GATCATGTAACCTGTGGA | 231 | 0 | 0 | 0 | Os07g40860 | Vegetative cell wall protein gp1 precursor |
| 14 | GATCCATGCTGGCGTACT | 206 | 0 | 2 | 0 | Os08g44020 | Lyase |
| 15 | GATCATGATGGCAGAAAC | 174 | 0 | 0 | 0 | Os12g4310 | 9,10-9,10 carotenoid cleavage dioxygenase 1 |
| 16 | GATCTCTGGCAATGGTT | 170 | 0 | 0 | 0 | Os03g22810 | Superoxide dismutase 1 |
| 17 | GATCGACTTTCCATCCC | 124 | 0 | 0 | 0 | Os06g24990 | Xylanase inhibitor protein 1 precursor |
| 18 | GATCGGCGCAGACGAC | 111 | 0 | 0 | 0 | Os07g01660 | Disease resistance response protein 206 |
| 19 | GATCCGATGCTGTTGTTG | 103 | 25 | 12 | 0 | Os08g04170 | Zinc finger C-x8-C-x5-C-x3-H type family protein |
| 20 | GATCAACAGAATTCGACGCC | 146 | 44 | 4 | 0 | Os02g41860 | Aquaporin Pip2.2 |
| Genes specifically induced in PLW |
| 21 | GATCTGTGGTATTACAGTA | 231 | 0 | 0 | 0 | Os07g40860 | Vegetative cell wall protein gp1 precursor |
| 22 | GATCCATGCTGGCGTACT | 206 | 0 | 2 | 0 | Os08g44020 | Lyase |
| 23 | GATCATGATGGCAGAAAC | 174 | 0 | 0 | 0 | Os12g4310 | 9,10-9,10 carotenoid cleavage dioxygenase 1 |
| 24 | GATCGACTTTCCATCCC | 124 | 0 | 0 | 0 | Os06g24990 | Xylanase inhibitor protein 1 precursor |
| 25 | GATCGGCGCAGACGAC | 111 | 0 | 0 | 0 | Os07g01660 | Disease resistance response protein 206 |
| 26 | GATCCGATGCTGTTGTTG | 103 | 25 | 12 | 0 | Os08g04170 | Zinc finger C-x8-C-x5-C-x3-H type family protein |
| 27 | GATCATGTAACCTGTGGA | 231 | 0 | 0 | 0 | Os07g40860 | Vegetative cell wall protein gp1 precursor |
| 28 | GATCATGATGGCAGAAAC | 174 | 0 | 0 | 0 | Os12g4310 | 9,10-9,10 carotenoid cleavage dioxygenase 1 |
| 29 | GATCATGTAACCTGTGGA | 231 | 0 | 0 | 0 | Os07g40860 | Vegetative cell wall protein gp1 precursor |
| 30 | GATCTGTGGTATTACAGTA | 231 | 0 | 0 | 0 | Os07g40860 | Vegetative cell wall protein gp1 precursor |

(Fig. 2; Table S5 of the Electronic Supplementary Material).
and Tables S4 and S5 of the Electronic Supplementary Material). Defense- or metabolism-related genes encoding vegetative cell wall protein gp1 (Os07g40860), 9,10 carotenoid cleavage dioxygenase (Os12g44310), superoxide dismutase (Os03g22810), fungal xylanase inhibitor (Os06g24990), and aquaporin PIP2.2 (Os02g41860) were specifically up-regulated in the beet armyworm-infested plants (Table 5 and Table S5 of the Electronic Supplementary Material). Up-regulation of some defense- or metabolism-related genes also occurred in the water weevil-infested plants; these genes encoded metallothionein-like protein type 3 (Os05g11320), glutathione-S-transferase (Os07g07320), zinc finger, C3HC4-type family protein (Os02g42690), leucine-rich repeat receptor protein kinase (Os02g40240), ankyrin repeat domain-containing protein (Os06g3800), and 4-coumarate-CoA ligase 2 (Os02g46970; Table 5 and Table S5 of the Electronic Supplementary Material).

Validation using RT-PCR and SBS

The expression pattern of 14 genes randomly selected from the PLA and PLW libraries were further evaluated using RT-PCR (see the gene list in Table S1 of the Electronic Supplementary Material). Four up-regulated and three down-regulated genes in PLA or PLW compared to the two controls were analyzed through RT-PCR. About 65% of the genes (nine genes) showed a similar expression pattern in both RT-PCR and MPSS data (Fig. 4a). It is noteworthy that the gene encoding allene oxide synthase (a marker gene for JA synthesis) and the gene encoding phenylalanine ammonia-lyase (a maker gene for SA synthesis) showed up-regulation in plants infested with both insects compared to the controls (Fig. 4a).

The SBS library SPLW was constructed using the same RNA used to construct the PLW MPSS library. A total of
three million signatures were obtained from the library, which is 3-fold greater than the number of reads in the PLW library (Table 1). The number of reliable significant signatures was about 2-fold greater in SPLW than in PLW (31,995 vs 15,912). Many of the low-copy signatures (1–100 TPM) were identified in the SBS library (96,570 in SPLW vs 17,865 in PLW). When the number of the annotated genes with reliable significant signatures was compared, the SBS library had about 57.4% more genes (13,964) than the MPSS library (8,871; Fig. 4b, Table 1), suggesting a much deeper coverage in the SBS library for transcriptome survey. Between the matched annotated genes in the two libraries, 7,349 (83%) genes were present in both libraries. The reliable significant signatures from SPLW and PLW libraries were compared using Pearson’s correlation coefficient. A moderate correlation coefficient (0.65) was observed when MPSS and SBS expression data were compared without removal of any outlier transcripts. After removal of four outlier transcripts, the correlation coefficient was high (0.85; Fig. 4c; Table S6 of the Electronic Supplementary Material).

Discussion

With expected changes in climate and rice cropping systems, insect pests on rice are likely to become more epidemic and destructive in the future. Although insecticides are effective, undesirable environmental effects of insecticides and insect resistance to insecticides are becoming serious concerns in rice growing regions. It is clear that development of highly resistant cultivars is essential for sustainable rice production. However, the molecular basis of host resistance to insects in rice remains largely unknown. Yuan et al. (2008) identified 196 rice genes whose expression was significantly up-regulated by fall armyworm (Spodoptera frugiperda) caterpillars using a half-genome rice oligo microarray. The current study used two high-throughput sequencing techniques to provide the first large-scale and deep transcriptome analysis of rice plants infested with two insect pests. The deep-sequencing capacity of both techniques assured the collection of most transcripts in the rice tissues. Although MPSS and SBS are two different platforms, the transcriptomes generated by the two methods were highly correlated in our study. Many genes commonly or specifically induced or suppressed in the plants infested by the two insects have been identified. Novel genes were also obtained with antisense and alternative transcripts that are specifically expressed in the infested tissues. In addition, many highly and specifically expressed TF genes were found in the infested rice plants, and these genes may play important roles in regulating or coordinating insect-defense pathways or networks in rice. Further elucidation of the function of these genes in host defense against insects will provide new insights into the molecular basis of insect resistance and novel genes for engineering insect-resistant rice.

We found that many genes involved in host-defense signaling pathways generate antisense transcripts after insect infestation. This kind of phenomenon was also observed in rice infected with the fungal pathogen Magnaporthe oryza (Gowda et al. 2007). However, the function of antisense genes in plant defense against pathogens and insects is unclear. In addition, we also observed alternative splicing in about 18% of the rice genes in the libraries of insect-infested rice and in about 14% of the rice genes in the library of uninfested rice. The importance of alternative splicing in the resistance to pathogens was found in tobacco and Arabidopsis; when the derivative of alternative splicing of the tobacco N gene and the Arabidopsis RPS4 genes was silenced, the level of the N- and RPS4-mediated resistance was reduced or abolished (Jordan et al. 2002). In addition, R gene alternative splicing was dynamic during the defense response (Gassmann 2008). The function of both antisense and alternative transcripts identified in this study requires further detailed analysis in the defense response of plants to insect attack.

Terpenes are an important class of defense compounds that accumulate in plants after pathogen infection or arthropod-induced injury. Previous research has shown that Lepidopteran herbivory and oral factors induced transcripts encoding novel terpene synthases in M. truncatula (Gomez et al. 2005; Bede et al. 2006). Recently, Yuan et al. (2008) confirmed the induction of expression of seven of the 11 terpene synthase genes after fall armyworm infestation that was identified through the microarray experiments. Enzymes encoded by three TPS genes, Os02g02930, Os08g07100, and Os08g04500, were also biochemically characterized. In the current study, terpene synthase genes were induced in the host after both beet armyworm and water weevil infestations. In addition, we observed the induction of the Bowman–Birk family of proteinase inhibitors (BBPI) in both PLA and PLW libraries. BBPIs might contribute to plant defense against insect attack by inhibiting digestive enzymes of various insects. Transgenic plants expressing a BBPI gene had enhanced resistance to herbivory (Hilder et al. 1987). Genetic manipulation of the BBPI genes in transgenic rice may lead to new methods for insect control in rice production.

Various transcriptome analyses indicated that insect feeding elicits defense response in the host through SA-, JA-, and ET-regulated genes (Walling 2000; Moran et al. 2002; de Vos et al. 2007). The feeding of brown plant hoppers on rice up-regulates several genes involved in phenylpropanoid biosynthesis and genes required for sesquiterpene synthesis (Zhang et al. 2004; Cho et al. 
In tomato, aphid infestation up-regulates SA signaling (Li et al. 2006) while in Arabidopsis SA has been shown to have a neutral and negative effect on aphid and silver leaf whitefly growth, respectively (Pegadaranu et al. 2005; Zarate et al. 2007). Chewing insects largely induce JA because of the extensive damage caused by chewing (Howe 2004; Kessler and Baldwin 2002; Halitschke et al. 2003). In our study, many JA and SA biosynthetic genes were up-regulated in both PLAs and PLW libraries, including the genes encoding phosphatidylcholine and linolenate 13(S)-hydroperoxylinolenic acid in the JA pathway and the genes encoding phenylalanine ammonia-lyase gene in the SA pathway. This up-regulation suggests an important role of both JA and SA in the response of rice to insect infestation. Endogenous ET has been shown to act as a cross-talk regulator with JA (Penninckx et al. 1998; Leon et al. 2001; Arimura et al. 2005, 2008). Enhanced production of ET has been reported in aphid-infested barley, which indicates active biosynthesis of this phytohormone in response to minimal wounding (Argandona et al. 2001). In the current study, however, the role of the ET-mediated signaling in insect-infested rice plants was unclear because the expression of the ACC synthase and ACC oxidase genes in the ET pathway was down-regulated. Nevertheless, the role of SA, JA, ET, and their cross-talks in the rice insect defense warrants further in-depth investigation.

MPSS has been used for whole genome transcription analysis in the last decade and has generated abundant data concerning expression in many organisms (Vega-Sanchez et al. 2007; Simon et al. 2009). Its complicated library construction procedure and high sequencing cost are two main limiting factors for the use in individual laboratories. As the cost of the next-generation sequencing methods has significantly decreased in the last few years, SBS sequencing has become a popular method for transcriptome analysis. To validate our MPSS results, we made and sequenced an SBS library using the same RNA sample that was used for the PLW library. Comparison analysis showed that about 83% of the genes were expressed in both MPSS and SBS libraries. Pearson’s correlation analysis showed a high level of similarity (coefficient = 0.85) in expression patterns of genes between these two platforms. However, SBS is a much better choice for transcriptome analysis because it costs 90% less than MPSS and generates 3-fold more transcripts. Furthermore, about 30% more transcripts have been found in the SBS library than in the MPSS library. Many of these additional signatures are low-copy transcripts, indicating that SBS is a powerful method for identifying race transcripts. As the sequencing cost for SBS is further reduced in the future, SBS will likely become a routine transcriptomic analysis for many biological experiments.

**Methods**

**Insect rearing, plant growth conditions, and insect infestations**

Beet armyworm larvae were reared in the laboratory, and neonates were maintained on rice plants before third-instar larvae were used in the experiment. Rice water weevils were collected as adults from the field and maintained on rice plants in the greenhouse. Nipponbare rice plants (Oryza sativa) were grown in a greenhouse. When the plants were 6 weeks old, they were individually placed in 24 cages. Insects (100 army worms or 500 weevils per cage) were added to 12 of the cages (six cages for each kind of insect). When the insects were added to cages, the plants in six other cages were mechanically damaged with a hole punch; 2–5 mm were removed from leaf edges, and care was taken to avoid damaging the mid-vein. Leaves were damaged at intervals of approximately 4 cm along the leaf edge, and the treatment was repeated 30 min after the initial damage. The plants in the six remaining cages were untreated controls, i.e., they did not experience insect infestation or mechanical damage. All leaf tissue from all 24 cages was collected 24 h after the insects had been added to the cages and after the leaves had been initially wounded. Conditions during this 24-h period were the same as described earlier in this section.

**RNA isolation and RT-PCR**

Total RNA was isolated using TRIzol reagent according to the manufacturer’s instructions. RT-PCR was performed as reported previously (Venu et al. 2007). PLA, PLW, PLC, and NLD refer to the libraries of plants infested with the beet armyworm, plants infested with the water weevil, mechanically wounded plants, and unwounded control plants, respectively (Table 1). Selected candidate genes that were up- or down-regulated in these four libraries were amplified by gene-specific primers, which are listed in Table S1 of the Electronic Supplementary Material.

**MPSS and SBS library construction and bioinformatics**

The total RNA isolated from beet armyworm-infested plants, water weevil-infested plants, mechanically wounded plants, and untreated control plants was used for the construction of MPSS libraries. In addition, the same RNA for the PLW library was used for the construction of the SBS (SPLW) library. The MPSS libraries were constructed and sequenced essentially as previously described (Brenner et al. 2000; Meyers et al. 2004a; b; Nobuta et al. 2007). The SBS library was constructed according to manufacturer’s (Illumina) instructions with minor modifications. All data from the
Identification of antisense, alternative, and novel transcripts

To identify the antisense orientation of the MPSS signatures for the rice reference sequences, we converted all signatures into antisense orientation by a reverse complementation procedure. The antisense signatures from all the MPSS libraries were independently matched against the rice reference sequences. For validating identified antisense signatures from these MPSS libraries, we matched the antisense MPSS signatures against longer antisense rice FL-cDNAs and TIGR-EST databases. These signatures were derived from the rice genome by extracting all occurrences of GATC plus the 14 nt sequence at the 3′ terminus (16 nt in case of SBS). These signatures were used for matching analysis with the experimental MPSS or SBS signatures obtained in this study. All the virtual genomic signatures derived from the rice genome were assigned a “class” based on the position of the signature relative to annotated genes (Meyers et al. 2004a). Signatures that did not match to the genome corresponded to the “Class 0” signatures and those that matched the genome corresponded to Classes 1 to 7. The SAGEspy program (http://www.osc.edu/research/bioinformatics/projects/sagespy/index.shtml) was used to match the experimental MPSS signatures with the target rice databases to identify the sense, antisense, novel, and alternative transcripts from the MPSS libraries. Clustering analysis was done using in-house programs and Microsoft Access. The bioinformatics pipeline for the SBS data analysis was performed similar to MPSS data analysis with few modifications (Brenner et al. 2000; Meyers et al. 2004a; b; Nobuta et al. 2007). Classification of genes was done using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/).

The reliable signatures that matched the rice genome but did not match rice gene expression databases like KOME FL-cDNAs and TIGR-EST databases were considered to be novel transcripts. Similarly, the genome-matched signatures were considered to be novel genes if they did not match the TIGR ESTs, KOME FL-cDNAs, and TIGR annotated rice genes.

Promoter analysis

To identify the targets/binding sites of insect-responsive transcription factors and the conserved cis elements among different up-regulated genes, we performed a promoter analysis of the genes commonly induced in both PLA and PLW. Regions 1.0 kb upstream of the expressed genes were extracted, and the cis elements within these DNA sequences were identified with the “PLACE Signal Scan Search” software (http://www.dna.affrc.go.jp/htdocs/PLACE/, Higo et al. 1999).

Acknowledgments

This work was supported by US National Science Foundation awards 0321437 and 0701745 to B.C.M. and G.L.W. and funding from the Arkansas Rice Research and Promotion Board to K.L.K. We thank Dr. Bruce Jaffee for his careful editing of the manuscript.

References

Arimura G, Tashiro K, Kuhara S, Nishioka T, Ozawa R, Takabayashi J. Gene responses in bean leaves induced by herbivory and by herbivore-induced volatiles. Plant Cell. 2000;19:1096–122.

Arimura G, Kost C, Boland W. Herbivore-induced, indirect plant defenses. Biochim Biophys Acta. 2005;1734:91–111.

Arimura G, Garms S, Maffei M, Bossi S, Schulze B, Leitner M, et al. Herbivore-induced terpenoid emission in Medicago truncatula: concerted action of jasmonate, ethylene and calcium signaling. Planta. 2008;227(2):453–64.

Argandona VH, Chaman M, Cardemil L, Munoz O, Zuniga GE, Coruera LJ. Ethylene production and peroxidase activity in aphid-infested barley. J Chem Ecol. 2001;27:53–68.

Bede JC, Musser RO, Felton GW, Korth KL. Caterpillar herbivory and salivary enzymes decrease transcript levels of Medicago truncatula genes encoding early enzymes in terpenoid biosynthesis. Plant Mol Biol. 2006;60:519–31.

Brenner S, Johnson M, Bridgham J, Golda G, Lloyd DH, Johnson D, et al. Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. Nat Biotechnol. 2000;18:630–4.

Broekgaarden C, Poelman EH, Steenhuis G, Voorrips RE, Dicke M, Vosman B. Genotypic variation in genome-wide transcription profiles induced by insect feeding: Brassica oleracea–Pieris rapae interactions. BMC Genomics. 2007;8:239.

Cho SK, Jung KW, Jeung JU, Kang KH, Ship KS, You MK, et al. Analysis of differentially expressed transcripts from plant hopper-infested wild rice (Oryza minuta). Plant Cell Rep. 2005;24:59–67.

de Vos M, Kim JH, Jander G. Biochemistry and molecular biology of Arabidopsis–aphid interactions. Bioessays. 2007;29:871–83.
De Vos M, Van Oosten VR, Van Poecke RMP. Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. Mol Plant Microb Interact. 2005;18:923–7.

Devoto A, Ellis C, Magusin A, Chang HS, Chilcott C. Expression profiling reveals COI1 to be a key regulator of genes involved in wound- and methyl jasmonate-induced secondary metabolism, defense, and hormone interactions. Plant Mol Biol. 2005;58:497–513.

Gao LL, Anderson JP, Klingler JP, Nair RM, Edwards OR, Singh KB. Involvement of the octadecanoid pathway in blue green aphid resistance in Medicagno truncata. Mol Plant Microb Interact. 2007;20:82–93.

Gassmann W. Alternative splicing in plant defense. Curr Top Microbiol Immunol. 2008;326:219–33.

Gowda M, Venu RC, Raghubathy MB, Nobuta K, Li H, Stahlberg E, et al. Deep and comparative analysis of the mycelium and appressorium transcriptomes of Magnaporthe grisea using MPSS, RL-SAGE, and oligoarray methods. BMC Genomics. 2006;8(7):310.

Gowda M, Venu RC, Huameng L, Jantasuriyarat C, Songbiao C, Bellizzi M, et al. Magnaporthe grisea infection triggers RNA variation and antisense transcript expression in rice. Plant Physiol. 2007;144:523–34.

Gomez SK, Cox MM, Bede JC, Inoue K, Alborn HT, Tumlinson JH, et al. Lepidopteran herbivory and oral factors induce transcripts encoding novel terpene synthases in Medicago truncatula. Arch Insect Physiol Bioch. 2005;58:114–27.

Halitschke R, Gase K, Hui D, Schmidt DD, Baldwin IT. Molecular interactions between the specialist herbivore Manduca sexta (Lepidoptera, Sphingidae) and its natural host Nicotiana attenuata. VI: microarray analysis reveals that most herbivore-specific transcriptional changes are mediated by fatty acid-amino acid conjugates. Plant Physiol. 2003;131:1894–902.

Harfouche AL, Shivaji R, Stocker R, Williams PW, Luthe DS. Ethylene signaling mediates a maize defense response to insect herbivory. Mol Plant Microb Interact. 2006;19:189–99.

Higo K, Ugawa Y, Iwamoto M, Korenaga T. Plant cis-acting regulatory DNA elements (PLACE) database. Nucleic Acids Res. 1999;27:297–300.

Hilder VA, Gatehouse AMR, Sheereman SE, Barker RF, Boulter D. A novel mechanism of insect resistance engineered into tobacco. Nature. 1987;330:160–3.

Howe GA. Jasmonates as signals in the wound response. J Plant Growth Regul. 2004;23:223–27.

Hudgins JW, Franceschi VR. Methyl jasmonate-induced ethylene production is responsible for conifer phloem defense responses and reprogramming of stem cambial zone for traumatic resin duct formation. Plant Physiol. 2004;135:2134–49.

Jordan T, Schornack S, Lahaye T. Alternative splicing of transcripts encoding toll-like plant resistance proteins—what’s the functional relevance to innate immunity? Trends Plant Sci. 2002;7(9):392–8.

Kempema LA, Cui X, Holzer FM, Walling LL. Arabidopsis transcriptome changes in response to phloem-feeding silver leaf whitefly nymphs. Similarities and distinctions in interactions. J Plant Growth Regul. 2007;26:201–9.

Kessler A, Baldwin IT. Plant responses to insect herbivory: the emerging molecular analysis. Annu Rev Plant Biol. 2002;53:299–328.

Korth KL. Profiling the response of plants to herbivorous insects. Genome Biol. 2003;4:221.

Leitner M, Boland W, Mithöfer A. Direct and indirect defenses induced by piercing-sucking and chewing herbivores in Medicagno truncata. New Phytol. 2005;167:597–606.

Leon J, Rojo E, Sanchez-Serrano JJ. Wound signaling in plants. J Exp Bot. 2001;52:1–9.

Li Q, Xie Q, Smith-Becker J, Navarre D, Kaloshian I. Mi-1-mediated aphid resistance involves salicylic acid and mitogen-activated protein kinase signaling pathways. Mol Plant Microb Interact. 2006;19:655–664.

Majer IT, Constabel CP. Molecular analysis of poplar defense against herbivory: comparison of wound- and insect elicitor-induced gene expression. New Phytol. 2006;172:617–35.

Mello OM, Silva-Filho CM. Plant-insect interactions: an evolutionary arms race between two distinct defense mechanisms. Braz J Plant Physiol. 2002;14:71–81.

Meyers BC, Tej SS, Vu TH, Hautenschild CD, Agrawal V, Edberg SB, et al. The use of MPSS for whole-genome transcriptional analysis in Arabidopsis. Genome Res. 2004a;14:1641–53.

Meyers BC, Vu TH, Tej SS, Matvienko M, Ghazal H, Agrawal V, et al. Analysis of the transcriptional complexity of Arabidopsis by massively parallel signature sequencing. Nat Biotechnol. 2004b;22:1006–11.

Morgan PJ, Cheng Y, Cassell JL, Thompson GA. Gene expression profiling of Arabidopsis thaliana in compatible plant-aphid interactions. Arch Insect Biochem Physiol. 2002;51:182–203.

Nobuta K, Venu RC, Lu C, Belo A, Vemaraju K, Kulkarni K, et al. An expression atlas of rice mRNAs and small RNAs. Nat Biotechnol. 2007;25:473–7.

Osato N, Yamada H, Satoh K, Ooka H, Yamamoto M, Suzuki K, et al. Antisense transcripts with rice full-length cDNAs. Genome Biol. 2003;5:R5.

Pegadoruva V, Knepper C, Reese J, Shah J. Premature leaf senescence modulated by the Arabidopsis PHYTOALEXIN DEFICIENT4 gene is associated with defense against the phloem-feeding green peach aphid. Plant Physiol. 2005;139:1927–34.

PENNINCKS IAM, Thomma BPH, Buchala A, Metraux JAP, Broekaeart WF. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensive gene in Arabidopsis. Plant Cell. 1998;10:2103–13.

Ralph S, Oddy C, Cooper D, Yueh H, Jancsik S. Genomics of hybrid poplar (Populus trichocarpa × deltoides) interacting with forest tent caterpillars (Malacosoma disstria): normalized and full-length cDNA libraries, expressed sequence tags, and a cDNA microarray for the study of insect-induced defenses in poplar. Mol Ecol. 2006;15:1275–97.

Reymond P, Weber H, Diamond M, Farmer EE. Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. Plant Cell. 2000;12:707–19.

Reymond P, Bodenhausen N, Van Poecke RM, Krishnamurthy V, Dicke M, Farmer EE. A conserved transcript pattern in response to a specialist and a generalist herbivore. Plant Cell. 2004;16:3132–47.

Ryan CA. The systemin signaling pathway: differential activation of plant defensive genes. Biochim Biophys Acta. 2000;1477:112–21.

Simon SA, Zhai J, Nandety RJ, McCormick KP, Zeng J, Mejia D, Meyers BC. Short-read sequencing technologies for transcriptional analyses. Annu Rev Plant Biol. 2009;60:305–333.

Stotz HU, Pittendrigh BR, Kroymann J, Weniger K, Fritsche J, Bauke SB, et al. Analysis of the transcriptional complexity of Arabidopsis by massively parallel signature sequencing. Nat Biotechnol. 2006;14:1007–17.

Thompson GA, Goggins FL. Transcriptomics and functional genomics of plant defense induction by phloem-feeding insects. J Exp Bot. 2006;57:755–66.

Vega-Sanchez ME, Gowda M, Wang GL. Tag-based approaches for deep transcriptome analysis in plants. Plant Sci. 2007;173:371–380.

Venu RC, Jia Y, Gowda M, Jia MH, Jantasuriyarat C, Stahlberg E, et al. RL-SAGE and microarray analysis of the rice transcriptome after Rhizoctonia solani infection. Mol Genet Genomics. 2007;278:421–31.
Voelckel C, Weisser WW, Baldwin IT. An analysis of plant–aphid interactions by different microarray hybridization strategies. Mol Ecol. 2004;13:3187–95.

von Dahl CC, Baldwin IT. Deciphering the role of ethylene in plant–herbivore interactions. J Plant Growth Regul. 2007;26:201–9.

Walling LL. The myriad plant responses to herbivores. J Plant Growth Regul. 2000;19:195–216.

Winz RA, Baldwin IT. Molecular interactions between the specialist herbivore Manduca sexta (Lepidoptera, Sphingidae) and its natural host Nicotiana attenuata: insect-induced ethylene reduces jasmonate-induced nicotine accumulation by regulating putrescine N-methyltransferase transcripts. Plant Physiol. 2001;125:2189–202.

Yuan JS, Köllner TG, Wiggins G, Grant J, Degenhardt J, Chen F. Molecular and genomic basis of volatile-mediated indirect defense against insects in rice. Plant J. 2008;55(3):491–503.

Zarate SI, Kempema LA, Walling LL. Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. Plant Physiol. 2007;143:866–75.

Zhang F, Zhu L, He G. Differential gene expression in response to brown planthopper feeding in rice. J Plant Physiol. 2004;161:53–62.

Zheng SZ, Dicke M. Ecological genomics of plant-insect interactions: from gene to community. Plant Physiol. 2008;146(3):812–7.