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Organ-specific transcriptome response of the small brown planthopper toward rice stripe virus

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ABSTRACT

Rice stripe virus (RSV) causes rice stripe disease and is transmitted by the small brown planthopper (*Laodelphax striatellus*, SBPH) in a persistent, circulative, and propagative manner. The alimentary canal and salivary gland of SBPH play important roles in viral replication and transmission. However, little is known about the underlying molecular functions of these two organs in the interaction between RSV and SBPH. In this study, organ-specific transcriptomes of the alimentary canal and salivary gland were analyzed in viruliferous and naïve SBPH. The number of differentially expressed unigenes in the alimentary canal was considerably greater than that in the salivary gland after RSV infection, and only 23 unigenes were co-regulated in the two organs. In the alimentary canal, genes involved in lysosome, digestion and detoxification were activated upon RSV infection, whereas the genes related to DNA replication and repair were suppressed. RSV activated RNA transport and repressed the MAPK, mTOR, Wnt, and TGF-beta signaling pathways in the salivary gland. The overall immune reaction toward RSV was much stronger in the salivary gland than in the alimentary canal. RSV activated the pattern recognition molecules and Toll pathway in the salivary gland but inhibited these two reactions in the alimentary canal. The responses from reactive oxygen and the immune-responsive effectors were stronger in the salivary gland than in the alimentary canal after RSV infection. These findings provide clues on the roles of the two organs in confronting RSV infection and aid in the understanding of the interaction between RSV and SBPHs.

1. Introduction

Many insects serve as vectors for the transmission of pathogens, such as bacteria or viruses, into plants to cause infection. Approximately 80% of viral diseases in the plant kingdom depend on insect vectors for their transmission; the majority of these vectors include hemipteran insects, such as aphids, whiteflies, leafhoppers, planthoppers, and thrips, which possess distinct piercing-sucking mouthparts (Hohn, 2007; Hogenhout et al., 2008). Rice stripe disease is caused by the rice stripe virus (RSV, belonging to *Tenuiviruses*) and transmitted by the small brown planthopper (*Laodelphax striatellus* Fallén, SBPH). RSV has caused severe disease in the rice fields of many East Asian countries, including China. In China, the rice stripe disease is particularly severe in Jiangsu Province, where rice field production is reduced by 30%–50%, even resulting in the lack of harvest in some instances (Sun and Jiang, 2006). Controlling this disease is a difficult task because the RSV is transmitted by SBPH in a persistent, circulative, and transovarial propagative manner (Ramirez and Haenni, 1994). Viruliferous rice cannot infect healthy rice through the mechanical inoculation of RSV (Ling, 1972), indicating the indispensability of SBPH in RSV spread and the complicated interactions between the vector and virus. However, the molecular mechanism underlying the complicated interactions between RSV and SBPH has not been well explored at the omic level. Only two studies reported the overall gene expression change in SBPH after RSV infection (Zhang et al., 2010; Lee et al., 2013).

The alimentary canal and salivary gland are two especially important organs for persistent-propagative viruses as the first and the last “battle field” in insect vectors, respectively. Persistent-propagative viruses usually first accumulate in the alimentary canal, transfer into the hemolymph or nervous tissues across the epithelial cell walls, propagate in specific organs, and arrive at the

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salivary gland, from which the viruses are inoculated back to the plant hosts during feeding (Ammar, 1994; Ammar and Hogenhout et al., 2008). The transcriptomes or proteomes of the salivary glands or saliva of several hemipterans, including the brown planthopper *Nilaparvata lugens* (Noda et al., 2008; Konishi et al., 2009), pea aphid *Acyrthosiphon pisum* (Carolan et al., 2009), peach aphid *Myzus persicae* (Ramsey et al., 2007; Harmel et al., 2008), whitefly *Bemisia tabaci* (Su et al., 2012), and potato leafhopper *Empoasca fabae* (DeLay et al., 2012) have been analyzed previously. These studies identified several hundred salivary proteins that can play key roles in viral transmission and host immune reaction. The alimentary canal protects insects against the invasion and spread of pathogens and maintains intestinal immune homeostasis while functioning in nutrient digestion and detoxification (Wang and Granados, 2001). The transcriptome or proteome analyses of the alimentary canal of several hemipterans, including the brown planthopper (Bao et al., 2012; Peng et al., 2011), plant bug *Lygus hesperus* (Wright et al., 2006), and stink bug *Apodiphus amygdali* (Ramzi and Zibaeea, 2013) have been reported. However, the responses of these two organs toward infection by persistent-propagative plant viruses have not been explored and compared with any insect vector to date. Whether RSV provokes different reactions in the alimentary canal and salivary gland of the SBPH is a vital issue in understanding how the virus is processed in these two organs.

In this study, the SBPH transcriptome was sequenced and *de novo* assembled. The gene expression patterns in the alimentary canal and salivary glands were compared before and after RSV infection. The gene expression changes common to both or specific to either of the two organs upon RSV infection were analyzed. Moreover, the genes of the immune system were given greater attention. The results provide clues on the roles of the two organs in confronting RSV infection and information for future functional studies of the candidate genes involved in the interaction between the SBPH and RSV.

2. Materials and methods

2.1. Insect rearing

The viruliferous and naïve SBPH strains used in this study were derived from a field population in Hai'an, Jiangsu Province, China, and maintained in the laboratory for nearly eight years. The planthoppers were reared on 2–3 cm seedlings of rice *Oryza sativa* L. spp. *japonica* var. *nippobare* in glass incubators, which were sealed with a nylon mesh at 25 °C and exposed to 16 h of light per day. The insects were transferred to fresh rice seedlings every 8 days to ensure sufficient nutrition. The presence of RSV was discerned via dot–enzyme-linked immunosorbent assay (ELISA) using the monoclonal anti-Cp antibody as described by Wang et al. (2004). The RSV-carrying rate of the viruliferous strain was maintained at no less than 80% through purification selection every three months using dot–ELISA.

2.2. RNA isolation and transcriptomic sequencing

Approximately 100 alimentary canals and 500 salivary glands from viruliferous and naïve adult planthoppers were dissected and ground in liquid nitrogen. Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The extracted RNA was used to establish a paired-end RNA-seq library for transcriptome sequencing using an Illumina Hiseq 2000 sequencer (Illumina, San Diego, CA, USA). Two biological replicates for each organ were prepared. The two biological replicates of salivary gland samples from the viruliferous strain were prepared at different times with different RSV-carrying rates (86% and 94%) and sequenced at different times. The RSV-carrying rate was 94% for the two biological replicates of alimentary canal samples, which were sequenced once. The sequencing depth was approximately 4 G for each library with 100 bp length per end. The reads of each library were deposited in the Short Read Archive of the National Center for Biotechnology Information (NCBI) with accession number of SRP048969.

2.3. De novo RNA-seq assembly and annotation

After sequencing, FastQC was employed to check the quality distribution of the raw data (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). The raw reads were preprocessed by filtering against low-quality reads and adaptor contamination using Trimmomatic (Lohse et al., 2012). Clean reads were *de novo* assembled with Trinity pipeline (Grabherr et al., 2011). To reduce redundancy, TGICL was used to cluster the assembly based on pairwise sequence similarities (Pertea et al., 2003). Consensus sequences were further filtered by cd-hit, and the longest sequence was selected as representative for every cluster (Li and Godzik, 2006). After annotation, only the coding sequences were further clustered using cd-hit. The combination of clustered sequences obtained from the second run of cd-hit and other non-coding sequences were regarded as the final unigene sequences.

To obtain the coding regions and functional annotations, all unigenes were aligned against sequences from the databases of NR, Swiss-Prot, TrEMBL, and COG using BLASTx, with a cut-off E-value of $<10^{-5}$. The best hit species for every unigene was extracted from the NR and *N. lugens* protein sequences (http://gigadb.org/dataset/100139) using BLASTx, with a cut-off E-value of 10^{-5}. The taxonomy information of the species was generated using Taxtastic software. The coding and protein sequences were extracted from the assembly based on the aligned region of the best hit. Interproscan was employed to identify protein domains by searching against the Interpro (IPR) protein domain database (Hunter et al., 2009). Gene ontology (GO) annotations were extracted from the IPR entries. Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation was performed using the KAAS webserver from the KEGG database (Moriya et al., 2007). The protein sequences of *L. striatellus* were aligned against the known immune proteins of *Drosophila melanogaster*, *Bombyx mori*, *Tribolium castaneum*, and *Anopheles gambiae* (Zou et al., 2007) using BLASTp, with a cut-off E-value of $<10^{-5}$.

2.4. Analysis of differentially expressed unigenes (DEGs) and correlation clusters

The RSEM program was used to calculate the RPKM (reads per kilo bases per million) expression value (Li and Dewey, 2011). The DEGs in the alimentary canal were assessed using the data of two biological replicates. The generated gene count file was inputted into edgeR (Robinson et al., 2010) to detect the DEGs. Both RSEM and edgeR pipelines have been integrated into the Trinity platform (Haas et al., 2013). The DEGs were determined by setting a fold change cutoff of at least 2 and FDR cutoff value of 0.01. The DEGs in the salivary gland were calculated independently for the two replicates. To minimize the influence of differences in RNA output size between samples, the number of total reads was normalized by multiplying with normalization factors, as suggested by Robinson and Oshlack (2010). The DEGs were detected using the method described by Chen et al. (2010). This method was formulated based on the Poisson distribution, and it eliminated the influences of RNA output size, sequencing depth, and gene length. The DEGs were determined by setting a fold change cutoff of at least 2 and FDR cutoff value of 0.01. The changed unigenes common between the