Genome-Wide Identification and Expression Profiling of the Wnt Gene Family in Three Rice Planthoppers: Sogatella furcifera, Laodelphax striatellus, and Nilaparvata lugens

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Abstract

The Wnt gene family plays essential roles in regulating many developmental processes, including the maintenance of stem cells, cell division, and cell migration. The number of Wnt genes varies among species. Due to the diversity and importance of their functions, the Wnt gene family has gained extensive research interest in various animal species from invertebrates to vertebrates. However, knowledge of the Wnt gene family is limited in rice planthoppers. Three planthopper species, the white-backed planthopper (Sogatella furcifera Horvath), the small brown planthopper (Laodelphax striatellus Fallén) and the brown planthopper (Nilaparvata lugens Stål) (Hemiptera: Delphacidae), are devastating specialist pests of rice and cause serious damage to rice plants. To better study the evolution and function of the Wnt gene family in rice planthoppers, we identified 8 Wnt family genes in three rice planthoppers with both genomic and extensive transcriptomic resources available. We conducted a systematic analysis of the three kinds of rice planthoppers and analyzed the dynamic patterns of gene conservation, as well as Wnt gene loss and duplication. The expression profiles in different developmental stages of S. furcifera and different adult organs and tissues of L. striatellus provide preliminary functional implications for the Wnt genes in rice planthopper. This study presents the first genome-wide study of the Wnt gene family in rice planthoppers, and our findings provide insights into Wnt function and evolution in rice planthoppers.

Key words: rice planthopper, Wnt, phylogenetic analysis, protein structure, expression pattern

The Wnt gene is a somite polarity gene that plays an important regulatory role in embryonic development (MacDonald et al. 2009). Wnt acts as a ligand in the Wnt signaling pathway (Huelsken and Behrens 2002). It binds to cell membrane surface receptors and regulates the expression of downstream genes (Guder et al. 2006). The Wnt/β-catenin pathway is a very important development-related signaling pathway, and it is also a popular topic in current biological research (Liu et al. 2022). This pathway determines cell fate, cell proliferation, cell polarity, tissue homeostasis, and disease occurrence during embryonic development (Rao and Kuhl 2010, Clevers and Nusse 2012).

The Wnt family is divided into 13 subfamilies and the 13 Wnt subfamilies were present in the common ancestor of cnidarians and bilaterally symmetric animals (Kusserow et al. 2005, Lengfeld et al. 2009). In Drosophila melanogaster, the molecular regulatory mechanism of the Wnt signaling pathway is most studied. In 1976, Sharma et al. found that a mutation in the gene Drosophila melanogaster wingless-1 (DmWnt1) produced wingless Drosophila melanogaster (Sharma and Chopra 1976). In the following 40 years, studies on Wnt genes achieved breakthrough progresses (Murat et al. 2010). The molecular functions of Wnts are highly conserved, but the number of Wnts varies greatly among species (Bolognesi et al. 2008a, Murat et al. 2010, Shigenobu et al. 2010a).

Compared with D. melanogaster, fewer studies have focused on the function of Wnt genes in other insects. It is reported that Wingless (Ghwg) in the Gryllus bimaculatus may function in the posterior sequential segmentation embryos (Miyawaki et al. 2004). In addition, in Tribolium castaneum, Wnt signaling plays an important role in the development of the foot during the embryonic stage. (Ober and Jockusch 2006, Shah et al. 2011). Silkworm Wnt1 also functions in regulating the development of posterior somites during embryonic development (Yamaguchi et al. 2011).

S. furcifera, L. striatellus and N. Lugens commonly known as rice planthoppers, belong to the same group of family, Delphacidae.
Plathoppers are one of the most destructive pests of rice in tropical and temperate regions of Asia (Horgan et al. 2017). At present, the Wnt gene family has been systematically identified in D. melanogaster, T. castaneum, Acrystosiphon pisum, Anopheles gambiae, Apis mellifera, and Bombyx mori (Dearden et al. 2006, Bolognesi et al. 2008a, Murat et al. 2010, Shigenobu et al. 2010b, Ding et al. 2019). In rice planthoppers, Wnt1 was reported to be involved in the development and growth of wings in S. furcifera as in Drosophila (Yu et al. 2014). However, studies on this family in S. furcifera, L. striatellus, and N. lugens are still limited. In view of the important role of the Wnt gene in insect development, we identified the Wnt gene families from the genomes of the three kinds of rice planthoppers, constructed a phylogenetic tree, and analyzed the expression profiles of S. furcifera and L. striatellus. This research provides a basis for further research on the function of the Wnt gene in rice planthoppers and its application to rice planthopper control.

Materials and Methods

Genome-Wide Identification of Sogatella furcifera, Laodelphax striatellus, and Nilapavarta lugens Wnt Genes

The whole genome sequences of S. furcifera (Wang et al. 2017), L. striatellus (Zhu et al. 2017), and N. lugens (Xue et al. 2014) were obtained from NCBI (https://www.ncbi.nlm.nih.gov). RNA-seq data from different developmental periods of S. furcifera (Wang et al. 2017) (NCBI SRA accession numbers: SRR3990920-SRR3990925, SRR3990981, SRR3990986) and RNA-seq data of L. striatellus from different periods and different tissues (Zhu et al. 2017) (SRR5816373, SRR5816375, SRR5816381, SRR5816382, SRR5816383, SRR5816394) were also downloaded from NCBI. Taking S. furcifera as an example, we first used HsAt2 (Kim et al. 2015) software to compare the reads from the SRA data to the S. furcifera reference genome, then used Samtools to sort the aligned data, followed by StringTie software to sort the stitched files, perform the initial assembly and then merge the transcripts (Pertea et al. 2015). The gffread tool in Cufflinks software was used to extract the sequence in the GTF file, and then the NCBI blast tool kit was used to build the S. furcifera genome and EST local database. The Wnt Pfam domain (PF00110) was obtained from the Pfam protein database (http://pfam.xfam.org/) and was searched against the previously constructed S. furcifera genome local database. The obtained FPKM value of each sample was imported into Mew 4.0 software, and a heat map was drawn.

Sequence Alignment and Phylogenetic Analysis

Human (Homo sapiens), pea aphid (A. pisum), and mosquito (A. gambiae) sequences were downloaded from the EMBL protein database (https://www.ebi.ac.uk/), along with T. castaneum, D. melanogaster, A. mellifera, Heliconius Melpomene, Danaus plexippus, Oporophila brunata, and B. mori Wnt protein sequences. Multisequence alignment was performed using ClustalW (Larkin et al. 2007) and then edited by BioEdit software. MEGA 7.0 software was used to construct a phylogenetic tree of Wnt genes from rice planthoppers and the above species. Parameters used: neighbor-joining, NJ; Bootstrap = 1,000; and Poisson Model; pairwise deletion.

Protein Structure and Conserved Motif Analysis

The domain prediction of Wnt proteins from the three rice planthoppers was obtained from the Simple Modular Architecture Research Tool (SMART, http://smart.embl-heidelberg.de/). Conserved motif analysis of the Wnts was performed by MEME (Multiple Expectation Maximization for Motif Elicitation, https://meme-suite.org/meme/) online analysis. Signal peptide analyses were performed using the SignalP - 6.0 server (https://services.healthtech.dtu.dk/service.php?SignalP).

Three-Dimensional Structural Analysis

SWISS-MODEL (https://swissmodel.expasy.org/) was used to perform homology modeling to obtain Three-Dimensional (3D) structures of the Wnt proteins of three rice planthoppers. PyMOL software version 2.5 was used to perform the structural alignment and the calculation of the root-mean-square deviations (RMSD).

Results

Identification of the Wnt Genes in the S. furcifera, L. striatellus, and N. lugens Genomes

To identify Wnt genes in the three rice planthoppers, we used the Wnt Pfam domain (PF00110) to search against genome local database of three rice planthoppers and validated the mRNA sequences by EST databases searching. We identified 8 Wnt genes in each of the genome of S. furcifera, L. striatellus, and N. lugens. All sequences contain full open reading frame (ORF) except for LsWnt1 due to some gaps in L. striatellus genome sequence. cDNAs of all three

| Table 1. Summary of the Wnt gene family in three rice planthoppers |
|---|---|---|---|---|
| Gene name | ORF size (bp) | ORF size (aa) | MW (kDa) | pl |
| SfWnt1 | 1,170 | 390 | 43.23 | 9.17 |
| SfWnt5 | 1,098 | 366 | 40.79 | 8.75 |
| SfWnt6 | 1,080 | 360 | 40.25 | 9.39 |
| SfWnt7 | 1,065 | 355 | 38.32 | 9.85 |
| SfWnt10 | 1,128 | 376 | 42.02 | 8.91 |
| SfWnt11 | 1,083 | 361 | 39.90 | 9.06 |
| SfWnt16 | 1,200 | 400 | 43.80 | 8.76 |
| SfWntA | 1,098 | 366 | 40.70 | 7.12 |
| LsWnt1α | 645 | 213 | NA | NA |
| LsWnt5 | 1,098 | 366 | 41.77 | 8.85 |
| LsWnt6 | 1,089 | 363 | 40.59 | 9.39 |
| LsWnt7 | 1,086 | 362 | 38.76 | 9.86 |
| LsWnt10 | 1,131 | 377 | 42.17 | 8.91 |
| LsWnt11 | 1,065 | 355 | 38.15 | 8.17 |
| LsWnt16 | 1,197 | 399 | 43.45 | 8.71 |
| LsWntA | 1,095 | 365 | 41.63 | 8.90 |
| NlWnt1 | 1,182 | 394 | 43.58 | 9.18 |
| NlWnt5 | 1,098 | 366 | 40.95 | 8.80 |
| NlWnt6 | 1,074 | 358 | 39.97 | 9.26 |
| NlWnt7 | 1,077 | 358 | 38.53 | 9.22 |
| NlWnt11 | 1,113 | 370 | 40.39 | 9.24 |
| NlWnt16 | 1,200 | 400 | 43.73 | 8.71 |
| NlWntA | 1,107 | 368 | 41.81 | 8.84 |

aa, amino acid; MW, molecular weight; pl, isoelectric point.

Partial sequence.
planthoppers Wnt genes have been submitted to GenBank and their accession numbers in GenBank are shown in Supp Table S3 [online only]. Detailed information on the Wnt genes of these three rice planthoppers is listed in Table 1 and Suppl Table S1 (online only), including gene length, amino acid length, theoretical isoelectric point (PI), and molecular weight. The predicted Wnt amino acid length of the S. furcifera was between 355 aa and 400 aa; the Wnt amino acid length of the L. striatellus was between 355 aa and 399 aa; and the Wnt amino acid length of N. lugens was between 358 aa and 400 aa. The predicted average molecular weight of S. furcifera, L. striatellus, and N. lugens was 38.32–43.80, 38.15–43.45, and 38.53–43.73 kDa, and the PI values were 7.12–9.81, 8.17–9.81, and 8.71–9.26, respectively.

Structural Analysis of rice Planthopper Wnt Proteins

The functional domain was predicted by SMART and the result indicated that all the proteins contain a conserved Wnt1 domain (Pfam domain: PF00110) (Fig. 1). At the same time, signal peptides were found at the N-terminus of all full length ORF of Wnts in rice planthopper by SignalP - 6.0 (Fig. 1).

The conserved motifs of the planthopper Wnt proteins were analyzed by the MEME program, and 5 conserved motifs were obtained with amino acid length range from 21 to 50 (Fig. 2). The 5 conserved motifs exist in all three species of rice planthoppers, and they have highly similar continuing compositions and distributions. This result suggests that these Wnts may share similar functions.

We obtained 3D structures of Wnt proteins of the three rice planthoppers by performing homology modeling. The 3D structures of the three rice planthopper Wnts showed that they shared similar patterns in the arrangement of components (Fig. 3). We also used PyMOL to calculate RMSD value of Wnts (Supp Table S2 [online only]). RMSD is used to compare the similarity and stability between proteins by calculating the mean square error of the positions of carbon atoms in two similar proteins. The lower value of RMSD means higher similarity between two structures. The results show that the RMSD values of Wnts were ranged from 0.009 Å to 2.011 Å among S. furcifera, L. striatellus, and N. lugens suggesting these Wnts were structurally conserved during evolution.

Phylogenetic Analysis of Rice Planthopper Wnt Proteins

We used the NJ method to construct an evolutionary tree in MEGA 7.0 using Wnt protein sequences from the three rice planthoppers, Homo sapiens, and 9 other insect species: A. pisum, A. gambiae, T. castaneum, D. melanogaster, A. mellifera, H. melpomeone, D. plexippus, O. brumata, and B. mori. The 8 Wnts from the rice planthoppers, Wnt1, Wnt5, Wnt6, Wnt7, Wnt10, Wnt11, Wnt16, and WntA, were clustered with those of other insects (Fig. 4). This result indicates that these 8 Wnt proteins are related to the subfamilies of the corresponding clusters of similar family members. The Wnts of the rice planthoppers are all orthologically clustered in one group and are independent compared with other insects, indicating that the three rice planthoppers are closely related and exhibit different evolutionary dynamics. The Wnt1, Wnt5, Wnt6, Wnt7, and Wnt10 proteins of L. striatellus and S. furcifera have the closest phylogenetic relationship, while the Wnt16 proteins of N. lugens and S. furcifera have the closest phylogenetic relationship. Wnt5, Wnt6, Wnt7, and Wnt11 were closely related to the orthologous sequences from Amyelois transitella. Wnt16 separated into a cluster with Wnt10 of B. mori.
in mammals (*Homo sapiens* and *Mus musculus*), 7 in Diptera (*D. melanogaster* and *A. gambiae*), 9 in Coleoptera (*T. castaneum*), and 8 in Lepidoptera (*H. melpomene*, *D. plexippus*, and *B. mori*) (Fig. 5). However, in Hemiptera, there are 8 *Wnts* in rice planthoppers, compared to 6 *Wnts* in *A. pisum* in previous reports (Shigenobu et al. 2010a).

**Expression Profile of the Rice Planthopper Wnt Genes**

*Wnt* genes play an important role in the embryogenesis of metazoans, including embryos, the formation of the body axis, organogenesis and the determination of cell fate, proliferation, migration, and regeneration (Fradkin et al. 1995). RNA-seq data from different developmental periods of *S. furcifera*, different periods, and different tissues of *L. striatellus* were processed by Hisat2, Samtools, and StringTie. FPKM values were retrieved from assembled RNA-seq data and were used to determine expression level. Expression profile of *Wnt* genes in eight developmental stages of *S. furcifera* was presented in a heatmap (Fig. 6). The eight developmental stages included embryos, first instar nymphs, second instar nymphs, third instar nymphs, fourth instar nymphs, fifth instar nymphs, 5-day-old adults, and 10-day-old adults. Compared with other periods, all *Wnt* genes had the highest expression levels in the embryonic period except *Wnt6*, which is most strongly expressed in first instar. All *Wnt* genes expression was generally decreased with development until the lowest expression in the 10-day-old adult. *Wnt5* expressed higher than other *Wnt* genes in every stage, while *Wnt11* was expressed at the lowest levels compared to other *Wnt* genes and was undetectable after the fourth instar stage.

Similarly, we also performed RNA-seq expression analysis on *L. striatellus* at different stages (Fig. 7). Similar to the expression profile of *S. furcifera*, the expression of all 8 *Wnts* was the highest in the egg stage, the expression of *Wnts* gradually decreased with increasing worm age, and the expression of *Wnts* was the lowest in the adult stage. *Wnt10* and *WntA* were expressed stronger than other *Wnts* in egg stage. The expression level of *Wnt11* was extremely low after adulthood, while *Wnt1* could not be detected.

Based on the RNA-seq data of *L. striatellus*, we characterized the expression profiles of four tissues: brain, fat body, gonad, and tentacle (Fig. 8). The eight *Wnts* were expressed in most tissues and were expressed at low levels to medium levels in most tissues.
The expression levels of Wnt6 and Wnt7 were low in brain, fat body, and gonad. Wnt11 was not detected in fat body and tentacle. Compared with other tissues, Wnt1, Wnt5, Wnt10, and WntA have higher expression levels in the brain, while Wnt16 has the lowest expression level in the brain. Wnt6 and Wnt7 had the highest expression in the tentacle.

Discussion

We identified 8 Wnt genes (Wnt1, Wnt5-Wnt7, Wnt10, Wnt11, Wnt16, WntA) in S. furcifera, L. striatellus, and N. lugens (Fig. 1; Table 1). The Wnt genes of these three rice planthoppers belong to the same superfamily and share high homology (Fig. 1). This result indicates that Wnt is evolutionarily conserved among these three rice planthoppers. According to the algorithm of the SignalP - 6.0 server, all full length ORF of Wnts contain signal peptides (Fig. 1) which may play an important role in the transmembrane transport of Wnt proteins (Banziger et al. 2006). In previous study, the C-terminal cysteine-rich domain of Xenopus Wnt8 formed a strong hydrophobic contact with a groove in the receptor Fz8 and a mini-Wnt comprising only the C-terminal domain is able to bind to the Fz8 (Janda et al. 2012). The C-terminus motif1 in planthopper Wnts is also cysteine-rich (Fig. 2), which may share the same function in Xenopus Wnt8, i.e., important for receptors binding. Homology modeling revealed similar 3D structures of each Wnt among rice planthopper species (Fig. 3), suggesting that these Wnts perform similar functions in different planthoppers.

According to previous studies, the ancient metazoan Wnt family is believed to contain 13 diverse members (Prud’Homme et al. 2002, Kusserow et al. 2005, Lengfeld et al. 2009, Cho et al. 2010). In bilaterally symmetrical animals, the continuous evolution of the genome has led to the deletion or duplication of multiple Wnt genes (Crow and Wagner 2006). Gene deletions occur more frequently in insects, while Wnt gene duplication events occur frequently in vertebrate genomes (Janssen et al. 2010). The orthologs of Wnt2 and Wnt4 seem to have been lost in all insects, and the Wnt16 gene has only been reported in A. pisum (Shigenobu et al. 2010c). In addition, Wnt11 may have been lost in Diptera insects (Murat et al. 2010). D. melanogaster and Caenorhabditis elegans lost 6 Wnts (Wnt2 to 4, 11, 16 and A) and 8 Wnts (Wnt1 to 3, 6 to 8, 11 and 16) (Bolognesi et al. 2008b). These results show that each animal lineage has its own Wnt gene pool. Among the insects compared in this study, neither A. mellifera nor Homopteran insects have Wnt9, while other insects including A. gambiae, T. castaneum, D. melanogaster, H. melpomene, D. plexippus, O. brunata, and B. mori contain the Wnt9 (Fig. 5). Except for D. melanogaster and T. castaneum, which contain Wnt8, no other insects contain this family. In addition, Wnt16 is unique to Homopteran insects. From the perspective of the phylogenetic tree in this study, Wnt16 of Hemiptera became a separate branch and was adjacent to the Wnt10 branch of other insects (Fig. 4). In addition, the three kinds of rice planthoppers all contain Wnt6, but A. pisum, which is also a homopteran insect, has lost this subfamily during its evolution. Similarly, Wnt11 was lost in A. gambiae.
and *A. mellifera*, and WntA was lost in *D. melanogaster*. Wnt16 is expressed in a typical segment polarity gene (SPG) like pattern both during anterior and posterior segment formation in all chelating species suggesting a conserved role in segmentation (Hayden and Arthur 2014, Constantinou et al. 2016, Hogvall et al. 2019, Janssen et al. 2021). Wnt16 has thus far not been in the focus of scientific studies due to its lost in most insects. Therefore, it will be interesting to investigate the evolutionary mechanism why Wnt16 in *A. pisum* and rice planthoppers retain this gene.

We analyzed the Wnts expression profiles of *S. furcifera* from embryonic to developmental stages. The expression trends of most Wnt families were surprisingly similar. They expressed at the highest level in the embryonic stage, decreased in the nymph stage, and were the lowest in the adult stage (Figs. 6 and 7). Previous research indicated that relative expression levels of Wnt1 mRNA of *S. furcifera* were significantly higher in nymphs than in adults (Yu et al. 2014). Differentially expressed genes (DEGs) analysis based on transcriptome of ontogenetic development of *S. furcifera* showed that Wnt10, Wnt16, and WntA were expressed the highest during the embryo stage (Wang et al. 2017). Our results showed the rice planthoppers have similar expression pattern compared to other insects. For example, expression of Wnts in *B. mori* also displayed the highest expression in the embryonic stage, and gradually decrease as the embryo develops to the hatching stage (Ding et al. 2019). Wnt signaling genes are involved in several key biological processes during embryonic development, such as cell polarity, body axis determination, and growth signal transmission (Wiese et al. 2018). Defects in Wnt signaling may cause embryo lethality or induce developmental defects. The expression of Wnt was the lowest in the adult stage (Figs. 6 and 7), indicating that the function of Wnt was weakened after maturity.

In intermediate embryonic insects, Wnt1 transcripts can be detected in the middle of the blastoderm and gradually extend back to the abdomen as the somite differentiates. Wnt1 also participates...
in the process of head formation, such as the development of the eye, the telencephalon, and the diencephalon (Friedrich 2003, Lekven et al. 2003, Rossi et al. 2007, Nakao 2010). *D. melanogaster* *Wnt5*,
Wnt1, and Wnt9 have been reported to be key molecules involved in regulating axon guidance and synapse formation (Russell et al. 1992, Yoshikawa et al. 2003, Inaki et al. 2007). In rice planthoppers, Wnt1 of S. furcifera was reported to play a key role in the development of wings (Yu et al. 2014). D. melanogaster Wnt5 is broadly expressed in the developing brain and regulates axonal development of mushroom body neurons (Shimizu et al. 2011) and regulates the growth of the neuromuscular junction (NMJ) (Liebl et al. 2008). The high expression of Wnt1 and Wnt5 in the S. furcifera and L. striatellus at the early developmental stage (Figs. 6 and 7) and the high expression of Wnt1 and Wnt5 in the L. striatellus brain and tentacle (Fig. 8) indicate that the rice planthoppers Wnt1 and Wnt5 may also be involved in similar complex functions as in D. melanogaster.

The Wnt6 of D. melanogaster expression pattern is similar to that of Wnt1 and can be detected in embryo stage. D. melanogaster Wnt10 is mainly expressed in the embryonic mesoderm, central nervous system, and gut and Wnt10 is also expressed in the ventral nerve chord and brain from stage 15 through the end of embryogenesis (Janson et al. 2001a). D. melanogaster is often used as a model animal to study Wnt function. For example, knock out of Wnt6 in D. melanogaster results in lack of maxillary palps absence of Wnt6 (Doumpas et al. 2013). A. pisum belonging to the order Hemiptera, a group that has lost maxillary palps absence of Wnt6 (Doumpas et al. 2013), indicating Wnt6 play an important role in the development of maxillary palps. However, in Hemiptera, both in rice planthoppers and Diaphorina citri contain Wnt6 (Vosburg et al. 2021). Thus, the function of Wnt6 in rice planthoppers would be interesting to further investigate. To sum, our results show that Wnts play important roles in the development and early embryo differentiation in the three rice planthoppers.

### Conclusions

In this study, we performed the first genome-wide analysis of the Wnt gene family in three rice planthoppers. We obtained Wnt1, Wnt5, Wnt6, Wnt7, Wnt10, Wnt11, Wnt16, and Wnt4 in each species of planthopper. We also described the information of domain, motifs, and 3D structure of Wnts in the three rice planthoppers. The phylogenetic analysis highlights the dynamic patterns of gene evolution and loss of Wnt genes among different species. In addition, we obtained Wnt gene expression profiling of different developmental stages in S. furcifera, and L. striatellus, and multiple tissues of L. striatellus. The results indicated that Wnt plays an important role in the development and early embryo differentiation. In conclusion, our results should be useful for further studies on the function of Wnt genes in rice planthoppers and will help with their application to rice planthopper control.

The three planthoppers Wnt genes identified in this study was submitted to GenBank and the accession numbers were shown in Supp Table S3 (online only) and all Wnt protein sequence were shown in Supp Table S1 (online only).

### Author Contributions

LP: Conceptualization, Methodology, Visualization, Investigation, Software, Validation, Data curation, Writing-Original draft preparation, Funding acquisition. YZ: Conceptualization, Supervision, Data curation, Validation, Visualization, Writing, Reviewing and Editing.

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