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Research

Glucose Utilization in the Regulation of Chitin Synthesis in Brown Planthopper

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Abstract

Glucose-6-phosphatase (*G6Pase*) and hexokinase (*HK*) are two key enzymes in the glycolysis and gluconeogenesis pathways, which catalyze the synthesis and degradation of glucose in insects, respectively. *G6Pase* and *HK* play an important role in insect growth by regulating the metabolism of glucose, leading to the efficient metabolism of other macromolecules. However, it is unclear whether these genes could be investigated for pest control through their actions on chitin metabolism. We studied the potential functions of *G6Pase* and *HK* genes in the regulation of chitin metabolism pathways by RNAi technology. Interference with *G6Pase* expression did not affect trehalose and chitin metabolism pathways in brown planthopper, *Nilaparvata lugens* (Stål). However, knockdown of the *HK* gene resulted in a significant decrease of expression of genes associated with the trehalose metabolic pathway but had no significant effect on trehalase activity, trehalose content, or glucogen content. Additionally, *HK* knockdown resulting in downregulation of the genes involved in chitin metabolism in the brown planthopper. These insects also showed wing deformities and difficulty in molting to varying degrees. We suggest that the silencing of *HK* expression directly inhibited the decomposition of glucose, leading to impaired chitin synthesis.

Key words: brown planthopper, glucose-6-phosphatase, hexokinase, trehalose metabolism, chitin metabolism

Rice (*Oryza sativa* L.) is the most important cereal crop in China and is the staple food for more than two-thirds of the world's population. Surprisingly, its annual yield loss due to pests and disease reaches levels of 24–41% (Boddupally et al. 2018). Among the most destructive of these pests is *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae). It is a single-feeding pest with strong migratory ability and can damage rice directly by sucking phloem sap through its mouthparts (Cheng et al. 2013). In addition, brown planthopper can also carry rice viruses, representing an additional threat to rice productivity (Lu et al. 2016). Although traditional insecticides have proven to be effective in controlling the population of brown planthopper (Sun et al. 2013, Yang et al. 2017), the excessive use of chemical pesticides has led to many problems, including increased production costs, toxicity to natural enemies of brown planthopper and their 'resurgence', and harm to agro-ecosystems and human health (Rola and Pingali 1993, Becker et al. 1996, Nauen and Denholm 2005, Wang et al. 2008, Bottrell and Schoenly 2012). Therefore, the development and utilization of biological pesticides have become an important avenue of research.

Manipulating the regulation of insect chitin synthesis has become a favorable avenue of investigation in the development of new pest control methods (Arakane et al. 2005, Wang et al. 2012, Mansur et al. 2014, Xu et al. 2017, Wu et al. 2019, Wang et al. 2019). The exoskeleton of insects is composed of the stratum corneum and plays an important role in the growth and development of insects, including protection, support, movement, and as a barrier against environmental stress (Merzendorfer and Zimoch 2003, Zhu et al. 2016). However, an exoskeleton with a rigid structure has limitations on the growth and development of insects. Insects regularly form a new stratum corneum to replace the old stratum corneum, and chitin plays a key role in this process (Nakabachi et al. 2010). Chitin is considered the second most abundant biomolecule. It is a polymer of *N*-acetylglucosamine linked by β-1,4 glycosidic bonds, synthesized by chitin synthase, a transmembrane protein (Merzendorfer and Zimoch 2003). Chitin is found in many organisms, including microorganisms such as fungi, protists and algae, arthropods such as insects, crustaceans and arachnids, and other invertebrates such as sponges, coelenterates, mollusks and nematodes (Sobala et al. 2015). In insects, chitin is the central component of the epidermis, the trachea and the peritrophic matrix (PM) (Zhuo et al. 2014).

The sugar present in insect's blood (hemolymph)—trehalose—is a nonreducing sugar consisting of two glucose molecules linked by alpha, alpha-1,1-glycosidic linkages, widely found in bacteria, yeast, fungi, plants and invertebrates, but not present in mammals (Becker et al. 1996, Elbein 1974, Wingler 2002, Elbein et al. 2003, Frison et al. 2007). Trehalose is not only an energy source but also an

important stress protection agent that shields the body from damage in various adverse conditions (Becker et al. 1996, Elbein et al. 2003, Tang et al. 2010, Shukla et al. 2015, Liu et al. 2016). Therefore, it plays a key role in insect development and physiological activities. It has been reported that the concentration of trehalose in the hemolymph of insects affects the rate of energy consumption during flight (Clegg and Evans 1961), the synthesis of chitin during molting (Candy and Kilby 1962), cold resistance (Wyatt 1967), food selection and feeding behavior (Thompson and Dahlman 1999), and the oogenesis of insects (Santos et al. 2012).

Glucose is the most widely used carbohydrate in animals for energy production, the provision of macromolecular precursors, and signaling molecules in liver and adipose tissue (Vaulont et al. 2000). Hexokinase (*HK*) is the first enzyme in the glucose metabolism pathway, as well as a multifunctional protein with roles in transcriptional regulation (Niederacher and Entian 1991, Herrero et al. 1995) and apoptosis (Gottlob et al. 2001, Bryson et al. 2002), as well as acting as a cytokine neurotransmitter interleukin (neurotrophic factor) (Gurney et al. 1986, Chaput et al. 1988, Faik et al. 1988). In addition, *HK* is also involved in pathogen-host interactions; for example, *HK* was first reported in *Nematocida parisii* (Cuomo et al. 2012) and has also been shown in *Paranosema (Antonospora) locustaes* by biochemical experiments (Reinke et al. 2017), and in *P. locustaes* (Senderskiy et al. 2014, Timofeev et al. 2017). Locustae *HK* is predicted to contain a signal peptide, while showing the presence of *HK* in the host's indirect immunofluorescence assay (IFA) (Senderskiy et al. 2014, Timofeev et al. 2017). The special function of secreted hexokinase has been studied in *Trachipleistophora hominis*, where it acts as a regulator to increase glycolysis or ATP production on the surface of parasites (Ferguson and Lucocq 2019). Hexokinase irreversibly catalyzes ATP-dependent glucose phosphorylation, producing ADP and glucose-6-phosphate (G-6-P), which is the initial step in the glycogen synthesis, glycolysis, or pentose phosphate pathways (Morris et al. 2006, Calmettes et al. 2013, Al-aryahi et al. 2014). Importantly, it is also the second process in the chitin synthesis pathway (Zhang et al. 2011). The gluconeogenesis-specific enzyme, glucose-6-phosphatase (*G6Pase*), first appeared in echinoderms but also found in echinoderms, molluscs, and vertebrates (Miyamoto and Amrein 2017). *G6Pase* hydrolyzes glucose-6-phosphate (G-6-P) to free glucose in the terminal step of gluconeogenesis and glycogenolysis. It is one of the rate-limiting enzymes in the gluconeogenesis pathway and its expression can be regulated by the IRS/PI3K/Akt signaling pathway (Barthel and Schmoll 2003).

Most of the current research in the field focuses on the regulation of chitin synthesis by trehalose. However, the question of whether the use of glucose has an effect on the synthesis of chitin remains unanswered. To address this question, we interfered with *HK* and *G6Pase*—the enzymes that directly regulate glucose synthesis and degradation—to explore the potential function of glucose utilization in chitin synthesis. If glucose metabolism is used to regulate the synthesis of chitin in brown plant hopper, a novel mechanism for the prevention and control of brown planthopper or other rice pests will be exposed.

Materials and Methods

Test Insect

N. lugens used in this study were from the laboratory breeding population, and the test insect source comes from the Hangzhou population of the China Rice Research Institute. Feeding conditions of brown planthopper: *O. sativa* is a susceptible strain TN1 (Taichung Native 1), temperature 25 ± 1°C, light–dark cycle 16:8 (L:D) h, relative humidity 70 ± 5%.

RNA Extraction and cDNA Preparation

The total RNA from brown planthopper was obtained by Trizol extraction under RNase free conditions. RNA integrity was assessed using gel electrophoresis, concentration and purity was checked on the NanoDropTM 2000 micro-assay spectrophotometer. RNA was stored at −80°C before use. cDNA was synthesized using the PrimeScript RT reagent kit (TaKaRa) with gDNA Eraser kit (TaKaRa) according to the manufacturer's instructions. The synthesized cDNA was stored at −20°C.

Synthesis of dsRNA

The dsRNA primers for the synthesis of dsHK, dsG6Pase, and dsGFP were designed using Primer 5 software (specific primer sequences are shown in Table 1). cDNA was first polymerase chain reaction (PCR) amplified with the primers of interest. The PCR products were subject to T cloning, followed by a subsequent amplification with primers containing the T7 promoter sequence. Cross-PCR reactions were performed using a T7 RiboMAXTM Express RNAi System kit (Promega) to synthesize dsRNA. The integrity of dsRNA was determined by gel electrophoresis, and the concentration and purity of synthesized dsRNA were measured using NanoDropTM 2000 microassay spectrophotometer.

Table 1. Primers used in the dsRNA synthesis and qRT-PCR detection

Gene	Application type	Primer set	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Length of target fragment (bp)
NlHK	dsRNA synthesis	dsNlHK	TGGTGCGAGAAGAAGTGAA	TCCAGATAGCAGGCGTTG	409
		dsNlHK-T7	T7-GTGGTGCGAGAAGAAGTGAA	T7-TCCAGATAGCAGGCGTTG	459
		qRT-dsNlHK	GGTGCGAGAAGAAGTGAAG	GTGAAACCCATTGGTAGAGT	148
NlG6Pase	dsRNA synthesis	dsNlG6Pase	GGGTGCTCTTATCGGTTGT	AGGGCATTGTCGGTTGG	414
		dsNlG6Pase-T7	T7-GGGGTGCTCTTATCGGTTGT	T7- AGGGCATTGTCGGTTGG	464
		qRT-dsNlG6Pase	TTTCGGCTCACTTCCCTC	GCAGTAATCAACATAGCACCT	132
GFP	dsRNA synthesis	dsNlGFP	AAGGGCGAGGAGCTGTTCACCG	CAGCAGGACCATGTGATCGCGC	
		dsNlGFP-T7	T7-AAGGGCGAGGAGCTGTTCACCG	T7-CAGCAGGACCATGTGATCGCGC	

T7 : GGATCCTAATACGACTCACTATAGG.

Microinjection of Brown Planthopper

The volume of dsRNA per injection was determined by a standard capillary under a microscope. The brown planthoppers were anesthetized with CO_2 and placed in the groove of a pre-prepared agarose gel. The dsGFP, dsHK, and dsG6Pase were injected into the lateral epidermis of the two pairs of hind paws in the chest of the brown planthopper. 50 ng of dsRNA was injected into each brown planthopper. Finally, the injected brown planthoppers were transferred to a glass tube containing fresh rice, and the surviving brown planthoppers were taken for subsequent experiments with 48 h after injection.

Tissue Collection

The material for exploring the developmental expression of brown planthopper was from 1st of 4th instar nymph to 3rd days after reaching adulthood. Sampling was carried out at 24 h intervals, and three samples were collected in parallel to assess developmental expression patterns. Material from the head, foot, wing, epidermis, ovary, and fat body were obtained through anatomical mirror dissection of LEICA EZ4. The samples were stored at −80°C.

Gene Expression by qRT-PCR

Total RNA was extracted from the brown planthopper material, and synthesized cDNA by reverse transcription as previously described. The relative expression levels of *G6Pase* and *HK* genes at different developmental stages and in different tissues were detected by qRT-PCR. The brown planthopper *18S* gene was selected as an internal reference gene. qRT-PCR primers are shown in Table 1. Gradient PCR was performed to verify the optimal annealing temperature of the primers, and agarose gel electrophoresis was performed to ensure the specificity of the PCR product.

To carry out fluorescence real-time quantitative PCR, the following reaction was set up: SYBR Premix Ex Taq 10 µl (TaKaRa), template cDNA 1 µl, forward primer 1 µl, reverse primer 1 µl, in 20 µl reaction. The PCR amplification procedure was: predenaturation at 95°C for 2 min, 39 cycles of denaturation at 95°C for 30 s and annealing at 55–60°C for 30 s, and dissolution curve at 65–95°C for 5 s.

The relative expression of the target genes and genes involved in the trehalose and chitin metabolism in the brown planthopper after RNAi: the brown planthopper that was injected 48 h, was divided into three parallel tubes, then the total RNA was extracted and reverse transcribed to obtain three tubes of cDNA in parallel. The cDNA was subjected to real-time PCR using the SYBR Premix Ex Taq kit. The quantitative primers used are shown in Table 2.

Determination of Total Trehalose, Glucose Content, and Trehalase Activity

PBS was added to the material for 200 µl; the mixture was thoroughly ground with a grinding rod and placed in a sonicator for sonication. After disruption, 800 µl of PBS was added, and the sample was centrifuged at 1,000 × g for 20 min at 4°C. The supernatant was taken for 350 µl and centrifuged at 20,800 × g for 1 h at 4°C. The supernatant after ultracentrifugation was used to measure glucose, protein concentration, and soluble trehalase activity, and the pellet was resuspended in PBS to determine glucose, protein concentration, and membrane-bound trehalase activity. The remaining supernatant was used for the determination of total protein and trehalose concentrations. Methods were previously described in Zhang et al. (2017).

The experimental and the control groups' materials were taken, 200 µl of PBS was added, and the mixture was thoroughly ground with a grinding rod and placed in a sonicator for sonication. After

Table 2. Primers used for qRT-PCR

Gene name	Genebank number	Forward primer (5′-3′)	Reverse Primer (5′-3′)	Length of target fragment (bp)
QNl18S		CGCTACTACCGATTGAA	GGAAACCTTGTTACGACTT	165
QNlPGM1	KU556839.1	TTCTCGGTTGGTGGTGC	CCTTCAGCCTGGGACAT	127
QNlPGM2	KU556840.1	CGTTACAGGCTACGGAAGT	GACCCAAAGCAGTCAAA	141
QNlGP	KU556838.1	GCTGCCTATGGCTATGGTATTC	TCTGAGTGTTGACCCACTTCTTG	202
QNlGS	KU556837.1	GCTCCAAAGCCTATGTTTCTACTG	TGGTAACCCCTGTCCCTCA	160
QNLUGPase	KU556842.1	ATACAAGATGGCGGCTAA	TTGTGGCAGTTGATAGAGC	136
QNlTPS1	GQ397450	AAGACTGAGGCGAATGGT	AAGGTGGAAATGGAATGTG	154
QNlTPS2	KU556826	AGAGTGGACCGCAACAACA	TCAACGCCGAGAATGACTT	161
QNlTPS3	KU556827	GTGATGCGTCGGTGGCTAT	CCGTTCATCATTGGGCATAGT	224
QNlTRE1-1	FJ790319	GCCATTGTGGACAGGGTG	CGGTATGAACGAATAGAGCC	132
QNlTRE1-2	KU556829	GATCGCACGGATGTTTA	AATGGCGTTCAAGTCAA	178
QNlTRE2	GQ397451	TCACGGTTGTCCAAGTCT	TGTTTCGTTTCGGCTGT	197
QNlHK	KU556830	GGTGCGAGAAGAAGTGAAG	GTGAAACCCATTGGTAGAGT	147
QNlGFAT	KU556833	CCTCCCAGTTCATCTCGC	CCAAGTTCTTCAAACCCTTTAT	105
QNlG6Pase	KU556841.1	AGACCCTGGCAGTAGAATAG	GGGAAGTGAGCCGAAAT	132
QNlG6PI1	KU556832.1	GTTCACGGTCGTCTGGAAAG	TGACTGCTCCGTTTCACTCT	82
QNlG6PI2	KU556831.1	AACAAGGCGACATGGAATCG	ACCATTTGTTCCTGGTTCGC	85
QNlG6PI3	XM_022345379.1	ATGTCACAGTGCATGTCGTG	ACCTGCTCTCATTGATGCCA	120
QNlGNPNA	KU556834	TGAGCTGCTGAAGACACT	CCTGAATAACGGTGATGTA	179
QNlUAP	JF330415	ACGACAGATTAAAGCCGATAC	TACCTTGTCCACCAGCCA	147
QNlCHS1	AEL88648	CCGCAAACGATTCCTACAGA	AGGTCCTTGACGCTCATTCC	222
QNlCHS1a	JQ040014	TGTTCTTGCTACAACTCAATAAA	ACACCAATCCGATAGGCTC	141
QNlCHS1b	JQ040013	GCTGTCTTTGCTTTCTTCAT	ACACCAATCCGATAGGCTC	187

disruption, 800 µl of PBS was added and centrifuged at 1,000 g for 20 min at 4°C. 350 µl of the supernatant was taken at 4°C, 20,800 × g for 1 h, and the remaining supernatant was used for the determination of total protein and trehalose concentrations. The supernatant after ultracentrifugation was used to measure glucose, protein concentration, and soluble trehalase activity assay, and the pellet was suspended in PBS for determination of glucose, protein concentration, and membrane-bound trehalase activity, the specific steps refer to the method of Zhang et al. (2017).

Statistical Analysis

The relative copy number of the gene was determined by qRT-PCR, using the $2^{-\triangle\triangle CT}$ method. The reactions were performed in triplicate and with three biological replicates. The control group was the results of the brown planthopper injected with dsGFP. The converted values were analyzed as follows (Chen et al. 2018):

$$2^{-\triangle\triangle CT} = 2^{-[(CT\ control\ group - CT\ control\ 18s) - (CT\ test\ group - CT\ test\ group\ 18s)]}$$

Difference analysis was performed by using SPSS software, and one-way analysis of variance (ANOVA) data analysis method was employed to analyze potential differences. Differences were considered significant if the P value was between 0.01 and 0.05, and extremely significantly different the P value was less than 0.01. The analyzed data was plotted using SigmaPlot 10.0 software.

Results

Developmental and Tissue Expression Patterns of *G6Pase* and *HK*

In order to explore the specific expression profiles of *HK* and *G6Pase* genes in different developmental stages of brown planthopper, we

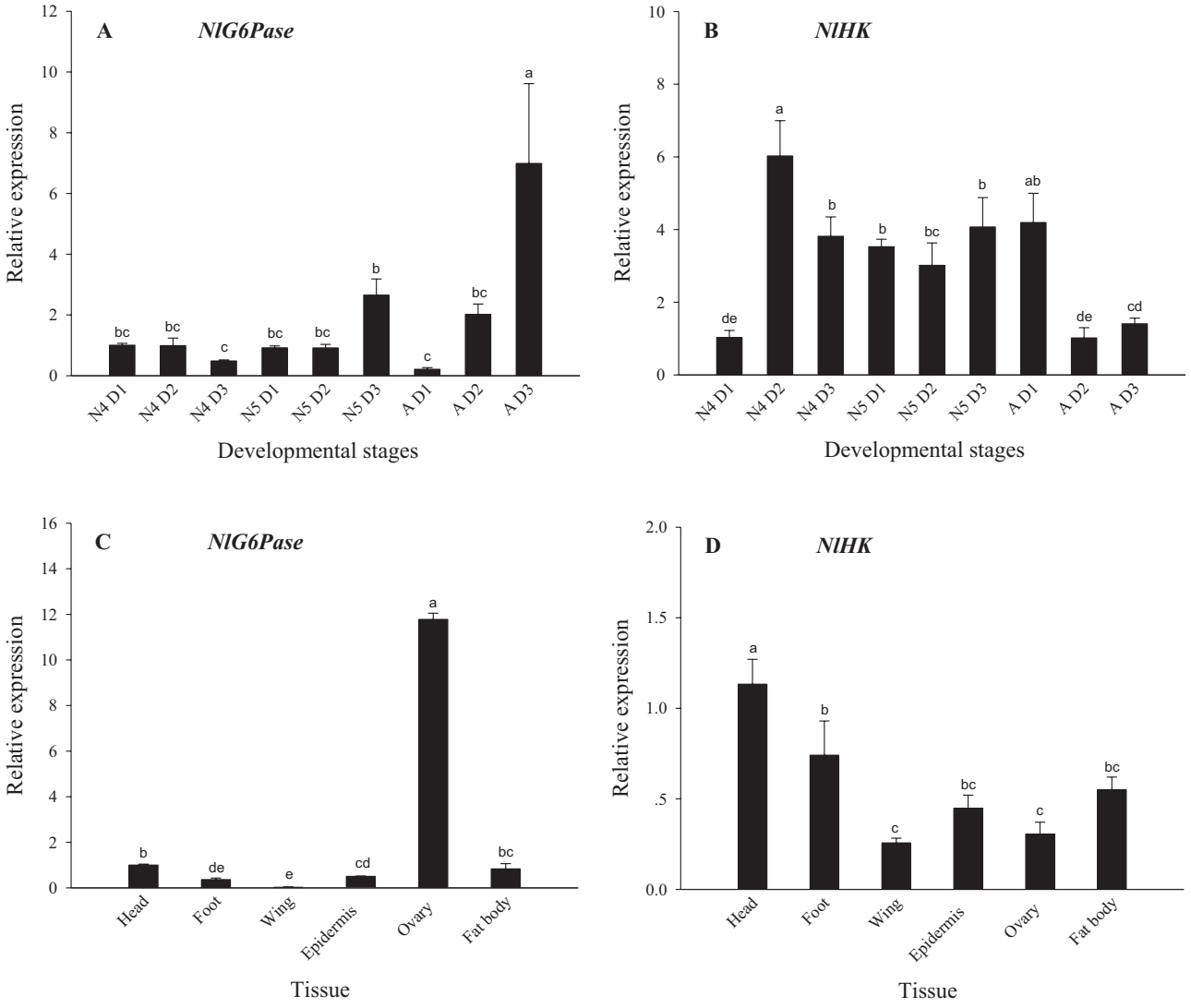

Fig. 1. Relative expression of *G6Pase* and *HK* in different tissues and developmental stages in brown planthopper. Expression patterns of *G6Pase* (A) and *HK* (B) at different developmental stages from first day of 5th instar nymph to third day of adulthood. Expression patterns of *G6Pase* (C) and *HK* (D) in various tissues including head, foot, wing, epidermis, ovary and fat body collected from adults. The mRNA level was normalized to the NI18S mRNA, and the relative expression of each tissue and developmental stage was measured in relation to that obtained in the head and first day of 5th instar nymph. A one-way analysis of variance (ANOVA) was performed to test for statistical significance. Data are presented as the means ± SD ($N = 3$). Means with different letters within the same parameter group differ significantly, $P < 0.05$.

examined the expression levels of *HK* and *G6Pase* genes from the fourth instar to the third day of adulthood. The results showed that the expression of *HK* and *G6Pase* was relatively stable in the nymphal stage. However, *HK* increased significantly in the early stage of the fourth instar nymph and then decreased significantly in the adult stage. The expression of *G6Pase* gradually increased across developmental time, reaching its peak at the final time point, while the expression of *HK* showed a significant downward trend (Fig. 1A and B).

We also examined the expression levels of *HK* and *G6Pase* genes in different tissues of the adult brown planthopper, namely, foot, wing, midgut, ovary, and fat. The tissue expression profiles showed that the *G6Pase* gene was highly expressed in the ovary of the brown planthopper, followed by the head and fat body, and its expression in the wing was relatively low. The expression level of *HK* gene was highest in the head, followed by foot, and the expression level in wings and ovaries was relatively low (Fig. 1C and D).

Expression of *G6Pase* and *HK* in Brown Planthopper After RNAi

The expression of *G6Pase* and *HK* genes were knocked down using RNAi technology. The results showed that compared to the control group, the expression levels of these genes were significantly decreased after 48 h of interference with the *G6Pase* or *HK* gene in brown planthopper. Which indicate that RNAi effectively inhibited the expression of *G6Pase* or *HK* genes. In addition, the results showed that the expression of *G6Pase* was significantly decreased after interference with *HK*, and there was no significant change in the expression of *HK* gene after interference with *G6Pase* (Fig. 2).

Expression of Trehalose Metabolism-Related Genes in Brown Planthopper Following *G6Pase* and *HK* Knockdown

To assess the effect of *G6Pase* and *HK* inhibition on trehalose metabolism in the brown planthopper, we analyzed the expression of key genes by qRT-PCR. After 48 h of *G6Pase* knockdown, the expression levels of trehalose metabolism-related genes in brown planthopper were mostly unchanged, except for the downregulation of *TPS1* and *TPS3* genes, and the upregulation of *TPS2*. After *HK* knockdown, the expression levels of trehalose metabolism-related genes in brown planthopper were all significantly or extremely significantly decreased, with the exception of *TPS2* and *UGPase* (Fig. 3).

Effects of *G6Pase* and *HK* Gene Inhibition on Trehalase Activity in Brown Planthopper

We next tested the enzymatic activities of soluble and membrane-bound trehalase. The results showed that there was no significant change in soluble trehalase activity and membrane-bound trehalase activity in the brown planthopper after inhibition of *G6Pase* or *HK* expression (Fig. 4).

Effects of *G6Pase* and *HK* Gene Knockdown on the Trehalose and Glucose in Content in Brown Planthopper

Interference with *G6Pase* or *HK* gene expression has little effect on the content of trehalose in the brown planthopper. Compared with the control group, there was no significant difference in the content of trehalose in the brown planthopper after *G6Pase* or *HK* gene inhibition (Fig. 5B). However, the glucose content in the brown planthopper was significantly increased after *G6Pase* knockdown, whereas there was no significant difference in the glucose content following *HK* knockdown (Fig. 5A).

Evaluation of Chitin Level in Brown Planthopper Following *G6Pase* and *HK* Inhibition

We next sought to investigate the effects of *G6Pase* and *HK* knockdown on chitin metabolism, and employed qRT-PCR to test the expression of related genes. Our results demonstrate that perturbation of *G6Pase* activity does not significantly impact the expression of genes involved in chitin metabolism in brown planthopper, except in the case of *G6PI2* and *UAP* (Fig. 6A). In contrast, inhibition of *HK* expression resulted in significant or extremely significant downregulation of the genes involved in chitin metabolism. In addition, in the brown planthopper also showed wing deformities (Fig. 6A) and difficulty in molting to varying degrees (Fig. 6B).

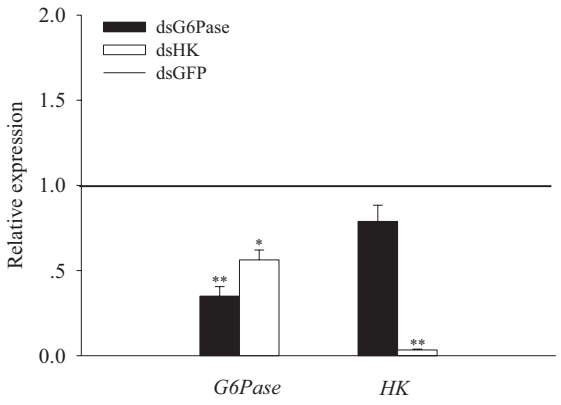

Fig. 2. Relative expressions of *G6Pase* and *HK* after injection for 48 h in brown planthopper. qRT-PCR data showing mRNA levels of NIG6Pase and NIHK relative to NL-18S mRNA level following RNAi targeting. First day of instar nymph were chosen as the targets for dsRNA injection. * indicates significant difference and ** indicates extremely significant difference, the same below.

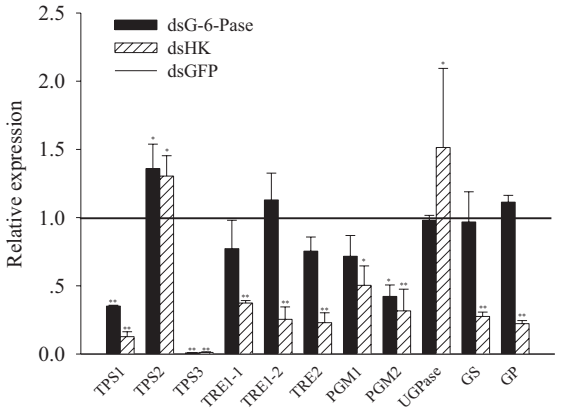

Fig. 3. Expression level of trehalose metabolic pathway genes in brown planthopper after RNAi. Expression of three trehalose-6-phosphate synthases (TPS), three trehalases (TRE), two phosphoglucomutase (PGM), UDP-glucose pyrophosphorylase (UGPase), glycogen synthase (GS), and glycogen phosphorylase (GP) at 48 h relative to the NL-18S mRNA level were measured using qRT-PCR.

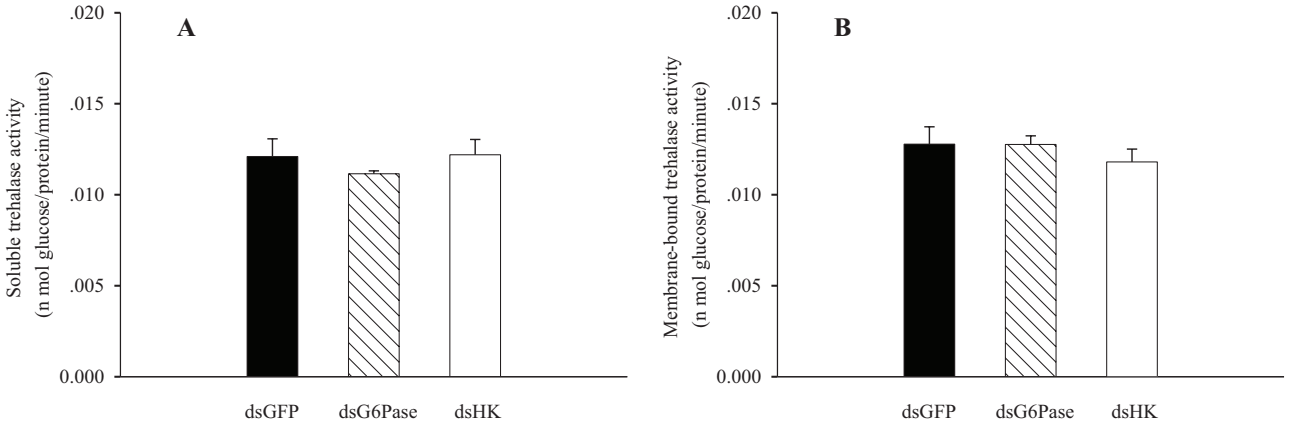

Fig. 4. Trehalase 1 (Soluble trehalase, A) and trehalase 2 (Membrane-bound trehalase, B) activities with *G6Pase* and *HK* RNAi for 48 h. Brown planthopper larvae were divided into three groups, and injected with dsGFP, dsNlG6Pase and dsNlHK, respectively. Insects were collected and used to detect trehalase activities after 48 h. Three replications were performed per group.

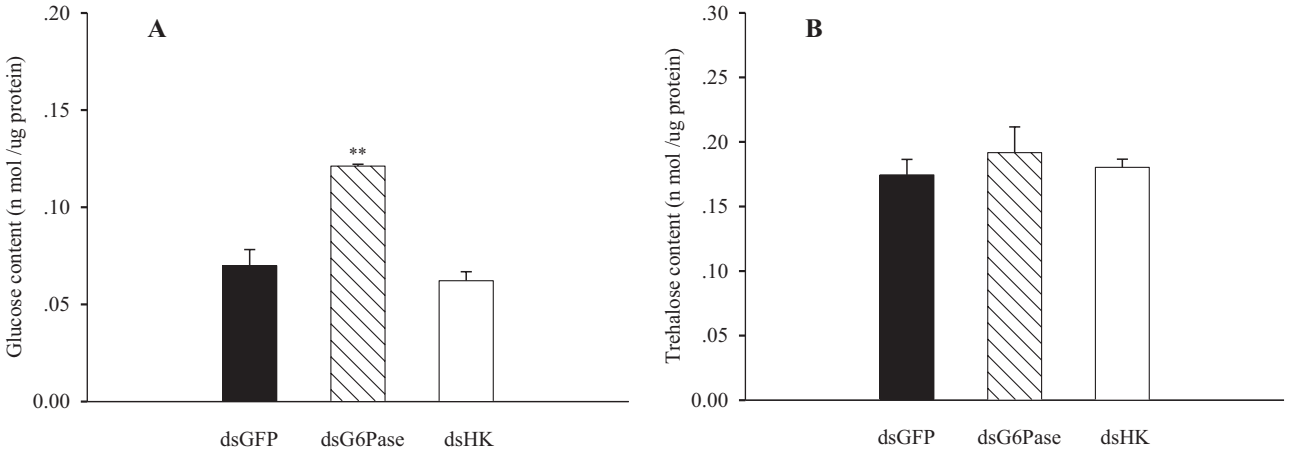

Fig. 5. Contents of glucose (A) and trehalose (B) in *N. lugens* after *G6Pase* and *HK* RNAi for 48 h. Brown planthopper larvae were divided into three groups, and injected with dsGFP, dsNlG6Pase and dsNlHK. Insects were collected and used to detect contents of glucose and trehalose after RNAi treatment for 48 h. Three replications were performed per group.

Discussion

G6Pase is a member of the nonspecific esterase family with polymorphism. The function of *G6Pase* is mainly to catalyze the hydrolysis of acetyl groups (Alterio et al. 2010). In the study of insects' *G6Pase*, *G6Pase* was expressed only in the brain in *Drosophila melanogaster* and *Anopheles gambiae*, suggesting that it has a neuronal function, and implicating its involvement in neuronal conduction (Miyamoto and Amrein 2017). Notably, there is a difference between the expression patterns of *N. lugens* to *Drosophila melanogaster* or *Anopheles gambiae*. In our tissue expression pattern of *G6Pase*, *G6Pase* was expressed in various tissues of the brown planthopper, and was highly expressed in the ovary, followed by brain and fat bodies (Fig. 1C). The expression of *G6Pase* in brown planthopper was maintained at a low level in the nymphal stage and increased in the adult stage (Fig. 1A). Interestingly, *G6Pase* has a sharp rise and fall at the end of the fifth and adulthood (Fig. 1A), suggesting that *G6Pase* may be involved in the emergence of brown planthopper.

Hexokinase is a rate-limiting enzyme that plays a key role in glucose homeostasis and energy metabolism through glucose (Glc) phosphorylation and Glc signaling (Ge et al. 2019). In the study of insect hexokinase, *Bombyx mori* muscle hexokinase is almost type I and is present in almost all tissues: hexokinase in testis and malphighian tube consisted of type I and type II, midgut contained type I, II, and IV hexokinases, while fat body tissues had types I, III and IV (Yanagawa 1978). Tadano (1987) detected *HK-1* in the head, chest, and abdomen of the adult *Aedes togoi*; in contrast, *HK* was concentrated in the chest in *Anopheles stephensi* (Gakhar and Nagpal 1996). Our tissue expression pattern shows that *HK* is ubiquitous in various tissues of brown planthopper, and has the highest expression level in the brain (Fig. 1D), which is consistent with the findings of mammals (Lawrence et al. 1984) and the findings of Ge et al. (Ge et al. 2019). Consistently, *HK* has shown to be evolutionarily conserved in the organic central nervous system (Lawrence et al. 1984, Ge et al. 2019). In relation to its expression throughout development, *HK-2* and *HK-3* existed at all developmental stages in the study of *An. stephensi* (Gakhar and Nagpal 1996). In addition, *HK-1* did not exist in all adult stages, its activity increased during larval growth, and was the highest at the last age observed (Gakhar and Nagpal 1996). Studies have also shown that the expression levels of *DM1* and *DM2* (two hexokinase isoenzyme sequences) are uniform in the 2nd and 3rd instar larvae, pupa and adult stages of *Drosophila* (Jayakumar et al. 2001). Our results demonstrate that the expression of *HK* is maintained at a relatively high level

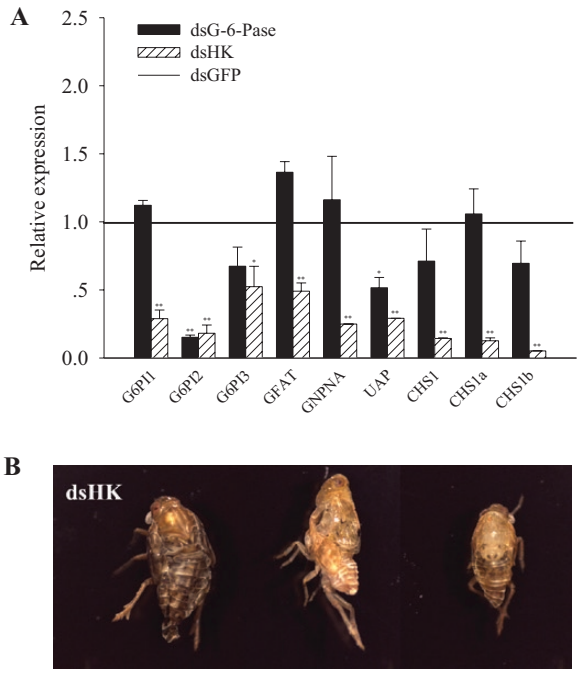

Fig. 6. Expression level of regulated genes of chitin metabolic pathway in brown planthopper after *G6Pase* and *HK* knockdown, and associated-abnormal phenotype (A) Expression of three glucose-6-phosphate isomerase (G6PI), glutamine: fructose-6-phosphate aminotransferase (GFAT), glucosamine-6-phosphate *N*-acetyltransferase (GNPNA), UDP-N-acetylglucosamine pyrophosphorylase (UAP) and three chitin synthase (CHS) at 48 h relative to the NL-18S mRNA level measured using qRT-PCR. (B) All abnormal insects in the larva-adult stage.

during the fourth and fifth ages, and decreases during adulthood in the brown planthopper (Fig. 1B), which is similar to the expression pattern of *An. stephensi* (Gakhar and Nagpal 1996).

RNAi is an effective and widely used tool to regulate gene expression in the field of gene function research (Lou et al. 2018). To verify the specificity and validity of dsG6Pase and dsHK activities in the brown planthopper, we examined the relative expression of *G6Pase* and *HK* after injection of dsG6Pase or dsHK. We found that the relative expression levels of *G6Pase* and *HK* gene were significantly decreased after RNAi ($P < 0.01$; Fig. 2), validating our experimental approach. Trehalose is the blood sugar of insects and plays an important role in their growth and development (Elbein 1974, Tang et al. 2010, Shukla et al. 2015). Studies have shown that after the *TRE* gene is interfered with the brown planthopper, there is an impaired synthesis of chitin, resulting in the phenomenon of wing malformation, and difficulty in molting, indicating that trehalose has a direct influence on chitin synthesis (Zhang et al. 2017). The aim of our study was to investigate the potential function of glucose utilization in the synthesis of chitin in brown planthopper. We first examined the expression of some genes related to trehalose and measured some biochemical indicators. Our study found that most of the genes were significantly downregulated after interference with *HK* expression, but there was almost no significant effect on the genes involved in trehalose metabolism after interference with *G6Pase* (Fig. 3). We next tested the trehalase activity and found an interesting phenomenon: inhibition of either with *G6Pase* or *HK* expression had no significant effect on trehalase activity (Fig. 4). Previous studies have shown that many genes that affect circulating glucose levels do not affect trehalose levels (Ugrankar et al. 2015), a notion that is consistent with our findings. Some studies have found that wild-type larvae raised on high-sugar diets become obese, and glucose and trehalose levels in the hemolymph of *Drosophila* are increased (Musselman et al. 2011). In contrast, some studies have reported that a high-sugar diet can induce hyperglycemia, while the level of trehalose in *Drosophila* unchanged by culture conditions or caloric restriction (Ugrankar et al. 2015, Pasco and Léopold 2012). These data support the idea that glucose is sensitive to environmental change, and in these environments, trehalose does not change significantly, indicating that glucose and trehalose levels are independently regulated.

To investigate these results further, we tested the trehalose and glucose content of the brown planthopper after *HK* and *G6Pase* knockdown and found that the content of trehalose did not change significantly (Fig. 5), consistent with reports by Ugrankar and Pasco (Pasco and Léopold 2012, Ugrankar et al. 2015). Unexpectedly, after interfering with the *HK* expression, there was no significant change in glucose content, while there was a significant increase following *G6Pase* knockdown. According to the expression of the binding gene (Fig. 2), the expression of *G6Pase* and *HK* genes were significantly decreased after knockdown of *HK*. This demonstrates that the synthesis and decomposition of glucose were inhibited, the glucose content was maintained at a normal level, and the *G6Pase* gene was inhibited. There was no significant change in the expression of *HK* gene, indicating that the decomposition of glucose was normal, but the glucose content increased, which may be due to feedback regulation by other pathways.

The chitin biosynthetic pathway is critical for insect development (Merzendorfer and Zimoch 2003, Zhu et al. 2016), and some studies have shown that insect *TRE* and *TPS* genes can regulate chitin synthesis by mediating trehalose metabolism (Chen et al. 2018; Chen et al. 2010a, b). Our results ruled out the interference of trehalose; therefore, we next sought to test the expression of genes involved in the chitin synthesis pathway in the brown planthopper after the *G6Pase* and *HK* knockdown. We found that after interference with the *G6Pase* gene, there was almost no significant change in the genes involved in the chitin metabolism pathway in the brown planthopper (Fig. 6A). This could be due to the involvement of other pathways (without *G6Pase*) in the process of glucose synthesis. After the *HK* gene was knocked down, the genes involved in the chitin metabolism pathway in brown planthopper were significantly reduced in expression, especially the *CHS1*, *CHS1a*, *CHS1b* (Fig. 6A). We also observed different degrees of wing deformity and molting difficulty (Fig. 6B), suggesting that silencing the *HK* gene can inhibit the expression of *CHS*, resulting in the inability of the brown planthopper to synthesize chitin normally. Previous studies have found that the nymphs of brown planthopper have high mortality and molting difficulty after RNAi of NlCHSA (Wang et al. 2012). Recently, in a study of *Acyrthosiphon pisum* nymph, it was also found that interference with *ApisCHS* can destroy embryonic development, leading to difficulty in nymphal molting (Ye et al. 2019). Moreover, Zhao et al. found that there were malformations and high mortality after *CHS1*, *CHS1a*, and *CHS1b* knockdown in whitebacked planthoppers (Wang et al. 2019). The above studies demonstrate that CHS plays an important role in chitin synthesis, consistent with our results. However, we did not detect changes in chitin content of brown planthopper (data not shown), which will be further explored in future research.

Conclusion

In our study of the developmental expression patterns of *G6Pase* and *HK* gene in brown planthopper, we found that *G6Pase* and

HK were expressed at all stages and were relatively stable in the nymphal stage. However, *G6Pase* was significantly decreased in the larva-adult stage, indicating that it may relate to the molting process. In terms of tissue-specific expression, we found that *G6Pase* is highly expressed in the ovary, followed by the brain; while *HK* is highly expressed in the brain.

We evaluated the possibility of the brown planthopper *HK* and *G6Pase* genes as new targets in pest control. The results showed that interference with *G6Pase* had little effect on chitin synthesis, while knockdown of *HK* showed strong interference. Our results indicate that the knockdown of *HK* inhibits chitin synthase *CHS1*, *CHS1a*, and *CHS1b* in the brown planthopper, which causes the chitin not to be synthesized normally.

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