A fragment of a 70 kDa *Heterodera glycines* heat shock protein (HgHSP70) interacts with soybean cyst nematode-resistant protein GmSHMT08[1]

LIU Zhi[1, 2], ZHANG Liu-ping[1], ZHAO Jie[1], JIAN Jin-zhuo[1], PENG Huan[1], HUANG Wen-kun[1], KONG Ling-an[1], PENG De-liang[1, *], LIU Shi-ming[1, *]

[1] *State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R.China*
[2] *MARA-CABI joint laboratory for bio-safety, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R.China*

Abstract
Soybean cyst nematode (SCN) *Heterodera glycines* is considered as the major constraint to soybean production. *GmSHMT08* at *Rhg4* locus on chromosome 08, encoding a serine hydroxylmethyltransferase, is a major gene underlying resistance against *H. glycines* in Peking-type soybeans. However, the molecular mechanism underpinning this resistance is less well characterized, and whether GmSHMT08 could interact with proteins in *H. glycines* remains unclear. In this study, yeast two-hybrid screening was conducted using GmSHMT08 as a bait protein, and a fragment of a 70kDa heat shock protein (HgHSP70) was screened from *H. glycines* that exhibited interaction with GmSHMT08. This interaction was verified by both GST pull-down and bimolecular fluorescence complementation assays. Our finding reveals *HgHSP70* could be applied as an essential candidate gene for further exploring the mechanism on *GmSHMT08*-mediated resistance against SCN *H. glycines*.

Keywords: soybean cyst nematode, *Heterodera glycines*, GmSHMT08, HgHSP70, interaction, resistance

1. Introduction

Soybean [*Glycine max* (L.) Merr.], an important source of proteins and oils essential for human and animal health, is one of the most economically important crops in the world. *Heterodera glycines*, commonly referred to as soybean cyst nematode (SCN), is a soil-borne obligate endoparasite of soybean roots. Due to its widespread distribution and ability to reduce seed yield, *H. glycines* is considered as the most damaging and economically important pest in soybean and poses serious threats to global soybean production (Koenning and Wrather 2010; Rincker *et al*. 2017; Peng, 2021, Peng *et al*. 2021). In the United States, the losses caused by *H. glycines* have been estimated to exceed 1 billion USD annually (Koenning and Wrather 2010). In Northeast China, one of major soybean production regions, *H. glycines* generally decreases soybean production by 5%–100% depending on the severity of infection (Duan 2011).

Once second stage juvenile (J2) of *H. glycines* emerges from eggs in the soil, it searches soybean roots and penetrates plant root tissue. Upon successful penetration, SCN J2s migrate in search for a suitable feeding site near the vascular cylinder, where it converts adjacent root cells into specialized, fused cells

[1] LIU Zhi, E-mail: liuzhi7667@hotmail.com; Correspondence LIU Shi-ming, E-mail: smliuhn@yahoo.com; PENG De-liang, E-mail: pengdeliang@caas.cn

that form a feeding site, termed as syncytium (Endo 1964; Ohtsu *et al*. 2017). Cyst nematodes secrete a group of proteins, commonly termed as effectors, into plant cells to modify host processes (Hussey 1989). Substantial researches are focused on identifying these effectors, and elucidating their complex functions (Ge, et.al., 2018). Over 80 *H. glycines* effectors have been identified and confirmed so far (Gao *et al*. 2003; Noon *et al*. 2015, Wang, et.al., 2019). Recently, a total of 431 effectors were predicted from the genome of 'TN10' (Masonbrink *et al*. 2019). Characterization of some known effectors has provided critical insights into the parasitic strategies of *H. glycines*, including defense suppression, plant hormone signaling alteration, cytoskeletal modification and metabolic manipulation (reviewed by Mitchum *et al*. 2013; Hewezi 2015; Juvale and Baum 2018). However, research has yet to provide a basic understanding of the molecular mechanism of virulence, i.e., the ability of some nematode populations to infect soybean plants with resistance genes, while other nematode populations are controlled by these resistance genes.

A major soybean quantitative trait locus (QTL) gene, *GmSHMT08* in *Rhg4* locus, which encodes an enzyme serine hydroxylmethyltransferase (GmSHMT08, EC2.1.2.1), confers resistance to *H. glycines* (Liu *et al*. 2012). The enzyme GmSHMT08 is ubiquitous in nature and structurally conserved across kingdoms. It catalyzes the reversible conversions of serine and glycine, providing one-carbon unit for a series of important biosynthetic processes such as the syntheses of methionine, thymidylates, and purines (Schirch 1982; Matthews and Drummond 1990; Bauwe and Kolukisaoglu 2003; Schirch and Szebenyi 2005). Alleles of *GmSHMT08* conferring resistance or susceptibility differ by two genetic polymorphisms that alter a key regulatory property of the enzyme. Compared to sensitive alleles, this resistant allele contains two critical point mutations resulting in two amino acid changes (P130R and N358Y), which are hypothesized to impair a key regulatory property of the encoded GmSHMT08 enzyme (Liu *et al*. 2012).

Though the GmSHMT08-mediated resistance against *H. glycines* is well observed, the molecular mechanism underpinning this genetic resistance remains unclear. So far, we hardly have any clue whether GmSHMT08 could interact with any proteins from *H. glycines* and if such interaction exists, whether it could subsequently induce GmSHMT08-mediated resistance. It is necessary to find out the proteins from *H. glycines* that interact with GmSHMT08.

In this study, an amino fragment from 310 to 673 of a HSP70 protein (hereafter referred as HgHSP70p) was identified to interact with GmSHMT08 using a yeast two-hybrid system. The interaction between HgHSP70p and GmSHMT08 was consolidated by GST pull-down and bimolecular fluorescence complementation (BiFC) assay. No signal peptide or transmembrane helices were predicted in HgHSP70 using bioinformatic tools; *in situ* hybridization showed that HgHSP70 was expressed in the intestinal cells.

2. Materials and methods

2.1. Nematodes and plant material

Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) race 4 was used in this study. Cysts were collected from the soybean (Williams 82) stock cultures maintained in the greenhouse. Soybean roots were washed through nested 850-μm-aperture and 250-μm-aperture sieves and cysts were collected from the 250-μm-pore sieve. Cysts were hatched in 3 mM $ZnCl_2$ solution at room temperature and pre-parasitic second-stage juveniles (Pre-J2) were collected on a 25-μm-aperture sieve after 3 to 7 days.

To isolate parasitic juveniles and adult life stages of *H. glycines*, 2-week-old soybean plants were inoculated with hatched J2s (parasitic J2s). Infected roots were harvested 4, 8, 12, and 18 days post inoculation (dpi) for the collection of parasitic J2, J3, J4, and female adults, respectively (de Boer *et al*. 1999). The soybean cultivar PI437654 was used for Gm*SHMT08* cloning, and Williams 82 was used for *H. glycines* propagation.

2.2. Gene cloning

Total RNA of soybean was isolated from roots of 10-day soybean cultivar PI437654 using MiniBEST universal RNA extraction kit (TaKaRa, Japan) according to the manufacturer's instruction. Total RNA of *H. glycines* was isolated from Pre-J2s using a RNeasy mini kit (Qiagen, Germany) according to the manufacturer's instruction, mRNA of *H. glycines* was isolated using a Dynabeads™ mRNA direct™ kit (Invitrogen, Norway) according to the manufacturer's instruction. The isolated RNA and mRNA were treated with DNase I to eliminate any genomic DNA contamination for future use. cDNA was obtained using reverse transcriptase SuperScript III (Invitrogen, USA) according to the manufacturer's instruction, and all cDNA aliquots were stored at -20 ℃ for future use. The coding sequence of *GmSHMT08* (*Glyma.08g108900*) was amplified from soybean cultivar PI437654 cDNA. HgHSP70 and HgHSP70p were amplified from *H. glycines* cDNA. All primers used in this study were synthesized by Invitrogen Biotechnology Co. Ltd and are listed in Appendix A.

2.3. SCN cDNA library construction and yeast two-hybrid screens

Total RNA of *H. glycines* isolated from Pre-J2 was used for cDNA library construction. cDNA library was constructed using the CloneMiner™ II cDNA library construction kit (Invitrogen, USA) according to the manufacturer's instruction and then integrated into pGADT7-Rec vector using the Make Your Own "Mate & Plate" Library System kit (Takara, Japan). The coding sequence of *GmSHMT08* was cloned into the multiple cloning sites (between *Eco*R I and *Sal* I) of pGBKT7 vector using In-fusion HD Cloning kit (Takara, China), and the recombinant pGBKT7-GmSHMT08 was used as a bait in the yeast two-hybrid screening. Yeast strain Y2H Gold was used in the yeast two-hybrid screening and 3 screenings were performed using the Clontech two-hybrid system on synthetic dropout nutrient medium (SD/-Ade-His-Leu-Trp) containing X-α-gal (40 mg/L) and AbA (200ng/mL) according to the manufacturer's yeast protocols handbook (Invitrogen, USA). Among all the positive clones, HgHSP70p was selected as a candidate prey. The full-length open reading frame (ORF) of the full-length HgHSP70 was obtained by RACE (rapid amplification of cDNA ends) approach using 5' RACE system for rapid amplification of cDNA ends kit (Invitrogen, USA) and 3' RACE system for rapid amplification of cDNA ends kit (Invitrogen, USA) according to the manufacturer's instruction.

2.4. Property analysis of HgHSP70

Hydrophobicity or Hydrophilicity analysis of HgHSP70 was analyzed by ProtScale tool (https://web.expasy.org/protscale/) using Kyte and Doolittle's amino acid scale (Kyte and Doolittle 1982). Prediction of signal peptide was performed using SignalP 5.0 tool (Almagro *et al*. 2019). Prediction of transmembrane helices was performed using an online tool TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/).

2.5. GST pull-down

The *GmSHMT08* was amplified and inserted into the vector pGEX-4T-1 to produce a GST-SHMT fusion

protein (77.46 kDa). The full-length ORF of HgHSP70 and the coding sequence of HgHSP70p was amplified and inserted into the vector pCzn1 to produce fusion proteins His-HgHSP70 (75.67 kDa) and His-HgHSP70p (39.82 kDa), respectively. GST pull-down assay was performed using a Pierce™ GST protein interaction pull-down kit according to the manufacturer's instruction. The interaction between GmSHMT08 and HgHSP70/HgHSP70p was analyzed by SDS–PAGE and Western blotting. A GST-Tag (12G8) Mouse mAb (primary antibody, Abmart, Shanghai, China), His-Tag (2A8) Mouse mAb (primary antibody, Abmart, Shanghai, China) and Goat Anti-Mouse lgG (Secondary antibody, Abmart, Shanghai, China) were used in Western blotting.

2.6. Bimolecular fluorescence complementation (BiFC) assays

The *GmSHMT08* was cloned into pCAMBIA1300-35S-YFPc- and pCAMBIA1300-35S-YFPn-, respectively. The full-length ORF of HgHSP70 was cloned into pCAMBIA1300-35S-YFPn-, the coding sequence of HgHSP70p was cloned into pCAMBIA1300-35S-YFPc-. All fusion constructs were verified by sequencing and then transformed to *A. tumefaciens*. Four leaves from four-week-old *N. benthamiana* plants were subjected to agroinfiltration with recombinant strains of *A. tumefaciens* as described by Zhao *et al*. (2019) used for fluorescence visualization. Images were captured by confocal microscopy (Zeiss LSM880) at an excitation wavelength of 488 nm for YFP.

2.7. Quantitative real-time polymerase chain reaction (qRT-PCR)

To quantify the transcript expression levels of target gene *HgHSP70*, mRNA of *H. glycines* was isolated from eggs, Pre-J2, parasitic J2, J3, J4 and adult females. The isolated mRNA was reverse transcribed, and all cDNA aliquots were stored at -20℃. The qRT-PCR was performed using the SYBR Premix Ex Taq^{TM} kit (Takara, China) in triplicate reactions. The relative expression level of *HgHSP70* was quantified using qRT-PCR at different development periods/stages. The *β-actin* gene was used as an internal control. The fold-change values were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001) and relative to the expression of egg stage (Appendix A). The final volume of reaction mixtures was 20 μL including 10 μL of SYBR Premix Ex Taq (2x), 0.4 μL of ROX, 0.5 μL of each primer, 2 μL of cDNA and 6.6 μL of double distilled H_2O.The PCR conditions were as follows: 95 ℃ for 30s, followed by 40 cycles of 95 ℃ for 5 s and 60 ℃ for 34 s, followed by a melting curve step (95 ℃ for 15 s, 60 ℃ for 60 s, 95 ℃ for 15 s and 60 ℃ for 15 s) using a 7500 fast real-time PCR system (Applied Biosystems).

2.8. *In situ* hybridization

In situ hybridization analysis was used to evaluate the spatial distribution of HgHSP70. *In situ* hybridization was performed essentially as described by de Boer *et al*. (1998). Antisense RNA probes labeled with digoxigenin were generated by in vitro transcription using target genes *HgHSP70* that had been cloned into the T-Vector pMD19 (TaKaRa, China) and a DIG RNA labeling kit (Roche, Germany). Freshly Pre-J2 were collected and then fixed using 3% paraformaldehyde (Sigma-Aldrich, MO, USA) for 16 h at 4 ℃ followed by an incubation for 6 h at room temperature. The nematodes were then sliced by random hand cutting using a razor blade and were permeabilized using proteinase-K, acetone and methanol. Subsequently, the nematodes were mixed with a hybridization solution (Roche, 11745832910, Germany) containing a sense or antisense probe at 55 ℃ overnight. After washing with 4 x SSC (0.6 M NaCl and 0.06 M sodium citrate, pH=7.0) and 0.1 x SSC, the nematodes were incubated with 1 x blocking solution for 1 h and then with a 1:1000 dilution of anti-digoxigenin-AP (Roche, 11745832910, Germany) in blocking solution for 2 h. Finally, the nematodes were visualized using an Olympus BX53 microscope

after nitro blue tetrazolium and 5-bromo-4-chloro-3'-indolyphosphate staining overnight.

3. Results

3.1. A 70 kDa heat shock protein (HSP70) screened from a *H. glycines* cDNA library

To identify proteins from *H. glycines* that could interact with GmSHMT08, a *H. glycines* cDNA library was screened in triplicate using the GmSHMT08 protein as a bait. Totally, 12 positive clones were screened on synthetic dropout nutrient medium (SD/-Ade-His-Leu-Trp) containing X-α-gal and AbA. HgHSP70p, a fragment from 310 to 673 of HgSP70, from the positive clones was found interacting with GmSHMT08 in all three rounds of screens and was selected for further investigation. The interaction between GmSHMT08 and HgHSP70p was verified by co-transformation into yeast using Yeast two-hybrid system (Fig. 1). Full length open reading frame (ORF) of HgHSP70 was obtained by 5'RACE and 3'RACE.

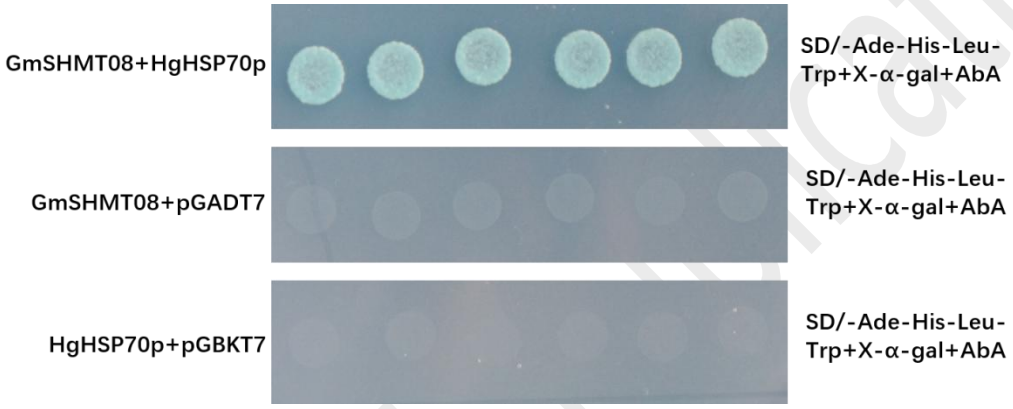

Figure 1 Yeast two-hybrid validation of the interaction between GmSHMT08 and HgHSP70p. Only co-transformed yeast cells containing pGBKT7-SHMT plus pGADT7-HgHSP70p could grow and turn blue on selective medium SD/-Ade-His-Leu-Trp+X-α-gal+AbA. Co-transformed yeast cells containing pGBKT7-SHMT plus pGADT7 vector and pGADT7-HgHSP70p plus pGBKT7 could not grow on selective medium SD/-Ade-His-Leu-Trp+X-α-gal+AbA.

3.2. Property analysis of HgHSP70

HgHSP70 encodes a typical 70 kDa HSP70. Hydrophilicity of HgHSP70 was analyzed by ProtScale tool. As shown in Fig. 2-A, the value of most amino acids were negative, indicating the majority amino acids of HgHSP70 were hydrophilic. Prediction of signal peptide was performed using SignalP tool. As shown in Fig. 2B, no peak was detected in C score, S score or Y score. As a result, no signal peptide was detected in HgHSP70. Prediction of transmembrane helices was performed using TMHMM Server v. 2.0. As shown in Fig. 2C, all amino acids were predicted locating outside the cell membrane and no transmembrane helices was predicted, suggesting HgHSP70 cannot be secreted outside the cell.

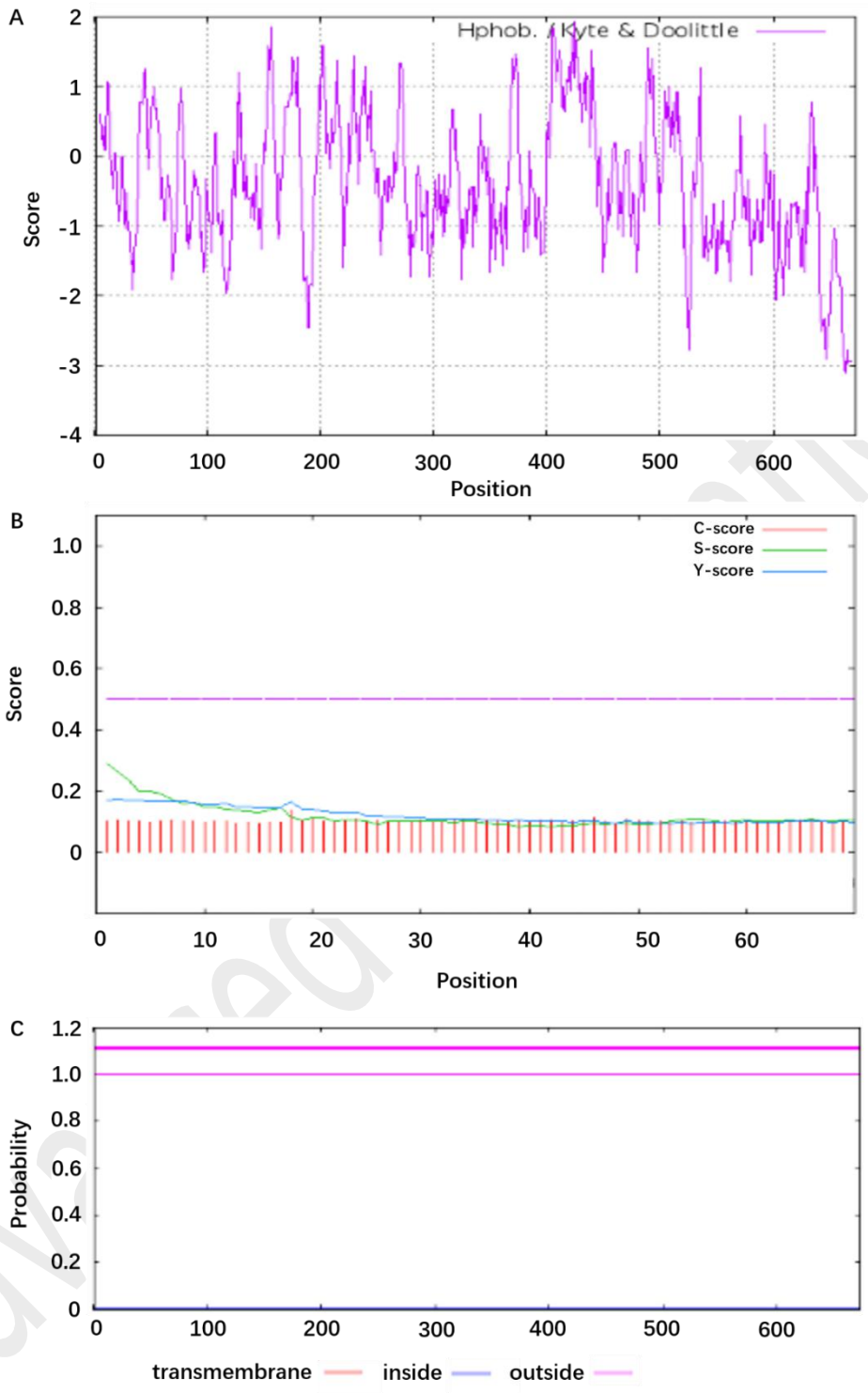

Figure 2 Property analysis of HgHSP70. (A) Hydrophilicity analysis of HgHSP70: Online tool ProtScale was used for hydrophilicity analysis. Data shown revealed that value of most amino acids were negative, indicating the majority amino acids of HgHSP70 were hydrophilic. (B): Signal peptide prediction of HgHSP70: SignalP was used for Signal peptide prediction. No peak was detected in C score, S score or Y score, suggesting no signal peptide existed in HgHSP70. (C): Transmembrane helices prediction of HgHSP70. all amino acids were predicted outside the cell membrane and no transmembrane helices was found.

3.3. HgHSP70p interacted with GmSHMT08

To confirm the solid interaction between HgHSP70/HgHSP70p and GmSHMT08, GST pull-down and

bimolecular fluorescence complementation (BiFC) assays were performed. For GST pull-down, GST-tagged GmSHMT08 was used as a bait protein to capture His-tagged HgHSP70p and His-tagged HgHSP70, respectively. The capture complexes were detected by Western blotting. Only one band was unveiled in His Western blotting at 40 kDa, which is the correct size of His-HgHSP70p (Fig.3B). This verified the interaction between GmSHMT08 and HgHSP70p *in vitro* but no interaction between GmSHMT08 and HgHSP70.

For BiFC, strong and frequent YFP fluorescence was observed in *N. tabacum* leaf cells when co-transformed with YFPn-GmSHMT08 and YFPc-HgHSP70p; indicating a solid interaction between GmSHMT08 and HgHSP70p (Fig. 4). However, the interaction between GmSHMT08 and HgHSP70 was negative as weak YFP fluorescence was observed in *N. benthamiana* leaf cells when co-transformed with YFPc-GmSHMT08 and YFPn-HgHSP70 (Fig. 4). The result confirmed the interaction between GmSHMT08 and HgHSP70p *in vivo* and was consistent with that obtained in GST pull-down.

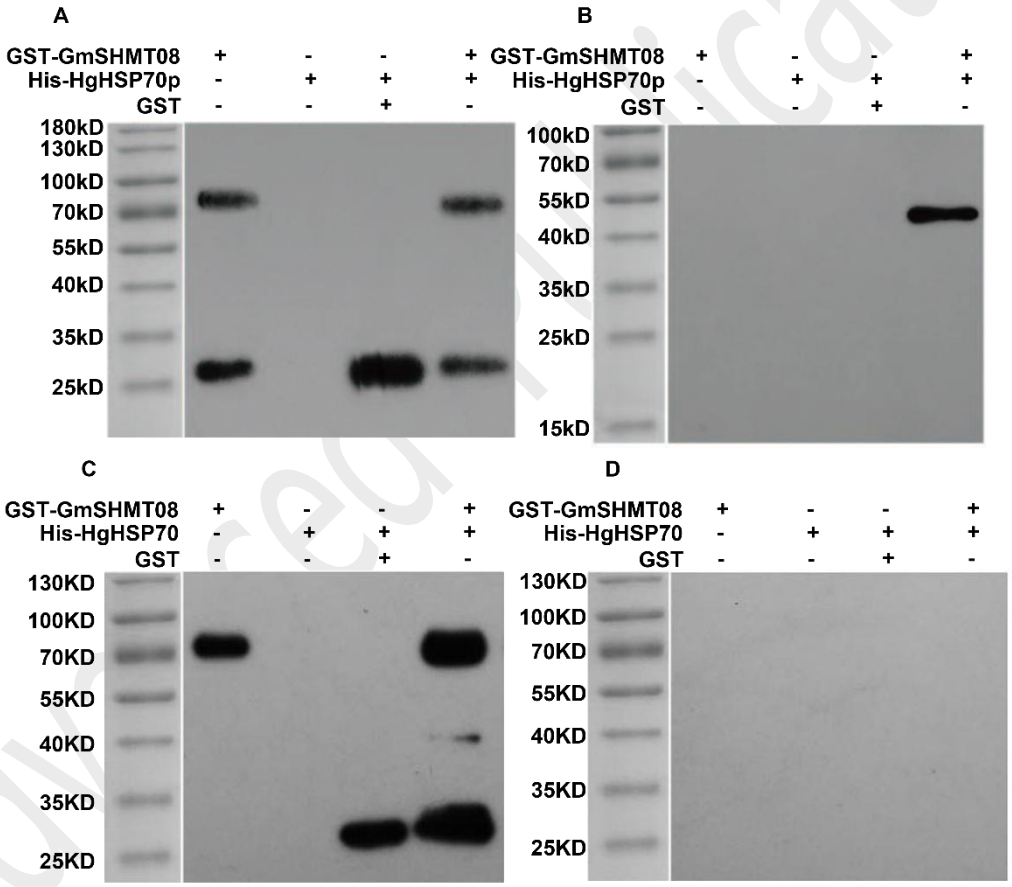

Figure 3 GST pull-down validation of interaction between HgHSP70p and GmSHMT08. (A-B) GST-GmSHMT08 pulled down the HgHSP70p; (A) proteins were detected using an anti-GST antibody; (B) proteins were detected using an anti-His antibody. (C-D) GST-GmSHMT08 failed to pull down the HgHSP70. (C) proteins were detected using an anti-GST antibody; (D) proteins were not detected using an anti-His antibody.

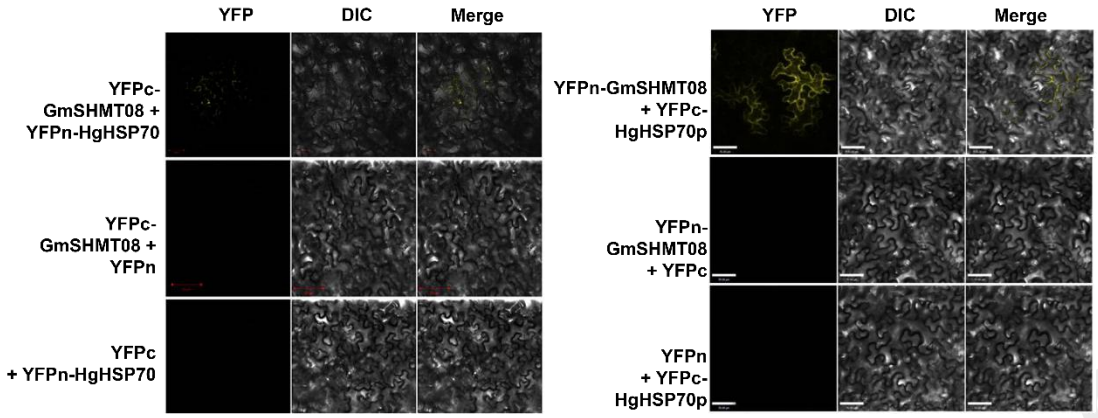

Figure 4 BiFC validation of interaction between HgHSP70/HgHSP70p and GmSHMT08. Left: weak YFP fluorescence was observed in *N. tabacum* leaf cells when co-transformed with YFPc-GmSHMT08 and YFPn-HgHSP70. Right: strong and frequent YFP fluorescence was observed in *N. tabacum* leaf cells when co-transformed with YFPn-GmSHMT08 and YFPc-HgHSP70p.

3.4. Expression pattern of HgHSP70 at different developmental stages of *H. glycines*

Transcriptional expression of *HgHSP70* was analyzed by qRT-PCR at different developmental periods/stages of *H. glycines*. The results showed that the expression of *HgHSP70* was decreased in Pre-J2 and then increased and reached a peak in parasitic J2. Subsequently, the expression of *HgHSP70* was gradually reduced in parasitic J3 and reached a nadir in parasitic J4. Eventually, the expression of *HgHSP70* increased again and reached almost the same expression level of parasitic J2 (Fig. 5).

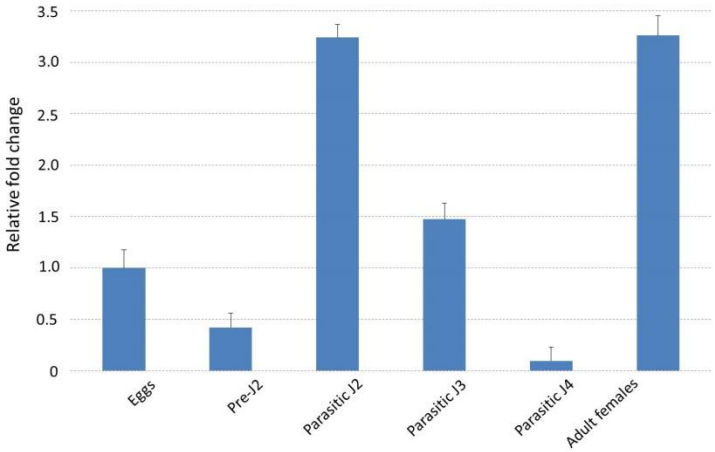

Figure 5 Developmental expression patterns of HgHSP70 in *H. glycines*. The relative expression level of HgHSP70 was quantified using quantitative reverse transcription PCR (RT-PCR) at different development periods/stages of *H. glycines*. Data shown were the means of three repeats plus standard deviation (SD), and three independent experiments were performed with similar results (Appendix B).

3.5. HgHSP70 transcripts were accumulated in intestinal cells

In situ hybridization was performed in triplicate using Pre-J2 *H. glycines* and a strong hybridization signal was observed in the intestinal cells (Fig. 6), indicating HgHSP70 transcripts were accumulated specifically in intestinal cells.

Figure 6 Localization of HgHSP70 in *H. glycines* Pre-J2. *In situ* hybridization was performed using Pre-J2 and *in vitro* transcribed RNA labeled with digoxigenin. Antisense probes of *HgHSP70* showed a strong hybridization signal in the intestinal cells. Bars = 100μm.

4. Discussion

Heat shock protein (HSP) family is a ubiquitous group of highly conserved proteins that have been characterized in a wide range of prokaryotic and eukaryotic organisms, from *Escherichia coli* through *Drosophila*, and from *Caenorhabditis elegans* to humans (Francesca *et al*. 2009). According to their molecular weights, HSPs are classified into several families: HSP100, HSP90, HSP70, HSP60, HSP40 and low molecular mass HSPs (Lindquist and Craig 1988). HSPs play multiple roles in the development, growth and survival of individual organism and are connected with the ability to respond to various stresses by synthesis, accumulation or reduction of protein components (Nakamoto and Vigh 2007). HSP response has been reported in nearly every cell and tissue type of multicellular organisms (reviewed by Usman *et al*. 2017).

HSP70 is the most structurally and functionally conserved protein family in HSPs (Usman *et al*. 2015). To whatever degree, all organisms show the profile of HSP70 (Lindquist, 1986). Prokaryotes only have a single HSP70 (the archetypical DnaK) isoform, whereas eukaryotes have an expanded number of genes that code for distinct HSP70 isoforms, which function in different physiological conditions and subcellular locations (Fernández-Fernández *et al*. 2017). HSP70s function as molecular chaperone that protect the cells to withstand stresses (e.g. heat, drought and oxidative stresses) challenging mostly protein machinery (Tompa and Kovacs 2010). The best characterized roles of HSP70s include engagement in normal folding and refolding of proteins, assistance of mis-folded or damaged proteins to attain or regain their native states, degradation of unstable and misfolded proteins, and transport of proteins between cellular compartments (Werner and Hinton 1999; Tomanek and Sanford 2003; Hartl and Hayer-Hartl 2002; Daugaard *et al*. 2007). As members of molecular chaperones that regulate the stress response system, HSP70s' primary function, protein folding and refolding, has been retained from bacteria to humans (Lindquist and Craig 1988; Kabani and Martineau 2008). Regardless of their functions in preventing aggregation of proteins and helping again in folding of proteins that are not native in environments that are not favorable, several HSP70s also play a crucial role in housekeeping activities under favorable conditions (Tompa and Kovacs 2010). Yet, in most eukaryotes certain HSP70s acquired specialized functions that allowed cells and organisms to adapt, survive, and thrive in various environments.

Recent researches reveal that HSP70s mediate responses to abiotic and biotic stresses in plants. An extensive number of evidences have demonstrated the involvement of HSP70s in thermotolerance and drought stress (Alvim *et al*. 2001; Sarkar *et al*. 2013; Lee *et al*. 2009; Koizumi *et al*. 2014; Guo *et al*. 2014; Usman *et al*. 2015; Ristic *et al*. 1991; Wang *et al*. 2014; Fei *et al*. 2015). Though less well characterized,

several reports have shown that HSP70s are involved in plant's resistance against diseases. Flora and fauna with respect to infection by virus are known to be correlated with HSP70s. At the time of infection, they regulate the response of host stress as well as infection cycle (Nagy *et al*. 2011; Mayer 2005). Plant HSP70 is involved in the intracellular movement of tomato yellow leaf curl virus (TYLCV). TYLCV DNA levels decreased due to inactivation of HSP70. This indicates the function of HSP70 in TYLCV multiplication in plants (Gorovits *et al*. 2013). In *Arabidopsis thaliana*, AtHsp70-15 plays an essential role during normal growth and in the heat response, and AtHsp70-15-deficient plants are more tolerant to infection by turnip mosaic virus (Jungkunz *et al*. 2011).

While most studies of HSPs have focused on mammals, plants and model organisms, molecular and functional studies in plant parasitic nematodes (PPNs) remain deficient. For studies concerning HSPs in PPNs, only a small number of studies have been focused on HSP90s. Gillan *et al* (2009) conducted a functional genomic study of HSP90 in parasitic and free-living nematodes and discovered that factors other than the level of sequence identity were important for determining whether parasite genes can functionally complement in *C. elegans*. Mt-HSP90 in *Meloidogyne artiellia* was revealed to provide a link between environmental conditions and nematode life cycle (Luca *et al*. 2009). A HSP90 in *Meloidogyne incognita* was found to be involved in plant resistance. Plant-mediated RNAi of *HSP90* led to significant level of resistance against *M. incognita* in transgenic *Nicotiana tobacum* plants (Tessutti *et al*. 2014). As for *H. glycines*, only one HSP90 gene has been cloned and characterized so far (Skantar and Carta 2004).

This study, for the first time, reported a fragment of a 70 kDa HSP (HgHSP70p) from *H. glycines* could interact with the soybean resistant protein GmSHMT08. The full-length ORF of HgHSP70 was obtained by 5'-RACE and 3'-RACE. The expression pattern and transcript accumulation pattern of HgHSP70 in *H. glycines* could enrich our understanding of the HSP in plant parasitic nematode. The interaction between HgHSP70p and GmSHMT08 was verified by both GST pull-down and BiFC assays. However, the interaction between full-length HgHSP70 and GmSHMT08 is negative in both GST pull-down and BiFC assays. In the GST pull-down assay, the GmSHMT08 failed to capture HgHSP70, indicating no interaction between these two proteins. While in BiFC assay, even though weak YFP fluorescence was observed in *N. benthamiana* leaf cells when co-transformed with YFPc-GmSHMT08 and YFPn-HgHSP70, the fluorescence was neither strong enough nor frequent enough to confirm a solid interaction between these two proteins. More evidence is required to further consolidate the interaction between HgHSP70 and GmSHMT08. Besides, it remains unclear whether this interaction actually happens and how it occurs in a natural eco-system in *H. glycines* infested soybean field. There are two possible mechanisms: (1) HgHSP70/HgHSP70p is secreted outside the *H. glycines* and GmSHMT08 interacts with it in soybean root cells; (2) *H. glycines* absorbs GmSHMT08 when feeding and HgHSP70/HgHSP70p interacts with GmSHMT08 inside *H. glycines*. Our finding enriches the understanding of HSP70 from plant parasitic nematode in terms of interacting with plant resistant protein and provides a clue for further investigation of the interaction mechanism between soybean and *H. glycines* and GmSHMT08-mediated resistance against *H. glycines*.

5. Conclusion

GmSHMT08 in Peking-type soybeans confers resistance against *H. glycines*. The mechnism behind

GmSHMT08-mediated resistance is still unclear and identifying proteins from *H. glycines* that could interact with GmSHMT08 would provide a clue to decrypt this resistance mechnism. In this study, a fragment of a 70 kDa HSP (HgHSP70p) from *H. glycines* was idenfitied to interact with GmSHMT08 using yeast two-hybrid screens. This interaction was verified by GST pull-down and BiFC. This evidence is a step forward in understanding the molecular mechanisms of GmSHMT08-mediated resistance against *H. glycines*.

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