INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–1889/2020/23–5–857–862
DOI: 10.17957/IJAB/15.1362
http://www.fspublishers.org

Full Length Article

Proteomic Analysis of *Heterodera glycines*: Comparison for White Females and Brown Cysts

B.X. Li, M. Luo and H.Y. Wu*
Guangxi Key Laboratory of Agric-Environment and Agric-products Safety, Agricultural College of Guangxi University, Nanning 530004, China
*For correspondence: whyzxb@gmail.com
Received 09 December 2019; Accepted 19 December 2019; Published 03 March 2020

Abstract

Soybean cyst nematode (SCN), *Heterodera glycines*, is recognized as the most destructive pathogen of *Glycine max* (soybean) causing severe economic damages to soybean crops worldwide. Cysts are an important stage in the life cycle of the nematode, can endure harsh environmental conditions and survive for a long time. This poses a challenging task for pest control. In order to understand the underlying mechanism of the change from white females to brown cysts, the proteome of white females and brown cysts from SCN were analyzed in our study, using a two-dimensional gel electrophoresis combined with mass spectrometry and Gene Ontology. Two hundred and fifty-four proteins spots were identified of which 176 were present in white female cysts whereas 78 proteins were observed in brown cysts. Seventy-eight proteins were analyzed by mass spectrometry while a total of 12 proteins were shown to be common for white and brown cysts, 22 and 43 proteins were exclusively detected in either white or brown cysts, respectively. For the 2-DE pattern of *H. glycines*, the proteins in white females were mostly associated with post-translational modifications, protein turnover, chaperones and RNA transport. In contrast, brown cyst proteins performed a wider range of functions including growth, cell proliferation and signal transduction. Two proteins were involved in the pathway of the melanin production. The results could provide a better understanding the changes from white female to brown cyst.

Keywords: Proteomics; *Heterodera glycines;* Two-dimensional polyacrylamide gel electrophoresis; Mass spectrometry

Introduction

The soybean cyst nematode (SCN), *Heterodera glycines*, was first described in Northeast China in 1899 (Wrather *et al.* 2001; Liu *et al.* 2011). The nematode is considered to be one of the most widespread and devastating soybean pathogen around the world. Similar to other plant nematodes, the life cycle of *H. glycines* consists of an egg stage, four juvenile stages, and the adult stage. Among these, the second-stage juvenile (J2) nematode moves through the soil and infects plants by penetrating the host plant roots, develops through the third and fourth juvenile stages. Vermiform, adult males fertilize lemon-shaped adult females and the adult females produce eggs. Once inside the root, the J2 become sedentary and establish syncytic feeding site, after three moults, reaches the adult stage, taking a lemon shape for the female or vermiform male, respectively. After the death of the female, the eggs are retained inside the hardened body (cyst), until suitable conditions arrive. The eggs inside cyst can remain viable for several years in the soil (Niblack *et al.* 2006; Yu 2011). These processes cause severe root damage in soybean plants and results in severe growth and development disruptions that lead to an extensive reduction of soybean production. *H. glycines* was responsible for greater soybean yield loss than any other pathogen in many major soybean-producing countries including the United States, China, Argentina, Brazil, Indonesia, India, Canada, Paraguay, Italy, and Bolivia (Wrather *et al.* 2001). Numerous chemical methods have been used to control SCN and nematicide is an important management tool, for example, fosthiazate exhibits strong toxicity against SCN, including increasing of J2 mortality, and reducing egg hatching and reproduction rates, but effective control of SCN in the field is still a challenging problem (Wu *et al.* 2019).

Proteomics is one of the foremost branches of science in the post-genomics era and is mainly focused on studying the expression, translational modification and interaction of proteins in cells, tissues and organs. In previous studies of plant-parasitic nematodes, more attention has been paid to the secretion of proteins by nematodes and the interaction between nematodes and hosts. For example, proteins secreted by *Meloidogyne incognita* juveniles were separated and seven abundant proteins were identified. A calcium binding protein called calreticulin involved in multiple functions including intracellular calcium homeostasis and protein maturation was identified in this process (Jaubert *et*

al. 2002). S-phase kinase-associated protein 1(SKP1) is a key component of the Skp1p-Cdc53p-F-box protein complex that provides ubiquitin-protein ligase activity required for cell cycle progression, and its homolog was identified in the dorsal gland of *H. glycines* (Gao *et al.* 2003). Previous research indicates *H. glycines* uses six subventral gland cell–synthesized β -1, 4-endoglucanases to hydrolyze the β-1, 4 glycosidic bonds of cellulose in the cell walls during penetration and intracellular migration within soybean roots (Boer *et al.* 1999; Wang *et al.* 1999). Through proteomics, the mechanism of infection with a pathogen can be studied in more detail and will facilitate control strategies to prevent infection of crop plants.

In addition, previous studies have also focused on the comparison of proteins among different *H. glycines* populations (Pozdol and Noel 1984; Donald *et al.* 2008). Two pathotypes of potato cyst nematodes (*G. rostochiensis*) were identified based on the proteomics combined with electrophoresis using polyacrylamide gel and larval measurements (Trudgill and Parrott 1972). Researchers discovered that 19 protein spots from cereal cyst nematode (*H. avenae*) were unique, with 11 in the non-diapause nematode and 8 in the diapause nematode. These proteins were mainly associated with signal transduction, energy production and cell proliferation during the development process (Wang *et al.* 2017). In addition, 426 proteins in *H. glycines* J2 were involved in metabolism, growth and development as identified by a previous study (Chen *et al.* 2011). Recently, a large number of parasitism genes involved were revealed in the genome (Masonbrink *et al.* 2019). However, the proteome in *H. glycines* nematode from white females to brown cysts remains unidentified. As mentioned earlier, the white female become brown cyst after death to protect the living eggs inside, until suitable conditions arrive, hatch and infect the host again. Why do white females turn brown and harden after death? And are there changes in the expression of related functional proteins? Therefore, the objective of the study is to (1) analyze the proteome of *H. glycines* white females and brown cysts and (2) identify the proteins and discover their functions in these organisms. The results will help better understand the process of SCN parasitism, biological information of this nematode and will make it possible to develop new strategies to control this devastating agricultural pest.

Materials and Methods

Nematode materials

H. glycines race 4 were grown on soybean (Cv. Ludou 4) in a greenhouse and the white females and brown cysts were separated using a sieving-decanting method on day 35 and day 45 post inoculation of cysts wdith eggs as described by Liu (2000). The fresh white females and brown cysts were cleaned using an ultrasonic cleaning machine for 1 min, and then were transferred to another 1.5 mL PCR tube, stored at -80ºC.

Total protein extraction of SCN

One hundred cysts/females were transferred into a 1.5 mL Eppendorf tube with 100 μL protein lysis solution (8 *M* urea, 4%CHAPS, 30 m*M* Tris-HCl, pH 8.0). EP tubes were placed in an ice-bath and homogenized with a micro homogenizer for 4 min and stored on ice for 30 min. Every 10 min the EP tubes were exposed to ultrasonic waves for 1 min before centrifugating samples at 15200 g for 30 min at 4°C. The supernatant was obtained and stored at -80°C.

Determination of protein concentration

A 100 μg/mL bovine serum albumin (BSA) standard solution was prepared and the standard curve of the protein was obtained by gradient dilution. The absorption value was determined at 595 nm using the Bradford method with a Microplate reader (Multiskan MK3, Thermo) (Bradford, 1976), and the protein concentration of the sample was determined and expressed as μg/μL.

Two-dimensional electrophoresis (2-DE)

The first isoelectric focusing electrophoresis (IEF) was performed using 13 cm pH 4–7 linear IPG Drystrips in the IPGphor system (GE Healthcare). The hydration was prepared on a tray, 400 μg protein was loading in each drystrip. Then replenish it with the rehydration solution to 450 μL and mix well. All IPG Drystrips were rehydrated with rehydration buffer (8 *M* urea, 4% (w/v) CHAPS, 1% (v/v) pharmalytes, pH 3–10, 2 mg/mL DTT, 0.002% 1% bromophenol blue stock solution) for about 14 h. Rehydration and isoelectric focusing was performed at 20°C as follows: maximum current 50 μA, 500 V/500 Vhs, 1000 V/6000 Vhs, 8000 V/13500 Vhs, 10000 V/40000 Vhs.

After IEF, strips were equilibrated in equilibration buffer I (50 m*M* Tris-HCl pH 8.8, 6 *M* urea, 30% (v/v) glycerol, 2% (w/v) SDS, 0.002% bromophenol blue, 1% DTT) for 15 min and washed with ultrapure water and then transferred to the same buffer containing 2.5% iodoacetamide instead of DTT for another 15 min. Then, the strips were sealed with agarose and run on 15% SDS-PAGE gels at the following parameters: 5 W/gel for 60 min, 15 W/gel for about 5 h.

After electrophoresis, the SDS-PAGE gels were visualized by staining with Colloidal Coomassie Blue G-250 according to the method of Newsholme *et al.* (2000). The gels were scanned with an Image Scanner III LabScan 6.0 (GE Healthcare). The protein spots were analyzed using the software ImageMaster™ 2D 6.0.

Mass spectrum identification and protein classification

The differentially expressed protein spots were excised from the stained gel (W and B represent protein spots from white females and brown cysts, respectively), protein was digested

as described by Hellman *et al*. (1995), the peptide segments were analyzed using an UltrafleXtreme (MALDI-TOF-TOF) mass spectrometer and the peptide mass fingerprinting (PMF) of protein spots was obtained. Then the protein sequence was identified using localized MASCOT software (Version 2.3.02 Matrix Science) in the NCBI Nematoda database (202362 sequences). For database searches, the following parameters were used: Unhydrolyzed enzyme cleavages with one missed cleavage site allowed, carbamidomethylation of cysteine as a fixed modification, Oxidation (M), Gln- > pyro-Glu (N-term Q) and Deamidated (NQ) as variable modifications, monoisotopic as mass values (MS/MS fragment ion masses) with peptide mass tolerance of 100 ppm and Fragment Mass Tolerance ± 0.6 Da, mass range of Mass Spectrogram between 500–3500 Da, resolution ratio of 50000. The identified proteins were searched by UniProt database (http://www.uniprot.org/help/uniprotkb) to determine biological function and classification.

Results

Proteomics analysis of differentially expressed proteins

2-DE protein patterns of white female and brown cyst are shown in Fig. 1. The distribution of protein spots in white female cysts differed from the brown cysts. The former had a molecular weight in the range of 44.3 kDa to 6.5 kDa, which was more uniform compared to brown cysts. In contrast, the protein spots of brown cysts mainly had a molecular weight between 24 kDa and 6.5 kDa, and concentrated on one side near the acid end. The protein spots of white females were remarkable less than those of brown cysts, and both cysts have two identical protein spots in common (S1 and S2) (Fig. 1, red arrow). Three protein spots (W43, W35 and W83) at 36 kDa and one spot (W19) at 16 kDa were present in the white female but were absent in the brown cysts. Five protein spots (B30, B28, B23, B24, B27) at 16 kDa were present in brown cysts but not in the white female ones (Fig. 1, red circle). There were 78 and 176 protein spots in the 2-DE gels of white female and brown cyst, respectively.

The distribution map of proteins (Fig. 2) shows that the molecular weight (MW) of brown cysts ranged from 0 kDa to 100 kDa, of which most of proteins were distributed within the range of the isoelectric point (pI) 4–11. Similarly, the MW and pI of the proteins in white females mainly ranged from 0 kDa to 100 kDa, and ranged between 4 and 11.4, respectively. The proportion of proteins with a lower molecular weight was higher compared to proteins with a higher molecular weight in both white females and brown cysts.

Functional identification of expressed proteins in female *H. glycines* white and brown cysts

A total of 78 spots from the 2-DE gels (50 from brown cysts, 28 from white females) were selected for identification, twelve identical proteins and 65 differentially expressed proteins were identified, due to the origin from the same protein, such as protein CRH-2, isoform A (B22, B27) (identified proteins are shown in Table 1). Furthermore, the different position proteins were also selected for further analysis based on visual inspection of the 2-DE gels (Fig. 1, red circle). Results showed that proteins in brown cysts are involved in cytoskeleton formation and metabolism. Additionally, compared with the map of brown cyst protein spots, there are two different protein spots (W19, W43) in white female protein map, which were associated with post-translational modification, protein turnover and chaperones. Moreover, detailed analysis showed that the proteins missing in white females were mainly involved in signal transduction mechanisms, general function prediction (Hypothetical protein CBG09071, spot B60) and metabolic function (Protein CRH-2, isoform A, spot B27), while the proteins missing in brown cysts were mainly associated with RNA transport (DC-STAMP domain-containing protein 2, spot W35), post-translational modification, protein turnover and chaperones (*C. briggsae* CBR-TAG-308 protein, spot W43). Table 2 shows the detailed information of proteins in each nematode with accession number, protein MW, pI, sequence coverage rate, protein score and peptides matched as well as function.

Functional annotation for SCN proteins

For insight into the functions of the identified proteins in the study, the gene function classification system ‘gene ontology’ (GO) database was used, which categorizes proteins into different groups based on biological processes (541 proteins), cellular components (255 proteins) and molecular functions (84 proteins). The main biological functions of the brown cyst proteins were: cellular processes (10.49%) and single-organism processes (10.49%). Based on cellular components proteins were categorized into cell (20.97%) and cell parts (20.97%). The percent of proteins classified into binding and catalytic activities were the highest among molecular functions, which were 54.79% and 27.40%, respectively. Similarly, for the white female proteins, the most represented GO terms were associated with biological processes including but not limited to single-organism processes (12.21%) and cellular processes (10.69%). The most represented GO terms in the cellular component category were associated with cell (20.29%) and cell part (20.29%). Furthermore, binding (72.73%) and structural molecule activity (18.18%) represented the majority of terms in the molecular functions category. The entire GO analysis of the identified proteins in the cysts is shown in Fig. 3.

Discussion

Proteomics can clarify the mechanism of biological changes under physiological and pathological conditions because proteins act as the direct embodiment of life. The pathogenicity and infection mechanism can be revealed by

Table 1: List of identical expressed proteins in the different position

Spot number	Protein ID	Protein name	Species
B10, B21	gi|308248833	Hypothetical protein CRE_20012	*Caenorhabditis remanei*
B75, B26	gi|18314323	Actin 1	*Heterodera glycines*
B19, B24	gi|380447939	Actin 2	*Heterodera avenae*
B53, B58	gi|212646510	Protein SMA-1, isoform B	*Caenorhabditis elegans*
B22, B27	gi|351058366	Protein CRH-2, isoform A	*Caenorhabditis elegans*
B52, B98, B173	gi|341879775	CBN-LET-721 protein	*Caenorhabditis brenneri*
B32, B30, B23, B97, B190	gi|341898605	CBN-DYF-14 protein	*Caenorhabditis brenneri*
W9, W13, W17, W18, W36, W40, W27, W38, W7	gi|18677188	Hypothetical protein Hgg-17	*Heterodera glycines*
W8, W34	gi|2055454	Prohibitin-like molecule TC-PRO-1	*Toxocara canis*
W87, W43, B161	gi|268530818	*C. briggsae* CBR-TAG-308 protein	*Caenorhabditis briggsae*
W53, B55, B39	gi|324546817	Polyubiquitin-A	*Ascaris suum*
W86, W5, B59, B5, B54	gi|324500174	227 kDa spindle- and centromere-associated protein	*Ascaris suum*

Table 2: Identification of expressed proteins in white females and brown cysts of *H. glycines*

Protein spots	Protein name	Protein ID	MW (Da)	Isoelectric point	Coverage rate (%)	Protein score	Peptides matched	Functions
Same position								
S1	Protein LGG-2, isoform b	gi|308071946	8696.3	7.52	22.97	28.7	1	Autophagy
S2	tropomyosin	gi|268619116	33174.5	4.29	43.31	75.0	14	Cytoskeleton
Different position								
B24	Actin 2	gi|380447939	39535.7	5.45	22.86	73.7	6	Cytoskeleton
B26	Actin 1	gi|18314323	42149.9	5.16	15.96	96.5	5	Cytoskeleton
B27	Protein CRH-2, isoform A	gi|351058366	25616.78	5.62	37.95	67.1	9	Metabolic function
B23	CBN-DYF-14 protein	gi|341898605	207041.6	5.43	21.15	82.7	41	Unknown
B28	Protein C24A3.1	gi|115535053	73507.3	9.59	17.08	58.7	8	
B30	CBN-DYF-14 protein	gi|341898605	207041.6	5.43	17.46	73.0	30	
W19	Hypothetical protein CAEBREN 04267	gi|341883408	54304.2	9.22	33.84	83.2	19	
W43	C. briggsae CBR-TAG-308 protein	gi|268530818	485092.9	4.87	16.53	108.0	70	Post-translational modification, protein turnover, chaperones
Proteins in white or brown cysts								
B60	Hypothetical protein BG09071	gi|268572011	53691.4	9.76	39.05	66.2	20	Transcription, general function
B9	Protein PLK-2	gi|17510519	72710.1	8.88	19.46	69.6	11	prediction only, transcription
B74	Hypothetical protein CBG20734	gi|268532860	195849.8	8.24	14.57	93.9	26	Transcription
W50	putative ubiquitin family protein	gi|339260924	8253.4	5.05	58.90	168.0	7	Post-translational modification, protein turnover, chaperones
W35	DC-STAMP domain-containing protein 2	gi|324504955	84813.3	9.17	24.38	82.5	17	RNA transport
W6	Protein ELKS-1	gi|351063094	95522.3	6.62	21.41	67.3	21	Unknown
W77	Polyubiquitin-C, partial	gi|324539232	16739.1	7.37	35.14	110.0	7	Post-translational modification, protein turnover, chaperones

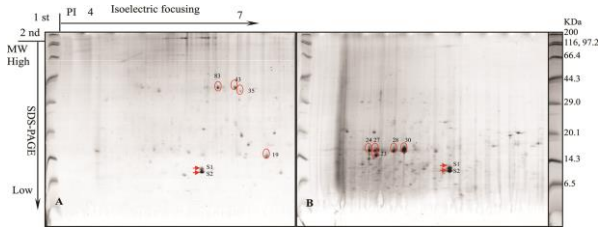

Fig. 1: Two-dimensional electrophoresis map of protein in white female (**A**) and brown cyst (**B**) of *H. glycines*. Red arrows were two identical protein spots (S1, S2) in white female and brown cyst. Red circles represent differential protein spots in white female and brown cyst, three protein spots (W43, W35 and W83) at 36 kDa and one spot (W19) at 16 kDa were present in the white female but were absent in the brown cysts, five protein spots (B30, B28, B23, B24, B27) at 16 kDa were present in brown cysts but not in the white female ones

analyzing proteins, understanding the function of proteins and biological pathways. A multifunctional protein, referred to as 'translationally controlled tumour protein' (TCTP) plays an important role for parasitism and a novel *M. enterolobii* TCTP effector (MeTCTP) molecule could suppress programmed cell death to promote parasitism (Zhuo *et al.* 2016). Major types of peptidases increased in the *Bursaphelenchus xylophilus* secretome play important functions in the parasite-host interaction including tissue penetration, digestion of host proteins and protection from the host immune system attack in nematodes (Cardoso *et al.* 2016).

In the present study 254 protein spots from *H. glycines* were detected, of which 78 occurred in white females, and 176 proteins spots in brown cysts, respectively. 78 proteins were identified by mass spectrometry. Some proteins were located in multiple spots due to modifications in the protein such as methylation, phosphorylation and glycosylation which then causes changes in the molecular weight (MW) and isoelectric point (pI) of protein. Similarly, a number of proteins from *Saccharomyces cerevisiae* located in multiple spots appeared on the gels at approximately the same

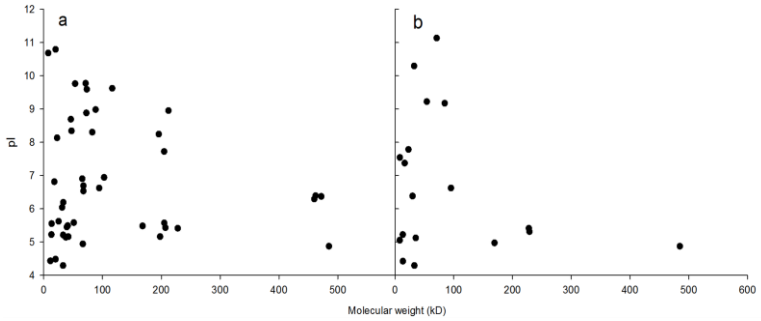

Fig. 2: Molecular weight and isoelectric point distribution map of proteins identified by mass spectrometry in brown cyst and white female **a**, brown cyst. **b**, white female

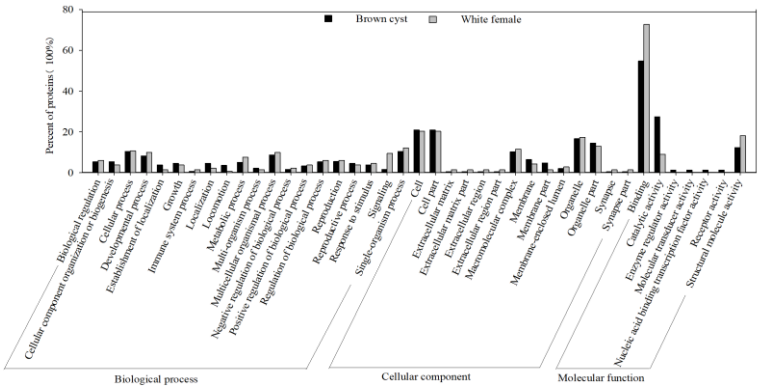

Fig. 3: Gene Ontology (GO) classification of identified proteins between brown cysts and white females in *H. glycines*

molecular mass but at a different pI position, which can be explained by differential post-translational protein processing such as processing of signal sequences, phosphorylation, acetylation or amidation (Kolkman *et al.* 2004). Previous results showed that more high-molecular-weight (>44.3 kDa) protein bands occurred in white cysts compared to the brown cysts in *H. glycines* and *H. avenae*. Soluble protein content in white cysts was also higher in white cysts than those in brown cysts of *H. glycines* (Mo *et al.* 2017). Similarly, when analyzing the entire protein pattern, more high-molecular-weight protein were found in the white females compared to the brown cysts, but the total number of protein spots in white females were remarkable less than those of brown cysts. The difference of the number and types of proteins between white females and brown cysts need to be further studied.

Based on the functional analysis, twelve proteins were common in white females and brown cysts, which were mainly associated with categories such as cytoskeleton (tropomyosin, Actin 1, Actin 2), replication, recombination and repair proteins (227 kDa spindle-and centromere-associated protein), post-translational modification, protein turnover and chaperones (*C. briggsae* CBR-TAG-308, Polyubiquitin-A). The same proteins were also found in the J2 of *H. glycines* (Chen *et al.* 2011). Therefore, we speculated that these proteins may be essential to maintain the growth and development of soybean cyst nematodes.

In addition, the missing proteins of white females were mainly involved in signal transduction and function prediction, hypothetical protein CBG09071 (spot B60) and protein PLK-2 (spot B9); metabolism, such as Protein CRH-2, isoform A (spots B27). The hypothetical protein CBG09071 showed similarity to glycogen synthase kinase-3β (GSK-3β), which regulates metabolic and signaling proteins, structural proteins and cell survival. Furthermore, GSK3β plays also one of the most critical roles in regulating a broad array of transcription factors, thereby controlling gene expression (Grimes and Jope 2001). Protein CRH-2, isoform A and hypothetical protein CBG20734 were absent in white females but were active at the transcription level. Moreover, the hypothetical protein CBG20734 is involved in the purine and pyrimidine metabolic pathway. Additionally, protein CRH-2, isoform A and hypothetical protein CBG09071 were also associated with the formation of melanin through the same pathway analysis. CRH can induce cell proliferation and act on epidermal melanocytes as shown in a previous study (Slominski *et al.* 2005). Based on our results we identified these two proteins (protein CRH-2, isoform A and hypothetical protein CBG09071) to be involved in the hardening and browning of soybean cyst nematode epidermis.

The missing proteins in brown cysts include DC-STAMP domain-containing protein 2 (spot W35), polyubiquitin-C, partial (spot W77), and putative ubiquitin

family protein (spot W50), which were mainly involved in RNA transport, post-translational modification and protein folding and chaperone, respectively. Ubiquitination was involved in proteasomal degradation, DNA repair, protein stability, and other various cellular events. It was also one of the most ubiquitous post-translational modifications in eukaryotes (Hemantha *et al.* 2014); previously, polyubiquitin-C has been reported to be present in *Schistosoma mansoni* egg secretions (Cass *et al.* 2007). Furthermore, protein ELKS-1 (spot W6), involved in the NFKB signaling pathway, and the NFKB family protein are important for regulating cell survival and apoptosis (Forman *et al.* 2016).

Conclusion

There were more proteins expressed in brown cysts than in white females based on the 2-DE pattern of *H. glycines*. The proteins in brown cysts were related to growth, cell proliferation, signal transduction, and other life activities and two proteins (protein CRH-2, isoform A and hypothetical protein CBG09071) participate in the pathway of melanin formation. The proteins in white females mainly regulate post-translational modification, protein turnover, chaperones and RNA transport of *H. glycines*.

Acknowledgements

The research was sponsored by the National Natural Science Foundation of China (31660511), the Special Fund for Agro-scientific Research in the Public Interest (201503114). We would like to thank IvyTrans USA for editing and reviewing this manuscript for English language.

References

Boer JMD, Y Yan, X Wang, G Smant, RS Hussey, EL Davis, TJ Baum (1999). Developmental expression of secretory β-1,4-endoglucanases in the subventral esophageal glands of *Heterodera glycines*. *Mol Plant-Microb Interact* 12:663–669

Bradford MM (1976). Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding. *Anal Biochem* 72:248–254

Cardoso JMS, SI Anjo, L Fonseca, C Egas, B Manadas, I Abrantes (2016). *Bursaphelenchus xylophilus* and *B. mucronatus* secretomes: A comparative proteomic analysis. *Sci Rep* 6; Article 39007

Cass CL, JR Johnson, LL Califf, T Xu, HJ Hernandez, MJ Stadecker, JR Yates, DL Williams (2007). Proteomic analysis of *Schistosoma mansoni* egg secretions. *Mol Biochem Parasitol* 155:84–93

Chen X, MH MacDonald, MG Wesley, BF Matthews, SS Natarajan (2011). Extraction and analysis of soybean cyst nematode (*Heterodera glycines*) proteins by two-dimensional gel electrophoresis. *Nematropica* 41:240–247

Donald PA, AN Patananan, CA Cerna, T Simmons, N Castro, JL Wilgar, SC Goheen (2008). Protein comparison of soybean cyst nematode populations using SDS-PAGE. *Nematropica* 38:137–144

Forman K, E Vara, C García, R Kireev, S Cuesta, D Acuña-Castroviejo, JAF Tresguerres (2016). Influence of aging and growth hormone on different members of the NFkB family and IkB expression in the heart from a murine model of senescence-accelerated aging. *Exp Gerontol* 73:114–120

Gao B, R Allen, T Maier, EL Davis, TJ Baum, RS Hussey (2003). The Parasitome of the Phytonematode *Heterodera glycines*. *Mol Plant-Microb Interact* 16:720–726

Grimes CA, RS Jope (2001). The multifaceted roles of glycogen synthase kinase 3 in cellular signaling. *Prog Neurobiol* 65:391–426

Hellman U, C Wernstedt, J Gonez, CH Heldin (1995). Improvement of an 'In-Gel' digestion procedure for the micropreparation of internal protein fragments for amino acid sequencing. *Anal Biochem* 224:451–455

Hemantha HP, SN Bavikar, Y Herman-Bachinsky, N Haj-Yahya, S Bondalapati, A Ciechanover, A Brik (2014). Nonenzymatic polyubiquitination of expressed proteins. *J Amer Chem Soc* 136:2665–2673

Jaubert S, JB Laffaire, C Piotte, P Abad, MN Rosso, TN Ledger (2002). Direct identification of stylet secreted proteins from root-knot nematodes by a proteomic approach. *Mol Biochem Parasitol* 121:205–211

Kolkman A, MMA Olsthoorn, CEM Heeremans, AJR Heck, M Slijper (2004). Comparative proteome analysis of *Saccharomyces cerevisiae* grown in chemostat cultures limited for glucose or ethanol. *Mol Cell Proteomics* 4:1–11

Liu DW, LJ Chen, YX Duan (2011). Differential proteomic analysis of the resistant soybean infected by soybean cyst nematode, *Heterodera glycines* race 3. *J Agric Sci* 3:160–167

Liu WZ (2000). *Plant Nematology*. China Agriculture Press, Beijing, China

Masonbrink R, TR Maier, U Muppirala, AS Seetharam, E Lord, PS Juvale, J Schmutz, NT Johnson, D Korkin, MG Mitchum, B Mimee, SEVD Akker, M Hudson, AJ Severin, TJ Baum (2019). The genome of the soybean cyst nematode (*Heterodera glycines*) reveals complex patterns of duplications involved in the evolution of parasitism genes. *BMC Genomics* 20:1–14

Mo AS, YR Long, ZQ Qiu, HY Wu, XB Zhou (2017). Comparison of biochemical components within white and brown cysts in the cereal cyst nematode *Heterodera avenae*. *Intl J Agric Biol* 19:335–340

Newsholme SJ, BF Maleeff, S Steiner, NL Anderson, LW Schwartz (2000). Two-dimensional electrophoresis of liver proteins: Characterization of a drug-induced hepatomegaly in rats. *Electrophoresis* 21:2122–2128

Niblack TL, KN Lambert, GL Tylka (2006). A model plant pathogen from the kingdom Animalia: *Heterodera glycines*, the soybean cyst nematode. *Annu Rev Phytopathol* 44:283–303

Pozdol RF, GR Noel (1984). Comparative electrophoretic analyses of soluble proteins from *Heterodera glycines* races 1–4 and three other *Heterodera* species. *J Nematol* 16:332–340

Slominski A, B Zbytek, A Pisarchik, RM Slominski, MA Zmijewski, J Wortsman (2005). CRH functions as a growth factor/cytokine in the skin. *J Cell Physiol* 206:780–791

Trudgill DL, DM Parrott (1972). Disc Electrophoresis and larval dimensions of british, dutch and other populations of *Heterodera Rostochiensis*, as evidence of the existence of two species, each with pathotypes. *Nematologica* 18:141–148

Wang DY, HY Wu, XB Zhou (2017). Biochemical changes of the cereal cyst nematode, *Heterodera avenae*, at low temperatures. *Nematology* 19:915–923

Wang X, D Meyers, Y Yan, T Baum, G Smant, R Hussey, E Davis (1999). In planta localization of a β-1,4-endoglucanase secreted by *Heterodera glycines*. *Mol Plant-Microb Interact* 12:64–67

Wrather JA, TR Anderson, DM Arsyad, Y Tan, LD Ploper, A Porta-Puglia, HH Ram, JT Yorinori (2001). Soybean disease loss estimates for the top ten soybean producing countries in 1998. *Can J Plant Pathol* 23:115–121

Wu HY, M Luo, LY Zhang, XB Zhou (2019). Nematicidal activity of fosthiazate against soybean cyst nematode *Heterodera glycines*. *J Nematol* 51; Article e2019-21

Yu Q (2011). Soybean cyst nematode (*Heterodera glycines* Ichinohe). *In: Soybean Physiology and Biochemistry*, pp:461–475 El-Shemy, H. (Ed.). InTech Open, Janeza Trdine 9, Rijeka, Croatia

Zhuo K, J Chen, B Lin, J Wang, F Sun, L Hu, J Liao (2016). A novel *Meloidogyne enterolobii* effector MeTCTP promotes parasitism by suppressing programmed cell death in host plants. *Mol Plant Pathol* 18:45–54