EXTRACTION AND ANALYSIS OF SOYBEAN CYST NEMATODE (*HETERODERA GLYCINES*) PROTEINS BY TWO-DIMENSIONAL GEL ELECTROPHORESIS

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ABSTRACT

Chen, X., M. H. MacDonald, M. G. Wesley, B. F. Matthews, and S. S. Natarajan. 2011. Extraction and analysis of soybean cyst nematode (*Heterodera glycines*) proteins by two-dimensional gel electrophoresis. Nematropica 41:240-247.

Soybean cyst nematode (*Heterodera glycines*, SCN) is the most destructive pathogen of soybean (*Glycine max* (L.) Merr.) worldwide. In this study, three different protein extraction methods including phenol/ammonium acetate (phenol method), thiourea/urea solublization (lysis method) and trichloroacetic acid/acetone (TCA method) were evaluated to determine their efficacy in separating *Heterodera glycines* proteins by two-dimensional polyacrylamide gel electrophoresis (2-DE). In all three methods, nematode proteins were well separated with minor variations in the intensity of the protein spots. The phenol method showed higher protein resolution and spot intensity of all proteins compared with the other two methods. In addition, in the high-pI region, proteins were clearly resolved and strongly detected using the phenol method. Protein spots obtained from the phenol method were subjected to further analysis to test their suitability for identification by mass spectrometry. Twenty protein spots were randomly selected, digested with trypsin, and analyzed using Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) or liquid chromatography mass spectrometry (LC-MS/MS). While these results suggest that phenol method and the direct lysis method are efficient and reliable for the 2D separation of *Heterodera glycines* proteins, the phenol extraction procedure is superior for the alkaline proteins.

Key words: Proteomics, *Heterodera glycines,* two-dimensional polyacrylamide gel electrophoresis, mass spectrometry

RESUMEN

Chen, X., M. H. MacDonald, M. G. Wesley, B. F. Matthews, and S. S. Natarajan. 2011. Extracción y análisis de proteínas del nematodo quiste de la soya (*Heterodera glycines*) por electroforesis bidimensional. Nematropica 41:240-247.

El nematodo quiste de la soya (*Heterodera glycines*, SCN) es el nematodo más dañino del cultivo de la soya en el mundo. En este estudio, se evaluaron tres métodos de extracción de proteínas: acetato de fenol/amonia (método de fenol) solubilizacón con tioúrea/úrea (método de lisis) y ácido tricloroacético/acetona (método TCA), para determinar su eficacia en la separación de proteínas de *Heterodera glycines* por electroforesis bidimensional en poliacrilamida (2-DE). Con los tres métodos se separaron bien las proteínas, con variaciones menores en la intensidad. Se obtuvo mayor resolución de las proteínas y mayor intensidad de éstas con el método de fenol que con los otros dos métodos. Usando el método de fenol también se resolvieron más claramente y con mayor intensidad las proteínas en la región de alto pI. Se analizaron las proteínas obtenidas por el método de fenol para evaluar la viabilidad de identificación por espectrometría de masas. Se seleccionaron 20 puntos de proteínas al azar, se hizo digestión con tripsina, y se analizó con espectrometría de masas (Matrix-assisted laser desorption/ionization time of flight mass spectrometry, MALDI-TOF-MS) o espectrometría de masas de cromatografía líquida (liquid chromatography mass spectrometry, LC-MS/MS). Estos resultados sugieren que los métodos de fenol y de lisis directa son confiables y eficientes para la separación bidimensional de las proteínas de *Heterodera glycines*, siendo el método de fenol superior para las proteínas alcalinas.

Palabras clave: electroforesis bidimensional en poliacrilamida, espectrometría de masas, *Heterodera glycines,* proteómica.

INTRODUCTION

The soybean cyst nematode (SCN), *Heterodera glycines*, is one of the major pathogens of soybean around the world, especially in the United States, causing severe yield losses to this valuable crop (Wrather and Koenning, 2006). Like many other plant nematodes, its life cycle comprises of an egg stage, four juvenile stages (J1 to J4) and the adult stage. The pre infective second-stage juvenile (pi-J2) emerges from the egg. The pi-J2 is the stage that can migrate toward the root stele (Niblack *et al.*, 2006). Even though there are reports on the physiological and histological basis of nematode-host interactions (Riggs *et al.*, 1982; Dong *et al.*, 1997), fewer reports are available on the molecular biology and gene expression of this pathogen (Klink *et al.*, 2007a; Alkharouf *et al.*, 2007; Elling *et al.*, 2007; Klink *et al.*, 2009). To devise strategies to control SCN and develop soybeans with broad resistance against SCN, basic information about the nematode's proteome would be useful. A proteome profile for SCN will provide a better understanding of SCN parasitism and its virulence.

Two-dimensional gel electrophoresis (2-DE) is one of the most powerful tools for proteomic profiling for its robustness, parallelism and unique ability to analyze complete proteins at high resolution. The upstream and downstream steps of this process, i.e. sample preparation and image analysis, are critical for the experiment (Rabilloud, 2002). The isoelectric focusing (IEF) step is especially important to 2-DE, because it is very sensitive to many interfering compounds that potentially exist in the protein samples (Rabilloud, 2009).

Various protein extraction methods have been developed and used for proteomic studies for a variety of nematodes (Kaji *et al.*, 2000; Schrimpf *et al.*, 2001; Jaubert *et al.*, 2002; Navas *et al.*, 2002; Bantscheff *et al.*, 2004; Chen *et al.*, 2006; Dangi *et al.*, 2009). Suitable protein extraction methods vary depending on the type of tissues and species. For the plant-parasite nematodes, Jaubert *et al.* (2002) used 2-DE to identify secreted proteins from *Meloidogyne incognita*, but only identified seven proteins with microsequencing. Bellafiore *et al.* (2008) employed multidimensional protein identification technology (MudPIT) to identify 486 secreted proteins from *M. incognita*. Navas *et al.* (2002) precipitated proteins with acetone and examined protein variation using 2-DE in 18 isolates from *Meloidogyne* spp.. The number of protein positions ranged from 86 to 203. Mbeunkui *et al.* (2010) concentrated *M. hapla* lysate with 10 kDa molecular weight cutoff and separated the protein with SDS-PAGE, and employed LC/MS^{E} to identify 516 proteins.

The objective of this study was to establish an efficient extraction protocol for the 2-DE analysis of *H. glycines* J2. Three commonly used protein extraction methods were tested: trichloroacetic acid (TCA)/ acetone precipitation, phenol/ammonium acetate precipitation, and direct solubilization in 2-DE lysis buffer. The efficiency of these extraction methods was compared. In addition, the quality of protein spots were evaluated using mass spectrometry and the proteins were identified by MALDI-TOF MS and LC-MS/MS.

MATERIALS AND METHODS

Nematode materials

Heterodera glycines was grown at the United States Department of Agriculture, Plant Sciences Institute, Beltsville, MD according to Klink *et al.* (2007b). Briefly, *H. glycines* population NL1-RHg was maintained in the greenhouse on *Glycine max*, cultivar 'Essex' in 6- inch pots (Meyer and Meyer, 1996). Females were separated from root debris further by sucrose flotation, and the females were crushed gently with a rubber stopper in a 7.5 cm dia, 250 µm dia sieve to release the eggs. The eggs flowed through the sieve into a small plastic tray. Debris smaller than the eggs was removed by washing and collecting the eggs on a 25 µm dia mesh sieve. The eggs were placed in a small plastic tray with one cm of water. The tray was covered with plastic wrap and placed on a rotary shaker at 25 rpm. After three days, the second stage juvenile nematodes (J2) were then separated from unhatched eggs by running them through a 41 µm mesh cloth. The J2 were collected by centrifuging in an IEC clinical centrifuge for 30 seconds at 1720 rpm and stored at -80°C prior to protein extraction.

Protein extraction methods

The J2 were ground to a fine powder in liquid nitrogen. Triplicate samples of J2 were used for each of three protein extraction methods. In this investigation, three different extraction methods were used to extract protein from the J2.

Thiourea/Urea solubilization method (lysis method): In this protocol, J2 nematodes were frozen in liquid nitrogen and ground into a fine powder. Protein was extracted by vortexing 200 mg of J2 nematodes powder with 600 µl of lysis solution (7 M urea, 2 M thiourea, 4% CHAPS, 50 mM DTT , 1% (v/v) IPG buffer (pH 3-10)) and sonicated for 30 min at room temperature. The extract was centrifuged at 12,000 g for 10 min. The supernatant was transferred to a new tube. An aliquot was used to determine the concentration of protein.

Phenol/ammonium acetate method (phenol method): This procedure was carried out according to the protocol described by Hurkman and Tanaka (1986) and Chen *et al.* (2006) with minor modifications. J2 nematodes were ground with liquid nitrogen, and 200 mg nematode powder was extracted by adding 4 ml extraction buffer [5% Sucrose, 2% SDS, 50 mM DTT, 2 mM PMSF, 100 mM Tris-HCl (pH 8.0) and complete protease inhibitor (Roche Diagnostics GmbH, Germany)], and incubated on ice for 15 min and then centrifuged at 12,000 rpm for

30 min. The supernatant was transferred to a new tube, 4 ml water saturated phenol was added for extraction, and kept on ice for 10 min prior to centrifugation at 12,000 g for 30 min at 4°C. The phenol fraction was transferred to a new tube, 4 ml extraction buffer was added to the tube, then kept on ice and shaken 10 min. It was centrifuged at 12,000 g for 30 min at 4°C, and the phenol fraction was transferred into a new tube. Twenty ml of 0.1M ammonium acetate in methanol (ice cold) was then added to the tube and kept at −20°C overnight. This was centrifuged at 12,000 g for 30 min at 4°C, and the pellet was washed with methanol containing 0.1M ammonium acetate three times, and finally washed with ice-cold acetone. After air dried, the pellet was dissolved in 600 µl lysis buffer [7 M urea, 2 M thiourea, 4% CHAPS, 50 mM DTT, 1% (v/v) IPG buffer (pH 3-10)].

Trichloroacetic acid/acetone method (TCA method): This protocol was performed according to Natarajan *et al.* (2005) with some modifications. For this method, 200 mg liquid nitrogen ground J2 nematode powder was homogenized with 10 ml of a solution containing 10% (w/v) TCA in acetone with 0.07% (v/v) 2-mercaptoethanol. Total protein was precipitated overnight at -20°C. The extract was centrifuged at 12,000 g for 20 min at 4°C. The pellet was washed 3 times with acetone containing 0.07% (v/v) 2-mercaptoethanol. The pellet was air dried and resuspended in 600 µl of lysis buffer [7 M urea, 2 M thiourea, 4% CHAPS, 50 mM DTT, 1% (v/v) IPG buffer (pH 3-10)] followed by sonication on ice for 15 min. Insoluble material was removed by centrifugation at 12,000 g for 15 min at 4°C and the supernatant was used in 2D-PAGE analysis.

Protein determination and electrophoresis

Protein concentration was measured using the Bradford method (Bradford, 1976) with the Bio-Rad Protein Assay kit. To determine the protein concentration, all samples were precipitated in 10% (w/v) TCA and re-solubilized in 1N NaOH (Natarajan *et al.,* 2005).

The first dimension IEF was performed using 13 cm pH 3-10 linear IPG strips in the IPGphor system (GE Healthcare, Piscataway, NJ). All IPG strips were rehydrated with 250 µl rehydration buffer [7 M Urea, 2 M thiourea, 4% (w/v) CHAPS, 50 mM DTT, 1% (v/v) IPG buffer (pH 3-10), 0.002% bromophenol blue] containing 400 µg of protein. The voltage settings for isoelectric focusing were 30V for 12 h, 500 V for 1 h, 1000 V for 1 h, and 8000 V to a total 25 kVh. After IEF, strips were equilibrated for 15 min in equilibration buffer (50 mM Tris-HCl pH 8.8, 6 M urea, 30% glycerol, 2% SDS, 0.002% bromophenol blue, 1% DTT) and then incubated with the same buffer containing 2.5% iodoacetamide instead of DTT for another 15 min. The strips were subsequently placed on to 12.5% polyacrylamide gel using the Hoefer SE 600 Ruby electrophoresis unit. The 2D-PAGE gels were visualized by staining with Colloidal Coomassie Blue G-250 as described by Newsholme *et al.* (2000). The gels were scanned with ImageScannerTM II (GE Healthcare). Image analysis was performed using Progenesis SameSpots (Nonlinear Dynamics, Durham, NC) with manual correction. Total numbers of protein spots were determined in nematode samples for the three extraction methods. Triplicate samples were used for protein extraction. Analysis of variance (ANOVA) was used to analyze our experimental data.

In-Gel digestion of protein spots

Protein spots were excised from the stained gel and washed first with distilled water to remove ammonium sulfate and then with water/acetonitrile (50:50, v/v) containing 25 mM NH_4HCO_3 to destain the gel plug. The gel plug was dehydrated with acetonitrile, dried under vacuum, and then rehydrated with 20 µl of 10 µg/ml trypsin (modified porcine trypsin, sequencing grade, Promega, Madison, WI) digestion solution. The digestion was carried out at 37°C overnight. Digested peptides were extracted with 50% acetonitrile and 5% trifluoroacetic acid with sonication, and dried with vacuum centrifuge.

Protein identification

MALDI-TOF-MS analysis of tryptic peptides: A Voyager DE-STR MALDI-TOF mass spectrometer (Applied Biosystems, Framingham, MA) operated in positive ion reflector mode was used to analyze tryptic peptides. Samples were co-crystallized with α-cyanohydroxycinnamic acid (CHCA) matrix, and spectra were acquired with 75 shots of a 337 nm Nitrogen Laser operating at 20 Hz. Spectra were calibrated using the trypsin autolysis peaks at m/z 842.51 and 2,211.10 as internal standards.

MS/MS analysis of tryptic peptides: A Thermo Fisher Scientific LTQ Orbitrap XL hybrid linear ion trap, Orbitrap mass spectrometer (ThermoFisher Scientific, San Jose, CA) was used to analyze proteins that were not positively identified by MALDI-TOF mass spectrometry. Peptides were separated by reverse phase chromatography on a 100 x 0.18 mm BioBasic-18 column using a 30 min linear gradient from 5 to 40% ACN in 0.1% formic acid at a flow rate of 3 µl/min. The instrument was operated in data dependent mode with a duty cycle that acquired and recorded MS/MS spectra in the linear ion trap of the five most abundant ions determined by a high resolution survey scan in the Orbitrap (r = 30,000 @ m/z 400) over the range of 400 to 1600 m/z. High mass accuracy of the survey scans was maintained by calibration in real time using the lock mass option. The polydimethylcyclosiloxane ion $(Si(CH_3)_2O)_6$ (protonated m/z 445.120025) generated during the electrospray process from ambient air was used for the calibration lock mass (Schlosser

and Volkmer-Engert, 2003). Dynamic exclusion was employed to prevent the continuous analysis of the same ions. Once two MS/MS spectra had been acquired from any given ion, the parent mass was placed on an exclusion list for the duration of 3 min. Electrospray voltage was set at 3.5 kV and desolvation was assisted with 10 units of sheath gas; the capillary transfer tube temperature was set to 200°C. The minimum ion count required to trigger an ms/ms spectrum was set to 5,000 and normalized collision energy was set at 30%. Mascot Distiller ver. 2.3.0.0 (www.matrixscience.com) was used to prepare searchable peak lists for both MALDI PMF data and LC-MS/MS data.

Database searching

Protein identification was performed by searching the NCBI non-redundant database using the Mascot search engine (http://www.matrixscience.com), which uses a probability based scoring system. The following parameters were used for database searches with MALDI-TOF peptide mass fingerprinting data: monoisotopic mass, 25 ppm mass accuracy, trypsin as digesting enzyme with 1 missed cleavage allowed, carbamidomethylation of cysteine as a fixed modification, oxidation of methionine, N-terminal pyroglutamic acid from glutamic acid or glutamine as allowable variable modifications. For database searches with MS/MS spectra the following parameters were used: peptide tolerance of 5 ppm, MS/MS mass tolerance of 1 Da, peptide charge of +1, +2, or +3, trypsin as digesting enzyme with one missed cleavage allowed, carbamidomethylation of cysteine as a fixed modification, oxidation of methionine, N-terminal pyroglutamic acid from glutamic acid or glutamine as allowable variable modifications. Taxonomy was limited to Metazoa (Animals) for both MALDI and MS/MS ion searches. For MALDI-TOF-MS data to qualify as a positive identification, a protein's score had to equal or exceed the minimum score of 76 for a significance level of ($p < 0.05$). Positive identification of proteins by MS/MS analysis required a minimum of two unique peptides, with at least one peptide having a significant ion score [> 36 for NCBInr or > 47 for Invertebrates EST for a significance level of ($p < 0.05$)].

RESULTS AND DISCUSSION

Three methods for protein extraction from *H. glycines* J2, namely TCA/acetone precipitation, phenol/ammonium acetate precipitation, and direct solubilization in 2-DE lysis buffer were evaluated and compared based on protein yield, spot numbers and intensity. J2 nematode protein obtained using these three methods were compared for protein content with the Bradford assay. Statistical analysis showed that the direct solubilization method (9.59 ± 0.386 mg g^{-1} fresh wt) and phenol method (8.74 ± 0.920 mg g^{-1} fresh wt) had significantly higher protein yield than the TCA method (4.93 ± 0.191 mg g^{-1} fresh wt). The direct solubilization method and phenol method did not show

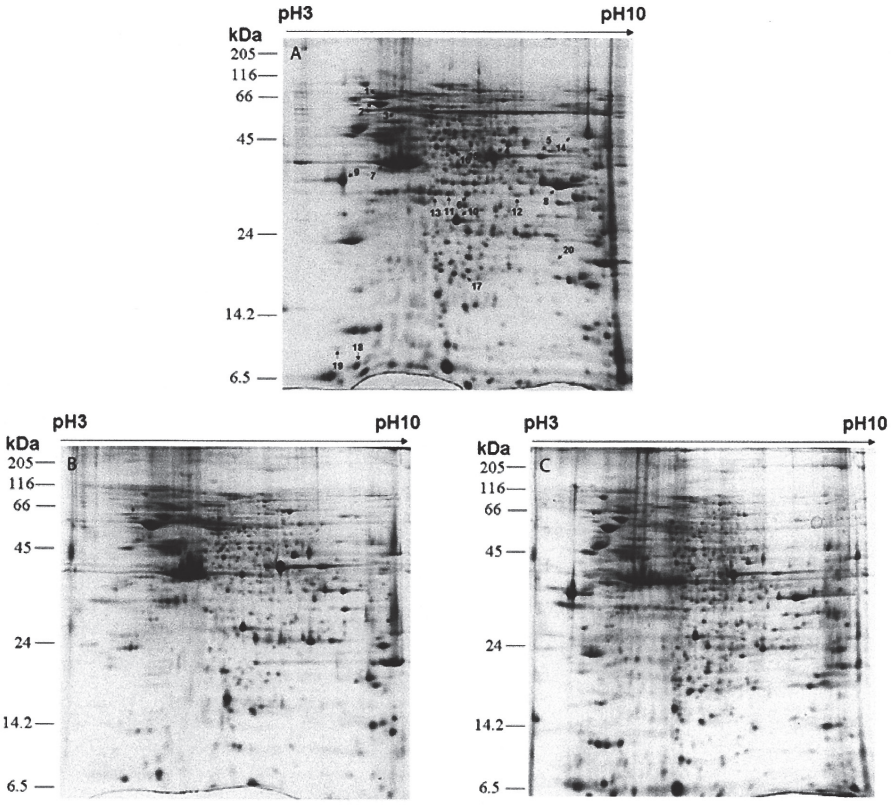

Figure 1. Two-dimensional electrophoretograms of *Heterodera glycines* extracted using various methods. (A) Phenol method, (B) TCA method, (C) Lysis method. 400 µg of proteins were focused in a rehydration buffers. The first dimension run using pH 3-10 IPG strips and the second dimension is a 12.5% SDS-PAGE. Gels were stained with Colloidal Coomassie Blue G-250.

significant variation from each other in protein yield.

The average number of protein spots resolved in 2-DE when using three extraction methods are presented in Table 1. The phenol extraction of SCN J2s resolved the highest numbers of protein spots in 2-DE among the three methods. The phenol extraction resolved more spots and showed 1.23 times and 1.10 times the number of protein spots procured by the TCA and the direct lysis method, respectively.

Protein patterns in 2-DE analysis for *H. glycines* J2 prepared from three different methods are presented in Figure 1. Although overall patterns of three methods were similar, the TCA method resolved the lowest amount of protein spots among the three methods. The TCA method has been found to be an efficient procedure to extract proteins from plant material samples (Yin *et al.*, 2007; Xu and Huang, 2008). Precipitation of protein with TCA method can remove interfering compounds, such as salt, lipid and polyphenols, but always causes protein loss due to insufficient resolubilization of protein (Gorg *et al.*, 2004). Although the direct lysis method showed good resolution in the pH 4-8 range, extraction above pH 8 produced fewer protein spots compared to the phenol method. The proteins direct extracted with lysis buffer was used in nematode protein extraction for 2-DE in the model organism *Caenorhabditis elegans* (Kaji *et al.*, 2000; Schrimpf *et al.*, 2001; Bantscheff *et al.*, 2004) and also in *Brugia malayi* (Dangi *et al.*, 2009). The phenol method detected more protein spots with high intensity in the high-pI region between pH 8.0 - pH 10.0. The phenol method was one of the most commonly used methods to extract proteins from plant samples for 2-DE analysis, and has proven to be highly efficient for extracting high-pI proteins (Wang *et al.*, 2003; Carpentier *et al.*, 2005). Chen *et al.* (2006) used the phenol method to extract proteins from *Steinernema feltiae* IS-6 IJs and demonstrated 2-DE profiling of this nematode. Our results were consistent with these studies and show that the phenol method is very efficient for protein extraction from *H. glycines* J2 nematodes, especially for those proteins with high pI.

To identify proteins following 2-DE, 20 randomly selected spots (labeled in Figure 1A) were excised from colloidal Coomassie blue stained gels containing proteins extracted using the phenol method and identified by MALDI-TOF MS and LC-MS/MS. MALDI-TOF MS and LC-MS/MS analysis showed that all the spots led to good quality spectra, indicating the compatibility of the phenol method with MS analysis. Although MALDI-TOF MS resolved good quality spectra of all the protein spots, only 7 out of 20 could be significantly identified by database searching against NCBInr. The other spots were subsequently identified after LC-MS/MS with NCBI non redundant and EST database searching. LC-MS/MS has been shown to be an alternative method to identify proteins from cross species databases. At present no annotated public genome sequence data of *H. glycines* is available, hence *H. glycines* EST database were used for MASCOT searching. Table 2 summarizes the detailed information of all the 20 spots.

Table 1. Protein yield and total number of spots using different protein extraction methods.

Methods	Protein yield[z] (mg g^{-1} fresh wt)	Spots number[z]
IEF	9.59 ± 0.386	765 ± 23.1
TCA	4.93 ± 0.191	679 ± 17.8
Phenol	8.74 ± 0.920	838 ± 18.9

[z]Mean ± SD (n = 3). Statistical analysis showed that there are no significant differences between IEF method and phenol method for protein yield, but both methods have significantly higher than the TCA method. For the spots numbers, all three methods have significant differences between each other

We were able to identify proteins from both major (spot 7) and minor (spot 20) spots. Spot 7 and 20 were identified as actin 1 and oxidoreductase, respectively. Spots 4 and 5 are related to unc-87, which is a calponin-related protein in *C. elegans* and required for maintaining the nematode body-wall muscle (Yamashiro *et al.*, 2007). Matthews *et al.* (2004) found that unc-87 was highly expressed in J2 juveniles, which is consistent with our proteomic results. Similarly, spots 11, 12, and 13, are related to troponin family proteins. Our results demonstrated that there are several troponin isoforms in *H. glycines* J2 stage. In *C. elegans*, there are 10 troponin isoforms, which are essential regulators of cytoskeletal activity in the somatic gonad (Ono and Ono, 2004). Those proteins have the same molecular weight but differ in isoelectric point. They are putative protein isoforms or have protein modifications. Another abundant protein which was identified as arginine kinase (spot 8), is also highly expressed in J2 stage at the RNA level (Matthews *et al.*, 2003). A less abundant protein (spot 14) is detected in samples prepared using the phenol method but is absent in sample using the TCA and direct lysis method. This protein spot was successfully identified as protein containing a KH domain by LC-MS/MS.

Based on these results, we found that the phenol extraction method and the direct lysis method are efficient methods to extract proteins of *H. glycines* J2s for 2-DE. In addition, the phenol extraction method is specifically more efficient to solubilize alkaline proteins. In the future we plan to use different pI range IEF strips to get more coverage of the *H. glycines* proteins. A combination of 2-DE and shotgun methods should enable us to develop a global database of total, developmentally and pathogenically expressed proteins from *H. glycines*.

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Table 2. Proteins identified by MALDI-TOF-MS and LC-MS/MS analysis in *Heterodera glycines.*

Spot number[z]	Protein identity	Gi number	MS method	Theoretical pI/ Mr	MOWSE score	Expected value	Sequence coverage (%)	peptides matched
1	heat shock protein 70-C	gi|22135552	MALDI-TOF	5.18/72898	199	2.40E-14	39%	24
2	heat shock protein hsp-90	gi|25987841	MALDI-TOF	6.11/8256	85	0.0066	82%	6
3	heat shock protein 70 A	gi|26422391	MALDI-TOF	5.26/70919	123	9.60E-07	32%	17
4	unc-87	gi|50086703	MALDI-TOF	7.04/41827	180	2.00E-12	53%	26
5	unc-87	gi|50086703	MALDI-TOF	7.04/41827	173	9.60E-12	58%	21
6	similar to filamin A interacting protein 1 isoform 2	gi|73973330	MALDI-TOF	8.15/109534	84	0.0078	21%	16
7	actin 1	gi|18314323	MALDI-TOF	5.20/42150	108	3.20E-05	32%	10
8	arginine kinase	gi|31247902	LC-MS/MS	8.49/40337	807		38%	15
9	tropomyosin	gi|33333845	LC-MS/MS	4.62/33320	1462		70%	26
10	galectin	gi|170583137	LC-MS/MS	5.96/31927	420		19%	8
11	Troponin family protein	gi|158594085	LC-MS/MS	4.95/32037	519		24%	6
12	Troponin family protein	gi|158594085	LC-MS/MS	4.95/32038	490		24%	6
13	Troponin family protein	gi|158594085	LC-MS/MS	4.95/32038	541		24%	6
14	KH domain containing protein	gi|158602012	LC-MS/MS	6.54/60844	397		35%	7
15	Aldehyde dehydrogenase protein 5	gi|17564084	LC-MS/MS	6.49/48818	365		27%	6
16	enolase	gi|86450230	LC-MS/MS	5.91/47375	267		5%	5
17	Superoxide dismutase	gi|224492522	LC-MS/MS	7.75/25137	219		23%	5
18	Late embryogenesis abundant protein 1	gi|24418520	LC-MS/MS	5.34/16028	202		20%	3
19	SUMO (ubiquitin-related) homolog family member (smo-1)	gi|17508217	LC-MS/MS	4.62/10216	100		19%	2
20	Oxidoreductase, short chain dehydrogenase/reductase family protein	gi|170592975	LC-MS/MS	9.11/28227	460		41%	7

[z]Spot number corresponds to the 2-D gel in Figs. 1 A.

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