PROTEIN COMPARISON OF SOYBEAN CYST NEMATODE POPULATIONS USING SDS-PAGE

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ABSTRACT

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Soybean cyst nematode (SCN, *Heterodera glycines*) represents one of the most serious threats to predictable soybean yield in the United States. Originally discovered in North Carolina during 1954, intraspecific SCN population variability was soon noted. To reduce SCN crop damage, multiple agriculture techniques have been exploited. Of these, resistant varieties and rotation to non host crops have been the most effective in reducing the SCN egg population density. However, no single strategy is effective due to variation in SCN populations, lack of complete resistance, and economics of non host crop production. Although it is well accepted that dissimilarities in virulence phenotypes are not associated with any morphological distinctions, knowledge of biochemical characterization of SCN populations is lacking. Therefore, the protein profiles of eggs from four SCN populations were differentiated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Out of 25 protein bands ranging in molecular weight from 40 to 200 kDa, no differences were observed among any of the populations. These results suggest that eggs from SCN populations differing in virulence phenotypes have similar proteins at the resolution of SDS-PAGE.

Key words: *Heterodera glycines*, SCN, variability.

RESUMEN

Donald, P. A., A. N. Patananan, C. A. Cerna, T. Simmons, N. Castro, J. L. Wilgar, and S. C. Goheen. 2008. Por esta razón, en este estudio caracterizamos el nematodo quiste de la soya SDS-PAGE. Nematropica 38:137-144.

El nematodo quiste de la soya (SCN, *Heterodera glycines*) es una de las principales causas de pérdida de rendimiento del cultivo de la soya en los Estados Unidos. Poco después de su descubrimiento en 1954, en Carolina del Norte, se observó la variabilidad intraespecífica de las poblaciones de este nematodo. Con el fin de reducir las pérdidas causadas por el nematodo quiste de la soya y su densidad de población en el suelo, se han utilizado varias estrategias. Las más efectivas han sido la siembra de variedades resistentes y la rotación con cultivos no hospedantes. Sin embargo, la efectividad de estas medidas se ve reducida debido a la variabilidad de las poblaciones, la falta de resistencia completa y los factores económicos asociados con la producción de cultivos no hospedantes. Aunque es bien sabido que no existe correlación alguna entre los fenotipos de virulencia y variaciones morfológicas, es poco lo que se ha hecho para caracterizar bioquímicamente a las poblaciones de este nematodo. Por esta razón, en este estudio caracterizamos los perfiles de proteínas de los huevos de cuatro poblaciones de nematodo quiste de la soya utilizando SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). No se observaron diferencias entre las poblaciones en 25 bandas de proteína con pesos entre 40 y 200 kDa. Estos resultados sugieren que los huevos de poblaciones con diferentes fenotipos de virulencia poseen proteínas similares al nivel de resolución que brinda la SDS-PAGE.

Palabras clave: Heterodera glycines, nematodo quiste de la soya, variabilidad.

INTRODUCTION

In 2006, soybeans comprised 57% of the world's oilseed production, with 38% of the soybean supply coming from the United States (American Soybean Association). Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe*)* is the most serious parasite of soybean, causing an estimated $1 billion in damage each year (Wrather and Koenning, 2006). Historically the use of resistant varieties and rotation to non host crops has been the most effective strategy in reducing the SCN egg population densities although economics sometimes preclude implementation of crop rotation (Reis *et al.*, 1983).

Diversity in SCN virulence was noted soon after the discovery of SCN in North Carolina during 1954 (Ross, 1962). Virulence is measured by the ability of SCN to reproduce on soybean genotypes with different sources of SCN resistance. The finding of SCN population variability eventually led to the development of the race test (Golden *et al.,* 1970) and the HG Type test, an expansion of the race test (Niblack *et al.*, 2002), to communicate biological differences in the populations. In general, knowledge of variability is important in reducing the risk of yield loss on soybean crops as a result of SCN infection and reproduction. However, because 86% of U S commercially available resistant varieties have a common source of resistance, rotation of sources of resistance is difficult to achieve (Shier and Ward, 2008).

Generic and species differences in cyst nematodes have been identified through gel electrophoresis (Bossis *et al.*, 1997; Franco, 1979; Pozdol and Noel, 1984; Trudgill and Carpenter, 1971; Trudgill and Parrott, 1972). Presently variability in SCN virulence phenotype is measured by the HG Type test. Soybean resistance to SCN however is reported using the race designation. Virulence phenotype differences have not been linked to specific proteins or genes (Lambert *et al.*, 2005; Ruben *et al.*, 2006; da Silva *et al.*, 2007). It appears that protein differences may reflect differences in virulence but a high level of variability confounded the results in most studies (Griffith *et al.,* 1982; Kalinski and Huettel, 1988; Pozdol and Noel, 1984; Silva *et al.,* 2000). Lambert *et al.*, (2005) identified *Hg-cm-1* alleles or a closely linked gene which may be associated with SCN population adaptability to different sources of resistance. The number of proteins present in SCN is not currently known nor is information readily available on the number of proteins involved in virulence. The nematode studied most extensively, *Caenorhabditis elegans*, has an estimated 19,762 protein coding genes (Schwarz, 2005). This number of genes is similar to the number of protein coding genes in humans (International Human Genome Sequencing Consortium, 2004). Proteins of females of *H. glycines* were investigated and minor differences were observed between the two groups (races 1 and 2) and (races 3 and 4) (Pozdol and Noel, 1984). Further investigation is warranted to determine if these differences can be observed for other *H. glycines* populations or if they might be related to the stage of development. In the present study the concept of differences in proteins was revisited using eggs instead of females, (b) proteins of molecular weight of 47 to 202 kDa vs. proteins of molecular weight 14 to 94 kDa, and (c) SCN populations which varied in virulence phenotype

from reproduction on three to seven sources of resistance.

MATERIALS AND METHODS

SCN populations representing HG Type 2.5.7 (race 1), two populations of HG Type 2.5.7 (race 5, subpopulations A and B) and two populations capable of reproducing on all commercially available sources of soybean resistance, HG Type 1.2.3.4.5.6.7 (LY1 and LY2), were grown under greenhouse conditions. HG Type 2.5.7 (race 1) was cultured on the soybean cultivar 'Hutcheson', HG Type 2.5.7 (race 5) on 'Bedford,' and HG Type 1.2.3.4.5.6.7 alternately on 'Hartwig' and Hutcheson. All seed was treated with mefenoxam + fludioxonil (148 mL/ cwt of seed). The SCN populations were increased in 9 cm diam clay pots containing steam sterilized loam soil: sand mixture (1:3 v/v). Each pot was inoculated with 1,000 to 4,000 eggs/ml of a population following the methodology of Niblack *et al.*, (1993). Seed specific for the populations were added to the pots and covered with the soil:sand mixture. The pots were watered as needed. Light was from natural source except on overcast days when supplemental light was supplied with SUN System I metal halide lights (EnviroCept Quality Products, Benton City, WA). The greenhouse temperature was set at 28°C with supplemental heat supplied under the bench as needed and evaporation pads used for cooling. The plants were grown for approximately 30 days. Plants were removed from the clay pots, cysts and females present on the roots were forcibly removed, and collected on a 250 µm mesh standard soil sieve. The nematodes were separated from root debris by sugar floatation centrifugation (Jenkins, 1964). Cysts and females were crushed, releasing eggs using the methodology of Faghihi and Ferris (2000). Cysts and females were collected from plants grown from May through July. HG Type characterization was done on each SCN population increase and the average FI for the indicators lines of each population is presented in Table 1. Eggs (over one million for each population) were collected on a 75 µm mesh sieve, transferred to a microcentrifuge tube, frozen at -80°C, and shipped overnight on dry ice to the Battelle Northwest Laboratory. Upon arrival, samples were stored at -80°C. SCN eggs were allowed to thaw completely

Table 1. Female indices of SCN populations used in study. Two samples of each population were included and three replications of each SCN population were analyzed.

Indicator Line	2.5.7 (Race 1)*	2.57 (Race 5)*	1.2.3.4.5.6.7 (LY1)*	1.2.3.4.5.6.7 (LY2)*
548402	1.4	2.7	21.2	12.4
88788	41.9	16.0	34.3	30.3
90763	1.0	1.0	71.7	21.3
437654	0.3	0.2	45.5	31.5
209332	57.5	37.0	39.0	59.6
89772	0.1	0.2	44.0	14.6
548316	65.8	16.6	48.3	49.4

*[(number of cysts on indicator line/number of cysts on PI 458658)*100].
Two samples of each population were included and three replications of each SCN population were analyzed.

to room temperature prior to homogenizing. Once thawed, the samples were centrifuged and the supernatant was removed. Each population run was repeated at least three times. The samples were homogenized at 0°C using a Dounce tissue homogenizer with a 2 cm diam Teflon pestle attached to a variable transformer controlled hand drill set at approximately 1000 rpm. A volume of 20% SDS approximately equal to the egg volume was added to the tube prior to homogenization and samples were homogenized for 15 seconds. The homogenate was transferred to a separate microcentrifuge tube. Another equivalent volume of 20% SDS was then added to the homogenizer to rinse off the residue. The residual deposit was homogenized for an additional 15 seconds. The two homogenates were combined and immediately prepared for electrophoresis as described below.

Chemicals and reagents used were of the highest purity available from Sigma-Aldrich (St. Louis, MO, USA) and Pierce (Rockford, IL, USA) unless otherwise indicated. The 2X sample buffer used for protein solubilizing consisted of 3.5 mL MilliQ water (Bedford, MA, USA), 20mM Trizma base (Sigma), 20% glycerol (Sigma), 2% SDS (Sigma), 0.5 mL β-mercaptoethanol (Sigma), 2mM ethylene diamine tetraacetic acid (EDTA, Sigma), and 0.05% bromophenol blue (Pierce). The 5X Running buffer was prepared by adding 30g Trizma base, 144g glycine, and 10g SDS to a two liter volumetric flask filled to volume with distilled water. Prior to SDS-PAGE, this solution was diluted 1:4 by adding 1200 mL MilliQ water to 300 mL 5X running buffer.

In all of the protein samples except those involving HG Type 2.5.7B, a 1:1 dilution of a 1:4 sample was used. For the initial 1:4 sample, one part homogenized SCN extract and four parts sample buffer were combined and vortexed. From this, one part of the 1:4 dilution was mixed with one part sample buffer. For HG Type 2.5.7B, a 2:1 dilution was made due to a lower volume of eggs, with two parts homogenized SCN and one part sample buffer. Protein samples were stored in a -10°C freezer until use.

One dimensional SDS-PAGE was carried out using Bio-Rad Criterion precast non-gradient 7.5% Tris HCl gels. A Bio-Rad Criterion Cell electrophoresis apparatus was used to separate the proteins on the precast gels. Bio-Rad prestained SDS-PAGE high range standards were used for calibration. A 30-40µl sample of extracted SCN protein was loaded into each well. Electrophoresis was carried out at 200V and 45 mA, with the current maintained constant using a LKB Bromma 2301 Macrodrive 1 power supply unit. For each gel, electrophoresis was performed for approximately 70 minutes. After each run, the gel was washed with MilliQ water for 15 min, stained using GelCode® Blue Stain Reagent (Pierce) for 3 hrs, and destained with MilliQ water for 1 hr. Gels were scanned using a Canon CanoScan LiDE 500F scanner. Protein band molecular weight identification and densitometric measurements were done using digitalized scientific software UN-SCAN-IT licensed from Silk Scientific Corporation (Orem, UT).

RESULTS

Two populations of *H. glycines* eggs of the HG Type 1.2.3.4.5.6.7, and two populations of HG Type 2.5.7 (race 1 and 5) were examined by one dimensional SDS-PAGE (Fig. 1). Twenty-five distinct bands were assigned a number and molecular weight. No differences were obvious between the populations within the resolution of the SDS-PAGE technique. The most distinct protein bands are identified as bands 1, 2,

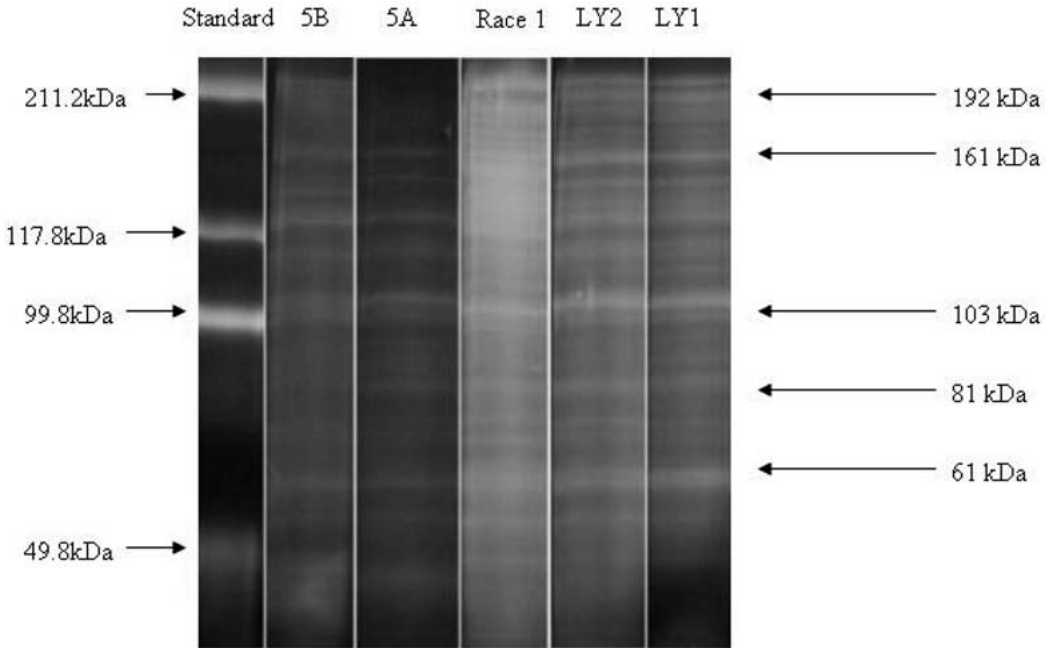

Fig. 1. SDS-PAGE comparison between HG Type 2.5.7 (races 5A and B, Race 1), and HG Type 1.2.3.4.5.6.7 (LY2, LY1), and a standard. Major proteins are shown and correspond to bands 1, 2, 7, 8, 15, 16, 19, 20, 21, 22, and 25 in Fig. 2.

7, 8, 15, 16, 19, 20, 21, 22, and 25 (Fig. 2). These were determined to have molecular weights of slightly greater than 200,000 to less than 50,000 daltons as shown in Fig. 2. Molecular weight assignments were based on the protein standards shown in Figure 1 with the assistance of the UN-SCAN-IT calibration program.

The proteins identified in the present study appear above what could be a large background of less prominent protein components in the SCN extract samples (see Fig. 2). The total number of proteins cannot be predicted from this method, but there are at least 25 distinguishable components that appeared in all populations. Pozdol and Noel (1984) examined bands in the region between molecular weights of 14 to 94 kDa and indicated approximately 45 proteins could be distinguished from white females. In the two studies there was an overlap in the area of molecular weight of the proteins between 50 and 94 kDa.

DISCUSSION

According to Lee and Atkinson (1976), proteins constitute 50-80% of the dry weight of nematodes. The major use of protein identification or detection has been for genetic or taxonomical relationships (Subbotin *et al.,* 1996; Andres *et al.,* 2001; and Holgado *et al.,* 2004) despite the fact that secretory proteins are considered to be essential to the mechanism by which plant parasitic nematodes infect (Davis *et al.,* 2000; Hussey and Grundler, 1998). Polyclonal antibodies were developed but were not sensitive enough to distinguish SCN eggs from eggs of other plant parasitic

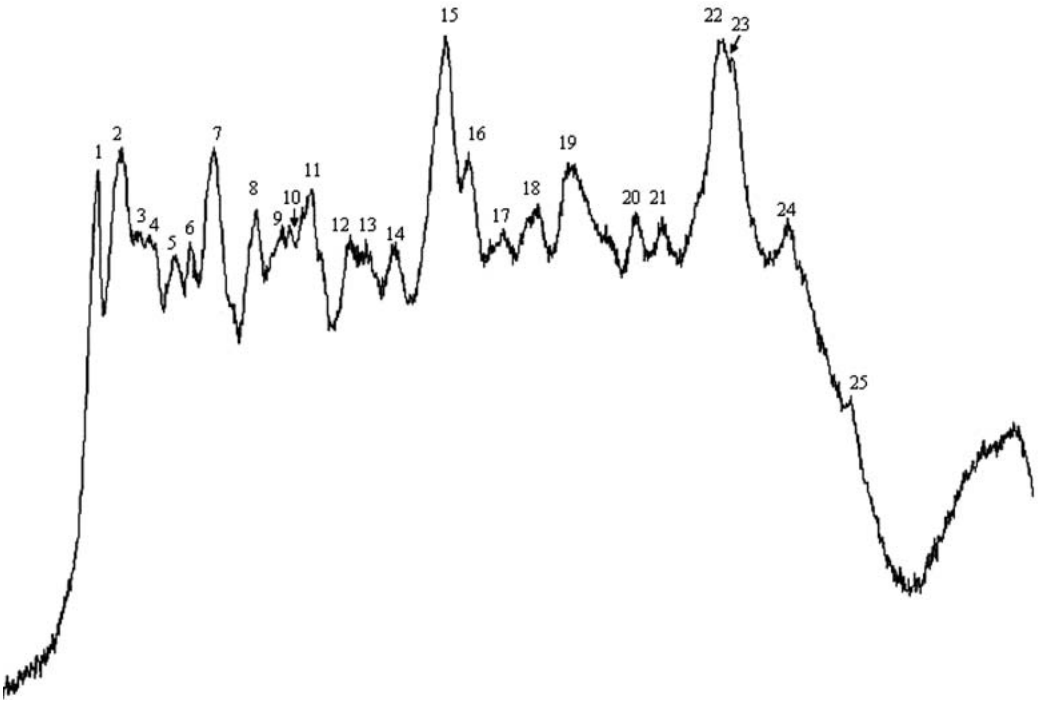

Fig. 2. Densitometry scan of population HG Type 1.2.3.4.5.6.7 (LY1) proteins from a SDS-PAGE gel. Molecular weight distribution from 202 to 47 kDa.

nematodes (Kennedy *et al.,* 1997). The proteins from the eggshells ranged from a molecular mass of 70 kDa or less. Masler (1999) identified vitellogenic proteins of molecular mass of 180 and 190 kDa from yellow and brown cysts.

There has been only one published study (Pozdol and Noel, 1984) of SCN proteins associated with virulence. Pozdol and Noel (1984) examined electrophoretic profiles of SCN females for differences related to virulence. Although they found similar results to our study, with no significant intraspecific differences in SCN populations related to virulence phenotype, differences in proteins at the species level were identified. Overall, Pozdol and Noel (1984) found 44 to 47 protein bands using SDS-Page, where we found 25 distinguishable bands. In the study by Pozdol and Noel (1984) proteins with a molecular mass at the lower end of the spectrum, 14 to 94 kDa were examined while most of the proteins in our study were of a higher molecular weight range, 50 to 200 kDa. Clearly Pozdol and Noel (1984) obtained greater resolution in their gels based on the number of bands observed within the molecular weight range they examined. Pozdol and Noel (1984) found one faint band of molecular weight 75 kDa, apparent only in HG Type 0- (race 3) and HG Type 1.2.3- (race 4), proved to be a distinguishing factor between the *H. glycines* races. Their hypothesis was that geographic origin might explain the differences they found as this band was not present in their HG Type 2- (race 1) or HG Type 1.2- (race 2) from North Carolina-Virginia area. This is consistent with our results in which a pro-

tein of 72 kDa was apparent in all examined races. All except our population LY1 originated from Tennessee field populations.

Pozdol and Noel (1984) also compared SCN populations which differed from each other in virulence phenotype by their ability to reproduce on three different sources of resistance. Our study compared SCN populations which were able to reproduce on three sources of resistance with populations which reproduce on four additional sources of resistance. Our populations also differed in FI among and between HG Types.

Although we observed no differences in the proteins of these populations, there remains a possibility that protein differences would be evident in other HG Type populations as suggested by Pozdol and Noel (1984). It is also reasonable to predict that weakly staining protein differences between the eggs of these populations exist, beyond the resolution of SDS-PAGE, and that weakly staining proteins are involved in the differing biological behavior of the SCN populations.

The precise nature of the relationship between the host plant and nematode is unclear. We conclude from this study that differences in virulence phenotypes cannot be correlated with protein bands from SCN eggs in the molecular weight range of 50 to 200 kDa as visualized with SDS-PAGE.

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DISCLAIMER

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