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Short Communication

Proteomic analysis of soybean cyst nematode

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The soybean cyst nematode (SCN, *Heterodera glycines*) is the major pest of soybean in the U.S. Infections of soybean by SCN include chlorosis, root necrosis, loss in seed yield and suppression of growth of root and shoots. Chemical control for soybean is not normally used because economic and environmental costs of the few effective treatments are prohibitive. Therefore, agricultural practices, such as crop rotation and the use of resistant cultivars, are used to mitigate the damage by SCN. There is usually a trade off between using the highest yielding soybean varieties, which are susceptible to SCN, and lower yielding SCN-resistant varieties. In the soil, SCN populations are diverse and are composed of numerous phenotypes or races to which soybean genotypes express selective susceptibility. Fourteen different races of SCN have been reported, and are defined by their interaction with soybean cultivars which differ in their response to different races of SCN. This variability in the SCN population complicates the effective use of cultivars resistant to SCN and confounds rotation schemes. Therefore, the limitations of current control practices with soybean dictate investigation of other avenues of protection of soybean against SCN and the prospect of genetically engineered, broad resistance of soybean to SCN is attractive. The life cycle for SCN includes egg, four juvenile (J-1, J-2 J-3 and J-4), and adult stages. The second stage of juvenile (J-2) hatched from egg enters the soil to reach the root tip along its entire length and migrates intracellularly to the vascular cylinder (Fig.1). There are not many reports available on changes of protein expression in SCN during its life cycle from J2 to adult. In recent years, the application of proteomic tools such as two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), matrix-assisted laser desorption /ionization time of flight mass spectrometry (MALDI-TOF-MS), and liquid chromatography - mass spectrometry (LC-MS) have become popular and powerful methodologies for accurately detecting and examining changes in protein composition.

Proteins were extracted via modified phenol extraction procedure. The J2 nematode proteins were precipitated with 4 ml extraction buffer with protease inhibitor and incubated on ice. Then the precipitate was centrifuged and the supernatant was transferred to a new tube with water-saturated phenol; this was repeated three times. Finally, ice cold ammonium acetate in methanol was added to the tube and kept at −20 °C over night. The mixture was centrifuged and the pellet was washed with methanol. The pellet was dried, dissolved in lysis buffer and used for 2D-PAGE analysis. In 2D-PAGE analysis, the proteins were separated based on the isoelectric point of the protein for the first dimension, and by molecular weight for the second dimension. The nematode protein samples were separated in the first dimension using 13cm pH 3-10 linear IPG strips via isoelectric focusing (IEF) according to Natarajan et al. (7). For the second dimension gel electrophoresis, the gel strips were reduced and alkylated and subsequently placed onto 12% polyacrylamide gel as described by Laemmli (5). After second dimension gel electrophoresis, the separated proteins were visualized using various fixations

and staining methods. For the protein identification, protein spots were isolated and excised from the stained gel. Then the spots were purified through various steps and digested using trypsin. The resulting tryptic fragments were extracted with acetonitrile with trifluoroacetic acid with sonication. The extract was dried and dissolved in acetonitrile and trifluoroacetic acid for mass spectrometry analysis. Protein identification was performed by searching the NCBI (National Center for Biotechnology Information) non-redundant database using the Mascot search engine (www.matrixscience.com), which uses a probability based scoring system (8).

Fig 1. Acid fuchsin staining of soybean cyst nematodes (SCN) in soybean root

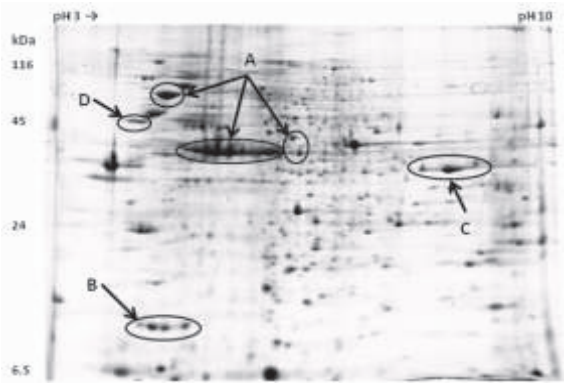

Fig 2. Two-dimensional electrophoresis profile of soybean cyst nematode proteins. The first dimension IEF was performed using pH 3.0-10.0 linear IPG strips. Numbered arrows indicate the polypeptides identified by mass spectrometry.

In this communication, we have selected four gel regions and the spots were identified by mass spectrometry using MALDI-TOF-MS. These regions are circled in Fig. 2 and labeled as A, B, C and D. Area A contained several abundant protein spots including one that was identified as Actin 1 with a theoretical isoelectric point (PI) of 5.30 and molecular weight of 42150. Actins are a family of highly conserved structural proteins that are expressed ubiquitously in eukaryotic cells. Actins are important for cell motility and are also involve in cell organization. The cellular functions of actin are broad and include cell division, cell shape and volume, phagocytosis, movement, secretion, and signaling (10). Actin filaments provide strength, and are integral to connecting cells, and the extracellular matrix, building paths for intracellular transport, and they provide a scaffold for generating force (3, 4). Area B contained four spots that were identified as myosin regulatory light chain (MRLC) with theoretical PI of 5.36 and molecular weight of 18989. MRLCs are distinct from myosin heavy chains and possess their own unique properties. MRLCs can be phosphorylated in skeletal and cardiac muscles. Phosphorylation modulates muscle contraction regulated by Ca2+-dependent troponin. In fast skeletal muscle, a dedicated Ca2+-dependent myosin light chain kinase (skMLCK) phosphorylates regulatory light chain (RLC). Biochemical RLC kinase and phosphatase together provides a biochemical memory for RLC phosphorylation and from this force is developed after activation (2). Areas C contained multiple spots that were identified as arginine kinase with theoretical PI of 8.49 and molecular weight of 40337. Arginine kinase (AK) is a phosphagen kinase and catalyzes the reversible transfer of phosphate from ATP and arginine to produce

phophoarginine. Phophoarginine plays a key role the energy in invertebrates and plays a key role in maintaining energy equilibrium and functions as a temporal energy buffer. AK can be involved in providing energy to the nematode for muscle movement. Through phosphoarginine, AK may also provide energy in the parasitic nematode *Steinernema carpocapsae* through phosphoarginine during the transition between anaerobic and aerobic metabolism that often occurs in soils saturated with water (9). These results suggest that, in addition to a role in muscle contraction during periods of high activity, AKs also may have an important role in providing a source of ATP during short intervals of stress (6). Area D contained an abundant spot that was identified as calreticulin (CRT) with a theoretical PI of 4.67 and molecular weight of 48596. Jaubert et al. (1) identified seven stylet secreted proteins from *Meloidogyne incognita* using 2D-PAGE and microsequencing. These included a MRLC, and a calreticulin (CRT) a calcium binding protein involved intracellular calcium homeostasis, protein maturation, and plays other roles in the cell. The authors cloned a gene for CRT and demonstrated that it was actively expressed in the esophageal glands of J2s of *M. incognita*.

The identification of important cyst nematode proteins in this investigation provides valuable information to the scientists who wish to develop nematode resistant soybeans.

Acknowledgment

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