GLYCOPROTEINS IN THE GELATINOUS MATRIX OF *ROTYLENCHULUS RENIFORMIS*[1]

Paula Agudelo[2], Robert T. Robbins[2], James McD. Stewart[3], and J. Brad Murphy[4]

Portion of Ph.D. dissertation by first author, published with the approval of the Director of the Arkansas Agricultural Experiment Station.[1] Graduate Research Assistant and Professor, Department of Plant Pathology, University of Arkansas, Nematology Lab, 2601 N. Young Ave., Fayetteville, AR 72704, U.S.A. Current address of senior author: 210 Long Hall, Department of Entomology, Soils, and Plant Sciences, Clemson University, Clemson, SC 29631.[2] Professor, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Plant Science Bldg., Fayetteville, AR 72701, U.S.A.[3] Professor, Department of Horticulture, University of Arkansas, Plant Science Bldg., Fayetteville, AR 72701, U.S.A.[4]

ABSTRACT

Agudelo, P., R. T. Robbins, J. M. Stewart and J. B. Murphy. 2004. Glycoproteins in the gelatinous matrix of *Rotylenchulus reniformis*. Nematropica 34:229-234.

The objective of this study was to test for the presence of glycoproteins in the gelatinous matrix of reniform nematode, *Rotylenchulus reniformis*. The proteinaceous components of freshly formed gelatinous matrix were analyzed following SDS-PAGE, using peroxidase-labeled plant lectins from soybean (*Glycine max*), wheat (*Triticum vulgaris*), asparagus pea (*Tetragonolobus purpureus*), castor seed (*Ricinus communis*), common gorse (*Ulex europaeus*), winged bean (*Psophocarpus tetragonolobus*), and *Bandeiraea simplicifolia*. Wheat germ agglutinin, which recognizes N-acetylglucosamine moieties, labeled two protein fractions between 60 and 80 kDa. In other studies, N-acetyl-glucosamine moieties have been detected in the gelatinous matrix of root-knot nematode. Glycosylation of nematode proteins has been implicated in host-parasite relationships and in protection against microorganisms. Thus, the confirmation of the presence of glycoproteins may be an important step towards the elucidation of the biological function of the gelatinous matrix in reniform nematode.

Key words: gelatinous matrix, glycoproteins, reniform nematode, *Rotylenchulus reniformis*.

RESUMEN

Agudelo, P., R. T. Robbins, J. M. Stewart, y J. B. Murphy. 2004. Glicoproteinas en la matriz gelatinosa de *Rotylenchulus reniformis*. Nematropica 34:229-234.

Este estudio se realizó con el objetivo de verificar la presencia de glicoproteínas en la matriz gelatinosa del nemátodo reniforme, *Rotylenchulus reniformis*. Se analizó el componente proteínico de la matriz gelatinosa recién formada, utilizando electroforesis en geles de poliacrilamida y lectinas de soya (*Glycine max*), trigo (*Triticum vulgaris*), *Tetragonolobus purpureus*, semillas de higuerilla (*Ricinus communis*), *Ulex europaeus*, *Psophocarpus tetragonolobus* y *Bandeiraea simplicifolia*. La aglutinina de trigo, la cual reconoce la presencia de N-acetil-glucosamina, detectó dos fracciones proteicas entre 60 y 80 kDa de peso. En otros estudios, se ha probado la presencia de N-acetil-glucosamina en la matriz gelatinosa del nemátodo del nudo radical. Existe alguna evidencia de la participación de proteínas glicosiladas de los nemátodos en relaciones de hospedante-patógeno y en protección contra microorganismos. Por tanto, la confirmación de la presencia de glicoproteínas en la matriz gelatinosa del nemátodo reniforme puede ser un paso importante en el descubrimiento de su función biológica.

Palabras clave: glicoproteínas, matriz gelatinosa, nemátodo reniforme, *Rotylenchulus reniformis*.

INTRODUCTION

The females of several sedentary plant-parasitic nematodes deposit their eggs in a gelatinous matrix. The matrix protects the eggs from antagonists and dehydration, and is considered an important survival mechanism (Bird and Rogers, 1965; Bird and Self, 1995; Orion, 1995; Sharon and Spiegel, 1993). However, information about the chemical composition of this matrix is very limited. Some work has been done to partially characterize the composition of the gelatinous matrix of root-knot nematode (*Meloidogyne* spp.) (Bird and Rogers, 1965; Bird and Self, 1995; Sharon and Spiegel, 1993; Spiegel and Cohn, 1985), but no information is available for other nematodes.

The reniform nematode (*Rotylenchulus reniformis*) produces a gelatinous matrix similar in aspect to that of the root-knot nematode, but with at least two fundamental differences. First, the substance forming the gelatinous matrix in the genus *Meloidogyne* is produced by rectal glands (Maggenti and Allen, 1960; Bird and Rogers, 1965), whereas in *R. reniformis* it is produced by vulval glands (Sivakumar and Seshadri, 1971). In addition, the root-knot nematode gelatinous matrix can be exposed or completely surrounded by plant tissue, whereas the reniform nematode gelatinous matrix is always completely outside the root. Only the anterior end of the body of the reniform nematode female is embedded in the root while the remaining portion of her body, along with the gelatinous matrix and eggs, is completely exposed. Bird and Rogers (1965) hypothesized that the chemical composition of the gelatinous matrix of *Tylenchulus* and *Heterodera* would be very similar to that of *Meloidogyne*, even though the origin of the matrix is different: a gland by the excretory pore in *Tylenchulus* (Maggenti, 1962), and cells of the uterine wall in *Heterodera* (Mackintosh, 1960). Because the chemical composition of the matrix has only been examined in the genus *Meloidogyne*, this hypothesis has not been tested.

Several studies have used lectins, proteins that bind to specific carbohydrate molecules (Rudiger and Gabius, 2001), to identify carbohydrates associated with nematode behavior (Davis *et al.*, 1989; Forrest and Robertson, 1986; Kaplan and Davis, 1991; Sharon and Spiegel, 1993). Using lectins, several proteinaceous components of the gelatinous matrix of root-knot nematode were found to be glycosylated (Sharon and Spiegel, 1993). These glycoproteins are thought to play a role in an agglutination phenomenon that could explain the ability of the gelatinous matrix to protect the eggs from surrounding microorganisms (Sharon and Spiegel, 1993). Confirmation of the presence of glycoproteins may be an important step towards the elucidation of the biological function of the gelatinous matrix in reniform nematode. The objective of this study was to test for the presence of glycoproteins in the gelatinous matrix of reniform nematode using plant lectins.

MATERIALS AND METHODS

Rotylenchulus reniformis was monoxenically cultured on tomato (*Lycopersicon esculentum*) cv. Rutgers. Seeds of tomato were surface sterilized by immersing them in 95% ethyl alcohol for 3 minutes and transferring them into 0.5% sodium hypochlorite for 10 minutes. The seeds were planted directly in autoclaved sand in sterile clear polypropylene Phytacon Vessels (Sigma, St. Louis, MO, USA). Each sealed vessel contained 250 cm^3 of sand, 100 cm^3 of Gamborg's B5 basal medium (Sigma, St. Louis, MO, USA) prepared according to manufacturer's instructions, and five tomato seeds.

Plants were inoculated one week after emergence. The vermiform stages of reniform nematode used as inoculum were washed three times in sterile streptomycin sulfate (10 ppm) solution. The tomato plants were inoculated near the root tip, with 0.1 ml of sterile aqueous suspension containing approximately 100 vermiform reniform nematodes. The containers were sealed and kept at 28°C, with a 12 h photoperiod. Periodic observations were performed to determine when females started gelatinous matrix production.

Freshly formed gelatinous matrix was harvested 18 to 21 days after inoculation with a micropipette, and collected in a microcentrifuge tube. The amount of gelatinous matrix in liquid state that can be collected from an individual depends on its stage of development, but approximately 1 µl of clean material can be obtained from a single female. For every replicate of this experiment, 100 to 120 µl of viscous liquid were pooled together. Samples were added to sodium dodecyl sulfate (SDS) sample buffer (1:1) (125 mM tris, pH 6.8, 4% SDS, 20% glycerol, 0.002% bromphenol blue) and mixed with 10% (v/v) b-mercaptoethanol, followed by heating at 90°C for 5 minutes. Proteins were analyzed by polyacrylamide gel electrophoresis (PAGE), in 12% gels containing 0.1% SDS, at 200 V, for 45 min. Each gel included eight lanes, one for each lectin tested and a negative control, with 40 µl of the denatured sample and buffer mixture per well. The molecular mass of sample polypeptides was calculated from mobility of Invitrogen See Blue® Plus2 (Carlsbad, CA) molecular weight standards.

Total protein extracts separated by SDS-PAGE were electrophoretically transferred at 30 V for 12 h to a Millipore Immobilon-P (Billerica, MA) polyvinylidene difluoride membrane. After transfer, the membrane was immersed in a blocking solution (150 mM NaCl, 10 mM Tris, pH 7.5, 10% Tween 20) for 2 h at room temperature. Individual lanes containing the transferred proteins were incubated separately with Sigma (St. Louis, MO) peroxidase-labeled lectins from the following plants: soybean (*Glycine max*), wheat (*Triticum vulgaris*) germ, asparagus pea (*Tetragonolobus purpureus*), winged bean (*Psophocarpus tetragonolobus*), common gorse (*Ulex europaeus*), castor bean (*Ricinus communis*), and *Bandeiraea simplicifolia*. Blocked membranes were incubated with the lectins (2 µg/ml Tris-buffered saline (TBS), pH 7.4, containing 0.1% bovine serum albumin (BSA)) for 1 h at room temperature, and then washed three times with TBS containing 0.1% BSA. To visualize labeling, 4-chloro-naphthol was used as a substrate. A gelatinous matrix solution unexposed to lectins was used as a control. The SDS-PAGE gel was stained with Coomassie Blue, after transfer, to detect possible remaining protein fractions. The experiment was repeated three times.

RESULTS AND DISCUSSION

Reniform nematode females started producing abundant gelatinous matrix 18 to 21 days after inoculation. The greatest challenge of this study was to collect the necessary amount of gelatinous matrix without eggs and other contaminants.

Wheat germ agglutinin labelled two protein fractions between 60 and 80 kDa (Fig. 1). The other lectins did not produce visible reactions. The protein fractions labeled with wheat germ agglutinin could also be detected in the polyacrylamide gels with Coomassie Blue staining, after transferring the samples to the polyvinylidene difluoride membranes, indicating fractions were only partially transferred. Results were identical in the three replicates.

Wheat germ agglutinin, which recognizes N-acetylglucosamine moieties, has

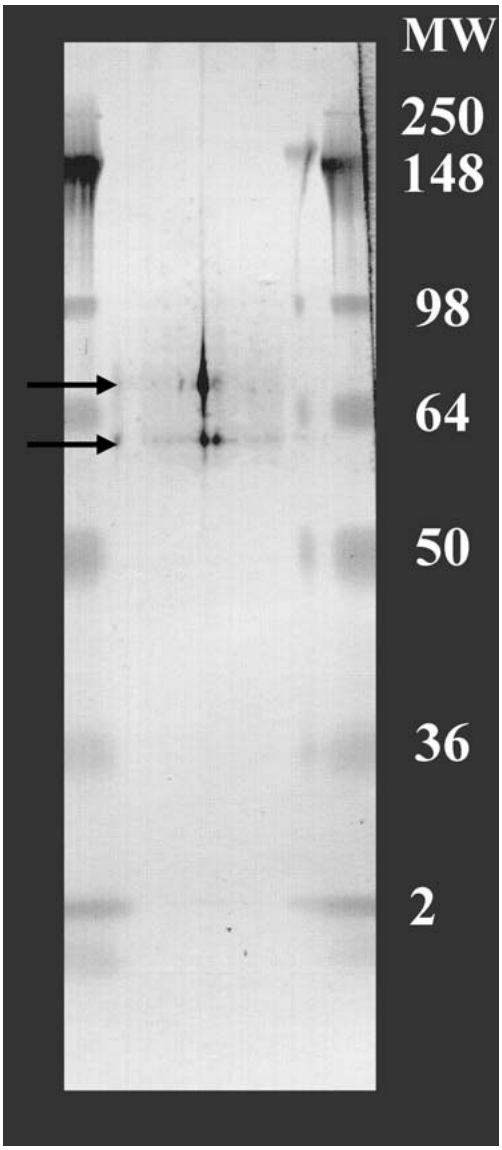

Fig. 1. Polyvinylidene difluoride membrane after incubation with peroxidase-labeled wheat germ agglutinin. Two of the transferred protein fractions (between 60 and 80 kDa) present in the gelatinous matrix of reniform nematode were bound to this lectin (MW: molecular weight in kDa).

also been found to label glycoproteins present in the gelatinous matrix of root-knot nematode (Sharon and Spiegel, 1993). However, Sharon and Spiegel (1993) found wheat germ agglutinin labeled much higher molecular weight protein fractions (150-200 kDa) of the root-knot gelatinous matrix than the ones found for reniform nematode in this study. In their results, the lower molecular weight fractions were labeled by soybean agglutinin and *Ulex europaeus* agglutinin, which in this study did not yield any positive reaction. These results suggest distinct glycoconjugation characteristics in these two nematodes, and indicate the need to test a wider variety of lectins in different genera of nematodes.

Geraert (1994) proposed that, in root-knot nematodes, the gelatinous matrix is "what is left of the plant sap after it has passed through the intestine", and that its function as an egg-sac "is accidental: it happens often because anal and vulval apertures are close together." We do not believe the role of the gelatinous matrix as an egg sac in any of the sedentary nematodes to be accidental. The hydrophobicity of the glycoproteins labeled, evidenced by the low transfer to the polyvinylidene difluoride membrane, may support the role of the gelatinous matrix as protection against desiccation of the eggs and female. Several studies propose this role of the matrix as a lubricant and as protection against dehydration (Orion and Kritzman, 1991; Sharon *et al.*, 1993; Orion *et al.*, 2001). Bird and Soeffky (1971) also suggest that the dehydrated egg mass exerts mechanical pressure on the egg shells and inhibits hatching under dry conditions.

The readily detectable presence of a proteinaceous component in the gelatinous matrix, and its glycosylation, suggest a greater role of the gelatinous matrix in the life history of reniform nematodes and root-knot nematodes than merely inhibiting desiccation or acting as a physical barrier. Orion *et al.* (1987) and Orion and

Franck (1990) showed that the gelatinous matrix of *M. javanica* digested host cells to form a canal through which eggs were forced outside the gall. This lysis of host cells suggested that the gelatinous matrix contained cellulytic and pectolytic enzymes. For reniform nematode, this function would be unlikely because the posterior end of the female and the entire egg mass are outside the roots.

Glycosylation of proteins of nematode origin has been implicated in host-parasite relationships (Davis *et al.*, 1989; Forrest and Robertson, 1986; Kaplan and Davis, 1991; Orion, 1995; Orion *et al.*, 1987). Gravato-Nobre *et al.* (1999) found a glycoprotein in the material left behind as root-knot nematode juveniles penetrate and migrate through the host tissue, and propose a functional role for the nematode surface coat in host-parasite recognition. Studies by Hu *et al.* (2000) showed that the surface coat of juveniles and the gelatinous matrix share common epitopes.

Another proposed role of the gelatinous matrix is protection against microorganisms (Orion and Kritzman, 1991; Sharon *et al.*, 1993; Orion *et al.*, 2001). Orion *et al.* (2001) concluded the gelatinous matrix retards microbial invasion of eggs, but do not elucidate whether this protective role of the gelatinous matrix is mechanical, biochemical or microbial in nature. Thus, studies that determine the composition of the gelatinous matrix are important for the elucidation of its function.

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