PHYSIOLOGY

Changes in Cotton Root Proteins Correlated with Resistance to Root Knot Nematode Development

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INTERPRETIVE SUMMARY

The southern root knot nematode is a serious pest of cotton. Previous research had produced germplasm (Aub634 source) essentially immune to this pest. The challenge has been to devise strategies for efficient incorporation of this source of resistance into commercial cotton cultivars. Toward this goal we are now conducting research into the developmental genetics of resistance to this serious pest. Although the resistant cotton lines pose no barrier to initial infection by root knot nematode, nematode development is arrested soon after root penetration. Results presented in this report show that a specific protein is produced in the resistant cotton roots at about the same time that development of the nematode is shut down. The temporal correlation of appearance of the protein and disruption of root knot nematode development opens the possibility that the protein is involved in the resistance response or is closely associated with the root knot nematode inhibitory effect. Detailed chemical characterization identified some sequences of amino acid building blocks of this protein. This information will be useful in the design of molecular probes needed to isolate the gene for this protein.

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Abbreviations: CNBr, cyanogen bromide; kDa, kiloDalton; PAGE, polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride;SDS, sodium dodecyl sulfate.

Isolation of this gene will yield clues to its role, if any, in the resistance to root knot nematode.

ABSTRACT

The cotton (*Gossypium hirsutum* L.) germplasm, Auburn 634 and others derived from this source, contain resistance genes that effectively inhibit reproduction of root knot nematode (*Meloidogyne incognita* [Kofoid and White] Chitwood, race 3). Although infective root knot nematode juveniles penetrate the resistant cotton lines in numbers similar to susceptible lines, nematode development is arrested in the resistant lines soon after infection. Analyses of root proteins via one- and two-dimensional polyacrylamide gel electrophoresis (PAGE) revealed a relatively abundant 14 kilodalton (kDa) polypeptide that was differentially expressed in resistant isoline 81-249 at 8 d after inoculation. Dissection of nematodes from equivalent root samples and their analysis separate from the root tissue showed that the 14 kDa was a plant protein. Expression of the 14 kDa protein in infected roots of 81-249 was localized to the nematode-induced galls. Digestion of the polypeptide with cyanogen bromide (CNBr) yielded two major fragments of 9 and 4 kDa from which partial amino acid sequences were obtained. Comparison of these partial sequences with gene databases did not reveal strong homologies with other sequences. Thus, the 14 kDa protein may represent the product of a novel, root knot nematode-inducible plant gene whose expression is temporally correlated with the resistance response to root knot nematode.

The cotton-breeding lines that Shepherd (1974a,b; 1987) developed, referred to as the Auburn 623/634 germplasm, are widely recognized as a strong source of resistance to root knot nematodes (Starr and Smith, 1993; Mueller, 1994). These cotton lines were derived from a breeding program spanning over 30 yr and originated from crosses between Clevewilt-6 (Jones et al., 1958) and a wild accession referred to as Mexico Wild (Minton et al., 1960). Because the trait is controlled by at least two genes that display only partial dominance in crosses with commercial cotton cultivars (Shepherd, 1986;

McPherson, 1993), this source of resistance remains difficult to incorporate rapidly by conventional breeding. Moreover, the only means of screening and identifying resistant progeny is by greenhouse bioassays of individual seedlings inoculated with root knot nematode and cultured for 40 to 50 d (Shepherd, 1979).

Use of this source of resistance was facilitated by studies undertaken to characterize the nature of the resistance and the interaction between the host plant and nematode at the biochemical/molecular level. Recent studies have shown that the cotton resistance genes do not alter root penetration by root knot nematode juveniles but ultimately inhibit the development of the juveniles into adult females (Creech et al., 1995; Jenkins et al. 1995). An apparent resistance response is initiated within 10 d after penetration of the roots since the root knot nematode juveniles develop no further than a swollen J2 to J4 stage and fail to establish and/or maintain giant cells (Jenkins et al., 1995).

The objective of this study is to compare protein patterns of roots from root knot nematode resistant and susceptible cotton isolines early after penetration of root knot nematode to see whether there are differences in gene expression correlated with this resistance response. The advantage of using such isolines for comparing protein patterns is that the overall genetic backgrounds of the parental line, ST213, and the backcrossed-resistant line, 81-249, approach similarity, except for those genes related to root knot nematode resistance.

MATERIALS AND METHODS

Plant Growth/Inoculation with Root Knot Nematode

The root knot nematode resistant cotton line 81-249 used in this study contained root knot nematode resistance genes from Auburn (AUB) 634 with a genetic background from the susceptible cv. Stoneville (ST) 213 (Shepherd et al., 1988). The 81-249 is a near-isogenic line derived through backcrossing two times with ST213 and selection following each backcross for seedlings with a high level of resistance to root knot nematode. Seedlings were grown in the greenhouse and were inoculated with 1000 root knot nematode second stage (J2)

juveniles per plant as described previously (Creech et al., 1995). Control (noninoculated) plants were grown under the same conditions. Root tissues for protein analyses were collected 8 d after inoculation. Whole roots were harvested and washed free of soil with a low pressure water spray. The small immature galls were excised with a scalpel and collected in disposable microtissue grinders (Kontes[1]). Typically, five galls were randomly selected from an individual plant with each plant representing an independent replicate. Equivalent size root sections were excised from noninoculated control plants. Samples were stored at -70°C until protein extraction. For comparison, leaf punches (5 mm diam.) were taken for protein analysis from the youngest fully expanded leaf of the same plants.

Additional plants were cultured for 40 d after inoculation to verify the resistance level of the isolines by quantitation of root knot nematode reproduction (Shepherd, 1979). The phenotypes of the roots of control and inoculated plants at 8 and 40 d after inoculation were captured digitally with an Olympus SZH10 stereo-microscope.

Protein Extraction

Tissue samples were removed from storage at -70°C and hand ground in a volume of 0.1 mL sodium dodecyl sulfate (SDS) sample buffer (Laemmli, 1970). Homogenates were heated at 95°C for 5 min then briefly centrifuged at 12 000 x g to pellet cellular debris. The resulting supernatants (total protein extracts) were stored at -70°C until analysis by PAGE. Prior to electrophoresis, protein concentrations in the samples were estimated from 1 µL aliquots (Marder et al., 1986) then equalized with addition of SDS sample buffer to approximately 500 µg protein µL^{-1}.

Protein Electrophoresis

One-dimensional SDS-PAGE was run with 4.5% (w/v) stacking and 15% (w/v) resolving gel acrylamide concentrations using the buffer system of

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[1]Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA, and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Laemmli (1970) and standard format (Hoefer Scientific Instruments, San Francisco, CA) or mini-gel (Bio-Rad) electrophoresis apparatus. Protein loads were 10 µg total protein per lane. Constant voltage of 50 V was applied for 1 h followed by 150 V for the duration of the run at room temperature.

Two-dimensional PAGE was based on O'Farrell's (1975) method. Total protein extracts were resolved in the first dimension by isoelectric focusing for 9600 V-h in glass tubes of 2 mm diam. containing 2.0% (v/v) Resolyte pH four to eight ampholines (BDH Laboratory Supplies, Poole, England). The protein load was 10 µg total protein per tube gel. The tube gel was then subjected to SDS-PAGE on a 4.5% (w/v) and 12.5% (w/v) acrylamide stacking and resolving gel, respectively, at constant current of 4 mA. Resolved proteins were stained with Coomassie R-250 and in some cases subsequently with ammoniacal silver (Dickens et al., 1995).

Polypeptide Purification and Amino-terminal Microsequencing

The 14 kDa polypeptide was isolated by excising Coomassie stained band from one-dimensional preparative SDS-gels (15% [w/v] acrylamide resolving). To clearly identify the 14 kDa band following brief (10 min) Coomassie staining, the preparative lane containing root protein extract from resistant-inoculated 81-249 (8 d after inoculation) was bordered by a single lane of sample from susceptible-inoculated ST213 (8 d after inoculation and lacking 14 kDa) and by molecular weight markers (BioRad). Excised gel slices containing the 14 kDa band were washed in distilled water then incubated for 30 min in 125 mM Tris-HCl, pH 6.8, 0.1% (w/v) SDS. The gel slices were pooled and loaded onto a second SDS-gel to concentrate the protein. The 4.5% (w/v) stacking gel was extended in length by twofold to allow uniform stacking of the protein following migration from the gel slices. The pooled slices were covered in the well with SDS sample buffer (Laemmli, 1970). The stacking voltage of this second gel was 25 V until the bromophenol blue tracking dye reached the resolving gel followed by 150 V for the duration of the run. Following electrophoresis, the protein was transferred to Immobilon PVDF blotting membrane

(Millipore) by electroblotting in a Hoeffer apparatus. The transfer buffer was 25 mM Tris-base, pH 8.5; 192 mM glycine without methanol. Transfer voltages were 10 V for 30 min followed by 25 V for 3 h, all at 4°C. The blot was washed for 10 s in distilled water then stained for 60 s with Coomassie (40% (v/v) methanol, 5% (v/v) acetic acid, 0.025% (w/v) Coomassie Blue R250). After destaining for 120 s (30% (v/v) ethanol, 5% (v/v) acetic acid), the blot was air dried. The 14 kDa band was cut from the PVDF membrane with a sterile scalpel.

Internal fragments were generated by digestion of the purified protein with CNBr (Scott et al., 1988). The 14 kDa band immobilized on PVDF blotting membrane was digested in 250 µL of 70% (v/v) formic acid containing 10 mg mL^{-1} CNBr for 6 h at room temperature. The CNBr digestion solution was collected and the peptides were then eluted from the membrane by extracting two times for 2 h each with 200 µL elution solvent (70% (v/v) isopropanol, 0.2% (v/v) trifluoroacetic acid, 0.1 mM lysine, 0.1 mM thioglycolic acid). The peptide elution solvent was pooled with the CNBr digest solution and concentrated just to dryness in a SpeedVac concentrator (Savant). The peptides were then resuspended in 50 µL distilled water and again dried before final resuspension in 35 µL SDS sample buffer. The sample was heated at 95°C for 300 s prior to loading and electrophoresis on a high resolution, Tris-Tricine peptide gel as described by Schagger and von Jagow (1987).

The resolved peptides were electroblotted as described above but onto Immobilon-P^{SQ} Sequencing Membrane (Millipore). The resulting blot was washed for 60 s in distilled water, 45 s in sequencing membrane stain (0.1% (w/v) Coomassie R-250, 45% (v/v) methanol, 5% (v/v) acetic acid), then 60 s (three times) in sequencing destain (45% (v/v) methanol, 5% (v/v) acetic acid). Finally, the membrane was washed three times with distilled water before air drying. The major CNBr fragments of approximately 9 and 4 kDa were cut from the membrane with a scalpel and submitted for N-terminal amino acid sequencing to the Center for Analysis and Synthesis of Macromolecules, State University of New York, Stony Brook, NY, a facility supported in part by NIH Grant RR02427 and the Center for Biotechnology. An Applied Biosystems model 475A protein sequencer equipped with a

phenylthiohydantoin-amino acid analyzer was employed using the manufacturers' protocols.

RESULTS

Phenotype of Resistance to Root Knot Nematode

The course of nematode infection in the nearly isogenic cotton lines was compared. At 8 d after inoculation, the susceptible (ST213) and resistant (81-249) lines showed virtually identical symptoms of initial gall formation along the root (arrows, 8 d after inoculation, Fig.1). Thus, as documented for other cotton lines (Shepherd and Huck, 1989; Jenkins et al., 1995; Creech et al., 1995), the resistance genes of isoline 81-249 do not inhibit initial J2 penetration. However, further development of root knot nematode dramatically differed in the susceptible and resistant lines as evidenced by the lack of egg masses on roots of 81-249 at 40 d after inoculation (Fig. 1). This difference was measured by quantitation of egg masses and total egg numbers per plant (Table 1). These data show that resistance mechanisms initiated in 81-249 inhibited root knot nematode development and reproduction by at least 98%, a level observed previously in other cotton lines containing these resistance genes (Shepherd et al. 1988; Jenkins et al., 1993).

### Differential Expression of Proteins in 81-249

Root knot nematode J2 infection apparently induces a resistance response in the resistant line, thereby, limiting continued nematode development. Differential gene expression in the 81-249 isoline during early stages of interaction with root knot nematode was assessed by comparing polypeptide profiles of root tissue from susceptible and resistant lines at 8 d after inoculation with corresponding profiles of noninoculated controls. The chances of detecting differences in proteins related to plant/nematode interaction were maximized by excising the galls from roots of individual plants at 8 d after inoculation (Fig. 2A, arrows). The developmental stage of the nematodes within galls of susceptible and resistant lines at 8 d after inoculation typically ranged from a J4 to swollen J2 stage root

Table 1. Reproduction of root knot nematode (*Meloidogyne incognita*, race 3) on cotton isolines ST213 and 81-249. Data are means (± SE, *n* = 10) from greenhouse bioassays at 40 d after inoculation of seedlings with 1000 root knot nematode second stage juveniles.

| Isoline	Egg masses/plant	Total eggs/plant
ST213	119.5 (± 39.3)	42 456 (± 14 330)
81-249	1.3 (±1.1)	378 (± 301)

knot nematode (Fig. 2B,C, respectively). For noninoculated control plants, root sections similar in length to the galls were excised for protein extraction. Resolution of polypeptides by SDS-PAGE with Coomassie staining revealed enhanced expression of a 14 kDa polypeptide in the inoculated resistant line (arrow, lane 4, Fig. 2D). Otherwise, the overall patterns of proteins from susceptible and resistant lines were similar, presumably reflecting their near-isogenic nature (lanes 1-4, Fig. 2D).

Because the total protein extracts of the galls would necessarily contain root knot nematode proteins as well, nematodes were dissected (i.e., as in Fig. 2B,C) from samples of galls equivalent to those of lanes three and four (Fig. 2D) for analysis separate from the plant tissue. As expected from the disparity in mass of the nematode vs. the root gall
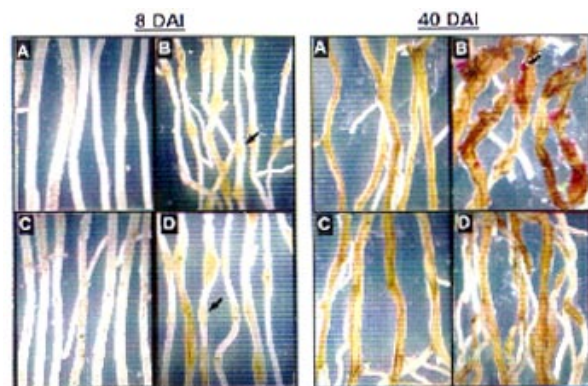
Fig. 1. Phenotype of root knot nematode-infected roots of ST213 (susceptible) and 81-249 (resistant) plants at 8 and 40 d after inoculation, compared to noninoculated controls. *A*, noninoculated susceptible plant; *B*, inoculated susceptible plant; *C*, noninoculated resistant plant; *D*, inoculated resistant plant. Arrows at 8 d after inoculation indicate early gall formation which is equivalent in susceptible and resistant lines at this stage of root knot nematode infection (*B* and *D*, 8 d after inoculation). Control and inoculated roots at 40 d after inoculation were stained with phloxine B (Creech et al., 1995) to visualize egg masses (arrow, *B*, 40 d after inoculation).
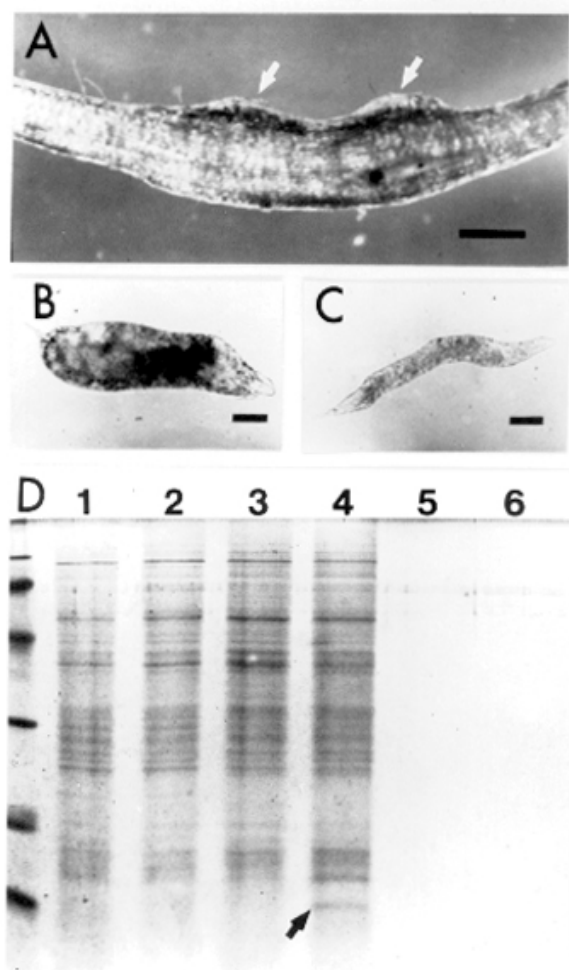
Fig. 2. One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of total proteins from root knot nematode infected root tissue of ST213 (susceptible) and 81-249 (resistant) at 8 d after inoculation as compared to noninoculated controls. *A*: example of young galls (arrows) at 8 d after inoculation that were excised from susceptible and resistant plants for extraction of total protein (scale bar = 4 mm); *B* and *C*: J4 and swollen J2 stage root knot nematode, respectively, representing the range of developmental stages observed in galls of either susceptible or resistant lines at 8 d after inoculation. Nematodes were dissected directly from the galls without fixation or staining (scale bars = 50 µm); *D*: Coomassie stained SDS-gel of total proteins of susceptible, noninoculated (lane 1); resistant, noninoculated (lane 2); susceptible, inoculated (lane 3); resistant, inoculated (lane 4); nematodes dissected from gall tissue of susceptible (lane 5) and resistant (lane 6) at 8 d after inoculation. The six stained molecular weight standards (BioRad) on the left correspond to proteins of 97, 66, 43, 31, 22, and 14 kDa.
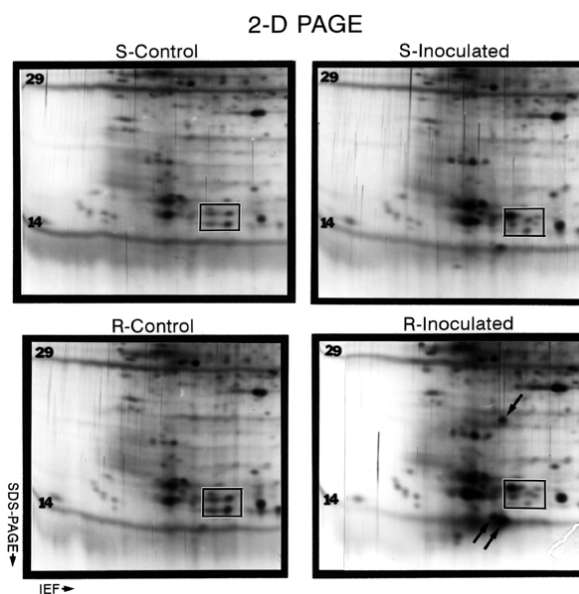
Fig. 3. Two-dimensional polyacrylamide gel electrophoresis (PAGE) of total proteins from root knot nematode infected root tissue of ST213 (susceptible, S) and 81-249 (resistant, R) at 8 d after inoculation as compared to noninoculated controls. Isoelectric focusing was run in the first dimension (basic side on right) followed by sodium dodecyl sulfate (SDS)-PAGE in the second dimension. Regions of the silver stained gels with dynamic changes in protein patterns have been enlarged for demonstration. Boxed regions indicate identical changes in protein patterns in S and R lines following inoculation with root knot nematode. Arrows indicate root knot nematode-induced changes in proteins unique to the R line. Numerals on the left side indicate protein standards (kDa) that were added to the agarose used to seal the isoelectric focusing gel to the second dimension slab gel and correspond to carbonic anhydrase (29 kDa) and lysozyme (14 kDa). The isoelectric focusing pH range for the portion of the gels shown was approximately 5 to 7.

(i.e., scale bars- B and C vs. A, Fig. 2), proteins from root knot nematode per se were not detectable (lanes 5 and 6, Fig. 2). Intense silver staining of the gel revealed only a faint background of protein for lanes 5 and 6 (data not shown). The 14 kDa polypeptide, therefore, appears to be a plant protein expressed in the resistant cotton line after root knot nematode infection.

The differential expression of the 14 kDa protein following root knot nematode infection of the resistant line was verified by two-dimensional PAGE (Fig. 3). Few differences in protein patterns were observed between the noninoculated controls (susceptible-control vs. resistant-control, Fig. 3). Following root knot nematode infection, the

susceptible and resistant lines displayed some changes in protein patterns that were identical (boxed areas, Fig. 3). The major difference between resistant and susceptible lines after inoculation was in the 14 kDa region (upward arrows, Fig. 3) where, in addition to the major 14 kDa protein, a minor, slower migrating spot was present. The downward arrow shows an additional protein of minor abundance (i.e., visualized by silver staining but not with Coomassie) at approximately 22 kDa unique to the infected resistant line. These results indicate that nematode infection has some common effects on gene expression in susceptible and resistant galls at 8 d after inoculation. However, beyond this shared response, the resistant line displayed unique changes in proteins in which the major difference was synthesis of the 14 kDa polypeptide at levels significantly higher than the levels of the minor products.

Localization of the Fourteen Kilodalton Polypeptide

To determine if the 14 kDa polypeptide was expressed throughout the infected root system of 81-249, galls present on susceptible and resistant lines at 8 d after inoculation and unswollen root tissue adjacent (acropetal) to the gall were collected for analysis by SDS-PAGE (Fig. 4). The 14 kDa protein was primarily localized in the gall tissue of the roots of the resistant line; a relatively low level of the protein was observable in the root tissue adjacent to the resistant galls (Fig. 4).
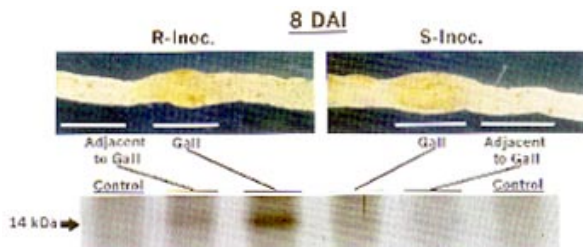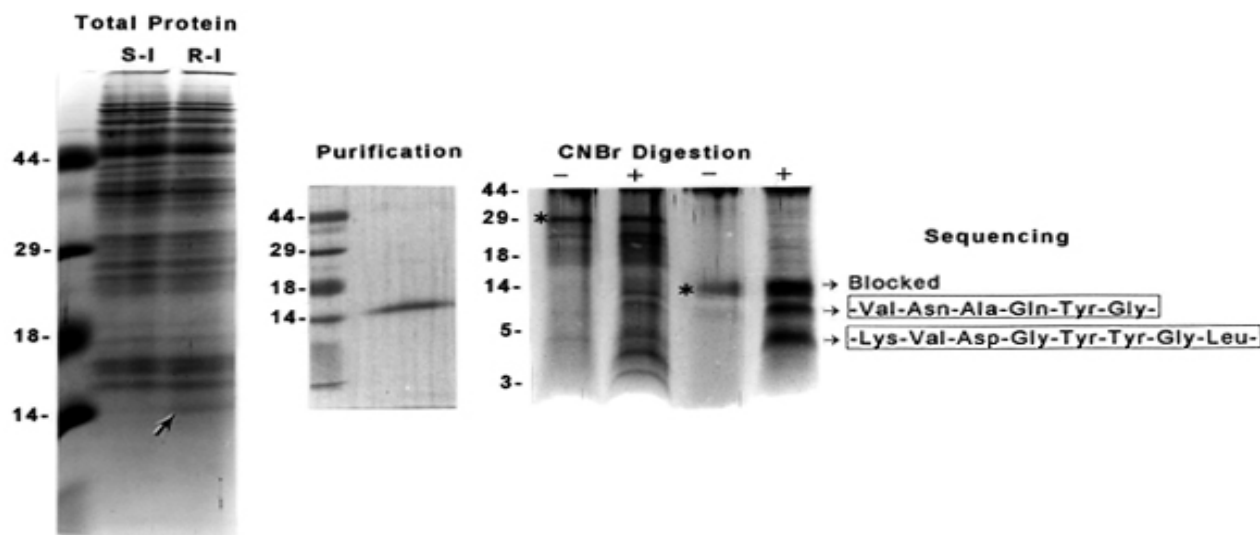

Fig. 4. Localization of the differentially expressed 14 kDa protein to the young galls of 81-249 (resistant, R) at 8 d after inoculation. Band absent in gall from ST213 (susceptible, S). In addition to gall tissue as analyzed in Fig. 2 and 3, root tissue adjacent (acropetal) to the galls was analyzed for the presence of the 14 kDa protein (arrow) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Control root tissue was from noninoculated plants. The gel was silver stained.
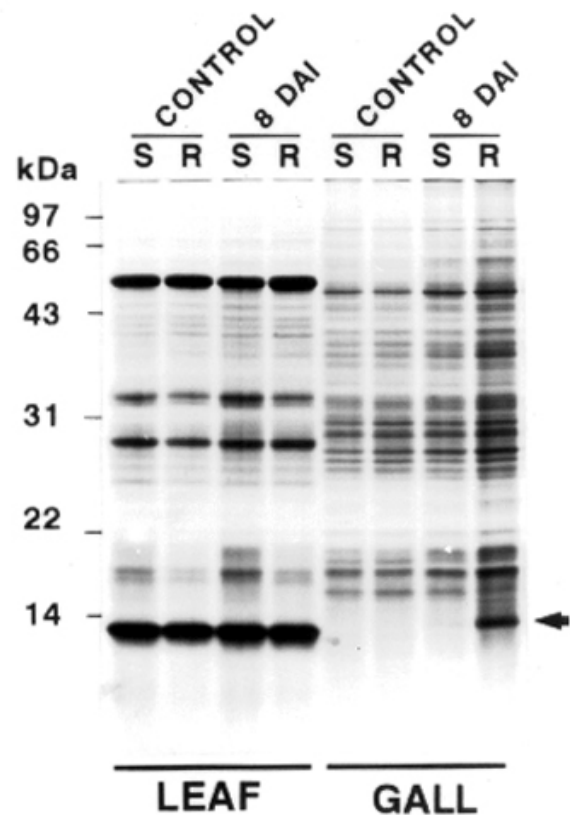
Fig. 5. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of total proteins of leaves vs. root galls of ST213 (susceptible, S) and 81-249 (resistant, R) plants at 8 d after inoculation. Control represents noninoculated plants. The arrow indicates the 14 kDa protein differentially expressed in the R plant galls. The positions of the protein standards are indicated on the left. The abundant polypeptide in all leaf samples just below the 14 kDa protein standard is the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase. The gel was silver stained.

The possibility of a more general systemic effect on protein expression following root knot nematode infection was investigated by analyzing leaf proteins vs. gall tissue of susceptible and resistant plants at 8 d after inoculation (Fig. 5). No major differences in polypeptide profiles of leaves were seen. The 14 kDa protein was again clearly differentially expressed in the galls of the inoculated resistant line. The major leaf polypeptide migrating just below the 14 kDa molecular weight marker was the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase. These results strongly suggest that the 14 kDa protein is a root-specific gene product with differential expression in the resistant line localized in the vicinity of the feeding nematode.

Fig. 6. Purification and amino acid microsequencing of internal fragments of the differentially expressed 14 kDa polypeptide. *Total Protein*: total protein extracts of resistant, R, plant galls at 8 d after inoculation (R-I) were run on preparative sodium dodecyl sulfate (SDS)-gels to resolve the 14 kDa protein (arrow). Only the end of the preparative R-I lane is shown with adjacent lanes containing protein of susceptible, S, plant galls at 8 d after inoculation (S-I) and prestained molecular weight standards (Bethesda Research Labs.) in kDa; *Purification*: the 14 kDa was excised from preparative gels, concentrated by re-electrophoresis, and then electroblotted onto polyvinylidene difluoride (PVDF) membrane. Coomassie staining of the blot revealed a single protein band of 14 kDa; *Cyanogen bromide (CNBr) Digestion*: A positive control, carbonic anhydrase (29 kDa), and the purified 14 kDa protein were digested in the presence (+) and absence (-) of CNBr. The digests were resolved on high-resolution peptide gels and blotted onto PVDF. The first two lanes are from the control protein, while the last two lanes are from the 14 kDa protein. The asterisks denote the original proteins recovered by digestion in the absence of CNBr; *Sequencing*: Amino acid sequences obtained for the 9 and 4 kDa fragments of the 14 kDa protein. The undigested 14 kDa band (- or + CNBr lanes) was chemically blocked on the N-terminus.

Microsequencing of the Fourteen Kilodalton Polypeptide

The 14 kDa protein was targeted for N-terminal amino acid microsequencing. The 14 kDa band, visualized by brief staining with Coomassie, was cut from preparative gels, concentrated by re-electrophoresis on a second SDS gel, and electroblotted onto polyvinylidene difluoride(PVDF) membrane, yielding a single sharp band of 14 kDa (Fig. 6). Initial attempts at N-terminal microsequencing of the purified, PVDF immobilized band failed because of chemical blockage of the protein. Internal fragments of the 14 kDa protein, generated by digestion of the PVDF immobilized protein with CNBr/formic acid, were resolved on a high-resolution peptide gel and then electroblotted onto PVDF (Fig. 6, +CNBr digestion, lane 4). The three major stainable bands obtained corresponded to undigested 14 kDa, and 9 and 4 kDa fragments. Omission of CNBr from the digest solution yielded only the original 14 kDa band (-CNBr digestion, asterisk, lane 3). The specificity of cleavage by CNBr was checked by digestion of a control protein, carbonic anhydrase, under identical conditions (Fig. 6). Digestion in the absence of CNBr yielded the intact 29 kDa protein (CNBr digestion, asterisk, lane 1), while digestion with CNBr yielded a distinct fragmentation pattern (+CNBr digestion, lane 2). The internal fragments generated from the 14 kDa protein, corresponding to the peptides of 9 and 4 kDa (+CNBr digestion, lane 4), were sequenced (Fig. 6). The amino acid sequences obtained for the 9 and 4 kDa fragments were used for homology searches against GENEMBL, PIR, and SWISS-PROT databases. No strong homologies were found.

DISCUSSION

The relationship between a host plant and an obligate endoparasite, such as root knot nematode, is a complex and highly evolved interaction

(Williamson and Hussey, 1996). After penetration of the root tip, the infective second stage juvenile (J2) migrates intercellularly to cells undergoing differentiation (Hussey, 1985). Subsequently, root knot nematode development and reproduction depends on root knot nematode induction of specialized feeding sites within the vascular cylinder of the root. In the absence of host resistance mechanisms, these initial feeding cells become enlarged as a result of repeated nuclear divisions that result in the root knot nematode feeding structures known as giant cells (Jones, 1981). Surrounding cortical cells then undergo hypertrophy leading to the characteristic galling along the root system of infected plants. Within these galls nematode development proceeds via several successive molts to the mature female form capable of reproduction (i.e., egg mass production).

Current evidence shows that sedentary nematodes alter patterns of plant gene expression in the cells destined to become feeding sites (Gurr et al., 1991; Hussey, 1989; Yamamoto et al., 1991). Recently, a root knot nematode-responsive element of the promoter of a root specific gene was isolated and shown to be independent of the *cis*-acting elements controlling tissue specific expression of the gene (Opperman et al.,1994; Opperman and Conkling, 1995). While these studies provide a glimpse of the complexity of evolved mechanisms of normal plant/nematode interactions, they do not address possible interactions with host resistance genes.

Plant resistance to root knot nematode, as to a diverse range of phytopathogenic organisms, may involve a hypersensitive response as a general component (Staskawicz et al., 1995; Williamson and Hussey, 1996). In cotton, phytoalexin synthesis was increased in the endodermis of the roots by infection with root knot nematode (Veech, 1978). Transcription of genes in the phenylpropanoid pathway was enhanced in resistant soybean [*Glycine max* (L.) Merr.] after infection by root knot nematode (Edens et al., 1995). However, in such cases the resistance level to root knot nematode has been intermediate.

In contrast to work on fungal and bacterial pathogens (Staskawicz et al., 1995), no gene for resistance to root knot nematode that confers high levels of resistance to the plant has been isolated and characterized. The single dominant allele at the *Mi* locus in tomato [*Lycopersicon lycopersicum* (L.)] confers strong resistance to root knot nematode, but the gene has not been isolated (Williamson et al., 1994). The gene for resistance to beet (*Beta vulgaris* L.)cyst nematode was recently cloned but does not affect root knot nematode (Cai et al., 1997).

The dynamics in plant protein expression, as reported here following infection of the susceptible and resistant cotton lines, are consistent with the emerging view of complexity in plant/nematode interaction. Alteration in plant gene expression during the initiation of root knot nematode feeding sites perhaps explains the identical changes in protein patterns seen in susceptible and resistant lines following infection. Beyond this shared response, we have shown that the resistant line differentially expressed several polypeptides in the root after root knot nematode infection. The relative abundance of the differentially expressed 14 kDa polypeptide and its localization to the root knot nematode induced galls led us to target this protein for additional characterization. Unfortunately, the N-terminus of the 14 kDa protein was chemically blocked and thus recalcitrant to amino acid sequencing by Edman degradation. We suspect that the blockage reflects a post-translational modification rather than an isolational artifact for the following reasons: (i) longer polymerization of the gels to decrease chances of interference from amino-reactive components and inclusion of the free-radical scavenger, thioglycolic acid, in cathode buffers did not alleviate the blockage; and, (ii) identical procedures and reagents were employed in sequencing the N-terminus of an antennal specific protein of the plant bug, *Lygus lineolaris* (Dickens et. al., 1995; Dickens and Callahan, 1996).

The lack of homology of the internal amino acid sequences with known gene products is not surprising, considering the limited sequence obtained. Still, the amino acid sequence data on the 14 kDa protein will be useful in the design of oligonucleotide probes for amplification of the complementary DNA by polymerase chain reaction. Cloning and sequencing of the cDNA corresponding to the 14 kDa protein should provide insight on the role, if any, of the protein in the resistance response of these cotton lines to root knot nematode.

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