Altered Amino Acid Metabolism in Root-Knot Nematode Inoculated Cotton Plants

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The root-knot nematode (RKN), *Meloidogyne incognita* (Kofoid and White) Chitwood, is a sedentary endoparasite that retards growth and development of cotton, *Gossypium* spp. L., by attacking the root system, causing galling, stunting, and other adverse effects. All commercial cultivars have susceptibility to RKN, although they vary in degree of susceptibility. Plant breeding has led to the development of resistant germplasm. Subsequent work has indicated that the mechanism of resistance may involve two major genes and that resistant lines may produce a unique 14 kDa protein. In the present study, the protein content of roots increased more in a susceptible line than in a resistant line after inoculation with RKN. The mole ratios of individual amino acids in RKN infected roots also were different from those of uninfected roots, and changes due to infection were greater in the susceptible line than in the resistant line. The results indicate protein synthesis in roots is modified by RKN. Alternatively, increases in protein content may be attributed in part to RKN protein. Cellulose was lower after infection in both susceptible and resistant lines.

Keywords: *Root-knot nematode; Meloidogyne incognita; cotton; Gossypium spp.; amino acids; protein; cellulose*

INTRODUCTION

The root-knot nematode (RKN), *Meloidogyne incognita* (Kofoid and White) Chitwood, is a sedentary endoparasite that retards growth and development of cotton *Gossypium* spp. by attacking the root system, causing galling, stunting, and other adverse effects. Shepherd et al. (1988a,b) reported that some RKN-resistant cultivars contained from 1200 to 5000 eggs per plant, whereas susceptible lines contained from 6000 to >100000 eggs per plant at 40 days after inoculation (DAI).

Production of a large number of RKN eggs in susceptible roots in a relatively short time is associated with a tremendous amount of damage inflicted upon young cotton seedlings by the nematode. As galls increase in size, the root cortex surrounding the galls splits, exposing a relatively large area of the central cylinder (Shepherd et al., 1988a,b).

The history and problems associated with breeding cotton for resistance to the RKN were reviewed by Fassuliotis (1982) and Sasser (1986). Recent research (Jenkins et al., 1993) indicates that cotton cultivars and most germplasm resources in the United States are susceptible to *M. incognita*, although cultivars vary considerably in their degree of susceptibility. McPherson et al. (1995) obtained evidence that suggested that resistance is determined by two major genes, one dominant and the other additive. Other genetic studies of cotton resistance to RKN include those of Tang et al. (1994), Creech et al. (1995), and Jenkins et al. (1995). Although infective RKN juveniles penetrate resistant cotton lines in numbers similar to those of susceptible lines, nematode development is arrested in the resistant lines soon after infection.

Callahan et al. (1997) carried out analyses of root proteins via one- and two-dimensional PAGE, which revealed a relatively abundant 14 kDa polypeptide that was differentially expressed in the resistant isoline 81-249 at 8 DAI. He reported that "dissection of nematodes from equivalent root samples and their analysis separate from the root tissue showed that the 14 kDa polypeptide had a plant origin". The 14 kDa polypeptide may be the product of a novel, RKN-inducible plant gene the expression of which is temporally correlated with a resistance response to RKN (Callahan et al., 1997).

Several other potential sources of resistance to RKN have been investigated, including methoxylated terpenoid aldehydes (Veech, 1978), terpenoid aldehydes (Hedin et al., 1984; Khoshkhoo et al., 1994), and sterols (Hedin et al., 1995). Although each of these provided some correlations, they did not lead to unambiguous conclusions. Consequently, a search for other factors or explanations of resistance was pursued.

As previously discussed, descriptions of the formation of giant cells in galls following infection with RKN suggested changes in protein metabolism of the root that were nucleic acid mediated, as well as changes resulting from massive increases of RKN within the root (Tang et al., 1994; Creech et al., 1995; Jenkins et al., 1995). Therefore, changes in the amounts of protein and structural components such as celluloses and hemicelluloses would be expected to be measurable and diagnostic. Accordingly, we have carried out studies to

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measure these components and their changes upon infection in RKN-susceptible and -resistant cotton roots.

MATERIALS AND METHODS

Plant Materials. Cotton near-isolines M249RNR (RKN highly resistant, 95-577, bulk selfs) and MS Sel. S213 (RKN highly susceptible, GH923-22-1,2,3,4, bulked selfs, 1996 seed) were utilized with 20 plants per treatment of each line for each inoculation treatment of three sampling dates.

Experimental Design. The experimental design was a repeated measures design arranged in a randomized complete block with three replications. Each replication consisted of 60 plants of each genotype. Whole plots were a factorial combination of isolines and inoculation treatments. Data were analyzed by the analysis of variance (The SAS System, SAS Institute, Cary, NC). Statistical analyses were performed among replications (three), inoculation and noninoculation (two), days after inoculation (three), resistant and susceptible lines (two), and laboratory duplicates (two) for the analyses of chemical constituents.

Treatments. Plants were uninoculated or inoculated with 5000 eggs per plant and sampled at 12, 22, and 42 DAI by employing 20 plants per treatment (replications).

Procedures. Seed of the two near-isolines (BC2) were soaked in tap water overnight. The presoaked seeds were planted in 10-cm pots in the greenhouse and inoculated with 5000 RKN eggs per pot at the time of planting. Twenty additional plants of each line were uninoculated. Roots of each treatment were gently washed with tap water to remove the soil. The roots of whole plants were stained with Phloxine B, and the presence or absence of egg masses was determined at 12, 22, and 42 DAI. No egg masses were present at 12 DAI in inoculated plant roots; however, numerous galls were present in both the resistant and susceptible lines. Galls were smaller in roots of resistant plants. Dates of harvesting of roots were at 12, 22, and 42 DAI. This test was repeated on three occasions over a one year period.

Analytical Procedures. Association of Official Analytical Chemists (AOAC) methods (Horwitz, 1975) were used for the following analyses: total solids (moisture), 14.083; crude fat, 14.019; crude fiber, 14.118; acid detergent fiber, 7.056, 7.057, ash, 14.114; total protein, 2.049 (% N × 6.25); nitrogen-free extract (NFE) by difference from 100%. AOAC methods were also used for the analysis of acid detergent fiber, 973.18, and lignin (by loss on ignition), 973.18C (Helrich, 1990).

Neutral detergent fiber was determined according to the methods of Van Soest and Wine (1967). From these procedures, lignin, cellulose, and hemicellulose were determined directly, and soluble cell wall contents were determined by difference from 100%.

Amino acid analyses were performed according to the AOAC Official Method "Protein Efficiency Ratio", 982.30 (Cunniff, 1997). The calculation of amino acid molar ratio is described in footnotes *c* and *d* of Table 1.

Computational Methods. Chemical analyses were performed in duplicate on freeze-dried root tissues collected in the above-described three tests. Tabular data are averages of the tests.

RESULTS AND DISCUSSION

Table 1 presents the results of an analysis of the major categories of constituents present in cotton roots from RKN-susceptible uninoculated (SU), RKN-susceptible inoculated (SI), RKN-resistant uninoculated (RU), and RKN-resistant inoculated (RI) plants 12, 22, and 42 DAI. These DAI are representative of when effects of the two genes for resistance have been visually observed to be manifested. The least significant difference (LSD 0.05) values are shown for each DAI entry group and also for overall at the bottom of Table 1.

Table 1. Root Constituents of an RKN-Susceptible and an RKN-Resistant Cotton Line before and after Inoculation[a,b]

	%, dry wt						
sample[c]	protein	lignin	hemi-cellulose	cellulose	ash	fat	NFS
12 DAI							
RU-12	18.5	10.2	9.4	27.6	12.4	3.0	18.9
RI-12	19.2	10.0	8.2	27.3	12.1	2.8	20.4
SU-12	17.1	9.9	11.0	28.5	12.2	2.9	18.4
SI-12	18.6	10.0	7.1	28.7	12.2	2.8	20.6
mean	18.4	10.0	8.9	28.0	12.2	2.9	19.6
LSD[d] 0.05	0.6	NS	1.2	NS	NS	NS	NS
22 DAI							
RU-22	13.0	10.0	12.1	29.3	16.8	2.6	16.2
RI-22	14.4	10.0	11.3	28.2	15.9	3.8	16.4
SU-22	13.2	10.0	10.0	33.7	11.1	2.8	19.2
SI-22	14.7	10.0	11.0	31.7	11.9	3.2	17.5
mean	13.8	10.0	11.1	30.7	13.9	3.1	17.3
LSD 0.05	0.4	NS	NS	NS	NS	0.4	0.1
42 DAI							
RU-42	7.2	10.0	12.9	36.6	9.1	1.9	22.3
RI-42	7.8	10.0	12.4	34.8	9.6	1.7	23.7
SU-42	7.3	9.8	12.8	38.5	7.9	2.1	21.6
SI-42	9.6	10.0	13.7	32.3	10.9	3.4	20.1
mean	8.0	10.0	13.0	35.6	9.4	2.3	21.9
LSD0.05	0.1	NS	NS	1.9	1.8	NS	NS
overall DAI							
mean	13.9	10.1	11.2	31.1	12.9	2.5	19.2
LSD 0.05	0.3	1.1	*e*	1.5	1.4	0.2	0.7

[a] Association of Official Analytical Chemists (AOAC) methods (Horwitz, 1975; Helrich, 1990). Analyses in duplicate. [b] Cotton near-isolines M249RNR [RKN highly resistant, 95-577, Blk(x)] and MS Sel. S213 [RKN highly susceptible, GH923-22-1,2,3,4, Blk(x), 1996 seed]. [c] SU, susceptible uninoculated; SI, susceptible inoculated; RU, resistant uninoculated; RI, resistant inoculated at 12, 22, and 42 days after inoculation. [d] LSD, least significant difference, $P = 0.05$, within DAI and across DAI, no interaction. NS, nonsignificant at $P = 0.05$. [e] Significant interaction of DAI × hemicellulose; therefore, an overall LSD is not appropriate.

Some trends were observed in levels of all treatments of root constituents sampled at 12, 22, and 42 DAI. With increasing age, protein was decreased while hemicellulose (8.9−13.0%) and cellulose (28.0−35.6%) were increased. No clear trends were apparent in the levels of lignin, ash, fat, and nitrogen-free solubles (NFS), although ash and fat were up at 22 DAI and then down at 42 DAI, whereas NSF was down at 22 DAI and then up at 42 DAI.

When treatments were compared, protein was higher in both SI and RI (inoculated) roots at all three dates (12, 22, and 42 DAI), and the trend toward higher levels of protein in SU and SI roots was most evident at 22 and 42 DAI. Cellulose was decreased in SI and RI roots at 42 DAI relative to SU and RU roots. This same trend was observed at 12 and 22 DAI, but LSD 0.05 values were not significant (Table 1).

Table 2 presents data on the amino acid compositions of SU, SI, RU, and RI root tissue at 42 DAI (Cunniff, 1997). It was not possible within the context of the study to harvest sufficient root tissues for analysis at the earlier dates.

The total amino acid contents as determined by summation was slightly lower in each category (SU, SI, RU, RI) than the Kjeldahl protein analyses, but they were parallel. Total amino acids were 27% higher in SI roots than in SU roots and 15% higher in RI roots than in RU roots. The lesser increase in RI roots may be indicative of a lower rate of protein biosynthesis in response to infection by the RKN.

Table 2. Amino Acid Compositions of an RKN-Susceptible and an RKN-Resistant Cotton Variety at 42 Days, before and after Inoculation; Percent and Molar Ratios (MR)[a,b]

	SU-42		SI-42		RU-42		RI-42	
amino acid	%[c]	MR[d]	%	MR	%	MR	%	MR
cystine	0.08	1.1	0.13	0.9	0.09	1.3	0.08	0.5
tyrosine	0.06	1.0	0.11	1.0	0.07	1.3	0.08	0.8
tryptophan	0.06	1.0	0.14	1.1	0.06	1.0	0.12	1.0
methionine	0.05	1.1	0.09	1.0	0.05	1.1	0.09	1.0
histidine	0.09	2.0	0.15	1.6	0.08	1.8	0.12	1.3
arginine	0.20	3.9	0.32	3.1	0.18	3.6	0.24	2.3
phenylalanine	0.20	4.2	0.29	2.9	0.19	4.0	0.21	2.1
isoleucine	0.19	5.0	0.29	3.7	0.19	5.0	0.20	2.6
proline	0.18	5.4	0.27	3.9	0.16	4.8	0.19	2.8
valine	0.23	6.8	0.30	4.3	0.22	6.5	0.24	3.4
threonine	0.25	7.2	0.22	6.3	0.33	4.6	0.25	3.5
leucine	0.34	8.9	0.43	5.5	0.32	8.4	0.43	4.9
serine	0.36	10.2	0.36	5.7	0.27	8.9	0.35	4.5
lysine	0.39	9.2	0.55	6.3	0.36	8.5	0.30	4.8
glycine	0.22	10.1	0.30	6.7	0.20	9.2	0.22	4.9
alanine	0.28	10.8	0.36	6.7	0.25	9.7	0.28	5.3
glutamic acid	0.54	12.7	0.69	7.8	0.49	11.5	0.54	6.1
aspartic acid	0.78	20.0	0.72	9.0	0.53	13.7	0.69	8.6
total amino acids	4.50		5.72		4.04		4.63	
total protein	7.3		9.6		7.2		7.8	

[a] Amino acid analyses performed in duplicate according to AOAC Official Method 982.30, Protein Efficiency Ratio. [b] SU, susceptible uninoculated; SI, susceptible inoculated; RU, resistant uninoculated; RI, resistant inoculated at 42 days after inoculation; see Table 1 for description of plant material. [c] Percent of root dry weight. [d] Step 1: %/mol wt = mol. Step 2: assign MR = 1 to amino acid with lowest molar concentration; MR of other amino acids are then relative to MR = 1.

Table 2 also presents mole ratios of the individual amino acids. Increased protein after infection may be inferred to be that which was biosynthesized as a response to the RKN. The molar ratios of Asp, Glu, Ala, Gly, Ser, and Leu were most evidently decreased in SI protein as compared with SU protein. Whether the higher level of protein in SI roots represents RKN-induced increased biosynthesis by the plant or is actually RKN protein derived from the increase in the RKN population cannot be determined from these data. The molar ratios of primarily the same amino acids were also decreased in the RI protein as compared with the RU protein. However, total amino acids in RI roots were increased by only slightly more than half of the increase in SI roots. Thus, it can be inferred that RKN had a lesser effect on the biosynthesis of protein in RI roots. Histological studies have shown a lesser effect of this nematode on root tissues resistant to the RKN. This evidence is somewhat similar to that of Callahan et al. (1998), who isolated a 14 kDa protein in RKN-resistant root tissue. They speculated that this protein is the product of a plant gene associated with resistance to the RKN.

In summary, the protein content of infected roots is higher than in uninfected roots in both susceptible and resistant lines at 42 DAI. Similar trends appeared to be developing at 12 and 22 DAI. Cellulose is also higher in SU and RU tissues relative to SI and RI tissues at 42 DAI. Amino acids in SI roots are higher than in SU roots, and the increase after infection is greater than in resistant tissue. The mole ratios of the individual amino acids in RKN infected roots are different from those in uninfected roots, indicative of stimulation of protein biosynthesis that is modified by RKN. Alternatively, this increase could be attributed in part to RKN protein itself.

LITERATURE CITED

Callahan, F. E.; Jenkins, J. N.; Creech, R. G.; Lawrence, G. W. Changes in cotton root proteins correlated with resistance to root knot nematode development. *J. Cotton Sci.* **1997**, *1*, 38−47.

Creech, R. G.; Jenkins, J. N.; Tang, B.; Lawrence, G. W.; McCarty, J. C. Cotton resistance to root-knot nematode: I. Penetration and reproduction. *Crop Sci.* **1995**, *35*, 365−368.

Cunniff, P., Ed. *Official Methods of Analysis of AOAC International*, 16th ed., 3rd revision; AOAC International: Gaithersburg, MD, 1997; Vol. II.

Fassuliotis, G. Plant resistance to root-knot nematodes. In *Nematology in the Southern Region of the United States*; Southern Cooperative Series Bulletin 267; Riggs, R. D., Ed.; U.S. GPO: Washington, DC, 1982; pp 33−49.

Hedin, P. A.; Callahan, F. E.; Dollar, D. A.; Creech, R. G. Total sterols in root-knot nematode infected cotton plant roots. *Comp. Biochem. Physiol.* **1995**, *111B*, 447−452.

Helrich, K., Ed. *Methods of Analysis of the Association of Analytical Chemists*, 15th ed.; Association of Official Analytical Chemists: Washington, DC, 1990; 1298 pp.

Horwitz, W., Ed. *Methods of Analysis of the Association of Analytical Chemists*, 12th ed.; Association of Official Analytical Chemists: Washington, DC, 1975; 1094 pp.

Jenkins, J. N.; Creech, R. G.; Tang, B.; Lawrence, G. W.; McCarty, J. C. Cotton resistance to root-knot nematodes: II. Post-penetration development. *Crop Sci.* **1995**, *35*, 369−373.

Khoshkhoo, N.; Hedin, P. A.; McCarty, J. C. Terpenoid aldehydes in root-knot nematode susceptible and resistance cotton plants. *J. Agric. Food Chem.* **1994**, *42*, 204−208.

McPherson, G. R.; Jenkins, J. N.; McCarty, J. C.; Watson, C. E. Combining ability analysis of root-knot nematode resistance in cotton. *Crop Sci.* **1995**, *35*, 373−375.

Sasser, J. N. Half a century dynamics and control of cotton diseases: Nematodes. In *Proceedings of the Beltwide Cotton Production Research Conference*, Las Vegas, NV, Jan 4−9, 1986; Brown, J., Ed.; National Cotton Council of America, Memphis, TN, 1986; pp 48−51.

Shepherd, R. L.; McCarty, J. C.; Jenkins, J. N.; Parrott, W. L. Registration of twelve nonphotoperiodic lines with root-knot nematode resistance primitive cotton germplasm. *Crop Sci.* **1988a**, *28*, 868−869.

Shepherd, R. L.; McCarty, J. C.; Parrott, W. L.; Jenkins, J. N. Resistance of cotton cultivars and elite breeding lines to root-knot nematodes. *Miss. Agric. For. Exp. Stn. Tech. Bull.* **1988b**, *No. 158*.

Tang, B.; Lawrence, G. W.; Creech, R. G.; Jenkins, J. N.; McCarty, J. C. Post-infection development of *Meloidogyne incognita* in cotton roots. *Miss. Agric. For. Exp. Stn. Tech. Bull.* **1994**, *No. 195*.

Veech, J. A. An apparent relationship between methoxy-substituted terpenoid aldehydesand the resistance of cotton to *Meloidogyne incognita*. *J. Nematol.* **1978**, *24*, 81−87.

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