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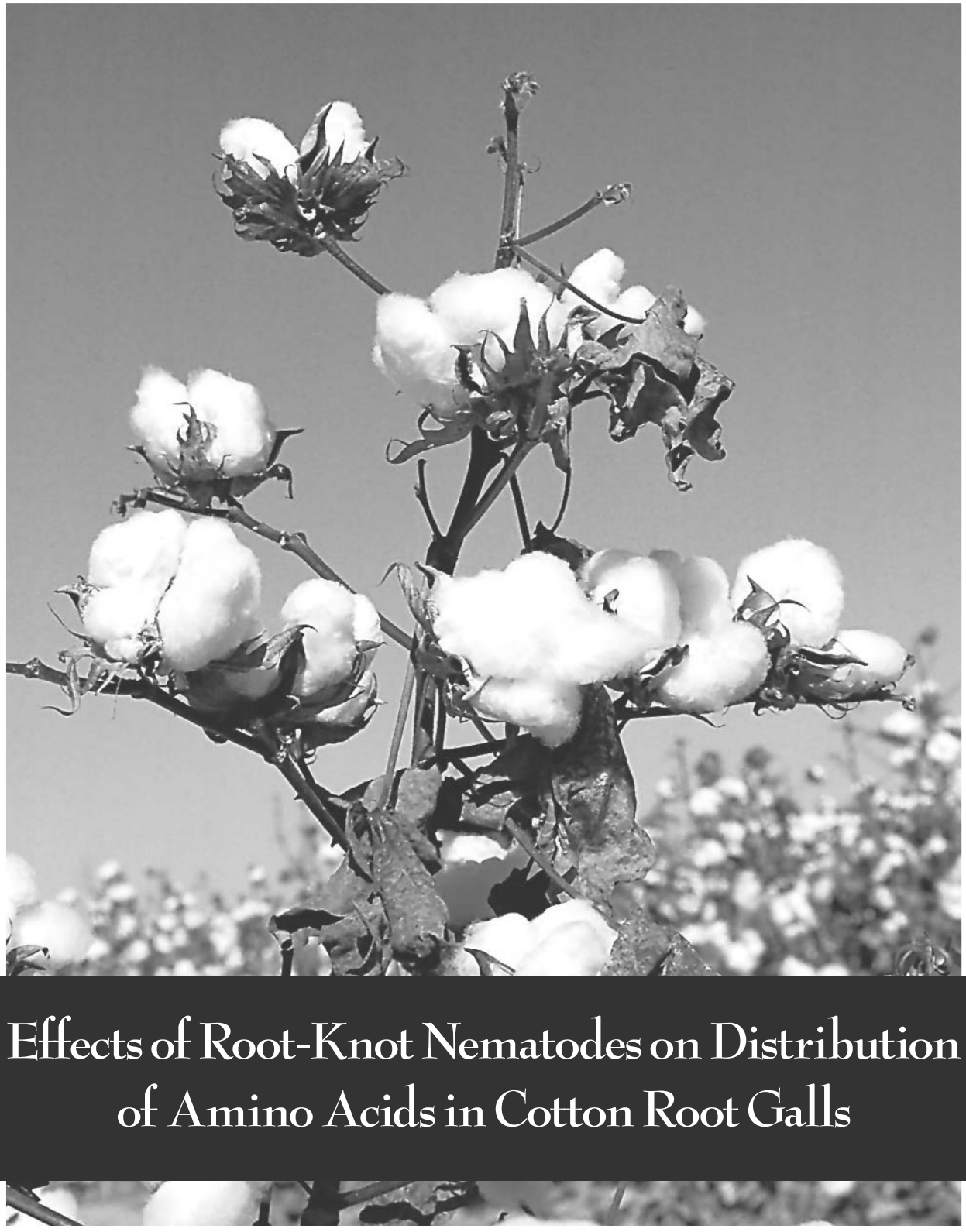

Effects of Root-Knot Nematodes on Distribution of Amino Acids in Cotton Root Galls

Experiment Station
Vance H. Watson, Director

Mississippi Agricultural & Forestry Experiment Station
Malcolm A. Portera, President • Mississippi State University • J. Charles Lee, Vice President

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Paul A. Hedin
Crop Science Research Lab
U.S. Department of Agriculture
Agricultural Research Service

Roy G. Creech
Department of Plant and Soil Sciences
Mississippi State University

For more information, contact Dr. Hedin by telephone at (662) 320-7428. This publication was published by the Office of Agricultural Communications, a unit of the Division of Agriculture, Forestry, and Veterinary Medicine at Mississippi State University. June, 2001

Abstract

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 are susceptible 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. This study indicated that 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 that of uninfected roots, and changes due to infection were greater in a susceptible line than in a resistant line. The amino acid contents in RKN eggs and their mole ratios were also determined. They were similar but not identical to the mole ratios of the total protein amino acids of SI roots.

Key Words: Cotton, *Gossypium* spp., Root-Knot Nematode, *Meloidogyne incognita*, Amino Acids, Protein

Effects of Root-Knot Nematodes on Distribution of Amino Acids in Cotton Root Galls

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, 1988b) reported that some RKN-resistant cultivars contained from 1,200 to 5,000 eggs per plant, whereas susceptible lines contained from 6,000 to more than 100,000 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, 1988b).

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. 1995) 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 suggesting 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 susceptible lines, nematode development is arrested in the resistant lines soon after infection.

The formation of giant cells in galls following infection with RKN suggested changes in protein metabolism of the root that was 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.

In a recent study (Hedin and Creech 1998), 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 suggest protein synthesis in roots was modified by RKN. Alternatively, increases in protein content may be attributed in part to RKN protein.

In the study described in this bulletin, root-knot nematodes were harvested in sufficient quantity to permit an analysis of their amino acids. To supplement the information previously obtained of the amino acid contents of susceptible and resistant lines of cotton at 42 DAI, similar analyses were performed at 22 days after inoculation. If the mole ratios of the amino acids in susceptible, inoculated roots (SI-22) were very similar to those of the root-knot nematode, it could suggest that the RKN had become closely associated with amino acid metabolism in the root galls.

MATERIALS AND METHODS

Plant Genotypes. Cotton near-isolines M249RNR (RKN highly resistant, 95-577, bulk selfs) and MS Sel. 213 (RKN highly susceptible, GH1923-22-1,2,3,4, bulked selfs, 1996 seed) were utilized with 20 plants per treatment of each line for each inoculation treatment of three sampling dates.

RKN Eggs. Surplus eggs from other activities were frozen as they became available. After thawing, they were separated from debris in water by repeated swirling and settling. The isolated eggs were inspected by transfer to a slide and viewed under magnification. The egg isolate was then freeze dried to yield 0.1799 grams.

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 analysis of variance (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 5,000 eggs per plant and sampled at 12, 22, and 42 days after inoculation (DAI), employing 20 plants per treatment (replications).

Procedures. Seed of the two related isolines (BC2) were soaked in tap water overnight. The presoaked seeds were planted in 10-centimeter pots in the greenhouse and inoculated with 5,000 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 1-year period.

Analytical Procedures. Association of Official Analytical Chemists (AOAC) methods (Horwitz 1975) were used for the several root 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 x 6.25); and nitrogen-free extract (NFE) by difference from 100%. AOAC methods were also used for analysis of acid detergent fiber, 973.18; and lignin (by loss on ignition), 973.18C (Helrich 1990).

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

Amino acid analyses were performed on RKN eggs and with plant root tissue according to the AOAC Official Method “Protein Efficiency Ratio,” 982.30 (Cunniff 1997). The calculation of amino acid molar ratio is described in footnote 4 of Table 2.

Computational Methods. Analyses were performed in duplicate on freeze-dried root tissues. Tabular data were averages of the tests.

RESULTS

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.

Some trends were observed in levels of all treatments of root constituents sampled at 12, 22, and 42 DAI. With increasing age, protein decreased while hemicellulose (8.9 - 13%) and cellulose (28 - 35.6%) increased. No clear trends were apparent in the levels of lignin, ash, fat, or nitrogen-free solubles (NFS). However, ash and fat levels 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 categories were compared, protein was higher in both SI and RI (inoculated) roots at all three

Table 1. Root constituents of a RKN-susceptible and a RKN-resistant cotton line, inoculated and uninoculated. [1, 2]

Sample [3]	Percent, dry weight						
	Protein	Lignin	Hemicellulose	Cellulose	Ash	Fat	NFS [6]
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 [4] 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-12	7.2	10.0	12.9	36.6	9.1	1.9	22.3
RI-12	7.8	10.0	12.4	34.8	9.6	1.7	23.7
SU-12	7.3	9.8	12.8	38.5	7.9	2.1	21.6
SI-12	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
LSD 0.05	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	– [5]	1.5	1.4	0.2	0.7

[1]Methods of Analysis of the Association of Analytical Chemists (Horwitz 1975; Helrich 1990), Analyses in Duplicate.
[2]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].
[3]SU = Susceptible Uninoculated; SI = Susceptible Inoculated; RU = Resistant Uninoculated; RI = Resistant Inoculated; and DAI = Days After Inoculation.
[4]LSD = Least Significant Difference, P=0.05; within DAI and across DAI – no interaction. NS = Nonsignificant at P=0.05.
[5]Significant interaction of DAI x Hemicellulose; therefore, an overall LSD is not appropriate.
[6]NFS = Nitrogen Free Solubles.

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 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 22 DAI and of the RKN eggs (Cunniff 1997).

The total amino acid contents as determined by summation was slightly lower in each category (SU, SI, RU, RI) than the Kjeldahl protein analyses. Total amino acids were 53% higher in SI roots than in SU roots, and they were 14% lower in RI roots than in RU roots. This finding indicated that metabolism in the SI roots was more affected in response to infection by the RKN.

Table 2 also presents the total amino acid contents of RKN as determined by summation. An explanation for the lower content in comparison with that of the plant root tissue is not evident. However, the major goal of this study was to determine if the ratios were more similar to those of SI roots than the SU, RU, and RI roots. Unfortunately, no consistent relationship was evident at either 22 or 42 DAI.

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).

Table 2. Root amino acid compositions of RKN-susceptible and RKN-resistant varieties at 22 days, inoculated and uninoculated, and also of isolated root-knot nematodes.[1, 2]

Amino Acid	SU-22		SI-22		RU-22		RI-22		RKN	
	% [3]	MR [4]	%	MR	%	MR	%	MR	%	MR
Cysteine	0.08	1.1	0.10	0.9	0.09	1.3	0.09	1.3	0.09	1.2
Tryptophan	0.06	1.0	0.06	1.1	0.06	1.0	0.06	1.0	0.06	1.0
Tyrosine	0.41	2.0	0.29	1.6	0.25	1.4	0.22	1.3	0.19	1.4
Methionine	0.09	0.6	0.18	1.2	0.12	0.6	0.08	0.5	0.06	0.6
Histidine	0.18	1.0	0.35	1.5	0.20	1.0	0.17	0.9	0.06	1.0
Arginine	0.39	2.0	0.75	2.7	0.46	2.0	0.44	2.0	0.21	2.7
Phenylalanine	0.33	1.8	0.66	2.6	0.44	2.0	0.34	1.7	0.15	2.0
Isoleucine	0.39	2.8	0.73	4.0	0.45	2.6	0.40	2.6	0.17	3.0
Proline	0.45	3.2	0.84	4.3	0.45	3.1	0.37	2.8	0.21	4.0
Valine	0.56	3.6	0.75	4.3	0.54	3.6	0.47	3.5	0.23	4.3
Threonine	0.46	3.8	0.46	5.0	0.48	3.2	0.46	3.3	0.24	4.7
Leucine	0.60	4.2	0.84	4.3	0.70	4.1	0.60	3.9	0.25	4.3
Serine	0.50	4.6	0.92	6.0	0.52	4.0	0.43	7.9	0.23	5.0
Lysine	0.57	2.2	1.15	5.1	0.60	3.1	0.58	3.3	0.28	4.0
Glycine	0.38	5.4	0.71	7.1	0.41	4.8	0.35	4.5	0.23	7.7
Alanine	0.48	5.5	0.92	7.4	0.50	4.6	0.45	4.6	0.22	6.0
Glutamic Acid	1.17	7.4	1.74	7.8	1.30	6.7	1.16	6.7	0.58	8.7
Aspartic Acid	1.25	8.8	1.35	6.8	1.79	7.6	1.40	9.1	0.41	6.7
Total Amino Acids	8.35	–	12.80	–	9.21	–	8.07	–	3.87	–
Total Protein	13.20	–	14.70	–	13.00	–	14.40	–	–	–

[1]Amino acid analyses performed in duplicate by Association of Official Analytical Chemists, AOAC Official Method 982.30, Protein Efficiency Ratio.
[2]SU = Susceptible Uninoculated; SI = Susceptible Inoculated; RU = Resistant Uninoculated; and RI = Resistant Inoculated. See Table 1 for description of plant material.
[3]Percent of root dry weight.
[4]Molar Ratios (MR) calculated in two steps: Step 1 – Percent ÷ molecular weight = moles; and Step 2 – Assign MR = 1 to amino acid with lowest molar concentration. Then, MR of other amino acids are relative to MR = 1.

Further efforts to confirm that the 14 kDa plant polypeptide may be the product of a novel RKN-inducible plant gene cannot be developed by amino acid study.

Table 3 presents data on the amino acid compositions of SU, SI, RU, and RI root tissue at 42 DAI (Cunniff 1997). 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 3 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 increases in 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 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.

Table 3. Amino acid compositions of a RKN-susceptible and a RKN-resistant cotton variety at 42 days.[1,2]

Amino Acid	SU-42		SI-42		RU-42		RI-42	
	% [3]	MR [4]	%	MR	%	MR	%	MR
Cysteine	0.08	1.1	0.13	0.9	0.09	1.3	0.08	0.5
Tryptophan	0.06	1.0	0.11	1.0	0.07	1.3	0.08	0.8
Tyrosine	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	7.30	–	9.60	–	7.20	–	7.80	–

[1]Amino acid analyses performed in duplicate by Association of Official Analytical Chemists, AOAC Official Method 982.30, Protein Efficiency Ratio.
[2]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.
[3]Percent of root dry weight.
[4]Molar Ratios (MR) calculated in two steps: Step 1 – Percent ÷ molecular weight = moles; and Step 2 – Assign MR = 1 to amino acid with lowest molar concentration. Then, MR of other amino acids are relative to MR = 1.

SUMMARY

The root-knot nematode (RKN) is a sedentary endoparasite that retards the growth, development, and yield of cotton by attacking the root system, causing galling and stunting. Although present commercial cultivars are generally susceptible to the RKN, research has led to the development of resistant germplasm.

The study discussed in this bulletin demonstrated that the protein content of susceptible, infected roots increased more than did that of resistant, infected roots. The protein content of infected roots was 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 was also higher in SU and RU tissues relative to SI and RI tissues at 42 DAI. Amino acids in SI roots were higher than in SU roots, and the increase after infection was greater than in resistant tissue. The mole ratios of the individual amino acids in RKN-infected roots were different from those in uninfected roots, indicative of stimulation of protein biosynthesis modified by RKN. Alternatively, this increase could be attributed in part to RKN protein itself.

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