

Free Amino Acids in Roots of Infected Cotton Seedlings Resistant and Susceptible to Meloidogyne incognita¹

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Abstract: Quantities of free amino acids in segments of cotton roots resistant and susceptible to *Meloidogyne incognita* were compared. Following infection, the root-knot susceptible cultivar, M8, had greater percentage increases of certain individual free amino acids than the resistant cultivar, Cleve-wilt, but the sum total of free amino acids was greatest in the resistant cultivar. More free amino acids were present in infected than in noninfected plants of both cultivars. The overall concn of glycine declined over the 10-day period following inoculation. The concns of the aromatic amino acids, tyrosine and phenylalanine, varied as functions of infection, cultivar, and time of harvest. Proline in susceptible M8 increased nearly 2000-fold 10 days after infection, when considerable thickening of syncytial walls is occurring. **Key Words:** resistance, root-knot nematode, cell-wall metabolism.

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Resistance in cotton, *Gossypium hirsutum* L., to *Meloidogyne incognita* (Kofoid & White) Chitwood has been characterized by three types of host-parasite reactions: retarded gall development, failure of most of the nematodes to reach maturity, and the development of fewer and smaller galls on resistant plants which bear fewer egg sacs than those on susceptible plants. Reduced pathogenesis also may result from nematode immobilization due to toxins produced in the

area of infection, and from the failure of nematodes to influence the formation of syncytia (2, 14, 18).

Several investigations of the biochemical and physiological relationships of *M. incognita* to its hosts have been conducted. Myuge (19) found higher concns of amino acids in root-knot-induced galls of tomato than in nongalled roots. Galls on tomato and cucumber roots contained four times as much protein as nematode-free roots. Infection by *Ditylenchus dipsaci* (11) and *Radopholus similis* (8, 9) also is known to cause alterations in the amino acid content of their respective hosts.

Sayre (22) proposed that enzymes from root-knot nematodes liberate tryptophan from protein complexes and that this compound, converted to indoleacetic acid (IAA) could be responsible for galling. Although it is not known whether amino acids such as tryptophan are important in galling or syncytial formation, tryptophan has long been established as a precursor of IAA (24).

Free amino acid content of tissues has been related to the susceptibility or resistance of plants to various other pathogenic organisms. Cystine (12) and alanine are thought to play a role in resistance to diseases caused by *Fusarium* sp. (10). A relationship also exists

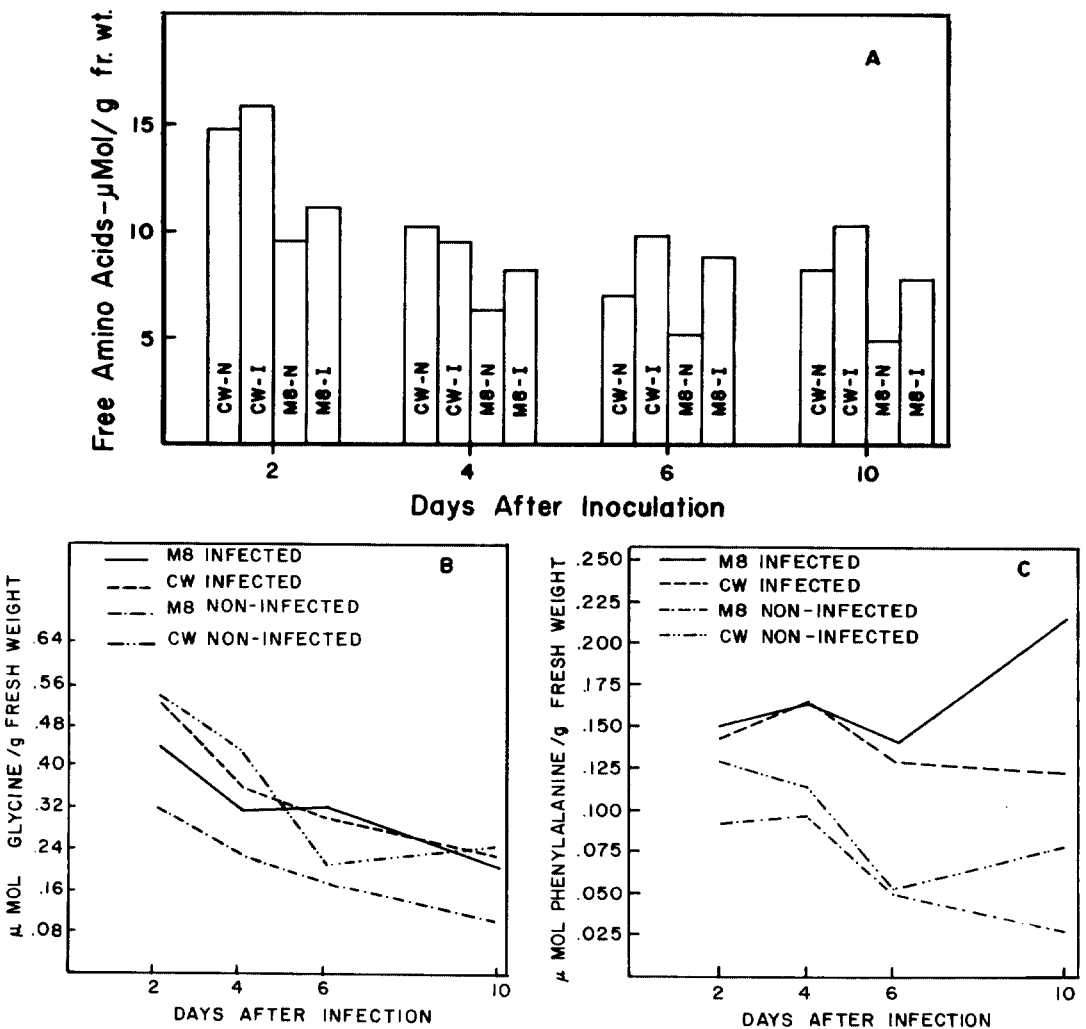


FIG. 1. Free amino acids of resistant (CW) and susceptible (M8) control cultivars following infection (I) with *Meloidogyne incognita*: A) Total free amino acids at intervals following inoculation. N = noninoculated controls, CW = Cleve wilt 6-3-5 cotton; B) Concentration of glycine in roots of susceptible (M8) and resistant (CW) cotton; C) Concentration of phenylalanine in roots of susceptible and resistant cotton.

between the level of alanine and susceptibility to *Verticillium* by cotton (1, 23). Chiang and Nip (3) observed higher levels of alanine, asparagine, and tyrosine in tissues of cabbages resistant to clubroot.

We report the occurrence of 17 free amino acids in infected and noninfected roots of cotton plants resistant and susceptible to the root-knot nematode, *M. incognita*.

MATERIALS AND METHODS

Meloidogyne incognita from a single egg mass (14) was propagated in the greenhouse on chile pepper, *Capsicum frutescens* L., and second stage larvae were obtained by Lownsbery and Viglierchio's method (13).

Larvae were treated for 12 h in a modified Baermann funnel containing 13 mg of Aretan (5) in 100 ml sterile distilled water. That was followed by a thorough rinsing of the larvae on a (400-mesh) sieve with sterile distilled water.

Seeds of resistant (Clevewilt 6-3-5) and susceptible (M8) cotton cultivars (14) were treated for 20 min in 20% household bleach and rinsed with sterile distilled water until the eluate was neutral to litmus paper. Germination and inoculation procedures of McClure and Robertson (17) were used to produce 2-cm segments of cotton roots containing 50-100 nematodes. Seedling

segments treated similarly but not infected served as controls.

One gram of fresh root tissue (40 segments from 40 roots) was harvested from each treatment 2, 4, 6, and 10 days after inoculation and ground to a fine powder in liquid nitrogen with a mortar and pestle. Three milliliters of sterile distilled water were added with further grinding and the mixture was filtered through cheesecloth. The filtrate was mixed with 0.75 ml of 15% sulfosalicylic acid, allowed to stand for 1 h at room temp, and centrifuged at 10,000 g for 30 min. Analyses of the supernatant were performed on a Beckman Model 120 C automatic amino acid analyzer according to the procedures given by the manufacturer. Each experiment was performed twice and the results averaged.

RESULTS

Total free amino acids of noninfected and infected root segments generally declined during the 10 days after inoculation (Fig. 1-A). During this period, the concn of free amino acids in infected segments usually exceeded that in noninfected segments. Differences in amino acid content between infected and noninfected roots were greater in the susceptible M8 cultivar than in the resistant Clevewilt cultivar. Maximum differences occurred 6 days after inoculation.

TABLE 1. Changes in amino acid concentration of resistant (CW = Clevewilt 6-3-5) and susceptible (M8) cotton cultivars expressed as amino acid concn ($\mu\text{mole/g}$ fresh weight) in healthy tissue relative to that in infected tissues. Negative values indicate percentage decreases following infection, positive values percentage increases.

	% Change - Infected/healthy							
	Days After Inoculation							
	2		4		6		10	
	CW	M8	CW	M8	CW	M8	CW	M8
Tryptophan	0	35	6	7	63	54	8	152
Lysine	- 6	16	95	64	96	64	64	242
Histidine	32	26	107	127	277	283	95	433
Arginine	6	23	52	38	15	31	- 42	3
Aspartic Acid	21	13	- 14	2	28	83	49	42
Threonine	19	- 5	- 19	- 23	187	28	118	40
Serine	6	18	- 31	28	16	97	5	- 20
Glutamic Acid	23	56	26	47	116	198	230	287
Proline	- 3	-44	- 4	32	110	93	52	1775
Glycine	- 3	32	- 10	35	41	85	- 4	88
Alanine	-13	67	77	105	138	339	29	277
Valine	-18	32	53	51	76	95	3	588
Methionine	-21	57	81	67	175	0	125	550
Isoleucine	-12	11	49	45	90	56	60	488
Leucine	-18	17	66	38	75	52	107	260
Tyrosine	-13	2	32	25	69	50	- 16	188
Phenylalanine	11	62	47	73	152	194	56	693

Concentrations of individual amino acids also changed after infection. The magnitude and direction of these changes were dependent upon the amino acid and the time of harvest following inoculation (Table 1). Initially, more than half of the amino acids decreased in resistant Cleve wilt infected with *M. incognita*. By the 6th day after inoculation, the concn of all amino acids in resistant roots had increased and exceeded those in their noninfected counterparts.

Changes in individual amino acids following infection were maximum 10 days after inoculation in both cultivars. However, for most amino acids, greater percentage increases occurred in susceptible M8 than in resistant Cleve wilt. After 10 days, the greatest changes in Cleve wilt were recorded for glutamic acid, methionine, threonine, and leucine; whereas, in M8, the amino acids which had increased most in the infected roots were proline, phenylalanine, valine, methionine, isoleucine, and histidine.

When absolute quantities of amino acids were compared, some amino acids (such as aspartic acid and arginine) were consistent within cultivars, and were relatively unchanged by infection or time of harvest. Others, such as glycine (Fig. 1-B) had an overall decline after inoculation, following the general trend of total free amino acids. In the case of glycine, the lowest values were recorded for noninoculated M8. Infection appeared to have little effect on the glycine content of Cleve wilt.

Concentrations of the aromatic amino acids, phenylalanine, and tyrosine responded as functions of both infection and time of harvest as well as the cultivar analyzed (Fig. 1-C). The greatest concn of phenylalanine was found in infected roots of the susceptible M8, 10 days after inoculation with the most pronounced increase occurring between the 6th and 10th days following inoculation. There was an overall decline in phenylalanine concn in the other treatments over the 10-day test period. However, the rates of decrease and the final concns differed markedly between treatments.

DISCUSSION

Calculations based upon known concns of free amino acids in larvae of *M. incognita* (16) show that larvae present in the infected roots would account for less than 0.1% of the amino

acids recovered in our analyses. Therefore, the direct contribution of the nematodes to the amino acid composition of the root is not significant, and consequently the greater quantity of free amino acids in infected than in noninfected tissues indicates a plant response to nematode infection. However, interpretation of biochemical changes in host-parasite complexes is difficult especially where obligate endoparasites are involved (11, 21).

Increased levels of free amino acids upon infection by root-knot nematodes has been noted by Owens and Specht (20), who found that free amino acids increased sharply in the galls of tomato. Increased levels of amino acids have also been found in galls of alfalfa caused by *Ditylenchus dipsaci* (11), and in galls initiated by *Longidorus africanus* on grapevine roots (6).

Changes in free amino acid content could occur in several ways. Howell and Krusberg (11) noted that increases in levels of free amino acids in galled vs. healthy tissues of alfalfa and pea could be due to increased translocation into the area of infection, increased rates of synthesis, decreased rates of translocation out of the gall, and/or decreased rates of breakdown. Cytochemical analyses by Owens and Specht (20) have shown that most of the increase of free amino acids in tomato infected with root-knot nematodes occurred in the syncytia rather than in the galls. Owens and Specht concluded that in tomato, accumulation of amino acids is due to synthesis in situ. Should this be true for cotton, the root-knot resistant Cleve wilt must synthesize proportionately more amino acids than the root-knot susceptible M8.

Some of the amino acids which increased in cotton roots following infection may be important in the host reaction to the nematode. The increase of phenylalanine in infected cotton roots may be due to its mobilization for phenol metabolism. Lower final concns of phenylalanine in all treatments except infected M8 10 days after inoculation could reflect its probable use in production of phenols. At least two phenols in cotton, scopoletin and gossypol, possess phytoalexin-like properties. However, the toxicity of these substances to nematodes is not known.

Increased production of tryptophan could result in greater quantities of auxin, since tryptophan is a known precursor of IAA (7). Cotton cultivars susceptible to *M. incognita*

form galls which consist of hypertrophic and hyperplastic cortical tissue. Associated with these galls and with nematode development is the presence of nematode-induced, multinucleate syncytia. These symptoms suggest the action of auxins or auxin-like materials, which are known to affect plant tissues in similar ways (24). Resistance of Cleve wilt to *M. incognita* is characterized by two reactions: (i) nematodes which penetrate but fail to develop; or (ii) galls which they induce are small or without detectable nematodes. Most nematodes which penetrate produce no visible swellings or syncytia (14). Production of galls and formation of giant cells are probably independent phenomena. However, both are initially characterized by cellular hypertrophy. Furthermore, many nuclei of the giant cells are enlarged. Such reactions are typical of auxin-mediated growth (4, 24, 26). This suggests that tryptophan levels could influence amounts of IAA present; however, it is not known from these studies whether tryptophan forms IAA in either cultivar or whether changes in tryptophan concn result from other perturbations in the host physiology.

Proline in some plants appears to be critically important in the formation of cell wall proteins and the extensibility of the cell wall may well be controlled by the amount of hydroxylation of the proline residues in it (25). In our analyses, thousandfold, and more, increases in the proline content of susceptible M8 were noted 10 days after inoculation with *M. incognita*. This interval corresponds to the time at which galls become macroscopically visible and during which secondary wall thickenings form around the nematode-induced syncytia (15). Since thin-walled syncytia and reduced galling are typical of the resistant Cleve wilt, our data suggest that the use of cotton cultivars infected with *M. incognita* could facilitate the understanding of cell-wall metabolism.

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