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SHORT COMMUNICATION

COMPARATIVE BIOCHEMISTRY OF COTTON RESISTANT AND SUSCEPTIBLE TO THE ROOT-KNOT NEMATODE*

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Key Word Index—*Gossypium hirsutum*; Malvaceae; comparative biochemistry; root constituents; *Meloidogyne incognita*; root knot nematode.

Abstract—Cotton varieties resistant and susceptible to the root-knot nematode, *Meloidogyne incognita*, have been compared by selected analyses of naturally occurring constituents of the roots. Quantitative data are presented on the occurrence of amino acids, free sugars, fatty acids, sterols, total lipids, total phenols and gossypol. No qualitative differences between varieties were detected.

THE USE of chemical compounds as specific indicators of the resistance of plants to nematodes offers several advantages to the plant breeder who is attempting to incorporate disease resistance into commercially desirable varieties.[1,2] Problems related to uniformity of inoculum or to incidence of 'escapes' could be avoided and, in many cases, screening and selection of resistant individuals could be accelerated by a rapid and specific chemical analysis. Chemical indicators of resistance to a wide variety of other pests and disease organisms have been demonstrated[3–5] but their usefulness in breeding programs has not been fully explored.

The purpose of the present investigation was to examine the presence of certain compounds which occur naturally in roots of cotton plants resistant and susceptible to the root-knot nematode, *Meloidogyne incognita*. With the exception of phenolic compounds, only non-infected roots were analyzed, since the primary objectives were to determine whether healthy plants differed sufficiently to establish a chemical basis for a rapid assay for resistance and to provide an insight into the chemical nature of resistance

RESULTS

Roots of healthy cotton plants resistant and susceptible to *M. incognita* differed little with respect to chemical composition (Tables 1 and 2 and text). Several minor quantitative differences occurred among the free amino acids, i.e. twice as much phenylalanine and 37% more alanine was found in the resistant than in the susceptible variety (Table 1). Conversely, the susceptible variety contained as much as 50% more lysine than did the resistant variety. No qualitative differences were apparent.

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Table 1. Concentration of total and free amino acids in roots of cotton plants susceptible and resistant to *M. incognita*

	Conc. (μM/g dry wt)			
	Clevewilt-6 (resistant)		Deltapine smooth leaf (susceptible)	
Amino acid	Total	Free	Total	Free
Lysine	77·0	2·4	73·7	3·6
Histidine	1·8	0·7	1·9	0·8
Arginine	43·0	1·0	45·5	1·5
Aspartic acid	124·3	5·2	135·5	5·5
Threonine	44·5	13·6	52·0	13·0
Serine	60·0	Trace	77·5	Trace
Glutamic acid	111·0	5·0	133·7	5·1
Proline	59·5	4·2	61·5	3·8
Glycine	101·2	4·0	105·3	3·3
Alanine	107·8	17·6	100·8	12·8
Cysteine	Trace	Trace	Trace	Trace
Valine	70·3	5·4	74·7	5·2
Methionine	Trace	Trace	Trace	Trace
Isoleucine	55·0	3·2	56·0	3·3
Leucine	92·5	4·5	90·0	4·5
Tyrosine	13·5	2·0	13·2	2·0
Phenylalanine	39·5	3·4	42·0	1·7

Sucrose was the primary sugar constituent of cotton roots (87 and 82 mg/g dry wt in resistant and susceptible respectively) and along with fructose (5·0 and 6·4 mg/g) and glucose (18·6 mg/g in each) accounted for the major portion of the free sugars. Five additional compounds in the free sugar fraction were detected but not identified since they accounted for less than 1% of the total sugars detected by GLC.

Visual examination of neutral lipids separated by TLC indicated that sterols were the major lipid constituent (Table 2). Triglycerides, diglycerides (1,3-and 1,2-diglycerides) sterol esters, free fatty acids and phospholipids were also detected but not determined quantitatively. No differences in lipid classes were noted between varieties. GLC and IR spectroscopy of unsaponifiable lipids showed that the sterol fraction consisted solely of campesterol and sitosterol. Of the total fatty acids, 21 GLC peaks were recorded and 13 identified. The predominant fatty acid in the roots of both varieties was palmitic followed by linolcic, linolenic and oleic.

Proteins, separated by acrylamide gel electrophoresis and visualized by staining with amido-black, were nearly indistinguishable with respect to origin. Entire root systems also were surprisingly similar with respect to total phenols and gossypol. Total phenols accounted for 1·1–1·3% of the dry weight regardless of variety and gossypol or gossypol-like substances 13% of the total phenols. There was little change on infection.

DISCUSSION

The present investigation was conducted to provide a basis from which to investigate further details of the biochemical nature of the resistance of plants to nematodes. Of particular interest was the striking similarity of the resistant and susceptible varieties. No

TABLE 2. CONCENTRATION OF FATTY ACIDS, STEROLS AND TOTAL LIPIDS IN ROOTS OF COTTON PLANTS SUSCEPTIBLE AND RESISTANT TO *M. incognita*

Fatty acid*, sterol or total lipid	Conc mg/g dry wt	
	Clevewilt-6 (resistant)	Delta pine smooth leaf (susceptible)
9:0	0·15	0·15
12:0	Trace	0·03
14:0	0·07	0·08
15:0	0·10	0·16
16:0	4·96	4·01
16:1	0·18	0·16
17:0	0·14	0·12
18:0	0·34	0·29
18:1	1·17	0·94
18:2	2·19	2·53
18:3	1·47	1·82
20:0	Trace	Trace
20:1	0·41	0·46
Campestreol	0·91	0·71
Sitosterol	0·91	0·91
Total lipid	33·10	32·40

* First number refers to the number of carbon atoms per molecule; second number to the number of double bonds.

qualitative differences were detected and the quantitative differences observed were of limited magnitude. The signifiacnce of these variations is, at present, a matter of speculation. The higher phenylalanine levels which occurred in the free amino acid fraction of the resistant variety could be indicative, for instance, of an altered phenylalanine metabolism. Phenylalanine is a known precurser for the phytoalexin, pisatin, which occurs in pea plants infected with *Monilinia fructicola.*[6]

If an altered phenolic metabolism is associated with resistance in cotton, however, it was not reflected in the gossypol and total phenolic content of healthy or infected roots. Since it is known that gossypol, a phytoalexin peculiar to cotton and its relatives, forms in all tissues of the cotton plant in response to pathogens, the above observation would appear to be an anomaly.

Root-knot nematodes, on the other hand, do not infect cotton roots systemically. More than 90% of the nematodes penetrate within the terminal 1–2 cm of the root tip. If single cells or small groups of cells respond as a unit to infection with the production of gossypol or other phenols then such a response may not be detected by the analysis of entire root systems. Histochemical analyses and chemical examination of root tips healthy and infected with *M. incognita* are now underway in this laboratory.

EXPERIMENTAL

Seeds of the resistant variety, *Gossypium hirsutum* L. var. Clevewilt-6 were increased by self pollination and bulk harvest. A single lot of the susceptible variety, *G. hirsutum* var. Deltapine Smooth Leaf was purchased from commercial sources. Roots for all analyses, except those for phenolic and protein determination were harvested from 4-week-old plants reared hydroponically in Hoagland's solution. After harvest, roots were washed in H_2O, frozen in liquid N_2, lyophilized and stored under dry N_2 at $-20°$. Prior to analysis, 1 g of root tissue was ground to pass a 40-mesh seive and immediately placed in the extracting solvent. Phenolic

[6] L. A. HADWIGER, *Phytopathol.* **57**, 1258 (1967).

determinations were made on fresh roots of 10-day-old seedlings grown in quartz sand and proteins were extracted from seeds or from radicals of 72-hr-old seedlings which had been germinated at 28° in rolled germination tubes. Roots were infected by the addition of a suspension of 5000 nematodes per plant.

Amino acid analyses were performed on a Beckman automatic amino acid analyzer.[7] Soluble sugars and lipids were analyzed by TLC and/or GLC chromatography.[8–10] Total phenols were examined by the method of Swain and Hillis,[11] gossypol according to Bell[12] and proteins by the method of Davis.[13] Two or more analyses were performed for each class of compounds. All replicates were consistent, and the results were averaged.

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