Biochemical Changes in Terminal Root Galls Caused by an Ectoparasitic Nematode, *Longidorus africanus*: Amino Acids[1]

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Abstract: The amino acids of terminal root galls caused by *Longidorus africanus* on bur marigold (*Bidens tripartita* L.) and grapevine (*Vitis vinifera* L.) were studied. The galled roots of bur marigold contained 73% more cell-wall protein and 184% more free amino acids. The main changes among the free amino acids of the galled tissue were a large increase (1900%) in proline and a decrease in aspartic acid (56%) compared with the respective check tissue. Hydroxyproline decreased in the wall protein fraction from 5.6% in the healthy tissue to 3.6% in the infected tissue.

Percent of hydroxyproline in total amino acids of the wall protein fraction of grapevine roots decreased from 0.7% in the healthy tissue to 0.3% in the galled tissue, and total proteins of this fraction decreased from 9.5 mg to 4.5 mg, respectively. Total protein in the protoplasmic fraction also decreased from 3.0 mg in healthy to 1.0 mg in infected roots. No change was noticed in total proteins in the free amino acids fraction but free proline decreased 40% in the infected roots.

The relationship of these differences to the specific reactions of the hosts to nematode feeding is discussed. *Key Words:* Proline, Hydroxyproline, Aspartic acid, Glutamic acid.

Studies of physiological activities in root galls caused by nematodes have been confined to those incited by endoparasites, particularly *Meloidogyne* spp. Unfortunately interpretations of results have been complicated by the presence of living sedentary nematodes (14). Similar research on root galls caused by ectoparasitic nematodes has not appeared, primarily because it has been difficult to culture the nematodes in a controlled system. Recently, however, culture techniques were developed for certain *Longidorus* spp. (4, 5), a genus which caused terminal swellings of host roots (3) which, however, are generally smaller than *Meloidogyne* galls (Fig. 1A, B). In preliminary studies on the physiology of terminal root galls caused by the ectoparasitic nematode, *L. africanus* Merny, unusually large quantities of amino acids were found in the galled tissues. This paper reports results of investigations on the kinds of amino acids found in such galled roots.

MATERIALS AND METHODS

Longidorus africanus was selected for its relatively short life cycle and ease of culture (4). An herbaceous host, bur marigold (*Bidens tripartita* L.), and a woody host, grapevine (*Vitis vinifera* L.) were used. Both plants are good hosts of the nematode (4) although their etiologies differ (6). Hand-picked specimens of *L. africanus* were reared on the host plants maintained at 21–24 C, alongside non-inoculated check plants, as previously described (4, 5).

Preliminary experiments with bur marigold showed that 30 days after inoculation the total free amino acids reach a maximum and symptoms were easily visible. Accordingly, 30-day-old infected and check plants of this host were used. After harvest, representative galled and healthy root tips (up to 1 cm) were excised and freeze-dried,

Received for publication 27 January 1971.

[1] Contribution No. 1802-E (1970 Series) from the Volcani Institute of Agricultural Research, Bet Dagan, Israel, supported in part by Grant No. FG-IS-223 from the U.S. Department of Agriculture.

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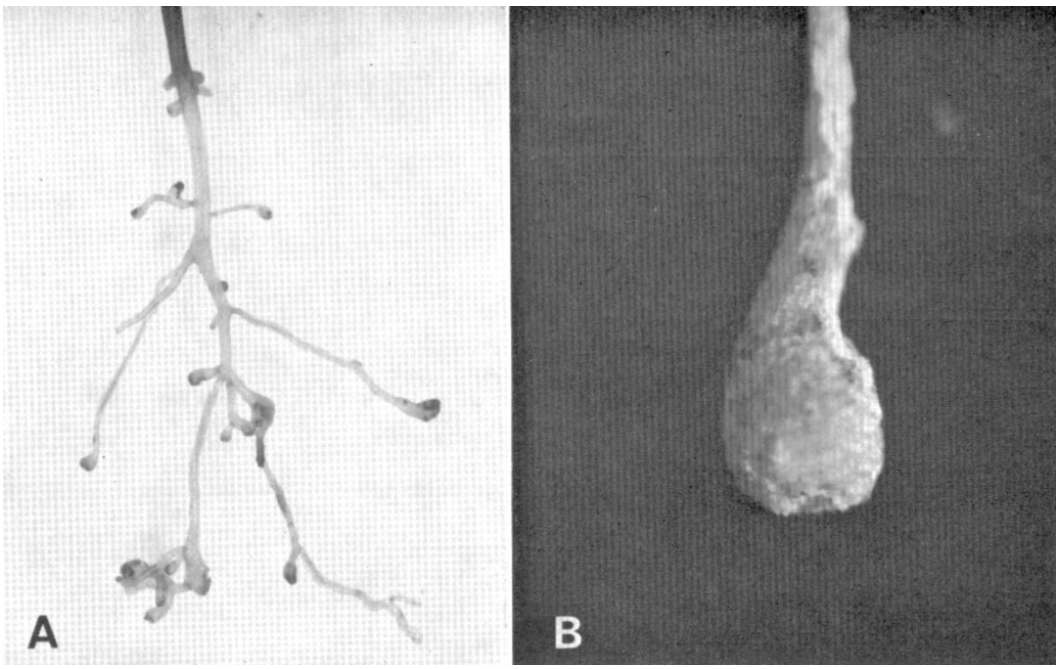


FIG. 1. Root galling of *Bidens tripartita* (bur marigold) by *Longidorus africanus*. **A.** Entire root system (×3); **B.** Closeup of single gall (×50).

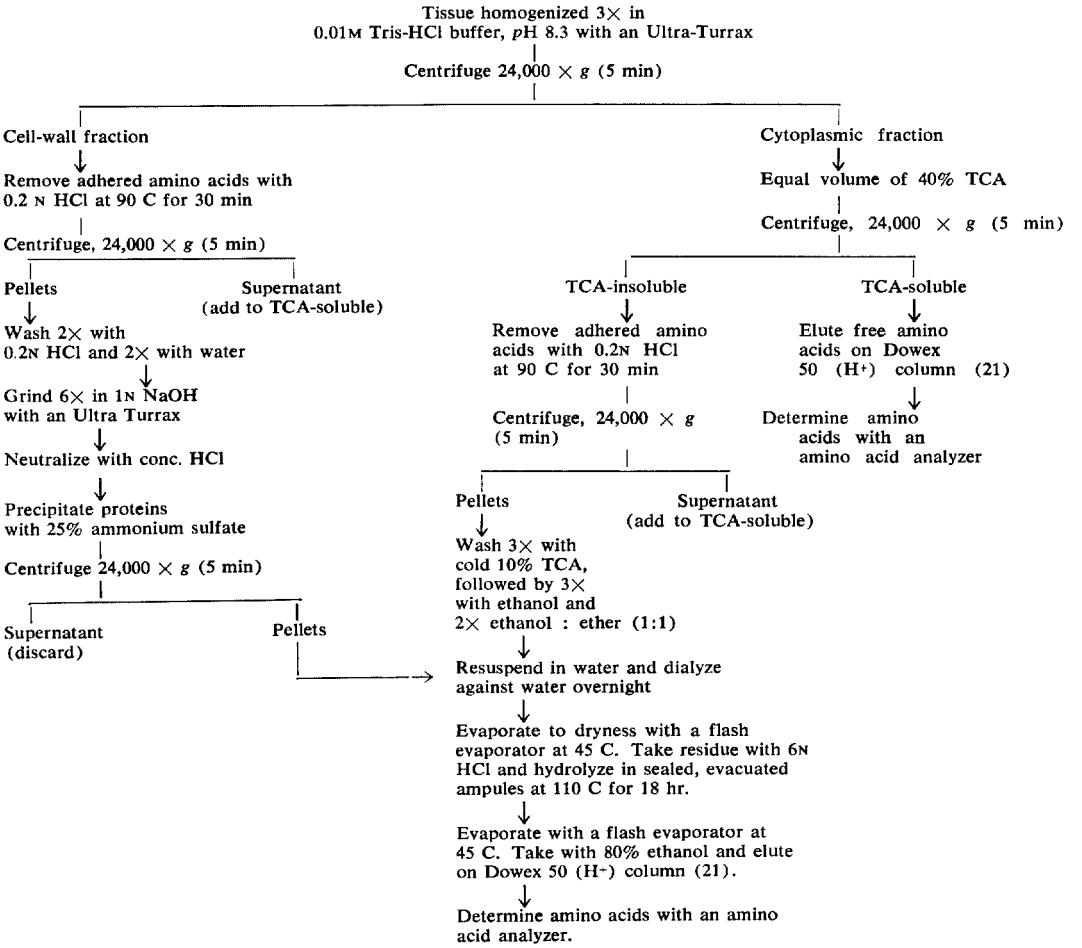
and 1 g of each extracted. The proteins were fractionated by the method of Olson (12) (Fig. 2). Amino acids were analyzed by a single-column accelerated method in a BC-200 (BioCal) amino acid analyzer. Hydroxyproline was determined separately on the same column at 30 C. The total amino acid content was calculated by summing the amino acids measured on the analyzer. Grapevines were harvested three months after rooted cuttings were inoculated, and root tip samples were taken and treated in the manner described above. Amino acids in the grapevine-root extracts were determined colorimetrically; total amino acids by the method of Baxter (2), proline by the method of Ough (13) and hydroxyproline according to Hutterer and Singer (9).

RESULTS

The total free amino acid content of the galled tissue of bur marigold exceeded that of the respective check tissue by 184% (Table 1). Proline was the most prominent amino acid in the infected tissue and showed an increase of 1900% over proline in the healthy tissue. Glutamic acid concentration increased by 270% in the infected roots. However, on the basis of percentage composition of the total amino acids, glutamic acid is 8.9% and 6.8% in healthy and infected roots, respectively, which was not a large difference. Aspartic acid was the only free amino acid which decreased (56%) in the infected roots.

In the protoplasmic protein fraction (Table 2) differences between healthy and infected tissues were small, both in total protein and individual amino acids. The cell-wall fraction of the galled tissue, on the other hand, showed an increase (73%) of protein content (Table 3). The largest increases among the wall protein amino acids were in methionine (167%), isoleucine (137%), leucine (127%) and tyrosine

FIG. 2. Scheme for extraction and fractionation of protein and free amino acids from roots.



(130%), and the smallest in hydroxyproline (19%). But on a basis of percent of total amino acids only methionine showed an increase (from 0.3 to 0.5%), while hydroxyproline decreased (from 5.5% in healthy tissue to 3.6% in infected tissue).

In grapevine (Table 4) the total protein decreased in the protoplasmic and cell-wall fractions of the infected tissue, but the amount of free amino acids remained unchanged. The quantity of proline in the free amino acid fraction, both in the healthy and infected tissue, was small and a decrease in all three fractions was detected in the infected tissue. However, on the basis of percent of total, proline increased from 3.0% to 5.2% in the protoplasmic fraction of the infected roots and only slightly decreased (from 1.0 to 0.6%) in the wall fraction. There was much less hydroxyproline (–84%) in the cell-wall protein of the galled tissue.

DISCUSSION

The outstanding feature of *L. africanus*-parasitized roots of *Bidens tripartita* is the

TABLE 1. Free amino acids in terminal galls caused by *Longidorus africanus* and in healthy root tips of bur marigold (μg/g dry weight).

Amino Acids	Galls		Healthy Root Tips		Increase or Decrease (%)
	% of total	μg/g	% of total	μg/g	
Aspartic acid	3.1	133	20.3	306	−56
Glutamic acid	8.9	382	6.8	103	271
Proline	43.0	1,840	6.1	92	1,900
Glycine	4.4	187	8.0	120	56
Alanine	14.4	614	17.7	267	130
Valine	6.3	269	10.1	152	77
Methionine	1.0	45	1.3	19	130
Isoleucine	2.8	118	4.1	62	91
Leucine	4.3	183	7.8	118	55
Tyrosine	4.2	181	9.0	136	33
Lysine	3.4	196	3.3	50	190
Histidine	2.5	108	3.1	46	133
Arginine	1.6	70	2.3	35	100
Total		4,276 mg		1,506 mg	184

large amount of free proline they contain (Table 1). Free proline in substantial quantities was also reported after infection by the nematode *Radopholus similis* in grapefruit seedlings (8), and by *Meloidogyne* spp. in tomato (14), in alfalfa tissue galled by *Agrobacterium tumefaciens* (17), in water-deficient wheat (7), Ladino clover (16) and Bermuda grass (1).

The simultaneous decrease of aspartic acid and increase of proline (Table 1) suggests a correlation between them. Work on

TABLE 2. Amino acids in protoplasm in protein hydrolyzate of terminal galls caused by *Longidorus africanus* and in healthy root tips of bur marigold (μg/g dry weight).

Amino Acids	Galls		Healthy Root Tips		Increase or Decrease (%)
	% of total	μg/g	% of total	μg/g	
Hydroxyproline	2.5	34	2.5	26	31
Aspartic acid	10.8	147	10.2	107	38
Threonine	6.3	86	5.5	57	51
Serine	6.2	84	5.7	59	42
Glutamic acid	11.6	158	11.9	124	27
Proline	3.7	51	4.3	45	13
Glycine	5.9	80	5.8	60	33
Alanine	6.5	88	6.5	67	31
Valine	6.6	89	5.7	60	48
Methionine	1.2	17	1.6	17	0
Isoleucine	6.3	85	6.1	64	33
Leucine	9.7	132	9.2	96	37
Tyrosine	2.3	31	3.7	39	−20
Phenylalanine	5.9	80	5.9	62	29
Lysine	7.8	106	8.9	93	14
Arginine	6.4	87	6.2	65	34
Total		1,355 mg		1,041 mg	30

TABLE 3. Amino acids in cell-wall protein hydrolyzate of terminal galls caused by *Longidorus africanus* and in healthy root tips of bur marigold (μg/g dry weight).

Amino Acids	Galls		Healthy Root Tips		Increase (%)
	% of total	μg/g	% of total	μg/g	
Hydroxyproline	3.6	85	5.5	71	19
Aspartic acid	11.4	265	12.4	161	65
Threonine	5.9	137	6.2	80	71
Serine	6.1	143	7.1	91	57
Glutamic acid	12.0	281	12.3	159	71
Proline	4.4	103	4.7	61	69
Glycine	6.4	150	6.4	83	81
Alanine	6.4	150	6.4	83	80
Valine	6.7	157	6.0	77	103
Methionine	0.5	12	0.3	4	167
Isoleucine	5.7	133	4.3	56	137
Leucine	9.8	228	7.8	100	127
Tyrosine	3.5	83	2.8	36	130
Phenylalanine	7.4	172	7.8	100	71
Lysine	5.8	136	5.5	71	91
Arginine	4.2	98	4.2	55	78
Total		2,333 mg		1,288 mg	73

the metabolism of radioactive proline in leaves showed that proline was converted to asparagine or glutamine (18) or to aspartic acid and glutamic acid probably via the tricarboxylic acid cycle (21). Stewart et al. similarly explained the accumulation of proline in wilting leaves (19), postulating a net synthesis from sugars via glutamic acid, and that proline serves as a readily-available storage compound in the moisture-deficient tissue. Gusev and Gordon (7), however, suggested that proline

TABLE 4. Free and protein-bound amino acids from different fractions of *Longidorus africanus*-infected and healthy grapevine roots (μg/g dry weight).

Fraction	Total amino acids (mg)	Proline			Hydroxyproline		
		μg/g	% of total	Decrease (%)	μg/g	% of total	Decrease (%)
Protoplasm infected	1.0	52.0	5.2		17.5	1.7	
Protoplasm healthy	3.0	90.0	3.0	–42	40.0	1.3	–81
Cell-wall infected	4.5	27.5	0.6		12.5	0.3	
Cell-wall healthy	9.5	92.5	1.0	–70	67.5	0.7	–84
Free amino acids, infected	0.85	15.0	1.8		0	0	
Free amino acids, healthy	0.80	25.0	3.1	–40	0	0	0

accumulation during drought is a result of protein hydrolysis. Since the water solubility of proline is greater than that of other amino acids, its accumulation enables osmotically-bound water to increase, which, in turn, enables water conservation during drought. The fact that in our work the protoplasmic protein content of galled and healthy tissues were similar (Table 2), whereas the cell-wall fraction of the galled tissue contained much more protein than the corresponding extract fraction of healthy tissue (Table 3), supports the hypothesis of Stewart, et al. (19) That is, that protein synthesis was stimulated in the infected tissue and that the amino acids were not simply released during hydrolysis of existing proteins.

Proline is a known precursor of hydroxyproline, an important constituent of the cell-wall protein (extensin) (10, 11). Enzyme inhibition in the pathway of extensin synthesis or a change in the rate of cell-wall formation might lead to proline accumulation. Our finding that hydroxyproline decreased in the wall fraction while free proline increased in the galls suggests that nematode activity may have interfered with the biosynthesis of the root cell walls.

Roberts and Baba (15) observed that coleus stem segments treated with proline after pretreatment with indole-3-acetic acid showed increased formation of xylem elements. They suggested that proline might act as a "xylogenic factor" that is immediately released by the ruptured vascular bundles when an intact plant is wounded. Cohn and Orion (6) found xylem elements scattered in galled tissue from *Longidorus*-infected plants. These observations, coupled with our finding that *L. africanus*-parasitized roots also contain more proline, support the contention that proline might play a role in xylogenesis.

The amino acid pattern of grapevine roots differed qualitatively and quantitatively from those of bur marigold. These differences are probably a result of the specific reactions of the two hosts to the feeding of the nematode. Cohn and Orion (6) observed that whereas *Longidorus* completely stopped growth in bur marigold and rendered the meristem non-functional, in grapevine the meristem continued to divide and produce a new apex. It appears, therefore, that in roots of grapevine the free amino acids may have been used for new growth, whereas in bur marigold these amino acids accumulated in the galls. Nevertheless, as in bur marigold, the concentration of hydroxyproline in the cell-wall protein of the infected grapevine roots was lower than in cell-wall protein of healthy roots. Thus, it appears likely that the nematode may interfere with proline metabolism and cell-wall biosynthesis in both host plants.

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