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

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Abstract: Changes in DNA and RNA in roots of bur marigold fed upon by *Longidorus africanus* were studied using analytical methods, radioactive precursors, and analytical CsCl density-gradient centrifugation. The analyses showed that almost twice as much RNA and DNA was present in parasitized root tips as in those of nonparasitized control plants. Studies on the rates of incorporation of labeled thymidine and uridine confirmed the DNA levels determined by analytical methods, but revealed a much higher incorporation rate of RNA in healthy root tips than in those attacked by *L. africanus*. However, ^{32}P- incorporation followed by DNase and RNase digestion showed that the seemingly greater amount of RNA in healthy root tips was due to a rapid formation of a pool of unlabeled uridine following infection.

The possibility that *L. africanus* injected DNA into roots during feeding was examined by the density-gradient centrifugation method, with negative results. However, the rapid increase of RNA precursors in the parasitized roots might have been caused by injection of plant virus particles during nematode feeding.

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Previous investigations on biochemical alterations in terminal root galls induced by *Longidorus africanus* Merny (5, 6) showed that some major changes take place following infection. Such alterations were expected in view of the great histological changes which occur in the infected root tips; i.e., retardation of meristematic activity and hyperplasia of the

cortical parenchyma (3). The swollen tip is densely packed with small parenchyma cells, and the amounts of DNA and RNA would be expected to increase, as reported for roots infected with *Meloidogyne* (12, 13) and *Heterodera* (4). Moreover, the observation that the root tip kept enlarging after *L. africanus* feeding ceased (1), might indicate that the terminal root gall is induced by the transfer of nematode DNA into the plant tissue during feeding. A similar phenomenon was reported for tumors induced by *Agrobacterium tumefaciens* (19). Characterization of DNA in the plant root tips was performed by means of CsCl density-gradient centrifugation. Such a study could also determine whether qualitative changes occur in the terminal root galls, as reported for a number of tumors (7).

MATERIALS AND METHODS

Incorporation of labeled thymidine, uridine, and phosphate: To determine the time-rate of increased DNA and RNA synthesis, plants of bur marigold (*Bidens tripartita L.*) were infected with handpicked specimens of *Longidorus africanus* as described by Cohn and Mordechai (2).

Three groups of 75 root tips (0.5 mm long) were excised from the parasitized plants at each sampling date into small vials containing 1 ml of 0.1-strength sterile Hoagland's solution. Root tips of healthy plants were excised at the beginning and end of the experiment and treated similarly. Radioactive chemicals (all from Radiochemical Centre, Amersham, England) were added as follows: thymidine (methyl-^{3}H), specific activity 23.3 Cie/mmole, and uridine-5-^{3}H, specific activity 27.0 Cie/mmole, both at a final concentration of 10 μCie/ml; ^{32}P-orthophosphate was given at a final concentration of 50 μCie/ml. The root-tip cultures were incubated 1 h in a 30-C water bath with continuous shaking. Incorporation was terminated by absolute ethanol:glacial acetic acid (3:1). Root tips were serially dehydrated in ethanol (70%, 85%, 90%, 100%; 30 min in each), defatted in two changes of acetone and once in ether, and homogenized with 3 ml of 0.05M tris-(hydroxymethyl)aminomethane-HCl (Tris-HCl) buffer (pH 7.0) + 0.005M $CaCl_2$ + 0.045M $MgSO_4$, as described by Jakob and Bovey (9). Samples of 0.3 ml of the homogenized root tips 72 h after infection which were incubated with ^{32}P, and of corresponding check plants, were taken for enzymatic digestion. For RNase treatment, 1 mg/ml of pancreatic RNase (A-grade, Calbiochem) was added to the homogenates. The RNase was dissolved in 0.005M acetate buffer (pH 5.5) and heated at 90 C for 15 min. For DNase treatment, 0.2 mg/ml DNase (B-grade, Calbiochem) was added to the homogenates. These homogenates were incubated for 2.5 h at 37 C. The addition of 10% trichloroacetic acid (TCA) plus thymidine 5'-monophosphate disodium 3½ H_2O, 8.6 mg/100 ml and deoxycytidine-5-monophosphate monohydrate, 6.6 mg/100 ml of TCA (to both enzyme-treated and untreated homogenates), facilitated termination of enzymatic digestion and nucleic acid precipitation. The precipitated nucleic acids were filtered and radioactivity was counted in a Packard Tricarb scintillation spectrometer (9).

Nucleic acid determinations: For all determinations freeze-dried root tips (about 0.5 mm long) of both parasitized (2 wk after infection) and healthy plants were first homogenized in cold 85% methanol with an all-glass homogenizer, centrifuged at 12,000 *g* and the pellets treated to give a cold acid-soluble lipid-free residue (8). This residue was used for the extraction of nucleic acids. Extraction of individual nucleic acids was performed by the method of Schmidt and Thannhauser (15). DNA and RNA contents were measured with diphenylamine and orcinol reagents (10), respectively, or by their absorption at 265 nm minus that at 320 nm. Total nucleic acids determination was done after extraction by the Schneider method (16) and by phosphorus determination (18).

DNA isolation: Freeze-dried root tips (300 mg) of healthy and parasitized (2 wk after infection) plants were homogenized in 5 ml of 0.15 M NaCl and 0.1 M (ethylene dinitrilo)tetraacetic acid (saline-EDTA), at pH 8.0. DNA was isolated by the method of Marmur (11) except that phenol saturated with Tris-HCl buffer pH 8.2 was used, and the lysozyme step was omitted.

Density-gradient centrifugation: Analytical CsCl density-gradient centrifugation was performed as described by Schildkraut et al. (14), in a Spinco Model E analytical ultracentrifuge. To 0.1 ml (3.0 μg) of the infected or 0.1 ml (2.6 μg) of the healthy DNA samples were added 0.01 ml of 50 μg/ml

TABLE 1. Effect of feeding of *Longidorus africanus* on nucleic acids of bur marigold root tips. Nucleic acids of healthy root tips and those fed upon by *Longidorus africanus* (data represent average of three replicates).

Method	Literature Reference	Procedure	Nucleic acids[a]		DNA		RNA	
			Infected	Healthy	Infected	Healthy	Infected	Healthy
Phosphorus	(18)	H_2SO_4 digestion and titration with NaOH + ascorbic acid	170	113				
Schneider	(16)	5% $HClO_4$ (90 C, 15 min)	225	150				
Schmidt and Thannhauser	(15)	KOH digestion and acid precipitation of DNA						
I Ultraviolet		Absorptivity at 265 nm minus that at 320 nm			38	24	220	104
II Ribose	(10)	Orcinol					334	256
III Deoxyribose	(10)	diphenylamine			18	7		

[a] µg/10 mg of cold acid-soluble, lipid-free residue.

Micrococcus lysodeikicus DNA, 0.89 g CsCl, and 0.6 ml distilled water, and the index of refraction was adjusted to 1.4 ± 0.001. Photographs were taken after 24 h and scanned with a microdensitometer.

RESULTS

The estimation of total nucleic acids, DNA, and RNA in *Longidorus*-infected and healthy root tips of bur marigold by several methods is presented in Table 1.

All analytical methods employed revealed an increase in both DNA and RNA content of infected root tips. However, estimation according to the orcinol method gave higher values than with other methods, probably as a result of contamination by inulin which was present in the roots in large amounts.

TABLE 2. Incorporation of thymidine (methyl-^{3}H) and uridine-^{3}H (in counts/min) into root tips which were fed upon by *Longidorus africanus* and healthy root tips of bur marigold (data represent averages of nine replicates).

Time infected h	Thymidine-^{3}H		Uridine-^{3}H	
	Infected	Healthy	Infected	Healthy
24	3,470	3146		
48	2,882		13,676	147,278
72	2,842		44,906	
96	3,351		42,264	
120	2,921		30,399	
168	5,682	3027	33,410	
288	17,474	2624	79,798	101,886

Experiments on the incorporation of thymidine-^{3}H and uridine-^{3}H into parasitized and healthy root tips of bur marigold showed that the rate of thymidine incorporation increased almost two-fold seven days after infection, whereas the rate of uridine incorporation was much higher in the healthy root tips (Table 2). On the other hand, the rate of ^{32}P incorporation into RNA of parasitized root tips was twice that of healthy root tips (Table 3), while only a small difference was noted in its incorporation into DNA.

Density-gradient centrifugation of DNA isolated from parasitized and healthy root tips did not reveal any qualitiative changes or a new type of DNA.

DISCUSSION

DNA and RNA contents increased and thymidine incorporation was doubled in the terminal root galls one week after infection with *L. africanus* as compared with check plants. Such nuclear and cytoplasmic changes and other biochemical changes which were reported previously (5, 6), reflect the interaction between the host and the parasite. Similar alterations were reported in tomatoes infected with *Meloidogyne incognita acrita* (12, 13) and in soybeans infected with *Heterodera glycines* (4). These are endoparasitic nematodes which penetrate into the host tissue where they induce many changes either by secretions through the stylet or by body excretion. *L. africanus*, on the other hand, is an ectoparasitic

root-tip feeder and the swelling is probably a result of secretion through the stylet. Since one nematode is capable of inducing such a swelling (1), some initial triggering mechanism must be amplified to stimulate the various cellular changes which follow. One possibility which was investigated in this study is that the changes in the root tip are induced by nematode DNA which is injected into the tissue during feeding. However, this was not shown, since no new type of DNA could be detected by the density-gradient centrifugation method.

The rate of thymidine incorporation into cells of *Longidorus*-infected root tips did not correspond to the rate of increase in the size of the root-tip, which began to swell as early as 20 h after feeding started (1); a change in incorporation rate was recorded one wk after infection. It is possible that accelerated cell division starts one wk after feeding and is preceded by an increase in number of parenchyma cells in the outer tissue layers (1).

Contrary to the results of thymidine incorporation, the rate of uridine incorporation was much slower in parasitized than in healthy root tips. I suspected that this was due to a large pool of "cold" uridine in these roots. This theory was verified by the ^{32}P-incorporation experiment, in which it was shown that infected (as compared with healthy) root tips did contain twice as much RNase-removable ^{32}P radioactivity. The uridine is synthesized rapidly, as shown by the fact that it is noticeable as early as 48 h after infection. Since some species of *Longidorus* are known to carry and transfer RNA viruses (17), it is tempting to speculate on the possibility that the cause of this rapid increase in uridine is the introduction of viral RNA precursors into the roots by the nematode during feeding. However, the uniform response of different plant species to *Longidorus* infection (17) is not typical of multiple virus etiology. Each plant species would be expected to have its own specific viral agent and reaction. Therefore, the increase is more likely to be a result of an overall increase of host RNA synthesis, stimulated at the infection site by nematode feeding. Both these hypotheses merit further investigation.

TABLE 3. Incorporation of ^{32}P-orthophosphate (in counts/min × 10^2 after 72 h) into root tips fed upon by *Longidorus africanus*, and healthy root tips of bur marigold; and radioactivity removed after DNase and RNase treatments (data represent averages of nine replicates).

Treatment	Before enzyme treatment		Enzyme-removed radioactivity	
	Infected	Healthy	Infected	Healthy
Control total	2537	1218		
DNase-treated	2164	922	373	296
RNase-treated	1462	690	1075	528

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