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CHANGES IN NUCLEOTIDE COMPOSITION OF THE PLANT RNA DURING INFECTION BY *MELOIDOGYNE INCOGNITA*

by

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Changes in the nucleic acid metabolism due to infection by plant pathogens has been well-documented (Heitefuss and Wolf, 1976; Chakravorty and Shaw, 1977). More specifically for phytopathogenic nematodes the reports (Bird, 1971; Rubinstein and Owens, 1964; Owens and Specht, 1966) indicate alteration in nucleic acid levels. Premachandran and Dasgupta (1983) have found a varying time course pattern of RNA and DNA synthesis in susceptible and resistant varieties of tomato plants consequent upon infection by *Meloidogyne incognita*. Increased concentrations of purines with a reduction in the pryrimidines of tomato roots infected with *M. incognita* has also been observed (Okopnyi, 1977).

In this investigation we examine the alterations in the proportions of the nucleotides during pathogenesis by the nematode and its correlation with the resistance response.

Materials and Methods

Stock cultures of the root-knot nematode, *M. incognita* raised on tomato (*Lycopersicon esculentum* Mill.) were used in these studies.

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The tomato cultivars « Pusa Ruby » and « SL-120 » were used as susceptible and resistance sources respectively to *M. incognita* (Singh and Choudhary, 1974). Thirty-seven day old seedlings of each cultivar in 10 cm pots were inoculated with axenized, active, second-stage larvae. Uninoculated plants of each cultivar served as controls.

Thirty days after inoculation, the plant roots were harvested, washed, surface-sterilised and again washed repeatedly with sterile water. The fresh plant material (2 g fresh weight) was then steamed in an autoclave for two minutes at 100°C, dried at 100°C for 3 hours, powdered and then stored at −12°C. The nucleotide composition was determined according to the method of Zscheile and Murray (1963). RNA was extracted from the powdered root material by steaming several times with 0.55 M NaCl at 100°C for 30 minutes. The supernatants were pooled and the RNA precipitated with an equal volume of 95% ethanol. The mixture was kept at 2°C overnight and RNA was separated by centrifugation at 6,000 g for 10 minutes. Hydrolysis of the residue was carried out at 30°C with N KOH for 40 hours and the pH of the hydrolysate was adjusted to 8.5 with 1.0 M HCl. The mixture was then centrifuged again at 1,000 g to remove the small amount of proteins precipitated. The supernatant was separated and chromatographed on columns of Dowex-1 × 8 (200-400 mesh) Cl^-. One millilitre of the hydrolysate was loaded on the column. After washing with distilled water, the columns were washed with 0.01 M ammonium chloride and then with distilled water again until the effluents were neutral. The nucleotides were eluted with varying concentration (pH 1.1 and 2.2) of HCl. The quantification of the nucleotides in the different fractions was done spectrophotometrically. The molar concentrations of the individual nucleotides were determined using the equations given by Zscheile and Murray (1963).

Results and Discussion

A marked increase in the total nucleotide content following infection by the nematode was apparent in both the cultivars. Comparison of figures 1 and 2 indicates the augmentation of each nucleotide peak at each wavelength consequent to nematode infection. The data provided in Table I show the changes in ratios between inoculated and control plants of the individual nucleotides. In the infected suscep-

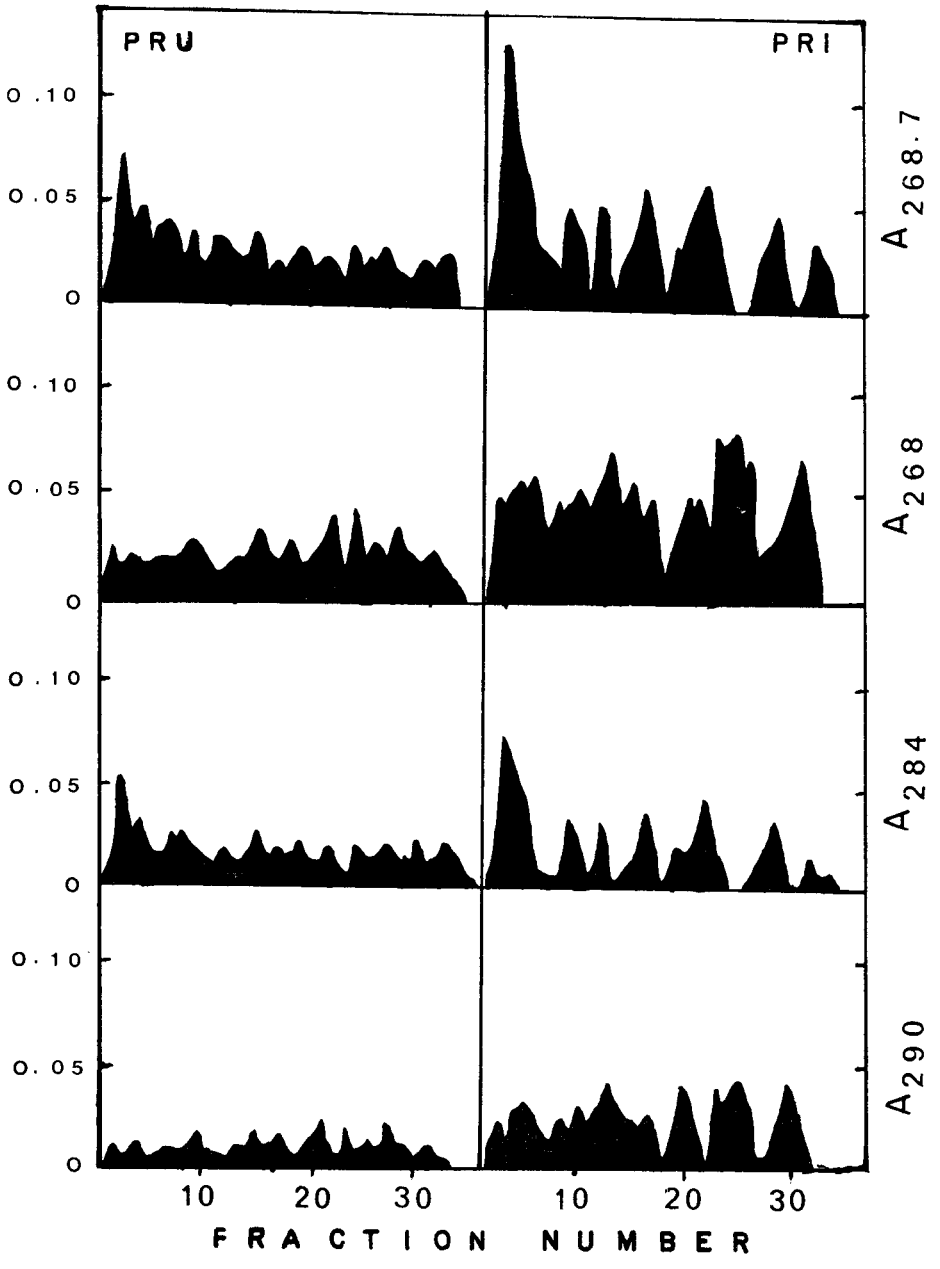

Fig. 1 - Comparison of chromatograms of nucleotides from roots of tomato cultivar « Pusa Ruby » from a 1x4 cm column of Dowex 1x8 (Cl^-) form ion-exchange resin. Fraction volume, 3 ml. Flow rate, 40 ml/hr.
Absolute units at different wavelengths plotted against fraction number.
PRU: Uninoculated Pusa Ruby; PRI: Inoculated Pusa Ruby.
Note the general increase in nucleotides in the inoculated Pusa Ruby.

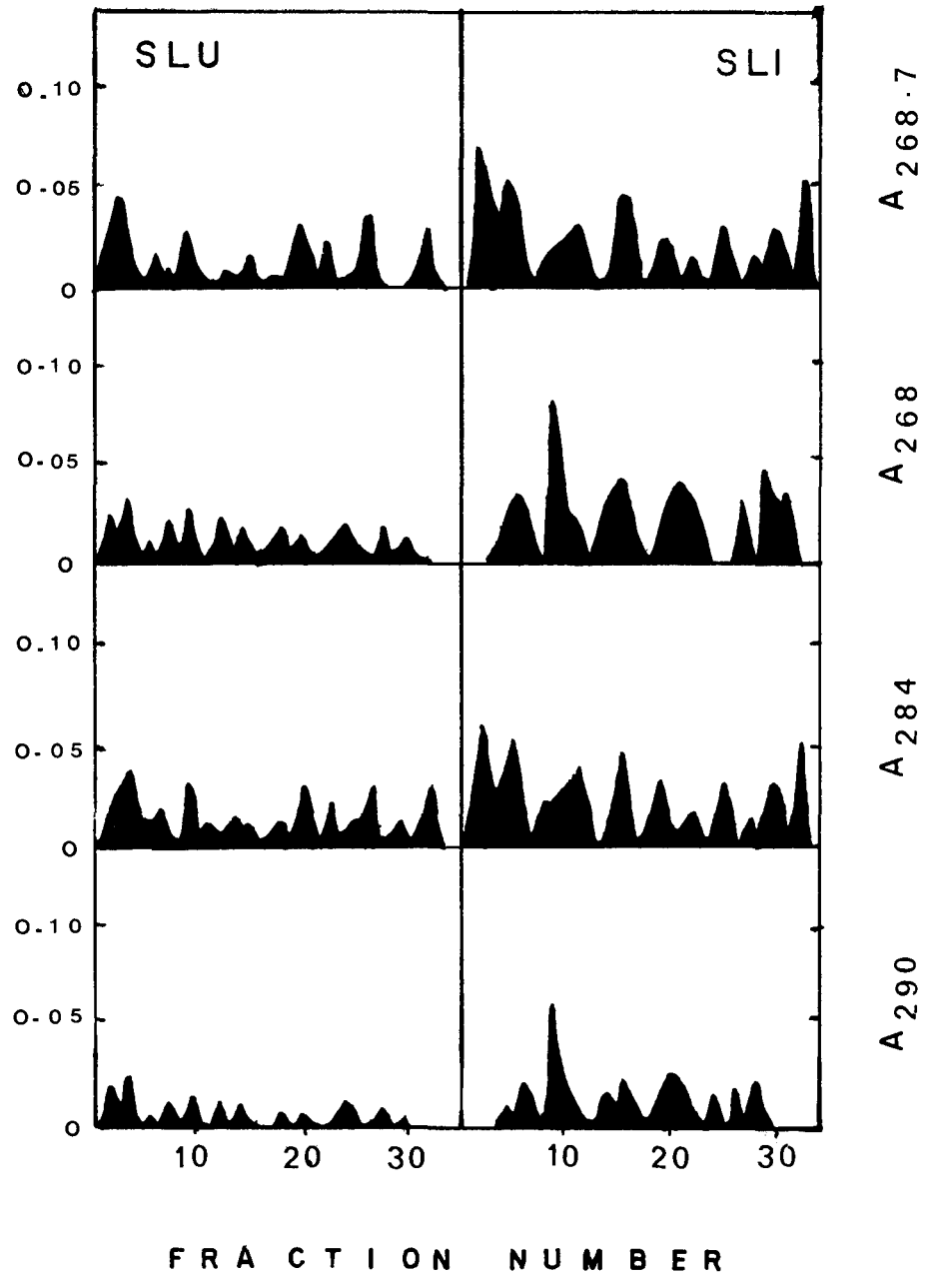

Fig. 2 - Comparison of chromatograms of nucleotides from roots of tomato cultivar « SL-120 » from a 1x4 cm column of Dowex 1x8 (Cl^-) form ion-exchange resin. Fraction volume, 3 ml. Flow rate, 40 ml/hr.
Absolute units at different wavelengths plotted against fraction number.
SLU: Uninoculated SL-120; SLI: Inoculated SL-120.
Note the general increase in nucleotides in the inoculated SL-120.

tible cultivar the increases observed were mainly in the quantities of adenylic, cytidylic and guanylic acids. The infected resistant cultivar responded in an essentially similar manner except in that their adenylic acid content was lower than the healthy ones.

Our data on quantitative changes in the nucleotides in the susceptible combination are in concordance with the observations of Okopnyi (1977). The shift in the relative composition of the adenylic acid compared with the other nucleotides may perhaps be indicative of a preferential synthesis of poly-adenylated sequences representing mRNA molecules (Manahan *et al.*, 1973; Van de Walle, 1973). The increased nucleotide pool in the plant cell may be utilized at this stage thus stepping up the protein bio-synthetic machinery accounting for the higher protein content observed in the inoculated plants.

The significance of these changes in the concentration of the individual nucleotides in the resistance/susceptible response of the plants is yet to be elucidated. It should be worthwhile to consider these changes, particularly that of adenylic acid, during the very early stages of nematode infection.

Table I - *Ratio of the changes in RNA nucleotides in tomato cultivars « Pusa Ruby » and « SL-120 » after inoculation with* Meloidogyne incognita.

Nucleotide	RATIO OF THE MOLAR CONCENTRATIONS OF NUCLEOTIDES (inoculated/control) (males/g fresh weight) x 10^6					
	Pusa Ruby			SL-120		
	Inoculated	Control	Ratio	Inoculated	Control	Ratio
Adenylic acid	10.32	20.82	2.02	2.49	2.0	0.80
Cytidylic acid	11.70	16.89	1.44	13.42	26.0	1.94
Guanylic acid	21.70	48.07	2.22	22.47	39.99	1.78
Uridylic acid	5.67	5.04	0.89	4.43	1.54	0.35
Total purines	32.02	68.89	2.15	24.96	41.99	1.68
Total pyrimidines	17.37	21.93	1.26	17.85	27.54	1.54
Total nucleotides	49.39	90.82	1.84	42.81	69.51	1.62

SUMMARY

Changes in the nucleotide composition of RNA in the roots of tomato infected by the root-knot nematode, *Meloidogyne incognita*, were studied. The data indicates that infected susceptible plants cv. Pusa Ruby contain more adenylic, cytidylic and guanylic acids than the uninfected ones which contained more of uridylic acid. In the resistant cultivar SL-120, the pattern was essentially similar except that the inoculated plants contained less adenylic acid than controls.

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