Nematol. medit. (1980), *8*: 51-57.

Department of Plant Pathology
University of Agricultural Sciences
Hebbal, Bangalore 560 024, India

THE ROLE OF NITROGEN, AMINO ACIDS AND PHENOLS IN RESISTANCE OF TOMATO TO ROOT-KNOT NEMATODES (1)

by

Y. D. NARAYANA and D. D. R. REDDY

Narayana and Reddy (1980) have shown that the tomato variety NTDR-1 exhibited a high degree of resistance to *Meloidogyne incognita* and *M. javanica* by limiting larval invasion and preventing development of any nematodes within the roots. Free amino acids content of the host tissue has been related to susceptibility or resistance of plants to various organisms (Lakshminarayan, 1955; Myuge, 1956). Increased phenolic content was considered to be contributory factor in the resistance to various nematode infections (Zuckerman, 1971; Singh and Choudhury, 1973; Sivapalan and Sivanatharajan, 1974). The present study was made to determine whether there are any differences in the total nitrogen, the quality and quantity of total amino acids, and the quantity of free total phenolics and chlorogenic acid in shoots and roots of tomato « NTDR-1 » resistant to root-knot nematodes, compared with the susceptible « Pusa Ruby ».

MATERIALS AND METHODS

Seeds of the two tomato varieties were sown in pans containing autoclaved soil. One month old seedlings were transplanted in 25 cm pots filled with autoclaved soil, one seedling per pot. Shoots and roots, 30 and 60 days after transplanting, were chemically ana-

(1) Adapted from M. Sc. thesis submitted to the University of Agricultural Sciences, Bangalore by the senior author.

lysed. Oven dried material (105° C in hot air oven) of each sample was ground separately into fine powder. The total nitrogen content was determined by Kjeldhal's method (Bal, 1925) on one gram aliquots (three/sample) of the powdered material. Total amino acids were estimated by an automatic aminoacid analyzer 60 days after transplanting. Ten grams of each oven dried sample were hydrolyzed in 10 ml of 6 N HCL at 110° C for 22 hours in a glass test tube. The extracts were filtered through Whatman 42 and evaporated to dryness. The residue was dissolved in 2 ml of citrate buffer at pH 2.2 before analysis.

Total phenols in the extract were determined by the method of Bray and Thorpe (1954) and chlorogenic acid in a Unicam Spectrometer read at 540 nm against a standard curve (Arnow, 1937).

RESULTS AND DISCUSSION

The shoots of susceptible Pusa Ruby had less total nitrogen than those of resistant NTDR-1. In the roots, however, nitrogen was markedly higher in the susceptible variety than in those of resistant, both at 30 and 60 days old plants. Sengupta (1964) recorded higher nitrogen content in both roots and shoots of susceptible tomato varieties than resistant ones. Possibly an increase in nitrogen content in the plant makes it more susceptible to root-knot nematode attack.

Eighteen amino acids were detected and identified in the shoots and roots of the two varieties. Number and quantity of amino acids

Table I - *Total nitrogen content of shoots and roots of a susceptible and a resistant variety of tomato.*

Variety		Per cent nitrogen on dry weight		
		Days after transplanting		
		30	60	Mean
Pusa Ruby (Susceptible)	Shoot	2.54	2.12	2.33
	Root	2.29	2.00	2.14
NTDR-1 (Resistant)	Shoot	3.89	3.34	3.60
	Root	1.54	1.70	1.62

in the shoots appear to be the same in both the susceptible and resistant varieties except for methionine which was not detected in resistant plants and cystine present only in traces. Arginine, alanine, leucine, lysine and valine were the most abundant amino acids in shoots of both susceptible and resistant varieties. More than double the quantity of total amino acids were present in the roots of susceptible Pusa Ruby compared with NTDR-1 (Table II). Percentage composition of individual amino acids remained more or less the same in both varieties but marked differences were noted in the absolute quantities. Free amino acids possibly serve as a readily available source of nutrition for the nematodes. Myuge (1956) reported that amino acids were of primary importance in the successful

Table II - *Absolute and relative amounts of total amino acids per gram dry weight in shoots and roots of a susceptible and a resistant variety of tomato 60 days after transplanting.*

Amino acids	Amount of total amino acids							
	Pusa Ruby (Susceptible)				NTDR-1 (Resistant)			
	Shoot		Root		Shoot		Root	
	µg/g	%	µg/g	%	µg/g	%	µg/g	%
Arginine	9756	12.99	5576	7.70	7542	9.94	2788	8.38
Asparticacid	1012	1.35	7653	10.57	1032	1.36	4325	13.00
Alanine	6950	9.26	4455	6.16	7128	9.40	2005	6.03
Cystine	961	1.28	Tr	—	Tr	—	Tr	—
Glutamicacid	1501	2.00	9195	12.71	1361	1.79	4783	14.38
Glycine	5254	7.00	3940	5.44	5630	7.42	2635	7.92
Histidine	4344	5.79	4344	6.00	4168	6.81	Tr	—
Leucine	9182	12.23	5248	7.25	9510	12.64	2623	7.89
Isoleucine	4984	6.64	3608	4.99	4920	6.49	1640	4.93
Lysine	9356	12.46	9356	12.93	9251	12.19	4680	14 07
Methionine	596	0.79	Tr	—	—	—	Tr	—
Norleucine	Tr	—	Tr	—	Tr	—	Tr	—
Phenylalanine	5286	7.04	3305	4.57	5783	7.62	1653	4.97
Proline	4606	6.14	2878	3.98	6333	8.35	2490	7.79
Serine	420	0.56	2890	3.99	525	0.69	Tr	—
Tyrosine	2174	2.90	2265	3.13	3170	4 18	950	2.72
Threonine	1430	1.90	2680	3.70	893	1.18	Tr	—
Valine	7264	9.68	4980	6.88	7615	10.04	2635	7 92
Total	75076	100.00	72373	100.00	75861	100.00	33262	100.00

Tr = Traces.

establishment of gall formation by root-knot nematodes. Owens and Novotny (1960) noticed comparatively large quantities of amino acids in nematode-susceptible plants; thus the reduced quantity of total amino acids in the roots of resistant NTDR-1 may be one a factor in preventing the establishment of root-knot nematode, as noted in our earlier investigations (Narayana and Reddy, 1980).

The total phenolic content of both shoots and roots of resistant « NTDR-1 » was markedly higher than that of suceptible « Pusa Ruby » (Table III). Total phenolics were found to be more at 60 days after transplanting than at 30 days. Several investigators have endeavoured to investigate the relation between phenolic content and disease resistance. In the present investigation the phenolic content and the resistant variety was distinctly higher than that of the susceptible variety. Similarly Singh and Choudhury (1973) observed a higher phenolic content in immune tomato cultivars than in susceptible ones. Wilski and Giebel (1966) proposed that resistance to *Globodera rostochiensis* in tomato depends on the phenolic glucosidases secreted by the nematode. It is likely that a similar type of interactions occurs in NTDR-1 resulting in inhibition of nematode development.

Table III - *Total phenols per g dry weight in shoots and roots of a susceptible and a resistant variety of tomato.*

Variety		Total phenols (mg/g)			Per cent increase with respect to Pusa Ruby
		Days after transplanting			
		30	60	Mean	
Pusa Ruby (Susceptible)	Shoot	0.029	0.041	0.035	
	Root	0.012	0.021	0.016	
NTDR-1 (Resistant)	Shoot	0.040	0.060	0.050	48.85
	Root	0.020	0.027	0.023	43.75

This variety NTDR-1 also had a relatively higher chlorogenic acid content in both shoots and roots (Table IV). Pi and Rhode (1967, 1973) found that chlorogenic acid is in higher concentration in the resistant tomato variety Nemared compared to the susceptible

« B-5 » and concluded that the root-knot resistance in related to higher content of this compound. Also, Milne *et al.*, (1965) found higher quantities of chlorogenic acid in tobacco varieties resistant to *Meloidogyne javanica.* Wallace (1961) working with the foliar nematode of chrysanthemum also found high amounts of chlorogenic acid in the resistant varieties and showed *in vitro* that the chemical inhibited the movement of the parasite.

Table IV - *Chlorogenic acid per g of dry weight in shoots and roots of a susceptible and a resistant variety of tomato 60 days after transplanting.*

Variety		Chlorogenic-acid (µg/g)	Per cent Increase with respect to Pusa Ruby
Pusa Ruby (Susceptible)	Shoot	330	
	Root	10	
NTDR-1 (Resistant)	Shoot	385	16.7
	Root	55	450.0

Polyphenol level may confer disease resistance, but the speed of parasitically induced phenol synthesis and oxidation of toxic quinones is more important. More often it is known that wherever a tissue shows a hypersensitive reaction, the polyphenol oxidases are rapidly and strongly activated and polyphenol synthesis is stimulated. It is therefore possible that the higher level of chlorogenic acid in resistant NTDR-1 is a contributory factor in the hypersensitive reaction that may correlated with the death of the nematodes observed in our earlier studies (Narayana and Reddy, 1980).

SUMMARY

Roots and shoots of healthy plants of tomato varieties NTDR-1 and Pusa Ruby resistant and susceptible respectively to root-knot nematodes were analysed for nitrogen, amino acids, free phenolics and chlorogenic acid. Nitrogen percentage was markedly higher in the roots of the susceptible variety. Eighteen amino acids were identified and the total amino acid content was more than double the quantity in the roots of the susceptible variety compared to resistant one. Total phenolic content in shoots and roots of the resistant variety was

48.85% and 43.75%, respectively, higher than in the susceptible variety. The resistant variety also had markedly higher chlorogenic acid content in both root (450%) and shoot (16.16%) than the susceptible one.

RIASSUNTO

Ruolo di azoto, amminoacidi e fenoli nella resistenza del Pomodoro nei confronti dei nematodi galligeni.

Le radici ed i germogli di piante di Pomodoro delle varietà NTDR-1 e Pusa Ruby, rispettivamente resistente e suscettibile nei confronti dei nematodi galligeni, sono stati analizzati per il loro contenuto in azoto, amminoacidi, fenoli liberi ed acido clorogenico. La percentuale di azoto ed il contenuto totale in amminoacidi (ne sono stati determinati 18) sono risultati molto più alti nelle radici della varietà suscettibile che non in quella resistente. Il contenuto totale di fenoli nei germogli e nelle radici è stato nella varietà resistente del 48,85 e del 43,75% rispettivamente, notevolmente più elevato che non nella varietà suscettibile. La varietà resistente aveva anche un maggior contenuto di acido clorogenico di quella suscettibile.

LITERATURE CITED

ARNOW'S L. E., 1937 - Colorimetric determination of the components of 3,4 dihydrophenylalanine Tyrosine mixtures. *J. Biol. Chem., 118*: 531-537.

BAL P., 1925 - Chemical analysis of samples. *J. Agri. Sci., 15*: 454.

BRAY H. G. and THORPE W. Y., 1954 - Analysis of phenolic compounds of interest in metabolism. *Methods of Biochemical Analysis* Vol. I. Ed. D. Glick, Interscience Publishing Inc. New York, pp. 27-52.

LAKSHMINARAYAN K., 1955 - Role of Cystine chelatin on the mechanism of fusarium wilt of cotton. *Experimentia, 9*: 338-389.

MILNE D., BOSHOFF D. and BUCHAN P., 1965 - The nature of resistance of *Nicotiana rependa* to root-knot nematode *Meloidogyne javanica. South Africa J. Aric. Sci., 8*: 557-570.

MYUGE S. G., 1956 - The trophic characters of *Meloidogyne incognita. Zh. Obshch. Biol., 17*: 396-399.

NARAYANA Y. D. and REDDY D. D. R., 1980 - Penetration, development and histopathology of root-knot nematodes in tomato 'NTDR-1'. *Nematol. medit., 8*: 43-49.

OWENS R. G. and NOVOTNY A. A., 1960 - Physiological and biochemical studies on the nematode galls. *Phytopathology, 50*: 650.

PI C. L. and RHODE R. A., 1967 - Phenolic compounds in tomato to injury caused by root-knot and lesion nematodes. *Phytopathology, 57*: 344 (Abstr.).

PI C. L. and RHODE R. A., 1973 - Phenol accumulation related to resistance in tomato infected by root-knot *Meloidogyne incognita. J. Nematol., 5*: 253-258.

SENGUPTA K., 1964 - Biochemical investigation on the mechanism of resistance to the root-knot nematode *Meloidogyne incognita* Chitwood in some economic crops. M. Sc. thesis submitted to the Indian Agricultural Research Institute, New Delhi.

SINGH R. S. and CHOUDHURY B., 1973 - The chemical characteristics of tomato cultivars resistant to root-knot nematodes (*Meloidogyne* spp.). *Nematologica, 19*: 443-448.

SIVAPALAN P. and SIVANATHARAJAN V., 1974 - Polyphenol content in the feeder roots of nematode tolerant and susceptible tea root clones in relation to infection by *Pratylenchus loosi*. *Tea Q.*, *44*: 173-174.

WALLACE H. R., 1961 - The nature of resistance in chrysanthemum varieties to *Aphelenchoides ritzemabosi*. *Nematologica*, *6*: 49-58.

WILSKI A. and GIEBEL J., 1966 - B-glucosidase in *Heterodera rostochiensis* and its significance in resistance of potato to this nematode. *Nematologica*, *12*: 219-224.

ZUCKERMAN M., 1971 - Gnotobiology. In *Plant Parasitic Nematodes*. Vol. II. Ed. B. M. Zuckerman, W. F. Mai and R. A. Rhode, Academic Press, New York, pp. 159-184.

Accepted for publication on 28 October 1979.