

Notes brèves

BIOCHEMICAL DIFFERENCES IN TOMATO CULTIVARS RESISTANT AND SUSCEPTIBLE TO *MELOIDOGYNE INCOGNITA* ⁽¹⁾

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Phenolic compounds have been implicated as disease resistance factors in a number of host parasite combinations (Bhatia, Uppal & Bajaj, 1972; Epstein, 1972; Feldman & Hanks, 1968; Pollock & Drysdale, 1976). Polyphenol oxidase and peroxidase, the enzymes involved in the oxidation of phenols to more toxic quinones, are known to increase in several infected plants (Arora, 1979; Conti *et al.*, 1974; Yamamoto, Hokin & Tani, 1978). Most of the studies conducted to assess the biochemical changes in plants have been confined to the responses activated by nematode invasion. Very little information is available on the inherent level of compounds/enzymes which have been implicated as resistance/incompatibility factors. Consequently, five cultivars of tomato, one susceptible and four resistant to a local population of *Meloidogyne incognita*, were analysed for total phenols, orthodihydroxy phenols and flavonols and the activity of enzymes indole acetic acid oxidase, peroxidase and polyphenol oxidase was assayed.

Material and methods

The high degree of resistance of the four cultivars was confirmed by inoculation. Punjab Chhuhara, a locally evolved cultivar was used as the susceptible check. Roots of five week old seedlings grown in

sterilised soil were uprooted and washed with distilled water to provide material for analysis.

Two grammes of roots were macerated with two grammes of sand and 10 ml of 0.1 M phosphate buffer (pH 7.0) containing 1 mm cysteine-Hcl and 0.01% ascorbic acid in a chilled mortar. The homogenates were filtered through four layers of cheese cloth and the filterates centrifuged in a refrigerated centrifuge (Remi K-24) at 10 000 g for ten minutes. The supernatant thus obtained was used to measure the activities of indole acetic acid (IAA) oxidase, peroxidase and polyphenol oxidase.

Indole acetic acid oxidase activity was estimated by the method of Gordon and Weber (1951). Peroxidase activity was measured by the method of Shanon, Key and Jew (1966) while polyphenol oxidase activity was assayed according to Taneja and Sachar (1974).

For the estimation of total phenols, orthodihydroxy phenols and flavonols, 0.5 g of the dried root sample was refluxed with 80% methanol for one hour. The extracted material was centrifuged, the supernatant taken and methanol evaporated under suction. 0.5 ml of methanol was added to the residue and after dissolving the residue completely, volume was made to 25 ml with 80% methanol.

Total phenols were determined by the method of Swain and Hillis (1959) and orthodihydroxy phenols by the method of Nair and Vaidyanathan (1964). The estimation of total flavonols was conduct-

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ed by following the method of Balbaa, Zaki and Elshamy (1974).

Results and discussion

The phenolic content in the roots of all resistant cultivars was substantially higher than that of the susceptible one. Orthodihydroxy phenols were also significantly higher in all resistant cultivars. Rohde (1972) indicated the possible role of preformed simple phenols in the incompatible host/parasite interaction. While phenols appear to have limited toxicity, some of their oxidation products like quinones are more toxic (Giebel, 1970; Hung & Rohde, 1973).

Although levels of preformed phenols in roots have also been positively correlated with resistance of certain plant cultivars to nematodes (Cohn, 1974; Rohde, 1972), their significance is not clear, as most of the experiments on relationship of host phenol composition to nematode resistance have considered such changes long after the expression of

resistance. However, orthodihydroxy phenolic compounds, such as chlorogenic and caffeic acids are present in much greater quantities in resistant tomato roots (Bajaj & Mahajan, 1977; Hung & Rohde, 1973) even before nematode invasion and their possible role in conferring a resistant reaction needs further investigation.

While the presence of total as well as orthodihydroxy phenols and their oxidation products has been reported in the uninfected tomato roots, no report is available on the presence of flavonols. Considering the fact that all resistant cultivars analysed presently possessed significantly higher amounts of these compounds, their possible role in resistance cannot be ruled out.

IAA oxidase is either identical with or associated with peroxidase (Huang, Lin & Huang, 1971). We found more IAA oxidase/peroxidase activity in the roots of the susceptible cultivar as compared to resistant ones. According to Wilski and Giebel (1971), the inherent activity of these enzymes by itself cannot be considered responsible for resistance or susceptibility because the chemical nature of the phenolic compounds which are produced

Table 1
Biochemical differences in tomato cultivars resistant and susceptible to *Meloidogyne incognita*.
Column values are means of three replications

	Total ⁽¹⁾ phenols	Orthodihydroxy ⁽¹⁾ phenols	Flavonols ⁽¹⁾	IAA oxidase ⁽²⁾	Peroxidase ⁽³⁾	Polyphenol ⁽⁴⁾ oxidase
Resistant cultivars						
Piersol	410 ± 7.48	31.0 ± 0.37	109 ± 3.20	2.85 ± 0.10	27.5 ± 6.10	33.3 ± 3.10
Punuui	375 ± 4.08	37.5 ± 1.00	120 ± 4.00	16.43 ± 0.50	65.0 ± 4.00	25.8 ± 3.80
Ronita	350 ± 8.17	30.0 ± 0.82	101 ± 5.20	9.71 ± 0.05	45.0 ± 2.40	18.3 ± 1.10
Rossol	350 ± 4.08	32.0 ± 0.33	96 ± 2.94	29.30 ± 0.28	72.5 ± 13.10	27.1 ± 1.50
Mean of resistant cultivars	371.25	32.62	106.50	14.57	52.50	26.10
Susceptible Control						
Punjab						
Chhuhara	145 ± 2.36	8.0 ± 0.41	27 ± 1.63	42.85 ± 0.04	107 ± 3.10	34.2 ± 5.10
SE of difference of resistant cultivars over susceptible	0.04	0.46	2.21	0.27	4.04	2.61

⁽¹⁾ : mg/100 g dry weight ; ⁽²⁾ : Activity expressed as μM of IAA Oxidised/g fresh weight ; ⁽³⁾ : Activity expressed as $\Delta \text{OD } 430 \text{ nm/min/g}$ fresh weight ; ⁽⁴⁾ : Activity expressed as units/g fresh weight.

in response to infection is supposed to determine their activity. However, this hypothesis remains unproven because the chemical analyses of infected tissue were conducted long after resistance was expressed (Kaplan & Keen, 1980).

We conclude that in resistant tomato cultivars, the level of total as well as orthodihydroxy phenols and flavonols is apparently important. The known resistance to root knot nematodes in tomato is usually based on major genes and, therefore, one gene products. The role of phenols and flavonols would presumably come later, following the formation of giant cells to which the genes give rise. From our study it appears that the role of factors like orthodihydroxy phenols and flavonols as well as the activity of the enzymes like IAA oxidase, peroxidase and polyphenoloxidase cannot be ruled out in conferring a resistant reaction in tomato.

REFERENCES

- ARORA, Y.K. (1979). *Changes in the enzymes involved in polyphenol metabolism in mung bean infected with Rhizoctonia solani*. Ph. D. thesis, Punjab Agricultural University, Ludhiana, India.
- BAJAJ, K.L. & MAHAJAN, R. (1977). Phenolic compounds in tomato susceptible and resistant to *Meloidogyne incognita* (Kofoid & White) Chitwood. *Nematol. mediterr.*, 5 : 329-333.
- BALBAA, S.T., ZAKI, A.Y. & ELSHAMY, A.M. (1974). Total flavonoid and rutin content of different organs of *Sophora japonica* L. *J. Assoc. Off. Anal. Chem.*, 57 : 752-755.
- BHATIA, I.S., UPPAL, D.S. & BAJAJ, K. L. (1972). Study of phenolic contents of resistant and susceptible varieties of tomato (*Lycopersicon esculentum*) in relation to early blight disease. *Indian Phytopath.*, 25 : 231-235.
- COHN, E. (1974). Relations between *Xiphinema* and *Longidorus* and their host plants. In : Lamberti, F., Taylor, C.E. Seinhorst, J.W. (Eds). *Nematode Vectors of plant viruses*, New York, Plenum Press: 365-386.
- CONTI, G.G., VAGETTI, M., FAVALI, M.A. & BASSI, M. (1974). Phenylalanine ammonia lyase and polyphenol oxidase activities correlated with necrogenesis in cauliflower mosaic virus infection. *Acta Phytopath. Acad. Sci. Hung.*, 9 : 185-193.
- EPSTEIN, E. (1972). Biochemical changes in terminal root galls caused by an ectoparasitic nematode *Longidorus africanus*: phenol, carbohydrate and cytokinens. *J. Nematol.*, 4 : 246-250.
- FELDMAN, A.W. & HANKS, R.W. (1968). Phenolic content in roots and leaves of tolerant and susceptible citrus cultivars attacked by *Radopholus similis*. *Phytochem.*, 7 : 5-12.
- GIEBEL, J. (1970). Phenolic content in roots of some solanaceae and its influence on IAA oxidase activity as an indicator of resistance to *Heterodera rostochiensis*. *Nematologica*, 16 : 22-32.
- GORDON, S.A. & WEBER, R.P. (1951). Colorimetric determination of indole acetic acid. *Pl. Physiol.*, 26 : 192-195.
- HUANG, C.S., LIN, L.H. & HUANG, S.P. (1971). Changes in peroxidase isoenzymes in tomato galls induced by *Meloidogyne incognita*. *Nematologica*, 17 : 160-167.
- HUNG, C.L. & ROHDE, R.A. (1973). Phenol accumulation related to resistance in tomato to infection by root knot and lesion nematodes. *J. Nematol.*, 5 : 253-258.
- KAPLAN, D.T. & KEEN, N.T. (1980). Mechanisms conferring plant incompatibility to nematodes. *Revue Nématol.*, 3 : 123-134.
- NAIR, P.M. & VAIDYANATHAN, C.S. (1964). A colorimetric method for the determination of pyrocatechol and related substances. *Analyt. Biochem.*, 7 : 315-321.
- POLLOCK, C.J. & DRYSDALE, R.B. (1976). The role of phenolic compounds in the resistance of tomato cultivars to *Verticillium albo-atrum*. *Phytopath. Z.*, 86 : 56-66.
- ROHDE, R.A. (1972). Expression of resistance in plants to nematodes. *A. Rev. Phytopath.*, 10 : 233-252.
- SHANNON, L.M., KEY, E. & JEW, J.Y. (1966). Peroxidase isoenzymes from horse radish roots. I. Isolation and physical properties. *J. Biol. Chem.*, 241 : 2166-2172.
- SWAIN, T. & HILLIS, W.E. (1959). The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *J. Sci. Fd Agric.*, 10 : 63-68.
- TANEJA, S.R. & SACHAR, R.C. (1974). Induction of polyphenol oxidase in germinating wheat seeds. *Phytochem.*, 13 : 2695-2702.
- WILSKI, A. & GIEBEL, J. (1971). *Searches for biochemical reasons of plant resistance to nematodes*. Final Technical Report (1966-1971), Institute of Plant Protection, Poland.
- YAMAMOTO, H.H. & TANI, T. (1978). Peroxidase and polyphenol oxidase in relation to the crown rust resistance of oat leaves. *Phytopath. Z.*, 91 : 193-202.

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