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ACID PHOSPHATASE (E.C.3.1.3.2.) ACTIVITY IN RESISTANT AND SUSCEPTIBLE CULTIVARS OF TOMATO INOCULATED WITH *MELOIDOGYNE INCOGNITA* RACE 1

by
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Summary. Sequential development of acid phosphatase activity from the root and shoot of resistant and susceptible tomato cultivars was investigated following inoculation with *Meloidogyne incognita*. Enzyme activity was lower in the susceptible inoculated cultivar (Pusa Ruby) than its control and higher in the resistant inoculated cultivar (Pusa Nemamukkt). Maximum change in activity was observed in shoot and root respectively of the resistant cultivar 24 and 48 hours after inoculation; thereafter, the per cent increase over control showed a gradual declining trend. Qualitatively, the enzyme separated into two isozymes in root and shoot samples, with no inter-cultivar difference.

Acid phosphatases, orthophosphoric monoester phosphohydrolases, (E.C.3.1.3.2.) are an important group of hydrolytic enzymes that catalytically break down a wide variety of phosphomonoesters. These enzymes are also involved in providing energy to the host plant for combating the attack of the pathogens (Sharma, 1991; Wagih, 1993) and high activity of acid phosphatase has been reported near the feeding sites of root-knot nematodes in different crop plants (Veech and Endo, 1969; Giebel *et al.*, 1977).

Evidence in the literature suggests that Aps-1', the allele encoding for APase-1', an electrophoretically separable isozyme of acid phosphatase-1 and one of the many forms of acid phosphatases present in tomato (*Lycopersicon esculentum* Mill), is closely linked to the *Mi* gene (Rick and Fobes, 1974). While this allele (Aps-1') is thought to be derived from the wild-type species *L. peruvianum*, the other allele Aps-1^+ is indigeneous to *L. esculentum* and is not linked with *Mi*. The relationship between *Mi* and Aps-1' resulting from either a close linkage of the genes or pleiotropic effect of a single gene, suggests that APase-1' (acid phosphatase-1') can be a diagnostic marker for screening of root-knot nematode resistant tomato cultivars (Medina Filho and Stevens, 1980; Tanaka *et al.*, 1990). However, limited information is available about qualitative and quantitative changes of this enzyme in relation to post-nematode infection. The present investigation was undertaken on sequential development of acid phosphatase in two tomato cultivars, resistant and susceptible to the root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw., race 1.

Materials and methods

In the present investigation two tomato cultivars, Pusa Ruby and Pusa Nemamukt (SL-120) were susceptible and resistant hosts, respective-

ly, to *M. incognita*, race 1. Preliminary tests confirmed the compatible and incompatible reaction of these hosts to the nematode population. Population of nematodes were cultured by the procedure of Ganguly and Dasgupta, (1979). Three weeks old seedlings in 4 inches pots of sterilized sand were inoculated with about 5000 active second-stage *M. incognita* juveniles that had been surface-sterilized with 0.1 percent mercuric chloride. Plants of each cultivar were left uninoculated to serve as control. The plants were harvested at 1, 2, 3, 7, 14, 21 and 28 days after nematode inoculation, and the roots washed thoroughly in running tap water to remove any adhering sand. The roots were surface-sterilized by immersion for 10 minutes in 0.1 per cent mercuric chloride, followed by four to five washings in sterile water. They were then blotted, weighed and used immediately for the biochemical studies or stored at -12 °C untill required (2-3 hrs).

All subsequent experimental procedures adopted in this investigation were carried out at 4 °C, unless stated otherwise. Fully expanded shoots and roots harvested at each time interval were chopped into small pieces and homogenized in a mortar and pestle with an equal volume of pre-chilled 0.1 M Tris-HCl extraction buffer, pH 7.5 containing 5 mM dithioetheritol (DTT). The slurry was strained through four layers of sterilized cheese cloth and the filtrate was centrifuged at 14,000 g for 20 minutes.

The procedure for assay of acid phosphatase activity was essentially the same as that described by Giebel *et al.* (1977). Enzyme solution (0.25 ml) was incubated at 30 °C for 1 hour in 0.25 ml of 16 mM p-nitrophenyl phosphate and sodium salt in acetate buffer, (pH 5.0) containing 0.2 percent 1 M magnesium chloride. After the incubation period, 0.75 ml of 1 N sodium bicarbonate was added to stop the reaction and to develop colour. The reaction mixture was then read at 400 nm by a spectrophotometer Model Spectromom 204. The units of enzyme were expressed as micromoles of p-nitrophenol liberated per hour under the standard assay conditions.

The enzyme was extracted from young roots and fully expanded top shoot of nematode inoculated and uninoculated resistant and susceptible plants, according to the procedure of Surrs *et al.* (1989). Tissue (1g) was ground in extraction buffer containing 10 ml of 0.05 M Tris, 2 ml glycerol, and 100 µl of 2-mercaptoethanol. The extracts were centrifuged at 14,000 g for 5 minutes and 30 µl of sample was loaded on to 7 percent polyacrylamide gels, pH 8.9. Electrophoresis was carried according to the procedure described by Davis (1964) at a constant 100 v until the tracking dye i.". bromophenol blue, had migrated to the end of the slab. The electrode buffer consisted of Tris 0.025 M and 0.076 M glycine solution, pH 8.3. Standard staining procedures for acid phosphatase were followed (Vallejos, 1983). Composition of the staining solution was 20 ml of 0.05 M sodium acetate, pH 4.5, 3 ml of 0.05 M magnesium chloride, 250 mg of Fast black potassium salt, 5 ml of 1 per cent B-napthyl acid phosphate (in 50 per cent acetone) and 172 ml distilled water. After 45 minutes, when bands had developed, the gels were washed with double distilled water, fixed in 50 per cent solution of glycerol and photographed immediately. Each treatment was replicated three times. The results were statistically analyzed by factorial complete randomized design.

Results and discussion

Tables I and II illustrate sequential changes in acid phophatase activity in the roots and shoots, respectively, of uninoculated and inoculated plants of both the cultivars at various time intervals. With regard to enzyme activity the results of the investigation show: (1) compared to uninoculated plants, irrespective of time interval, there was a decrease in enzyme activity in the roots and shoots of the inoculated suscepti-

ble cultivar; (2) in contrast to the inoculated susceptible cultivar, there was a considerable increase in the enzyme activity in the roots and shoots of the resistant cultivar; (3) the magnitude of percent increase in enzyme activity in the inoculated plants over the control was generally maximum one day after inoculation in the shoots (138.1) and two days after inoculation in the roots (59.9) in resistant cultivar, thereafter, the percent increase over control showed a gradual decline; (4) the percent decrease or increase of enzyme activity over control in the susceptible and resistant cultivars was greater in shoot than in roots. Statistical analysis, showed that the effect of cultivar, treatment and days after inoculation were significantly different, except one day after inoculation in the case of Pusa Ruby.

Electrophoretic analysis revealed no difference between uninoculated and inoculated plant samples of susceptible and resistant cultivars. The enzyme resolved into two bands of Rf values, 0.62 and 0.19 in the shoots, and 0.61 and 0.43 in the roots.

The resistant cultivar showed an increase in acid phosphatase in the post-inoculation period while in the susceptible cultivar the activity decreased after inoculation compared to the uninoculated controls. This observation is identical to that of Giebel *et al.* (1977) and Sharma (1991), but contrasts with the report by Williamson and Colwell (1991) who recorded an increase in enzyme activity in root tips of both susceptible and resistant cultivars.

From these observations it is reasonable to assume that increase in acid phosphatase activity in the nematode-inoculated resistant plant indicates the stimulation of cytolysis at the infection site. Whereas the lesser activity of acid phosphatase in the nematode-inoculated sus-

TABLE I - *Sequential development of acid phosphatase activity (µM/hr/g fresh weight) in roots of susceptible and resistant tomatoes inoculated with* Meloidogyne incognita.

Days after inoculation (DAI)	Pusa Ruby (susceptible)		% change over control	Pusa Nemamukt (resistant)		% change over control
	Uninoculated	Inoculated		Uninoculated	Inoculated	
1	105.9	105.9	0.0	94.6	136.6	44.4
2	215.6	211.8	-1.7	204.3	326.7	59.9
3	227.0	223.2	-1.6	211.8	285.7	34.8
7	109.7	107.4	-2.0	109.7	139.6	27.2
14	74.1	60.5	-18.3	71.8	90.7	26.2
21	52.9	42.4	-19.9	60.5	68.1	12.5
28	45.4	36.0	-20.7	59.0	65.0	10.2

For acid phosphatase activity (µM/hr/g fresh weight) in roots

* Variety:	Mean	* Treatment:	Mean	* DAI(s)	Mean
Pusa Ruby	115.60	Uninoculated	117.35	1.	110.80
Pusa Nemamukt	137.48	Inoculated	135.73	2.	239.66
CD (5%)	0.48	CD (5%)	0.48	3.	236.98
				7.	116.62
				14.	74.33
				21.	54.01
				28.	51.38
				CD (5%)	0.91

TABLE II - *Sequential development of acid phosphatase activity (µM/hr/g fresh weight) in shoots of susceptible and resistant tomatoes inoculated with* M. incognita.

Days after inoculation (DAI)	Pusa Ruby (susceptible)		% change over control	Pusa Nemamukt (resistant)		% change over control
	Uninoculated	Inoculated		Uninoculated	Inoculated	
1	90.8	60.5	-33.3	79.4	189.1	138.1
2	124.8	94.6	-24.2	121.8	249.7	105.0
3	149.8	105.9	-29.2	185.4	261.0	40.8
7	117.3	96.1	-18.0	95.3	138.0	44.8
14	90.8	87.0	-4.1	69.6	98.3	41.3
21	75.6	68.1	-10.0	64.3	83.5	29.3
28	64.3	52.9	-17.6	60.5	77.8	28.6

For acid phosphatase activity (µM/hr/g fresh weight) in shoots

* Variety:	Mean	* Treatment:	Mean	* DAI(s)	Mean
Pusa Ruby	91.35	Uninoculated	99.30	1.	105.00
Pusa Nemamukt	126.74	Inoculated	118.79	2.	147.75
CD (5%)	0.43	CD (5%)	0.43	3.	175.57
				7.	111.71
				14.	86.46
				21.	72.90
				28.	63.93
				CD (5%)	0.80

ceptible plant ensures an increased level of ATP, necessary for protein co-polymerization which favours a successful host-parasite relationship. The early induced APase activity in resistant cultivar following nematode invasion suggests that the response is pathogen triggered. The relative decrease in enzyme activity with time, in both of the cultivars, inoculated as well as uninoculated, may be attributed to the catabolic nature of the enzyme, which is developmentally regulated (Medina Filho and Stevens, 1980) and very active in the early growth stages of the plant.

Acid phosphatase has been reported in the exudates of many nematodes (Van Gundy *et al.*, 1967) which suggests that the increase in enzyme activity is due to the pathogen itself. However, no acid phosphatase activity could be detected with the number of the second stage juveniles of *M. incognita* used in this investigation and the possibility that the nematodes themselves enhance the level of acid phosphatase can be disregarded. Besides, the decrease in the enzyme activity in susceptible plant harbouring large number of the nematodes would then be difficult to be accounted. It has been shown that in the area invaded by *M. incognita acrita* and *Globodera rostochiensis*, hydrolases, oxidase and dehydrogenase activity increase, (Veech and Endo, 1969; Giebel *et al.*, 1971). This may be due to the exuded hydrolytic nematode enzymes that destroy the host cells, activate plant endohydrolases and thus increase the total hydrolytic activity at the feeding site (Veech and Endo, 1969; Giebel *et al.*, 1971).

The results of our investigation also suggest that nematode infection of the root system induces systemic metabolic changes in the shoot, with a higher percent change in acid phosphatase activity in relation to the control, when compared to the root. The results suggest that, invasion of the root by nematodes causes the

transmission of biochemical messages to the host which are possibly reflected either in induction or acceleration of specific enzyme components. It may be due to the fact that, the youngest fully expanded top shoots were used in this study, where the enzyme is relatively very active.

The initial change in enzyme activity in both the cultivars during early phase of infection plays a vital role in deciding the course of disease development as suggested by various earlier workers. The initial increase in acid phosphatase activity in the resistant plant during the post-infectional period leads to a restriction in parasitic activity, the decrease of phosphatase activity in susceptible plant favouring the nematodes.

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