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Induction of Isoperoxidases in Resistant and Susceptible Tomato Cultivars by *Meloidogyne incognita*[1]

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Abstract: Isoperoxidases were detected in resistant Rossol and susceptible Roma VF tomato roots uninfected and infected by *Meloidogyne incognita.* Syringaldazine, guaiacol, p-phenylenediamine-pyrocatechol (PPD-PC), and indoleacetic acid (IAA) were used as substrates, and the corresponding peroxidative activities were detected either in cytoplasmic or in cell wall fractions, except for IAA oxidase, which was measured in soluble and microsomal fractions. Isoperoxidase activities and cellular locations were induced differently in resistant and susceptible cultivars by nematodes. Nematode infestation markedly enhanced syringaldazine oxidase activity in cell walls of the resistant cultivar. This isoperoxidase is involved in the last step of lignin deposition in plants. Conversely, the susceptible cultivar reacted to *M. incognita* infection with an increase in cytoplasmic PPD-PC oxidase activity, which presumedly is involved in ethylene production; no changes in cell wall isoperoxidases were observed. IAA oxidase was inhibited in susceptible plants after nematode inoculation, whereas in resistant plants this activity increased in the soluble fraction and decreased in the microsomal fraction.

Key words: auxin, ethylene, isoperoxidase, lignification, *Lycopersicon esculentum, Meloidogyne incognita,* peroxidase, resistance, tomato.

Peroxidase involvement in biotic and abiotic stresses of plants has been reported frequently (3,10). Little is known, however, about the role of peroxidase in specific nematode-plant interactions. Root-knot nematodes enhance synthesis of at least five isoperoxidases detected by electrophoresis (12). Ganguly and Dasgupta (4) claimed that peroxidase activity increased in both resistant and susceptible tomato cultivars attacked by root-knot nematodes. On the other hand, Zacheo et al. (21) and Bajaj et al. (1) reported that *Meloidogyne incognita* induced an increase of peroxidase activity in resistant tomato cultivars but a significant decrease in susceptible cultivars. Thus, the involvement of peroxidase in root-knot nematode infection cannot be evaluated conclusively.

In this study I estimated peroxidase activities with different substrates and ascertained the effect of nematode infection on the isoperoxidases and their compartmentalization. Isoperoxidases involved in lignification, auxin catabolism, and probably in ethylene biosynthesis are chosen because these physiological processes are probably involved in plant responses to root-knot nematodes (6-8).

MATERIALS AND METHODS

Growth and inoculation procedures: Seeds of tomato (*Lycopersicon esculentum* Mill.) cultivars Roma VF and Rossol, respectively susceptible and resistant to *Meloidogyne incognita,* were germinated in sterilized quartz sand. Seven days after germination, groups of five seedlings were transplanted into 3-cm-d clay pots containing quartz sand. Eight days later, half of the pots received 80 active second-stage juveniles (J2) of *M. incognita* race 2 per seedling. The remaining, uninoculated seedlings were controls. Five days after inoculation, enzymatic fractions were extracted from roots. Plants were maintained in a growth chamber at 26 C, illuminated for 12 hours per day, and watered with Hoagland's solution twice daily. Second-stage juveniles of *M. incognita* were extracted from highly infected eggplant roots as described by Chapman (2) and collected on a 38-μm-pore d (400 mesh) sieve.

Preparation of enzymatic fractions: Seedlings were washed with distilled water; then the roots (ca. 50-60 g per experiment) were

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TABLE 1. Isoforms of peroxidase from cytoplasmic fractions of resistant (Rossol) and susceptible (Roma VF) tomato roots uninfected and infected by *Meloidogyne incognita* race 2.

Substrate	Rossol			Roma VF		
	Uninfected	Infected	% of control	Uninfected	Infected	% of control
Guaiacol	5.4 ± 0.2	6.4 ± 0.5	+ 26 ± 6	6.5 ± 0.1	5.4 ± 0.1	− 17 ± 2
Syr.	39.6 ± 0.4	52.9 ± 2.7	+ 33 ± 6	48.6 ± 1.1	47.0 ± 4.3	− 3 ± 7
PPD-PC	23.6 ± 0.6	26.7 ± 1.1	+ 13 ± 3	20.9 ± 0.3	24.9 ± 1.5	+ 19 ± 9

Oxidation rates are expressed for guaiacol as ΔA_{470}/minute × mg protein, for syringaldazine (Syr.) as ΔA_{530}/minute × mg protein, for *p*-phenylenediamine-pyrocatechol (PPD-PC) as ΔA_{557}/minute × mg protein. Values are the averages of three experiments ± standard deviation.

separated from the shoots and kept in an ice bath. Roots were placed in ice-cold 0.05 M phosphate buffer (pH 6.0) and cut with scissors to obtain a coarse homogenate. This homogenate was ground by hand in a porcelain mortar and filtered through four layers of gauze. The filtrate was centrifuged for 10 minutes at 500 *g*. After two washings with the grinding medium pellets were used as cell wall fractions. The supernatant was further centrifuged for 15 minutes at 12,000 *g*. The pellet of this second centrifugation was discarded, and the supernatant was treated with ammonium sulfate to 70% saturation. After standing overnight at 4 C, the ammonium sulfate precipitate was collected by centrifugation at 30,000 *g* for 20 minutes, redissolved in a minimal volume of 0.05 M phosphate buffer (pH 6.0), and dialyzed against 0.05 M phosphate buffer (pH 7.0) containing 0.1 M KCl for ca. 12 hours at 4 C. No precipitation occurred with dialysis at this pH, so the dialyzate was designated the cytoplasmic fraction. When a microsomal preparation was required, the supernatant of the second centrifugation was recentrifuged at 100,000 *g* for 150 minutes; the pellet was used as the microsomal fraction, and the supernatant was precipitated with ammonium sulfate. Enzymatic fractions were stored at −20 C until analyzed; loss of the peroxidase activities caused by this treatment was negligible.

Spectrophotometric enzyme assays: Guaiacol oxidation was monitored in 1 ml of an assay mixture containing 0.05 M phosphate buffer (pH 6.0), 5 mM guaiacol, 2 mM H_2O_2, and 20 μl enzyme extracts at 470 nm. For syringaldazine oxidation the same assay mixture was used in the presence of 50 μM syringaldazine, with absorbance monitored at 530 nm. Peroxidase activity was also detected by oxidation of 0.35 mM *p*-phenylenediamine (PPD) in the presence of 4.5 mM pyrocatechol (PC) in 1 ml of 0.1 M Tris-HCl buffer (pH 7.6) and 2 mM H_2O_2, monitored at 557 nm.

IAA oxidation: Indoleacetic acid (IAA) oxidation was measured in an oxygraph cell as oxygen uptake in the presence of 800 μM IAA, 250 μM dichlorophenol (DCP), 100 μM $MnSO_4$, 100 μM H_2O_2, 50–100 μl enzyme extracts, 75 mM sodium succinate buffer (pH 4.0), final volume 1 ml, at 25 C. This activity was tested in cytosolic and microsomal fractions because IAA oxidase has been reported to be strictly soluble or bound to cellular membranes (10).

Protein determination: Protein was estimated by the Lowry method (15) with bovine albumine as a standard.

Replication: Spectroscopic and polarographic determinations of the peroxidase activities tested were replicated three times each experiment. Three experiments per cultivar were conducted. Thus, data reported in the tables are the means of values derived from three different root samples ± standard deviation.

RESULTS

Isoperoxidase activities from cytoplasmic fractions of resistant (Rossol) roots differed from similar activities in susceptible (Roma VF) roots infected by *Meloidogyne incognita* (Table 1). PPD-PC oxidase activity increased by about 20% in susceptible roots, whereas increases of syringaldazine and guaiacol oxidase activities occurred in

TABLE 2. Isoforms of peroxidase from cell wall fractions of resistant (Rossol) and susceptible (Roma VF) tomato roots uninfected and infected by *Meloidogyne incognita*.

Substrate	Rossol			Roma VF		
	Uninfected	Infected	% of control	Uninfected	Infected	% of control
Guaiacol	0.7 ± 0.02	1.0 ± 0.1	+ 53 ± 9	0.5 ± 0.02	0.5 ± 0.02	
Syr.	6.0 ± 0.5	10.5 ± 0.1	+ 75 ± 15	6.0 ± 0.5	6.4 ± 0.4	+ 7 ± 12
PPD-PC	6.1 ± 0.4	7.9 ± 0.6	+ 30 ± 17	6.0 ± 0.2	5.9 ± 0.1	− 0.5 ± 4

Oxidation rates are expressed for guaiacol as ΔA_{470}/minute × mg protein, for syringaldazine (Syr.) as ΔA_{550}/minute × mg protein, for *p*-phenylenediamine-pyrocatechol (PPD-PC) as ΔA_{557}/minute × mg protein. Values are the averages of three experiments ± standard deviation.

resistant roots. High syringaldazine oxidase activity occurred in cytoplasmic fractions of both cultivars.

In cell walls, activities of all three isoperoxidases tested increased in infected, resistant roots with respect to the uninfected controls (Table 2). Syringaldazine oxidase activity was more markedly enhanced (75%) after infestation. No apparent variations of isoperoxidase activities were found in cell walls of susceptible, infected roots relative to the uninfected controls.

Generally, activity moved from cytoplasmic to cell wall fractions in resistant, infected tomato roots (Table 3). Conversely, in susceptible roots the cytoplasmic percentage of PPD-PC peroxidase activity increased after infestation; no changes in percentage of total activity were detected for the other two isoperoxidases.

Distribution of IAA oxidase activity between the soluble and microsomal fractions was greatly changed in resistant tomato roots after *M. incognita* attack (Table 4). This activity decreased in microsomes (− 45%) and increased in the soluble fraction (+ 49%) which could also be representative of extracellular spaces. On the contrary, IAA oxidase activity was depressed in soluble fraction from susceptible, infected roots.

DISCUSSION

The results provide evidence that root-knot nematode infection differentially induces various plant isoperoxidases. Thus, reports in which changes in peroxidase activity due to nematode stress are based on only one substrate might result in an inaccurate representation of events.

Goldberg et al. (9) reported that intracellular (cytosol + membranes) peroxidase activity was maximal with PPD-PC as substrate. PPD-PC isoperoxidase could be a cytoplasmic modulating certain growth processes, e.g., ethylene biosynthesis (5). PPD-PC peroxidase activity is specifically enhanced in cytoplasmic fractions of susceptible roots 5 days after inoculation with *M. incognita* and possibly contributes to the rise in ethylene production found in the compatible response of tomato roots to root-knot nematodes (8). A corresponding decrease of guaiacol oxidase activity in the same fractions also occurs.

TABLE 3. Percentages of total activity of peroxidase isoforms in cytoplasmic (cyt.) and cell wall (c.w.) fractions of resistant (Rossol) and susceptible (Roma VF) tomato roots uninfected and infected by *Meloidogyne incognita*.

Substrate†	Rossol				Roma VF			
	Uninfected		Infected		Uninfected		Infected	
	cyt.	c.w.	cyt.	c.w.	cyt.	c.w.	cyt.	c.w.
Guaiacol	92 ± 2	8 ± 2	89 ± 1	11 ± 1	95 ± 0	5 ± 0.3	94 ± 0.2	6 ± 0.2
Syr.	90 ± 2	10 ± 2	88 ± 1	12 ± 1	92 ± 1	8 ± 1	92 ± 1	8 ± 1
PPD-PC	86 ± 2	14 ± 2	81 ± 3	19 ± 3	83 ± 1	17 ± 1	87 ± 1	13 ± 1

† Syr. = syringaldazine; PPD-PC = *p*-phenylenediamine-pyrocatechol.

TABLE 4. IAA oxidation in soluble and microsomal fractions of resistant (Rossol) and susceptible (Roma VF) tomato roots uninfested and infested by *Meloidogyne incognita.*

	Uninfected	Infected	% of control
Rossol			
Soluble fr.	261 ± 40.4	380.9 ± 3.0	+ 49 ± 28
Microsomal fr.	326.4 ± 20.6	179.5 ± 5.9	− 45 ± 5
Roma VF			
Soluble fr.	638.6 ± 61.4	466.3 ± 15.4	− 23 ± 2
Microsomal fr.	669.0 ± 45.1	653.6 ± 41.2	− 2 ± 1

Values, expressed as nmoles O_2/minute × mg protein, are the average of three experiments ± standard deviation.

Syringaldazine isoperoxidase has been reported generally to be specific for the lignification process and localized in cell walls (11), probably in their free spaces (16). However, the high syringaldazine oxidase activity present in the cytoplasmic fractions tested could derive from the solubilization of this isoperoxidase during the extraction procedures (14). Cell walls of the resistant cultivar reacted to *M. incognita* invasion with a large increase in syringaldazine oxidase activity, thus indicating an increase in lignin deposition. Lignification is generally considered a mechanism of resistance to fungal infection (19), but there has been no prior evidence associating lignification with nematode infection. Because of the apparent induction of the isoperoxidase that catalyzes the condensation of lignin precursors in the incompatible response, lignification could also be a crucial process in resistance to root-knot nematodes. Cell wall isoperoxidases do not appear to be induced in susceptible plants by *Meloidogyne* infection.

Frequently, higher concentrations of auxin occur in *Meloidogyne*-infected galled tissue than in gall-free tissue (13). However, there is no convincing evidence for the origin of the increased concentration of auxins in nematode-infected plant tissues (20). Data presented here suggest that, at least in the tomato–*Meloidogyne* compatible interaction, the inhibition of IAA oxidase could cause an increase in the auxin level, which could be involved in the development of the compatible response. An inhibition of IAA destruction occurs in the compatible reaction of potato to the cyst nematode *Globodera rostochiensis* (6,7); in this case, however, the authors proposed that the inhibition was caused by a low monophenol-to-polyphenol ratio. For the susceptible tomato–*Meloidogyne* interaction, I suggest that the high level of ethylene (8) could inhibit IAA oxidase (5,18).

In conclusion, further investigations are needed to clarify the possible role in pathogenesis of the peroxidases involved in detoxification, generation of H_2O_2 in cell walls, cell wall genesis, etc. (5,9,17). In future work I will focus on the factors that presumably elicit a different induction of peroxidase isoenzymes by susceptible and resistant varieties.

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