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SEQUENTIAL DEVELOPMENT OF POLYPHENOL OXIDASE (E.C. 1.14.18.1) IN RESISTANT AND SUSCEPTIBLE TOMATOES INOCULATED WITH THE ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*

by
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Although phenolics and their oxidation products are reported to be toxic to invading pathogens (Schall and Johnson, 1955), the role of polyphenol oxidase on the expression of disease symptoms caused by plant parasitic nematodes is ill-defined. A number of workers have demonstrated accumulation of phenolics after host-infection with plant parasitic nematodes (Epstein, 1972; Acedo and Rohde, 1971; Huang *et al.*, 1971; Brueske and Dropkin, 1973). These observations suggested a need to examine the role of polyphenol oxidase in the plant response to nematode activity. It has been shown that the sequence of events initiated with nematode infestation are of importance in understanding the mechanism of plant response to the nematode (Ganguly and Dasgupta, 1979, 1979a; Mote and Dasgupta, 1979). The objective of this investigation was to examine some of the qualitative and quantitative changes in polyphenol oxidase activity with reference to the root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw. infesting resistant and susceptible tomato varieties.

Materials and Methods

Five weeks old seedlings of tomato varieties Pusa Ruby (susceptible) and SL-120 (resistant) grown individually in pots containing 500 g of sterilized river sand, were inoculated with 5000 second stage larvae of *M. incognita* per seedling. The growing seedlings received

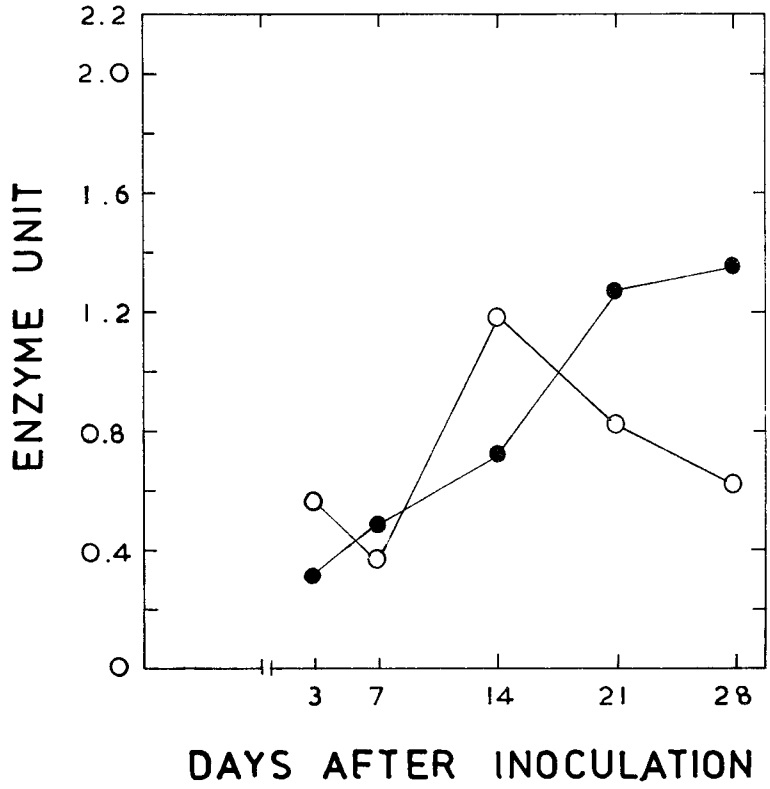

Fig. 1 - Changes in polyphenol oxidase activity in roots of *M. incognita* - inoculated and uninoculated tomato var. Pusa Ruby. O, uninoculated; ●, inoculated. Enzyme unit is defined as change in optical density per min per ml at 546 nm.

Hoagland nutrient solution. One set of seedling of each variety was left uninoculated to serve as controls. Seedling sets, infested and non-infested controls were harvested at 3, 7, 14, 21 and 28 days after inoculation. Four grams of root and shoot material was used for preparing crude extracts in pre-chilled 0.05 M tris-HCl buffer, pH 8.0 containing 0.1% ascorbic acid, 0.1% cystein-HCl, 17% sucrose and 0.002% of $MgCl_2$. The crude extracts were strained through four layers of sterilized cheese cloth and centrifuged at 16,000 g for 20 min. at 1° C. The supernatant from shoot material was dialyzed against 40 volumes of the same buffer while the root extract was subjected to sephadex G-25 column chromatography.

The protein was estimated by the method of Lowry *et al.* (1951).

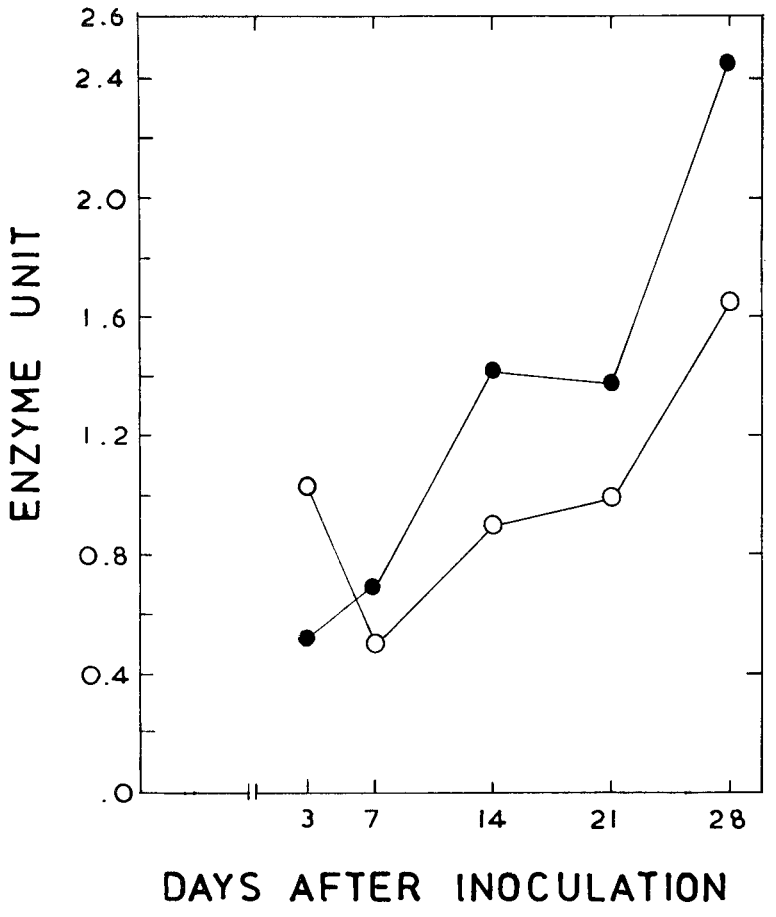

Fig. 2 - Changes in polyphenol oxidase activity in roots of *M. incognita* - inoculated and uninoculated tomato var. SL-120. O, uninoculated; ●, inoculated. Enzyme unit is defined as change in optical density per min per ml at 546 nm.

The amount of protein in the sample was calculated from a standard curve using bovine serum albumin as a protein standard.

The enzymic activity of polyphenol oxidase (catechol oxidase : O-diphenol; O_2 oxido-reductase E.C. 1.14.18.1) was determined following the method of Jennings *et al.* (1969) with slight modifications. The reaction mixture contained 0.2 ml of enzyme solution; 1.5 ml 0.02 M sodium potassium phosphate buffer, pH 6.0; 0.5 ml proline (5 mg/ml) and 0.5 ml catechol (2 mg/ml). The mixture was aerated by vigorous

shaking using a Vortex mixer, before addition of catechol. Enzyme activity was expressed in units as Δ O.D. (change in optical density) per min. per ml at 546 nm.

The extracts of root as well as shoot were subjected to polyacrylamide disc-electrophoresis under the alkaline system of Davis (1964) for the analysis of polyphenol oxidase. A sample containing 50-100 μg protein in 20% sucrose was loaded at the top of a gel together with 20% sucrose under buffer. The runs were made at room temperature with a current of 4 mA per tube. The runs were completed when the tracking dye, bromophenol blue, moved to within 5 mm of the anodic end of the gel. The gels were removed and the protein bands were stained overnight with 1% amido-schwartz in 7% acetic acid and de-stained in 7% acetic acid. To determine polyphenol oxidase isozymes, gels were stained by immersing them in DL-DOPA (1.5 mg/ml in 80% ethanol) for 15 minutes and then de-stained and stored in 30% ethanol (Constantinides and Bedford, 1976). Gels were stained separately for peroxidase (Shannon *et al.*, 1971) and polyphenol oxidase (Constantinides and Bedford, 1976). The relative electrophoretic mobility of protein bands and polyphenol oxidase isozymes was calculated as the ratio of the movement of the band to that of the tracking dye.

To check whether or not invading nematodes as contaminants contributed towards increased enzyme activity, equivalent numbers of second stage larvae used as initial inoculum were analyzed for the detection of polyphenol oxidase. In addition to second stage larvae, embryonated egg masses were also processed for the detection of polyphenol oxidase. Axenized nematodes were homogenized by an electrically operated Potter-Elvehjem type teflon glass micro tissue homogenizer at 14,000 rpm for 3 minutes in 0.05 M Tris-HCl buffer, pH 8.0 at 2° C. The homogenate was centrifuged at 16,000 g for 25 minutes at 1° C. The clear supernatant solution was subjected to qualitative and quantitative analyses for polyphenol oxidase activity as done in case of root and shoot extracts.

Results and Discussion

Protein analyses of shoots of infested and non-infested plants showed no differences which could be attributed to nematode activity.

In roots, after an initial decline, activity of polyphenol oxidase increased, though not consistently, in inoculated plants of both the

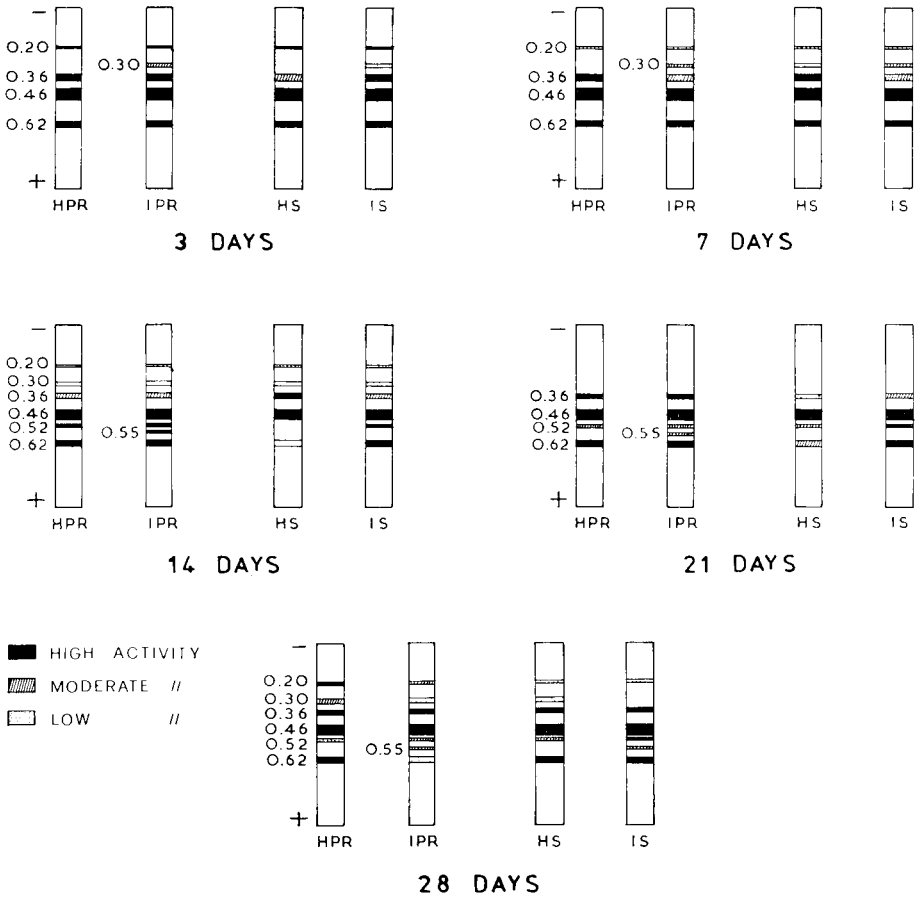

Fig. 3 - Zymogramme patterns of polyphenol oxidase isozymes of roots of tomato varieties at 3, 7, 14, 21 and 28 days after inoculation with *M. incognita*. HPR = Healthy Pusa Ruby; IPR = Inoculated Pusa Ruby; HS = Healthy SL-120; IS = Inoculated SL-120.

varieties (Figs. 1 and 2). By seven days after inoculation, the enzyme activity in the resistant variety increased by 30%, by 14 days 40%, by 21 days 54% and by 28 days 120%.

The isozymic pattern of healthy shoot extracts for all intervals of observations were same as that of diseased ones for both varieties.

Three days after inoculation, five bands of polyphenol oxidase were resolved for nematode-infested roots of Pusa Ruby as well as SL-120, as against four bands of polyphenol oxidase detected from

healthy roots of both the varieties. The newly appeared band in infested roots is apparently distinct from the existing ones as judged on the basis of R_f value ($R_f = 0.30$; Fig. 3). This particular band with an R_f value 0.30, also appeared in uninoculated SL-120, at 7 days after inoculation, and was absent from healthy Pusa Ruby. Fourteen days after inoculation, the number of bands in uninoculated and inoculated Pusa Ruby were 6 and 7 respectively. The polyphenol oxidase band with an R_f value 0.55 in inoculated Pusa Ruby appeared to be lacking in healthy counterparts. The polyphenol oxidase of SL-120, for the same interval is depicted in Figure 3. It is noteworthy to mention here that 21 days after inoculation, the polyphenol oxidase isozyme patterns were identical in the inoculated and uninoculated resistant reacting variety of SL-120. However, this was not true for the susceptible var. Pusa Ruby which showed differences in polyphenol oxidase isozymic pattern between uninoculated and inoculated.

Evidence presented in this investigation suggests that increase in polyphenol oxidase activities in the infested plants was due, at least in part, to synthesis of new enzyme protein. Indeed, the appearance of new polyphenol oxidase isozyme during the early period of post-infection (3-14 days) supports this view. The initial decrease in enzyme activity in the root during early infestation might arise from mechanical injury caused by nematodes or injection of inhibitory substances. The increase in enzyme activity at later stages may result from activation of latent enzyme by pectic enzyme produced by the pathogen (Deverall and Wood, 1961; Balasubramani *et al.*, 1971).

Possible reason to account for increased enzyme activity is the additive effect of the metabolite molecules originating from invading pathogen. Indeed, Maraite (1973) reported that one of the isozymes of polyphenol oxidase of an infested host was of pathogen origin. Whether or not the pathogen itself contributed directly to increase polyphenol oxidase activity in the host by contaminating some of the isozymes, was also examined in this investigation. We could not detect any polyphenol oxidase activity with the number of larvae used as initial inoculum. It may be mentioned here that Wallace (1961) also failed to detect polyphenol oxidase in *Aphelenchoides ritzemabosi*. We reject the possible contribution of nematode directly as contaminants towards increased enzyme activity observed during the post-infection period.

From the qualitative changes in polyphenol oxidase in two varieties after the infection, it was seen that the newly detected isozymes which

appeared in the diseased plants appeared at a later stage in the healthy plants. This phenomenon of accelerated senescence was true for both the varieties. The enzyme band with R_f value 0.30 from the diseased plant appeared in susceptible healthy variety at later stage than the resistant variety. Also the band with R_f value 0.52 appeared later in the healthy resistant variety than in healthy susceptible variety. The band with R_f value 0.55 which appeared in the infested susceptible variety at 14 days after inoculation, could be seen in the infested resistant variety 28 days after inoculation. Hence, the late appearance of isozyme with R_f value 0.55 in the inoculated resistant variety and its early appearance in the infested susceptible variety might be an important difference to account for resistance of SL-120. Therefore, we assume that a possible reason to explain the phenomenon of plant resistance to the nematode, is the ability of an incompatible host to resist premature aging associated with isozymes. Increased senescence with reference to polyphenol oxidase isozymes in a host following the invasion of pathogens has been observed by Maraite (1973) and Farkas (1968). However, this could be one of many events which decide the final course of plant-nematode-interaction.

SUMMARY

Sequential development of polyphenol oxidase activity was studied in tomato vars. Pusa Ruby (susceptible) and SL-120 (resistant) after inoculation with the root-knot nematode, *Meloidogyne incognita*. Nematode infestation did not result in interference of enzymic protein synthesis in the shoot region. Initial decrease in the enzyme activity after nematode infestation was consistent with time in both the varieties. Quantitative changes in enzyme activities were accompanied by qualitative changes in isozyme patterns in both the varieties. The nematodes did not contribute as contaminants towards increased polyphenol oxidase activity. Accelerated senescence of PPO isozymes was observed in the susceptible variety but not in the resistant variety.

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