RELATIONSHIP BETWEEN INDOLE ACETIC ACID OXIDASE, POLY PHENOL OXIDASE, INDOLE ACETIC ACID AND RESPONSE OF TOMATOES TO Meloidogyne incognita INFECTION

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ABSTRACT: Two tomato cultivars, Pusa Ruby and Mangala, (susceptible and resistant, respectively, to the populations of Meloidogyne incognita) were assayed for poly phenol oxidase (PPO), IAA oxidase activities and indole-acetic acid (IAA) contents under inoculated and uninoculated conditions. Polyphenol oxidase activity and IAA contents were higher in infected roots of both the cultivars compared to their respective healthy counterparts, although, the magnitudes of both the parameters were much higher in susceptible Pusa Ruby compared to the resistant Mangala. IAA oxidase activity was observed to be lower in healthy and infected roots of Pusa Ruby compared to the healthy and infected roots of Mangala.

Key Words: Host plant resistance, indole-acetic acid, indole-acetic acid oxidase, Meloidogyne incognita, poly phenol oxidase, tomato.

INTRODUCTION

Several scientists conclusively demonstrated that the root galls produced by different species of Meloidogyne contained indole compounds (auxin precursors) (Balasubramanian and Rangaswami, 1962; Bird, 1974; Yu & Viglierchio, 1966). Geibel (1974) emphasized that the pathogenic changes at the site of infection in susceptible plant were due to the auxins released by the complex enzymatic action. Two of the important enzymes involved in this complex enzymatic system were identified to be polyphenol oxidase and IAA oxidase that have a specific role in host expression. Poly phenol oxidase activity modifies the plant phenolics and was reported to increase both in giant cells (induced by nematodes) and in necrotic cells as compared to the healthy cells (Geibel, 1970). In the present investigation the relationship between auxins, IAA oxidase, PPO activity and the response of tomato cultivars, Pusa Ruby (susceptible to M. incognita) and Mangala (resistant to M. incognita) were studied.

MATERIALS AND METHODS

Three-week old seedlings of the root-knot nematode resistant and susceptible tomato (Lycopersicon esculentum (L) Mill.) cultivars, Mangala and Pusa Ruby, respectively, were
transplanted in earthen pots containing autoclaved soil. Two weeks later, they were inoculated with *M. incognita* juveniles @ 21g soil. Uninoculated sets served as checks. Ninety-six hours after inoculation, both inoculated and uninoculated Pusa Ruby and Mangala were harvested. The roots were cleared of soil, washed, blotted and weighed. One set of root samples was estimated for auxin contents and another set was assayed for poly phenol oxidase (PPO) and Indole Acetic acid oxidase activities.

I. Estimation of auxin contents:

i. Extraction and purification of auxins:

Five grams of infected and healthy root samples of Pusa Ruby and Mangala, each, was homogenized in excess of methanol. The homogenized tissue samples were used for extraction and purification of auxins as described by Wurt et al (1980).

ii. Derivatisation and quantification by G.L.C.:

The purified extract of each sample was reduced to dryness and stored in desiccator. The samples were derivatized with N,O-bis (methyl silyl) acetamide (BSA) for 30 minutes at room temperature. The derivatized sample product, thus obtained, was injected individually into gas chromatograph HP 5840 A. The operation conditions included temperature programming from 60 to 220°C with an increment rate of 12.8°C per minute. The injection and FID temperatures were estimated through ESTD.

II. Estimation of polyphenol oxidase activity:

The estimation of PPO activity was done essentially as described by Malik and Singh (1980). Five grams each of infected and healthy roots of Pusa Ruby and Mangala were homogenized in 0.1 M Phosphate buffer (pH 6.0) in pestle and mortar. The catecholase activity of the extract was assayed in a cuvette containing 0.01 M catechol in 0.1 M phosphate buffer (pH 6.0) and crude extract, in a final volume of 3.0 ml. The change in absorbance was recorded at 495 nm in spectrophotometer SPEKOLL 11. The enzyme activity is expressed in terms of rate of increase of absorbance/minute.

III. Estimation of Indole-3-acetic acid oxidase activity:

The enzymatic activity of IAA oxidase was determined following the method of Gortner & Kent (1953).

i. Preparation of enzyme extract:

Five grams of infected and healthy root samples (frozen) of Pusa Ruby and Mangala, each, were homogenized in excess of cold acetone. Homogenate for each sample was collected by Buchner filtration through Whatman No.1 filter paper. The homogenate was air-dried until it was free from acetone odour, the resulting dry powder was weighed and stored at -20°C. One gram of acetone powder was ground in two successive 20 ml aliquots of 25mM phosphate buffer (pH 6.2) in a mortar chilled in an ice bath. Extracts were collected by Buchner filtration using Whatman No.1 filter paper after each grinding. Filtrate collected from each sample was made up to 50 ml with phosphate buffer.

ii. Assay of enzyme activity:

Enzyme extract of 0.1ml was incubated in a reaction mixture of 2.5 ml 0.05M phosphate buffer (pH6.2) containing 0.1mM MnCl2, 0.1mM DCP and 0.1mM IAA (indole-3-acetic acid) for 15 minutes at 37°C. The reaction was stopped by adding 3 ml of 20% perchloric acid. Remaining IAA was determined spectrophotometrically after the addition of modified Salkowski reagent as given by Gordon & Weber (1951). The optical density of the pink coloured solution was taken after 25 minutes at 540 nm to determine residual IAA on a spectrophotometer. Enzyme activity was expressed as 5g IAA destroyed or oxidised/ min/mg protein. Total protein content in the
enzyme extract was estimated following the method of Lowry et al. (1951).

**RESULTS AND DISCUSSION**

The comparative study on the activities of PPO and IAA oxidase enzymes and IAA contents in healthy and nematode-infected root tissues of resistant and susceptible tomato cultivars clearly (Table 1 & Fig1) indicated that,

(i) the PPO activity and IAA contents were higher while IAA oxidase activity was less in infected roots of both Pusa Ruby and Mangala compared to their respective healthy root samples,

(ii) the healthy and nematode-infected Pusa Ruby roots (susceptible) had higher PPO activity and IAA contents than their healthy and infected Mangala (resistant) roots. IAA oxidase activity was lower in healthy and infected Pusa Ruby roots than that of healthy and infected roots of Mangala,

(iii) the nematode infection in both Pusa Ruby and Mangala induced higher PPO activity,
IAA contents, although, it was much higher in susceptible compared to the resistant tomato. Further, nematode infection in resistant Mangala, induced higher IAA oxidase activity compared to that in Pusa Ruby, and 

(iv) there was an inverse relationship between PPO activity and IAA oxidase activity in infected and healthy roots.

In a similar study on Fusarium-infected tomato plants, Malta and Gentile (1968) arrived at a linear relationship between PPO activity and ability to form IAA from tryptophan in the presence of chlorogenic acid. They observed that the pathological status was accompanied by an increased ability of leaf and stem extracts to produce IAA through the action of a PPO system. The PPO enzyme oxidises various ortho-diphenols which react with tryptophan to form auxin.

This mechanism of IAA formation may not be operative in healthy plants but may become operative after activation of the PPO complex by wounding or infection, and thus be responsible for the increase in IAA concentration in injured or diseased tissue (Gordon & Paleg, 1961). In the context of present study, the increase in IAA contents in infected tissues is primarily due to the PPO mediation, especially in susceptible root tissue. The higher activity of PPO enzyme and relatively lower activity of IAA oxidase in the infected susceptible roots accounts for their higher IAA contents. Gordon & Paleg (1961) hypothesised that the concentration of other compounds like, phenols and tryptophan, involved in the process of IAA formation, also increased as a result of infection.

Resistant and susceptible root tissues differ in their nature of phenols viz., mono and poly phenols, their relative contents and thus, in their PPO activity. On the other hand, lower peroxidase and higher IAA oxidase activities and their isozyme contents in a resistant root tissue compared to its susceptible counterpart, as observed in a resistant tomato cultivar, SL-120, could account for the rapid oxidation of IAA (Ganguly and Dasgupta (1979). Relating these observations, the lower accumulation of IAA in resistant infected tissue could be attributed to (i) the differential mediation of PPO and (ii) the activity of IAA oxidase.

It is, therefore, summarised that there is a dynamically interrelated mechanism of host expression to nematode infection with PPO and IAA oxidase enzyme systems and IAA accumulation.

REFERENCES


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