Asian J. Exp. Sci., Vol. 21, No. 2, 2007, 351-355

Interaction of *Trichoderma harzianum* with *Fusarium solani* During its Pathogenesis and the Associated Resistance of the Host

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Abstract : Changes in the activities of peroxidase and polyphenol oxidase in healthy and infected plants of *Solanum melongena* Linn., caused by *Fusarium solani* (Martius) Appel and Wollenweber were assayed under different periods. The enzymes activities appeared to be increased with increase in the period of infection and reached a maximum level at the end of an incubation period, which is directly correlated with the resistance of the plant against infection. It was noticed that the biocontrol agent, *Trichoderma harzianum* (Rifai) significantly interacts with the activities of phenol oxidizing enzymes of the pathogen. The reduction in the activities of phenol oxidizing enzymes after 21st day incubation was recorded when plants treated with both pathogen and antagonist. Such interrelationship between these two enzymes plays a key role to stimulate the defense mechanism of the host plant against infection.

Key words : *Solanum melongena*, *Fusarium solani*, *Trichoderma harzianum*, Wilt, Peroxidase, Polyphenol oxidase, Resistance

Introduction

Solanum melongena Linn., belongs to the family Solanaceae having immense commercial value for its fruits. Wilt disease of *S. melongena* caused by *Fusarium solani* (Martius) Appel and Wollenweber brings about a serious threat to its cultivation. Different species of *Trichoderma* are well known to antagonize other pathogenic fungi and success has been achieved in a number of crop diseases. *Trichoderma harzianum* (Rifai) is a most potent biocontrol agent which inhibits the growth of *F. solani*, causal organism of wilt of brinjal, efficiently both in vitro and in field condition (Chakraborty, 2005). Increased activity of polyphenol oxidase and peroxidase in response to infection by the pathogen has been reported by many workers (Jennings *et al.*, 1969; Vidhyasekaran, 1988) and are considered to play an active role in contributing to disease resistance in certain plant host- pathogen interaction following infections. In the present communication, an attempt was made to study the activity of these enzymes of brinjal plants during *T. harzianum* and *F. solani* interaction to find any correlation that may exist in host defense mechanism.

Materials and Methods

F. solani was isolated from wilted brinjal plants and maintained in pure line on potato dextrose agar (PDA) slants at 4°C till used. The identification of the pathogen was confirmed by Indian Agricultural Research Institute (IARI), New Delhi (ITCC No.4124.2K). The antagonists used *T. harzianum* was procured from IARI, New Delhi.

For studying the in vivo enzyme activity, fresh and healthy seedlings were taken and planted in earthen pots. After that soils and

plants were inoculated with *F. solani*. Spore suspension and mycelial mats in liquid culture of *F. solani* were directly applied to the pot soils where brinjal plants were planted and plants were injected with spore suspension by double sterilized syringe. After mixing the spores and mycelial mats of the pathogen with the soils and injecting the pathogen spores to the plants, plants were kept with proper supervision for using it as source of in vivo experiments. Liquid culture (spore suspension) and mycelial mats of *T. harzianum* was directly applied to another sets of *Fusarium* treated pots by using previously sterilized wheat bran as substrate so as to get *Fusarium* + *Trichoderma* treated pots.

Assay of polyphenol oxidase

For extraction of the enzyme, polyphenol oxidase, 2.0g each of healthy and infected tissues were homogenized separately with a pinch of neutral sand in 6.0 ml of phosphate buffer (0.1M at pH 7.0) at 0°C. The extracts were obtained by filtering off the debris with a clean cloth and centrifuged at 3000 rpm for 15 minutes in cold centrifuge. The supernatants were recovered and collected which served as the enzyme source.

Polyphenol oxidase activity was measured following the method described by Sadasivam and Manickam (1996). 2.0 ml of the enzyme extract and 3.0 ml of 0.1 (M) phosphate buffer were mixed together in a cuvette and the sample was adjusted to zero absorbance in a Spectrophotometer (Systronics, uv-vis, 117) at 495 nm. 1.0 ml of 0.01 M catechol in 0.1 M phosphate buffer (0.4 mg/ml) was added to the above mixture and the reactants were quickly mixed. The enzyme activity was measured as the change in absorbance per minute upto 30 minute (/min) at 495 nm immediately after the addition of catechol solution, which initiated the reaction. Control in similar manner was maintained at different times by heating the extract at 100°C for 10 minutes. The activity was always measured zero indicating complete inactivation of the enzyme by this heat treatment.

Assay of peroxidases

For extraction of the enzyme, peroxidase, 4.0g each of health and infected tissues were separately homogenized in 20ml of chilled distilled water at 0°C. A pinch of neutral sand was added to facilitate crushing. The extracts were obtained by filtering off the debris with a clean cloth and centrifuged at 3000 rpm for 15 minutes in cold. The supernatants were recovered serving as the enzyme source and held in tubes separately in ice-bath until assay.

5 ml of freshly prepared pyrogallol reagent (prepared by mixing 10ml of 0.5M pyrogallol solution and 12.5 ml of 0.66 M phosphate buffer and volume was made to 100 ml with distilled water) and 1.5 ml of the enzyme extract were mixed in a cuvette of a spectrophotometer and the mixture was immediately adjusted to zero absorbance. 0.5 ml of 1% H_2O_2 solution was added to it and inverting the tube mixed the content. The reaction was initiated by the addition of H_2O_2. Enzyme activity was recorded as the change in absorbance per minute (mu/min) at 430nm immediately after the addition of substrate. Similarly, control on non-enzymatic oxidation was maintained at different times by heating the extract at 100°C for 10 minutes. The activity was always measured zero indicating its complete inactivation by the heat treatment. The results are graphically represented in fig 2.

Results

From the result it is evident that following incubation after 7 days, the polyphenol oxidase and peroxidase activities show considerable variation in respect of its production. With increase in the period of incubation after inoculation with the pathogen, *F. solani*, the activities tend to increase. It was noticed that with prolongation of time of incubation, the activities gradually reached maximum on 21st day in case of healthy plant after which the activities slow down. On the other hand, the maximum activities of the enzymes were recorded on 14th day of incubation in plant infected with both the pathogen, *F. solani* and the antagonist, *T. harzianum* after which the activities slowed down. But in case of plants infected with *F. solani* alone, the activity showed gradual increase and reached maximum on 28th day. When plants infected with *T. harzianum* only, the enzyme activities remained slightly lower compared to *F. solani* treated plants while the activities recorded higher than the healthy plants. On the other hand, the activities remain unchanged during the period of 21st and 28th day of incubations.

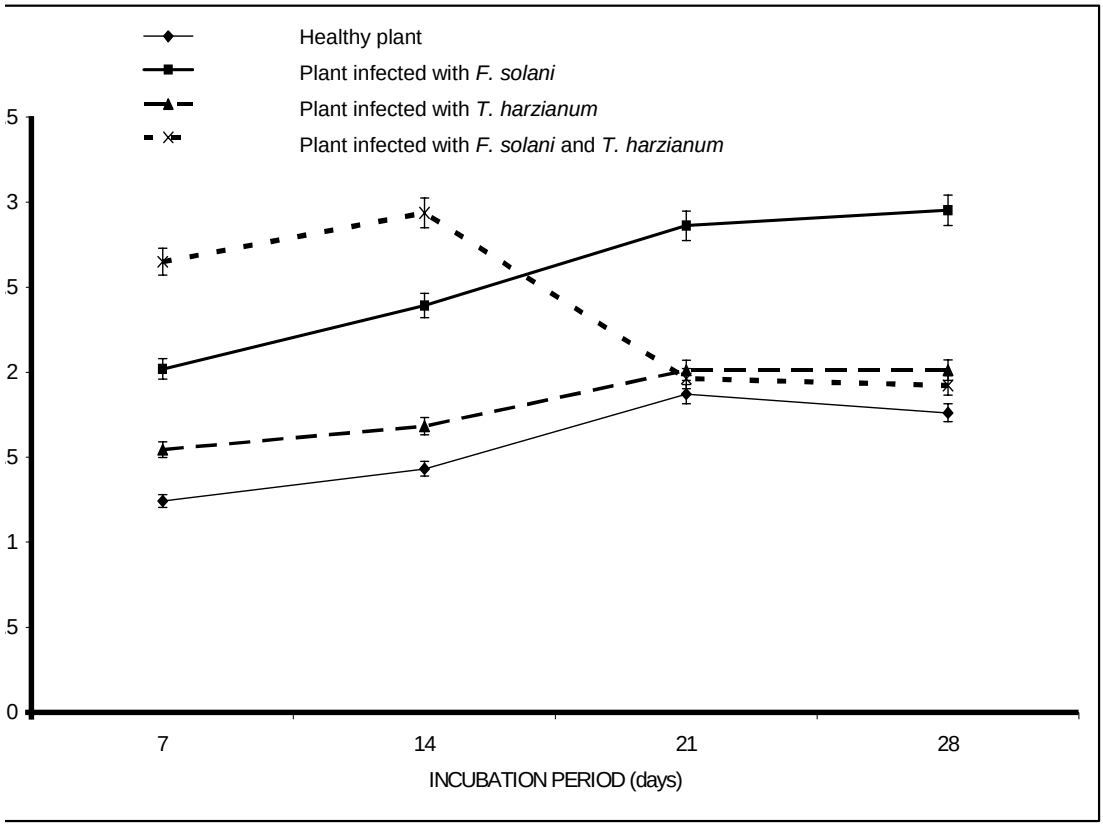

Fig.1: Polyphenol oxidase activity in brinjal plant infected by *F. solani* and *T. harzianum* at different incubation periods (days). Bar represents standard errors.

Discussion

Increase polyphenol oxidase activity in host tissues in response to infection by the pathogen has been reported by a numbers of workers (Karthikeyan and Bhaskaran, 1992; Banik and Chatterjee, 1993, Ojha *et al*., 2005). Polyphenol oxidase might function as an alternate electron transport chain and serve as terminal oxidases in plant tissues (Jennings *et al*., 1969). It is quite probable that the toxic metabolites of the pathogen may activate phenol-oxidizing enzymes where *F. solani* was found to produce such a toxic metabolite.

The phenol-oxidizing enzyme plays a vital role in tissue browning by way of its capacity to oxidize phenols to quinines. The toxic substances, quinones, which are more reactive and have more antimicrobial activity than the phenols already existing in plants (Karthikeyan and Bhaskaran, 1992; Dutta, 1998), account for the increased host resistance against the invading pathogen. Polyphenol oxidase which has been recorded to be increased as a result of infection by the fungal pathogen as is evident in the present experiment, considered to play an active role in contributing resistance to certain plant host-fungi interactions following infections (Esposito *et al*., 1993). Increased peroxidase activity upon infection might be required for an additional deposition of lignin around the lesions induced by pathogens. Changes in the rate of synthesis of peroxidase enzyme as a result of pathogenic infection might be responsible for determining the resistance or susceptibility of the host (Gahukar and Jambhale, 2004) or might be a reflection of the symptoms response associated with degenerative process.

Triggering off of such increased peroxidase activity may be due to the activation of the latent enzymes in hosts or appearance of new isozymes or may be due to the production of the enzymes by the infecting fungi (Dutta, 1998). Phenolic compounds and related oxidative enzymes are mostly considered as one of the important biochemical parameters for disease resistance, and also that the accumulation of total phenols is usually higher in resistant genotypes as compared to susceptible ones (Pradeep and Jambhale, 2002). This correlates the present findings where *F. solani* infected plant having higher peroxidase activity on 28th day of infection than the plant infected with both the pathogen and the antagonist wherein antagonist adversely affects the pathogen.

In the present experiment, when brinjal plant was infected with both the pathogen, *F. solani* and the antagonist, *T. harzianum*, polyphenol oxidase and peroxidase activities remained much higher compared to both healthy plant and *F. solani* infected plant up to 14th day of infection. This might be due to the fact that the host plant when challenged with both the pathogen and the antagonist, the host plant ought to secrete more phenol enzymes for defense but at the steady state of infection the antagonist itself deters the activity of the pathogen resulting in the decline of enzyme activity.

Since the increase in the activity of the enzyme was noticeable from early stages of infection, this may well be due to the increased synthesis of the enzyme and not due to the degenerative process, which should have taken longer time. The enzyme activity enhances as a general metabolic response against pathogen invasion. The

secondary metabolites such as glycosidic substances produced in response to pathogen invasion thus produced may act as a precursor for actual defense mechanism (Sreedhar *et al*., 1995). Even though genotypes (plants) show high polyphenol oxidase and peroxidase activity which have an inhibitory function, plants in field encounter with many natural forces that may alter the enzyme action. Hence for preliminary resistance screening these activities can be used (Gahukar and Jambhale, 2004).

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