Peroxidase and chitinase activities in brinjal inoculated with *Meloidogyne incognita* (Kofoid & White) Chitwood and endomycorrhiza

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ABSTRACT: Studies were conducted to observe the development of peroxidase and chitinase activity in brinjal cv.Co-2 inoculated with Vesicular arbuscular mycorryzae (VAM) and the root knot nematode, *Meloidogyne incognita.* Peroxidase activity was increased and a decrease in chitinase activity was observed which is a defense mechanism of the host to invading pathogen.

KEY WORDS: Chitinase, *Meloidogyne incognita*, peroxidase, VAM

There are many reports where enzymes, like peroxidase are involved in defense mechanism of plants (Nidiry *et al.*, 1992). A potential but indirect role of chitinase in plant pathogen interactions is an elicitor of defense reaction. Chitinase is a lytic enzyme, which degrade chitin. This enzyme is produced by both microorganisms and plants, which trigger defense reaction within the plants (Boller, 1987; Ryan, 1988). Vesicular arbuscular mycorrhizae (VAM) suppress root pathogen through morphological, physiological and biochemical alteration in the host plants (Sharma and Dohroo, 1996). Plants inoculated with VAM were less susceptible to RKN (Sikora and Schonbeck, 1975). The objective of the present investigation is to study the changes in peroxidase and chitinase activity in VAM and root knot nematodes affected plants.

MATERIALS AND METHODS

One-month-old brinjal, (*Solanum melongena* L.) seedlings cv. Co-2 were transplanted into two kg capacity pots filled with sterile pot mixture (red soil: sand: farm yard manure-2: 2: 1). Ten g/ kg soil of VAM inoculum, *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. and Trappe, *G.mosseae* (Nicol & Gerd). Gerd and Trappe, *G. intraradices* Schenck and Smith, and *G. fulvum* (BK. And BR.) Trappe and Gerd. were mixed according to the treatments in each pot containing two seedlings per pot. Fifteen days after transplanting freshly hatched J_2 @ one nematode/g of soil were inoculated near the rhizosphere by making small holes. The treatments (Table 1) were replicated three times and the data were statistically analyzed. One g of fresh leaves and roots were taken from each replicate and

homogenized in a glass pestle and mortar in an ice bath using individual extraction buffer. The enzyme activity of peroxidase was determined using pyrogallol as substrate following the method given by Raja and Dasgupta (1986). The enzyme solution (1ml) was added to a reaction mixture consisting of 2.0 ml of 5 percent pyrogallol (freshly prepared is water), 1.0 ml of 147m, M H_2O_2, 2.0 ml of 0.1 ml phosphate buffer, pH 6.0 and 14.0 ml of water, After exactly 1 minute the reaction was stopped by adding 1.0 ml of 2N $H_2 So_4$ and extracted twice with 5.0 ml portion of ether and optical absorbency was recorded at 420nm using ether.

One g of plant sample was collected and was homogenised in three ml of 0.1mM sodium citrate buffer (pH 5) with a mortar and pestle at 4°C. The homogenate was centrifuged for 15 minutes at 10,000 g. The supernatant was used as an enzyme source and 0.4 ml of this enzyme solution was taken into a 1.5 ml Expender tube and was added with 10μl sodium acetate buffer (pH 5) and 0.1 ml of colloidal chitin. This was incubated in water bath at 37°C for two hours and then centrifuged at 1000 g for three minutes. An aliquot of 0.3 ml was taken into a glass tube containing thirty ml of phosphate buffer and twenty ml of snailgut enzyme (30 mg/ml) and incubated for one hour. To the samples, blank and standard, seventy ml of borate buffer was added. The tubes were heated in a boiling water bath for exactly three minutes and rapidly cooled in ice water. Into the tubes, two ml of p-dimethyl amino benzaldehyde (DMAB) was added and immediately after mixing, the tubes were incubated for twenty min at 37°C. After twenty minutes the tubes were cooled in tap water and read without delay at 585 nm in Hitachi model 200-20 spectrophotometer. The chitinase in leaf and root was expressed as n-mole N-acetyl glucosamine released per minute per g of fresh tissue (Boller and Mauch, 1988).

RESULTS AND DISCUSSION

In the case of peroxidase, the results showed an elevate level of enzyme activity. In plants inoculated with nematodes, *G. mosseae* recorded the highest peroxidase activity (94.5) followed by *G. fasciculatum* with 76.5, which is in agreement with Ganguly and Dasgupta (1979), who reported that increased activity of peroxidase in tomato inoculated with *M. incognita.*

Increased peroxidase activity is associated with resistant reaction due to increased phenol concentration and hence influence the resistance (Giebel, 1974). The elevated peroxidase activity in the diseased plants may be due to the synthesis of new isozymes as a response to the parasitic invasion of host (Mohanty *et al.*, 1986). Peroxidase activity was observed to be more in nematode inoculated plants than in untreated control (Mohanty *et al.*, 1986; Ganguly and Dasgupta, 1987; Sujatha and Mehta, 1998). The resistance is due to the oxidation of phenolic compounds to quinone, which are known to be more toxic to microorganisms

The role of chitinases in higher plants is a defense mechanism against attack by pathogens. Highest chitinase activity was observed in *G. mosseae* in both shoot and root. In all the nematode inoculated VAM species, the chitinase activity was found lesser and least in nematode alone. Krebs and Grumet (1991) and Masuta *et al.* (1991) reported that chitinase in plants was induced by chitosan. Chitinases is involved directly in plant defense reactions; they require a substrate - chitin, in the pathogen. Chitin is known to be a structural element in the egg shell of nematode (Bird and Bird, 1991). Chitinase is a hydrolytic element which is responsible for degrading chitin, in the eggshell during embryonic development and thereby damage the development of embryo (Zamir *et al.*, 1993).

It can be concluded that peroxidase increased after infection of nematodes. This increase in due to defense mechanism of the host to the invading pathogen and also responsible for increased lignin phenol contents of the crop (Okey *et al.*, 1997). The chitin should be exposed to the effect of the chitinase. The eggs that are laid with in gelatinous matrix may be protected against enzymatic activity. Studies are needed to determine the effect of partial or totally purified chitinase of plant or microbial origin on nematode egg shall integrity and larval emergence and growth.

Table 1. Effect of peroxidase and chitinase activity due to interaction of *Meloidogyne incognita* and VAM

Treatment	Peroxidase activity				Chitinase activity (n mol of N-acetyl glucosamine/min/g)			
	Fresh Shoot	Increase over nematode alone (%	Fresh Root	Increase over nematode alone (%)	Fresh Shoot	Increase over nematode alone (%)	Fresh shoot	Increase over nematode alone(%)
G. fasciculatum	57.0	5.0	57.0	-19.1	21.5	77.6	25.9	82.3
G. fasciculatum + *M. incognita*	76.5	27.5	63.0	-10.6	19.7	62.8	20.7	45.7
G. mosseae	64.5	7.5	55.0	-21.9	26.4	118.1	28.9	103.5
G. mosseae + *M. incognita*	94.5	57.5	67.0	-4.9	20.7	71.0	23.5	65.4
G. intraradices	4.5	-92.5	15.0	-78.7	22.3	84.2	24.4	71.8
G. intraradices + *M. incognita*	37.5	-37.5	75.0	6.3	19.7	62.8	21.2	49.2
G. fulvum	45.0	-25.0	27.0	-61.7	14.3	18.1	18.5	30.2
G. fulvum + *M. incognita*	46.5	-22.5	52.5	-25.5	13.4	10.7	15.3	7.7
M. incognita alone	60.0	–	70.5	–	12.1	–	14.2	–
Control	40.5	–	49.5	–	19.6	–	16.0	–
CD (P=0.05)	2.9	–	3.0	–	2.1	–	1.3	–

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