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Defence responses of moderately resistant and susceptible rice varieties induced by rice root knot nematode, *Meloidogyne graminicola*

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Abstract
A pot culture experiment was carried out to quantify the induction of defence enzyme *viz*., peroxidase (PO), polyphenoloxidase (PPO) and Phenylalanine ammonialyase (PAL) in response to infection by *M. graminicola* with moderately resistant rice varieties (ADT 41, ADT 45, TRY 1, TPS 3, Swarna and GEB 24) and susceptible varieties (Co 47, Pusa Basmathi, ASD 19, Co 43, Co 2 and Co 19). Total phenol and defence enzymes *viz*., peroxidase, polyphenol oxidase and phenylalanine ammonialyase induction were found to be higher in *M. graminicola* infested moderately resistant varieties. The susceptible varieties infested by the root knot nematode showed reduction in total phenols, PO, PPO and PAL activity.

Keywords: Rice, Root knot nematodes, Defence response, resistant varieties

Introduction
The rice root-knot nematode, *Meloidogyne graminicola* belongs to the family Heteroderidae is one of the most economically important nematodes affecting rice.
It has been reported to cause significant yield losses of 20-50 per cent in many regions of rice production areas including India. While feeding the roots, it will penetrate cells and inject their salivary gland secretions into host tissues. There is number of digestive enzymes like amylase, proteases etc., are released during the process of feeding resulting in hydrolysis of host components and leading to altered host metabolism. The primary symptom of root-knot nematode infection is formation of typical root galls on the roots of susceptible host plants. Nutrient and water uptake by plants are considerably reduced because of the damaged root system, resulting in weak and low-yielding plants (Abad *et al*., 2003) [1] and the host plants is therefore exposed to a variety of host defence responses (Jones *et al*., 2007) [11]. Characterization of defence response is essential to advance our understanding of plant-nematode interaction. In recent past, some progress has also been made in this direction to understand, the basic biochemical mechanism of plant nematode interactions by several workers (Ganguly and Dasgupta, 1983; Mohanty *et al*., 1995; Darsana *et al*., 2015) [8, 18, 16]. This information would be of greatly helpful in breeding works for development of cultivars resistant to root-knot nematode. Hence, the present study was focused on quantification of defence enzyme *viz*., peroxidase, polyphenoloxidase phenylalanine ammonialyase and total phenol in response to infection by *M. graminicola* was studied with each seven varieties of moderately resistant and susceptible rice varieties.

Materials and Methods
Seeds of moderately resistant rice varieties (ADT 41, ADT 45, TRY 1, TPS 3, Swarna and GEB 24) and susceptible varieties (Co 47, Pusa Basmathi, ASD 19, Co 43, Co 2 and Co 19) were sown in 10 cm diameter plastic pots containing 250 g sterilized sandy loam (five seeds per pot). One week after germination, two seedlings of equal height were maintained in the pots while the others were removed. After 15 days about 500 freshly hatched second-stage juveniles were introduced into four holes made around the roots of the plants. The variety PY 1 and TN 1 served as resistant and susceptible check respectively. The pots were placed in a completely randomized design in the greenhouse. Pots were watered daily. Biochemical analysis was performed 45 days after nematode inoculation.

Estimation of total phenol
Paddy roots were collected from both resistant and susceptible varieties.
The ethanol extracts were prepared with 80% ethanol. One ml of the extract was taken into two test tubes and the volume was made upto 3 ml with distilled water. To this, one ml of

Folin ciocalteau's reagent was added to each test tube followed by addition of 2 ml 20 per cent sodium carbonate solution. The tubes were kept in hot water bath for a minute, cooled and diluted to 10 ml with distilled water. The intensity of blue color developed was measured at 660 nm wave length in a spectrophotometer. From the standard graph, the amount of phenol present was calculated and expressed as µg per g root (Sadasivam and Manickm, 1992) [25].

Quantification of peroxidase (PO)

Root samples (1 g) maintained at -40 °C were homogenized in 2 ml of 0.1 M phosphate buffer, pH 7.0 at 4 °C. The homogenate was centrifuged at 16,000 rpm at 4 °C for 15 min and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1 per cent H2O2.

The reaction mixture was incubated at room temperature (28 ± 2 °C). The changes in absorbance at 420 nm were recorded at 30 seconds intervals for 3 min. The enzyme activity was expressed as changes in the absorbance min-1 mg-1 protein (Hammerschmidt *et al*., 1982) [10].

Quantification of polyphenol oxidase (PPO)

The freeze dried root samples (1 g) were homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 16,000 rpm for 15 min at 4 °C. The supernatant was used as the enzyme source. The reaction mixture consisted of 200 µl of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction, 200 µl of 0.01M catechol was added and the activity was expressed as changes in absorbance at 495 nm min^{-1} mg^{-1} protein (Mayer *et al.*, 1965) [16].

Quantification of Phenylalanine ammonialyase (PAL)

Root samples (1g) stored at -70 °C were homogenized in 3 ml of ice cold 0.1 M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinyl pyrrolidine. The extract was filtered through cheesecloth and the filtrate was centrifuged at 16,000 rpm for 15 min. The supernatant was used as enzyme source. PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm and expressed as nmol trans-cinnamic acid min^{-1} mg^{-1} protein (Dickerson *et al*. 1984) [7].

Results

Studies on total phenol content in moderately resistant and susceptible rice varieties revealed highest phenol content in moderately resistant varieties compared to susceptible varieties. Among the moderately resistant varieties, total phenol content was found to be highest in PY 1 (2.73 mg g^{-1}) followed by ADT 45 (2.69 mg g^{-1}). Total phenol content was ranged from 1.58 to 2.00 mg g^{-1} in susceptible varieties and it was lowest in TN 1 (1.58 mg g^{-1}) followed by Co 47 (1.68 mg g^{-1}).

The induction of defence enzyme activity in response to infection by *M. graminicola* was studied with each seven varieties of moderately resistant and susceptible.

The peroxidase activity was found to be enhanced in *M. graminicola* infested moderately resistant varieties compared to susceptible varieties. The highest activity of PO was observed in PY 1 (7.98) followed by ADT 45 (7.73). Peroxidase activities in susceptible varieties ranged from 5.28 to 6.32. Lowest peroxidise activity was recorded in susceptible check TN 1 followed by Co 47 (5.53). Investigation on the PPO activity was undertaken in moderately resistant and susceptible rice varieties infected with *M. graminicola*. The PPO activity in roots of the moderately resistant varieties was highest compared to susceptible varieties, at 45 days after inoculation (DAI). The highest PPO activity was observed in PY 1 (2.17) followed by ADT 45 (2.06). Comparatively reduced activity of PPO was seen in susceptible varieties which ranged from 0.96 (TN 1) to 1.06 (Co 19) (Table. 1).

Table 1: Induction of total phenol, PO, PPO and PAL activity in moderately and susceptible rice varieties in response to infection by *M. graminicola*

	Rice varieties	Total Phenol content (mg/g)	Peroxidase (Change in absorbance m^{-1} mg^{-1} protein)	Polyphenoloxidase (Change in absorbance m^{-1} mg^{-1} protein)	Phenylalannine ammonialyase (n mol trans-cinnamic acid $m^{-1}mg^{-1}$)
Moderately resistant	ADT 41	2.60	7.37	1.89	2.65
	ADT 45	2.69	7.73	2.06	2.79
	TRY 1	2.24	7.42	1.94	2.67
	TPS 3	2.39	6.91	1.87	2.72
	Swarna	2.52	7.15	1.97	2.71
	GEB 24	2.28	7.57	1.93	2.56
Resistant check	PY 1	2.73	7.98	2.17	2.80
SEd		0.19	0.17	0.12	0.15
CDP = 0.05)		0.38	0.36	0.25	0.31
Susceptible varieties	Co 47	1.68	5.53	0.99	1.80
	Pusa Basmathi	2.00	5.94	1.03	1.85
	ASD 19	1.97	5.70	1.01	1.81
	Co 43	1.92	6.32	1.02	1.92
	Co 2	1.78	6.26	1.00	1.82
	Co 19	1.91	6.04	1.06	1.94
Susceptible check	TN 1	1.58	5.28	0.96	1.74
SEd		0.20	0.21	0.09	0.14
CD (P = 0.05)		0.42	0.43	0.18	0.28

PAL activity in rice varieties infested with *M. graminicola* was measured as changes in n mol transcinnamic acid m^{-1} mg^{-1}.

It is clearly evident from table 21 that PAL activity was higher in moderately resistant rice varieties than susceptible v

arieties. The PAL activity was almost similar in moderately resistant rice varieties with slight difference. But it was significant among the susceptible varieties. The level of PAL activity recorded in moderately resistant rice varieties *viz*., ADT 41, ADT 45, TRY 1, TPS 3, Swarna, GEB 24 and PY 1 were 2.65, 2.79, 2.67, 2.72, 2.71, 2.56 and 2.80 respectively. PAL activity of susceptible rice varieties was lower and it ranged from 1.74 to 1.94 (Table.1).

Discussion

Phenolic compounds are known to play a major role in the defense mechanisms of plants against pathogens. The accumulation of phenolic compounds in the nematode injured area and the activity of associated oxidative enzymes have been reported by Mountain (1965) [22]. The post - inflectional increase in phenolic contents could be due to their release from glycosidic esters by the enzymatic activity of host or pathogen (Noveroske *et al*., 1964) [24] or due to migration of phenols from non-infected tissues (Farkas and Kiraly, 1962) [9].

The present investigation revealed that total phenol content increased in nematode infested moderately resistant rice varieties. Similar observations were made by Bajaj *et al*. (1983) [2] while working on the biochemical alteration in tomato susceptible and resistant cultivars infested by *M. incognita*. In nematode infested resistant plants, phenolic content increased much faster than that of a susceptible one, as observed by earlier workers in other crops (Mote *et al*., 1990; Chakrabarti and Mishra, 2002) [21, 4].

The results are in accordance with the findings of Malli *et al*. (2002) [15], who reported that there was more reduction in total phenols in susceptible genotypes than in resistant genotypes of moth bean following yellow mosaic virus infection. Phenols directly or indirectly interfere with several metabolic systems of organisms. Based on these findings, it could be concluded that rapid accumulation of phenolic compounds occured in incompatible (resistant) host pathogen interactions than the compatible (susceptible) ones.

Peroxidases are regarded as detoxifying agents for H_2O_2 and have a precise metabolic function in the defense mechanism. In the present investigation, the PO activity decreased in nematode infested susceptible roots compared to moderately resistant roots. Localized necrosis and cell wall thickening were produced around the nematode infestation court in moderately resistant varieties (Moore *et al*., 1978). Langrimini and Rothstain (1987) [20, 14] stated that these two responses occurred in plants due to accumulation of PO following nematode infestation.

The present finding supports the observation of Montes *et al*. (2004) [19] that the peroxidase activity in the roots of resistant cultivars increased more than in susceptible cultivars after nematode infection (Kim *et al*., 1990). Zacheo *et al*. (1990) found that PO activity in the resistant pea accession MG 103738 showed a greater increase following infection with *Heterodera goettingiana*. Enhanced peroxidise activity may play a role in the lignification of cell walls, which assists in delaying the penetration by the nematodes as mechanism of resistance.

The moderately resistant rice varieties infested by the root knot nematode in the present study showed a general increase in PPO activity and it was in line with investigation of Nagesh *et al*. (1999) [23] who revealed that there was increased PPO activity in the China aster resistant line AST - 5 which prevented the nematode penetration and further development. Enhanced activities of defence enzymes have been suggested to have a direct or indirect role in the induction of systemic resistance in plants against pathogens (Dalisay and Kuc, 1995) [5].

The enzyme estimated in the present investigation showed that moderately resistant rice varieties possessed higher PAL activity than susceptible varieties indicating the inherent higher content of PAL in resistant varieties. Plant resistance to many pests and diseases can be affected by the activity of PAL enzymes. Higher activity of the enzymes was recorded in the roots of Ramakrishna (resistant) variety of rice than in the susceptible Annapurna variety. This clearly indicates that nematode induced a new synthesis of PAL enzymes in the feeding cells. Trans cinnamic acid, the product of PAL are lignin precursors and co factors of IAA oxidase and hence play a significant role in resistance reaction (Mishra and Mohanty, 2007) [17].

The constitutive levels of PAL activities in roots of moderately resistant rice varieties were found to be greater than susceptible varieties at 45 days after inoculation. The PAL activity results reported in this study agree with those of Kathiresan and Metha (2005) [12], who observed the infestation of lesion nematode, *Pratylenchus zeae* increased PAL activity in resistant clones of sugarcane compared to susceptible clones. Brueske (1980) [3] also reported that the PAL activity decreased in susceptible tomato and carrot roots infected with *Meloidogyne* spp. The increased activity of PAL may associate with the production of t-cinnamic acid which act as substrate for initiation of lignin synthesis pathway towards protection against nematode penetration.

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