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Analysis of defense related enzymes in Chilli Genotypes Infected with Root-Knot Nematode (*Meloidogyne incognita*)

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Abstract

An experiment was laid out to determine the defence enzyme activity in chilli varieties against *M. incognita*. The enzyme (PO, PPO, PAL and SOD) activity was analyzed in resistant variety (*Pusajwala* and NP 46-A) and susceptible variety (S-5 and Ghoomar) of chilli. The roots of inoculated and un-inoculated plants were analyzed after 7, 14, 21, 28 and 60 days after transplanting. Enzyme activity was observed maximum in resistant compared to susceptible varieties, which increased after root-knot nematode infection. The highest activity of enzymes was found between 21 and 28 days after transplanting. The plant growth characters (shoot weight, shoot length, root weight and root length) were reported maximum in resistant varieties compared to susceptible varieties. While the nematode population (number of galls, number of eggs, number of egg masses and nematode juveniles) was reported less in resistant varieties as compare to susceptible varieties.

Key words: Defence enzymes, root-knot nematode, *Meloidogyne incognita*, chilli

Introduction

Chilli (*Capsicum frutecens*) belongs to the family Solanaceae. Chilli is grown for its pungent fruits, which are used in both green and ripe red form (the latter in the dried form) to add taste to the food. Many chilli varieties are available in India and most of them have gained popularity and are under commercial cultivation. The conditions required for growing these crops also suites to many pathogens. Hence the production of chilli is tremendously reduced by pest and diseases which are considered as major biological constraints to low productivity. Plant parasitic nematodes cause approximately 21.3% losses in crops, it's amounting to Rs.102,039.79 million (1.58 billion USD) annually in India (Kumar *et al.,* 2020).

Root-knot nematodes that parasitize plants generally confine themselves to the roots of a wide range of plants and cause significant reduction quality and quantity of produce. Nematode

infection leads to oxidative stress in plants due to the production of reactive oxygen species (ROS) such as the superoxide radical, hydrogen peroxide, hydroxyl radical and alkoxyl radical. These ROS produced in the cell are detoxified by both enzymatic and nonenzymatic antioxidant systems. Reactive oxygen species (ROS) play an important role in plant defence during pathogen attack, levels of ROS detoxifying enzymes like peroxidase (PO) and catalase (CAT) are often suppressed in resistant plants (Klessing *et al.,* 2000). As a result, plants produce more ROS and accumulation of these components leads to HR in plant cells. For example, hydrogen peroxide plays a major role in triggering HR in incompatible interactions. Antioxidant enzymes such as PO, SOD, POD and CAT are considered to be the main protective enzymes engaged in the removal of free radicals and activated oxygen species (Blokhina *et al.,* 2003; Chawla *et al.,* 2013; Chandrawat *et al.,* 2018). While, phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) are reported to be involved in the mechanism of disease resistance. PAL is key enzyme for the biosynthesis of lignin, suberin, phytoalexins, stilbenes, coumarins and other flavonoids (Mahatma *et al*., 2019). So far substantial work has been done on various aspects of *M. incognita* on chilli. However, there is not much information available on determining the role of defence enzymes activity in plants against root-knot nematode, *M. incognita*. Keeping this in view, the present investigations were undertaken to assess the defence enzymes against root-knot nematode, *M. incognita* infecting chilli to evolve eco-friendly and economically feasible methods for the management of nematodes.

Materials and methods

The studies were conducted at Department of Nematology, Rajasthan College of Agriculture, MPUAT, Udaipur. The details of material required and methods applied were as follows:

Preparation and multiplication of pure culture of M. incognita:

Chilli plants infected with *M. incognita* were uprooted from the pure culture plots and brought to the laboratory. Egg masses, collected from the infected roots were kept in distilled water in watch glasses at laboratory temperature for hatching. Freshly hatched 2nd stage juvenile was then inoculated on one-month-old plants grown and maintained in 6" earthen clay pots containing steam sterilized soil in order to get adequate population of *M. incognita* on the plants as well as in soil to carry out further experiments.

Experiment on effect of Meloidogyne incognita on plant growth characters and enzyme activity:

Earthen pots (6" diameter) were washed, cleaned and disinfected before use by rinsing them with 4 per cent formalin solution. Pots were filled with 1 kg of infested soil. Uniform size 30 days old seedlings of chilli were transplanted in pots, one healthy plant in each pot was maintained and others were uprooted carefully without disturbing the one to be maintained. To avoid insect damage, spray of Malathion (0.05%) was given as and when required. The pots were randomly rotated to eliminate the effect of sun and shade. The care was taken from sowing to harvest including watering of plant during the course of experimentation.

***Observation on plant growth characters, enzyme activity and nematode reproduction parameter*:**

The defence enzymes *viz.,* PO, PPO, PAL and SOD in *M. incognita* resistant (Pusa Jwala and NP-46 A) and susceptible (S-5 and Ghoomar**)** varieties of chilli were assessed on every 7days interval after transplanting (7, 14, 21, 28) and 60 days after transplanting. The plants were uprooted 60 days after transplanting, while uprooting, the care was taken to avoid losses of both roots and nematodes in adhering soil. Observation on plant growth parameters *viz*., fresh root and shoot weight, shoot and root length were recorded. For studying nematode infestation, the roots were stained in 0.1% acid fuchsin in lacto phenol at 80ºC for 2-3 minutes (McBeth *et al.,* 1941). Then after gentle wash, roots were kept in clear lacto phenol for 24 hrs and then examined under microscope for nematode infection and observation of number of galls/plant, number of egg masses/plant and number of eggs/egg mass were recorded. The juveniles were extracted from 200 cc of each pot by Cobb's decanting and sieving method (Cobb, 1918), followed by modified Baermann funnel technique (Christie and Perry, 1951) total population in each pot was calculated.

Enzyme analysis

Determination of peroxidase enzymes in chilli roots.

The method proposed by Hammerschmidt *et al.*, (1982) was adopted for assaying the activity of peroxidase (EC 1.11.1.7). A 20% homogenate was prepared in 0.1M phosphate buffer (pH 7.0) from the root parts of the plant. The homogenate was centrifuged at 16,000 rpm at 4°C for 15 min and the supernatant was used for the assay. 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 H_2O_2. The reaction mixture was incubated at room temperature (28 ± 2 °C). The changes in absorbance at 420 nm were recorded at 30s intervals for 3 min. The enzyme activity was expressed as changes in the absorbance min^{-1} mg^{-1} protein

Determination of polyphenol oxidase enzymes in chilli roots.

Polyphenol oxidase (EC 1.10.3.1) activity was estimated simultaneously by the method of Mayer *et al.,* (1965). The enzyme extract was prepared by homogenizing 0.5 g of root tissue in 2.0 ml of the extraction medium 0.1M sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 16,000 rpm for 15 min at 4 °C and the supernatant was used for the assay. 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 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 nm min^{-1} mg^{-1} protein.

Determination of phenylalanine ammonia lyase enzymes in chilli roots.

The activity of phenylalanine ammonia lyase (EC 4.3.1.5) was measured following the method of Dickerson *et al*., (1984). Root samples (1g) 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 cheese cloth 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. A sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30°C. The amount of cinnamic acid synthesized was calculated using its extinction coefficient of 9630 m^{-1}. Enzyme activity was expressed as nmol cinnamic acid min^{-1} mg^{-1} protein

Determination of super oxide dismutase enzymes in chilli roots.

SOD was assayed according to the method of Beauchamp and Fridovich (1971). The activity of SOD (EC 1.15.1.1) was assayed by measuring its ability to inhibit the photochemical reduction of NBT. The roots (0.5g) were homogenized with 3.0ml of potassium phosphate buffer, centrifuged at 2000 rpm for 10 minutes and the supernatants were used for the assay. The 3 ml reaction mixture contained 50 mM phosphate buffer pH 7.8, 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 1.0 mM EDTA and 20 µl enzyme extract. Riboflavin was added last and the reaction was initiated by placing the tubes 30 cm below 15 W fluorescent lamps. The reaction was started by switching on the light and was allowed to run for 10 min. Switching off the light stopped the reaction and the tubes were covered with black cloth. Non-illuminated tubes served as control. The absorbance at 560 nm was read. One unit of SOD is the amount of extracts that gives 50% inhibition the rate of NBT reduction.

Results and discussion

An experiment was carried out under cage house condition in pots filled with naturally infested soil with an initial inoculum level 2 J_2/g soil of root-knot nematode, *M. incognita* to analyze the defence related enzymes PO, PPO, PAL, and SOD in resistant and susceptible variety of chilli at the different time intervals to understand the degree of disease-resistance.

The enzyme activity was analyzed in resistant variety (*Pusajwala* and NP 46-A) and susceptible variety (S-5 and Ghoomar) of chilli. The roots from inoculated and uninoculated plants were evaluated for their enzyme activity after 7, 14, 21, 28 and 60 days after transplanting. It was observed that enzyme activity increased in nematode infected resistant varieties as compared infected susceptible varieties. The enzyme activity showed gradual increase in resistant as well as susceptible verities from 7 DAI onwards to 28 DAI in inoculated plants as compared to un-inoculated ones. Among enzymes the PO was found highest in chilli roots during different time of observations followed by SOD, while PPO and PAL was observed in low quantity as compared to PO in resistant as well as susceptible verities (Table 1 to 4).

Data revealed that the plant growth parameter (shoot length, shoot weight, root length and root weight) were increased in both resistant varieties as compared to susceptible varieties. The maximum plant growth parameters, shoot length (21.15 cm), shoot weight (9.38 g), root length (30.44 cm) and root weight (24.87 g) were recorded in Pusa jwala followed by NP-46A. However, minimum plant growth parameters were observed in Ghoomar variety (Table-5).

While, *M. incognita* reproduction was reported minimum in resistant varieties as compared to susceptible varieties of chilli. Among different varieties, minimum number of galls per plant (7.50), egg masses per plant (3.30), eggs per egg mass (82.70), and nematode population/200cc soil (127.40) was observed in Pusa jwala. However, maximum nematode reproduction were observed in Ghoomar variety (Table-6)

Among all the treatments resistant variety Pusa jwala was found to be the best treatments in respect to highest enzyme activity, improving plant growth characters and had a less nematode reproduction. Among all the treatments resistant varieties were found to be the best treatments to improve plant growth characters and decrease nematode population. The results obtained are similar with the findings Shukla and Chakraborty (1988) who also found significantly higher peroxidase enzyme activity than susceptible varieties of tobacco and tomato. Qui *et al.* (1997) also observed that resistant cultivar had higher chitinase activity than the susceptible cultivar at every sample time beginning at 3 DAI. The results indicated that higher chitinase activity and early induction of specific chitinase isozymes may be associated with resistance to root-knot nematode in soybean. Mishra and Mohanty (2007) also observed that rice root-knot nematode infected plant roots produced greater amount of phenolics, PAL, Tyrosine ammonia lyase (TAL) respectively compared to their healthy counterparts in rice. Kalaiarasan, P. (2009) also evaluated the plants inoculated with *M. incognita* showed a substantial rise in PO and PPO activity in both susceptible and resistant genotypes; however, variety Hisar lalit exhibited resistance response to root knot nematode with more enzyme activity and less number of galls per plant. Chawla *et al.* (2013) also evaluated that antioxidative enzyme activities increased after infection in tomato roots with root-knot nematode in the resistant varieties as compared to that of susceptible varieties. Bhau *et al*. (2016) found increased peroxidise and phenolic content as a means of defence against nematode infestation but no changes in polyphenol oxidase enzyme activity were observed. Thagaria *et al*. (2016) screened sixteen castor varieties in which, six varieties found resistance, eight were moderately resistance and two varieties were found susceptible. Kumar *et al.* (2017) screened the reaction of eleven fennel varieties against root-knot nematode, *M. incognita* in which, only three varieties *viz.* hisar swarup, rf 125 and gf 2 were found resistant, while five varieties were found moderately resistant reaction. Gurjar *et.al.* (2021) Screened ten varieties of tomato for their reactions against root-knot nematode *M. incognita* under poly house condition. Results revealed that out of 10 varieties of tomato Arka-rakshak, Sikandar, Eemrald and Sarathi found moderate resistant. Badshah, Dev, Subriyano and Shanshah found susceptible and varieties Kanak and Navoday observed as highly susceptible to root-knot nematode. Kumawat *et al.,* 2024 screened thirty varieties/germplasm among them highest root rot incidence was recorded in RF-125 and UF-33 while, root-galls incidence found minimum in RF-101 and maximum in UF-23.

The resistance or susceptible response of screened varieties against nematode found differ with findings of other researchers, it may be due to the nature of variety in particular environment or may be due to soil conditions. The findings also showed that level of defence enzymes may be increased after infection which may be increased the plant growth parameters and reduced nematode reproduction.

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Author contribution:

BSC performed the research and wrote the manuscript, AUS and VS conceived and designed the research work and edited manuscript. SSB and HS assisted in research work and wrote manuscript.

Table 1. Peroxidase activity in roots of *Meloidogyne incognita* resistant and susceptible varieties of chilli.

Varieties	Days after transplanting Unit min^{-1} gm^{-1} protein									
	Inoculated					Un-inoculated				
	7	14	21	28	60	7	14	21	28	60
	RESISTANT VARIETIES									
Pusa	21.738	32.904	35.977	40.714	28.731	18.101	28.364	27.998	24.050	9.332
Jwala	(27.79)	(35.00)	(36.85)	(39.65)	(32.41)	(25.18)	(32.18)	(31.94)	(29.36)	(17.78)
NP-46 A	21.118	32.058	37.612	39.445	27.913	19.652	28.505	28.956	23.035	6.851
	(27.35)	(34.48)	(37.83)	(38.90)	(31.89)	(26.32)	(32.27)	(32.55)	(28.68)	(15.17)
	SUSCEPTIBLE VARIETIES									
S-5	8.092	7.922	24.107	20.610	16.719	24.586	23.768	21.992	14.943	7.161
	(16.53)	(16.35)	(29.40)	(27.00)	(24.13)	(29.72)	(29.17)	(27.96)	(22.74)	(15.52)
Ghoomar	9.981	9.727	25.629	15.704	12.208	27.659	25.770	25.009	10.657	4.680
	(18.42)	(18.17)	(30.41)	(23.34)	(20.44)	(31.73)	(30.51)	(30.00)	(19.05)	(12.49)
SEm ±	0.003	0.004	0.003	0.004	0.003	0.008	0.010	0.005	0.002	0.003
CD at 5%	0.009	0.011	0.009	0.011	0.010	0.023	0.031	0.016	0.007	0.010
CV	0.03	0.03	0.02	0.02	0.03	0.06	0.07	0.04	0.02	0.08

Note: (1) Data are average value of five replications.
(2) Initial inoculums level 2 J2/g soil.

Table 2. Polyphenol oxidase activity in roots of *Meloidogyne incognita* resistant and susceptible varieties of chilli

Varieties	Days aftertransplanting Specific activity of PPO unit min^{-1} gm^{-1}protein									
	Inoculated					Un-inoculated				
	7	14	21	28	60	7	14	21	28	60
	RESISTANT VARIETIES									
Pusa	0.217	0.344	0.469	0.656	0.171	0.132	0.224	0.257	0.237	0.086
Jwala	(2.63)	(3.35)	(3.90)	(4.63)	(2.37)	(2.07)	(2.69)	(2.88)	(2.77)	(1.61)
NP-46 A	0.180	0.314	0.451	0.648	0.163	0.116	0.187	0.249	0.231	0.076
	(2.42)	(3.19)	(3.84)	(4.61)	(2.30)	(1.92)	(2.46)	(2.82)	(2.72)	(1.53)
	SUSCEPTIBLE VARIETIES									
S-5	0.075	0.139	0.186	0.140	0.099	0.121	0.167	0.178	0.170	0.057
	(1.55)	(2.10)	(2.44)	(2.16)	(1.75)	(1.99)	(2.31)	(2.38)	(2.36)	(1.33)
Ghoomar	0.092	0.144	0.205	0.168	0.108	0.129	0.174	0.196	0.180	0.060
	(1.71)	(2.18)	(2.59)	(2.31)	(1.84)	(1.99)	(2.36)	(2.50)	(2.44)	(1.40)
SEm ±	0.002	0.003	0.003	0.003	0.002	0.004	0.004	0.004	0.004	0.003
CD at 5%	0.006	0.009	0.008	0.008	0.006	0.013	0.011	0.012	0.011	0.008
CV	2.50	2.18	1.43	1.19	2.43	6.14	3.57	3.12	3.14	6.97

Note: (1) Data are average value of five replications.
(2) Initial inoculums level 2 J2/g soil.

Table 3. Phenylalanine ammonia lyase activity in roots of *Meloidogyne incognita* resistant and susceptible varieties of chilli

Varieties	Days after transplanting Specific activity of PAL µ mol cinnamic acid min^{-1} gm^{-1} protein									
	Inoculated					Un-inoculated				
	7	14	21	28	60	7	14	21	28	60
RESISTANT VARIETIES										
Pusa	0.098	0.165	0.238	0.293	0.092	0.047	0.072	0.098	0.093	0.019
Jwala	(1.72)	(2.30)	(2.75)	(3.09)	(1.72)	(1.20)	(1.49)	(1.75)	(1.72)	(0.63)
NP-46 A	0.086	0.152	0.231	0.281	0.088	0.049	0.076	0.086	0.071	0.018
	(1.65)	(2.23)	(2.73)	(3.04)	(1.64)	(1.21)	(1.53)	(1.62)	(1.51)	(0.54)
SUSCEPTIBLE VARIETIES										
S-5	0.044	0.067	0.087	0.084	0.042	0.034	0.051	0.069	0.063	0.006
	(1.14)	(1.42)	(1.67)	(1.63)	(1.14)	(1.06)	(1.30)	(1.46)	(1.42)	(0.30)
Ghoomar	0.046	0.079	0.100	0.091	0.038	0.038	0.059	0.077	0.069	0.008
	(1.21)	(1.56)	(1.80)	(1.72)	(1.05)	(1.05)	(1.34)	(1.56)	(1.43)	(0.32)
SEm±	0.004	0.004	0.002	0.003	0.003	0.002	0.003	0.003	0.004	0.002
CD at 5%	0.011	0.011	0.007	0.010	0.009	0.007	0.008	0.008	0.012	0.006
CV	9.95	5.77	2.60	3.24	8.24	9.74	7.92	6.15	9.62	48.26

Note: (1) Data are average value of five replications.
(2) Initial inoculums level 2 J2/g soil.

Table 4. Super oxide dismutase activity in roots of *Meloidogyne incognita* resistant and susceptible varieties of chilli

Varieties	Days aftertransplanting Specific activity of SOD unit gm^{-1} protein									
	Inoculated					Un-inoculated				
	7	14	21	28	60	7	14	21	28	60
RESISTANT VARIETIES										
Pusa	2.19	2.98	4.08	4.26	1.18	2.03	2.21	2.4	2.49	0.74
Jwala	(8.51)	(9.94)	(11.65)	(11.91)	(6.24)	(8.19)	(8.55)	(8.91)	(9.08)	(4.93)
NP-46 A	1.86	2.57	3.62	3.93	1.07	1.62	1.74	1.85	1.96	0.63
	(7.84)	(9.23)	(10.97)	(11.43)	(5.94)	(7.31)	(7.58)	(7.81)	(8.05)	(4.55)
SUSCEPTIBLE VARIETIES										
S-5	1.21	1.34	1.62	1.81	0.415	1.13	1.26	1.36	1.54	0.18
	(6.31)	(6.65)	(7.32)	(7.73)	(3.69)	(6.11)	(6.45)	(6.70)	(7.13)	(2.43)
Ghoomar	1.19	1.28	1.58	1.855	0.36	1.04	1.24	1.48	1.56	0.205
	(6.26)	(6.50)	(7.22)	(7.83)	(3.44)	(5.85)	(6.39)	(6.98)	(7.18)	(2.57)
SEm±	0.002	0.004	0.003	0.003	0.003	0.003	0.003	0.004	0.003	0.003
CD at 5%	0.006	0.011	0.009	0.008	0.008	0.008	0.010	0.013	0.008	0.008
CV	0.22	0.32	0.20	0.16	0.61	0.33	0.36	0.41	0.23	1.11

Note: (1) Data are average value of five replications.
(2) Initial inoculums level 2 J2/g soil.

Table 5. Plant growth characteristic of *Meloidogyne incognita* resistant and susceptible varieties of chilli after 60 days of transplanting

Varieties	Shoot length (cm)			Shoot weight (g)			Root length (cm)			Root weight (g)		
	Ist Year	IInd Year	Pooled	Ist Year	IInd Year	Pooled	Ist Year	IInd Year	Pooled	Ist Year	IInd Year	Pooled
T1 Pusa Jwala	21.24	21.06	21.15	9.24	9.52	9.38	30.32	30.56	30.44	24.96	24.78	24.87
T2 NP- 46 A	19.32	19.56	19.44	8.12	8.58	8.35	28.12	27.68	27.90	22.04	22.26	22.15
T3 S-5	8.55	8.81	8.68	3.50	3.77	3.63	10.08	9.94	10.01	8.20	8.10	8.15
T4 Ghoomar	6.71	7.11	6.91	2.04	2.26	2.15	7.30	7.64	7.47	4.06	3.90	3.98
SEm±	0.300	0.258	0.262	0.196	0.231	0.199	0.377	0.363	0.344	0.208	0.212	0.163
CD at 5%	0.899	0.775	0.786	0.588	0.692	0.598	1.130	1.088	1.030	0.625	0.636	0.49
CV	4.81	4.09	4.18	7.66	8.55	7.58	4.45	4.28	4.05	3.14	3.21	2.47

Note: (1) Data are average value of five replications.
(2) Initial inoculums level 2 J2/g soil.

Table 6. Nematode reproduction parameters in *Meloidogyne incognita* resistant and susceptible varieties of chilli after 60 days of transplanting.

Varieties	No. of galls/ plant			No. of egg masses / plant			No. of eggs and larvae / egg mass			Juvenile (J2) population / 200cc soil		
	Ist Year	IInd Year	Pooled	Ist Year	IInd Year	Pooled	Ist Year	IInd Year	Pooled	Ist Year	IInd Year	Pooled
T_1 Pusa Jwala	7.20	7.80	7.50	3.00	3.60	3.30	83.20	82.20	82.70	126.00	128.80	127.40
T_2 NP- 46 A	8.80	9.20	9.00	4.20	4.40	4.30	96.20	95.20	95.70	189.20	185.60	187.40
T_3 S-5	41.00	43.00	42.00	32.80	34.20	33.50	132.40	137.00	134.70	686.20	690.80	688.50
T_4 Ghoomar	46.00	47.80	46.90	40.60	42.20	41.40	141.00	143.80	142.40	933.80	939.20	936.50
SEm±	0.658	0.747	0.610	0.524	0.539	0.434	1.674	1.526	1.525	1.544	2.321	1.671
CD at 5%	1.972	2.241	1.829	1.572	1.614	1.300	5.018	4.574	4.573	4.629	6.957	5.010
CV	5.71	6.20	5.18	5.82	5.71	4.70	3.31	2.98	2.99	0.71	1.07	0.77

Note: (1) Data are average value of five replications.
(2) Initial inoculums level 2 J2/g soil.

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