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Original Research Article

Induction of Systemic Resistance in Tomato by *Pseudomonas* spp. and *Bacillus* spp. Against Root Knot Nematode *Meloidogyne incognita*

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A B S T R A C T

The present study aimed to find *Pseudomonas* spp. and *Bacillus* spp. to protect tomato plants from root knot nematode. Induction of defense mechanisms in present research revealed that *Pseudomonas* spp. and *Bacillus* spp. inoculation significantly induced peroxidase (POX), polyphenoloxidase (PPO) and phenylalanine amonialyase (PAL) activities in root tissues. These bacterial isolates were found to possess the highest ISR activity PO, PPO, PAL and phenolics at third day after inoculation in tomato treated with bacterial isolates challenge inoculated with *M. incognita* under pot culture condition. The present study implies that earlier and higher accumulation of phenols and defense enzymes *viz.*, PO, PPO and PAL in tomato root resulted in significant reduction in nematode infection.

Keywords

Induced systemic resistance, Tomato and root knot nematode

Introduction

The root knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites and are among the most damaging agricultural pests attacking crop plants (Sahebani and Hadavi, 2008; Mai and Abawi, 1987). It causes high levels of economic loss in a multitude of agricultural crops worldwide. They are capable of severely damaging a wide range of crops, in particular vegetables, causing dramatic yield losses mainly in tropical and sub-tropical agriculture (Sikora and Fernandez, 2005; Kiewnick and Sikora, 2006).

Several control measure such as regulatory, physical, chemical, cultural and biological methods have been employed for the control of nematodes on crop plants but these control measures have their own merits and demerits. Due to the problems caused by chemical control, mainly their deleterious effects on human health and environment, development of alternative control methods is of great importance.

The use of nematicides is still an effective measure to control root knot nematodes, but the nematicides available are few, expensive and extensive application may lead to i) reduction in beneficial organisms in soil rhizosphere; ii) health hazards to human beings and animals; and iii) pollution of environment. An alternate strategy is to exploit the use of biological antagonists to

manage the nematodes. Biocontrol appears to offer an environmentally safe and ecologically feasible option for plant protection with great potential for promoting sustainable agriculture.

Though studies on induction of systemic resistance by PGPR against nematode pests in crop plants are few, PGPR strains have been used successfully as biological control agents for sugar beet and potato cyst nematode (Sikora, 1992).

P. fluorescens induced systemic resistance against *Heterodera schachtii* and inhibited early root penetration in sugar beet (Oostendorp and Sikora, 1990). Application of the bacterium *P. chitinolytica* reduced the root knot nematode infection in tomato crop (Spiegel *et al.*, 1991), while the level of infestation of root knot nematode *Meloidogyne incognita* on tomato was reduced with fewer galls and egg masses in the soil following root dipping with *P. fluorescens* strain Pf1 (Santhi and Sivakumar, 1995).

With this background, therefore, we intend to study the Induction of Systemic Resistance in tomato by *Pseudomonas* spp. and *Bacillus* spp. against root knot nematode *Meloidogyne incognita*.

Materials and Methods

Biochemical changes induced by *Pseudomonas spp.* and *Bacillus spp.* in tomato challenged with *M. incognita*

Biochemical analysis was made with tomato root samples collected from the evaluation of liquid formulation of bacterial isolates under pot culture condition. Tomato plants were carefully uprooted without causing damage to root tissues daily from 0 to 5 DAI.

Treatment details

T_1-*Pseudomonas fluorescens* (Pfpv1)
T_2-*Pseudomonas* spp. (Pfks 23)
T_3-*Pseudomonas* spp. (Pfpv12)
T_4 - *Pseudomonas fluorescens* (Pf1)
T_5- *Bacillus subtilis* (Bsvn11)
T_6- *Bacillus pumilus* (Bsvn12)
T_7- *Bacillus cereus* (Bsks 2)
T_8- *Bacillus subtilis* (Bbv57)
T_9- Control

Estimation of total phenol

The procedure developed by Malik and Singh (1980) was followed for the estimation of total phenol from the tomato plant samples collected in the present study.

One gram root sample was ground in 10 ml of 80% ethanol using pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant dried and dissolved in 5 ml distilled water.

The aliquots (2 ml) taken in test tubes were made to the volume of 3 ml with water and 0.5 ml of Folin-Ciocalteau reagent. After three min two ml of 20% Na_2Co_3 was added to each tube and placed in boiling water for a min and cooled. The absorbance was measured at 650 nm.

Estimation of peroxidase (PO)

The peroxidase activity was assayed spectrophotometrically (Hartee, 1955). Reaction mixture consists of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1 % hydrogen peroxide. The reaction mixture was incubated at room temperature (28 ± 2ºC). The change in absorbance at 420 nm was recorded at 30 sec intervals for three min. Boiled enzyme preparation served as blank. The enzyme activity was expressed as change in the

absorbance of the reaction mixture per min on fresh weight basis (Hammerschmidt *et al.,* 1982).

Estimation of polyphenoloxidase (PPO)

The polyphenoloxidase activity was determined following the procedure as described by Bryant and Forrest (1979).

The enzyme extract was prepared by homogenizing one g root tissue in 100 ml aliquots of cold acetone. The homogenate was filtered through Whatman No.1 filter paper and air dried. The resulting dry powder was used for the estimation of activity of PPO.

One g dry powder prepared was ground with two successive 20 ml aliquots of 25 mM phosphate buffer (pH 6.2) in a mortar chilled in ice bath. Then filtered through Whatman No.1 filter paper and diluted to 50 ml with phosphate buffer. Each two ml of phosphate buffer and enzyme extract was taken in a test tube and to this 1 ml of paracoumaric acid and 1 ml of manganese chloride were added and incubated in dark at 30 ºC. Before and after 50 min of incubation 2 ml of the mixture, were taken and 5.2 ml of perchloric acid and 0.5 ml of ferric nitrate solution were added and diluted to 10 ml with water. After incubation period of 60 min in dark the absorbance was measured at 535 nm.

Estimation of phenylammonialyase (PAL)

One gram of plant sample was homogenized in 3 ml of ice cold 0.1 M sodium borate buffer, pH 7.0, containing 1.4 mM of 2-mercaptoethanol and 50 mg of insoluble polyvinylpyrrolidone (PVP). The resultant extract was filtered through cheese cloth and the filtrate was centrifuged at 15,000 rpm for 15 min at 4ºC and the supernatant was used as the enzyme source. The PAL activity was determined as the rate of conversion of L-Phenylalanine to trans-cinnamic acid at 290 nm. Sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml. of 0.1 M borate buffer (pH 8.8) and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30ºC. The amount of transcinnamic acid synthesized was calculated using its extinction coefficient of 9630 M^{-1} cm^{-1} (Dickerson *et al.,* 1984). The enzyme activity was expressed in fresh weight basis as nmol trans-cinnamic acid min^{-1} mg^{-1} of sample.

Results and Discussion

Effect of *Pseudomonas spp.* and *Bacillus spp*. isolates on biochemical changes in tomato plants infested with *M. incognita*

Phenol

Increase in accumulation of total phenol was observed in liquid formulations of bacterial isolates treated tomato plants (Table 1). The phenol accumulation reached highest at 3 DAI and it was significantly higher (4.762 min^{-1} g fresh root) in Pfpv1 treated plants. It was followed by Bsvn 11 (4.573 min^{-1}g fresh root). The untreated control has the lowest accumulation of total phenol content of (1.992 min^{-1}g fresh root). Phenolic compounds are known to play a major role in the defense mechanism of plants against various external infectious agents. *P. fluorescens* releases antimicrobial factors including lytic enzymes which lead to the accumulation of phenolics (Meena *et al.,* 2000) by secretion of indole acetic acid that induced phenol metabolism in plants (Shabaev *et al.,* 1999).

Activity of peroxidase (PO)

Assay of peroxidase activity in tomato plants inoculated with *M. incognita* showed differences among the treatments of liquid formulations of *Pseudomonas* spp. and *Bacillus* spp. isolates.

Table.1 Effect of liquid formulation of *Pseudomonas* spp. and *Bacillus* spp. isolates on total phenol content in roots of tomato infested with *M. incognita*

Isolates	Total phenols (mg/g fresh weight)					
	0 DA1	1 DA1	2 DA1	3 DA1	4 DA1	5 DA1
Pfpv 1	3.749 (55.26)	3.876 (56.08)	4.337 (54.3)	4.762 (58.16)	2.776 (44.45)	1.934 (35.83)
Pfks23	3.394 (50.58)	3.591 (52.6)	3.882 (48.94)	4.392 (54.64)	2.431 (36.56)	1.734 (28.43)
Pfpv12	3.003 (44.15)	3.126 (45.55)	3.675 (46.06)	3.986 (50.02)	2.215 (30.38)	1.528 (18.78)
Pf1	3.285 (48.94)	3.354 (49.25)	3.763 (47.32)	4.221 (52.8)	2.332 (33.87)	1.628 (23.77)
Bsvn11	3.581 (53.16)	3.732 (54.39)	4.115 (51.83)	4.573 (56.43)	2.573 (40.06)	1.831 (32.22)
Bsvn12	2.967 (43.47)	3.003 (43.32)	3.554 (44.23)	3.820 (47.85)	2.169 (28.9)	1.429 (13.15)
Bsks2	2.843 (41.01)	2.965 (42.59)	3.276 (39.49)	3.621 (44.98)	1.982 (22.19)	1.312 (5.41)
Bbv 57	2.856 (41.28)	2.924 (41.79)	3.429 (42.19)	3.753 (46.92)	2.005 (23.09)	1.418 (12.48)
Control	1.677	1.702	1.982	1.992	1.542	1.241
SEd	0.05	0.052	0.059	0.064	0.036	0.026
CD(P=0.05)	0.106	0.109	0.124	0.136	0.077	0.054

Note: Figures in parentheses are per cent increase over control

Table.2 Effect of liquid formulation of *Pseudomonas* spp. and *Bacillus* spp. isolates on PO content in roots of tomato infested with *M. incognita*

Isolates	PO (Change in absorbance min^{-1} g^{-1})					
	0 DA1	1 DA1	2 DA1	3 DA1	4 DA1	5 DA1
Pfpv 1	0.789 (92.01)	0.846 (93.97)	0.924 (92.96)	1.341 (93.81)	1.312 (95.19)	1.287 (97.9)
Pfks23	0.573 (89)	0.628 (91.87)	0.765 (91.5)	1.165 (92.87)	1.149 (94.51)	1.127 (97.6)
Pfpv12	0.421 (85.03)	0.501 (89.82)	0.548 (88.13)	0.943 (91.19)	0.921 (93.15)	0.865 (96.87)
Pf1	0.462 (86.36)	0.532 (90.41)	0.637 (89.79)	1.005 (91.74)	1.087 (94.2)	1 (97.3)
Bsvn11	0.643 (90.2)	0.734 (93.05)	0.853 (92.37)	1.276 (93.49)	1.264 (95.01)	1.193 (97.73)
Bsvn12	0.392 (83.92)	0.423 (87.94)	0.562 (88.43)	0.835 (90.05)	0.725 (91.31)	0.654 (95.87)
Bsks2	0.375 (83.2)	0.269 (81.04)	0.427 (84.77)	0.689 (87.95)	0.539 (88.31)	0.436 (93.8)
Bbv 57	0.381 (83.46)	0.365 (86.02)	0.512 (87.3)	0.723 (88.52)	0.634 (90.06)	0.527 (94.87)
Control	0.063	0.051	0.065	0.083	0.063	0.027
SEd	0.0081	0.0088	0.0103	0.015	0.015	0.014
CD(P=0.05)	0.0169	0.0185	0.0216	0.033	0.032	0.03

Note: Figures in parentheses are per cent increase over control

Table.3 Effect of liquid formulation of *Pseudomonas* spp. and *Bacillus* spp. isolates on PPO content in roots of tomato infested with *M. incognita*

Isolates	PPO (Change in absorbance min^{-1} g^{-1})					
	0 DA1	1 DA1	2 DA1	3 DA1	4 DA1	5 DA1
Pfpv 1	0.489 (71.16)	0.738 (78.72)	1.253 (80.76)	1.673 (80.57)	1.547 (86.1)	1.321 (76.38)
Pfks23	0.367 (61.58)	0.534 (70.59)	1.002 (75.94)	1.437 (77.38)	1.362 (84.21)	1.154 (72.96)
Pfpv12	0.271 (47.97)	0.326 (51.84)	0.735 (67.21)	1.297 (74.94)	1.192 (81.96)	0.964 (67.63)
Pf1	0.321 (56.07)	0.452 (65.26)	0.987 (75.58)	1.352 (75.96)	1.267 (83.03)	1.001 (68.83)
Bsvn11	0.431 (67.28)	0.631 (75.11)	1.134 (78.74)	1.563 (79.2)	1.421 (84.86)	1.263 (75.29)
Bsvn12	0.223 (36.77)	0.302 (48.01)	0.623 (61.31)	1.172 (72.26)	1.002 (78.54)	0.814 (61.67)
Bsks2	0.191 (26.17)	0.212 (25.94)	0.412 (41.5)	0.829 (60.79)	0.723 (70.26)	0.623 (49.91)
Bbv 57	0.2 (29.5)	0.274 (42.7)	0.521 (53.74)	0.923 (64.78)	0.824 (73.9)	0.742 (57.95)
Control	0.141	0.157	0.241	0.325	0.215	0.312
SEd	0.0051	0.0074	0.0136	0.02	0.018	0.015
CD(P=0.05)	0.0107	0.0155	0.0287	0.042	0.038	0.032

Note: Figures in parentheses are per cent increase over control

Table.4 Effect of liquid formulation of *Pseudomonas* spp. and *Bacillus* spp. isolates on PAL content in roots of tomato infested with *M. incognita*

Isolates	PAL (Change in absorbance min^{-1} g^{-1})					
	0 DA1	1 DA1	2 DA1	3 DA1	4 DA1	5 DA1
Pfpv 1	0.197 (38.57)	0.288 (32.63)	0.492 (59.55)	0.863 (74.39)	0.523 (75.71)	0.489 (71.57)
Pfks23	0.178 (32.02)	0.261 (25.67)	0.453 (56.07)	0.625 (64.64)	0.356 (64.32)	0.262 (46.94)
Pfpv12	0.163 (25.76)	0.243 (20.16)	0.336 (40.77)	0.427 (48.24)	0.2 (36.5)	0.197 (29.44)
Pf1	0.171 (29.23)	0.254 (23.62)	0.442 (54.97)	0.537 (58.84)	0.276 (53.98)	0.241 (42.32)
Bsvn11	0.186 (34.94)	0.273 (28.93)	0.471 (57.74)	0.726 (69.55)	0.438 (71)	0.431 (67.74)
Bsvn12	0.159 (23.89)	0.239 (18.82)	0.266 (25.18)	0.392 (43.62)	0.198 (35.85)	0.183 (24.04)
Bsks2	0.138 (12.31)	0.211 (8.05)	0.225 (11.55)	0.298 (25.83)	0.139 (86.33)	0.156 (10.89)
Bbv 57	0.143 (15.38)	0.222 (12.61)	0.244 (18.44)	0.312 (29.16)	0.163 (22.08)	0.172 (19.18)
Control	0.121	0.194	0.199	0.221	0.127	0.139
SEd	0.002	0.003	0.006	0.008	0.004	0.004
CD(P=0.05)	0.005	0.008	0.012	0.018	0.01	0.009

Note: Figures in parentheses are per cent increase over control

The root analysis results showed high level of PO activity (1.341 $min^{-1}g^{-1}$root) on third day after challenge inoculation of the nematode and thereafter the activity declined slowly. It was followed by Bsvn11 (1.276 $min^{-1}g^{-1}$ root) (Table 2). The ISR studies revealed that PO activity was high on 3 DAI in all bacterial isolates challenge inoculation of the nematode, when compared to the control. Similar results were found in many studies. Peroxidase is a key enzyme in the biosynthesis of lignin (Bruce and West, 1989). Increased activity of peroxidases has been elicited by PGPE in different plants such as rice (Gnanamanickam *et al.,* 1999). Cotton (Rajendran *et al.,* 2003) and banana (Harish, 2005). Peroxidases have been implicated in a number of physiological and biochemical functions that may contribute to resistance including exudation of hydroxyl cinnamyl alcohol into free radical intermediates (Gross, 1980), phenol oxidation (Schmidt and Feucht, 1980), polysaccharide cross linking (Fry, 1986), cross linking of extensin monomers (Everdeen *et al.,* 1988) and lignification, and are also associated with deposition of phenolic compounds into plant cell walls during resistance interactions (Graham and Graham, 1991).

Activity of polyphenoloxidase (PPO)

PPO activity was significantly higher with liquid formulations of bacterial isolates of Pfpv1 and Bsvn11 in tomato plant infested by *M. incognita* than the control plants. PPO activity reached its highest level at 3 day after challenge inoculation and it was significantly higher (1.673 and 1.563 change $min^{-1}g^{-1}$ root), when compared to control plants. The untreated control showed the lowest PPO activity of 0.042 $min^{-1}g^{-1}$ root (Table 3). PPO are enzymes which use molecular oxygen to catalyze the oxidation of monophenolic and orthodiphenolic compounds. PPO is a copper containing enzyme that oxidises phenolics to highly toxic quinines and is involved in the terminal oxidation of diseased plant tissue and role in disease resistance. Chen *et al.,* (2000) reported that PGPR induced PPO activity in cucumber root tissues upon challenge inoculation with *P. aphanidermatum*.

Activity of phenylalanine ammonia lyase (PAL)

Increase in the activity of PAL was observed in liquid formulations of bacterial isolates of Pfpv1 and Bsvn11 in tomato plant infested by *M. incognita* than the control plants. The root analysis results showed high level of PAL activity (0.863 $min^{-1}g^{-1}$ root) on third day after challenge inoculation of the nematode and thereafter the activity declined slowly. It was followed by Bsvn11 (0.726 $min^{-1}g^{-1}$ root) (Table 4). Untreated control showed lowest activity of PAL (0.221 $min^{-1}g^{-1}$ root). Phenylalanine ammonia lyase (PAL) plays an important role in the biosynthesis of various defense chemicals in phenyl propanoid metabolism (Daayf *et al.,* 1997). PAL activity could be induced during plant pathogen interactions (Ramanathan *et al.,* 2000).

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