Madras Agric. J., 98 (7-9): 255-257, September 2011
https://doi.org/10.29321/MAJ.10.100290

Short Note

Systemic Resistance in Tomato against *Meloidogyne incognita* Induced by Plant Growth Promoting Rhizobacterium, *Pseudomonas fluorescens*

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Resistance-related enzymatic activities due to selected strains of *Pseudomonas fluorescens* were assayed by split root bioassay in tomato under glass house conditions. Application of *P. fluorescens* as seedling root dip increased the level of peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) in tomato roots indicated the systemic resistance induced by the bacterial formulations against root knot nematode, *Meloidogyne incognita*. Among the four *P.fluorescens* isolates tested alone and in consortium with *Bacillus subtilis*, Bbv 57, consortial application of *P. fluorescens*, Pf 128 with *B. subtilis,* Bbv 57 recorded the highest enzymatic activity in tomato roots compared to other strains either alone or in consortium. Results indicated that *P. fluorescens* was capable of inducing systemic resistance against *M. incognita* in tomato by the accumulation of the defense enzymes like PO, PPO and PAL.

Key words: Tomato, Plant Growth Promoting Rhizobacterium, *Meloidogyne incognita*, systemic resistance

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetables across the globe seriously affected by root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood. Economic loss in India due to this nematode was estimated to be 27.21 per cent (Jain *et al.,* 2007). *Pseudomonas fluorescens,* a promising rhizo bacterium was reported to have the ability to colonize the roots of wide range of crops (Kloepper *et al.,* 1980) and induce systemic resistance against the plant pathogens by producing defense enzymes (Leeman *et al.,* 1995). The defense enzymes were reported to reinforce the cell wall structure and cause biochemical and physiological changes in the plant system (Chen *et al.*, 2000), which are directly inhibitory to nematodes (Paul and Kumar, 2003). *P. fluorescens*, in consortium with *Bacillus subtilis* was reported to be effective against root-knot nematode, *M. incognita* in tomato (Jonathan *et al.,* 2009). Banana plants treated with *P. fluorescens* enhanced the production of defense enzymes which are responsible for induced systemic resistance and suppression of nematode infestation (Senthilkumar *et al.,* 2008). In the present study, induction of defense enzymes by *P. fluorescens* against *M. incognita* infestation on tomato was studied by split root bioassay under glass house conditions.

Materials and Methods

Split root bioassays were performed for a better understanding of the mechanism of nematode

suppression by the bacteria. In this method, a single root was split into equal halves and the treatments were given to one half of the root and the other half was maintained as control. About 30 days old healthy and uniform sized tomato cv. Co3 seedlings were uprooted carefully from the seedling pan and their root system was split into equal halves and one half of the root was dipped in 100 ml of the bacterial suspension (2.5 x 109 cfu/ml) for about 30 min. Other half of the root was maintained as control without any treatment. The seedlings were planted in a polybag containing 250 g of sterilized pot mixture in such a way that each half of the root system was planted in a separate polybag (Plate 1) apposed to each other. Carbofuran treated (1 kg a.i/ha) and a control plant were also maintained for comparison. Care was taken that the separated halves of the root system do not come into contact with each other. Watering was done using rose can in order to avoid cross contamination between the two separate root portions. Five days after planting, both the halves of the root system were inoculated with the second stage juveniles of *M. incognita* @ 250 nematodes per root system. The treatments were arranged in a completely randomized design (CRD) with ten treatments and three replications and seedlings were maintained for two months.

After two months, the plants were uprooted and about one gram of root sample was collected from the two split halves of the root system and homogenized in 1 ml of 0.1 M phosphate buffer (pH

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7.0) at 4°C for 15 min. and the supernatant was collected. It served as an enzyme source. Peroxidase, Polyphenol-oxidase and Phenylalanine ammonia lyase activities were analysed from the samples spectrophotometrically. Nematode incidence in the treated and control plants was recorded by taking gall index. The gall indices were graded on 0 to 5 scales (Taylor and Sasser, 1978).

Assay of Peroxidase (PO)

Reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of one per cent H_20_2. Reaction mixture was incubated at room temperature (28±2°C). Changes in absorbance at 420 nm were recorded at 30 sec intervals for 3 min. Enzyme activity was expressed as changes in the absorbance $min^{-1}g^{-1}$ (Hammer schmidt *et al.,* 1982).

Assay of Polyphenol oxidase (PPO)

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} g^{-1} (Mayer *et al.,* 1965).

Assay of Phenylalanine ammonia lyase (PAL)

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 polyvinylpyrrolidine. The extract was filtered through cheese cloth and the filtrate was centrifuged at 16,000 g for 15 min. The supernatant was used as enzyme source. PAL activity was determined at the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. Samples containing 0.4 ml of enzyme extract were 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 Trans - cinnamic acid synthesized was calculated. Enzyme activity was expressed as nmol trans -cinnamic acid $min^{-1}g^{-1}$ (Dickerson *et al.,* 1984).

Results and Discussion

In the split root bioassay, enhanced activity of peroxidase in tomato roots was observed in *P. fluorescens* treated root half than the control root half portion. Among the treated root portions, high level of PO activity was observed in the plants treated with consortium, Pf 128 + Bbv 57 (0.737 $min^{-1}g^{-1}$

Table1. Efficacy of liquid formulation of *P. fluorescens* isolates on PO, PPO and PAL activities in tomato roots by split root bioassay

Treatment	Peroxidase (Change in absorbance min^{-1} g^{-1})			Poly Phenol Oxidase (Change in absorbance $min^{-1}g^{-1}$)			Phenylalanine Ammonia Lyase (Change in cinnamic acid $min^{-1}g^{-1}$)			
	Treated root half	Untreated root half	% increase over control	Treated root half	Untreated root half	% increase over control	Treated root half	Untreated root half	% increase over control	Gall index
Pf 128	0.641	0.500	28.20	0.079	0.056	41.07	0.420	0.318	32.08	1.7
Pfbv 22	0.553	0.407	35.87	0.072	0.051	41.18	0.412	0.308	33.77	2.5
Pf 1	0.482	0.331	45.62	0.067	0.047	42.55	0.388	0.287	35.19	3.0
Pf 223	0.473	0.324	45.99	0.058	0.04	45.00	0.378	0.276	36.96	3.2
Pf 128+ Bbv 57	0.737	0.592	24.49	0.088	0.071	23.94	0.519	0.451	28.38	1.3
Pfbv 22 + Bbv 57	0.688	0.548	25.55	0.087	0.067	29.85	0.458	0.353	29.75	1.4
Pf 1+Bbv 57	0.643	0.500	28.60	0.082	0.062	32.26	0.426	0.329	29.48	1.7
Pf 223 +Bbv 57	0.552	0.431	28.07	0.079	0.057	38.60	0.414	0.32	29.38	2.5
Carbofuran 3G 1kg ai/ha	0.421	0.420	0.24	0.048	0.047	2.13	0.309	0.307	1.63	3.6
Control	0.418	0.416	0.48	0.047	0.047	0.00	0.307	0.303	1.32	4.3
CD (p=0.05)	0.019	0.0041			0.0095	0.0023		0.0119	0.0032	

root). Its untreated root half portion also recorded the PO activity of 0.592 $min^{-1}g^{-1}$ root. Per cent increase of PO activity of treated portion over untreated control portion was recorded as 24.49 per cent. It is followed Pfbv 22 + Bbv 57 treated root portion (0.688 $min^{-1}g^{-1}$ root) with 25.55 per cent increase over its untreated root portion. Lowest PO activity was recorded in carbofuran treated plants and control plants (Table 1).

Similarly, polyphenol oxidase activity was also observed to be higher in Pf 128 + Bbv 57 treated root half (0.088 $min^{-1}g^{-1}$ root) over its untreated control (0.071$min^{-1}g^{-1}$root) with 23.94 per cent increase over control. It was followed by Pfbv 22 + Bbv 57 treated root half (0.087 $min^{-1}g^{-1}$ root) which recorded 29.85 per cent increase over its control. PPO activity was in a negligible level in carbofuran treated and untreated control plants (Table 1).

Increased activity of PAL was observed in Pf 128 + Bbv 57 (0.519 $min^{-1}g^{-1}$ root) treated root half over its untreated control (0.451) with significant increase of 28.38 per cent over control root portion. It was

followed by Pfbv 22 + Bbv 57 applied root halves (0.458 $min^{-1}g^{-1}$root) with 29.75 per cent increase over its control root portion. Carbofuran treated and untreated control plants recorded the lowest PAL activities when compared to other treatments (Table 1).

A significant decrease in the nematode population in the soil and root ultimately resulted in the lowest gall index (1.3) in the Pf 128 + Bbv 57 treated plants followed by Pfbv 22 + Bbv 57 which recorded (1.4). In contrast the highest gall index (4.3) was recorded in the control.

This study was in accordance with the findings of Siddiqui and Shaukat (2004) who observed the application of bacterial cell suspension to one-half of the root system lowered the populations of root-knot nematode in non-bacterized nematode-treated sections indicating enhanced defense in the non-bacterized half. It is ascertained that fluorescent pseudomonads induce systemic resistance against root-knot nematode *viz*., a signal transduction pathway, which is independent of SA accumulation in roots. These results were also in confirmation with the findings of Niknam and Dhawan (2002), who documented *P. fluorescens* and *B. subtilis* induced systemic resistance in tomato against *R. reniformis* in split root bioassay.

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Received: March 2, 2011; Accepted: July 20, 2011