BIOLOGIA PLANTARUM 55 (2): 317-322, 2011

Pseudomonas fluorescens mediated systemic resistance in tomato is driven through an elevated synthesis of defense enzymes

S.C. VANITHA and S. UMESHA*

Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore-570006, Karnataka, India

Abstract

Pseudomonas fluorescens was used as a biological control agent against bacterial wilt disease caused by *Ralstonia solanacearum*. Activities of phenylalanine ammonia lyase (PAL), guaiacol peroxidase (POX), polyphenol oxidase (PPO) and lipoxygenase (LOX) increased in *P. fluorescens* pretreated tomato seedlings, which were later inoculated with *R. solanacearum* and activities of PAL, POX, PPO and LOX reached maximum at 12, 9, 15 and 9 h, respectively, after inoculation. Reverse transcription - polymerase chain reaction (RT-PCR) confirmed the maximum induction of all these enzymes in *P. fluorescens* pretreated seedlings.

Additional key words: gene expression, lipoxygenase, *Lycopersicon esculentum*, peroxidase, phenylalanine ammonia lyase, polyphenol oxidase, *Ralstonia solanacearum*.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable grown worldwide. The major constraint to tomato production in India is bacterial wilt caused by *Ralstonia solanacearum*. The control of bacterial wilt has been difficult due to the high variability of the pathogen, high ability to survive in diverse environments and its extremely wide host range. Chemical control of plant diseases is usually expensive and may have a negative impact on the environment and on public health. Biological control makes management of plant diseases less dependent on the use of high-risk chemicals and it is environmentally friendly.

Fluorescent pseudomonads, non-pathogenic rhizobacteria, are among the most effective biological control agents against soil-borne plant pathogens. Several isolates of *Pseudomonas fluorescens*, *P. putida,* and *P. aureofaciens* suppress the soil-borne pathogens through rhizosphere colonization, antibiosis and iron chelation by siderophore production. Certain fluorescent pseudomonads are also found to promote plant growth by production of plant growth-promoting substances and thus they are called plant growth promoting rhizobacteria (PGPR). PGPR are known to induce resistance against fungal, bacterial and viral diseases. In addition to plant growth-promotion and direct antimicrobial activity, activation of defense genes by PGPR application is a novel strategy in plant protection.

PGPR activates systemically the plant's latent defense mechanisms against pathogens so called induced systemic resistance (ISR; Hammerschmidt and Kuc 1995). This mechanism operates through the activation of multiple defense compounds at sites distant from the point of pathogen attack. Recent investigations on mechanisms of biological control by PGPR revealed that several strains protect the plants from pathogen attack by strengthening the epidermal and cortical cell walls with deposition of newly formed barriers beyond infection sites including callose, lignin and phenolics and by activating defense genes encoding chitinase, POX, PPO and PAL (M'Piga *et al*. 1997, Chen *et al*. 2000).

The potential of *P. fluorescens* in providing disease resistance and plant growth promotion has been proved in a variety of crops and pathogen interaction, as in sheath blight, sheath rot, blast of rice and bacterial blight

Received 6 July 2009, *accepted* 20 May 2010.

Abbreviations: CA - *trans*-cinnamic acid, ISR - induced systemic resistance; LOX - lipoxygenase; PAL - phenylalanine ammonia lyase; PGPR - plant growth promoting rhizobacteria; POX - guaiacol peroxidase; PPO - polyphenol oxidase; RT-PCR - reverse transcription - polymerase chain reaction.

Acknowledgements: The present work is the result of research work entitled, "Molecular Studies on Bacterial Wilt of Tomato and Its Management" awarded by Rajiv Gandhi National Fellowship Scheme, University Grants Commission, Government of India, New Delhi, India.

* To whom all correspondence should be addressed; fax: (+91) 821 2419884, e-mail: pmumesh@gmail.com

of cotton (Nandakumar *et al*. 2001, Radjacommare *et al*. 2002, Rajendran *et al*. 2006); ground nut (Meena *et al*. 2000); wilt disease of tomato (Ramamoorty *et al*. 2002a) and *Pythium* disease of tomato and hot pepper (Ramamoorty *et al*. 2002b). The induction of systemic resistance by the *Pseudomonas* strains was demonstrated in bean, carnation, rice, and cucumber (Alstrom 1991, Wei *et al*. 1991, Nandakumar *et al.* 2001). These strains of *Pseudomonas* spp. were found to induce resistance against different pathogens in cucumber (Wei *et al*. 1991) and radish (Hoffland *et al*. 1996). Success of induced systemic resistance influenced by PGPR strains was demonstrated under field conditions in cucumber against *C. orbiculare* (Wei *et al*. 1991). The induction of defense genes against various pathogens in different hosts has been well documented earlier (Anand *et al*. 2007, Ganeshamoorthy *et al*. 2008).

Earlier, we reported that seed treatment and soil application of *P. fluorescens* isolate DABBV4 significantly improved the seed germination and seedling vigour. The bacterial wilt disease incidence was significantly reduced in plants raised from *P. fluorescens* treated seeds followed by challenge inoculation with *R. solanacearum* (Vanitha *et al*. 2009). Hence the objectives of the present study deals with the induction of defense enzymes such as phenylalanine ammonia lyase (PAL), guaiacol peroxidases (POX), polyphenol oxidase (PPO) and lipoxygenase (LOX) by *P. fluorescens* against challenge inoculation with *R. solanacearum*. The changes in the gene expression patterns were also studied using specific primers for these enzymes.

Materials and methods

Tomato cultivars of two category such as resistant (R) and highly susceptible (HS) cultivars were procured from private seed agencies (*Vikram Agro Centre*, *Kaveri Agro Traders* and *Annadatha Kendra*) in Mysore, India. All seed samples used in the experiment were surface sterilized with 3 % (v/v) sodium hypochlorite solution for 2 - 5 min and washed with distilled water three times. *R. solanacearum* isolate DABBV1 was isolated from the infected plants and characterized using biochemical/ physiological tests. *P. fluorescens* isolate DABBV4 was isolated from the native soil and confirmed by various tests specific to *P. fluorescens* (Vanitha *et al*. 2009).

Bacterial inoculum was prepared by growing bacteria on Kelman's TTC (2,3,5 triphenyl tetrazolium chloride, *Sigma,* St. Louis, USA) agar medium (10 g dm^{-3} glucose, 10 g dm^{-3} peptone, 1 g dm^{-3} casein hydrolysate, 18 g dm^{-3} agar) and 5 cm^3 of 0.005 % filter-sterilised TTC was added to the 1 dm^3 autoclaved medium) for 48 h at 30 °C. The bacterial cells were collected in sterile distilled water and pelleted by centrifugation (*UniCen, 15 DR, Herolab,* Wiesloch*,* Germany) of the suspension at 12 000 *g* for 10 min. The pellet was resuspended in distilled water and bacterial concentration was adjusted to 1×10^8 cfu cm^{-3} at absorbance of 610 nm using an UV-visible spectrophotometer (*Hitachi U-2000,* Tokyo*,* Japan) according to Ran *et al*. (2005).

The different strains of *P. fluorescens* were isolated from native soil. The bacteria were isolated by adding approximately 1 g of soil to 9 cm^3 of sterile distilled water. The solution was vortexed, allowed to settle for at least 20 min, and then vortexed again. The bacterial fraction was collected from the supernatant following centrifugation at 8 000 *g* and aliquots plated onto King's B medium agar plates (KMB) (King *et al*. 1956). The isolated *P. fluorescens* strains were further evaluated for their antagonistic activity by the dual culture technique. The identity of the isolate based on inhibition zone with the best antagonistic activity was confirmed by performing various tests specific to *P. fluorescens*. The 48-h old cultures grown on King's medium B broth were centrifuged at 8 000 *g* for 10 min using bench top centrifuge. Inoculum was prepared by adjusting the bacterial concentration with sterile distilled water to 1×10^8 cfu cm^{-3} (for detail see Vanitha *et al*. 2009).

Tomato (*Lycopersicon esculentum* Mill.) seeds of resistant cv. Golden were treated with the *P. fluorescens* suspension for 12 h and then were germinated on moist blotter discs placed in 9-cm Petri dishes, at 25 seeds per plate following standard procedure of the International Seed Testing Association (ISTA 2003). The plates were incubated at 28 ± 2 ºC for 8 d until cotyledons were completely opened. The roots of 8-d-old seedlings were dip-inoculated by pouring *R. solanacearum* suspension into the Petri dishes. A set of three controls were maintained, *i.e*., only *P. fluorescens* treated seeds, water and *R. solanacearum* treated seeds. The inoculated and uninoculated seedlings were harvested at 0, 3, 6, 9 and 72 h post inoculation and stored at -80 ºC for subsequent analysis.

One gram of tomato seedling fresh mass was homogenized to fine paste in a pre-chilled mortar with 25 mM Tris-HCl buffer (pH 8.8) (m/v; 1:1). The homogenate was centrifuged at 8 000 *g* for 30 min at 4 °C and the supernatant was directly used as enzyme source. Phenylalanine ammonia lyase activity was assayed by a modified procedure of Lisker *et al*. (1983). The enzyme activity was determined by measuring the production of *trans*-cinnamic acid (CA) from L-phenylalanine using spectrophotometer *Hitachi U-2000*. The reaction mixture contained 1 cm^3 enzyme extract, 0.5 cm^3 50 mM L-phenylalanine and 0.4 cm^3 25 mM Tris-HCl buffer (pH 8.8). After incubation for 2 h at 40 ºC, the activity was stopped by the addition of 0.06 cm^3 5 M HCl and the absorbance was read at 290 nm against the same volume of reaction mixture without L-phenylalanine which served as blank.

Further, 1 g of fresh mass was homogenized in 1 cm^3 of 10 mM phosphate buffer (pH 6.0) and centrifuged at

12 000 *g* for 20 min at 4 °C and the supernatant served as enzyme source. Peroxidase (POX) assay was carried out as described by Hammerschmidt *et al*. (1982) with minor modifications. The reaction mixture (3 cm^3) consisted of 0.25 % (v/v) guaiacol in 10 mM potassium phosphate buffer (pH 6.0) containing 10 mM H_2O_2. Addition of 0.1 cm^3 of crude enzyme extract initiated the reaction and absorbance at 470 nm was measured for 1 min. Polyphenol oxidase (PPO) activity was determined according to Mayer *et al*. (1965). The reaction mixture consisted of 1.5 cm^3 of 0.1 M sodium phosphate buffer (pH 6.5) and 0.2 cm^3 of the enzyme extract. The reaction was started with the addition of 0.2 cm^3 of 10 mM catechol. The increase in absorbance was measured at 420 nm for 1 min. Lipoxygenase activity was estimated according to Borthakur *et al*. (1987). The activity was determined spectrophotometrically by monitoring the appearance of conjugated diene hydroperoxide, absorbing at 234 nm. The reaction mixture contained 2.7 cm^3 of 0.2 M sodium phosphate buffer (pH 6.5), 0.3 cm^3 of 10 mM linoleic acid in *Tween 20* and 0.05 cm^3 of the enzyme extract. Protein contents of the extracts were determined according to standard procedure of Bradford (1976) using BSA (*Sigma, USA*) as standard.

Total RNA was extracted from resistant cv. Golden and from hyper sensitive cv. Quality using phenol/ chloroform extraction procedure (Sambrook *et al*. 1989). Total RNA was quantified spectrophotometrically and equal loading of RNA samples were confirmed by visualizing RNA stained with ethidium bromide under UV radiation. Reverse transcription reaction were carried out as recommended by the manufacturer (*Bangalore Genei,* Bangalore, India). The products of reverse transcription were used as templates for PCR analysis. The primers used for PCR were designed using the sequence reported in the gene bank (www.ncbi.nlm.nih.gov). PCR reactions were carried out in a 0.025 cm^3 mixture containing 0.001 cm^3 of cDNA, 3 units of Taq DNA polymerase, 10 mM dNTP, 5 pmoles of primers (*Bangalore Genei*) of each target and reference genes. The cycle numbers were optimized to ensure that amplification of reference gene and the gene of interest remained within the amplification range thereby giving an accurate representation of transcript abundance. Amplification for PAL, POX and PPO was carried out according to the following temperature profile: 94 ºC for 2 min for denaturation, followed by 22 cycles for 94 ºC for 30 s, 58 ºC for 30 s, 72 ºC for 1 min with final elongation at 72 ºC for 10 min. The amplification temperature profile LOX consisted of 29 cycles and for β-actin 17 cycles. The relative amount of each obtained PCR products were readily quantified by direct scanning of the ethidium bromide stained 1.2 % TAE-agarose gels. The experiments were repeated four times using independent samples. The primers used are in Table 1.

All the Quantitative estimation of enzymes were based on three separate experiments, each with three replicates. The data were subjected to analysis of variance (*ANOVA*) using *SAS* (*JMP* software; *version 9.0*; SAS Institute Inc., Cary, NC, USA). Significant effects of pathogen inoculation were determined by the magnitude of the *F*-value (P = 0.05). The mean values were compared using Fisher's LSD test.

Table 1. Forward and reverse primers used.

Gene product	Forward primer (5'→3')	Reverse primer (3'→5')
Phenylalanine ammonia lyase	TTCAAGGCTACTCTGGC	CAAGCCATTGTGGAGAT
Peroxidase	GCTTTGTCAGGGGTTGTGAT	TGCATCTCTAGCAACCAACG
Polyphenol oxidase	CATGCTCTTGATGAGGCGTA	CCATCTATGGAACGGGAAGA
Lipoxygenase	TTTCTGCGACTTGAGGTTCGG	ATTAGTCTTTACCTTCTTGTCCAGT
β-actin	GGGGAGGTAGTGACAATAAATAACAA	GACTGTGAAACTGCGAATGGC

Results and discussion

Plants have endogenous defense mechanisms that can be induced in response to attack by pathogens. Inducing the plant's own defense mechanisms by prior application of a biological inducer is thought to be a novel plant protection strategy. In our previous study, *P. fluorescens* isolate DABBV4 was found to reduce the incidence of bacterial wilt in tomato under greenhouse conditions (Vanitha *et al.* 2009). *P. fluorescens* could act as strong elicitors of plant defense reactions (M'Piga *et al*. 1997). In the present study, varying patterns of enzymes activities were studied before and after inoculation of seedlings. Higher activities of all the enzymes estimated were detected in *P. fluorescens* pretreated seedlings, which were later inoculated with *R. solanacearum* when compared to all the other three treatments.

Maximum PAL activity was 12 h after inoculation (hpi). PAL activity increased in *P. fluorescens* pretreated seedlings challenged with the pathogen while seedlings inoculated with the pathogen alone had lower PAL activity. PAL activity in seedlings treated with only *P. fluorescens* remained almost unchanged throughout the experiment but compared to control it was

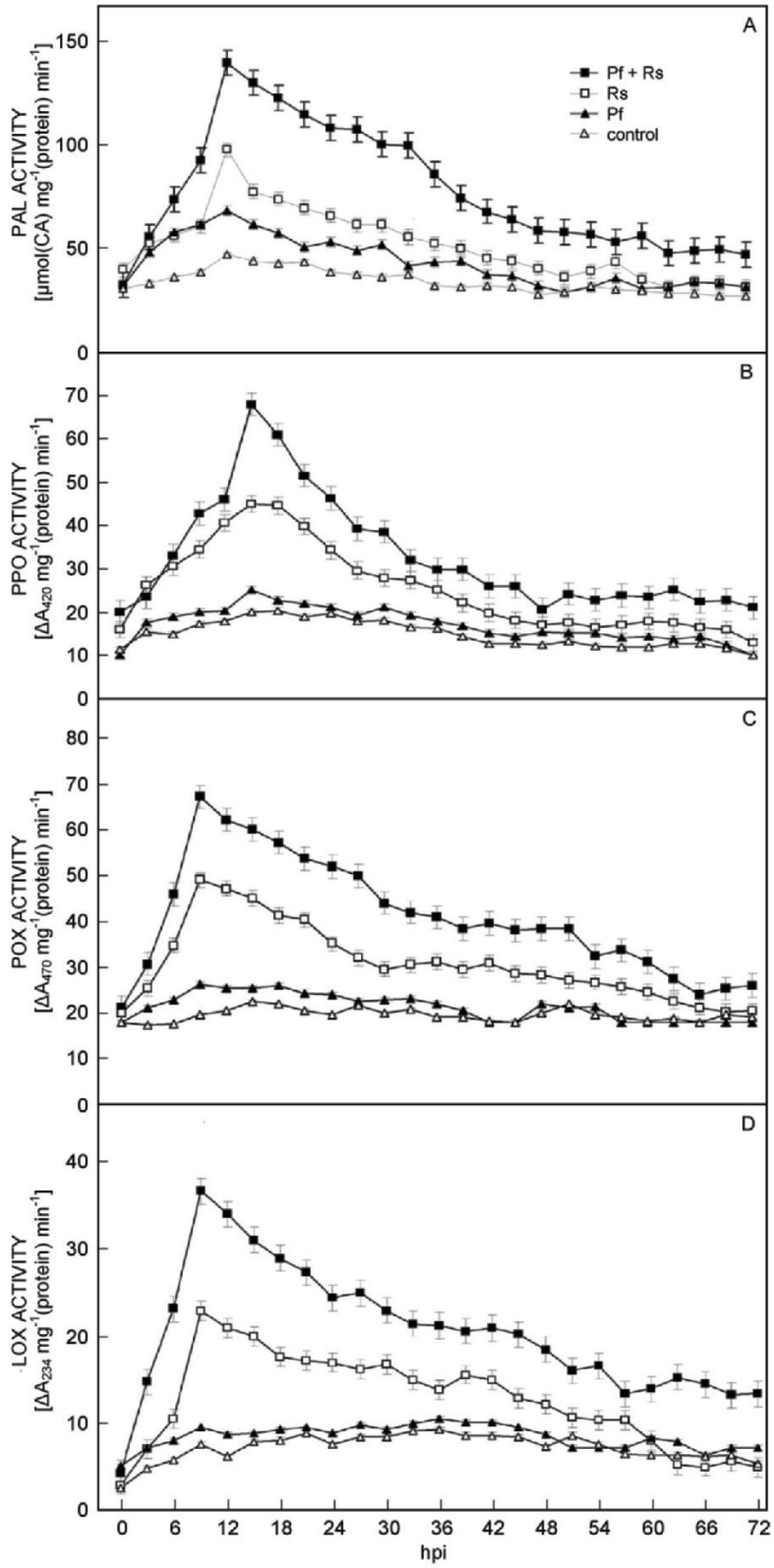

Fig. 1. The effect of different treatments on PAL activity (*A*), PPO activity (*B*), POX activity (*C*), and LOX activity (*D*) in resistant tomato cultivar Golden during 72 h after inoculation (hpi). Pf+Rs - *P. fluorescens* pre-treated seeds challenge inoculated with *R. solanacearum*; Rs - seeds inoculated with *R. solanacearum*; Pf - seeds treated with *P. fluorescens*; control - seeds treated with distilled water. The means of three independent experiments, *bars* indicate SE.

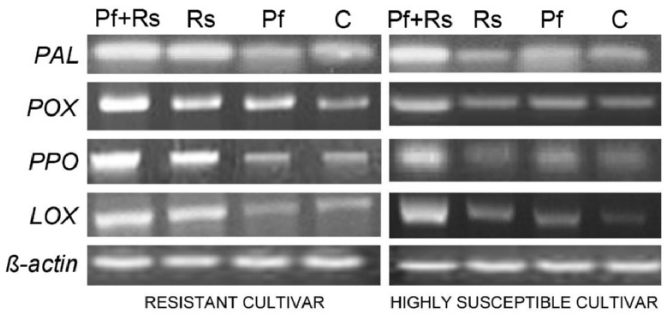

Fig. 2. Induced gene expression pattern of defense-related enzymes and β-actin in highly susceptible tomato cultivar Quality when compared to resistant cultivar Golden. Pf+Rs - *P. fluorescens* pre-treated seeds challenge inoculated with *R. solanacearum*; Rs - seeds inoculated with *R. solanacearum*; Pf - seeds treated with *P. fluorescens*; C - control seeds treated with distilled water.

slightly higher (Fig. 1*A*). Induction of PAL by fluorescent pseudomonads was reported also in cucumber (Chen *et al*. 2000), tomato (Ramamoorthy *et al*. 2002a, Anand *et al*. 2007) and mulberry (Ganeshmoorthi *et al*. 2008).

POX is a key enzyme in the biosynthesis of lignin. Increased activity of cell wall bound peroxidases has been elicited in different plants due to pathogen infection. In our study, POX activity increased after inoculation and reached its maximum at 9 hpi. Similarly to PAL activity, tomato seedlings inoculated with the pathogen alone recorded lower POX activity than pre-treated seedlings but higher that the seedlings treated with *P. fluorescens* alone and untreated control seedlings (Fig. 1*C*). Increased activity of POX has been elicited by *P. fluorescens* in different plants such as cucumber (Chen *et al*. 2000), rice (Nandakumar *et al*. 2001), tomato (Ramamoorthy *et al*. 2002a) and mulberry (Ganeshmoorthi *et al*. 2008).

PPO catalyses the oxidation of phenolic compounds to highly toxic quinones which play an important role in plant disease resistance. Also in our study with tomato seedlings treated with *P. fluorescens*, the induced systemic resistance was associated with enhanced PPO activities. At 15 hpi, the activity of PPO was maximal in seedlings pretreated with *P. fluorescens* and later challenge inoculated with *R. solanacearum*. Seedlings treated with *R. solanacaerum* alone also showed increased PPO activity but the increase was comparatively less. The PPO activity in seedlings treated with *P. fluorescens* alone and distilled water never reached to the level of activity seen in the seedlings treated with *P. fluorescens* and inoculated with *R. solanacearum* (Fig. 1*B*). Chen *et al*. (2000) reported that various rhizobacteria and *P. aphanidermatum* induced the PPO activity in cucumber root tissues. Accumulation of PPO was increased in *P. fluorescens* treated banana plants (Harish 2005), tomato (Anand *et al*. 2007) and mulberry (Ganeshmoorthi *et al*. 2008).

High LOX activity may be constitutive in plants resistant to pathogens, but with additional increase upon infection to pathogen. In our study, the LOX activity was maximum at 9 hpi in seedlings treated with *P. fluorescens* and challenged with *R. solanacearum*. Similarly to above mentioned enzymes, LOX activities were lower in other treatments (Fig. 1*D*). Increase in LOX activity was detected in leaf extracts from tomato plants, whose seeds had been treated with rhizobacteria (Silva *et al*. 2004).

It is well known that the defense genes are inducible genes and appropriate stimuli or signals are needed to activate them. Gene expression patterns were compared between the treatments and also between the cultivars. In resistant cv. Golden treated with *P. fluorescens* and inoculated with *R. solanacearum*, we detected maximum transcripts of *PAL*, *POX*, *PPO* and *LOX* genes when compared to its control and also to the hypersensitive cv. Quality. *PAL* gene expression in cv. Quality was equal to that of cv. Golden when the seeds pretreated with *P. fluorescens* before inoculation. Similar results were observed in the *POX* gene expression where the seedlings showed a significantly stronger gene expression when had been pre-treated with *P. fluorescens*, while weak expression was not noticed in the other treatments. *PPO* and *LOX* gene expression was also found to be significantly induced in the seedlings of cv. Quality which were pretreated with *P. fluorescens*. The expression of *β-actin*, the house keeping gene, was found to be unaltered in both the resistant and sensitive cultivars in all the treatments (Fig. 2). Goswami and Punja (2008) reported the up-regulation of number of genes involved in host defense responses in ginseng (*Panax quinquefolius*) roots challenged with *Fusarium equiseti.* Peng *et al.* (2005) reported the maximum gene expression of PAL, POX, PPO and LOX in tomato seedlings when exposed to wounding. Liu *et al.* (2010) reported that the *Bacillus cereus* induced the systemic resistance by expressing defense-related genes in *Lilium formosanum* against leaf blight caused by *Botrytis elliptica.*

In conclusion, the present study proved that *P. fluorescens* induced resistance against *R. solanacearum* in tomato seedlings is associated with the enhanced expression of genes for defense-related

enzymes. Pre-treatment of tomato plants with *P. fluorescens* triggered the increased PAL, POX, PPO and LOX activities in response to invasion by *R. solanacearum*.

References

Alstrom, S.: Induction of disease resistance in common bean susceptible to halo blight pathogen after seed bacterization with rhizosphere pseudomonads. - J. gen. appl. Microbiol. **37**: 495-501, 1991.

Anand, T., Chandrasekaran, A., Kuttalam, S., Raguchander, T., Prakasam, V., Samiyappan, R.: Association of some plant defense enzyme activities with systemic resistance to early leaf blight and leaf spot induced in tomato plants by azoxystrobin and *Pseudomonas fluorescens*. - J. Plant Interact. **2**: 233-244, 2007.

Borthakur, A.B., Bhat, B., Ramasoss, C.S.: The positional specifications of the oxygenation of linoleic acid catalysed two forms of lipoxygenase isolated from Bengal gram (*Cicer arietinum*). - J. Biosci. **11**: 257-263, 1987.

Bradford, M.M.: A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein due binding. - Anal. Biochem. **72**: 248- 254, 1976.

Chen, C., Belanger, R.R., Benhamou, N., Paullitz, T.C.: Defense enzymes induced in cucumber roots by treatment with plant-growth promoting rhizobacteria (PGPR). - Physiol. mol. Plant Pathol. **56**: 13-23, 2000.

Ganeshmoorthi, P, Anand, T., Prakasan, V., Bharani, M., Ragupathi, N., Samiyappan, R.: Plant growth promoting rhizobacterial (PGPR) bioconsortia mediates induction of defense-related proteins against infection of root rot pathogen in mulberry plants. - J. Plant Interact. **3**: 233-244, 2008.

Goswami, R.S., Punja, Z.K.: Molecular and biochemical characterization of defense responses in ginseng (*Panax quinquefolius*) roots challenged with *Fusarium equiseti*. - Physiol. mol. Plant Pathol. **72**: 10-20, 2008.

Hammerschmidt, R., Kuc, J.: Induced Resistance to Disease in Plants. - Kluwer Academic Publishers, Dordrecht 1995.

Hammerschmidt, R., Nuckles, E.M., Kuc, J.: Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. - Physiol. Plant. **20**: 73-82, 1982.

Harish, S.: Molecular biology and diagnosis of *Banana bunchy top virus* and its management through induced systemic resistance. - Ph.D. Thesis. Tamil Nadu agricultural University, Combatore 2005.

Hoffland, E., Hakulinen, J., Van Pelt, J.A.: Comparison of systemic resistance induced by avirulent and nonpathogenic *Pseudomonas* species. - Phytopathology **86**: 757-762, 1996.

King, E.O., Ward, M.K., Raney, D.E.: Two simple media for the demonstration of pyocyanin and fluorescein. - J. Laboratory clin. Med. **4**: 301-307, 1956.

Lisker, N., Cohen, I., Chalutz, E., Fucus, Y.: Fungal infections suppress ethylene-induced phenylalanine ammonia lyase activity in grape fruits. - Physiol. Plant Pathol. **22**: 331-338, 1983.

Liu, Y.-H., Huang, C.-J., Chen, C.-Y.: Identification and transcriptional analysis of genes involved in *Bacillus cereus*-induced systemic resistance in *Lilium*. - Biol. Plant. **54**: 697-702, 2010.

Mayer, A.M., Harel, E., Shaul, R.B.: Assay of catechol oxidase: a critical comparison of methods. - Phytochemistry **5**: 783-789, 1965.

Meena, B., Radhajeyalakshmi, R., Marimuthu, T., Vidhyasekaran, P., Doraisamy, S., Velazhahan, R.: Induction of pathogenesis related proteins, phenolics and phenylalanine ammonia lyase in groundnut by *Pseudomonas fluorescens*. - J. Plant Dis. Protect. **107**: 514 - 527, 2000.

M'Piga, P., Belanger, R.R., Paulitz, T.C., Benhamou, N.: Increased resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63-28. - Physiol. mol. Plant. Pathol. **50**: 301-320, 1997.

Nandakumar, R., Babu, S., Viswanathan, R., Raguchander, T., Samiyappan, R.: Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. - Soil Biol. Biochem. **33**: 603-612, 2001.

Peng, J.N., Lie, Z.H., Xiang, H., Haung, J.H., Jia, S.H., Miao, X.X., Huang, Y.P.: Preliminary studies on differential defense responses induced during plant communication. - Cell Res. **15**: 187-192, 2005.

Radjacommare, R., Nandakumar, R., Kandan, A., Suresh, S., Bharathi, M., Raguchander, T., Samiyappan, R.: *Pseudomonas fluorescens* based bioformulation for the management of sheath blight and leaffolder in rice. - Crop Protect. **21**: 671-677, 2002.

Rajendran, L., Saravanakumar, D., Raguchander, T., Samiyappan, R.: Endophytic bacterial induction of defence enzymes against bacterial blight of cotton. - Phytopathol. Mediterr. **45**: 203-214, 2006.

Ramamoorthy, V., Raguchander, T., Samiyappan, R.: Induction of defense-related proteins in tomato roots treated with *Pseudomonas fluorescens* Pf1 and *Fusarium oxysporum* f. sp. *lycopersici*. - Plant Soil **239**: 55-68, 2002a.

Ramamoorthy, V., Raguchander, T., Samiyappan, R.: Enhancing resistance of tomato and hot pepper to *Pythium* diseases by seed treatment with fluorescent pseudomonads. - Eur. J. Plant Pathol. **108**: 429-441, 2002b.

Ran, L.X., Liu, C.Y., Wu, G.J., Van Loon, L.C., Bakker, P.A.H.M.: Suppression of bacterial wilt in *Eucalyptus urophylla* by fluorescent *Pseudomonas* spp. in China. - Biol. Control **32**: 111-120, 2005.

Sambrook, J., Fritisch, E.F., Maniatis, T.: Molecular Cloning: a Laboratory Manual. 2nd Ed. - Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1989.

Silva, H.S.A., Da Siva Romeiro, R., Macagnan, D., De Almeida H.V.B., Pereira, M.C.B., Mounteerd, A.: Rhizobacterial induction of systemic resistance in tomato plants: non-specific protection and increase in enzyme activities. - Biol. Control **29**: 288-295, 2004.

Vanitha, S.C., Niranjana, S.R., Mortensen, C.N., Umesha, S.: Bacterial wilt of tomato in Karnataka and its management by *Pseudomonas fluorescens*. - Biocontrol **54**: 685-695, 2009.

Wei, G., Kloepper, J.W., Tuzun, S.: Induction of systemic resistance in cucumber to *Colletotrichum orbiculare* by selected strains of plant growth promoting rhizobacteria. - Phytopathology **81**: 1508-1517, 1991.