East African Scholars Journal of Agriculture and Life Sciences
Abbreviated Key Title: East African Scholars J Agri Life Sci
ISSN 2617-4472 (Print) | ISSN 2617-7277 (Online)
Published By East African Scholars Publisher, Kenya
Volume-7 | Issue-10 | Oct-2024 | DOI: https://doi.org/10.36349/easjals.2024.v07i10.003

OPEN ACCESS

Original Research Article

PRP5 Induction by Infection of *Pythium Sp.* in Tomato

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Article History
Received: 15.03.2024
Accepted: 23.04.2024
Published: 31.10.2024

Journal homepage:
http://www.easpublisher.com

Quick Response Code

Abstract: The *Pseudomonas fluorescens* isolate FP 7 was found to protect the tomato plants against soil borne fungus, *Pythium aphanidermatum*. The ability to induce defense proteins *viz.* β-1,3 glucanase, peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) by this *P. fluorescens* isolate FP 7 against *P. aphanidermatum* fungus was further studied. Increased activity of PO, PPO and β-1,3 glucanase were observed in plants pretreated with FP 7 isolate. Native PAGE (polyacrylamide gel electrophoresis) of PO showed the single isoform in all the treatment including the control and difference is by the increased intensity of the band in the inoculated control and FP 7 treatment in the tomato plant. Isoform analysis of the PPO showed the induction of the PPO in the *P. fluorescens* treated plants challenged with *P. aphanidermatum*. β-1,3 glucanase in tomato cultivar, Co 3 with and without challenge inoculation of *P. aphanidermatum*, revealed changes in the isoform pattern after staining the gel with 2,3,5-triphenyl tetrazolium chloride. Moreover, higher accumulation of phenolics was noticed in plants pretreated with *P. fluorescens* isolate FP 7 challenged with *P. aphanidermatum*. The relevant function of Thaumatin like defense proteins and secondary metabolites involved in the phenylpropanoid pathway collectively contributing to enhanced resistance is discussed.

Keywords: PR Proteins, *Pythium*, Pseudomonas, Peroxidase, PGPR.

INTRODUCTION

Soil borne plant pathogens are major yield-limiting factors in the production of field and horticultural crops. The genetic resistance in crop plants to many soil borne pathogens is rare and hence other practices such as soil fumigation, soil solarization and crop rotation has been successfully followed to control these diseases. Our studies have focused on pre and post-emergence damping off disease caused by *Pythium* spp., which is the most important root disease of tomato worldwide. (Osburn *et al*., 1989). Although fungicides have shown promising results in controlling the damping off disease, development of fungicide resistance discourages its use for disease control (Whipps and Lumsden, 1991). The need for agriculture to became more sustainable and less dependent on chemical pesticides has necessitated the development of alternative approaches to control *Pythium* and other diseases caused by soil borne pathogens. Sanitation and application of organic compost and regulation of water and temperature during seedling growth contributed to the management of the disease to some extent. Thus, these control measures are not effective for the control of damping-off disease. Utilization of fluorescent pseudomonads has received much attention to replace the existing method of controlling damping-off disease.

Some potential antagonistic rhizobacteria, which are commonly called plant growth promoting rhizobacteria, interact with plant root and protect the roots against pathogenic microorganisms. *Pseudomonas fluorescens* are common members of the plant growth promoting rhizobacteria micro flora in the rhizosphere of protected plants (Nandakumar *et al*., 2001; Ramamoorthy *et al*., 2002; Kloepper *et al*., 1993. The use of fluorescent pseudomonas is gaining importance for plant growth promotion and biological control. Fluorescent pseudomonads control *Pythium* spp. through different modes of action such as competition for nutrients and space (Elad and Chet, 1987), antibiosis (Howie and Suslow, 1991), production of siderophores (Loper, 1988) and lytic enzymes (Frindlender *et al*., 1993). In addition, induction of resistance by fluorescent pseudomonads is the effective mechanism by which these bacteria protect several crops against pests and diseases (Chen *et al*., 2000; Pieterse *et al*., 2001; Ramamoorthy *et al*., 2002; Zehnder *et al*., 2001). The ability of pseudomonads to suppress soil borne fungal

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pathogens is to trigger induced systemic resistance in the host.

Despite the importance of induced systemic resistance by biological agents, little is known about the frequency and ecology of naturally occurring fluorescent pseudomonads. ISR, once expressed, activates multiple potential defense mechanisms that include enhanced activity of PR-proteins such as chitinases, β-1,3-glucanases, peroxidases (PO), other defense enzymes and secondary metabolites like phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), phenolics (Nandakumar *et al*., 2001; Ramamoorthy *et al*., 2002; Dalisay and Kuc, 1995; Kandan *et al*., 2002), accumulation of antimicrobial low molecular weight substances phytoalexins (Van Peer and Schippers, 1992) and formation of protective biopolymers, eg. Lignin, callose and hydroxy proline–rich glycoprotein (Kandan *et al*., 2002; Hammerschmidt and Kuc, 1982). The role of these defense enzymes and secondary metabolites in pest and disease resistance were well established (Nandakumar *et al*., 2001; Stout *et al*., 1999; Tscharntke *et al*., 2001; Morimoto and Komai, 2000).

MATERIALS AND METHODS

Plant Material, Pathogen and Bacterial Strains

Tomato cultivar Co3 (obtained from Horticultural College and Research Institute, Coimbatore, INDIA) and the fluorescent pseudomonads strains FP 7 (obtained from culture collection of Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, INDIA) were used in all experiments. The bacterial culture was maintained in King's B broth (KBB) (King *et al*., 1954) in 30% glycerol at -80°C. From the stock, fresh cultures were prepared on plates of King's B medium (KBM).

Preparation of_Talc-Based Formulation of PGPR Strains

A loopful of bacterium was inoculated into the KBB and incubated in a rotary shaker at 150 RPM for 48 h at room temperature (28±2°C). After 48 h of incubation, the broth containing 9 x 10^8 cfu/ml was used for the preparation of talc-based formulation. To the 400 ml of bacterial suspension, 1 kg of the talc powder (sterilized at 105℃ for 12 h), calcium carbonate 15 g (to adjust the pH to neutral) and carboxymethyl cellulose 10 g (adhesive) were mixed under sterile conditions, following the method described by Vidhyasekaran and Muthamilan (1995). After shade drying for overnight under sterile conditions, it was packed in polypropylene bag and sealed. At the time of application, the population of bacteria in talc formulation was 2.5 to 3 10^8 cfu/g.

Greenhouse Studies

Pathogen Inoculation and Disease Incidence in the Bacterized Plants

The bacterial strain FP 7 was assessed for their efficacy in inducing the defense enzymes against soil borne pathogen *P. aphanidermatum* incidence in tomato under greenhouse conditions. Potting soil (red soil: sand: decomposed cow dung manure at 1:1:1 w/w/w; available N, P, K, Ca and Mg of red soil were 160, 18, 280, 0.28 and 0.2 kg ha^{-1}, respectively and pH was 7.0) was autoclaved and sterilized for 1 h and placed in earthen pots (diameter 0.3 m, height 0.4 m, volume of soil 0.4m^3). The tomato seeds were surface sterilized with 2% sodium hypochlorite for 30 seconds and soaked in double sterile distilled with talc-based formulation (10 g kg^{-1} of seed). After 24 h, the bacterial suspension was drained off and the seeds were dried under shade for 30 min. pathogen inoculated and pathogen un-inoculated control (healthy) was maintained. The virulent strain of *Pythium* aphanidermatum, mass multiplied in the sand-maize medium, was mixed with the sterilized potting medium at the ratio of 19:1 w/w. Watering was done regularly, and damping-off disease incidence was recorded at 25 days after sowing. Three pots per replication and in all the treatments three replications in factorial completely randomized design (CRD) were maintained.

Assay of PGPR Induced Proteins

Sample Collection and Enzyme Extraction

The PGPR treated tomato plants subjected to fungal infection was collected at 0, 24, 48, 72, 96 and 168h intervals by uprooting without causing damage. The samples were collected from all the three replication separately and were homogenized with liquid nitrogen and stored under refrigerated condition at -80°C. One g of powdered sample was extracted with 2 ml of sodium phosphate buffer 0.1M, pH 7.0 at 4. The homogenate was centrifuged for 20 min at 10000 rpm. Protein extract prepared from samples were used for estimation of at β-1,3 glucanase, peroxidase (PO), polyphenol oxidase (PPO) and L-phenylalanine ammonia-lyase (PAL) by spectrophotometer and electrophoresis method.

Assay of β-1,3 Glucanase

β-1,3-glucanase activity was assayed by the laminarin-dinitrosalicylic acid method (Pan *et al*., 1991). The reaction mixture consisted of 62.5 µl of 4% laminarin and 62.5 µl of enzyme extract. The reaction was carried out at 40°C for 10 min. The reaction was stopped by adding 375µl of dinitrosalicylic acid and heating for 5 min on boiling water, vortexed and its absorbance was measured at 500 ηm. The enzyme activity was expressed as µg glucose released min^{-1} g^{-1} of sample.

Detection of β-1,3 Glucanase Activity After PAGE Under Non – Denatured Conditions

β-1,3 glucanase isozymes and protein pattern were detected after a single separation using native polyacrylamide gel electrophoresis (PAGE) by the methods of Pan *et al*., (1989). To stain for β-1,3 glucanase, the PAGE gels were washed, then incubated at 40º C for 30 min in a mixture containing 75 ml of 0.05M sodium acetate (pH 5.0) and 0.6 g of laminarin (sigma) dissolved in 75 ml of water. The gels were then

incubated in a mixture of methanol, water and acetic acid (5:5:2) for 5 min, washed with water and stained with 0.3 g of 2,3,5-triphenyltetrazolium chloride in 200 ml of 1.0 M NaOH in a boiling water bath until red bands appeared (approx. 10 min).

Assay of PO and PPO

Peroxidase and polyphenol oxidase activity were assayed spectrophotometrically as described by Hammerschmidt *et al*., (1982) and by Mayer *et al*., (1965) respectively. The enzyme activity was expressed as change in the absorbance of the reaction mixture/min/g on fresh weight basis.

Activity Gel Electrophoresis for PO and PPO

To study the expression pattern of different isoforms of peroxidases in different treatments, activity gel electrophoresis was carried out. For native anionic polyacrylamide gel electrophoresis, resolving gel of 8% acrylamide concentration and stacking gel of 4% acrylamide concentration were prepared. After electrophoresis, the gels were incubated in the solution containing 0.15% benzidine in 6% NH_4Cl for 30 min in dark and then drops of 30% H_2O_2 was added with constant shaking till the bands appear for peroxidase (Sindhu *et al*., 1984). For PPO, gel was equilibrated for 30 min in 0.1% *p*-phenylene diamine in 0.1M potassium phosphate buffer (pH 7.0) followed by 10 mM catechol in the same buffer. The addition of catechol followed by a gentle shaking resulted in appearance of dark brown discrete protein bands (Jayaraman *et al*., 1987). After staining, the gel was washed with distilled water and photographed. Fig.1

Estimation of Phenylalanine Ammonia-Lyase and Phenol

The PAL assay was conducted as per the method described by Ross and Sederoff (1992) using L-phenylalanine as substrate. Standard curve was drawn with graded amounts of cinnamic acid in toluene as described earlier. The enzyme activity was expressed as ηmoles of cinnamic acid/min/g fresh tissue. Phenol content of tomato was estimated according to Zieslin and Ben-Zaken (1993). The content of the total soluble phenols was calculated from a standard curve obtained from a Folin-Ciocalteau reagent with a phenol solution (C_6H_6O) and expressed as catechol equivalents g^{-1} tissue weight. Fig.2

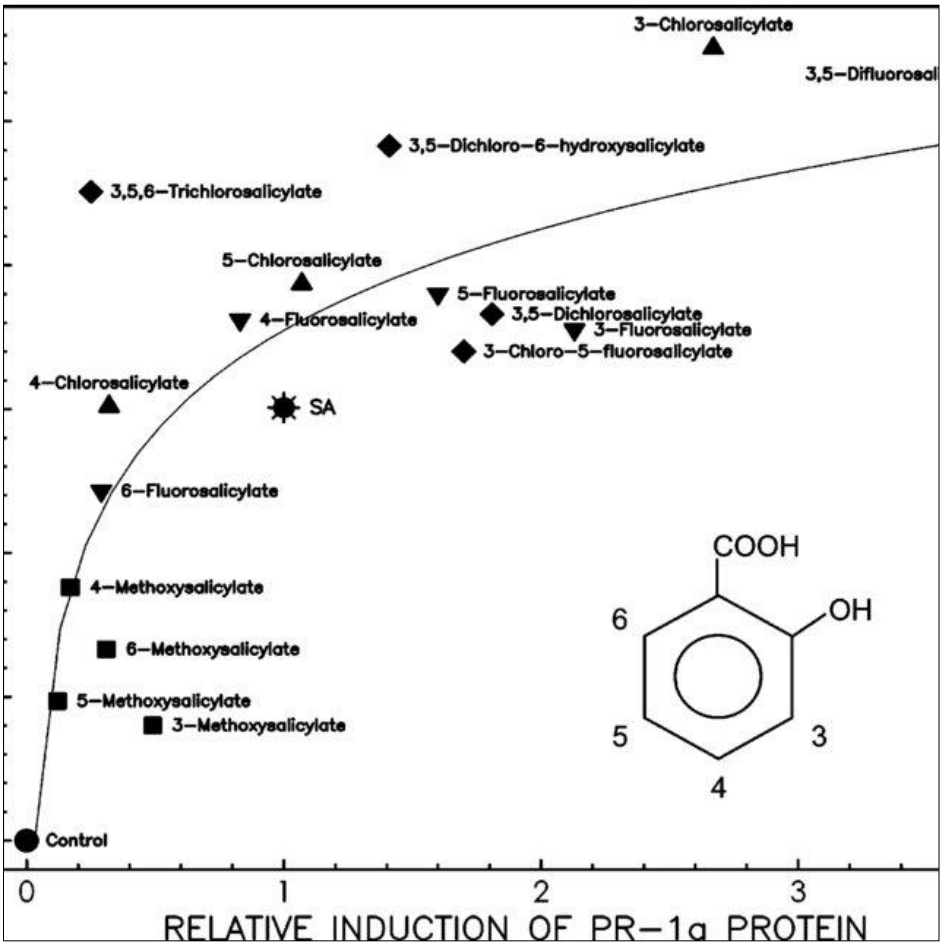

Fig.1

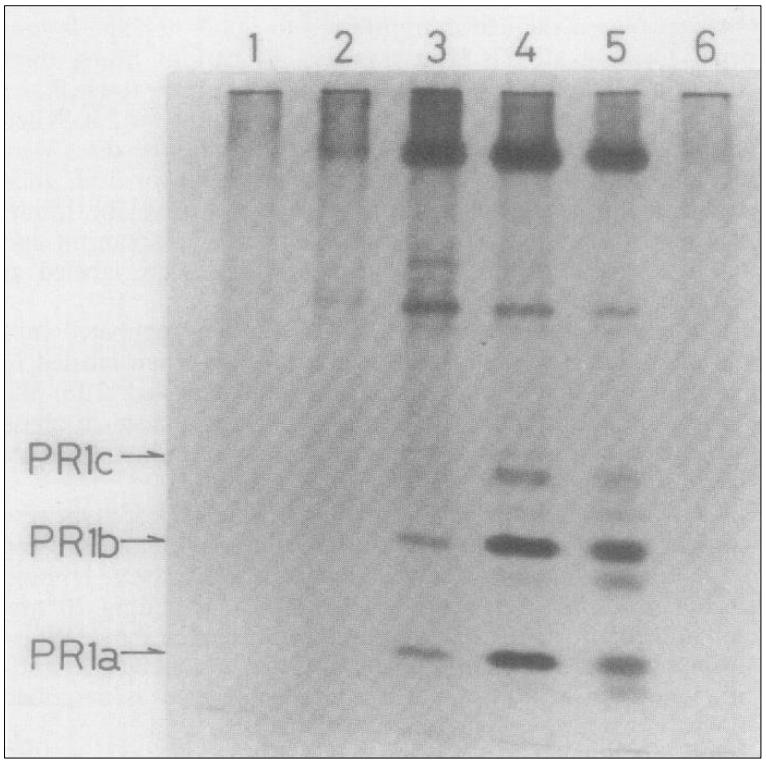

Fig. 2

Statistical Analysis

The data were analyzed independently for studies under glasshouse condition. The data were analyzed as completely randomized design (CRD) using the IRRISTAT version 92-1 programme developed by biometrics unit at the International Rice Research Institute, The Philippines. The treatment means were compared by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

RESULT

There was no significant difference in the suppression of damping-off of tomato among the formulations when they were applied at 0^{th} day of storage period compared with Ridomil fungicide as a standard treatment. All the formulations containing different carriers effectively reduced the disease incidence when applied at 0^{th} day of storage period. Generally, formulations mixed with *P. fluorescens* FP 7 mass multiplied in KMB amended with chitin recorded less disease incidence when compared with formulation mixed with *P. fluorescens* FP 7 multiplied in KMB without chitin amendment. During the time course of storage period the formulation containing talc as a carrier mixed with *P. fluorescens* FP 7 mass multiplied in KMB showed maximum biocontrol activity even after 75 days of storage period by recording the disease incidence of 38.67 per cent in tomato and the efficacy was comparable with ridomil used as a standard treatment for comparison. When compared to fungicide treatment, this formulation was effective up to 75 days in tomato. (Tables).

β-1, 3-Glucanase

The activities of β-1, 3-glucanase was found at higher level in the *P. fluorescens* pretreated plants and challenged with *P. aphanidermatum.* The activity reached maximum level on the 5^{th} day after challenge inoculation in tomato. In plants inoculated with *P. aphanidermatum* the increased activity was lasted only for a period of 2-4 days thereafter declined drastically (Fig.1)

Peroxidase

Earlier and increased activities of peroxidase (PO) were observed in *Pseudomonas*-pretreated tomato plants challenge inoculated with *P. aphanidermatum* and remained at higher levels throughout the experimental period. The activity reached maximum levels at 3^{rd} day after challenge inoculation (Fig. 1,2).

In bacterized tomato plants challenged with *P. aphanidermatum*, the expression of PO isoforms designated as PO1, PO2, PO3, PO4 and PO5 was more prominent and the expression of PO1 was higher when compared with untreated plants and also plants treated with *P. aphanidermatum* alone or plants treated with *P. fluorescens* alone (Plate). Plants inoculated with *P. aphanidermatum* alone also had higher level expression of PO2-PO5 isoforms. In tomato plants only one PO isoform (PO1) was noticed in all induction treatments except untreated plants in which the expression of PO1 was very weak. However, higher level expression of PO1 was observed in *P. fluorescens* FP 7 treated tomato plants challenge inoculated with *P. aphanidermatum* (Plate).

Polyphenol Oxidase

Similar to peroxidase activity, higher and increased activities of polyphenol oxidase (PPO) were noticed in *P. fluorescens* FP 7 treated tomato plants challenged with *P. aphanidermatum* when compared to other induction treatments and also untreated control plants (Fig. 3).

Three PPO isoforms designated as PPO1, PPO2 and PPO3 were prominently noticed in tomato plants treated with *P. fluorescens* and challenge inoculated with *P. aphanidermatum* (Plate 11A). In tomato, only one PPO isoform (PPO1) was expressed at higher level in all the induction treatments. In untreated plants, this constitutive PPO1 was expressed at a weak level.

Phenylalanine Ammonia Lyase

In tomato, seed treatment with *P. fluorescens* FP 7 induced the plants to synthesize phenylalanine ammonia lyase (PAL) whereas an additional increase in the synthesis was observed in *P. fluorescens* FP 7 - pretreated plants challenge inoculated with *P. aphanidermatum*. The activity reached the maximum level at 3rd day after challenge inoculation and thereafter the activity remained at higher levels throughout the experimental period of 10 days. In plants inoculated with *P. aphanidermatum* alone, increased activity of PAL was observed for a period of 2-4 days thereafter declined drastically in both tomato (Fig. 2).

Phenols

Higher level accumulation of phenolics was observed in bacterized roots challenge inoculated with *P. aphanidermatum* both at 5th and 10th day after challenge inoculation. In plants inoculated with *P. aphanidermatum* alone increased phenolic content was noticed at 5th day whereas at 10th day it was drastically reduced.

DISCUSSION

It was reported that fluorescent pseudomonads protected the host plants against plant pathogens through mechanisms other than direct antagonism by Voisard *et al*., (1989). The first evidence on Induced systemic resistance (ISR) by fluorescent pseudomonads was reported in cucumber against anthracnose (Wei *et al*., 1991), carnation against fusarium wilt (Van Peer *et al*., 1991) and bean against halo blight (Alstrom, 1991). The mechanism behind the induced resistance by fluorescent pseudomonads was not clearly explained. Moreover, ISR by rhizobacteria is relatively new area of research with regard to crop protection. Fluorescent pseudomonads are known to aggressively colonize roots therefore, they are efficient competitors and persist throughout the crop season (Kloepper and Schroth, 1981).

Phenylalanine ammonia lyase plays an important role in the biosynthesis of various defense chemicals in phenylpropanoid metabolism (Daayf *et al*., 1997). PAL activity could be induced in plant-pathogen interactions and fungal elicitor treatment (Ramanathan *et al*., 2000). De Meyer *et al*., (1999) reported that rhizosphere colonization by P. aeruginosa 7NSK2 activated PAL in bean roots and increased the salicylic acid levels in leaves. In the present study, increased activity of PAL was recorded in *P. fluorescens* FP 7-treated plants challenged with the pathogens. The time required to activate the defense mechanisms is important for the suppression of pathogen. Earlier and higher level of expression of defense enzymes and accumulation of chemicals at the infection site certainly prevent the fungal mycelial colonization. In tomato seedlings, the activity of PAL reached maximum at 3rd day after *P. aphanidermatum* inoculation. The activity of PAL was maintained at higher level throughout the experimental period. In plants inoculated with the pathogen alone the activity declined drastically 4 days after challenge inoculation. Invasion of root tissues by the pathogen might have resulted in decreased activity of PAL whereas earlier and increased activity of PAL due to *P. fluorescens* FP 7 treatment might have prevented the fungal invasion and thus the activity maintained at higher levels during the experimental period. Induction of PAL by fluorescent pseudomonads was reported in cucumber against *P. aphanidermatum* (Chen *et al*., 2000) and bean against Botrytis cinerea (Zdor and Anderson, 1992).

Peroxidase represents another component of an early response in plants to pathogen attack and plays a key role in the biosynthesis of lignin which limits the extent of pathogen spread (Bruce and West, 1989). The products of this enzyme in the presence of hydrogen donor and hydrogen peroxidase have antimicrobial activity and even antiviral activity (Van Loon and Callow, 1983). Increased activity of cell wall bound peroxidases has been elicited in different plants such as cucumber (Chen *et al*., 2000), rice (Reimers *et al*., 1992), tomato (Mohan *et al*., 1993) and tobacco (Ahl Goy *et al*., 1992) due to pathogen infection. In bean, rhizosphere colonization of various bacteria induced the peroxidase activity (Zdor and Anderson, 1992). In the present study, earlier and increased peroxidase activity has been recorded in *P. fluorescens* FP 7-treated plants challenged with the pathogen. Chen *et al*., (2000) reported the higher PO activity in cucumber roots treated with P. corrugata challenged with *P. aphanidermatum*. In this study expression of five PO isoforms.

Similar to PO, PPO activity was increased by *P. fluorescens* FP 7 against the challenged pathogens. Expression of four PPO isoforms, PPO1, PPO2, PPO3 and PPO4 was very clear in bacterized tomato seedlings challenge inoculated with *P. aphanidermatum*. Similarly, in bacterized tomato seedling expression of PPO1 isoform was at higher level. The induced PPO1 isoform and a higher level expression of PPO2 isoform might have also been implicated in induced defense responses against the pathogen invasion. In tomato, expression of PPO1 isoform was more prominent in

bacterized plant challenged with *C. capsici* compared to plants inoculated with *C. capsici* alone or plants treated with *P. fluorescens* alone. Meena *et al*., (2000) reported that *P. fluorescens* induced the activities of PPO in response to infection by *C. personata* in groundnut. Chen *et al*., (2000) reported that various rhizobacteria and *P. aphanidermatum* induced the PPO activity in cucumber root tissues.

The phenolic compounds may contribute to enhance the mechanical strength of host cell wall and may also inhibit the fungal growth, as phenolics are fungitoxic in nature. Seed treatment with *P. fluorescens* 63-28 induced the accumulation of phenolics in tomato root tissues (M'Piga *et al*., 1997). The hyphae of the pathogen surrounded by phenolic substances exhibited considerable morphological changes including cytoplasmic disorganization and loss of protoplasmic content. Accumulation of phenolics by prior application of *P. fluorescens* in pea has been reported against *P. ultimum* and *F. oxysporum f. sp. pisi* (Benhamou *et al*., 1996). The present study also indicates that the higher level accumulation of phenolics was observed in *P. fluorescens*, FP 7 treated tomato challenged with pathogen. Similar findings were reported in sugarcane against *C. falcatum* (Viswanathan and Samiyappan, 1999) and in groundnut against *C .personata* (Meena *et al*., 2000). Benhamou *et al*., (2000) reported that an endophytic bacterium, *Serratia plymuthica* induced the accumulation of phenolics in cucumber roots following infection by *P. ultimum*.

PR-proteins are host-coded proteins induced by different types of pathogens and abiotic stresses (Van Loon *et al*., 1998). Synthesis and accumulation of PR-proteins have been reported to play an important role in plant defense (Maurhofer *et al*., 1998; Van Loon *et al*., 1998). Colonization of bean roots by rhizobacteria was correlated with induction of PR proteins resulting in induced systemic resistance against B. cinerea (Zdor and Anderson, 1992). Similarly in tobacco, induction of two PR proteins namely β-1,3-glucanase and chitinase was noticed due to application of *P. fluorescens* isolate CHAO in response to infection by tobacco necrosis virus (TNV). Induction of these hydrolytic enzymes was also reported in pea against *P. ultimum* and *F. oxysporum f. sp. pisi* (Benhamou *et al*., 1996) and in tomato against *F. oxysporum f. sp. radicis-lycopersici* (M'Piga *et al*., 1997). Moreover, the activity of β-1,3-glucanase was higher in the bacterized plants challenged with the pathogen.

Thus induction of defense enzymes involved in phenylpropanoid pathway by *P. fluorescens* FP 7 in tomato against pathogen infection leads to induced protection by synthesizing various defense compounds. Earlier and enhanced levels of PAL, PO and PPO suppressed the further colonization of soil-borne pathogens in crop plants.

REFERENCES

- Abel, F. B., Morgan, P. W., & Saltveit M. F. (1992). Ethylene in plant biology. 2nd Ed. Academic Press Inc., San Diego, USA.
- Ahl Goy, P., Felix, G., Metraux, J. P., & Meins Jr, F. (1992). Resistance to disease in the hybrid Nicotiana glutinosa x Nicotiana debneyi is associated with high constitutive levels of β-1, 3-glucanase, chitinase, peroxidase and polyphenoloxidase. *Physiological and molecular plant pathology*, *41*(1), 11-21.
- Alleweldt, G. (1987). The contribution of grapevine breeding to integrated pest control. In: Cavalloro R. (ed.). Integrated pest control in viticulture, 369-377. A.A. Balkema, Rotterdam, The Netherlands.
- ALSTRÖM, S. (1991). Induction of disease resistance in common bean susceptible to halo blight bacterial pathogen after seed bacterization with rhizosphere pseudomonads. *The Journal of General and Applied Microbiology*, *37*(6), 495-501.
- Benhamou, N., Belanger, R. R., & Paulitz, T. C. (1996). Induction of differential host responses by Pseudomonas fluorescens in Ri T-DNA-transformed pea roots after challenge with Fusarium oxysporum f. sp. pisi and Pythium ultimum. *Phytopathology*, *86*.
- Benhamou, N., Gagné, S., Le Quéré, D., & Dehbi, L. (2000). Bacterial-mediated induced resistance in cucumber: beneficial effect of the endophytic bacterium Serratia plymuthica on the protection against infection by Pythium ultimum. *Phytopathology*, *90*(1), 45-56.
- Boller, T., & Mauch, F. (1988). Colorimetric assay for chitinase. In *Methods in enzymology* (Vol. 161, pp. 430-435). Academic Press.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1-2), 248-254.
- Bruce, R. J., & West, C. A. (1989). Elicitation of lignin biosynthesis and isoperoxidase activity by pectic fragments in suspension cultures of castor bean. *Plant physiology*, *91*(3), 889-897.
- Chen, C., Belanger, R. R., Benhamou, N., & Paulitz, T. C. (2000). Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and Pythium aphanidermatum. *Physiological and molecular plant pathology*, *56*(1), 13-23.
- Commare, R. R., Nandakumar, R., Kandan, A., Suresh, S., Bharathi, M., Raguchander, T., & Samiyappan, R. (2002). Pseudomonas fluorescens based bio-formulation for the management of sheath blight disease and leaffolder insect in rice. *Crop Protection*, *21*(8), 671-677.
- Daayf, F., Bel-Rhlid, R., & Bélanger, R. R. (1997). Methyl ester of p-coumaric acid: a phytoalexin-like compound from long English cucumber leaves. *Journal of Chemical Ecology*, *23*, 1517-1526.

- Dalisay, R. F., & Kuć, J. A. (1995). Persistence of reduced penetration by Colletotrichum lagenarium into cucumber leaves with induced systemic resistance and its relation to enhanced peroxidase and chitinase activities. *Physiological and Molecular Plant Pathology*, *47*(5), 329-338.
- De Meyer, G., Capieau, K., Audenaert, K., Buchala, A., Métraux, J. P., & Höfte, M. (1999). Nanogram amounts of salicylic acid produced by the rhizobacterium Pseudomonas aeruginosa 7NSK2 activate the systemic acquired resistance pathway in bean. *Molecular Plant-Microbe Interactions*, *12*(5), 450-458.
- Elad, Y., & Chet, I. (1987). Possible role of competition for nutrients in biocontrol of Pythium damping-off by bacteria. *Phytopathology*, *77*(2), 190-195.
- Elad, Y., & VOLPIN, H. (1991). Heat treatment for the control of rose and carnation grey mould (Botrytis cinerea). *Plant Pathology*, *40*(2), 278-286.
- Fridlender, M., Inbar, J., & Chet, I. (1993). Biological control of soilborne plant pathogens by a β-1, 3 glucanase-producing Pseudomonas cepacia. *Soil Biology and Biochemistry*, *25*(9), 1211-1221.
- Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research*. John wiley & sons.
- Hammer P. (1992). Mechanisms of resistance to infection by *Botrytis cinerea* in rose flowers. Ph.D. Thesis. Pennsylvania State University, University Park, USA.
- Hammerschmidt, R., & Kuć, J. (1982). Lignification as a mechanism for induced systemic resistance in cucumber. *Physiological Plant Pathology*, *20*(1), 61-71.
- Hammerschmidt, R., Nuckles, E. M., & Kuć, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to Colletotrichum lagenarium. *Physiological Plant Pathology*, *20*(1), 73-82.
- Howie, W. J., & Suslow, T. V. (1991). Role of antibiotic biosynthesis in the inhibition of Pythium ultimum in the cotton spermosphere and rhizosphere by Pseudomonas fluorescens. *Mol. Plant-Microbe Interact*, *4*, 393-399.
- Jayaraman, K. S., Ramanuja, M. N., Vijayaraghavan, P. K., & Vaidyanathan, C. S. (1987). Oxidative enzyme in pearl millet. *Food Chem*, *24*(3), 203-206.
- Kandan, A., Commare, R. R., Nandakumar, R., Ramiah, M., Raguchander, T., & Samiyappan, R. (2002). Induction of phenylpropanoid metabolism by Pseudomonas fluorescens against tomato spotted wilt virus in tomato. *Folia microbiologica*, *47*, 121-129.
- King, E. O., Ward, M. K., & Raney, D. E. (1954). Two simple media for the demonstration of pyocyanin and fluorescin. *The Journal of laboratory and clinical medicine*, *44*(2), 301-307.
- Kloepper, J. W., & Schroth, M. N. (1981). Development of a powder formulation of rhizobacteria for inoculation of potato seed pieces. *Phytopathology*, *71*(6), 590-592.
- Kloepper, J. W., Tuzun, S., Liu, L., & Wei, G. (1993). Plant growth-promoting rhizobacteria as inducers of systemic disease resistance. *Pest management: biologically based technologies. American Chemical Society Books, Washington, DC*, 156-165.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *nature*, *227*(5259), 680-685.
- Loper, J. E. (1988). Role of fluorescent siderophore production in biological control of Pythium ultimum by a Pseudomonas fluorescens strain. *Phytopathology*, *78*(2), 166-172.
- Maurhofer, M., Reimmann, C., Schmidli-Sacherer, P., Heeb, S., Haas, D., & Défago, G. (1998). Salicylic acid biosynthetic genes expressed in Pseudomonas fluorescens strain P3 improve the induction of systemic resistance in tobacco against tobacco necrosis virus. *Phytopathology*, *88*(7), 678-684.
- Mayer, A. M., Harel, E., & Shaul, R. B. (1965). Assay of catechol oxidase, a critical comparison of methods. *Phytochemistry, 5*, 783-789.
- Meena, B., Radhajeyalakshmi, R., Marimuthu, T., Vidhyasekaran, P., Doraisamy, S., & Velazhahan, R. (2000). Induction of pathogenesis-related proteins, phenolics and phenylalanine ammonia lyase in groundnut by *Pseudomonas fluorescens*. *J. Plant Dis. Protect*, *107*, 514-527.
- Mohan, R., Vijayan, P., & Kolattukudy, P. E. (1993). Developmental and tissue-specific expression of a tomato anionic peroxidase (tap1) gene by a minimal promoter, with wound and pathogen induction by an additional 5′-flanking region. *Plant molecular biology*, *22*, 475-490.
- Morimoto, M., & Komai, K. (2000). Plant secondary metabolites as plant defense systems, *Recent Research Developments in Phytochemistry*, *4*, 9-14.
- M'piga, P., Belanger, R. R., Paulitz, T. C., & Benhamou, N. (1997). Increased resistance toFusarium oxysporumf. sp. radicis-lycopersiciin tomato plants treated with the endophytic bacteriumPseudomonas fluorescensstrain 63-28. *Physiological and molecular plant pathology*, *50*(5), 301-320.
- Nandakumar, R., Babu, S., Viswanathan, R., Raguchander, T., & Samiyappan, R. (2001). Induction of systemic resistance in rice against sheath blight disease by Pseudomonas fluorescens. *Soil Biology and Biochemistry*, *33*(4-5), 603-612.
- Osburn, R. M., Schroth, M. N., Hancock, J. G., & Hendson, M. (1989). Dynamics of sugar beet seed colonization by Pythium ultimum and Pseudomonas species: effects on seed rot and damping-off. *Phytopathology*, *79*(6), 709-716.
- Pan, S. Q., Ye, X. S., & Kuć, J. (1991). Association of β-1, 3-glucanase activity and isoform pattern with systemic resistance to blue mould in tobacco

induced by stem injection with Peronospora tabacina or leaf inoculation with tobacco mosaic virus. *Physiological and Molecular Plant Pathology*, *39*(1), 25-39.

- Pieterse, C. M., Van Pelt, J. A., Van Wees, S. C., Ton, J., Léon-Kloosterziel, K. M., Keurentjes, J. J., ... & Van Loon, L. C. (2001). Rhizobacteria-mediated induced systemic resistance: triggering, signalling and expression. *European Journal of Plant Pathology*, *107*, 51-61.
- Ramamoorthy, V., Raguchander, T., & Samiyappan, R. (2002). Enhancing resistance of tomato and hot pepper to Pythium diseases by seed treatment with fluorescent pseudomonads. *European journal of plant pathology*, *108*, 429-441.
- Ramanathan, A., Vidhasekaran, P., & Samiyappan, R. (2000). Induction of defense mechanisms in greengram leaves and suspension-cultured cells by Macrophomina phaseolina and its elicitors/Induktion von Resistenzmechanismen in Blättern von Mungbohnen und in Suspensionszellkulturen durch Macrophomina phaseolina und seinen Elicitoren. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection*, 245-257.
- Reimers, P. J., Guo, A., & Leach, J. E. (1992). Increased activity of a cationic peroxidase associated with an incompatible interaction between Xanthomonas oryzae pv oryzae and rice (Oryza sativa). *Plant physiology*, *99*(3), 1044-1050.
- Sidhu, J. S., Ravi, J. S., & Minocha, J. L. (1984). Peroxidase isozyme patterns in primary trisomics of pearl millet. *Theoretical and Applied Genetics*, *68*, 179-182.
- Singh, U. P., Ram, D., & Tewari, V. P. (1990). Induction of resistance in chickpea (Cicer arietinum) by Aegle marmelos leaves against Sclerotinia sclerotiorum/Durch Blätter von Aegle marmelos induzierte Resistenz gegen Sclerotinia sclerotiorum in Kichererbsen (Cicer arietinum). *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection*, 439-443.
- Stout, M. J., Fidantsef, A. L., Duffey, S. S., & Bostock, R. M. (1999). Signal interactions in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato, Lycopersicon esculentum. *Physiological and Molecular Plant Pathology*, *54*(3-4), 115-130.
- Suslow, T. V. (1980). Growth and yield enhancement of sugarbeets by pelleting seed with specific Pseudomonas spp. *Phytopathological News*, *12*, 40.
- Tscharntke, T., Thiessen, S., Dolch, R., & Boland, W. (2001). Herbivory, induced resistance, and interplant signal transfer in Alnus glutinosa. *Biochemical Systematics and Ecology*, *29*(10), 1025-1047.
- Van Loon, L. C., & Callow, J. A. (1983). Transcription and translation in the diseased plant. In: *Biochemical Plant Pathology*. (Ed. J. A. Callow) John Wiley and Sons, Chichester, UK.
- Van Loon, L. C., Bakker, P. A. H. M., & Pieterse, C. M. J. (1998). Systemic resistance induced by rhizosphere bacteria. *Annual review of phytopathology*, *36*(1), 453-483.
- Van Peer, R., & Schippers, B. (1992). Lipopolysaccharides of plant-growth promoting Pseudomonas sp. strain WCS417r induce resistance in carnation to Fusarium wilt. *Netherlands Journal of Plant Pathology*, *98*, 129-139.
- Van Peer, R., Niemann, G. J., & Schippers, B. (1991). Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by Pseudomonas sp. strain WCS 417 r. *Phytopathology*, *81*(7), 728-734.
- Vidhyasekaran, P., & Muthamilan, M. (1995). Development of formulation of *Pseudomonas fluorescens* for control of chick pea wilt. *Plant Dis*, *79*, 782- 790.
- *Viswanathan, R., & Samiyappan, R. (1999). Induction of systemic resistance by plant growth promoting rhizobacteria against red rot disease caused by Colletotrichum falcatum Went in sugarcane. Sugar Tech, 1, 67-76.*
- Voisard, C., Keel, C., Haas, D., & Dèfago, G. (1989). Cyanide production by Pseudomonas fluorescens helps suppress black root rot of tobacco under gnotobiotic conditions. *The EMBO journal*, *8*(2), 351-358.
- Wei, G., Kloepper, J. W., & Tuzun, S. (1991). Induction of systemic resistance of cucumber to Colletotrichum orbiculare by select strains of plant growth-promoting rhizobacteria. *Phytopathology*, *81*(11), 1508-1512.
- Whetten, R. W., & Sederoff, R. R. (1992). Phenylalanine ammonia-lyase from loblolly pine: purification of the enzyme and isolation of complementary DNA clones. *Plant physiology*, *98*(1), 380-386.
- Whipps, J. M., & Lumsden, R. D. (1991). Biological control of Pythium species. *Biocontrol Science and Technology*, *1*(2), 75-90.
- Zdor, R. E., & Anderson, A. J. (1992). Influence of root colonizing bacteria on the defense responses of bean. *Plant and Soil*, *140*, 99-107.
- Zehnder, G. W., Murphy, J. F., Sikora, E. J., & Kloepper, J. W. (2001). Application of rhizobacteria for induced resistance. *European journal of plant pathology*, *107*, 39-50.
- Zieslin, N., & Ben-Zaken, R. (1993). Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. Plant Physiol. *Biochem*, *31*, 333-339.

Cite This Article: Senthilmurugan Palanisamy (2024). PRP5 Induction by Infection of *Pythium Sp.* In Tomato. *East African Scholars J Agri Life Sci*, *7*(10), 141-148.