IJIE Int. J. Indust. Entomol. 35(2) 63-70 (2017)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2017.35.2.63

Fluorescent Pseudomonas Induced Systemic Resistance to Powdery Mildew in Mulberry (*Morus* spp.)

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Abstract

Native fluorescent pseudomonas bacteria were isolated from rhizosphere soil of mulberry and were evaluated against powdery mildew. *In vitro* conidial germination study showed significant (P<0.05) variation in conidial germination by bacterial strains *Pf1* and *Pf3*. Mildew incidence was significantly varied due to treatment with various pseudomonas strains *in vivo*. Significantly (P<0.05) less mildew incidence was in plants treated with the bacterial strain *Pf1* (9.11%) followed by *Pf3* (13.48%) controlling 69.40% and 54.75% respectively compared with untreated control. Similarly, mildew severity was least (8.51%) in plants treated with strain *Pf1* followed by *Pf5* (9.23%) and *Pf3* (9.72%) controlling the severity by 84.51%, 77.01% and 71.96% respectively compared with control. The bacterial strains significantly influenced biochemical constituents such as chlorophyll, protein and soluble sugar content of the mulberry leaf. Similarly, bacterial strains significantly increased the activity of the peroxidase (PO) and Polyphenol oxydase (PPO) activity from 7th day up to the 28th day after treatment. The strain *Pf1*, *Pf3* and *Pf5* exhibited a marked enhancement in the peroxidase at different periods of infection. Significant (P<0.01) negative correlation was found between powdery mildew severity with phenol content (R^2= 0.67) as well as peroxidase (R^2= 0.92) and polyphenol oxidase (R^2=0.72) activity thus confirms induction of systemic resistance in mulberry by pseudomonas bacteria. The study shows scope for exploration of rhizosphere fluorescent pseudomonas bacteria for induction of systemic resistance in mulberry to contain powdery mildew disease effectively.

Int. J. Indust. Entomol. 35(2), 63-70 (2017)

Received : 11 Aug 2017
Revised : 1 Sep 2017
Accepted : 4 Sep 2017

Keywords:
Induced systemic resistance,
mulberry,
powdery mildew,
rhizosphere pseudomonas bacteria

Introduction

Powdery mildew deteriorates nutritive value of mulberry leaf due to disruption of biochemical activities. Feeding these inferior quality leaves to silkworm leads to production of poor silk in terms of quality and quantity (Qadri *et al*., 1999). Therefore control of mildew is a serious concern among sericulture farmers. Studies were conducted in the past (Philip *et al*., 1994; Sharma *et al.,* 2009) and recommended various chemicals for mildew management. However farmers restrict use of plant protection chemicals due to harmful impact of plant protection measures on environment and hence control of powdery mildew warrant alternative methods.

Induced systemic resistance (ISR) by plant growth promoting

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rhizobacteria (PGPR) has been achieved in large number of crops against broad spectrum pathogens including fungi (Leeman *et al*., 1995) bacteria (Liu *et al*, 1995) nematodes (Paul and Kumar, 2003) and viruses (Maurhofer *et al*., 1994). These natural bio-resources provide essential nutrients to plants and improve growth, competitiveness and responses to external stress factors by an array of mechanisms under different agro-ecosystems. ISR is defined as enhancement of the plant's defensive capacity against a broad spectrum of pathogens and pests that is acquired after appropriate stimulation. Van loon *et al*. (1998) referred induction of systemic resistance by rhizobacteria as induced systemic resistance also referred as systemic acquired resistance or immunization because of the resemblance in mechanism of these phenomena (Tuzun and Kloepper, 1995; Purkayastha, 1998). Production of a large number of defense enzymes such as phenylalanine ammonia lyase (PAL), chitinase, b-1, 3-glucanase, peroxidase, polyphenol oxidase and proteinase inhibitors are associated with ISR (Koch *et al.*, 1999).

Though fluorescent pseudomonads are used for management of diseases in many crops by inducing systemic resistance, beneficial effects of these microbes are yet to be explored in mulberry disease management.

Materials and methods

Isolation of native *Pseudomonas fluorescence* bacteria

Soil samples were collected from experimental garden of Central Sericultural Research & Training Institute, Mysore, where popular mulberry variety V1 is grown under recommended package of practices. The soils were collected from a depth of one foot from mulberry rhizosphere. The soil adhered to a root was separated and transferred into sterile polythene bags and carried to laboratory. The soil samples were then spread and allowed to air dry. The dried soil samples were powdered and sieved to get the fine powder and stored. These soil samples were processed for isolation of native *Pseudomonas fluorescens* bacteria. The isolation was done by following war-cup method (Aneja, 2003). The bacterial colonies were observed in UV Chamber (365 nm). The bacterial colonies emitting green fluorescent colour were identified as *P. fluorescence* and were further purified by placing these green fluorescent colonies on King's B medium (Proteose peptone 20 g, $K_2HPO_4 \cdot 3H_2O$ 1.908 g, $MgSO_4 \cdot 7H_2O$ 1.5 g, glycerol 15 ml, Bacto agar 15 g, distilled water 985 ml), and incubated at 28 ±2°C for 48 hours. The colonies were selected and further purified and sub cultured in King's B media for further experiment. Ten strains of fluorescent pseudomonads were finally selected to study their efficacy to induce systemic resistance in mulberry.

In vitro conidial germination studies

The *in vitro* conidial germination studies were conducted in the laboratory. Individual suspensions of all the bacterial strains were prepared approximately 10^8 cells/ ml ($OD_{620\ nm}$ 0.8~0.9) in 0.1% carboxy methyl cellulose (CMC) separately and treated by this on mulberry leaves 45 days after pruning using a hand held sprayer. The plants treated with only distilled water served as control. Three leaves of mulberry from 7th, 8th and 9th position from top of branch were detached from each treated and control plants 24 hours after inoculation. These leaves were brought in the laboratory in polythene bags separately. The leaves were transferred separately, keeping abaxial surface up in Petri plates (200mm dia.) lined with moistened filter paper. The leaves were then inoculated with conidia of *Phyllactinia corylea* by gently taping fully infected mulberry leaves to dislodged infective conidia on the treated leaves in such a way that each leaf receives 200~300 conidia/ cm^2. The Petri plates were then kept overnight (8 hr) under fluorescent light. The conidia were then harvested using transparent cello tape by gently adhering the cello tape on the treated leaves. This cello tape was then placed on the microscopic slides and viewed under the microscope for germination. Six observations were made from each replication. The total number of conidia and number of conidia germinated were enumerated and calculated the germination percentage from each treatment and control.

Assessment of disease incidence and severity

To evaluate the bacterial strains for their efficacy to induce systemic resistance in mulberry against powdery mildew, the experiment was conducted using mulberry plants (variety V-1) grown in earthen pots following recommended package of practices. The plants were pruned and after 45 days treated with

bacterial suspension. The bacterial suspensions were prepared by growing *P. fluorescens* on King's B agar medium for 48 h at 25 ± 2°C. Individual suspensions of all the strains of approximately 10^8 cells/ ml ($OD_{620\ nm}$ 0.8~0.9) in 0.1% carboxy methyl cellulose (CMC) were prepared separately. These bacterial suspensions were sprayed using a hand atomizer on the mulberry plants separately keeping three replications against each treatment and control in glass house in randomized block design. The plants were inoculated with *Pyllactinia corylea* 24 hours after treatment with *P. fluorescens* suspension by gently tapping severely mildewed mulberry leaves on the treated plants in such a way that each leaf receives 200-300 conidia/ cm^2.

The incidence and severity of mildew was recorded 30 days after inoculation visually on a 0-7 scale based on area covered by powdery mildew (Krishnaprasad and Siddaramaiah, 1979) as follows.

Disease incidence (%) =

$$\frac{\text{Number of leaves infected}}{\text{Total number of leaves}} \times 100$$

Disease severity (%) =

$$\frac{\sum \text{all numerical values}}{\text{Total number of leaves observed x Maximum grade}} \times 100$$

Where 0 = No mildew; 2=1-10%; 3=11-20%; 4=21-30%; 5=.31-50%; 6=.51-75% and 7=76-100% leaf area covered with mildew. ∑ of all numerical values were obtained after multiplying the number leaves infected under a particular grade with that grade. The maximum grade is 7 in the disease severity scale.

Estimation of biochemical constituents

The chlorophyll content in leaves was estimated following the method of Arnon (1949). Finely cut leaves (1 g) were taken and ground with 20-40 ml 80% acetone. It was then centrifuged at 5000-10000 rpm for 5minutes. The supernatant was transferred and the procedure was repeated till the residue become colorless. The absorbance of the solution was red at 645 nm and 663 nm against the solvent acetone. The amount of chlorophyll is calculated using the absorption coefficients.

Total soluble sugars were determined as described by Yemm and Willis (1954). About 100 mg of leaf powder was ground in 20 ml of 80% ethanol and incubated at 95°C for 10 minutes. To 1 ml of the supernatant sample, 4 ml of anthrone reagent was added. The reaction mixture shook gently and kept over a boiling water bath for 10 minutes and allowed to cool. The OD of blue green solution was measured at 625 nm.

Total protein content was estimated using the protocol of Lowry *et al*. (1951). A stock solution (1 mg/ml) of bovine serum albumin was prepared in 1N NaOH; five concentrations (0.2, 0.4, 0.6, 0.8 and 1 ml) from the working standard solution were taken in series of test tubes. In another set of test tubes 0.1 ml and 0.2 ml of the sample extracts were taken and the volume was raised up to 1 ml in all the test tubes. To each test sample, 5 ml of freshly prepared alkaline solution (prepared by mixing 50 ml of 2% Na_2CO_3 in 0.1 N NaOH and 1 ml of 0.5% $CuSO_4\ 5H_2O$ in 1% sodium potassium Tartrate) was added at room temperature and left undisturbed for a period of 10 minutes. Subsequently, to each of these mixture tubes 0.5 ml of Folin- Ciocaltcau reagent (diluted with equal volume of distilled water just before use) was rapidly added and incubated at room temperature (about 25ºC) for 30 minutes until the blue colour developed. The spectronic colorimeter was adjusted at wavelength of 750 nm and set at 100% transmittance using blank before taking the readings of the standard and the test samples respectively. Five replicates were examined in each case and their mean values were recorded.

Phenol content of mulberry leaves was determined using Folin-Ciocalteu reagent (Singleton and Rossi 1965). Freshly collected leaves (2 g) were homogenized in 80% aqueous ethanol with a pinch of white sand to facilitate crushing and the mixture was filtered using cheese cloth. The filtered extract was centrifuged at 10,000 rpm for 15 minutes and the supernatant was preserved. The residue was re-extracted twice with 80% ethanol and supernatant was collected, the residue was evaporated in an evaporation pan to dryness at room temperature. The residue was then dissolved in 5 ml distilled water. The extract was diluted to 3 ml with water and 0.5 ml of Folin-Ciocalteau reagent was added. After 3 min, 2 ml of 20% sodium carbonate was added and the contents were mixed thoroughly. The colour was developed and absorbance was measured at 650 nm in a spectrophotometer after 60 minutes using Catechol as a standard. The phenolic content was expressed in mg catechol/100 g of fresh weight of mulberry leaves.

Peroxidase and poly phenol activity were assessed from 0, 7, 14, 21 and 28 days after inoculation. For determination of peroxidase, 4 g mulberry leaves were homogenized in 20 ml of chilled distilled water at 0°C. A pinch of white sand was added

to facilitate crushing. The extracts were obtained by filtering off the debris with a cheese cloth and centrifuged at 3000 rpm for 15 min. The supernatants were collected and used as enzyme source and kept in an ice-bath until assayed following the method of Mahadevan and Sridhar (1982). Freshly prepared 5 ml of pyrogallol reagent (10 ml 0.5 M pyrogallol and 12.5 ml 0.66 M phosphate buffer at pH 6.0) and 1.5 ml of enzyme extract were mixed in the tube of spectrophotometer and the mixture was immediately adjusted to zero absorbance, and H_2O_2 solution (0.5 ml of 1%) was added to it. The content was mixed by inverting the tube, and adding of H_2O_2 initiated the reaction. Enzyme activity was recorded as the change in absorbance at 430 nm after the addition of the substrate.

For polyphenol oxidase quantification, mulberry leaves (2 g) were collected and washed thoroughly with running tap water followed by sterile distilled water and the surface was wiped off with filter paper. The leaves were ground separately with a pinch of neutral sand in 6.0 ml of sodium phosphate buffer (0.1 M at pH 7.0) at 0°C. The extracts were obtained by filtering off the debris with a cheese cloth and centrifuged at 3,000 rpm for 15 minutes. The supernatants were recovered and kept in a tube, in an ice bath until assayed. Enzyme assay was done following the method of Sadasivam and Manickam (1996). Sodium phosphate buffer (0.1 M) at pH 7.0 (3.0 ml) and 2.0 ml of the enzyme extract were mixed in a cuvette in a spectrophotometer. The mixture was immediately adjusted to zero absorbance. Catechol of 0.01 M (1.0 ml) in 0.1 M phosphate buffer (0.4 mg/ml) was added to the above mixture and the reactants were quickly mixed. Enzyme activity was recorded as the change in absorbance at 495 nm up to 30 minutes after the addition of catechol.

Statistical analysis

Data on conidial germination, disease severity, disease incidence and biochemical changes were subjected to Analysis of Variance (ANOVA) and the means were compared for significant difference at P<0.05. In case of enzyme activity, the mean data were analyzed by two way ANOVA and the difference among the means of treatments separated by Duncan's Multiple Range Test (DMRT) at P<0.05 and indicated in the table (a, b, c, d, e, f, g), the data followed by same letter in each column do not significantly differ.

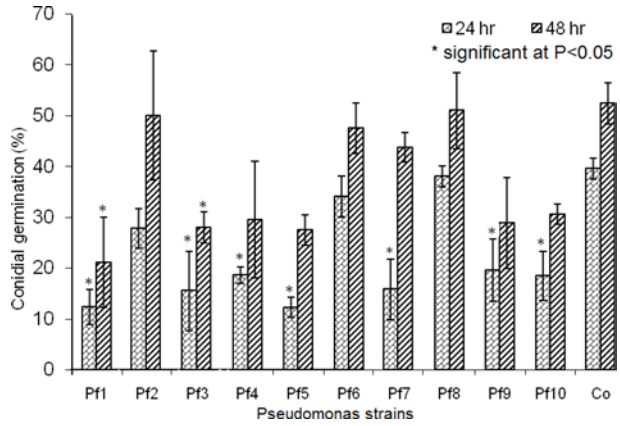

Fig. 1. Influence of fluorescent pseudomonas on conidial germination of *P.corylea*

Results and Discussion

Conidial germination

The conidial germination was least on mulberry leaves treated with bacterial strain *Pf1* (11.8%) 28 hr after and 48 hr after (20.10%) treatment followed by on the leaves treated with bacterial strain *Pf5* with 12.6% and 26.8% germination respectively 24 and 48 hr after treatment. The germination was observed higher on untreated control leaves with 42.20% and 56.70% after 24 hr and 48 hr respectively. Conidial germination on leaves treated with bacterial strains *Pf2, Pf6* and *Pf8* did not vary significantly either at 24 hr or 48 hr after treatment (Fig. 1).

Mildew incidence and severity

Mildew incidence was significantly least (9.11%) in plants treated with *Pf1* with 69.40% control compared with mildew incidence on untreated control plants. Mildew incidence in mulberry treated with bacterial strains *Pf3* (13.40%), *Pf4* (16.52%) and *Pf5* (15.01%) were also significantly low and at par controlling 54.75%, 44.54% and 49.61% respectively. However, mildew incidence did not significantly vary among plants treated with bacterial strain *Pf7*, *Pf9* and *Pf10* compared with mildew (29.70%) incidence in untreated control (Table 1).

Significantly lower mildew severity was found in plants treated with bacterial strains (*Pf1* (8.50%), *Pf3* (9.72%), *Pf5* (9.23%) and *Pf8* (9.80%) with >70% control of mildew severity with highest by *Pf1* (84.51%). However severity did not vary significantly among plants treated with strains *Pf2*, *Pf7* and *Pf10* with control.

Table 1. Effect of fluorescent pseudomonas on incidence and severity of mildew

Bacterial isolates	Mildew Incidence (%)	Control (%)	Mildew severity (%)	Control (%)
Pf1	9.11 ±0.97**	69.409	8.50±0.40**	84.51
Pf2	25.92 ±2.09	12.991	17.71±0.5	00.00
Pf3	13.48±0.65**	54.750	9.72±0.54**	71.96
Pf4	16.52±0.59**	44.545	10.15±0.31**	67.62
Pf5	15.01±1.95**	49.614	9.23±0.97**	77.04
Pf6	20.41±1.14*	31.487	14.43±0.68*	23.58
Pf7	29.09 ±3.09	2.350	16.45±2.11	2.82
Pf8	18.31±0.94**	38.536	9.80±0.59**	71.21
Pf9	29.31 ±4.67	1.611	14.35±1.10*	24.41
Pf10	28.65 ±2.47	3.827	16.80±0.59	00.00
Control	29.79 ±2.20	0.000	16.73±0.77	00.00

*Significant at P <0.05; ** Significant at P<0.01

Table 2. Influence of fluorescent pseudomonas on biochemical parameters of mulberry

Bacterial isolates	Chlorophyll (mg/g fr. wt)	Sugars (mg/g fr. wt)	Protein (mg/g fr. wt)	Phenol (mg/g fr. Wt)
Pf1	2.12**	43.76**	37.63**	0.36**
Pf2	1.70	40.20*	28.20*	0.23**
Pf3	1.72	41.51*	32.40**	0.35**
Pf4	1.77	38.03	30.02*	0.23**
Pf5	2.02**	45.43**	32.80**	0.35**
Pf6	1.57	37.00	30.90*	0.18
Pf7	1.60	40.29*	30.15*	0.21*
Pf8	1.90*	42.62**	33.58**	0.33**
Pf9	1.64	41.27*	30.00*	0.18
Pf10	1.65	39.16*	31.31**	0.22**
Control	1.69	38.06	28.04	0.17

*Significant at P <0.05; ** Significant at P<0.01

Biochemical changes

The amount of chlorophyll was found significantly higher in the leaves treated with bacterial strains *Pf1* (2.12 mg/g/fr.wt.) and *Pf5* (2.02 mg/gfr.wt.). Chlorophyll contents in leaves treated with other strains showed statistically on par with that of untreated control (1.69 mg/g fr. wt.). Total soluble sugars was significantly higher in all treatments except in the leaves of plants treated with bacterial strain *Pf4* and *Pf6*. The higher amount of soluble sugar was obtained from the leaves of plants treated with bacterial starin *Pf5* (45.43 mg/g fr.wt.) followed by in leaves treated with *Pf1* (43.76 mh/g fr. wt.) and *Pf3* (41.51 mg/g fr.wt.). Similarly, protein content in all the treatment was significantly higher compared with the same in untreated leaves. The highest amount of protein (37 mg/g fr.wt.) was in leaves treated with bacterial strain *Pf1* followed by in *Pf5* (32,80 mg/g fr. wt.) and *Pf3* (32.40 mg/g fr.wt.). Phenol content was significantly higher in leaves treated with bacterial strain *Pf1* (0.36 mg/g fr. wt.) and on par with the phenol content due to treatment with bacterial strains *Pf3* (0.35 mg/g fr. wt.), *Pf5* (0.35 mg/g fr. wt.) and *Pf8* (0.33 mg/g fr. wt.). However compared with control (0.17 mg/g fr.wt.), the phenol content did not vary significantly in case of *Pf6* and *Pf8* treated leaves (Table 2).

Bacterial strains significantly increased the activity of the peroxidase from 7th day up to the 28th day after *treatment*. The strain *Pf1*, *Pf3* and *Pf5* exhibited a marked enhancement in the peroxidase at different periods of infection. A visible increase in peroxidase activity was observed on the 14th day in the infected mulberry plants (Table 3).

Polyphenol oxidase activity (Table 4) increased considerably with the increase in period of pathogen inoculation and bacterial treatment. Compared with control, except in *Pf2*, increase in enzyme activity was more in bacteria treated plants with peak in *Pf1* followed by in *Pf3* and *Pf5* treated plants. In general, activity of both the enzyme was higher during 7-14 day after treatment. The correlation analysis showed significant ($P<0.01$) negative correlation between powdery mildew severity with phenol content ($R^2= 0.67$) as well as peroxidase ($R^2= 0.92$) and polyphenol oxidase ($R^2=0.72$) activity.

Fungicides carbendazim and sulphur are recommended for control of powdery mildew in mulberry. Shafat (2010) reported high inhibition of conidial germination and 68% control of mildew severity with the use of carbendazim @ 0.05%. Similar inhibition of conidial germination and 55.93% control of mildew (Gangwar *et al*., 2000) were obtained with the use of wettable sulphur @0.01%. The result of the present study shows control of mildew severity by bacterial strains *Pf1*, *Pf3* and *Pf5* higher than that of these fungicides.

In the past three decades numerous strains of florescent pseudomonas have been isolated from the soil and plant roots by several workers and their biocontrol activity against soil borne and foliar pathogens have been reported (Austin *et al*., 1977; Mew and Rosales 1986; Rabindran and Vidhyasekharan, 1996; Ramamoorthy *et al*., 2002). Two peroxidase isoforms were induced in the PGPR treated rice plants inoculated with the sheath blight pathogen *R. solani* (Nandakumar *et al*., 2001). High level expression of peroxidase was reported in *P.fluorescens* (*Pf1*) treated chilli plants challenged with *C.capsici* (Bharathi *et al*., 2004). Likewise, maize plants raised from *P. fluorescens* treated seeds showed higher activity of peroxidase and polyphenoloxidase when leaf was inoculated with the pathogen *R. solani*. The bacterized seeds with fluroscent bacteria lead to accumulation of higher phenolic compounds and higher activity of PO and PPO that may play defense mechanism in plants against pathogen (Shivakumar and Sharma *et al.,* 2003). Klopper *et al.* (1992) reported that among the PGPR, fluorescent pseudomonas are the most exploited bacteria for biological control of soil borne and foliar plant pathogens.

Enhanced activities of defense enzymes have been suggested to have a direct or indirect role in the induction of systemic

Table 3. Peroxidase activity in fluorescent pseudomonas treated mulberry leaves

Pseudomonas isolates	Peroxidase activity min/g fresh weight of mulberry leaves				
	Days after inoculation				
	0	7	14	21	28
Pf1	0.66^{a}	3.05^{a}	4.00^{a}	4.58^{a}	4.86^{a}
Pf2	0.56^{d}	1.50^{f}	1.73^{e}	2.07^{f}	2.22^{fg}
Pf3	0.62^{b}	2.97^{a}	3.48^{b}	3.88^{c}	4.35^{b}
Pf4	0.56^{d}	2.29^{c}	3.38^{b}	3.28^{d}	3.97^{c}
Pf5	0.63^{b}	2.67^{b}	3.48^{b}	3.66^{d}	4.35^{b}
Pf6	0.67^{a}	1.63^{e}	2.96^{c}	2.64^{e}	3.46^{d}
Pf7	0.67^{a}	1.63^{e}	2.26^{d}	2.64^{e}	2.97^{e}
Pf8	0.63^{b}	2.67^{b}	3.38^{b}	3.66^{cd}	3.97^{c}
Pf9	0.61^{b}	2.29^{c}	2.96^{c}	3.28^{d}	3.46^{d}
Pf10	0.59^{bc}	1.50^{f}	2.26^{d}	2.30^{ef}	2.97^{e}
Control	0.59^{bc}	1.54^{ef}	1.86^{de}	2.30^{ef}	2.56^{f}

Data followed by same letter (a, b, c, d, e, f, g) in each column do not significantly differ after Duncan's *t*-test ($P<0.05$).

Table 4. Polyphenol oxidase activity in fluorescent pseudomonas treated mulberry leaves

Pseudomonas isolates	Polyphenol oxidase activity min/g fresh weight of mulberry leaves				
	Days after inoculation				
	0	7	14	21	28
Pf1	0.47^{b}	2.55^{a}	3.49^{a}	3.73^{a}	4.65^{a}
Pf2	0.43^{bc}	0.87^{f}	1.05^{g}	1.84^{f}	1.34^{f}
Pf3	0.42^{a}	2.03^{b}	3.00^{bc}	3.53^{ab}	4.14^{bc}
Pf4	0.56^{a}	2.02^{b}	2.93^{bc}	3.38^{b}	3.89^{b}
Pf5	0.42^{a}	2.02^{b}	3.00^{bc}	3.53^{ab}	4.14^{b}
Pf6	0.38^{d}	1.07^{de}	1.82^{ef}	2.17d^{e}	2.23^{de}
Pf7	0.46^{b}	1.27^{d}	2.03^{e}	2.43^{d}	2.64^{d}
Pf8	0.56^{a}	2.03^{b}	2.93^{bc}	3.38^{b}	3.89^{bc}
Pf9	0.43^{c}	2.02^{b}	2.72^{c}	3.03^{c}	3.48^{c}
Pf10	0.44^{c}	1.27^{d}	2.03^{e}	2.43^{d}	2.64^{d}
Control	0.44^{c}	1.86^{c}	2.72^{c}	3.03^{c}	3.40^{c}

Data followed by same letter (a, b, c, d, e, f, g) in each column do not significantly differ after Duncan's t-test ($P<0.05$).

resistance in plants against pathogens (Dalisay and Kuc, 1995). Different studies conducted on cereals associated ISR showed increased accumulation of plant defense proteins at the site of pathogen infection (Chithrashree *et al*., 2011; Saikia *et al*., 2006; Sari *et al*., 2008) and also proved increased activity of phenolics or phytoalexins (Chithrashree *et al*., 2011). In the present study, strong induction of defense enzymes in bacterized plants challenge inoculated with the powdery mildew induced systemic resistance, which reduced development of the disease. Greater activity for peroxidase and polyphenol oxidase in treated plants suggests that quantitative differences in the isoforms associated with induced resistance. This is corroborates with the quantitative type of ISR observed by Nandakumar *et al*. (2001) in rice against sheath blight. Radjacommare *et al*. (2003) also detected unique PO and PPO isoforms in rice treated with *P. fluorescens* against *Rhizoctonia solani*. The efficacy of plant growth-promoting rhizobacteria was tested against the powdery mildew of grapevine caused by *Uncinula necator* (Schw.) Burn. Vaithiyanathan*, et al*. (2007) demonstrated induction of peroxidase and polyphenoloxidase with the application of a talc-based *Pseudomonas fluorescens* strain and significant reduction of powdery mildew in grapevine. Furthermore, increased plant growth, fresh weight and protection against powdery mildew and angular leaf spot in melon were reported due to treatment of melon seedlings with selected strains of *P. fluorescens* (Laura *et al*., 2012).

Present study shows higher accumulation of phenols and defense enzymes peroxidase, polyphenoloxidase in mulberry treated with *P. fluorescens* against powdery mildew. The highly negative correlation of these plant defense indicators with the disease severity shows induction of systemic resistance by *P. fluorescens* in mulberry against invading pathogen and hence could be used for management of mildew as alternative to chemicals.

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