



Induction of Systemic Resistant Molecules in Phylloplane of Rice Plants against *Magnaporthe oryzae* by *Pseudomonas fluorescens*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SS and GK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SS and SP managed the analyses of the study. Author SP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Rice blast caused by ascomycetes fungus *Magnaporthe oryzae*, is mostly considered the utmost significant disease of rice worldwide since its widespread dissemination and destructiveness under conducive conditions. Experiments were conducted in phylloplane of rice plants to study the induction of various defense enzymes and accumulation of phenol by three biocontrol agents viz., *P. fluorescens* talc (Pf1), liquid formulation with TNAU - Pf1 and Biocure - B under pot culture. The application of bioformulations of *P. fluorescens* triggered the activity of three defense related enzymes viz., peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) and SOD were induced and the accumulation of phenol was also noticed in phylloplane rice plant upon challenge inoculation with *Magnaporthe oryzae* the causal agent for leaf blast disease in rice. The activities of defense enzymes reached a peak at three days after inoculation (DAI) with the pathogen. Native PAGE analysis revealed the expression of an additional isoforms of PO, PPO, SOD and catalase were observed in biocontrol agents treated seedlings due to induced systemic resistance (ISR) induction.

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is the second most important staple food crop in the world followed by maize. Approximately 90% of global rice production is contributed by Asia alone [1]. Globally, it occupies 167 million hectares with an annual production of around 780 million tonnes and average productivity of 4.25 t/ ha [2]. Among the rice diseases that hamper the production, blast incited by *Magnaporthe oryzae* previously called as *Pyricularia grisea* (perfect stage *Magnaporthe oryzae*) is the most destructive and notorious disease worldwide inflicting yield losses of staggering dimension [3]. At present disease has been found in more than 85 countries in the world [4]. It is noteworthy to mention that yield losses up to 100 per cent have been documented in recent outbreak in Brazil on newly released upland cultivar Colosso [5]. Each year, rice blast causes losses between 10 and 30% of the rice harvest [6]. Yield losses in rice due to blast ranged from 35-50 per cent [7]. Blast is reported to be prevalent during both Southwest and Northeast monsoon period [8]. Biological control makes management of plant diseases less dependent on the use of high risk chemicals and is environmentally friendly. Fluorescent *Pseudomonads* are amongst the most effective biological control agents against soil borne plant pathogens. Several isolates of *P. fluorescens*, *P. putida* and *P. aureofaciens* suppress the soil borne pathogens through rhizosphere colonization, antibiosis and iron chelation by siderophore production. 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 systemically activates the plant's latent defense mechanism against pathogens called Induced Systemic Resistance (ISR) [9]. The induction of systemic resistance against various disease causing pathogens in crops such as banana, bean, carnation, rice, mango and cucumber by *Pseudomonas* and *Bacillus* strains have been reported widely [10,11].

The strains of plant growth promoting rhizobacteria are known to survive both in rhizosphere and phyllosphere [12]. Recent investigations on mechanisms of biological control by plant growth promoting fluorescent pseudomonads 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 [13] and by activating defense genes encoding chitinase, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase [14] and enzymes which are involved in the synthesis of phytoalexins [15] and expression of stress-related proteins [16].

2. MATERIALS AND METHODS

2.1 Collection of Pathogens and Antagonists

Rice cultivar IR 50 susceptible to blast was obtained from Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore was used throughout the studies. *P. fluorescens* strain Pf1 talc and liquid formulations were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore and Biocure - B commercial *P. fluorescens* liquid obtained from Ms. T. Stanes Pvt. Ltd., Coimbatore, were used for all the studies conducted in this investigation.

2.2 Efficacy of *P. fluorescens* against *M. grisea*

The effective bio-formulations of *P. fluorescens* selected based on *in vitro* and pot culture studies were formulated using talc as a carrier. Experiments were conducted in completely randomized design with three replications in each treatment. The biocontrol agents were sprayed in 30 day old plants and challenge inoculated with pathogen after two days. The treatments also included seedling treatment followed by foliar spray of biocontrol agents at 30 DAS without challenge inoculation. Sample collection samples were collected from individual treatments to study the induced systemic resistance in response to pathogen inoculation in rice plants under glass house (95% R.H. and 32°C). Leaf tissues from bioformulations treated plants inoculated with and without pathogen *viz.*, *M. oryzae* but maintained under the same conditions leaves were collected at 0, 1, 3, 5, 7 and 9th day and stored at -80°C until used. To assay the changes in activities of defense related enzymes *viz.*, phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase

Table 1. Treatment details

T. no.	Treatment details
T ₁	Biocure- B liquid formulation - ST+ SD+ SA+ FS @ 2 ml/ litre
T ₂	Biocure- B liquid formulation - ST+ SD+ SA+ FS @ 4 ml/ litre
T ₃	Biocure- B liquid formulation - ST+ SD+ SA+ FS @ 6 ml/ litre
T ₄	Biocure- B liquid formulation - ST+ SD+ SA+ FS @ 8 ml/ litre
T ₅	Biocure- B liquid formulation - ST+ SD+ SA+ FS @ 10 ml/ litre
T ₆	TNAU-Pf1 liquid formulation - ST+ SD+ SA+ FS @ 2 ml/ litre
T ₇	TNAU-Pf1 liquid formulation - ST+ SD+ SA+ FS @ 4 ml/ litre
T ₈	TNAU-Pf1 liquid formulation - ST+ SD+ SA+ FS @ 6 ml/ litre
T ₉	TNAU-Pf1 liquid formulation - ST+ SD+ SA+ FS @ 8 ml/ litre
T ₁₀	TNAU-Pf1 liquid formulation - ST+ SD+ SA+ FS @ 10 ml/ litre
T ₁₁	TNAU-Pf1 talc formulation - ST- 10 g/kg of seed + SD @ 2.5 kg/ ha + SA @ 2.5 kg/ ha + FS @ 0.2% @ 30 DAT and 10 days later
T ₁₂	Carbendazim - ST@ 2 g/ kg of seed + FS@ 0.2%
T ₁₃	Healthy control
T ₁₄	Control

ST – Seed treatment SD – Seedling dip SA – Soil application FS – Foliar spray

(PPO), b-1-3-glucanase and phenol. The plants inoculated only with *Magnaporthe oryzae* Pg12 isolated from rice cultivar IR 50 and also healthy plants were maintained for comparison.

2.3 Assay of Defence Related Enzymes by Colorimetric Method

2.3.1 Enzyme extraction

The leaf and sheath tissues were collected from bacterized and control rice plants and immediately homogenized with liquid nitrogen. One gram of powdered sample was extracted with 2 ml of 0.1 M sodium citrate buffer (pH 5.0) at 4°C. The homogenate was centrifuged for 20 min at 10,000 rotations per minute. Protein extracts prepared from rice plant tissues were used for estimation of defense enzymes. Sodium phosphate buffer 0.1 M (pH 7.0) was used for the extraction for the assay of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase enzymes, superoxide dismutase, catalase and total phenols. All the assays were experimented thrice for confirmation.

2.3.2 Assay of Peroxidase (PO)

Assay of PO activity was carried out as per the procedure [17]. The reaction mixture consists of 2.5 ml of a mixture containing 0.25 per cent (v/v) guaiacol in 0.01 M sodium phosphate buffer, pH 6.0 and 0.1 M hydrogen peroxide. Enzyme extract (0.1 ml) was added to initiate the reaction and observations were taken colorimetrically at 470 nm were taken. Crude enzyme preparations were diluted to give changes in absorbance at

470 nm of 0.1 to 0.2 absorbance units/min. The boiled enzyme was used as blank. Activity was expressed as the increase in absorbance at 420 nm min⁻¹ g⁻¹ of fresh tissue.

2.3.3 Assay of Polyphenoloxidase (PPO)

One gram of sample was homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) at 4°C. The homogenate was centrifuged at 20,000 g for 15 min at 4°C. The supernatant served as enzyme source and polyphenoloxidase activity was determined as per the procedure [18]. The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 200 µl of 0.01 M catechol was added and the activity was expressed as change in absorbance at 490 nm min⁻¹ g⁻¹ of fresh tissue.

2.3.4 Assay of Phenylalanine Ammonia-Lyase (PAL)

One gram of plant sample was homogenized in 3 ml of ice cold 0.1 M sodium borate buffer, pH 7.0, containing 1.4 mM of 2-mercaptoethanol and 50 mg of insoluble polyvinylpyrrolidone (PVP). The resulting extract was filtered through cheese cloth and the filtrate was centrifuged at 20,000 rpm for 15 min at 4°C and the supernatant was used as the enzyme source. The PAL activity was determined as the rate of conversion of L-phenylalanine to *trans*-cinnamic acid at 290 nm. Sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer was added and inoculated for 30

min at 30°C. The amount of *trans*-cinnamic acid synthesized was calculated using its extinction coefficient of 9630 M⁻¹ cm⁻¹ [19]. Enzyme activity was expressed in fresh weight basis as nmol of *trans*-cinnamic acid release min⁻¹ g⁻¹ of fresh tissue.

2.3.5 Assay of Superoxide Dismutase (SOD)

The enzyme extract was prepared by homogenizing 1 g tissue of rice in 2 ml of 0.2 M citrate phosphate buffer (pH 6.5) at 4°C. The homogenate was centrifuged at 15,000 g at 4°C for 30 min. The supernatant served as enzyme source and SOD activity was determined as its ability to inhibit the photochemical reduction of NBT [20]. The assay mixture (3 ml) consists of 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin. 0.1 mM EDTA and 100µl of the enzyme extract and the riboflavin was added at the end. Tubes were shaken and placed under a 40-W fluorescent lamp at 25°C. The reaction was initiated and terminated by turning the light on and off respectively. The absorbance at 560 nm was measured against identical non-illuminated in parallel to the sample tubes for blank. Each extract was subtracted from the blank and mathematical difference was then divided by blank and multiplied by 100 to obtain the percentage inhibition of NBT photo-reduction. The SOD activity was expressed in SOD units g⁻¹ tissue (50% NBT inhibition = 1 unit) [21].

2.3.6 Assay of catalase

Assay of catalase was experimented by following the procedure [22] using spectrophotometer. 3 ml of assay mixture was used; it contains 100 mM potassium phosphate buffer pH 7.5, freshly prepared 2.5 mM H₂O₂ and 100 µl enzyme extract. The catalase activity was measured by monitoring the degradation of H₂O₂ using UV-Visible Spectrophotometer (Varian Cary 50) at 240 nm over one min against plant extract free blank. The decrease in H₂O₂ was followed by decline in optical density at 240 nm, catalase activity was calculated using the extinction coefficient ($\epsilon_{240\text{ nm}} = 40\text{ mM}^{-1}\text{ cm}^{-1}$) for H₂O₂ and expressed in µmol min⁻¹ g⁻¹ of sample.

2.3.7 Estimation of total phenols

Phenol content was estimated as per the procedure [23]. 1 gram root tissue was homogenized in 10 ml of 80% methanol with pestle and mortar, agitated for 15 min at 70°C.

One ml of the methanolic extract was added to 5 ml of distilled water and 250 µl of Folin-Cicalteau reagent (1N) and the solution was kept at 25°C. After 3 min 1ml of saturated solution of sodium carbonate and 1 ml of distilled water was added and the reaction mixture was incubated for 1h at 25°C. The absorption of the blue colour was measured using UV-Visible Spectrophotometer (Model - Varian Cary 50, Victoria, Australia) at 726 nm. The content of the total soluble phenols was calculated according to a standard curve obtained from a Folin-Cicalteau reagent with a phenol solution (C₆H₆O) and expressed as catechol equivalents g⁻¹ tissue weight.

2.4 Activity Gel Electrophoresis

2.4.1 Peroxidase (PO)

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% and stacking gel of four per cent were prepared. After electrophoresis, the gels were incubated in the solution containing 0.15 per cent benzidine in six per cent NH₄Cl for 30 min in dark. Then drops of 30% H₂O₂ were added with constant shaking till the bands appear. After staining, the gel was washed with distilled water and photographed using DSLR camera [24].

2.4.2 Polyphenol Oxidase (PPO)

Enzyme was extracted by homogenizing one g of tissue in 0.01 M potassium phosphate buffer (pH 7.0). The homogenate was centrifuged at 20,000 rpm for 15 min at 4°C and the supernatant was used as enzyme source. After native electrophoresis, the gel was equilibrated for 30 min in p-phenylene diamine 0.1% in 0.1 M potassium phosphate buffer (pH 7.0) followed by 10 mM catechol in the same buffer. The addition of catechol was followed by a gentle shaking which resulted in appearance of dark brown discrete bands [25].

2.4.3 Catalase (CAT)

Electrophoresis was carried out under native condition in 8 per cent polyacrylamide gels for CAT activity staining. Electrophoresis running conditions were as described [26] and buffers and gels were prepared as described [27] lacking sodium dodecyl sulfate. Equal amounts of protein (40 µg) were loaded onto each lane. The activity

was assayed as described [28]. Gels were incubated in 0.003% H₂O₂ for 10 min and developed in a 1% (w/v) FeCl₃ and one per cent (w/v) K₃Fe (CN)₆ solution for 10 min.

2.4.4 Super Oxide Dismutase (SOD)

Electrophoresis was carried out under native condition in 8% polyacrylamide gels for SOD activity staining. Electrophoresis running conditions and gels were prepared as described [27] lacking SDS. Equal amounts of protein (40 µg) were loaded on to each lane. SOD activity was determined on native PAGE gels as described [29] and modified [30]. The gels were rinsed in deionized water and incubated in the dark for 30 min at room temperature in an assay mixture containing 50 mM potassium phosphate buffer (pH 7.8), 1 mM EDTA, 0.05 mM riboflavin, 0.1 mM nitroblue tetrazolium and 0.3% (v/v) N,N,N',N'-tetramethyl ethylene diamine (TEMED). At the end of this period, the gels were rinsed with deionized water and placed in deionized water and exposed on a light box for 5 to 10 min at room temperature until the development of colourless bands of SOD activity in a purple-stained gel was visible. The reaction was stopped by transferring the gels to 6% (v/v) acetic acid.

2.5 Statistical Analysis

The data were statistically analysed and the treatment means were compared by Duncan's Multiple Range Test (DMRT). The package used for analysis was IRRISTAT version 92 developed by the International Rice Research Institute, Biometrics Unit, Philippines.

3. RESULTS

3.1 Induction of Systemic Resistance Molecules in Rice Phylloplane

In the present study revealed enhanced activities of defense related enzymes PO, PPO, SOD and CAT in rice phylloplane plants treated with bioformulations challenged with *M. oryzae*. In general elevated levels of PO, PPO, SOD and CAT activity were observed in plants treated with biocontrol agents when inoculated with the pathogen. PO, PPO, SOD and CAT activity reached maximum at third days after inoculation (DAI) with the pathogen and declined thereafter in all the treatments (Figs. 1, 2 and 3). However, activity of SOD was at its peak at 3 DAI in bioformulations of *P. fluorescens* challenged with

challenged with *M. oryzae*. Activity of catalase was at its peak at 5 DAI in bioformulations of *P. fluorescens* challenged with challenged with *M. oryzae* (Fig. 4). PAL activity was shown to increase significantly up to three days in all the treatments and there after declined. The maximum PAL activity was induced in the rice plants treated with *P. fluorescens* talc (Pf1) with the treatment combination of seed treatment @ 10 g/ kg + seedling dip @ 2.5 kg/ ha + soil application @ 2.5 kg/ ha + foliar spray @ 0.2% (21.480 n-mol of *trans*-cinnamic acid min⁻¹ g⁻¹ of fresh tissue). The activity of the enzyme increased up to 5th day and declined thereafter (Fig. 3). The control (healthy plant) recorded lesser PAL activity. Phenol accumulation increased from third day and attained peak on 5 DAI. Maximum accumulation of phenol was noticed in bio-formulations of *P. fluorescens* with *M. oryzae* at 5 DAI when compared to plants inoculated with the pathogen alone (Fig. 5). Deposition of more phenolics in rice plants is seen in combined application of the endophytes including all methods of application.

3.2 Native PAGE Analysis for Isozyme Induction

3.2.1 Peroxidase

Native PAGE analysis showed three isoforms (PO1 to PO3) of peroxidase was observed in all treatments on 3rd day and five isoforms (PO 1 to PO5) of peroxidase was observed in all treatments on 5th day (Fig. 7) except healthy control. However, the intensity of the isoforms was more in plants treated with bioformulations of *P. fluorescens* than those challenged with the pathogen.

3.2.2 Polyphenol oxidase

Native PAGE analysis showed six isoforms (PPO1 to PPO6) of polyphenol oxidase was observed in all treatments on 3rd day and 4 isoforms (PPO1 to PPO6) of polyphenol oxidase was observed in all treatments on 5th day (Fig. 8) except healthy control. However, the intensity of the isoforms was more in plants treated with bioformulations of *P. fluorescens* than those challenged with the pathogen.

3.2.3 Superoxide dismutase

Native PAGE analysis showed four isoforms (SOD1 to SOD4) of superoxidase dismutase was observed in all treatments on 3rd day and 2

isoforms (SOD1 to SOD2) of superoxidase dismutase was observed in all treatments on 5th day (Fig. 9) except healthy control. However, the

intensity of the isoforms was more in plants treated with bioformulations of *P. fluorescens* than those challenged with the pathogen.

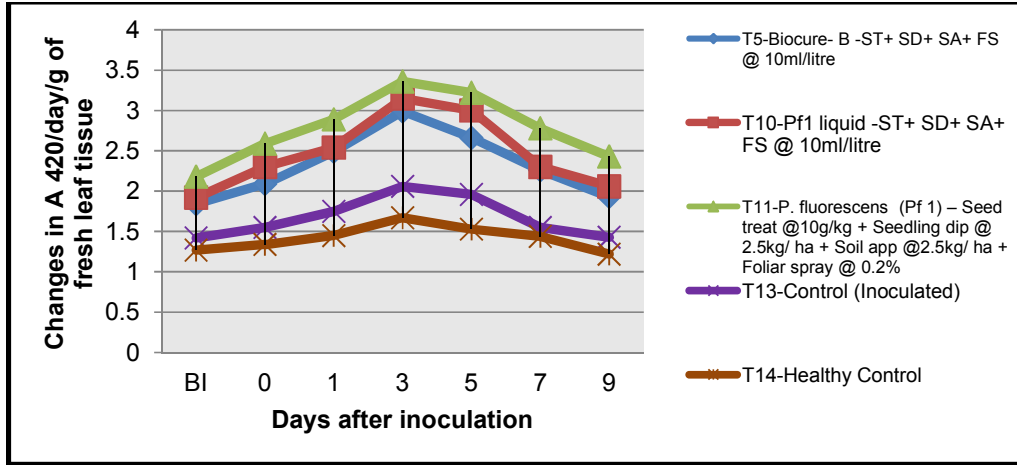


Fig. 1. Induction of peroxidase in rice plants upon treatment with bioformulations of *P. fluorescens* challenged with *M. oryzae*

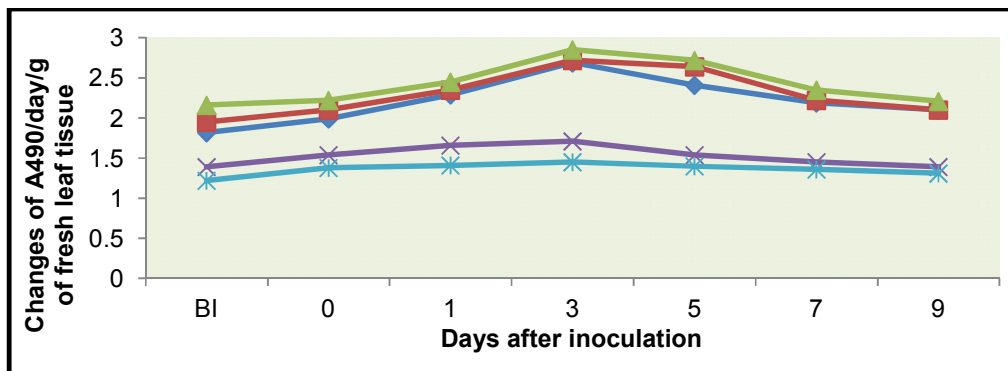


Fig. 2. Induction of polyphenol oxidase in rice plants upon treatment with bioformulations of *P. fluorescens* challenged with *M. oryzae*

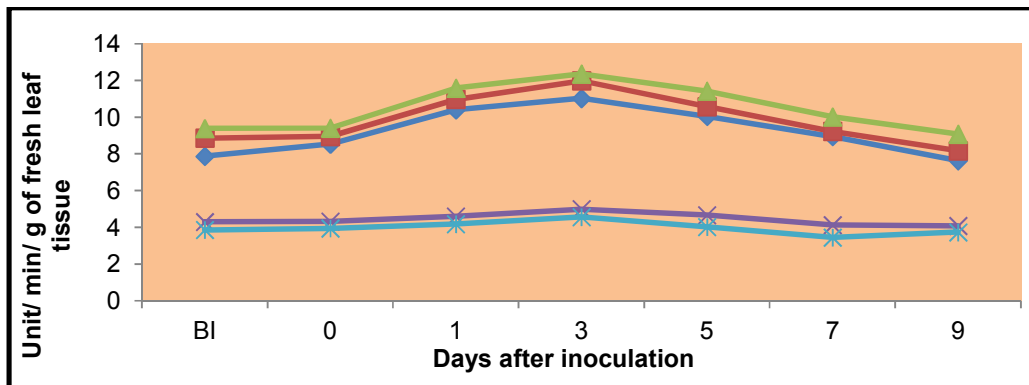


Fig. 3. Induction of superoxide dismutase in rice plants upon treatment with bioformulations of *P. fluorescens* challenged with *M. oryzae*

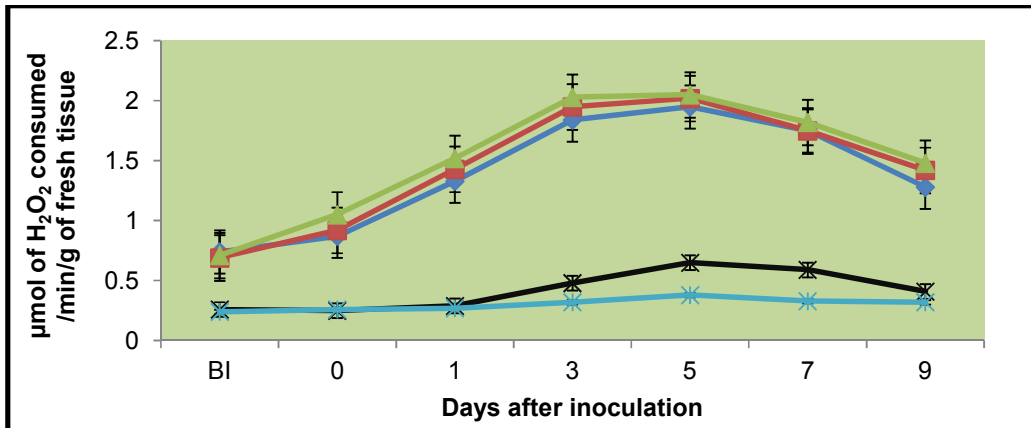


Fig. 4. Induction of catalase in rice plants upon treatment with bioformulations of *P. fluorescens* challenged with *M. oryzae*

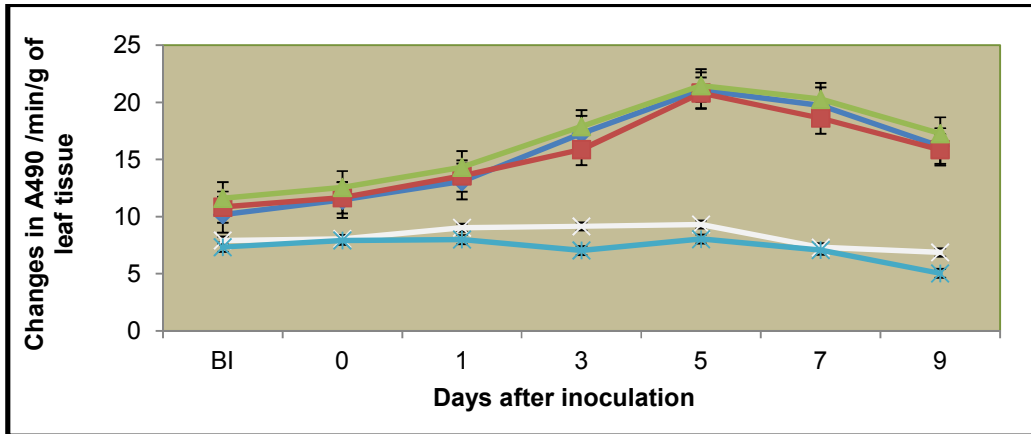


Fig. 5. Induction of phenylalanine ammonia lyase in rice plants upon treatment with bioformulations of *P. fluorescens* challenged with *M. oryzae*

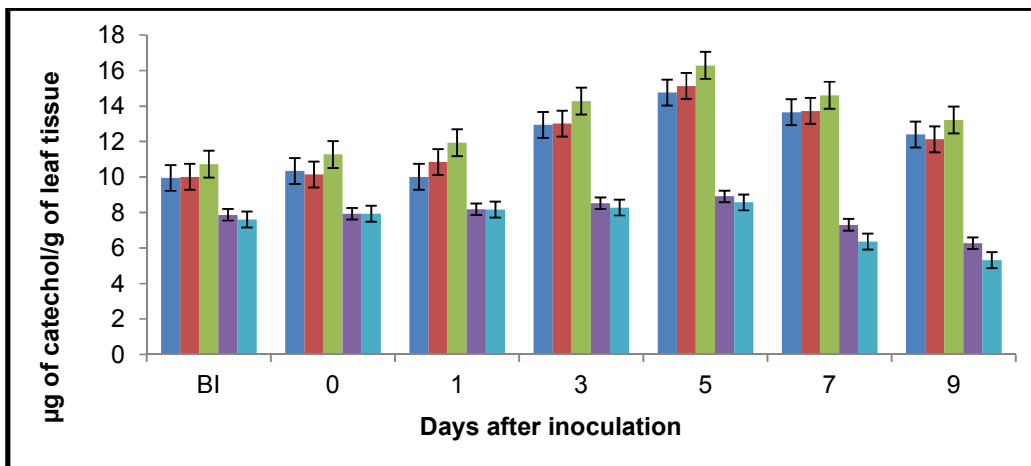


Fig. 6. Induction of total phenols in rice plants upon treatment with bioformulations of *P. fluorescens* challenged with *M. oryzae*

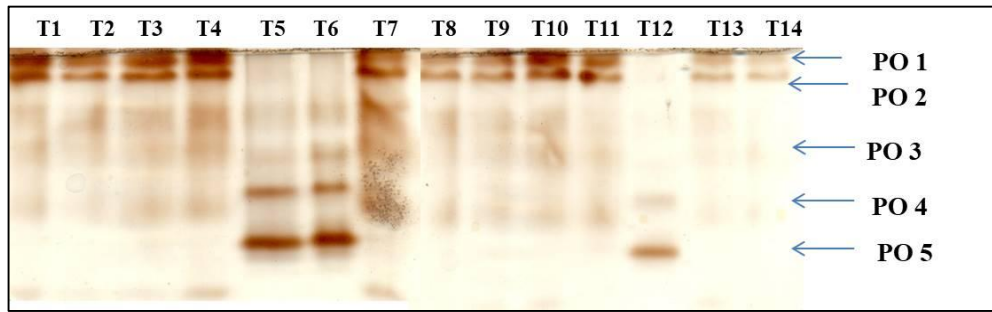


Fig. 7. Induction of peroxidase in rice by *P. fluorescens* challenged with *M. grisea*

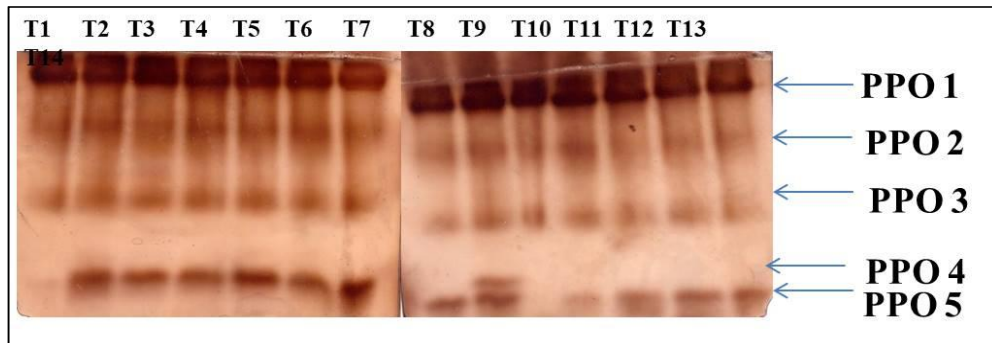


Fig. 8. Induction of polyphenol oxidase in rice by *P. fluorescens* challenged with *M. grisea*

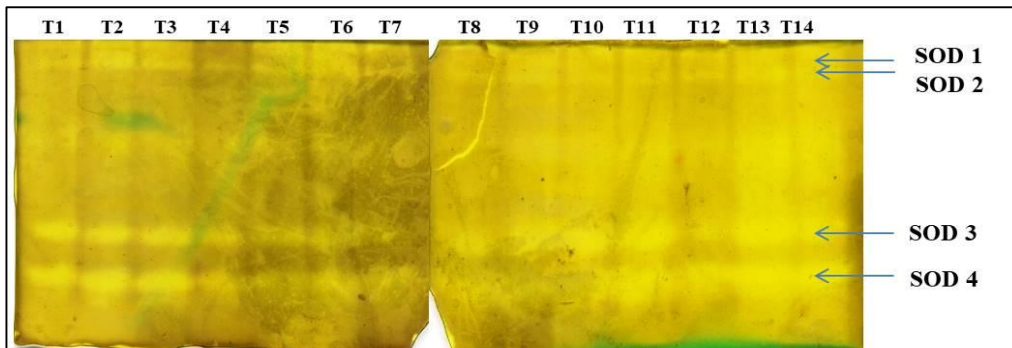


Fig. 9. Induction of superoxide dismutase in rice by *P. fluorescens* challenged with *M. grisea*

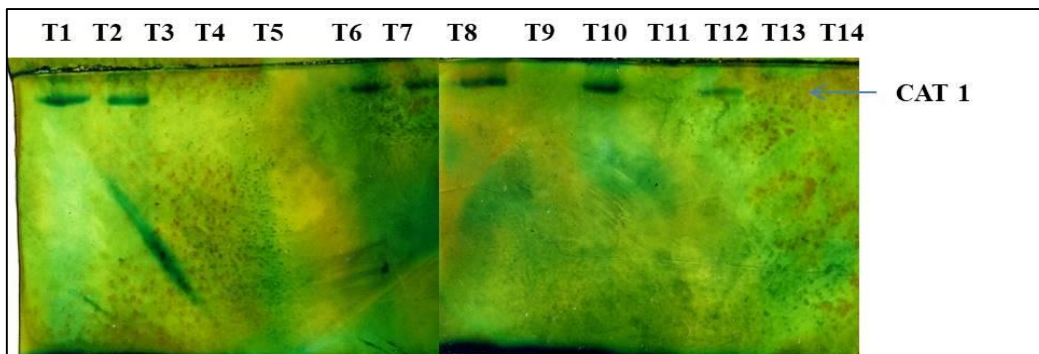


Fig. 10. Induction of superoxide dismutase in rice by *P. fluorescens* challenged with *M. grisea*

3.2.4 Catalase

Native PAGE analysis showed one isoform (CAT1) of catalase was observed in some treatments on 3rd day and one isoform (CAT1) of catalase was observed in some treatments on 5th day (Fig. 10) except healthy control. However, the intensity of the isoforms was more in plants treated with bioformulations of *P. fluorescens* than those challenged with the pathogen.

4. DISCUSSION

Induction of systemic resistance by PGPR against various diseases was considered as the most desirable approach in crop protection. There are major differences in ISR when compared to other mechanisms. First, the action of ISR is based on the defense mechanism that is activated by inducing agents. Second, ISR expresses multiple potential defense mechanism that include increased activity of chitinase, β -1-3 glucanase and peroxidase [31,32] and accumulation of antimicrobial low molecular substances- phytoalexins and formation of protective biopolymers viz., lignin, callose and hydroxyproline rich glycoprotein [9]. Third, an important aspect of ISR is the wide spectrum of pathogens that can be controlled by single inducing agent [33]. In the present investigation revealed enhanced activities of defense related enzymes PO, PPO, PAL, SOD, CAT and accumulation of phenol in phylloplane rice plants treated with bioformulations of *P. fluorescens* and challenged with *M. oryzae*. Induction of PAL is very important as the biosynthesis of lignin originate from L-phenylalanine. General phenyl propanoid metabolism is defined as the sequence of reactions involved in the conversion of L-phenylalanine to activated cinnamic acids [34]. The first enzyme of this path way is PAL that catalyzes the *trans*-elimination of ammonia from L-phenylalanine to form *trans*-cinnamic acid which in turn enters different biosynthetic path ways leading to lignin. PAL activity could be induced during plant-pathogen interactions [35]. In our study, PAL activity reached maximum at six days after inoculation (DAI) with the pathogen and declined thereafter in all the treatments. Plants that received only biocontrol agents as treatment also exhibited higher levels of PAL activity when compared with healthy control.

In plant systems, peroxidase is likely to play a role in synthesis of the plant cell wall and the enzyme cross-links phenolic residues of cell wall polysaccharides and glycoproteins, which serve

to strengthen the cell wall components. This action may represent part of a wound-healing response because some peroxidase isoenzymes are induced by stress such as that caused by high salt, physical wounding and microorganisms. Increased activity of peroxidases has been elicited by fluorescent pseudomonads in plants such as rice [11], black gram [36], groundnut [37], sugarcane [38], chillies [35] cucumber [13] and mango [10]. Polyphenol oxidases (PPO) are enzymes which use molecular oxygen to catalyze the oxidation of monophenolic and ortho diphenolic compounds. PPO usually accumulated upon wounding in plants. The present study revealed that the peroxidase (PO) and polyphenol oxidase (PPO) activity also increased significantly up to 3 days in all the treatments and there after declined. Similarly, soil and foliar application of *P. fluorescens* (Pf1) induced the accumulation of phenolics and enhanced the activities of peroxidase, phenylalanine ammonia lyase and polyphenol oxidase in black gram against Urdbean leaf crinkle virus (ULCV) in black gram [36]. Similarly, plants produce active oxygen species (AOS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH). It is one of the earliest responses of plants to attempt infection by pests and pathogens [39]. Scavengers of active oxygen species like catalase (which catalyzes the decomposition of H_2O_2) [40], superoxide dismutase (which scavenges O_2^-) [41] and peroxidase (which scavenges H_2O_2) suppress the oxidative burst [42] and inhibit tissue necrotization. Catalase and peroxidase are of particular interest because of their role in binding salicylic acid (SA), which plays an important role in induced resistance [43, 32].

Naturally, phenolic compounds enhance the mechanical strength of host cell wall and also inhibit the invading pathogenic organisms. Seed treatment with *P. fluorescens* induced the accumulation of phenolics in tomato root tissue [44]. The hyphae of the pathogen surrounded by phenolic substances made 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 *Pythium ultimum* and *Fusarium oxysporum* f. sp. *pisi* [45]. Similar findings were reported in sugarcane against *Colletotrichum falcatum* [46] in tomato and hot pepper against *Pythium aphanidermatum* [47] and in rice against *Rhizoctonia solani* and *Cnaphalocrosis medinalis*

[48] and in mango against *Colletotrichum gloeosporioides* [49] and *Lasiodiplodia theobromae* [10].

5. CONCLUSION

Overall, application of bioformulation of *P. fluorescens* bio-control agent on phylloplane gave the protection against blast pathogen throughout the crop stand as the different methods provide sufficient load of *P. fluorescens* than the untreated control plants. It also triggered the activity of the defense related enzymes viz., peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) and SOD were induced and the accumulation of phenol was also noticed in phylloplane rice plant upon challenge inoculation with *M. oryzae* the causal agent for leaf blast disease in rice. In addition, application of *P. fluorescens* provides prolonged protection against the *M. oryzae* in the rice phylloplane and induces innate immune response against various pathosystems.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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