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Defense Responses of Grapevine to *Plasmopara viticola* Induced by Azoxystrobin and *Pseudomonas fluorescens*

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Abstract: The induction of systemic resistance was studied to elucidate the role of azoxystrobin and *Pseudomonas fluorescens* in disease management of grapevine against *Plasmopara viticola*. The activity of defense enzymes *viz.*, peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) was found to be increased in azoxystrobin 23 SC and *P. fluorescens* treated grapevine seedlings compared to control. Increases in the activities of enzymes were more in *P. fluorescens* treated grapevine seedlings compared to azoxystrobin 23 SC. In addition to this increased expression of specific isoforms of PO and PPO were observed in azoxystrobin 23 SC and *P. fluorescens* treatred seedlings due to Induced Systemic Resistance (ISR) induction.

Key words: Grapevine seedlings · PO · PPO and PAL

INTRODUCTION

Grapevine (*Vitis vinifera* L.), is a non-climateric fruit crop, that grows as perennial and deciduous woody vines. Grapevine contributes to 50 per cent of the total production of the fruits in the world. The crop production is being challenged by an array of opportunistic pathogens. Among all downy mildew, caused by the oomycete, *Plasmopara viticola*, especially leads to massive damage and yield losses in this crop if no protective measures are taken. Although some grape cultivars (e.g., Solaris and Regent) are resistant against downy mildew, most traditional cultivars, planted over 90 per cent of the vine-growing area, are susceptible to this disease, necessitating an intensive use of plant protection chemicals to limit the damage in the vineyards.

Induced resistance may provide an alternative approach to plant protection especially for problems not satisfactorily controlled by various fungicides [1]. Induced systemic resistance is a phenomenon whereby resistance to the infectious disease is systemically induced by localized infection or treatment with microbial components or products or by a diverse group of structurally unrelated inorganic or organic compounds [2]. Vallad *et al.* [3] stated that Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR) are two forms of induced resistance; in that plant defenses are preconditioned by prior infection or treatment that results in resistance (or tolerance) against subsequent challenge by a pathogen or parasite.

Induction of systemic resistance by chemicals and Plant Growth Promoting Rhizobacteria (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, ß-1,3 glucanase and peroxidase [4,5 and 6] and accumulation of antimicrobial low molecular substances-phytoalexins and formation of protective biopolymers *viz.,* lignin, callose and hydroxyproline rich glycoprotein (7). Third, an important aspect of ISR is the wide spectrum of pathogens that can be controlled by single inducing agent [8, 9 and 10]. Thus ISR appears to be the result of several mechanisms, which together are effective against wide range of fungal, bacterial and viral pathogens. Induced responses and induced resistance have been well documented and found in many plant taxa [11].

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SAR is enhanced by SAR genes coding for pathogenesis-related (PR) proteins [12]. Some of these proteins have demonstrated antifungal activity [13]; therefore, it is thought that they contribute to protection and they commonly are used as markers for SAR. SAR normally requires a lapse period between treatment and inoculation [14], allowing for signal transduction and gene activation to take place [15]. The earlier authors also implied that the expression of genes of the phenylpropanoid pathway in grapevine was induced by SAR activators and suggested a role for *S*-adenosyl-L-methionine: transcaffeoyl-coenzyme A 3-*O*-methyltransferase (CCoAOMT) and stilbene synthase in the disease-resistance response [15]. The objective of the present study is to unravel the induction of various defense related genes encoding proteins implicated in strengthening of plant cell walls by various chemical and *P. fluorescens* treatments in response to infection by *P. viticola.*

MATERIALS AND METHODS

Source of Pathogen, PGPR strain, Chemicals and Planting Material: Downy mildew infected grapevine leaves were collected from Thondamuthur and the orchard of Tamil Nadu Agricultural University, Coimbatore, India. Conidia were harvested from 14-day-old mildew colonies on *V. vinifera* plants by shaking infected leaves in 50 ml of distilled water containing 0.05 % (v/v) Tween 20. The concentration of the suspension was adjusted to approximately 10^5 conidia per ml and was applied by hand-held atomizer (Preval Model 268; Precision Valve Corp., Yonkers, NY) within 30 min of its preparation [16].

P. fluorescens isolate, Pfl isolated from the rhizosphere of rice ecosystem was obtained from the Department of Plant Pathology, Coimbatore, TNAU. Pfl was kept at -80 °C in 44 per cent glycerol and cells from stocks were first grown in King's B broth. Inoculum was produced by transferring two loopful from the culture to 100 ml of KB broth in a 250 ml Erlenmeyer flask and incubated at room temperature on a shaker at 150 rpm for 48 h. The strain was sub cultured once in a month and maintained.

The grapevine *var.* Muscat was raised in pots by vegetative propagation under glass house conditions. The cuttings were collected from mature canes of healthy and virus-free vines. Depending upon the length of internodes in a cultivar, mature wood was cut into pieces of about 25-30 cm long which contained at least four buds.

The thickness of cuttings was 7-8 mm diameter (pencil thickness). The lower end of the cuttings was soaked in 50 ppm of Rootex (Indole Butyric Acid) for 24 h. Then the cuttings were planted in mud pots containing red soil, sand and farmyard manure in the 3:1:1 ratio. The pots were watered periodically.

Azoxystrobin 23 SC was obtained from Krishi Rasayana Company, New Delhi. The other chemicals used in the study were purchased from the pesticide market at Coimbatore, India.

Preparation of Talc-Based Formulation of PGPR Strains: A loopful of bacterium was inoculated into the KB broth 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^{-1} was used for the preparation of talc-based formulation. To 400 ml of bacterial suspension, 1kg of the purified talc powder (sterilized at 105°C for 12h), calcium carbonate 15 g (to adjust the pH to neutral) and carboxy methyl cellulose 10g (adhesive) were mixed under sterile conditions following the method described by Vidhyasekaran and Muthamilan [17]. After shade drying for over night it was packed in polypropylene bag and sealed. At the time of application, the population of bacteria in talc formulation was found to be 2.5 to 3 X 10^8 cfu g^{-1}.

Foliar Spray of PGPR and Chemicals and Artificial Inoculation of Pathogen: The talc-based product of Pf 1 was dissolved in water (20 g l^{-1}) and allowed to settle for 10 min, filtered through muslin cloth and the filtrate was sprayed on 30 days old sedlings [18]. Different chemicals namely, Azoxystrobin 23 SC (125 g ai ha^{-1}), Azoxystrobin 25 SC (125 g ai ha^{-1}), Miclobutanil (0.4 kg ha^{-1}), Metalaxyl (4 kg ha^{-1}), Mancozeb (2 kg ha^{-1}) were applied on the thirty days old seedlings. One day after treatment, treated seedlings were challenge inoculated with *P. viticola.* The seedlings neither treated with chemicals and *P. fluorescens* nor challenged by the pathogen were kept as control. The seedlings inoculated with pathogen alone served as inoculated control.

Three replications were maintained in each treatment; each replicate consisted of six pots. The experiments were conducted using complete randomized block design on a glasshouse bench. The humidity in the glasshouse was maintained at around RH 80 per cent. The temperature was adjusted to 26 °C (day) / 20 °C (night). Leaves from sprayed and unsprayed seedlings were collected at 0, 24 and 72 h intervals.

Enzyme Extraction: The leaf tissues were collected from seedlings and immediately homogenized with liquid nitrogen. One gram of powdered sample was extracted with 2 ml of sodium phosphate buffer, 0.1 M (pH 7.0) at 4 °C. The homogenate was centrifuged for 20 min at 10,000 rpm. Protein extract prepared from leaves were used for the estimation of peroxidase, polyphenol oxidase and L-phenylalanine ammonia-lyase.

Spectrophotometric Assay

Peroxidase (PO): Peroxidase activity was assayed spectrophotometrically as per the method described by Hartee [19]. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1 per cent H_2O_2. The reaction mixture was incubated at room temperature (28 ± 1 °C). The change in absorbance at 420 nm was recorded at 30 seconds intervals for 3 min and while the boiled enzyme preparation served as blank. The enzyme activity was expressed as change in the absorbance of the reaction mixture min^{-1} g^{-1} on fresh weight basis.

Polyphenoloxidase (PPO): Polyphenoloxidase activity was determined as per the procedure given by Mayer *et al.* [20]. 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 expressed as changes in absorbance at 495 nm $min^{-1}g^{-1}$ fresh weight of tissue.

Assay of L-phenylalanine Ammonia-lyase (PAL): The PAL assay was done as per the method described by Ross and Sederoff [21]. The assay mixture containing 100 µl of enzyme, 500 µl of 50 mM Tris HCl (pH 8.8) and 600 µl of 1 mM Lphenylalanine was incubated for 60 minutes. The reaction was arrested by adding 2 N HCl. Later 1.5 ml of toluene was added, vortexed for 30 seconds, centrifuged (1000 rpm, 5 min) and trans-cinnamic acid containing toluene fraction separated. The toluene phase was measured at 290 nm against toluene blank. Standard curve was drawn with graded amounts of cinnamic acid in toluene as described earlier. The enzyme activity was expressed as n moles of cinnamic acid $min^{-1}g^{-1}$.

Native Gel Electrophoresis

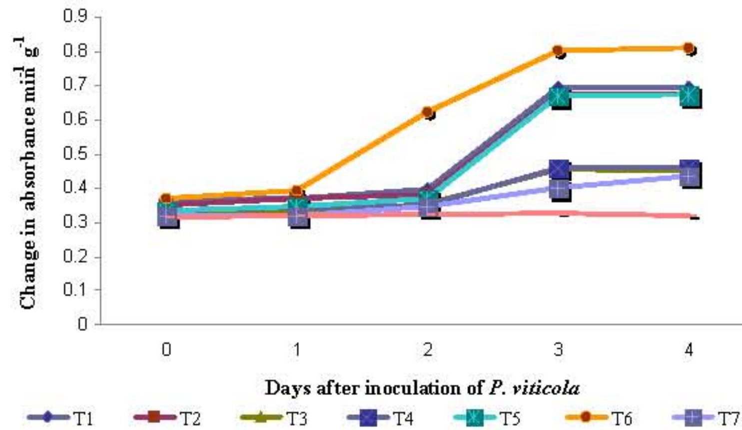
Peroxidase: To study the expression pattern of different isoforms of peroxidases in different treatments, activity gel electrophoresis was carried out. For native anionic polyacrylamide gel electrophoresis, resolving gel of 8 per cent acrylamide and stacking gel of 4 per cent acrylamide were prepared [22]. After electrophoresis, the gels were incubated in the solution containing 0.15 per cent benzidine in 6 per cent NH_4Cl for 30 min in dark. Then drops of 30 per cent H_2O_2 were added with constant shaking till the bands appear. After staining, the gel was washed with distilled water and photographed immediately [23].

Polyphenol Oxidase (PPO): The PPO was extracted by homogenizing one g of tissue in 0.1 M potassium phosphate buffer (pH 7.0). The homogenate was centrifuged at 20,000 g for 15 min at 4 °C in a centrifuge and the supernatant was used as the enzyme source. After native electrophoresis the gel was equilibrated for 30 min in 0.1 per cent *p*-phenylene diamine in 0.1 M potassium phosphate buffer (pH 7.0) followed by addition of 10 mM catechol in the same buffer. Then gentle shaking was given which resulted in appearance of dark brown discrete protein bands.

Statistical Analysis: The data were statistically analyzed [24] and the treatment means were compared by Duncan's Multiple Range Test (DMRT). The package used for analysis was IRRISTAT version 92-a developed by International Rice Research Institute, Biometrics Units, The Philippines

RESULTS

Activity of Peroxidase: Azoxystrobin 23 SC (125 g ai ha^{-1}) along with, azoxystrobin 25 SC (125 g ai ha^{-1}), miclobutanil (0.4 kg ha^{-1}), mancozeb (2 kg ha^{-1}), metalaxyl (4 kg ha^{-1}) and *P. fluorescens* (Pf 1) (2 kg ha^{-1}) were sprayed on grapevine and the changes in the activities of peroxidase was observed. Results indicated that, in all the treatments, there was no significant difference in the activity of peroxidase at zero days after inoculation; however, from first day onwards it started to increase. It was inferred that application of azoxystrobin and *P. fluorescens* led to an increase in peroxidase activity up to the third day after challenge inoculation with *P. viticola* when compared to control and declined there after. The increased activity of peroxidase was observed very early in *P. fluorescens* pretreated grapevine seedlings and challenge inoculated with *P. viticola* followed by azoxystrobin 23 SC and azoxystrobin 25 SC spray. In addition to this among all the treatments, the higher induction was recorded in *P. fluorescens* treated followed by azoxystrobin 23 SC. The enzyme activity in azoxystrobin 25 SC treated seedlings was statistically on par with the azoxystrobin 23 SC treated seedlings (Fig. 1).



T1 - Azoxystrobin 23 SC @ 125 g ai / ha
T2 - Azoxystrobin 25 SC @ 125 g ai / ha
T3 - Miclobutanil @ 0.4 kg / ha
T4 - Mancozeb @ 3 kg / ha
T5 - P. fluorescens (Pf 1) @ 2 kg / ha
T6 - Metalaxyl @ 4 kg / ha
T7 - Healthy control
T8 - Inoculated control

Fig. 1: Changes in the activity of peroxidase (PO) in grapevine seedlings challenge inoculated with *P. viticola*

Isoforms of Peroxidase: Native gel electrophoretic separation of crude peroxidase enzyme extract from the seedlings treated with azoxystrobin 23 SC along with standard fungicides azoxystrobin 25 SC, miclobutanil, mancozeb, metalaxyl and *P. fluorescens* showed different peroxidase isoforms patterns. The intensity of peroxidase was more in *P. fluorescens* and azoxystrobin 23 SC treated seedlings when compared to other treatments after challenge inoculation with *P. viticola*. There were two isozyme pattern detected in *P. fluorescens and* azoxystrobin 23 SC treated seedlings. Low induction of peroxidase isozymes was observed in the case of other chemicals treated and control both in inoculated and non-inoculated seedlings [Plate1].

Activity of Polyphenol Oxidase: The increased activity of polyphenol oxidase was observed in grapevine seedlings challenge inoculated with downy mildew pathogen. Application of *P. fluorescens* and azoxystrobin led to increased polyphenol oxidase activity up to the third day when compared to control. In all the day induction of polyphenol oxidase in *P. fluorescens* treated seedlings was more and was followed by seedlings treated with azoxystrobin 23 SC. In the study it was inferred that grapevine seedlings inoculated with pathogen alone recorded comparatively less polyphenol oxidase activity (Fig. 2).

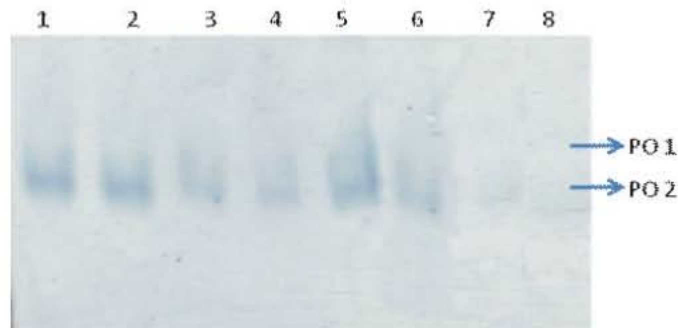
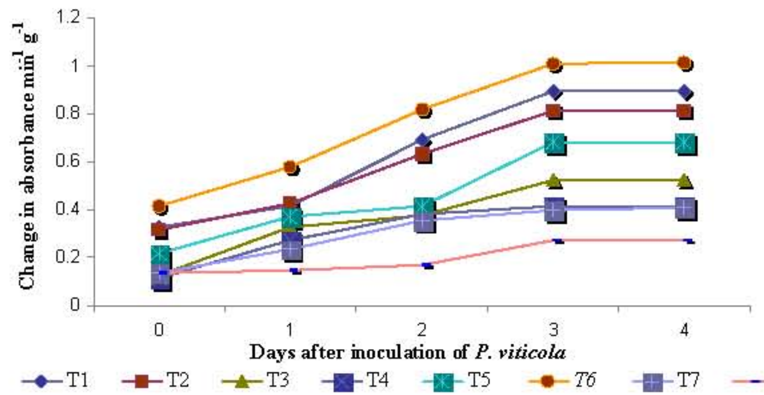


Plate 1: Native PAGE profile of peroxidase isoforms induced in grapevine seedlings in response to challenge inoculation of *P. viticola*



T1 - Azoxystrobin 23 SC @ 125 g ai / ha
T2 - Azoxystrobin 25 SC @ 125 g ai / ha
T3 - Miclobutanil @ 0.4 kg / ha
T4 - Mancozeb @ 3 kg / ha
T5 - P. fluorescens (Pf 1) @ 2 kg / ha
T6 - Metalaxyl @ 4 kg / ha
T7 - Healthy control
T8 - Inoculated control

Fig. 2: Changes in the activity of poly phenol oxidase (PPO) in grapevine seedlings challenge inoculated with *P. viticola*

Isoforms of Polyphenol Oxidase: Isoform pattern of the polyphenol oxidase in grapvine seedlings challenge inoculated with *P. viticola* was studied. Azoxystrobin 23 SC and *P. fluorescens* treated tissue taken after challenged with pathogens revealed the presence of isozyme of polyphenol oxidase. The unique enzyme polyphenol oxidase was not even visible from tissue of control seedlings. However, the isozyme appearance with higher intensity was induced only in azoxystrobin 23 SC and *P. fluorescens* treated seedlings compared to control [Plate 2].

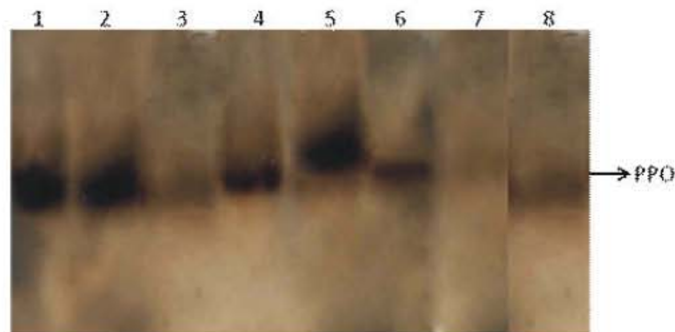
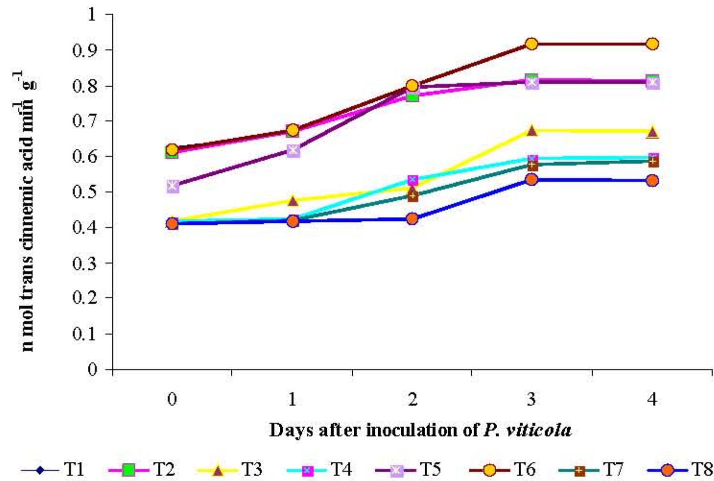


Plate 2: Native PAGE profile of polyphenol isoform induced in grapevine seedlings in response to challenge inoculation of *P. viticola*

Plate 1 and 2

Lane-1. Azoxystrobin 23 SC @ 125 g ai / ha
2. Azoxystrobin 25 SC @ 125g ai / ha
3. Miclobutanil @ 0.4 kg / ha
4. Mancozeb @ 3 kg / ha
5. *P. fluorescens* (Pf 1) @ 2 kg / ha
6. Metalaxyl @ 4 kg / ha
7. Healthy control
8. Inoculated control



T1 - Azoxystrobin 23 SC @ 125 g ai / ha
T2 - Azoxystrobin 25 SC @ 125 g ai / ha
T3 - Miclobutanil @ 0.4 kg / ha
T4 - Mancozeb @ 3 kg / ha
T5 - P. fluorescens (Pf 1) @ 2 kg / ha
T6 - Metalaxyl @ 4 kg / ha
T7 - Healthy control
T8 - Inoculated control

Fig. 3: Changes in the activity of phenylalanine ammonia lyase (PAL) in grapevine seedlings challenge inoculated with *P. viticola*
T1-Azoxystrobin 23 SC @ 125 g ai / ha T2-Azoxystrobin 25 SC @ 125 g ai / ha; T3-Miclobutanil @ 0.4 kg / ha T4-Mancozeb @ 3 kg / ha
T5-*P. fluorescens* (Pf 1) @ 2 kg / ha T6-Metalaxyl @ 4 kg / ha; T7-Healthy control T8-Inoculated control

Activity of Phenylalanine Amonia Lyase: It was observed that application of fungicide enhanced the phenylalanine ammonia lyase activity in different treatments. The PAL activity was induced from first day of inoculation and reached maximum at three days after inoculation with *P. viticola*. Among the treatments, PAL activity was maximum with azoxystrobin 23 SC and was almost statistically on par with *P. fluorescence* sprayed seedlings. There was almost two fold increase in PAL activity in the above treatment over pathogen inoculated and healthy control (Fig. 3).

DISCUSSION

Induced Systemic Resistance (ISR) of plants against pathogens is a widespread phenomenon that has been intensively investigated with respect to the underlying signaling pathways as well as to its potential use in plant protection. In many plants, enhanced disease resistance is frequently accompanied by the activation of genes encoding pathogenesis related proteins (PR). Because some of these proteins display antimicrobial activity, their accumulation has often been assumed to contribute to acquire disease resistance. Resistance to disease can be induced systemically in a number of plant species by biological and chemical means.

Induced Systemic Resistance by Azoxystrobin: Chemical fungicides often act as inducers of systemic resistance in plants against pathogens. The present study revealed that azoxystrobin showed remarkable changes in PO, PPO and PAL. Native gel electrophoresis also showed induction of isoforms in PO and PPO.

These results are in conformity with the findings of Sendhil Vel [25], who reported that the activity of PO, PPO, PAL, β-1, 3 glucanase and total phenols were higher in azoxystrobin treated grapevine seedlings. In a similar findings Hewitt [26] reported, that the systemic fungicide, probenazole treatment induced peroxidase, polyphenol oxidase, phenylalanine ammonialyase, tyrosine ammonialyase and catechol-*o*-methyl transferase accumulation in the treated leaves which indicated that the disease controlling effect of probenazole was

attributed to a host-mediated reaction. The accumulation of phenylalanine ammonia lyase in Fosetyl-Al treated tomato leaves for the control of *Fusarium* wilt was reported [27].

Anand *et al.* [28] reported that azoxystrobin at three different concentrations *viz.*, 31.25, 62.50 and 125 g ai ha^{-1}, mancozeb (1 kg ha^{-1}) and *P. fluorescens* (2 kg ha^{-1}) were found to increase the activity of defense enzymes peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, β-1, 3 glucanase, chitinase, catalase and defense-inducing chemicals (total phenols) in tomato against *Alternaria solani* (Ellis and Mrtin) Jones and Grout. and *Septoria lycopersici* Speg. The activity of these defense enzymes and chemicals was higher in azoxystrobin (125 g ai ha^{-1}) treated tomato plants challenge inoculated with the pathogens compared to other treatments.

The association between resistances induced by azoxystrobin and increased deposition of cell wall phenolics and enhanced enzyme activity of peroxidase is apparent in apple infested by fire blight [29 and 30].

Peroxidase and β-1, 3 glucanase are related to cross-linking of cell wall components, polymerization of lignin and suberin monomers and subsequent resistance to pathogen in several host-pathogen interactions [31], Mustard leaves sprayed with BTH (benzothiadiazole) three days prior to inoculation with *Albugo candida* (Lev.) Kunze showed elevated levels and enhanced activity of peroxidase at 11 days after inoculation [32]. Earlier several workers have shown that the peroxidase enzyme is involved in lignin biosynthesis, production of toxic quinones and phytoalexins with the onset of resistance [33 and 34]. Shahina Kalim *et al.* [35] reported that there was an increase in the specific activity of PO, PPO and reduction in specific activity of catalase in the roots of plants raised from carbendazim treated seeds in comparison to untreated ones. Therefore the increased activities of PO and PPO along with higher amount of total phenol might have contributed enhanced host resistance.

Induced Systemic Resistance *P. fluorescens:* Plants are bestowed with various defense related genes. It is well known that the defense genes are sleeping genes and appropriate stimuli or signals are needed to activate them. Inducing the plant's own defense mechanisms by prior application of biological inducer is thought to be a novel plant protection strategy. Induction of systemic resistance by *P. fluorescens* has earlier been reported by several workers [5]. Seed treatment with *P. fluorescens* suppressed the foliar pathogen by inducing systemic resistance [36 and 10]. The lipopolysaccharides present in the bacterial cell wall act as signal molecules and elicit various defense compounds.

The Pf1 isolate of the *P. fluorescens* and the secondary metabolite of *Strobilurus tenacellus* (Pers. ex FR.) *i.e.*, azoxystrobin were found to protect the grapevine downy mildew (*P. viticola*). In the present study, *P. viticola* in grapevine seedlings pretreated with *P. fluorescens* spray strongly induced synthesis and accumulation of PO, PPO and PAL. Sendhil Vel [25] has also made similar observations in grapevine seedlings pretreated with *P. fluorescens* and challenge inoculated with downy mildew and powdery mildew pathogens. The activity of these defense enzymes and chemicals were pronounced in the compatible reaction (control seedlings) of grapevine seedlings with downy mildew pathogen infection than the incompatible interaction (PGPR treated plant). *P. fluorescens* could act as a strong elicitor of plant defense reactions [37]. Recent studies indicated that prior application of *P. fluorescens* strengthens host cell wall structures resulting in restriction of pathogen invasion in plant tissue [38 and 39]. However, only limited information is available on plant mediated defense reactions induced by *P. fluorescens* in plants against invasion by obligate parasites.

The present study clearly indicated that the activities of enzymes of phenylpropanoid metabolism in grapevine seedlings induced by *P. fluorescens* isolate Pf1 in response to challenge inoculation with *P. viticola* might have prevented further infection by pathogens. This may be attributed to the availability of specific receptors for the inducers in seedlings.

In the present study, increased PO activity has been recorded in *P. fluorescens* (Pf1) treated grapevine seedlings challenge inoculated with *P. viticola.* The maximum PO activity was observed on the third day after challenge inoculation and the activity was maintained at higher levels throughout the experimental period. Seedlings inoculated with pathogens alone recorded comparatively less PO activity. A similar pattern of increased activity of PPO was observed in *P. fluorescens* treated grapevine seedlings. Sendhilvel *et al.* [40] reported that foliar spraying of Pf 1 significantly reduced the incidence of downy mildew through the induction of defense gene products *viz.,* peroxidase (PO). PO is a key enzyme in the biosynthesis of lignin and other oxidised phenols [41]. PO catalyzes the oxidation of hydroxy-cinnamyl alcohols into free radical intermediates, which subsequently are coupled into lignin polymers [42]. Besides the oxidized phenols, the oxidation of which is mediated by PPO and PO they are also highly toxic to the pathogens [43].

PO and PPO catalyze the oxidation of phenolic compounds through a PPO-PO-H_2O_2 system [44]. A number of studies have correlated the induction of PPO with a resistance response [45]. Besides, PO itself was found to inhibit the spore germination and mycelial growth of certain fungi [46].

Increased PAL activity was recorded in *P. fluorescens* pretreated grapevine seedlings challenged with *P. viticola* when compared to untreated control. Induction of defense enzymes like PAL is one of the responses of the host after treatment with biocontrol agents. Increase in mRNAs encoding for PAL and chalcone synthase could be recorded in the early stage of the infection in bean roots after treatment with various rhizobacteria [47]. Sendhil Vel [25] has also made a similar observation in grapevine seedlings inoculated with *U. necator* and *P. viticola.* The present results also revealed that seedlings treated with *P. fluorescens* increased the PAL activity and *P. fluorescens* pretreated grapevine seedlings challenged with *P. viticola* showed an additional increase in the PAL activity. PAL is a key enzyme of phenylpropanoid metabolism which leads to the synthesis of phenols [48]. The biocontrol agent *P. fluorescens* induced the host defense mechanism by increasing PAL activity with a consequent increase in phenol levels.

In conclusion, prior treatment of grapevine seedlings with azoxystrobin and *P. fluorescens* triggered the plant defense mechanism in response to infection by *P. viticola*. Earlier studies revealed that azoxystrobin and *P. fluorescens* are effective against various diseases of grapevine. Thus, it has been found that azoxystrobin and *P. fluorescens* treatment shows broad-spectrum protection against different pathogens attacking the same crop.

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