

Studies On The Host Defense Enzymes Produced In Rice By Application Of Organic Formulations And Sheath Blight Pathogen Inoculation

Thamarai Selvi, M.¹, Darwin Christdhas Henry L.², Sutha Raja Kumar³, R., Jaiganesh V.⁴, Kannan C.⁵

Department of Plant Pathology, Faculty of Agriculture,
Annamalai University, Annamalai Nagar – 608 002,
Cuddalore DT, Tamil Nadu.

¹Corresponding author E-mail: mthamaraiselviprabakaran@gmail.com

Abstract- Organic formulations against rice sheath blight disease are gaining momentum and encompasses all available control methods with each method compensating the deficiencies of other disease management practices. Combined use of biological and seaweed extracts are an important effective against sheath blight disease caused by *Rhizoctonia solani*. Pot culture experiments were conducted with various organic formulation alone. The different organic formulations (viz., T₁- *Pseudomonas fluorescens* (ST), T₂- *Sargassum wightii* (ST), T₃ = T₁+ T₂ (ST), T₄ - *Sargassum wightii* (spray), T₅= *Sargassum wightii*+ *Pseudomonas fluorescens* (spray), T₆=T₃+ T₅, T₇- control) were tested against sheath blight disease incidence and growth and yield attributes in rice under greenhouse condition. Among the treatments, (T₆ = T₃ +T₅) recorded the least sheath blight incidence and increase the bio metrics and yield characters. Rice plants treated with T₆ followed by challenge inoculation with *R. solani* gave a maximum induction of β-1, 3-glucanase activity on 5th day after pathogen inoculation and declined thereafter, maximum peroxidase activity at 7th day after pathogen inoculation then declined slowly, polyphenol oxidase activity on the 7th day and thereafter declined. Maximum activity of phenylalanine ammonia lyase activity was observed on the 7th day of inoculation.

Keywords- Sheath Blight, Rice, Enzymatic changes, Peroxidase, Poly Phenol Oxidase, Phenylalanine ammonia lyase

1. INTRODUCTION

Rice sheath blight caused by *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris* (Frank) Donk), is a destructive disease worldwide that causes significant yield loss and quality degradation. Presently, sheath blight disease management is mainly achieved through systemic fungicides. The indiscriminate use of fungicides leads to toxic residues, development of fungicide resistance and environmental pollution. Therefore, under intensive agriculture, there is an urgent need to develop disease control measures alternative to chemicals. Under these circumstances the use of organic formulations becomes the ultimate way of combating this disease. Organic formulations against rice sheath blight are gaining momentum and encompasses all available control methods with each method compensating the deficiencies of others. The present paper investigate the effect of host defense enzymes by application of Bio protectant, sea weed extract and Sheath blight pathogen inoculation.

2. MATERIALS AND METHODS

Enzyme Extraction

The plant tissues collected from plants were 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. Plighting extract prepared from leaves was used for the estimation of peroxidase (PO), polyphenoloxidase (PPO) and L-phenylalanine ammonia-lyase (PAL).

Peroxidase (PO) (Hammerschmidt *et al.*, 1982)

Peroxidase activity was assayed spectrophotometrically. 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 H₂O₂ which was incubated at room temperature (28 ± 1°C). The change in absorbance at 420 nm was recorded at 30 sec. interval for 3 min and 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) (Mayer *et al.*, 1965)

The reaction mixture consisted of 1.5 ml of 0.1M 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 changes in absorbance at 495 μm min⁻¹ g⁻¹ fresh weight of tissue.

Phenylalanine Ammonia-Lyase (PAL) (Ross and Sederoff, 1992)

The assay mixture containing 100 μl of enzyme, 500 μl of 50 mM Tris HCl (pH 8.8) and 600 μl of 1mM-phenylalanine was incubated for 60 min. The reaction was arrested by adding 2 N HCl. Later, 1.5 ml of toluene was added and vortexed for 30 sec. The centrifuged (1000 rpm, 5 min) toluene fraction

containing trans-cinnamic acid was separated. The toluene phase was measured at 290 nm against the blank of toluene. Standard curve was drawn with graded amounts of cinnamic acid in toluene as described earlier. The enzyme activity was expressed as μ moles of cinnamic acid min⁻¹ g fresh tissue⁻¹.

β -1, 3-glucanase (Pan et al., 1991)

Crude enzyme extract of 62.5 μ l was added to 62.5 μ l of 4 per cent laminar in and incubated at 40°C for 10 min. The reaction was stopped by adding 375 μ l of dinitrosalicylic acid (DNS) and heated for 5 min on boiling water bath (DNS prepared by adding 300 ml of 4.5 per cent NaOH to 880 ml containing 8.8 g of DNS and 22.5 g potassium sodium tartarate). The resulting colored solutions were diluted with distilled water, vortexed and the absorbance was read at 500 nm. The crude extract preparation mixed with laminar in at zero time incubation served as blank. The enzyme activity was expressed as μ g equivalents of glucose min⁻¹ g fresh weight⁻¹.

Compatibility Of Seaweeds With *P. Fluorescens* (Utpal Dey et al., 2015)

The compatibility was determined for *P. fluorescens*, seaweed extract, by using PDA and KB medium, respectively. The medium was mixed with the *P. fluorescens* each at 2 ml/18ml of PDA. Each milliliter of fungal and bacterial suspension contained 1×10^8 cfu/ml. the medium containing biocontrol agents were poured into Petri plates and immediately after solidification, two sterile paper discs were placed at equidistance to each other on the seeded medium. 5 ml of the 20 per cent seaweed extract after passing through bacterial filters was added to first sterile filter paper disc and 5 ml of 0.01 ml of sterile distilled water was added to second filter paper disc as control, respectively. The plates were incubated at room temperature 72 h (*P. fluorescens*) and observed for the inhibition zone. Absence of inhibition zone around the disc indicates the compatibility with respective bacterial and fungal isolates and the presence of inhibition zone indicates the incompatibility.

3. INDUCTION OF DEFENSE ENZYMES

Green house study was conducted to test the induction of defense enzyme on rice plants with different application of organic formulation.

β -1, 3-glucanase

β -1,3-glucanase activity was observed in the leaf samples of rice at different day intervals. Among the various's treatment, the plants treated with *S. wightii*, *P. fluorescens* (seed treatment, prophylactic spraying at 15, 30 and 45DAT) and followed by challenge inoculated with *R. solani* (T₆) recorded a maximum induction of β -1,3-glucanase activity 250.8 μ g of Glucose released/ min/g of fresh tissue on 5th day after pathogen inoculation. It was followed by the plants treated with application of *S. wightii* (prophylactic spray at 15, 30 and 45DAT) (T₅) recorded 240.5 μ g of Glucose released/ min/g of fresh tissue on 5th day after pathogen inoculation.

The enzyme activity significantly increased up to 5th day from the pathogen inoculation and then declined slowly in all the treatments (Fig 1).

Peroxidase (PO)

The activity of PO was observed in leaf sample of rice at different days interval. Among the various treatment, the plants treated with *S. wightii*, *P. fluorescens*, (seed treatment, prophylactic spraying at 15, 30, and 45 DAT) and followed by challenge inoculated with *R. solani*(T₆) recorded maximum induction of Peroxidase activity (62.50 changes in absorbance/min/g of fresh tissue) at 7th day after pathogen inoculation. It was followed by the plants treated with *S. wightii* (prophylactic spray at 15 and 30.45 DAT) (T₅) recorded a maximum induction of (59.96 changes in absorbance/ min/g of fresh tissue) respectively at the 7th day after pathogen inoculation. The enzyme activity significantly increased up to 7th day from the pathogen inoculation and then declined slowly in all the treatments (Fig 2).

Polyphenoloxidase (PPO)

Application of *S. wightii*, *P. fluorescens*(seed treatment, prophylactic spraying at 15, 30, and 45 DAT) and followed by challenge inoculated with *R. solani* (T₆) recorded maximum induction of PPO activity (3.38 changes in absorbance/min/g of fresh tissue) at 7th day, which decreased further. Without inoculation of pathogen and organic combination, a minimum poly phenol activity was recorded when compared to all other treatments. In all the treatments, enzyme activity increased up to 7th day and there after declined (Fig 3).

Phenylalanine Ammonialyase (PAL)

PAL activity was found to increase significantly in plants treated with *P. fluorescens*, *S. wightii* (seed treatment, prophylactic spraying at 15, 30 and 45 DAT) inoculated with *R. solani* (T₆). Maximum induction of PAL activity (5.10 changes in absorbance/min/g of fresh tissue) at 7th day. There after it decreased. It was followed by the application of *S. wightii* (prophylactic spray at 15, 30 and 45 DAT) (T₅) recorded a maximum induction at the 7th day of (5.03 changes in absorbance/min/g of fresh tissue) respectively. The enzyme activity significantly increased up to 7th day from the pathogen inoculation and then declined slowly in all the treatments (Fig 4). tissue on 5th day after pathogen inoculation.

4. DISCUSSION:

Sheath blight of rice caused by *Rhizoctonia solani* is one of the most important fungal diseases affecting rice plant and assuming greater importance all over the rice growing areas. In India, Yield losses due to sheath blight infection ranges from 8 to 50% depending on severity of the disease, stage of the crop at which it was infected by the fungus and overall environmental conditions (Savary et al., 2000; Singh et al., 2004). Hence this study is carried out to assess the yield loss due to infection and formulate the effective scope of organic formulation management practices.

Induction Of Defense Mechanism

Plants are bestowed with various defense related genes and it is well known that the defense genes are sleeping genes and appropriate stimuli or signals are needed to activate them. Inducing the plants own defense mechanisms by prior application of biological inducer is thought to be a novel plant protection strategy. Inductions of systemic resistance by marine products and organic amendments have been reported earlier by several workers (Poonpolgul and Kumphai, 2007; Jayaraj *et al.*, 2008; Ngullieet *al.*, 2010; Flora and Maria Victorialrani, 2012).

In the present study, rice plants pretreated with *S. wightii*, *P. fluorescens*, (seed treatment + prophylactic spraying at 15, 30 and 45 DAT) (T₆) significantly induced the synthesis and accumulation of β -1,3-glucanase, PO, PPO and PAL, against *R. Solani* when compared to all the other treatments.

The enzyme activity significantly increased from 7th day after the pathogen inoculation and then declined in all the treatments. Similar results were shown by Jayaraj *et al.* (2008), who reported that, *Ascochyta blight* + chlorothalonil treatment recorded a maximum induction of PO, PPO, PAL, β -1,3-glucanase, and total phenols than the application of *A. nodosum* alone in carrot against *Alternaria* leaf spot disease.

One application of extract at 0.8 or 1.6 l ha⁻¹ stimulated PO activity and two applications caused an eight-fold increase in PO activity. The treated leaves accumulated highest capsidiol (a phytoalexin in peppers) concentrations when compared to the control (Lizziet *al.*, 1998). Incorporation of *A. nodosum* extract into the planting medium caused a delayed and reduced incidence of Verticillium wilt of pepper plants. These plants also contained higher levels and early accumulation of phenolics (Garcia-Mina *et al.*, 2004).

Activity of certain defense – related enzymes, including peroxidase, polyphenyl oxidase, phenylalanine ammonia- lyase, chitinase and β - 1,3-glucanase were significantly increased in plants treated with seaweed (Jayaraj *et al.*, 2008, Manoj Kumar Solankiet *al.*, 2012) this is in accordance with our present findings.

Increase in PO and PPO activity at a later stage may contribute to cross linking of hydroxyproline rich glycoproteins (HRGPs), lignifications that will act as barriers against pathogen entry. PO-generated hydrogen peroxide may function as an anti-fungal agent in disease resistance. Hydrogen peroxide inhibits pathogens directly or it may generate other free radicals that are antimicrobial in nature (Chen *et al.*, 2000). PO is a key enzyme in the biosynthesis of lignin and other oxidised phenols (Bruce and West, 1989). PAL plays an important role in the biosynthesis of phenolics and phytoalexins (Daayfet *al.*, 1997). The increase in PAL activity indicates the activation of the phenyl propanoid pathway. The product of PAL is trans-cinnamic acid, which is an immediate precursor for the

biosynthesis of salicylic acid; a signal molecule in systemic acquired resistance (SAR) (Klessig and Malamy, 1994). PAL is a key enzyme of phenylpropanoid metabolism which leads to the synthesis of phenols (Massalae *al.*, 1980). These enzymes act upon the fungal cell wall resulting in degradation and loss of inner contents of cells (Benhamou *et al.*, 1996). The enzymatic degradation of the fungal cell wall may release non-specific elicitors (Hammerschmidt *et al.*, 1982) which in turn elicits various defense reactions. The fungal cell wall elicitors have been reported to elicit various defense reactions in green gram (Ramanathan *et al.*, 2000). The phenomenon of h free-proline accumulation in plants exposed to diverse environmental and biological stresses have considerable physiological significance.

In conclusion, prior treatment of combined application of *S. wightii*, *P. fluorescens* (seed treatment + prophylactic spraying at 15, 30 and 45 DAT) (T₆) followed by challenge inoculation with *R. solani* triggered the plant mediated defense mechanism that in turn reduce the sheath blight incidence in rice.

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Figure 1. β -1,3-glucanase activity σ in rice plants treated with different organic formulations under green house condition

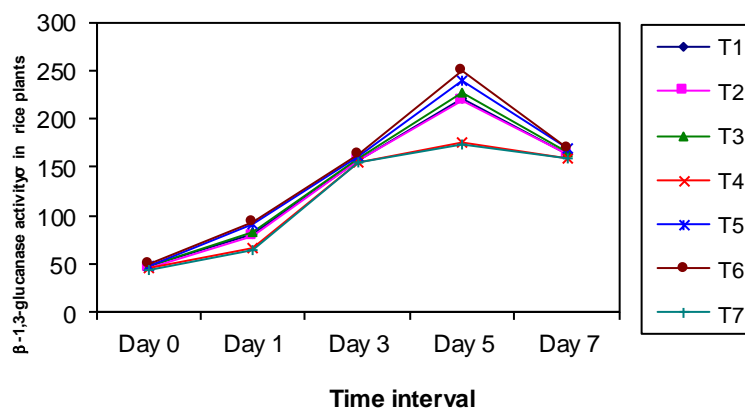


Figure 2. Peroxidase activity σ in rice plants treated with different organic formulations under green house condition

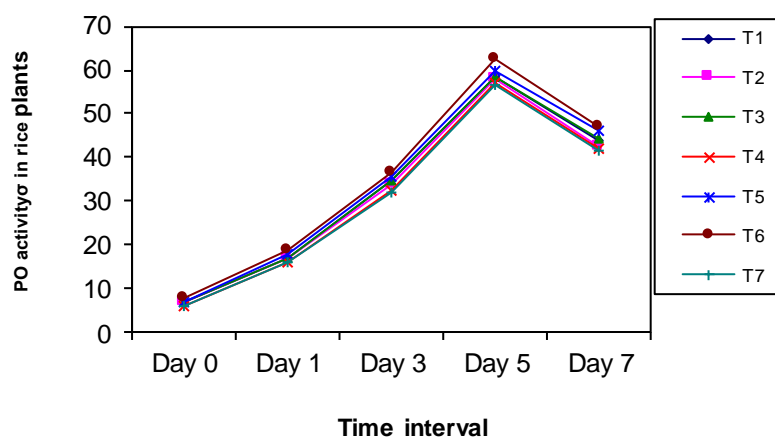


Figure 3. polyphenoloxidase activity^σ in rice plants treated with different organic formulations under green house condition

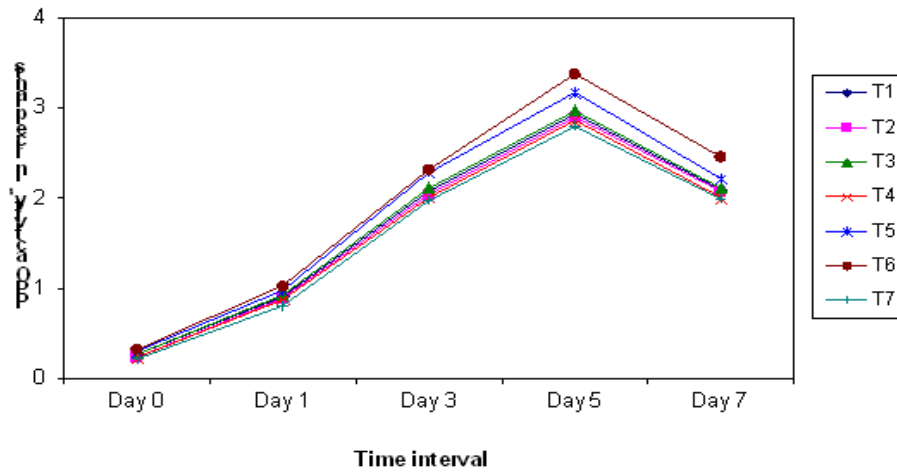


Figure 4. Phenylalanine ammonia-lyase activity^σ in rice plants treated with different organic formulations under green house condition

