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STUDIES ON THE INDUCTION OF HOST DEFENSE ENZYMES IN RICE DUE TO THE APPLICATION OF SILICON NUTRIENT, ORGANIC PRODUCT AND *BIPOLARIS ORYZAE* INOCULATION

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Abstract

The present studies were undertaken to investigate the changes in host defense enzymes in rice due to application of silicon nutrient, organic product *Panchakavya* and brown spot pathogen inoculation. Foliar application of silicon based nutrient potassium silicate @ 3 percent on 15 DAT (Days After Transplanting) along with foliar spray of organic product *Panchakavya* @ 5 percent on 30 DAT reduces the brown spot incidence and increased biometric characters of rice *var.* ADT 36. There is an increased the activity of host defense-related enzymes such as Peroxidase (PO), Polyphenol oxidase (PPO) and Phenylalanine ammonia lyase (PAL) were noticed due to application of potassium silicate and organic product. The activity of peroxidase increased up to 14th day of sampling and then decreased in all the treatments. Also, the induction of PAL reached the maximum on the 14th day and thereafter gradual decline was observed. Whereas, the PPO activity increased from the 7th day of sampling and maximum was observed on 21st day of sampling in all the treatments. Inoculation of brown spot pathogen also significantly increased the polyphenol oxidase (PPO) activity.

Key words: rice, brown spot, peroxidase, poly phenol oxidase, PAL activity

Introduction

Rice (*Oryza sativa* L.) is the second most cultivated crop worldwide and it has been estimated that half the world's population survives wholly or partially on this crop (Van Nguyen and Ferrero, 2006) and rice provides more calories per ha than any other cereal food grains. Also, rice crop has been under cultivation from time immemorial, being grown under varying climatic conditions in different parts of the world. With less land available to expand rice-growing areas due to competing demands from urbanization and industrialization on existing rice lands, production increases should come from intensive agriculture in existing lands of favourable and less favourable areas. Besides, the diseases caused by various pathogens continue to be the major constraint for enhancing the rice production.

Rice crop is widely affected by a number of diseases caused by fungi, bacteria, viruses and mycoplasma which results in considerable yield losses (Ou, 1985). Among the various fungal diseases of rice, brown spot or sesame leaf spot incited by *Helminthosporium oryzae* (Breda de Haan) Subram. and Jain (Syn: *Bipolaris oryzae* (Breda de Haan) Shoemaker) is found to occur in most rice growing areas. The disease is also referred as fungal blight or Helminthosporiose. In India, the first occurrence of the disease was perhaps during 1918-1919 in the deltaic tracts of Godavari and Krishna (Sundaraman, 1922). This disease was very much in news when the famine enquiry commission (1945) concluded that the main cause of the Bengal famine of 1942-1943 was brown spot or sesame leaf spot incited by *H. oryzae* and the losses then amounted to 50-90 percent (Ghose *et al.,* 1960; Padmanabhan, 1973a). Another epidemic of the brown spot disease occurred in Thanjavur district of Tamil Nadu during 1970-1971 (Padmanabhan, 1973b).

Normally fungicides are primary means of controlling plant diseases. But the use of chemical fungicides is under special scrutiny for posing potential environmental threat as the indiscriminate use of chemical fungicides resulted in environmental pollution and ill-health to biotic community as a whole. Even if acceptable fungicides

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are applied the pathogen often develops resistance and produce new biotypes. The increased consumer preference for healthy agricultural products and environmental risks associated with chemical residues in food are the major driving forces for the search of new safer control methods. Therefore, the present studies were undertaken to investigate the changes in host defense enzymes in rice due to application of silicon nutrient, organic product *Panchakavya* and brown spot pathogen inoculation.

Materials and Methods

Crop, Variety and Source

Crop : Paddy (*Oryza sativa* L.)

Variety : ADT 36

Source : Tamilnadu Rice Research Institute (TRRI), Aduthurai, Tamilnadu

"Panchakavya" (Modified)

The following ingredients were used to prepare approximately 20 liters of *Panchakavya* stock solution. Cow dung (5 kg), cow's urine (3 liters), cow's milk (2 liters), cow's curd (2 litres) and cow clarified butter/ghee (1 litre). In addition sugarcane juice (3 litres), tender coconut water (3 liters) and ripe banana (1 kg) were added to accelerate the fermentation process (Natarajan *et al.,* 1999).

All the materials were added to a wide mouthed mud pot and kept open under shade. The contents were stirred twice a day for about 20 minutes, both in the morning and evening to facilitate aerobic microbial activity. Fifteen days after the preparation, from the stock solution three percent concentration was prepared. The spray solution (500 liters ha^{-1}) was sprayed using hand-operated sprayer with cone type nozzle four times for each crop as per the treatment schedule. The bio gas slurry and cow dung slurry were collected from Annamalai University experimental farm.

Integrated management of brown spot of rice (Pot culture)

Treatment schedule

T_1 – PS_1

T_2 – PS_2

T_3 – PK_1

T_4 – PK_2

T_5 – $PS_1 + PS_2$

T_6 – $PK_1 + PK_2$

T_7 – $PS_1 + PK_2$

T_8 – $PK_1 + PS_2$

T_9 – Carbendazim 50 WP @ 0.1 percent as foliar spray (comparison)

T_{10} – Control

(Where, PS means Potassium silicate @ 3 percent and PK means organic product *Panchakavya* @ 5 percent; PS_1, PK_1 – Foliar application on 15 DAT (Days After Transplanting); PS_2, PK_2 - foliar application on 30 DAT).

Enzyme assays - Enzyme extraction

One g of the leaf material cut into small bits was crushed in chilled 0.1 M sodium phosphate buffer at pH 7.1. The volume was made up to 5 ml with the buffer, centrifuged at 2,100 rpm. for 30 min. and the supernatant was used as the enzyme source and all the assays *viz*., polyphenol oxidase, peroxidase, phenylalanine ammonia lyase and ascorbic acid oxidase were performed in a UV Spectrophotometer at 28±2°C (Sridhar *et al.,* 1969). Enzyme activity of PO and PPO was stated in terms changes in absorbance /minute/mg of protein (Anonymous, 1965). The activity of PAL was mentioned as nmol transcinnamic acid min^{-1} mg $protein^{-1}$.

Results and Discussion

Peroxidase

The activity of peroxidase was found more in T_8 ($PK_1 + PS_2$). The minimum peroxidase was recorded in healthy control treatment (26.78 n mol transcinnamic acid min^{-1} mg $protein^{-1}$). Generally, the treatments with PK showed increased peroxidase activity when compared to other treatments and control Fig. 1. The activity of peroxidase increased up to 14th day of sampling and then decreased in all the treatments.

Phenylalanine ammonia lyase

The data presented in Fig. 2 showed significant increase in the activity of PAL in rice plants treated with PK and PS. The induction of PAL reached the maximum

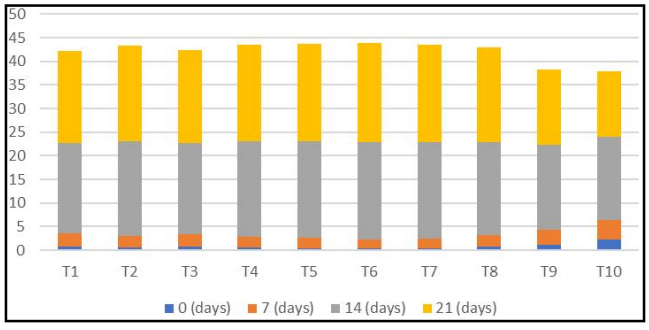

Fig. 1: Changes in Peroxidase of rice var. ADT 36 as influenced by application of PS, PK and *B.oryzae* inoculation.

on the 14th day and thereafter gradual decline was observed. Generally, the treatments with PK showed increased Phenylalanine ammonia lyase activity when compared to other treatments and control. Among the different treatments, T_8 recorded the maximum activity with 77.26 n mol transcinnamic acid min^{-1} mg $protein^{-1}$ of PAL on 21st day of sampling Fig. 2.

Polyphenol oxidase

The results revealed increased activity of polyphenol oxidase due to treatment with combined application of silicon-based nutrient, organic product and pathogen alone inoculated control. Among the treatments, T_8 ($PK_1 + PS_2$) recorded the maximum PPO activity (22.31 changes in absorbance/min/mg of protein) on 21st day after inoculation Fig. 3. The PPO activity increased from the 7th day of sampling and maximum was observed on 21st day of sampling in all the treatments. Inoculation of *B.oryzae* also significantly increased the polyphenol oxidase (PPO) activity.

An imperative feature of crop response to invading pathogens is thought to be the rapid production of host defense-related enzymes such as Peroxidase (PO), Polyphenol oxidase (PPO) and Phenylalanine ammonia lyase (PAL). In the present study also were proved to increased the activity of host defense-related enzymes such as Peroxidase (PO), Polyphenol oxidase (PPO) and Phenylalanine ammonia lyase (PAL) were noticed due to application of $PK_1 + PS_2$ (T_8). The combined treatment could have exerted a synergism mechanism among them

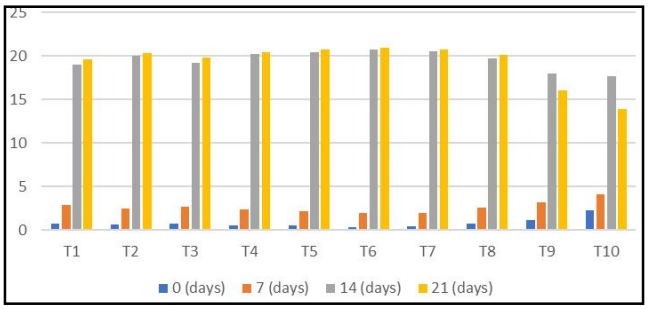

Fig. 2: Changes in PAL of rice var. ADT 36 as influenced by application of PS, PK and *B.oryzae* inoculation.

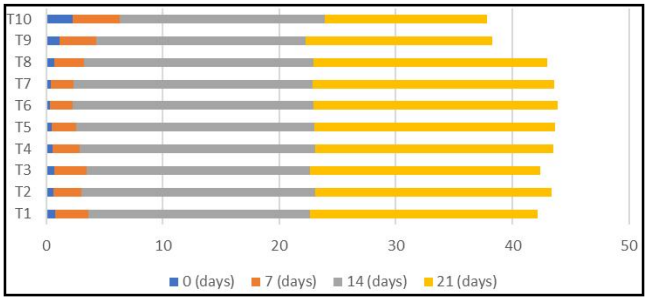

Fig. 3: Changes in PPO of rice var. ADT 36 as influenced by application of PS, PK and B.*oryzae* inoculation.

and resulted in the maximum induction of host defense related enzymes which could have been ascribed as the reason for the enhanced suppression of the brown spot pathogen.

Peroxidases (PO) participate into cell-wall reinforcement. They are involved in the final steps of lignin biosynthesis and in the cross-linking of cell wall defense proteins (Kombrink and Somssich, 1995). They are usually related to local host defense responses but they have been associated with systemic resistance in several crop species *viz.,* cucumber (Hammerschmidt *et al.,* 1982), potato (Chai and Doke, 1987) and tobacco (Ye *et al.,* 1990).

PPO is a copper containing enzyme, oxidizing phenolics to highly toxic quinines and involved in the terminal oxidation of diseased host which was attributed for its role in pathogen resistance (Kosuge, 1969). Higher PPO activity was related to increased contents of phenolic compounds, which have been shown to provide resistance against pathogens (Sharma *et al.,* 1994). The Peroxidase and Polyphenol oxidase are mentioned to have important roles in crop disease resistance (Zhang *et al.,* 2007). Sridhar and Mahadevan (1968) stated that Pyricularia oryzae and Bipolaris oryzae infected tissue exhibited an increase in host defense enzymes such as ascorbic acid oxidase and peroxidase. Increased activity of PO upon infection might be required for an additional deposition of lignin around the lesions induced by pathogens.

Phenylalanine Ammonia Lyase (PAL) is the first enzyme of the phenylpropanoid pathway and is involved in the biosynthesis of host defense compounds (Mitchell and Walters, 2004; Qin and Tian, 2005).

Vimala and Suriachandraselvan (2009) stated that earlier and increased activities of PAL in plant activator salicylic acid pre-treated Okra plants challenge inoculated with powdery mildew pathogen. Cherif *et al.,* (1994) mentioned that soluble silicon-activated defense responses to damping-off infection in cucumber, leading to increased activities of host defense enzymes and accumulation of biochemical compounds.

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