Changes in PAL and Ascorbic acid Oxidase activity of rice var. ADT 36 as influenced by application of Integrated approaches and *Bipolaris oryzae* inoculation

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Abstract

The Pot culture studies were undertaken to investigate the changes of Total phenol and O.D. phenol compound in rice as influenced by application of Zinc sulphate and foliar application of salicylic acid and Potassium silicate and Brown spot pathogen *Bipolaris oryzae* inoculation. The results revealed that Soil application of Zinc sulphate @ 25 kg/ ha along with foliar application of plant activator Salicylic acid @ 50 ppm on 15 days after transplanting and foliar spray of silicon based nutrient potassium silicate @ 3 % recorded the minimum disease incidence and maximum biometrics of rice. Also, The results revealed that the same treatment increased activity of PAL enzyme activity and Ascorbic acid oxidase activity due to treatment with combined application of resistance inducing chemical, macro-micro nutrient, silicon based nutrient and pathogen alone inoculated control when compared to comparison fungicide and control treatments.

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. In India 136.5 million tonnes of rice was produced from an area of 44.0 million ha with the productivity of 2915 kg per ha (Anonymous, 2008). In Tamil Nadu, rice is cultivated in an area of 2.05 million ha with a production of 7.2 million tonnes (Tamil Nadu Statistical Report, 2007).

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 (Current name: *Bipolaris oryzae* (Breda de Haan) Shoemaker) is found to occur in most rice growing areas.

Currently the disease is being managed by application of fungicides. Due to pesticides hazards, pollution effect, fungicide resistant, bio control agent resistant strains, lack of bioprotectant knowledge which required the integrated component approach in Indian farmer's level which will be improve growth and disease suppression.

In general defence reaction occurs due to accumulation of enzymes and PR proteins *viz*., peroxidase, phenylalanine ammonia lyase, chitinase, *β*-1, 3 glucanase, chalcone synthase, callose, lignin and phytoalexins. Plant defence enzymes *viz*., peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), β-1,3 glucanase, chitinase, catalase and defence inducing chemical (total phenols) was found to be increased in resistance inducing chemical treated plants (Sun *et al.,* 2010). Inoculation of *Erysiphe graminis* and Potassium Phosphate treatment in barley leaves led to significant increase in activities of phenylalanine ammonia lyase (PAL), peroxidase and lipoxygenase enzymes (Mitchell and Walters, 2004).

Systemic chemical inducers increased the activity of peroxidase, phenylalanine ammonia lyase, chalcone isomerise and reactive oxygen species (Silva *et al.,* 2010).

Therefore, with an aim to develop an integrated strategy involving the use of certain macro-micro nutrients, silicon based nutrients and resistance inducing chemicals for the successful sustainable management of rice brown spot. Hence, the present studies were undertaken to investigate the changes of Peroxidase and Poly Phenol Oxidase content by application of Macro-micro nutrient, Salicylic acid, potassium silicate along with pathogen inoculation.

Materials and Methods

Crop, Variety and Source

Crop : Rice (*Oryza sativa* L.)
Variety : ADT 36
Source : Tamil Nadu Rice Research Institute (TRRI), Aduthurai, Tamil Nadu.

-Pot culture studies

The pot culture studies was conducted to test the efficacy of certain macro-micro nutrients, silicon based nutrients and certain resistance inducing chemicals for assessing their influence on the incidence of brown spot of rice with various treatment and combinations. The brown spot susceptible variety ADT 36 grown in rectangular pots of size, 30x45 cm was used for the study. The plants were given artificial inoculation by spraying the spore suspensions with adequate spore load (50,000 spores/ml) at 15 DAT in the evening hours. The crop was maintained in a poly house with frequent spraying of water to provide adequate moisture and relative humidity to enable successful infection by the pathogen. The experiments were conducted in a randomized block design with three replications for each treatment and a suitable control. The fungicide carbendazim 50 WP @ 0.1 per cent was used for comparison and the standard agronomic practices as recommended by the State Agricultural Department were followed.

The effective treatments observed in different experiments conducted under pot and field conditions were pooled together and a new schedule of treatments in combination was evolved for the effective management of brown spot disease of rice. Also, zinc sulphate @ 25 Kg/ha was applied as basal application to the entire treatments (ZSS) except control and comparison. The treatment details are given below;

Treatment schedule

T_1 – ZSS + ZSF_1 + ZSF_2
T_2 – ZSS + SA_1 + SA_2
T_3 – ZSS + PS_1 + PS_2
T_4 – ZSS + ZSF_1 + SA_2
T_5 – ZSS + SA_1 + ZSF_2
T_6 – ZSS + SA_1 + PS_2
T_7 – ZSS + PS_1 + SA_2
T_8 – ZSS + PS_1 + ZSF_2
T_9 – ZSS + ZSF_1 + PS_2
T_{10} – Carbendazim 50 WP @ 0.1 per cent as foliar spray (comparison)
T_{11} – Control

$ZnSO_4$ @ 25 Kg/ha was applied as basal application to the entire treatments (ZSS) except control and comparison. The treatment details are given below;

T_1 – ZSS + Two sprays of zinc sulphate @ 3 % on 15 and 30 DAT

T_2 - ZSS + Two sprays with salicylic acid @ 50 ppm on 15 and 30 DAT.

T_3 - ZSS + Two sprays with potassium silicate @ 3 % on 15 and 30 DAT.

T_4 - ZSS + First spray with zinc sulphate @ 3 % on 15 DAT + second spray with salicylic acid @ 50 ppm on 30 DAT.

T_5 - ZSS + Second spray with zinc sulphate @ 3 % on 30 DAT

T_6 - ZSS + First spray with salicylic acid @ 50 ppm on 15 DAT + second spray with potassium silicate @ 3 % on 30 DAT

T_7 - ZSS + First spray with potassium silicate @ 3 % on 15 DAT + second spray with salicylic acid @ 50 ppm on 30 DAT

T_8 - ZSS +First spray with potassium silicate @ 3 % on 15 DAT + second spray with zinc sulphate @ 3 % on 30 DAT

T_9 - ZSS + First spray with zinc sulphate @ 3 % on 15 DAT + second spray with potassium silicate @ 3 % on 30 DAT

T_{10} – Carbendazim (0.1 %) – Comparison

T_{11} - Un treated control.

Phenolic changes - Method of sampling

Samples of plant materials from each treatment were taken at 0, 7, 14 and 21 days after inoculation both in healthy and inoculated plants for estimating the changes in the biochemical constituents *viz.*, reducing sugars, non-reducing sugars, total sugars, starch, ortho dihydroxy phenols, total phenols, amino nitrogen, protein and enzymes like peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and ascorbic acid oxidase.

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).

Enzymes	References
Phenylalanine ammonia lyase (PAL)	Southern and Deverall, 1990
Ascorbic acid oxidase	Oberbachner and Vines, 1963

The activity of PAL was expressed as nmol transcinnamic acid min^{-1} mg $protein^{-1}$.

Results and Discussion

Post infectional enzymatic changes: Phenylalanine ammonia lyase

The data depicted in table 1 showed significant increase in the activity of PAL in rice plants treated with ZS, SA and PS. The induction of PAL reached the maximum on the 14^{th} day and thereafter gradual decline was observed. Generally the treatments with SA showed increased Phenylalanine ammonia lyase activity when compared to other treatments and control. Among the different treatments, T_6 recorded the maximum activity with 74.58 n mol transcinnamic acid min^{-1} mg $protein^{-1}$ of PAL on 21^{st} day of sampling.

Ascorbic acid oxidase

Ascorbic acid oxidase activity was significantly influenced by ZS (Zinc sulphate), SA (Salicylic acid) and PS (Potassium silicate) application (Table 2). Generally the treatments with SA showed increased Ascorbic acid oxidase activity when compared to other treatments and control. Among the different treatments, T_6 recorded the maximum activity with 91.36 units/min/mg of protein on 21st day of sampling. Ascorbic acid oxidase content increased up to 7th day of sampling and then decreased in all the treatments.

The results depicted in Table 1 and 2 revealed rapid production and increased activity of defense-related enzymes such as PAL and Ascorbic acid oxidase activity respectively due to treatment with ZSS + SA_1 + PS_2 (T_6) .

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

Cherif *et al.* (1994) reported that soluble silicon-activated defense responses to *Pythium* infection in cucumber, leading to increased activities of chitinases, POD, PPO and accumulation of phenolic compounds. Vimala and Suriachandraselvan (2009) reported that earlier and increased activities of PAL in salicylic acid pre-treated bhendi plants challenge inoculated with *Erysiphe cichoracearum*.

Yu *et al.* (2011) proved that foliar application of silicon in cucumber plants inoculated with *Pseudoperonospora cunbensis* enhanced the defense related enzymes like Peroxidase, Polyphenol oxidase and Phenylalanine ammonia lyase.

SA treated plants showed an increase in Peroxidase, PPO and Ascorbic acid oxidase when *Cercosporidium personatum* was inoculated in groundnut (Meena *et al*., 2001). SA was found to enhance the activities of antioxidant enzymes, CAT, Peroxidase (PO) and Superoxide dismutase (SOD), when sprayed exogenously to the drought stressed plants of tomato (Hayat *et al.,* 2008) or to the salinity stressed plants of *Brassica juncea* (Yusuf *et al.,* 2008). Krantev *et al.* (2008) reported the exogenous application of salicylic acid enhanced the activities of antioxidant enzymes ascorbate peroxidase (APX) and SOD with a concomitant decline in the activity of CAT in maize plants. All these reports corroborates with the present findings.

The combination treatment consisting of ZSS, SA_1 and PS_2 (T_6) increases the PAL enzyme activity and Ascorbic acid activity when compared to control and fungicide treatments.

References

Anonymous, (2008). Enzyme nomenclature, Elsevier Publishing Co. Amsterdam, The Netherlands.

Cherif, M., Asselin, A. and **Belanger, R.R.** (1994). Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. *Phytopathol.,* **84**: 236–242.

Hayat, S., Hasan, S.A., Fariduddin, Q. and **Ahmad, A.** (2008). Growth of tomato (*Lycopersicon esculentum*) in response to salicylic acid under water stress. *J. Plant Int*., **3** (**4**): 297–304.

Krantev, A., Yordanova, R., Janda, T., Szalai, G. and **Popova, L**. (2008). Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. *J. Plant Physiol*., **165**: 920–931.

Meena, B., Marimuthu, T. and **Velazhahan, R**. (2001).Salicylic acid induces systemic resistance in groundnut against late leaf spot caused by *Cercosporidium personatum. Journal of Mycology and Plant Pathology*, **31 :** 139-145.

Mitchell, A.F. and **Walters, D.R.** (2004). Potassium phosphate induces systemic protection in barley to powdery mildew infection. *Pest Manag Sci.,* **60**: 126-134.

Ou,S.H. 1985, Rice Diseases, 2nd Edition, Common Wealth Mycological Institute, U.K. 380p.

Qin, G.Z. and **Tian, S.P.** (2005). Enhancement of biocontrol activity of Cryptococcus laurentii by silicon and the possible mechanisms involved. *Phytopathology,* **95**(**1**): 69-75.

Silva, R.V., Oliviera, R.D.L., Nascimento, K.J.T. and **Rodrigues, F.A.** (2010). Biochemical responses of coffee resistance against *Meloidogyne exigua* mediated by silicon. *Plant Pathol.,* **59**: 586-593.

Sridhar, R., Chandramohan, D. and **Mahadevan, A.** (1969). The role of parasite and its metabolism in triggering host physiology, *Phytopath. Z.* **69** : 21-27.

Sun, W., Zhang, J., Fan, Q., Xue, G., Li, Z. and **Liang, Y.** (2010). Silicon enhanced resistance to rice blast is attributed to silicon mediated defense resistance and its role as physical barrier. *Eur J Plant Pathol.,* **128**: 39-49.

Tamil Nadu Statistics Report, (2007). Department of Economics and statistics, Government of India, Season, Area and Crop production report 2006-2007. (Ref.: www.tn.gov.in)

Van Nguyen, N. and **Ferrero, A.** (2006). Meeting the challenges of global rice production. *Paddy water Environ.,* **4**: 1-9.

Vimala, R. and **Suriachandraselvan, M**. (2009). Induced resistance in Bhendi against powdery mildew by foliar application of salicylic acid. *Journal of Biopesticides,* **2**(**1**): 111-114.

Yu, Y., Schjoerring, J.K. and **Du, X.** (2011). Effects of silicon on the activities of defense-related enzymes in cucumber inoculated with *Pseudoperonospora cubensis*. *Journal of Plant Nutrition,* **34**: 2, 243 – 257.

Yusuf, M., Hasan, S.A., Ali, B., Hayat, S., Fariduddin, Q. and **Ahmad, A.** (2008). Effect of salicylic acid on salinity induced changes in *Brassica juncea*. *J. Integrative Plant Biol.* **50** (**8**): 1–4.

Table 1. Changes in Phenylalanine ammonia lyase of rice var. ADT 36 as influenced by application of ZS, SA, PS and *B.oryzae* inoculation

T.No.	Treatments	Phenylalanine ammonia lyase (n mol transcinnamic acid min^{-1} mg $protein^{-1}$)			
		0 (days)	7 (days)	14 (days)	21 (days)
1	ZSS + ZSF_1 + ZSF_2	19.51	98.83	108.46	70.19
2	ZSS + SA_1 + SA_2	21.06	100.59	113.72	72.77
3	ZSS + PS_1 + PS_2	19.67	99.47	109.78	70.48
4	ZSS + ZSF_1 + SA_2	21.39	101.18	117.65	72.95
5	ZSS + SA_1 + ZSF_2	21.55	101.44	119.10	73.67
6	ZSS + SA_1 + PS_2	22.14	102.80	123.56	74.58
7	ZSS + PS_1 + SA_2	21.73	101.78	119.73	73.88
8	ZSS + PS_1 + ZSF_2	19.83	99.71	110.83	70.67
9	ZSS + ZSF_1 + PS_2	20.04	99.94	111.52	70.94
10	Carbendazim	17.46	88.74	93.65	64.58
11	Control (inoculation)	12.53	81.68	86.77	53.45
12	Control (Healthy)	11.25	70.21	74.26	48.62

Table 2. Changes in Ascorbic acid oxidase of rice var. ADT 36 as influenced by application of ZS, SA, PS and *B.oryzae* inoculation

T.No.	Treatments	Ascorbic acid oxidase (Units/min/mg of protein)			
		0 (days)	7 (days)	14 (days)	21 (days)
1	ZSS + ZSF_1 + ZSF_2	20.38	104.42	97.52	79.66
2	ZSS + SA_1 + SA_2	21.52	113.62	99.92	83.65
3	ZSS + PS_1 + PS_2	20.52	106.58	97.99	80.02
4	ZSS + ZSF_1 + SA_2	21.94	115.44	100.76	86.62
5	ZSS + SA_1 + ZSF_2	22.47	115.92	101.33	87.19
6	ZSS + SA_1 + PS_2	23.12	116.99	101.80	91.36
7	ZSS + PS_1 + SA_2	22.96	116.70	102.46	89.92
8	ZSS + PS_1 + ZSF_2	20.74	108.30	98.36	80.18
9	ZSS + ZSF_1 + PS_2	20.92	111.17	98.74	80.78
10	Carbendazim	18.56	100.02	89.42	73.23
11	Control (inoculation)	13.60	81.06	63.58	49.27
12	Control (Healthy)	11.48	70.17	53.84	41.37