Changes in Sugar compounds of rice var. ADT 36 as influenced by application of Integrated disease management 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. The same treatment recorded lowest reducing, non-reducing and total sugars content when compared to comparison fungicide and control treatments. The sugar content gradually decreased with the sampling period.

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.

The pathogen that invades the host utilizes the sugar present in the host as they form the major sources of energy for the growth and development of the pathogen. Deranged carbohydrate metabolism of the host in response to infection was investigated by several workers (Sindhan *et al.*, 1996; Paul, 1998; Dagade, 2003). Sugars form the major source of energy for the development of pathogens inside

the host plant. Sugar content was altered when rice leaves were infected with foliar pathogens (Asada, 1962). Sridhar and Mahadevan (1979) observed higher amount of reducing and non-reducing sugars in the brown leaf spot susceptible rice varieties. Vidhyasekaran (1974) found increase in soluble sugars particularly glucose, fructose and sucrose after inoculation with *H. nodulosum* in susceptible cultivar than in the resistant cultivar. Sreerama kumar (1990) reported decreased reducing, non reducing sugar, total phenol and OD phenol content with increasing fungitoxicty of plant extracts in *H.oryzae* infected rice plants.

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 Phenol 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.

Preparation of ethanol extracts (Mahadevan and Sridhar, 1986)

Plant materials of both healthy and infected were collected and 4 g samples were taken. They were chopped and then extracted in 16 ml of boiling 80 per cent ethanol for 5 min. and cooled in running tap water. The material was homogenized by grinding in a porcelain pestle and mortar and squeezed through two layers of cheese cloth. The residue was transferred back to 5 ml of boiling 80 per cent ethanol and reextracted for 5 min. cooled and filtered. Both the extracts were pooled and filtered through Whatman No.41 filter paper. A jet of ethyl alcohol was used to wash the filter paper and the final volume was adjusted to 20 ml with 80 per cent ethyl alcohol, so as to get 5 ml of the extract representing every g of plant tissue. The ethanol extract was used for the estimation of sugars, phenols, amino nitrogen and protein. The biochemical constituents were assessed based on standard procedures.

Biochemical constituents	References
Reducing sugars	Nelson, 1944
Total sugars	Nelson, 1944
Non reducing sugars	Inman, 1965

Results and Discussion

Post infectional biochemical changes - **Reducing sugars**

From the results depicted in table 1, a general reduction was observed in the quantity of reducing sugars due to combination treatment with ZS, SA and PS. Also, decreasing levels of reducing sugar content was observed with increase in time. In inoculated plants the level of reducing sugar was higher than in control. Application of SA along with ZS or PS (T_6, T_7, T_5 and T_4) reduced the reducing sugar content in ADT 36 significantly when compared to control (30.82 mg/g). The reduction in reducing sugar content was maximum (19.25 mg/g) in T_6 (ZSS + SA at 15 DAT + PS at 30 DAT).

Non-reducing sugars

Combinations of spray with ZS, SA and PS significantly influenced the non-reducing sugars content when compared to control. In inoculated plants, the non-reducing sugar content was more in control (4.79 mg/g) on 21st day of sampling. The non-reducing sugars content decreased as the sampling periods increased. Minimum non-reducing sugar content (0.42 mg/g) was observed in -T_6 (ZSS + SA at 15 DAT + PS at 30 DAT) at all the sampling periods (Table 2).

Total sugars

Similar to the trend observed in reducing and non-reducing sugar content, the treatments with combinations of ZS, SA and PS reduced the total sugar content when compared to control. Maximum total sugar was recorded in control (36.08 mg/g). Minimum content was recorded in T_6

(ZSS + SA at 15 DAT + PS at 30 DAT) (16.91 mg/g). An increase in sampling period gradually decreased the total sugar content in all the treatments (Table 3).

From the results depicted in table 1, 2 and 3, a general reduction was observed in the quantity of reducing sugars, non-reducing sugars and total sugars whereas a significant increase was noticed due to combination treatment with ZS (Zinc sulphate), SA (Salicylic acid) and PS (Potassium silicate).

Carbohydrates are the basic building blocks for the synthesis of various defense chemicals such as phenolics, phytoalexins and lignin. Hence, the quality and quantity of sugars play an important role in disease resistance (Vidhyasekaran and Kandasamy, 1972; Vidhyasekaran, 1974). Altering the sugar content of leaves has been shown to be a possible way to control diseases (Sondeep singh *et al.,* 2009) and interfering with the physiology of the host could potentially offer an exciting opportunity to control diseases.

Pathogen infection leading to decreased sugar contents has been reported in sweet corn (Levy and Cohen, 1984). Kalim *et al.* (2003) reported significant decrease in the quantity of total soluble sugars in zinc and manganese treated roots of plants inoculated with *Rhizoctonia solani* and *R. bataticola*. The reducing, non and total sugar content were decreased with increasing conc. of resistance inducing chemicals in *H. oryzae* infected plants (Vengadesh Kumar, 2005). The exogenous SA application enhanced the carbohydrate content in maize (Khodary, 2004). Reduction of sugars and accumulation of starch due to the application of combination of lignite fly ash with potash in blast infected leaves was reported by Mallika and Ramabadran (1995) and Karpagavalli (1999). These reports lend support to the present findings.

The combination treatment consisting of ZSS, SA_1 and PS_2 (T_6) reduces the sugar compounds (reducing, non reducing and total sugars) compound when compared to control and fungicide treatments.

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

T. No.	Treatments	Reducing sugars (mg/g)			
		0 (days)	7 (days)	14 (days)	21 (days)
1	ZSS + ZSF_1 + ZSF_2	34.71	33.42	29.98	29.09
2	ZSS + SA_1 + SA_2	34.27	26.12	23.94	23.44
3	ZSS + PS_1 + PS_2	34.67	32.70	29.65	28.89
4	ZSS + ZSF_1 + SA_2	34.10	25.98	21.92	21.74
5	ZSS + SA_1 + ZSF_2	33.96	25.91	21.50	21.24
6	ZSS + SA_1 + PS_2	33.75	25.70	19.69	19.25
7	ZSS + PS_1 + SA_2	33.82	25.76	20.32	20.18
8	ZSS + PS_1 + ZSF_2	34.60	33.14	28.86	28.13
9	ZSS + ZSF_1 + PS_2	34.52	30.98	27.53	27.29
10	Carbendazim	34.94	33.64	30.36	29.65
11	Control	35.38	33.99	31.47	30.82

Table 2. Changes in Non - reducing sugars of rice var. ADT 36 as influenced by application of ZS, SA, PS and *B.oryzae* inoculation

T. No.	Treatments	Non - reducing sugars (mg/g)			
		0 (days)	7 (days)	14 (days)	21 (days)
1	ZSS + ZSF_1 + ZSF_2	5.55	3.98	2.74	1.89
2	ZSS + SA_1 + SA_2	5.39	3.42	2.29	1.07
3	ZSS + PS_1 + PS_2	5.52	3.96	2.69	1.80
4	ZSS + ZSF_1 + SA_2	5.33	3.30	2.14	0.82
5	ZSS + SA_1 + ZSF_2	5.30	2.97	2.10	0.69
6	ZSS + SA_1 + PS_2	5.19	2.13	0.93	0.42
7	ZSS + PS_1 + SA_2	5.26	2.56	1.70	0.65
8	ZSS + PS_1 + ZSF_2	5.49	3.92	2.53	1.57
9	ZSS + ZSF_1 + PS_2	5.42	3.80	2.42	1.35
10	Carbendazim	5.63	4.24	2.92	2.02
11	Control	5.97	5.11	4.97	4.79

Table 3. Changes in Total sugars of rice var. ADT 36 as influenced by application of ZS, SA, PS and *B.oryzae* inoculation

T. No.	Treatments	Total sugars (mg/g)			
		0 (days)	7 (days)	14 (days)	21 (days)
1	ZSS + ZSF_1 + ZSF_2	39.75	35.04	30.67	22.90
2	ZSS + SA_1 + SA_2	38.78	30.19	24.93	21.53
3	ZSS + PS_1 + PS_2	39.68	34.82	30.32	22.73
4	ZSS + ZSF_1 + SA_2	36.09	26.73	19.45	20.42
5	ZSS + SA_1 + ZSF_2	36.22	26.38	18.77	18.98
6	ZSS + SA_1 + PS_2	36.08	25.96	17.06	16.91
7	ZSS + PS_1 + SA_2	36.57	26.24	18.36	18.64
8	ZSS + PS_1 + ZSF_2	39.12	31.44	28.46	22.47
9	ZSS + ZSF_1 + PS_2	38.96	30.85	27.57	22.38
10	Carbendazim	39.98	37.64	33.28	28.04
11	Control	41.76	40.12	36.96	36.08