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Biochemical alterations in respect of total phenol, sugar and protein contents in *Rhizoctonia solani* Kuhn. inoculated rice plants

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Performance of sheath blight susceptible rice cultivars Indrasail, Swarna Masuri and IET-6141 (Kunti) and a resistant cultivar IR-20 along with their metabolic and biochemical changes in respect of total phenol, sugar and protein levels in uninoculated and *R. solani* inoculated plants were investigated. Of the susceptible cultivars, Indrasail showed the best performance of all regarding yield components and accumulation of phenols and proteins. The resistant cultivar IR-20 had higher level of phenols, sugars and proteins than susceptible cultivars tested. Post-infectional increase in phenol, sugar and protein contents seemed to be associated with disease resistance. Phenol content was high 14 d after inoculation whereas sugar and protein contents were high 21 d after inoculation.

Key words: Rice, sheath blight, *Rhizoctonia solani*, phenol, sugar, protein

INTRODUCTION

Sheath blight of rice incited by *Rhizoctonia solani* Kuhn. is a serious problem to the rice growers of all over India causing heavy reduction in yield. Cultivation of some popular rice cultivars has practically been ceased due to severe occurrence of the disease in the rice growing tracts. Inspite of widespread prevelance of the disease and heavy yield loss caused by it, the biochemical changes in rice plants in relation to disease development are important findings (Zuber and Manibhusanrao, 1984). Post-infection changes in host metabolism lead to the development of dynamic defence. Higher levels of phenol and protein and huge oxidase activities have often been found to play a vital role in disease resistance following pathogen infection (Stahman, 1967 ; Kosuge, 1969 ; Seevers and Daly, 1970). In the present communication an attempt has been made to find out the performance of four rice cultivars to sheath blight infection and the corresponding alterations brought about in total phenol, sugar and protein contents in uninoculated and *R. solani* inoculated susceptible and resistant rice plants with the advancement of the disease.

MATERIALS AND METHODS

Plant materials

Four rice cultivars, viz., Indrasail, Swarna Masuri, IET-6141 (Kunti) and IR-20 were used

in this experiment. The first three are highly susceptible and the last one is resistant to sheath blight pathogen. Rice grains were soaked in tap water for 24 h and were sown thereafter in 12 cm pots with garden soil and the seedlings were raised under green house condition. After 25 d of sowing, 5 seedlings were transplanted in 16 cm pot with garden soil maintaining 5 replications of each treatment.

Pathogen, inoculation and disease scoring

A highly virulent isolate of *R. solani* was used in the present investigation. Chopped paddy straw in conical flasks was sterilized by autoclave and inoculated with 10 d old *R. solani* culture aseptically and incubated at 27± 1°C for 10 d. A few bits of inoculated paddy straw containing 3 to 5 selerotia were used to inoculate the rice plants during booting stage. Inoculation was done by inserting the sclerotia containing paddy straw inbetween stem and sheath towards the base of the plant. Disease scoring was done 14 d and 21 d after inoculation following IRRI (International Rice Research Institute) scale.

Sample collection and extraction

Samples (sheaths and leaves) were collected twice from both healthy as well as inoculated plants first during 14 d after inoculation (just with the initiation of symptom) and second during 21 d after inoculation. Only blighted tissues from sheaths and leaves were used for all analyses. Levels of total phenol, sugar and protein were estimated as follows with the aid of Bausch and Lomb Spectronic-20 colorimeter and the results represented the mean of observations from five separate samples in each case.

Total phenols

One g each of healthy and infected tissue was cut into small pieces and extracted with 5 ml of 80% ethanol on boiling water bath for 5 minutes. The liquid was decanted and another 5 ml of 80% ethanol was added for further extraction. The extracts were combined, filtered and then evaporated under reduced pressure to dryness at 40°C. The residue was dissolved in 10 ml of glass distilled water, acidified with 1 ml/l HCl to pH 4.5 and then extracted three times with equal volume of diethyl ether. The diethyl ether fractions were pooled and evaporated to dryness and the residue was taken up in 5 ml of ethanol. Total phenol was estimated using Folin-Ciocalteau reagent (Bray and Thorpe, 1954) with catechol as the standard.

Total sugars

One g each of healthy and infected tissue was cut into small pieces and extracted with 5 ml of 80% ethanol on boiling water bath for 5 minutes. The liquid was decanted and another 5 ml of 80% ethanol was added for further extraction. The extracts were pooled, filtered and then evaporated under reduced pressure to dryness at 40°C. The residue was then dissolved in 10 ml of glass distilled water. Total sugar was estimated using Anthrone reagent (Morris, 1984) with dextrose as the standard.

Total proteins

Four g each of healthy and infected tissues were ground in a glass mortar in 20 ml of 0.1 mol/l Tris-HCl buffer (pH 8.0) at 0°C. The extract was centrifuged at 30,000 g for 20

minutes at 0°C. Equal volume of cold 40% trichloroacetic acid was added to the supernatant and after standing for 30 minutes at 0°C, the precipitate was pelleted by centrifugation at 15,000 g for 20 minutes at 0°C. The pellet was subsequently extracted by suspending it first in 5 ml of ice-cold 95% ethanol containing 0.1 mol/l potassium acetate and after centrifugation in 5 ml of isopropyl ether to remove lipids and pigments. The protein precipitate was pelleted by centrifugation and dissolved in 20 ml of 0.1 mol/l sodium hydroxide solution. Total protein was estimated following the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

RESULTS

Performance of rice cultivars to sheath blight infection

Of the three susceptible cultivars tested against sheath blight infection, Swarna Masuri showed maximum disease development followed by IET-6141 (Kunti) and Indrasail. The resistant cultivar IR-20 showed much less disease development as compared to susceptible cultivars. In susceptible cultivars, the pathogen frequently spread upto flag leaves and caused death of new tillers but in resistant cultivar the pathogen was mostly localized in lower leaf sheath region, its upward spread to the uppermost leaf sheath and leaf lamina was restricted to a great extent which was clearly evidenced by the results of relative lesion height. Relative lesion height was least in IR-20 followed by Indrasail, IET-6141 (Kunti) and Swarna Masuri. Of the three susceptible cultivars, Indrasail contributed maximum yield attributes followed by IET-6141 (Kunti) and Swarna Masuri in respect of their number of panicle bearing tillers, panicle length and 100 grain weight. The yield attributes of resistant cultivar IR-20 were greater than that of susceptible cultivars Indrasail, IET-6141 (Kunti) and Swarna Masuri. A negative correlation also exists in between mean disease index per plant and plant height at maturity.

Biochemical changes in response to sheath blight infection

Total phenols

All the cultivars accumulated phenols significantly during 14 d and 21 d after inoculation. Phenol contents, however, was much more in the tissues collected from 14 d after inoculation. Total phenol content of *R. solani* inoculated plants was greater than uninoculated plants irrespective of the cultivars tested as well as at both the stages of disease development 14 d and 21 d after inoculation. The resistant cultivar IR-20 showed much more phenol content than susceptible cultivars Indrasail, IET-6141 (Kunti) and Swarna Masuri. Of the susceptible cultivars, Indrasail had greater phenol content followed by Swarna Masuri and IET-6141 (Kunti).

Total sugars

All the cultivars showed a steady increase in sugar level with the progress of the disease and the sugar content differed significantly among the treatments. Of the susceptible cultivars, Swarna Mausuri had highest sugar content followed by Indrasail and IET-6141 (Kunti). However, the resistant cultivar IR-20 showed maximum accumulation of sugar over susceptible cultivars. *R. solani* inoculated rice plants showed greater level of sugar than uninoculated rice plants irrespective of the cultivars tested.

Table 1. Performance of sheath blight susceptible (Indrasail, Swarna Masuri and IET-6141) and resistant (IR-20) rice cultivars in response to infection by *R. solani*

Cultivars	Mean disease index/plant		RLH (%)	Plant height at maturity (cm)	Productive tillers (no./hill)	Panicle length (cm)	100-grain weight (g)
	14 DAI	21 DAI					
Indrasail	5.17	6.26	84.25 (66.62)*	90.52	12.85	21.87	2.11
Swarna Masuri	5.95	7.16	91.30 (72.84)	83.95	12.19	21.63	1.69
IET-6141 (Kunti)	5.61	6.33	89.85 (71.42)	86.92	11.10	21.77	1.90
IR-20	3.53	4.65	50.73 (45.42)	85.94	13.34	22.78	2.23
C.D. at 5%	0.24	0.31	2.82	NS	0.44	0.23	0.07

Figures (*) in parentheses indicate angular transformed values

DAI : Days after inoculation

RLH : Relative lesion height

NS : Not significant

Table 2. Total phenols in uninoculated and *R. solani* inoculated susceptible (Indrasail, Swarna asuri and IET-6141) and resistant (IR-20) rice cultivers

Cultivars	Total phenols (mg/g fresh weight of tissue)			
	14 DAI		21 DAI	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Indrasail	3.87	5.59	3.57	4.68
Swarna Masuri	3.77	4.90	3.33	4.13
IET-6141(Kunti)	1.25	2.07	1.50	1.83
IR-20	7.51	8.73	6.68	7.94
C.D. at 5%	0.057		0.082	

C.D. to compare between any two means between uninoculated and inoculated columns and also within columns

DAI : Days after inoculation

Table 3. Total sugars in uninoculated and *R. solani* inoculated susceptible (Indrasail, Swarna Masuri and IET-6141) and resistant (IR-20) rice cultivers

Cultivars	Total sugars (mg/g fresh weight of tissue)			
	14 DAI		21 DAI	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Indrasail	10.43	10.91	10.85	11.97
Swarna Masuri	10.73	12.68	10.96	14.92
IET-6141 (Kunti)	10.13	10.93	10.82	12.54
IR-20	16.57	19.13	16.90	21.17
C. D. at 5%	0.123		0.072	

C.D. to compare between any two means between uninoculated and inoculated columns and also within columns.

DAI : Days after inoculation

Table 4. Total proteins in uninoculated and *R. solani* inoculated susceptible (Indrasail Swarna Masuri and IET-6141) and resistant (IR-20) rice cultivers

Cultivars	Total proteins (mg/g fresh weight of tissue)			
	14 DAI		21 DAI	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Indrasail	3.32	4.48	3.88	5.03
Swarna Masuri	3.24	4.11	3.72	4.76
IET-6141 (Kunti)	3.12	3.23	3.28	3.57
IR-20	7.44	8.84	7.86	10.11
C.D. at 5%	0.090		0.092	

C.D. to compare between any two means between uninoculated and inoculated columns and also within columns.

DAI : Days after inoculation

Total proteins

Both resistant and susceptible cultivars differed significantly in the synthesis and accumulation of total proteins in response to *R. solani* infection. Inoculated plants always showed higher level of protein contents than uninoculated healthy ones in case of all the cultivars tested as well as at both the stages of disease development 14 d and 21 d after inoculation. Of the susceptible cultivars, Indrasail had higher level of protein content followed by Swarna Mausri and IET-6141 (Kunti). The resistant cultivar IR-20 accumulated much more protein than susceptible cultivars. Like sugars, protein level was much more in the tissues collected from 21 d after inoculation than the tissues collected from 14 d after inoculation.

DISCUSSION

The experimental findings provided evidence in support of the earlier idea that the phenolics play a vital role in disease resistance by inhibiting fungal growth through the development of brown necrotic lesion (Kosuge, 1969). Sheath blight susceptible cultivars Indrasail, Swarna Masuri and IET-6141 (Kunti) were found to have reduced level of phenol, sugar and protein than resistant cultivar IR-20. Increase in protein level indicated increased enzyme and metabolic activity in the rice plants. The post-infectional changes in the phenol, sugar and protein contents clearly proved increased dynamic response. The pre-infectional increase in protein and phenol levels was correlated with the protective effect derived upon challenge with biotic agent. Inter-estingly, both uninoculated and *R. solani* inoculated plants of all the cultivars showed an initial decrease (14 d after inoculation) in total sugar and protein content which increased later (21 d after inoculation) with the progress of the disease. Besides, *R. solani* inoculated plants always had higher level of phenol, sugar and protein contents than the uninoculated ones diseased condition (Kuc, 1972). Phenolics are synthesized in huge amount due to disease (Seevers and Daly, 1970 ; Daly *et al.*, 1971). Disintegration of tissues may result in the accumulation of phenols in susceptible plants (Edreva, 1977). In the present study, the same trend was noticed regarding the synthesis and accumulation of phenols in susceptible cultivars. Zuber and Manibhusanrao (1983) showed that increase in phenolies in the sheath blight

susceptible cultivar TKM-9 was correlated with the corresponding decrease in sugar content. Reddy and Sridhar (1975) stated that major portion of sugar is shunted to polyphenol synthesis. In the present stydy, systhesis and accumulation of sugars and phenols in the rice plants in response to *R. solani* infection support the views of Zuber and Manibhusanrao (1983) and Reddy and Sridhar (1975). As the phenolics are synthesized from sugars through shikimic acid pathway, reduction in sugar contents may be responsible for decreased accumulation of phenols (Lukens, 1968). In all the experiments, it was strongly evidenced that a negatively high correlation exists between total sugar, phenol and protein contents in plants and mean disease index per plant.

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