Influence of Biochemical Composition of Rice Leaf Sheaths on Sheath Rot Incidence

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SUMMARY

Boot leaf sheath contained almost three times more carbohydrate than non-boot leaf sheaths in all the three rice cultivars tested. The maximum amount of carbohydrate was recorded in healthy boot leaf sheath of ADTRH 1(306.3 mg/g), followed by ADT 39 (281 mg/g) and CO 43 (271.7 mg/g). There was a significant reduction in carbohydrate content of boot leaf sheath due to *S.oryzae* infection. The *S.oryzae* infected boot leaf sheath of ADTRH 1 recorded 182.0 mg/g of carbohydrate followed by ADT 39 (167.3 mg/g) and CO 43 (132.0 mg/g). The decrease in carbohydrate content of infected boot leaf sheath over healthy boot leaf sheath varied from 40.46 per cent to 51.12 per cent. The per cent decrease in protein content of infected boot leaf sheath over healthy boot leaf sheath was 41.67, 41.38 and 33.33 in ADTRH 1, CO 43 and ADT 39, respectively. The healthy boot leaf sheath recorded significantly higher amount of protein than healthy non-boot leaf sheath and infected boot leaf sheath. The per cent increase in phenolic content of infected boot leaf sheath over healthy boot leaf sheath was 37.71, 29.79 and 43.10 in ADTRH 1, CO 43 and ADT 39, respectively.

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The fungal sheath rot incited by *Sarocladium oryzae* is present in all the rice growing countries worldwide. The disease is considered a highly destructive disease in Tamil Nadu and other rice growing states of India (Chakravarty and Biswas, 1978; Lakshmanan, 1993). The fungus, *S.oryzae* primarily attacks the flag leaf sheath through stomata or injuries and ramifies intercellularly in the vascular bundles and mesophyll tissues of some susceptible cultivars (Shahjahan *et al*., 1977).

Lesions start at the uppermost leaf sheath enclosing young panicles as oblong or irregular spots, with brown margins and gray centre or brownish gray throughout. Panicles remain within the sheath or may partially emerge with discoloured and ill filled grains. Affected leaf sheaths have abundant whitish powdery mycelium. The pathogen infects rice plants at all growth stages, but it is most destructive after the booting stage (Milagrosa, 1987). In some of the tillers of the infected plants, panicles do not develop at all (Singh and Mathur, 1992; Mew and Gonzales, 2002). The present study was carried out to find out the biochemical composition of healthy boot leaf sheaths, healthy non boot leaf sheaths and changes due to *S.oryzae* infection, since the pathogen manifests only on boot leaf sheaths during flowering.

MATERIALS AND METHODS

Both healthy and sheath rot infected boot leaf sheath (5 g each) of three ruling varieties of rice in Tamilnadu *viz*., ADTRH 1, CO-43 and ADT 39 were collected, shade dried and powdered. The ethanol extract was prepared and used for further analysis. Four healthy leaf sheaths immediately below the boot leaf were also collected and used for bio-chemical analysis. They were used for estimation of total carbohydrate, total soluble protein and total phenols.

Total soluble protein:

The seed sample (1g) was homogenated in 10 ml of acetate buffer (0.1 M, pH 4.7), centrifuged at 5000 g for 15 minutes and the supernatant was saved. The reaction mixture consisted of 0.5 ml of enzyme extract, 0.5 ml of distilled water and 5 ml of dye solution (Coomassic brilliant blue). The intensity of colour developed was read at 595nm in a Hitachi Spectrophotometer. The protein content was estimated as BSA equivalent (Sadasivam and Manickam, 1996).

Total carbohydrate :

One hundred mg of the leaf sheath sample was weighed and transferred to a boiling tube. The sample hydrolyzed by keeping in a boiling water bath for 3 hour with 5 ml of 2.5 N HCl and cooled to room temperature. It was neutralized with sodium carbonate until the effervescence ceased and the volume was made up to 100 ml and centrifuged. The supernatant was collected and 1 ml aliquot was taken for analysis. Working standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml. Standard '0' served as blank. The volume was made up to 1 ml with distilled water. Then added 4 ml of anthrone reagent and heated for 8 minutes in a boiling water bath, cooled rapidly and read the green to dark green colour at 630 nm in Spectrophotometer (Sadasivam and Manickam, 1996).

Estimation of total phenolics:

The total phenol content of leaf sheaths was estimated as per the procedure given by Spies (1955).

RESULTS AND DISCUSSION

The carbohydrate content of both infected and healthy boot leaf sheath and healthy non-boot leaf sheath of three cultivars namely, CO 43, ADT 39 and ADTRH 1 were analyzed and furnished in Table 1.

The results indicated that boot leaf sheath contained significantly higher amount of carbohydrate than non-boot leaf sheath in all the three cultivars tested. The maximum amount of carbohydrate was recorded in healthy boot leaf sheath of ADTRH 1(306.3 mg/g), followed by ADT 39 (281 mg/g) and CO 43 (271.7 mg/g). Non – boot leaf sheaths recorded significantly less amount of carbohydrate than boot leaf sheaths. The maximum amount of carbohydrate was recorded in healthy non-boot leaf sheath of ADTRH 1(125.7 mg/g), followed by ADT 39 (117.7 mg/g) and CO 43 (108.7 mg/g). The per cent decrease in carbohydrate content of healthy non-boot leaf sheath over healthy boot leaf sheath was 58.96, 59.99 and 58.11 in ADTRH 1, CO 43 and ADT 39, respectively. There was a significant reduction in carbohydrate content of boot leaf sheath due to *S.oryzae* infection. The *S.oryzae* infected boot leaf sheath of ADTRH 1 recorded 182.0 mg/g of carbohydrate followed by ADT 39 (167.3 mg/g) and CO 43 (132.0 mg/g). The decrease in carbohydrate content of infected boot leaf sheath over healthy boot leaf sheath varied from 40.46 per cent to 51.12 per cent.

The healthy boot leaf sheath recorded significantly higher amount of protein than healthy non-boot leaf sheath and infected boot leaf sheath in all the three cultivars tested. The maximum amount of protein (5.8 mg/g) was recorded in the case of CO 43 followed by both ADT 39 and ADTRH 1(4.8 mg/g). In the case of healthy non-boot leaf sheath also, CO 43 contained more protein (5.0 mg/g), followed by ADT 39 (4.6 mg/g) and ADTRH 1(3.6 mg/g). The same trend was observed in infected boot leaf sheath also. The per cent decrease in protein content of infected boot leaf sheath over healthy boot leaf sheath was 41.67, 41.38 and 33.33 in ADTRH 1, CO 43 and ADT 39, respectively. The per cent decrease in protein

Table 1 : Biochemical changes in rice leaf sheath composition due to sheath rot

Sr. No.	Variety	Type of leaf sheath	Carbohydrate* (mg g $^{-1}$ FW)	% decrease over HBLS	Protein* (mg g $^{-1}$ FW)	% decrease over HBLS	Phenols* (mg g $^{-1}$ FW)	% increase over HBLS
1.	ADTRH 1	Boot leaf sheath (Healthy)	306.3d	-	4.8c	-	3.8a	-
		Boot leaf sheath (Infected)	182.0b	40.58	2.8a	41.67	6.1c	37.71
		Non-boot leaf sheath (Healthy)	125.7a	58.96	3.6b	25.00	3.4a	-10.53
2.	CO 43	Boot leaf sheath (Healthy)	271.7c	-	5.8d	-	3.3a	-
		Boot leaf sheath (Infected)	132.0a	51.12	3.4b	41.38	4.7b	29.79
		Non-boot leaf sheath (Healthy)	108.7a	59.99	5.0c	13.79	3.2a	-3.03
3.	ADT 39	Boot leaf sheath (Healthy)	281.0cd	-	4.8c	-	3.3a	-
		Boot leaf sheath (Infected)	167.3b	40.46	3.2ab	33.33	5.8c	43.10
		Non-boot leaf sheath (Healthy)	117.7a	58.11	4.6c	4.17	3.2a	-3.03

* Mean of three replications
Means followed by a common letter are not significantly different at 5% level by DMRT

content of healthy non-boot leaf sheath over healthy boot leaf sheath was 25.00, 13.79 and 4.17 per cent in ADTRH 1, CO 43 and ADT 39, respectively.

The results indicated that infected boot leaf sheath accumulated more phenol than non-boot leaf sheath and healthy boot leaf sheath in all the three cultivars tested. The maximum total phenol was observed in infected leaf sheath of ADTRH 1 (6.1 mg/g) followed by ADT 39 (5.8 mg/g) and CO 43 (4.7 mg/g). In the case of healthy boot leaf sheath, ADTRH 1 recorded maximum amount of total phenols (3.8 mg/g), followed by CO 43 and ADT 39 (3.3 mg/g). The same trend was also observed in case of healthy non-boot leaf sheath (Table 1).The per cent increase in phenolic content of infected boot leaf sheath over healthy boot leaf sheath was 37.71, 29.79 and 43.10 in ADTRH 1, CO 43 and ADT 39, respectively. The per cent decrease in phenol content of healthy non-boot leaf sheath over healthy boot leaf sheath was 10.53, 3.03 and 3.03 per cent in ADTRH 1, CO43 and ADT 39, respectively.

The present study also revealed that the boot leaf sheath contained significantly higher amount of carbohydrate than the leaf sheaths just below the boot leaf sheath. Similar trend was observed in the case of protein and phenols also. The results revealed that healthy boot leaf sheaths contained more than double the quantity of carbohydrate than the healthy non boot leaf sheaths. At the same time, the carbohydrate content of healthy boot leaf sheath was drastically reduced due to *S.oryzae* infection in all the three cultivars tested.

When the starch content was compared between different leaf sheath positions on the main stem of rice, the fourteenth leaf sheaths counted from the bottom, which elongated just before anthesis, showed about a four fold higher value than the tenth leaf sheath. The starch content, which is the major portion of total carbohydrate in leaf sheaths, was examined in four different leaf sheath positions on the main stem from forty-five day old seedlings. In the tenth and eleventh leaf sheaths, the starch content was relatively low, whereas in the 12^{th} and 14^{th} leaf sheath, the starch content increased rapidly about two weeks before anthesis and then decreased following anthesis (Perez *et al*., 1971).

Yoshito *et al*. (1997) referred the upper most leaf sheaths as temporary sink organs for the accumulation of starch. The same trend was noticed in the case of protein and phenols also in the present investigation. Total sugars, reducing sugars, starch, soluble proteins and phenolic contents were significantly higher in rice leaves during the flowering than other growth stages (Rajkumar, 2001; Nisha, 2002). These are temporarily stored in boot leaf sheaths during flowering and translocated to grains during maturity stage.

In the present experiment, it was observed that carbohydrate and protein contents were reduced due to *S.oryzae* infection in the infected leaf sheaths and due to utilization by *S.oryzae*, while the phenolic content increased. The results are similar to earlier studies of Mohan and Subramanian (1977). Bhavani, a moderately resistant variety and Kannagi, a highly susceptible variety to sheath rot were studied for biochemical composition of leaf sheaths. In healthy plants the quantity of reducing and non- reducing sugar was slightly higher in Kannagi than in Bhavani. *S. oryzae* inoculation caused a slight reduction in reducing and non – reducing sugar levels in both the varieties tested. It is clear from the study that *S. oryzae* utilized sugars present in the boot leaf sheath for its growth which resulted in the expression of sheath rot symptom at booting stage.

It has been reported that the rice seeds and the boot leaf sheaths are known as sink organs which implies its nutrition rich nature and hence, the sheath rot pathogen infects and manifests conspicuously on these two parts. It might be assumed that *S.oryzae* infection in rice seed is transmitted to the seedlings and plants and remained in the plant without conspicuous symptom unless the boot leaf emerges for want of required nutrition. After the emergence of boot leaf sheaths the fungus tends to grow profusely by utilizing the nutrients accumulated in the boot leaf sheaths to produce typical sheath rot symptoms.

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