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Endorsement of factors associated with the development of sheath rot pathogen in rice

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Abstract: A rice field trial against the sheath rot pathogen was conducted at Paddy Breeding Station, Coimbatore. The study proved that sheath rot was incited by *Sarocladium oryzae* and not due to *Fusarium* which is a saprophyte organism. The involvement of insect pests *viz.*, stemborer, leaf hoppers, earchead bug, leaf folder and mite in favoring the sheath rot infection was also proved. Among the insects stem borer paved the maximum way for the sheath rot infection followed by hoppers. The method of spread depicted that the pathogen spread was faster in a vertical manner (movement within the plant) followed by horizontal manner (plant to plant). The yield loss study also indicated that the reduction in growth parameters *viz.*, plant height, healthy tillers/ hill and panicle length were 16.84,100 and 20.17 percent over control respectively. The loss in yield parameters *viz.*, the per cent reduction in 1000 grain weight (11.22 percent over control) and grain yield (29.3 percent over control) were recorded in highly susceptible cultivar CO43.

Key words: Sarocladium oryzae, rice, yield loss, insect pests, disease spread

Introduction

Rice *(Oryza sativa* L) is one of the most important cereal crops of the world, affected by sheath rot incited by *Sarocladium oryzae* (Sawada) Gams and Hawkshworth. It has now become an economically serious menace due to the widespread occurrence in many rice growing countries. The yield loss up to 85 per cent has been reported by various workers. Almost all the improved varieties are susceptible to this disease, particularly the hybrids. The disease could be diagnosed by non-and partial-emergence of rice panicle along with irregular or oblong grey spots surrounded by brown margin on leaf sheath. The grains become dark brown and remain chaffy (Gill *et al.*, 1993). Though a fund of information are available on the disease, the controversial report regarding the causal organism, association of other factors, yield loss due to infection have not been investigated thoroughly. Keeping in view of the above facts, attempts were made in the present study to (i) confirm the pathogen asspciated with symptoms (ii) assess the role of insect pests on sheath rot symptom manifestation (iii) observe the method of spread (iv) estimate the yield loss caused by the pathogen.

Materials and Methods

Isolation and pathogenicity of the pathogen

The rice sheath rot pathogen *Sarocladium oryzae* was isolated from infected boot leaf sheath of CO 43 collected from Paddy Breeding Station, Tamil Nadu Agricultural University (TNAU), Coimbatore, India. Portions of the boot leaf sheath showing typical lesions were cut into small pieces and used for the isolation

Table 1. Pathogenicity test for the fungi isolated from rottened rice sheath

Fungus	CO 43			IR 50			C 20 R		
	Lesion length (cm)	Lesion width (cm)	Lesion colour	Lesion length (cm)	Lesion width (cm)	Lesion colour	Lesion length (cm)	Lesion width (cm)	Lesion colour
S.oryzae	8.63	1.31	Greyish black	6.72	0.74	Greyish brown	8.84	0.97	Greyish black
Fusarium sp.	0.00	0.00	-	0.00	0.00	-	0.00	0.00	-
S.oryzae + *Fusarium* sp.	8.51	1.16	Greyish black	6.56	0.68	Greyish brown	8.56	0.95	Greyish black
CD (p=0.05)	Fungus -NS* Varieties-NS* Fungus X Varieties X Lesion length and width - NS*								

* Values are mean of ten replications

of the fungus. The fungus was purified by using the single hyphal tip technique (Riker and Riker, 1968). A pure culture of the pathogen was maintained on potato dextrose agar slants (Riker and Riker, 1968) and was used for further studies. The pathogen was multiplied on paddy chaffy grain (200g) in 250 ml conical flask. Twenty five to fifty millilitres of water was added and sterilized in an autoclave at 1.4 kg cm^{-2} pressure for 20 min. The flasks were inoculated with mycelial discs (15 mm) of *S. oryzae.* Thirty days later, the chaffy grains fully covered with the fungus was used for inoculating the CO43 and IR 50 plants. The infected grains were placed in between boot leaf sheath and panicle in each tiller and covered with moist cotton. The inoculated plants were observed for development of symptom under glass house conditions. The plants were protected against insects by giving a spray with monocrotophos @ 0.25 per cent.

Confirmation of the pathogen associated with symptoms

In order to check whether the sheath rot symptom produced on CO 43 is only due to *S. oryzae* or any other fungus is being associated with it, isolations were made as mentioned earlier. The fungi *viz., S. oryzae* and *Fusarium* sp. were isolated from the infected CO 43 plants. Both the fungi were purified, maintained and multiplied in a similar manner as mentioned earlier. The rice cultivar CO 43 was sown in a field with the plot size of 5m x 5m. The plants were inoculated by inserting the *Fusarium* sp. and *S. oryzae* multiplied on chaffy grains separately on 80 days after sowing. The inoculated plants were observed for symptom expression daily.

Role of insect pests on sheath rot manifestation

To study the relationship between insect pest damage and intensity of sheath rot disease under artificially inoculated conditions, seedlings of highly susceptible CO 43 rice was used. Three seedlings were maintained for individual insect pest in the cage with the height of 1 meter and 15 cm in diameter. The insect pests were released first on 50 days after planting (boot leaf stage) except earhead bug which was released 90 days after planting and the plants were inoculated with the *S. oryzae* pathogen by single grain method. The treatments were replicated three times. The treatments were as follows.

Release of stem borer *(Scirpophaga incertulas Walk.)* @ 5 moths per hill	+ Artificially inoculated with *S. oryzae* after 24 hours
Release of brown plant hopper *(Nilaparvata lugens* Stal.) @ *10* insects per tiller	+ Artificially inoculated with S. *oryzae* after 24 hours
Release of green leaf hopper *(Nephotettix virescens* Dist.) @ *10* insects per tiller	+ Artificially inoculated with *S. oryzae* after 24 hours
Release of leaf folder *(Cnaphalocrocis medinalis* Guen.) @ 5 moths per tiller	+ Artificially inoculated with *S. oryzae* after 24 hours
Release of mite *(Steneotarsonemus spinki* Smiley.*)* 15 insects per tiller	+ Artificially inoculated with *S. oryzae* after 24 hours
Release of earhead bug *(Leptocorisa acuta* Thumb.) @ 5 bugs per tiller	+ Artificially inoculated with *S. oryzae* after 24 hours
Sarocladium oryzae alone	
Uninoculated control	

The percentage of sheath rot incidence was recorded 20 days before harvest.

Table 2. Role of insect pests on disease expression by *S. oryzae*

Treatment	Sheath rot incidence (PDI)
S.oryzae + Stem borer *(Scirpophaga incertulas)*	95.17 (77.33)[a]
S.oryzae + Green leaf hopper *(Nephotettix virescens)*	93.0 (74.68)[b]
S.oryzae + Leaf folder *(Cnaphalocrocis medinalis)*	89.0 (70.64)[c]
S.oryzae + Brown plant hopper *(Nilaparvata lugens)*	81.83 (64.80)[d]
S.oryzae + Mite *(Steneotarsonemus spinki)*	81.83 (64.80)[d]
S.oryzae + Earhead bug *(Leptocoria acuta)*	71.00 (57.42)[e]
S.oryzae alone	64.67 (53.53)[f]
Control (Without insect pests and *S.oryzae)*	0.0 (5.85)[g]

* Values are mean of five tillers
* Values in parentheses are arc sine transformed
* In a column, means followed by common letter are not significantly different at 5% level by DMRT.

Table 3. Method of spread of rotting symptoms causes by *S. oryzae* on different rice accessions.

Variety	Symptom formation in vertical manner (Days after inoculation)	Symptom formation in horizontal manner (Days after inoculation)	
		Tiller-Tiller	Hill-Hill
CO 43	8.00e	10.00ef	11.6e
IR 50	8.20e	10.70e	11.7e
C 20R	7.9e	09.90ef	10.8e
Gandasala	14.9a	16.9a	17.9a
Jergasala 1	14.8a	16.8a	17.8a
Jergasala 2	14.8a	16.8a	17.8a
Jergasala 3	15.2a	17.2a	18.2a
Kothandam selection	13.2bc	15.2bc	16.2bc
Mallikuruvai	14.4ab	16.5ab	17.4ab
Kothandam	13.1bc	15.1bc	16.1bc
Kudavalian	12.8c	14.8c	15.8c
Karunkrurvai	13.2bc	13.2d	13.2d
Maranel	13.8abc	138cd	13.8d
G3 B-3	10.5d	10.50e	10.5e
G3A	8.9e	00.89f	8.9f

* Values are means of fifty plants
* In a column, means followed by common letter are not significantly different at 5% level by DMRT.

Disease spread

Since the *Fusarium* sp. did not cause any symptoms of the sheath rot, *S. oryzae* alone was taken for further studies. For observing the method of spread, either in a horizontal manner (disease spread from plant to plant) or in a vertical manner (disease spread within the plant), a field trial was conducted during at Paddy Breeding Station. The trial was laid out under RBD replicated thrice with CO 43 as test variety. The plot size was 4m x 3m and the plants were raised with a spacing of 15x 20 cm. Individual plants were inoculated with the inoculum as described earlier and the spread of disease within the tiller was measured by measuring the increase in the lesion size from the initial stage. The horizontal spread from plant to plant and from hill to hill was observed and recorded.

Assessment of disease intensity

The disease intensity was assessed 15 days after inoculation by using a score chart of grades *viz.*, 0, 1, 3, 5, 7 and 9 based on the percentage of affected tillers (Anon, 2000).

Estimation of yield loss

For estimating the yield loss, a field trial was laid out by planting highly susceptible CO 43 variety with spacing of 15x 20 cm. The plot size was lm x 2 m. The treatment was replicated fifteen times. The plants were inoculated by inserting the chaffy grains with mycelium between boot leaf sheath and panicle in each tiller/ plant and one tiller/ plant separately. The control plants were maintained without inoculation. The disease intensity was assessed 20 days before harvest by using the scale described earlier. The yield loss was assessed in terms of plant height, lesion number, lesion length and width ratio, number of infected tillers/plant, number of productive tillers/plant, number of grains/panicle, number of chaffy grains/panicle and 1000 grain weight after harvest.

Results and Discussion

Confirmation of the pathogen associated with sheath rot symptoms

The sheath rot symptoms on CO 43 under natural conditions were selected and the isolation of the fungi was made. Two fungi were isolated in pure culture from the infected sheath. Based on the morphology and conidial characters, one fungal culture was identified as *S. oryzae.* This fungal culture was pale, saffron coloured, compact and cottony when grown on PDA medium. Hyphae measured 1.0 to 1.5 μm wide, conidiophores were verticillate, abundant and bearing conidia singly. Conidia are cylindrical with rounded ends, hyaline, one celled and 3.5-7.0 x 0.8 -1.5 μm. The second fungus culture was identified as *Fusarium* sp. This culture was white to pink coloured and scanty on PDA medium. Microconidia were numerous and arranged in chains. Macroconidia gradually attenuated towards the apex, distinctly pedicellate and uniformly curved.

Isolation and proving the pathogenicity

Rice cultivars CO 43 and IR 50 were inoculated with the *S. oryzae* and typical symptoms of sheath rot dieseae was produced within fifteen days after inoculation. Initial symptoms *viz.*, minute specks of discoloured lesions appeared on the upper most leaf sheath enclosing young panicles even within four days after inoculation. The infected flag leaf sheath recorded oblong or irregular greyish brown spots in advanced stages; they enlarged and developed grey centres and brown margins covering major portions of the leaf sheath. Failure (or) partial emergence of panicle was also noticed. The grains were discoloured and covered with a white fungi growth in later stages. In contrast, *Fusarium* sp. did not cause any disease to any one of the above mentioned rice cultivars. When *S. oryzae* and *Fusarium* sp. were co-inoculated on to the above mentioned cultivars, there was not much variation in the damage caused to the rice plants when compared plants inoculated with *S. oryzae* alone (Table 1). Thus confirming that *Fusarium* sp. encountered often with *S. oryzae* on isolation from the rottened sheath is only saphrophytic. For the present study, *S. oryzae* alone was used.

Role of insect pests on sheath rot manifestation

S. oryzae culture on chaffy grain and the insect pests were artificially inoculated on CO 43 rice variety in an insect proof cage house as described in materials and methods. The insects were allowed to feed on the plants first and then the pathogen was inoculated. The data revealed that the Per cent Disease Index (PDI) of sheath rot was significantly higher in stem borer infested plus *S. oryzae* inoculated plants (95.17) followed by green leaf hopper plus *S. oryzae* inoculated plants (93.0), leaf folder plus *S. oryzae* inoculated plants (89.0), brown plant hopper plus *S. oryzae* inoculated plants (81.83), mite plus *S. oryzae* inoculated plants (81.83) and earhead bug plus *S. oryzae* inoculated plants (71.0). *S. oryzae* alone inoculated plants exhibited 64.67 per cent infection (Table 2).

Disease spread

An experiment was conducted to find out the method of spread of disease in the following varieties *viz.,* CO 43, IR 50 and Cuddalore land races. Fifty plants were selected from each variety and the results are presented in Table 3.

The results revealed that the disease spread was faster in vertical manner followed by tiller to tiller and plant to plant or hills to hills. Among the cultivars tested, the short stature plant type varieties/ accessions *viz.,* CO 43, C 20 R, G3 A and G3 B-3 exhibited significantly faster disease spread within plants and from tiller to tiller as against the other, rice cultivars tested. The vertical spread was 8.7 days (10 cm), the spread from tiller to tiller was 8.8 days and the spread from hill to hill was 8.9 days in G3A Cuddalore land races.

Estimation of yield loss

The effect of sheath rot disease on growth parameters was studied in susceptible CO 43 plants and the results are presented in Table 4. The data revealed that the loss in growth parameters due to sheath rot disease was significantly higher in the diseased plants when compared to the control plants. The reduction in plant height, healthy tillers/hill and panicle length was 16.84, 100 and 20.17 per cent over control respectively. Similarly, the effect of sheath rot disease on yield parameters was studied and the results are presented in Table 5. The data revealed that the loss in yield parameters was significantly higher in diseased plants and the per cent disease index was 95.5 per cent. The per cent reduction in 1000 grain weight was 11.22 per cent over control and grain yield was 29.3 per cent over control.

A recent report states that the productivity of rice in Tamil Nadu keeps on changing. One of the reasons attributed to the changing trends in the productivity is the biotic factors. In future to sustain the productivity and to increase the productivity standard, we have to look in for more and more of high yielding varieties and hybrids. The fertilizer response high yielders are always prone to attack by biotic stresses. Among the biotic stresses, the sheath rot disease caused by *S. oryzae* is one which warrants an urgent attention, particularly in the hybrids. Since its first report from 1973by Agnihothrudu in India, the management of this disease is being attempted with limited success. The reason for the limited success is that proper understanding on the disease is still lacking.

The factors like the association of the major pests of rice with the rotting of the sheath, the spread of the disease, identification of resistant genotypes, use of biocontrol agents and botanicals *etc.* are essential to manage this disease. With this in view, investigations were carried on this problem and the results are discussed hereunder.

Confirmation of the pathogen associated with symptoms

The morphological characters of the two fungal cultures *viz.*, *S. oryzae* and *Fusariurn* sp. from CO 43 resembled those described by CMI (Anon, 1980) and the repeated occurrence of *Fusarium* sp. along with *S. oryzae* on isolation from sheath rot symptoms prompted to see whether *Fusarium* could also cause the rotting on the uppermost leaf sheath. From the Table 1, it is clear that *S. oryzae* alone could cause the typical rotting and not *Fusarium* sp. Inoculation of the sheath with both the fungi did not aggravate the symptoms, and the damage caused to the sheath did not increase, indicating that *Fusarium* sp. was associated only as a saprophyte.

The symptom produced by *S. oryzae* upon artificial inoculation was characterized by greyish brown lesions with dirty white powdery masses of conidia from the rottened portions of leaf sheath. Reisolation of *S. oryzae* was made and thus the pathogenicity was proved. A similar description of the symptoms and association of *S. oryzae* with the symptoms have been reported by Agnihothrudu (1973), Amin *et al.* (1974) and Ram Singh and Dodan (1995). Thus confirming that *S. oryzae* alone is responsible for the rotting of sheath in rice in Tamil Nadu.

Role of insect pests on disease manifestation

The role of insect pests in sheath rot disease manifestation has well been established under artificially inoculated condition. The disease was very severe on Stem borer affected plants (95.2) followed by Green leaf hopper (93.0), Leaf folder (89.0), Mite (81.8) and Brown plant hopper (81.8). All the plants affected by insect pests showed the higher disease incidence than *S. oryzae* alone. Thus confirming the fact that *S. oryzae* infection might be more devastating if associated with stem borer. The ear head bug a late comer on the rice plants can also aggravate the situations when compared to the control wherein the fungus alone was inoculated.

In general, wounding of plants by insects enhanced the disease incidence. Similarly, injury caused by mite *Steneotarsonemus spinki* (Hseih *et al.*, 1977), brown plant hopper *Nilaparvata lugens* (Naidu, 1983), leaf folder *Cnaphalocrocis medinalis* (Saroja *et al.*, 1987), ear head bug *Leptocorisa acuta* (Lakshmanan *et al.*, 1992) have been reported to enhance the sheath rot infection. The present finding is also in line with the earlier ones. In addition to the commonly occurring pests, one more important pest, the mite is also found to help the pathogen to cause more damage.

Disease spread

The study on preventing the disease spread is an essential criteria for the management

Table 4. Effect of sheath rot incidence on growth parameters of CO 43 rice plants.

Treatment	Plant height (cm)	Percent reduction over control	Number of infected tillers/hill	Percent reduction over control	Panicle length (cm)	Percent reduction over control
Inoculated plants	68.13	16.84	8.5 (3.0)	100	16.43	20.17
Control	81.93		0.0 (1.0)		20.58	
CD (p=0.05)	11.13		0.2		1.78	

* Values are mean of fifteen plants
* Values in parentheses are square root transformed values.

Table 5. Effect of sheath rot incidence on yield parameters of CO 43 rice plants.

Treatment	Number of infected panicles/ hill	Percent increase over control	Number of grains/ panicle	Percent reduction over control	1000 grain weight (g)	Percent reduction over control	Grain yield (kg/ ha)	Percent reduction over control	Percent disease index (PDI)
Inoculated plants	7.50 (2.83)	10000	56.13	50.24	17.80	11.22	2174.4	29.30	95.54*
Control	0.00 (1.00)		112.8		20.05		3079.33		
CD (p=0.05)	0.21		18.55		0.39		259.91		

* Values are mean of fifteen plants
* Values in parentheses are square root transformed

of disease (Singh, 1984). In the present study, the methods of spread in horizontal and vertical manner was studied. The sheath rot disease spread was faster within the plant followed by tiller to tiller and hill to hill. The symptom formation was observed within 9.6 days in vertical manner while 13.8 days and 14.5 days for tiller to tiller and hill to hill respectively. In the dwarf varieties with numerous tillers *viz.*, CO 43, IR 50, G3 A and G3 B-3 disease spread was faster. Amin *et al.*, (1974) found that the high yielding dwarf varieties were severely affected by sheath rot pathogen than taller varieties. The similar method of disease spread was reported in sheath blight of rice by Hashiba (1984); Damicone *et al.* (1993); Takushi and Ikeda(1956).

Estimation of yield loss

The study on the loss caused by the disease is a crucial factor for the management of disease (Singh, 1984). The present yield loss estimation studies revealed that the disease is capable of causing yield losses to the tune of 29.3 per cent in CO 43 variety. A higher disease index of 95.5 per cent was recorded in the variety CO 43. The reduction in growth and yield parameters *viz.*, plant height (16.84 per cent) and 1000 grain weight (11.22 per cent) were more pronouncing in CO 43 variety.

Three to twenty per cent and rarely as much as 85 per cent damage has been reported by Chen (1957) from Taiwan. Prabhakaran *et al.* (1975) and Mohan (1976) reported yield loss of 85 per cent and 57 per cent in Tamil Nadu respectively. Chakravarthy and Biswas (1978) reported that the reduction in grain weight due to sheath rot disease was 79 per cent. Estrada *et al.* (1984) observed that sheath rot incidence affected the yield components like thousand grain weight, total spikelets and filled spikelets. Ten to twenty per cent damage of tillers was recorded in the rice growing areas (Ou, 1985). Roddy *et al.* (2000) observed the effect of sheath rot on yield components of rice. *S. oryzae* increased the number of discoloured grains per panicle (172.9 per cent and 290.7 per cent) and reduced the grain filling (42 per cent and 47 per cent) and grain yield (38.7 per cent and 46.2 per cent) in the variety Prakash under *in vitro* and *in vivo* conditions respectively. The reduction in plant height and panicle length due to sheath rot disease was reported by Sckhar and Prasad (1989). The present study also indicated that there was a reduction in yield and it was to the tune of 29.3 per cent.

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