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An Efficient Inoculation Method for Artificial Screening of Sheath Blight in Rice

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ABSTRACT

Sheath blight (Rhizoctonia solani) of rice is an economically important pathogen of rice worldwide. The unavailability of a robust screening method in the greenhouse has hampered studies on the genetics of resistance and plant breeding efforts to improve resistance. In the current study, rice hull medium, rice grains medium, and maize grain medium were used at 5%, 10% and 15% as a medium of inoculum for sheath blight infection under artificial screening. Significant difference was observed in the infection pattern of sheath blight between the different treatments. The appearance of the first lesion was observed in 6 days after sowing (DAS) in 15% rice hull medium and 16 days with rice grains medium (5%). Rice hull medium (15%) enabled the formation of sclerotia at 13 DAS and the disease incidence reached up to 90% at 21 DAS. The results suggests rice hull medium as an effective medium for rapid disease screening of rice genotypes for sheath blight resistance under greenhouse conditions.

Key words: *Sheath blight, Rice, Inoculum, Disease Incidence, Sclerotia*

INTRODUCTION

Sheath blight disease caused by the fungal pathogen *Rhizoctonia solani* Kuhn [Teleomorph *Thanatephorus cucumeris* (Frank) Donk], is one of the main constraints in rice production especially under irrigated agro-ecosystems with input-intensive crop management and favorable environmental conditions [2, 10]. The losses caused due to sheath blight can result in 30-76% reduction in yield and affects the quality of produce and rice straw [9]. The severity of the disease depends on inoculum load, favorable climate, and host genotype. The pathogen is recognized as polyphagous competitive saprophyte with a wide host range and is able to survive for long periods in the soil. This disease is becoming severe due to intensive cultivation of high yielding varieties along with the application of heavy doses of nitrogenous fertilizers. Many studies reported that the source of inoculum of pathogen mainly comes from soil-borne sclerotia or the infected plant debris [1, 5, 8]. Numerous studies have been involved to identify host resistance but without any satisfactory control.

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The field trials are labor intensive and require a large amount of seed material, inoculum, and high humidity conditions up to 3 to 5 months to complete evaluations [3]. A rapid reproducible method with reduced environmental effect is required for screening rice against sheath blight. Herein, a study was conducted to study the effect of different inocula of *R. solani* on sheath blight incidence in rice.

MATERIAL AND METHODS

Preparation of pathogen inocula

The pure culture of a highly virulent strain of *R. solani* was maintained on Potato Dextrose Agar (PDA) at 4°C at the Department of Biotechnology, UAS, Dharwad. This culture was used as mother culture for preparing different media for the current study.

a) Rice hull medium: The 100 g medium contained three parts rice hull, one part rice grain and 50 ml of water was added per bottle[7].
b) Rice grains medium: The 100 g medium contained nineteen parts sand, one part rice grains and 10 ml of water was added per bottle[4].
c) Maize grains medium: The 100 g medium was composed of nineteen parts sand, one part crushed maize grains and 10 ml of water was added per bottle[11].

All the mixtures were filled separately in 500 ml bottles and autoclaved in three cycles at 15 psi for 30 min at 121 °C for three alternate days. The following day, agar discs from the periphery of 3-days old culture of *R. solani* on PDA were inoculated aseptically to the medium and incubated at 28°C for two weeks.

Plant material

A sheath blight susceptible rice cultivar BPT-5204 was used for the current study. The seeds were surface sterilized using 1.5 % sodium hypochlorite for 5 min, and then rinsed three times with sterile distilled water.

Effect of different inocula on disease incidence

For greenhouse experiments, the soil was autoclaved to ensure that the experiment was not affected by any soil-borne microorganisms. Each pot was filled with 200 g soil. The inocula were grown on different media *viz.*, rice hull, rice grains, and maize grains and were added to sterile soils at the rate 5%, 10% and 15% by weight. Pots were covered with polythene bags for 3 days to maintain high humidity and allowed for the fungal establishment. The control condition was maintained without the addition of any inoculum. Six seeds were sown per pot after 3 days of fungal establishment and pots were covered with moistened polythene bags. The experiment was repeated twice under glasshouse conditions. Pots were monitored for plant emergence and sheath blight incidence and observations were recorded up to 31 days after sowing (DAS).

Statistical analysis:

The statistical analysis of the disease response was based on a completely randomized design of nine different treatments and five pots were maintained per treatment.

RESULTS AND DISCUSSION

Rhizoctonia culture was well established in all the different inocula (Fig. 1). Rice hull medium showed prominent growth of mycelia during two weeks of incubation. Rice grains medium followed by maize grains medium showed poor mycelia growth but with the formation of sclerotial bodies at the end of two weeks of incubation.

Effect of different inocula on disease incidence

The earliest symptoms of sheath blight were observed 6 days after sowing in 15% rice hull inoculum treatment; while rice grains and maize grains inocula at 5% did not show any disease symptoms even 14 days after sowing (Table 1, Fig. 2). The 15% rice inoculum treatment showed highest disease incidence of 84.70% and 95.75% at 14 and 21 days after sowing respectively. All the treatments showed disease incidence 21 days after sowing; wherein 5% maize grains inoculum showed the lowest incidence at 19.4%. Different inoculation techniques for *R. solani* were studied by Kumar *et al.*, 2013 where mycelial suspension showed highest disease severity (65.3%) followed by sclerotia (45.8%) and sorghum grains (34.1%).

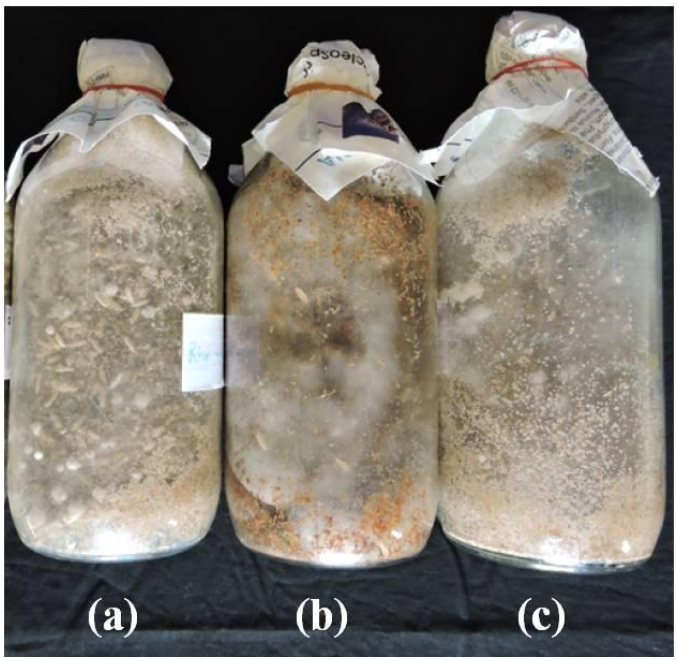

Fig. 1: The cultural features of *R. solani* grown on different inocula after two weeks of incubation: (a) Rice grains medium; (b) Rice hull medium; (c) Maize grains medium

Table 1: Effect of different inocula on sheath blight incidence in rice

Sr No	Medium (% inoculum)	Occurrence of first symptom (DAS)	Disease incidence (%) (14 DAS)	Disease incidence (%) (21 DAS)	Initiation of sclerotia formation (DAS)	No of sclerotia formed (31 DAS)
1	Rice hull 5%	12	49.31	90.00	17	7
2	Rice hull 10%	7	73.53	93.20	14	9
3	Rice hull 15%	6	84.70	95.75	13	16
4	Rice grains 5%	16	NA*	23.23	NA*	0
5	Rice grains 10%	14	32.26	52.23	26	5
6	Rice grains 15%	13	42.05	53.58	24	8
7	Maize grains 5%	15	NA*	19.40	NA*	0
8	Maize grains 10%	13	17.57	33.86	31	6
9	Maize grains 15%	13	34.31	39.30	30	8
	SEM ±	0.43	0.56	0.63	0.52	0.31
	LSD (5%)	1.25	1.64	1.84	1.51	0.90

NA*- No appearance

More than 90% plants were infected in all the rice hull treatments 21 days after sowing (Fig. 3), and other treatments showed 50% disease incidence or less even at 21 DAS and no further increase in disease incidence. The rice grains and maize grains medium had sclerotia formation within 14 days of incubation, which could be the reason for inconsistent results observed in these treatments. Similar findings of inconsistent results with sclerotia inoculum have been reported[3]. This could be because sclerotia vary in size within inoculum, and they are resting structures which may require different germination period. Hence, rice hull can be recommended an efficient medium for rapid disease development in two weeks towards effective screening of rice genotypes for sheath blight resistance. The greenhouse studies can help in ruling out environmental effect involved in field trials and independent of growing season requirements.

The sclerotia formation was observed 13 days after sowing in 15% rice hull inoculum treatment; whereas no sclerotial formation was observed in rice grains and maize grains inocula at 5% even 31 days after sowing. The highest number of sclerotia was recorded in 15% rice hull inocula treatment at 31 days after sowing. The early formation of sclerotia serves as a source of inoculum for infection in escaped plants and can allow for more efficient screening for identification of resistant genotypes.

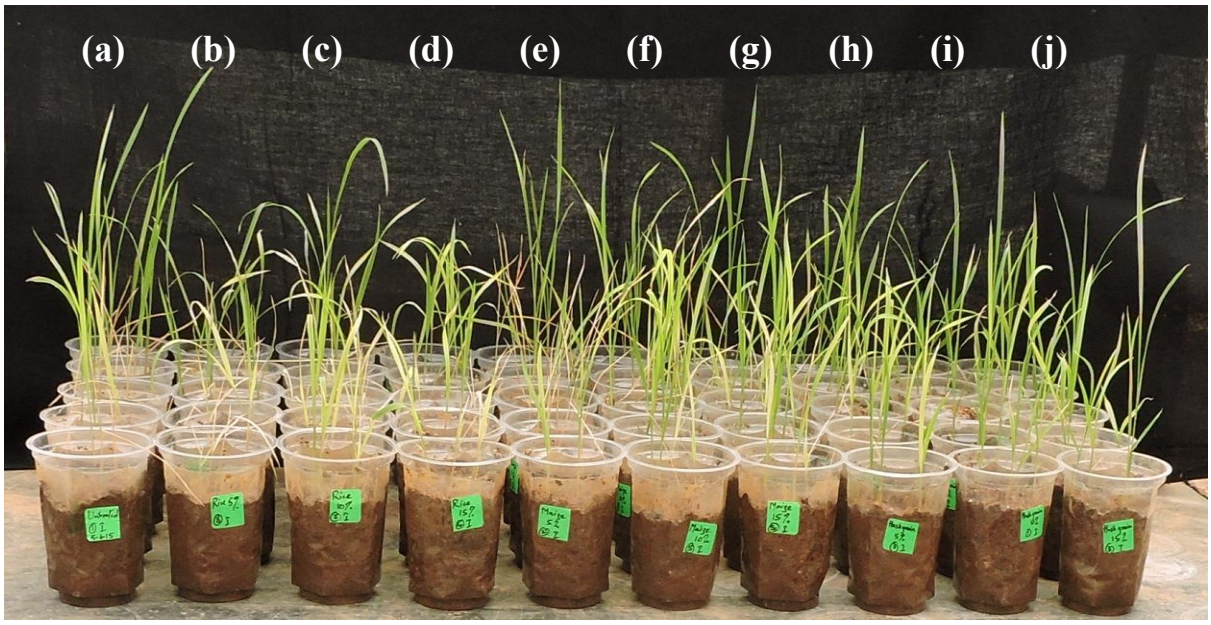

Fig. 2: Sheath blight incidence 14 days after sowing in rice with different inocula

(a) Control; (b) Rice grains 5%; (c) Rice grains 10%; (d) Rice grains 15%; (e) Maize grains 5%; (f) Maize grains 10%; (g) Maize grains 15%; (h) Rice hull 5%; (i) Rice hull 10%; (j) Rice hull 15%

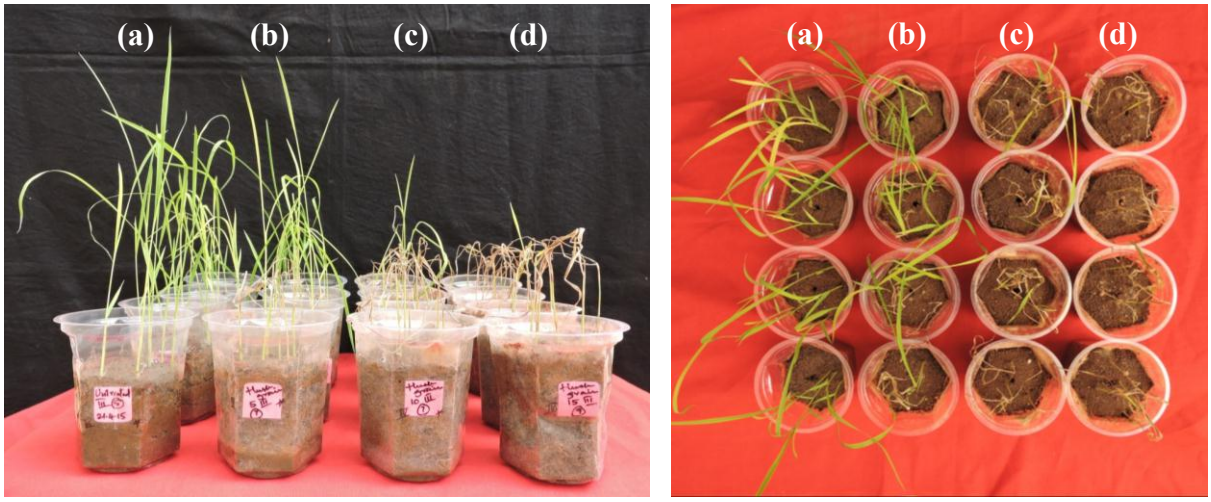

Fig. 3: Sheath blight incidence in rice at 21 DAS. (a) Control; (b) Rice hull 5%; (c) Rice hull 10%; (d) Rice hull 15%

CONCLUSION

Rice hull was found to be an efficient medium for rapid disease development within two weeks which can be used in screening of rice genotypes for sheath blight resistance under controlled conditions.

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