Evaluation of Sheath Blight Resistance in Diverse Rice Varieties under Artificial Inoculation and Field Conditions

ABSTRACT

Rice (*Oryza sativa* L.) is a vital food crop for nearly half of the global population, facing significant threats from various biotic and abiotic stresses. Among biotic stresses, diseases pose the most common challenge. Sheath blight, caused by the soil-borne fungal pathogen Rhizoctonia solani Kühn (R. solani), is a major concern, leading to substantial damage to rice and cereal crops worldwide. This study evaluated sheath blight resistance across diverse rice varieties, including short grain, long grain, aromatic, hybrid, and wild types, under both artificial inoculation and field conditions. Ten host plant species were assessed for resistance to sheath blight caused by Rhizoctonia solani, using physiological screening one-week post-inoculation. The highly virulent R. solani isolate (AG-1 IA anastomosis group) was cultured on PDA media, covering the entire plate within 3 to 5 days. Screening of cultivated rice varieties, wild rice accessions, and hybrid rice was conducted using field conditions and humidified chamber methods, with results confirmed through molecular characterization. Among the ten cultivated rice varieties, Arize 6444 and Dhanya 748 exhibited the lowest disease index, classifying them as moderately resistant. In contrast, Kalanamak and Pusa Basmati were highly susceptible, displaying high disease indices. Among wild rice accessions, Oryza rufipogon showed a disease index of five or less. Visual ratings indicated the highest susceptibility in Kalanamak, followed by Pusa Basmati and Swarna sub-1, while the lowest visual ratings were observed in wild rice accessions Oryza australiensis and Oryza rufipogon across all tested hosts. The findings underscore the importance of identifying resistant rice varieties to manage sheath blight effectively. The moderately resistant cultivars and wild rice accessions identified in this study offer valuable genetic resources for breeding programs aimed at enhancing sheath blight resistance in rice.

Key words

Cherry; Sheath blight resistance; Rhizoctonia solani; Rice varieties; Biotic stress;

INTRODUCTION

Rice (*Oryza sativa* L.) is a crucial food crop for nearly half of the world's population, playing a significant role in the diet of many (Sellamuthu et al., 2011; Venkataramani, 2002). Containing 8-9% protein, it stands as the world's single most important food crop and the primary food source for more than a third of the global population (Khush, 1997). In India, rice is cultivated on approximately 43.50 million hectares with an annual production of about 159.20 million tons and an average productivity of 3659.8 kg per hectare (Anonymous, 2013a). Millions of people rely on rice for food and income, necessitating continuous improvements in productivity and profitability within the rice farming system. Nutritionally, rice comprises 76-85% carbohydrates, around 8% protein, and several essential minerals like potassium, chlorine, phosphorus, calcium, magnesium, sodium, and iodine (Juliano, 1985; Grist, 1986). However, rice productivity is impacted by various biotic and abiotic stresses due to its global cultivation under diverse agro-climatic conditions. Among biotic stresses, insect pests such as the brown planthopper (Nilaparvata lugens Stål), green rice leafhopper (Nephotettix cincticeps), and stem borer (Chilo suppressalis), along with diseases like sheath blight, bacterial leaf blight, and tungro virus, significantly affect rice production. More than 70 diseases caused by fungi, bacteria, viruses, or nematodes have been recorded in rice, with rice blast (Magnaporthe grisea), bacterial leaf blight (Xanthomonas oryzae pv. oryzae), and sheath blight (Rhizoctonia solani) being the most severe constraints on high productivity (Ou, 1985).

Rice sheath blight, caused by Rhizoctonia solani, is a significant global disease, causing considerable yield losses (Sudhakar et al., 1998). The adoption of susceptible, high-yielding cultivars with numerous tillers and changes in cultural practices has favored the development of sheath blight, increasing its incidence and severity in rice-producing areas worldwide (Groth et al., 1991; Rush and Lee, 1992). First recorded in Japan by Miyake in 1910, sheath blight affects both the sheaths and laminar portions of leaves, with the disease being the most prominent symptom. Rhizoctonia solani Kühn (teleomorph: *Thanatephorus cucumeris* is a widespread soil-borne pathogen causing economically significant diseases in many crops (Adams, 1988). Given this context, the present investigation aims to study several physiological parameters among popular rice varieties in India to identify potential sources of resistance to sheath blight.

MATERIALS AND METHODS

Experimental Plant materials

Seeds of rice, wild rice, and hybrid rice were procured from NBPGR, Pusa New Delhi, C.R.R.I, Cuttack (Orissa), K.V.K., Masodha Faizabad and Department of Genetics and Plant Breeding, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad, India (Table 1a and 1b).

Table 1a: List of cultivated rice varieties, wild and hybrid used in present investigation:

S.No.	Varieties Name	Source of collection	Characteristic Features
1	Swarna Sub-1	GPB Department, NDUA&T, Kumarganj, Faizabad	Short grain, long duration, High amylose rice variety, Dwarf variety, highly susceptible to Sheath blight
2	BPT	GPB Department,	Drought tolerant and highly susceptible to Sheath

	5204	NDUA&T, Kumarganj, Faizabad	blight
3	CSR-13	GPB Department, NDUA&T, Kumarganj, Faizabad	Semi dwarf, salt tolerant, coarse grain, ShB susceptible
4	NDR-118	GPB Department, NDUA&T, Kumarganj, Faizabad	Drought tolerant, ShB susceptible
5	Kalanamak	GPB Department, NDUA&T, Kumarganj, Faizabad	Highly ShB susceptible
6	Pusa Basmati	GPB Department, NDUA&T, Kumarganj, Faizabad	Long grain, short duration, Aromatic rice variety, highly susceptible to Sheath blight

Table 1b: List of wild Rice and hybrid rice used in present

Sr.no	**Hybrid**	**Source of collection**	**Characteristics features**
7	Arize 6444	KVK, Masodha, Faizabad	Moderately resistant to ShB
8	Dhanya 748	KVK, Masodha, Faizabad	Moderately resistant to ShB
	Wild Accession	**Source of collection**	**Characteristics features**
9	*O. australiensis*	CRRI, Cuttack, Orissa	Long duration, highly resistant to BLB, Blast and Sheath Blight
10	*O. rufipogan*	GPB Department, NDUA&T, Kumarganj, Faizabad	Short duration, moderately resistant to BLB, Blast and Sheath Blight

***R. solani* isolates:**

A multinucleate compatible, highly virulent strain of *R. solani*, D-14 belonging to AG1-IA anastomosis group was obtained from the Rice Pathology Laboratory, G.B. Pant University of Agriculture and Technology, Pantnagar

(U.K.) India. Single tips of mycelia were then transferred onto fresh Potato dextrose Agar (PDA) and maintained at 28 ± 1ºC for 6 days and used as the inoculation purpose (Rangaswami and Mahadevan (2004). (Fig 1)

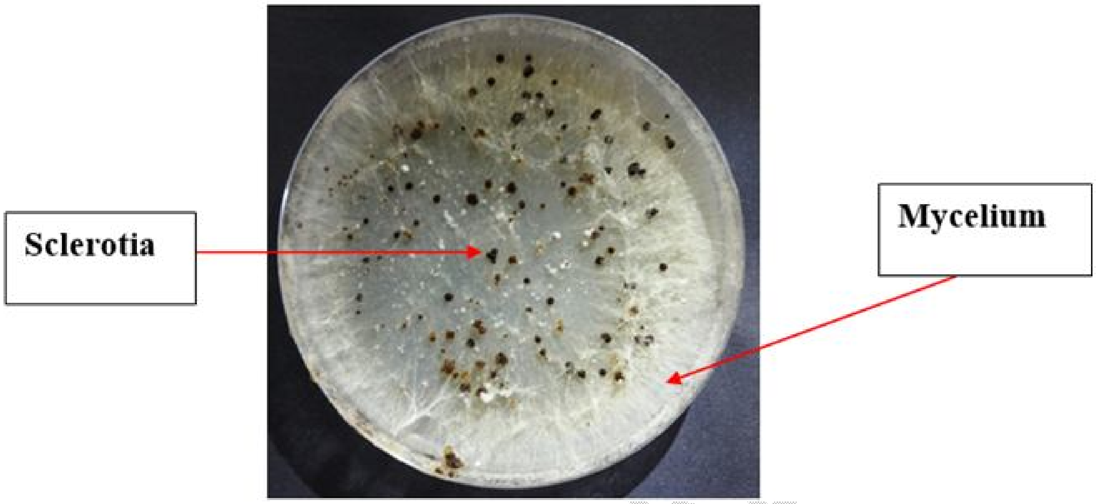

Fig. 1: Morphology of *R.solani* (D-14 belonging to AG1-IA)

Screening for resistance after the inoculation to different hosts with *R. solani* in different conditions:

Six-week-old rice plants were inoculated by slightly opening the leaf sheaths to expose the stem. Immature sclerotia (six days old) were placed beneath the leaf sheath, and a few drops of sterile water were added. The mycelium was then covered with cotton soaked in sterile water. For control plants, the leaf sheaths were also slightly opened, and sterilized water was added to the cotton without sclerotia, following the method described by Singh et al. (2002). Inoculation for rice, wild rice, and hybrid rice was conducted six weeks after transplanting.

Screening Conditions

The screening for sheath blight resistance in host plants was conducted from July to September during the 2015-16 growing season under two different conditions:

1. Field condition
2. Humidified chamber condition

Field Condition Screening for Resistance:

The field condition trial was conducted at the experimental field site of the Department of Plant Molecular Biology and Genetic Engineering. Experimental blocks were divided into three stripes. Each stripe consisted of a random selection of two hills from a susceptible check, two hills from a resistant check, and three hills from the test material in each replication. Data recording involved randomly selecting three tillers per hill and three leaves per tiller. The resistant check utilized cultivated rice varieties O. australiensis, while the susceptible check employed Pusa Basmati 1.

Humid Chamber Condition Screening for Resistance

The inoculation for the control condition trial was conducted in pots within a greenhouse environment. All plant material was initially raised in a greenhouse maintained at a temperature of 25°C ±3°C. Seeds were sown in pots of 15 cm diameter, with a planting depth of 1 cm in soil amended with nutrients: 30 mg nitrogen, 9.7 mg phosphorus (from single superphosphate), and 18.5 mg potassium (from muriate of potash) per kg of soil. Seedlings that emerged were thinned to seven per pot to standardize growth conditions. Assessment of the number and size of lesions was performed one week after inoculation.

Inoculation of Various Hosts with Rhizoctonia solani under Humid Chamber Conditions

The inoculation of different hosts followed the field condition method described by Singh et al. (2002). After inoculation, the plants were transferred to hot humid chambers measuring 140 cm in length and 120 cm in width. These chambers contained two 100-liter tubs and were covered with 0.03 mm thick transparent plastic. The transfer to humid chambers occurred in the evening (5:00 to 6:00 PM) for a duration of 24 hours to maintain optimal humidity levels. Non-inoculated control plants were also placed in the humidified chamber under similar conditions to serve as negative controls. One week after inoculation, the number and size of lesions were assessed for all plants.

$$\text{Disease index} = \frac{\text{Stem lesion length}}{\text{Plant height}} \times 9$$

Assessment of Disease Severity in the Field and Cluster Analysis

A disease index ranging from 0 to 9 was determined for each infected leaf with sheath. Infected leaves were cut from the base, and the lesion length along the culm base was measured relative to the total culm length. The disease index was calculated by dividing the lesion length by the culm length and multiplying by 9 (Jia et al., 2002). Disease severity categories were defined as follows: <4 indicated moderate resistance, 5 to 6 indicated moderate susceptibility, and 7 to 9 indicated high susceptibility. Additionally, each seedling was visually rated on a scale from 0 to 9: 0 indicated no lesions, and 9 indicated lesions covering 90 to 100% of the culm area. Ratings of 1 to 8 corresponded to 10 to 80% of the diseased plant area. Average lesion counts per individual plant were calculated in both field and humid chamber conditions for rice and wild rice one week after inoculation. (Fig 2 and 3).

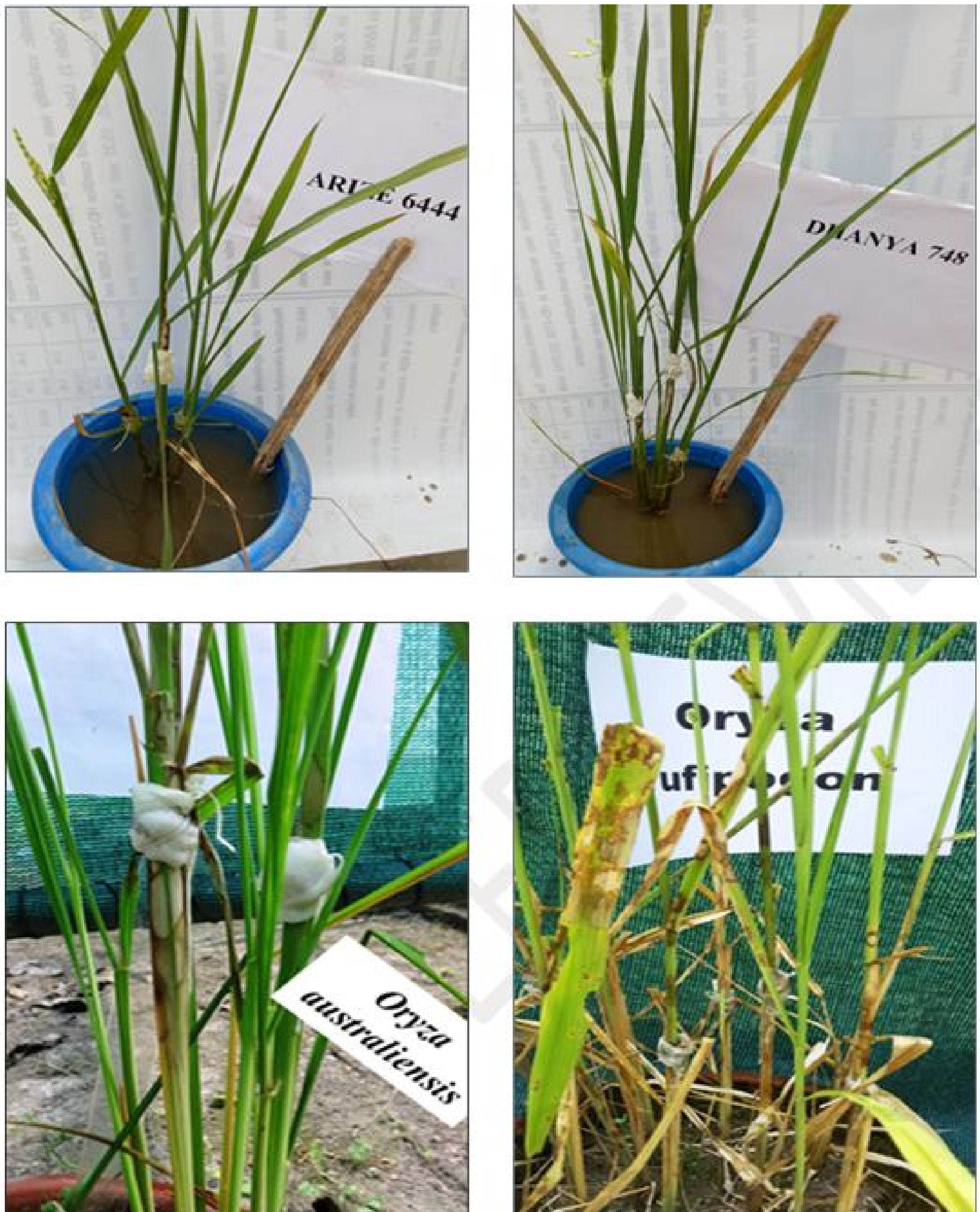

Fig: 2. Diagram showing disease symptoms in hybrid and wild rice plants

RESULTS AND DISCUSSION

Culture Multiplication of R. solani and Inoculation of Different Hosts

R. solani isolates (AG-1 IA anastomosis group) were successfully multiplied on PDA media. Mycelium transferred to PDA plates and incubated at 28°C showed initial growth within 48 to 72 hours, and the entire plate was colonized within 3 to 5 days post-inoculation. This rapid growth indicates that the R. solani isolate used is highly virulent. These findings are supported by Sharma et al. (2004), who also observed optimal growth of R. solani on PDA media with significant mycelial development within 72 hours at 28°C. The establishment of disease symptoms under artificial inoculation conditions was critical for evaluating disease severity accurately. Despite extensive research, no rice cultivar has been identified that confers complete resistance against sheath blight (Rush and Lee, 1992; Pinson et al., 2005). Studies have shown that early infection in healthy plants can occur within 12 hours when using immature mycelium of the pathogen instead of sclerotial bodies (Singh et al., 2003; Park et al., 2008). The results of the current study indicate that R. solani isolates (AG-1 IA anastomosis group) are among the most aggressive pathogens affecting rice. The rapid growth and high virulence of this strain underscore the challenge of managing sheath blight. Furthermore, the effectiveness of PDA as a growth medium for R. solani highlights its utility for laboratory studies of this pathogen. The establishment of reliable and precise evaluation methods for quantifying disease severity is essential for advancing sheath blight management strategies. These efforts will contribute to the development of more resistant rice cultivars and ultimately improve rice productivity in regions affected by sheath blight.

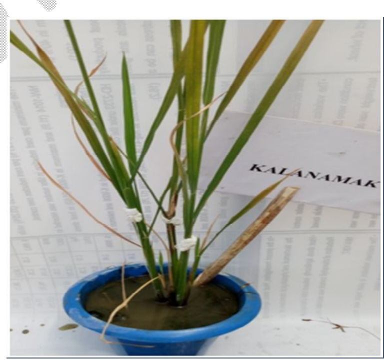

Fig: 3 Diagram showing disease symptoms in cultivated rice plants

Screening of Different Rice Varieties against Sheath Blight in Field & Humidified Chamber Conditions

The humidified chamber created a highly favorable environment for the development of sheath blight, with temperatures ranging from 30 to 35°C and relative humidity around 90%, promoting rapid disease progression after inoculation with R. solani. Although the temperature and humidity were slightly lower than in typical field conditions, significant differences were observed among the tested hosts for disease index, visual rating, and lesion number under humidified chamber conditions. Among the cultivated rice varieties, Kalanamak and Pusa Basmati exhibited higher disease indices of 7.56 and 7.55, respectively, indicating high susceptibility. In contrast, O. rufipogon, Arize 6444, and Dhanya 748 showed moderate resistance, with disease indices of 5.09, 4.97, and 4.69, respectively. The disease indices of wild rice accessions ranged from 1.61 to 5.09, with O. rufipogon and O. australiensis showing notable resistance (Table 2). In the present study, field and humidified chamber conditions were used to screen different hosts. Disease index, visual rating, and lesion number per leaf were measured according to the 0 to 9 scale of the IRRI-SES protocol. All hosts and non-host plants achieved 100% infection using immature sclerotia of R. solani (AG1-IA). The lowest disease index was recorded in the wild rice accession O. australiensis. These findings align with Eizenga et al. (2002), who reported that wild rice species contain potential genes for improving sheath blight resistance in the rice gene pool. Similarly, Bala and Goel (2007) identified seven accessions from 200 rice accessions representing 15 Oryza species that were resistant or moderately resistant to sheath blight and sheath rot. Among the cultivated rice varieties in this study, Dhanya 748, Arize 6444, and CSR 13 were found to be moderately resistant to sheath blight, while Kalanamak and Pusa Basmati were highly susceptible. These results are consistent with Park et al. (2008), who tested the efficiency of R. solani infection on two resistant (Tetep and Jasmine 85) and two susceptible (Chucheongbyeo and Junambyeo) cultivars using various inoculum types. The findings also concur with Hossain et al. (2014), who identified six moderately resistant cultivars and one susceptible local cultivar using micro-chamber and mist-chamber methods. When combining all screening results for cluster analysis, the resistant wild rice accession O. australiensis was separated into Cluster II. Moderately resistant cultivars Dhanya 748 and Arize 6444 were also included in Cluster II, while highly susceptible cultivars Kalanamak and Pusa Basmati 1 were grouped in Cluster I. Cluster III contained all susceptible hosts. These results are in close agreement with Prasad and Eizenga (2008), who used 73 different wild rice accessions for resistance screening against sheath blight. Their analysis also separated accessions into clusters based on susceptibility and resistance, highlighting the importance of wild Oryza species as sources of resistance against sheath blight.

Table 2: Summary of cultivated rice evaluated for sheath blight resistance using a disease index, visual rating and lesion number in field condition & humidified chamber condition:

S.No.	**Scoring in field condition**				**Humidified Chamber Condition**		
	Varieties	Disease index	Visual rating	Number of lesion/ leafs	Disease index	Visual rating	Number of lesion/ leafs
1	**Swarna sub-1**	6.07	6.33	5.67	6.71	6.33	7.67
2	**BPT- 5204**	6.25	5.33	5.38	6.49	5.33	7.83
3	**CSR-13**	5.64	5.67	3.67	6.19	5.33	5.17
4	**NDR- 118**	6.02	4.65	4.65	6.61	6.33	5.33
5	**Kalanamak**	7.13	7.00	7.33	7.56	7.67	8.33
6	**Pusa Basmati**	7.12	6.67	6.33	7.55	7.33	7.67
7	**Arize- 6444**	4.75	4.33	4.33	4.97	4.33	4.67
8	**Dhanya -748**	4.24	4.00	3.67	4.69	4.65	3.50
9	***O.australiensis***	1.25	2.00	2.35	1.61	2.33	2.50
10	***O.rufipogon***	4.54	2.65	2.50	5.09	3.33	3.50
CD 5%		0.65	0.94	0.94	0.70	1.03	1.00
SEm ±		0.23	0.33	0.33	0.24	0.36	0.35
CV		6.77	10.29	10.64	6.71	10.69	10.38

CONCLUSION

The study revealed that Wild rice accessions *O. australiensis* and *O.rufipogon* are the potential resistance source against *R. solani* that was proved by physiological screening. The study demonstrates the high virulence of R. solani (AG-1 IA) and underscores the utility of wild rice accessions, particularly O. australiensis, in enhancing sheath blight resistance. While some cultivated varieties like Dhanya 748 and Arize 6444 showed moderate resistance, others like

Kalanamak and Pusa Basmati were highly susceptible. Effective screening and cluster analysis methods are essential for identifying and developing resistant rice cultivars. These wilds may be exploited in the breeding programme and developing transgenic to enhance the resistance against sheath blight.

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