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RESEARCH ARTICLE

Prevalence, characterization and cross infectivity of *Rhizoctonia* species causing rice sheath blight complex in Tamil Nadu

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Abstract

Sheath blight of rice is a significant biotic stress caused by various *Rhizoctonia* species, including *Rhizoctonia solani* (sheath blight), *Rhizoctonia oryzae-sativae* (aggregate sheath spot) and *Rhizoctonia oryzae* (sheath spot). The presence of these diverse *Rhizoctonia* species highlights the complexity of sheath diseases and emphasizes the need for management approaches to mitigate their impact on rice production. In the current study, 37 isolates of *Rhizoctonia* spp. were collected from various rice-growing regions of Tamil Nadu, India during 2021-22. The isolates were characterized phenotypically and for their virulence pattern. The cultural characteristics including mycelial and sclerotial morphology of the isolates were recorded, aiding in the identification and characterization of the pathogens. An amplicon size of 265 bp was obtained in PCR analysis of *R. solani* isolates which confirmed their belonging to anastomosis group AG1-IA. Further differentiation using species-specific primers GMRS-3, GMRO-3 and GMROS-2 revealed that 30 isolates belonged to *R.* solani and 7 to *R. oryzae-sativae.* The highly virulent isolates of *R. solani* and *R. oryaze-sativae* were selected for host range studies in different hosts and the results indicated that all the plant and weed species were found to be infected by both *R. solani* and *R. oryzae-sativae* except pig weed and khaki weed.

Keywords: host range; *R. solani*; *R. oryzae-sativae*; sheath blight; virulence pattern

Introduction

Rice (*Oryza sativa* L.) is a staple food for two-thirds of the global population, particularly in the Asian region (1). Among biotic constraints, fungal diseases are a major hindrance to sustaining rice production. Sheath blight holds the second most prominent position after rice blast (2). Yield losses due to this disease range between 4-50 % depending on the stage of crop, time of infection, pathogen's virulence and climatic conditions (3-5).

Rhizoctonia species are soil inhabitants, fulfill diverse ecological roles as saprophytes, symbionts and pathogens (6). Sheath blight complex caused by 3 species of *Rhizoctonia* including *R. solani*, *R. oryzae-sativae* and *R. oryzae* cause rice sheath blight, aggregate sheath spot and sheath spot, respectively. *R. solani* is assigned into 14 reproductively incompatible anastomosis groups *viz.,* AG1 to AG13 and AGB1, the bridging isolate (7). Among these groups, AG1-1A was identified as the predominant anastomosis group responsible for causing sheath blight in rice (8, 9).

Rhizoctonia has an extensive host range and can infect several crops and weed species without rice plants and serve as inoculum source during hostile conditions (10). *Rhizoctonia* causes several diseases in crops, including banded sheath and leaf blight in sorghum, maize and rice, as well as stem rot and aerial blight in mung bean and soybean. It also results damping off in cotton, heart rots in cabbage, sheath rot in sugarcane, foliar blights in fruits and plantation crops and sprout canker and black scurf in potato (11).

Even though these three pathogens have been documented in India, detailed information is not available about the prevalence in Tamil Nadu, one of India's major rice growing states. Therefore, the present investigation was aimed to ascertain the existence and assortment of the *Rhizoctonia* spp. inciting rice sheath blight complex in Tamil Nadu, its morphological and molecular characterization as well as their host range.

Materials and Methods

Collection and isolation

A roving survey was carried out during 2021-22 across major rice growing regions of Tamil Nadu *viz*., Ariyalur, Erode, Cuddalore, Thanjavur, Trichy, Thiruvannamalai, Villupuram, Kallakuruchi and Madurai districts to estimate the prevalence of rice sheath blight complex. Plants exhibiting typical sheath blight symptoms-characterized by grayish-green, water-soaked lesions with dark brown margins on the leaf sheaths were collected, labelled and brought to the laboratory for isolation. A lesion of 2-3 mm portion was carefully excised, surface sterilized using 1 % sodium hypochlorite for a minute and rinsed in sterilized distilled water three times and transferred to Petri dishes containing potato dextrose agar (PDA) medium. The plates were then incubated under laboratory conditions at 28±2 °C for 5-7 days (12). The emerging mycelia were purified by the single hyphal tip method and identified based on morphological and microscopic characteristics.

Morphological characterization

Pure culture of *Rhizoctonia* isolates obtained during isolation were cultured on PDA medium at 28 ± 2 °C for a period of 10 days, during which mycelial and sclerotial characters of each isolate were recorded. These included the assessment of colony color and growth pattern, as well as sclerotial characteristics *viz*., number of sclerotia per Petri plate, their diameter (mm), color, texture and pattern.

Virulence spectrum of *Rhizoctonia* isolates

The virulence of all *Rhizoctonia* isolates were evaluated by conducting pathogenicity tests in a glass house using susceptible rice cultivar TN-1 during *Kharif* season (2022). The experiment involved 37 earthen pots, with each pot containing three hills that served as replicate. Inoculation was done by placing the mycelial disc beneath the leaf sheath of fifty day old rice plants and wrapping it with moist cotton to impart humidity for the germination of the fungus (13). The disease severity was measured 10 days after inoculation in accordance with the Relative Lesion Height (RLH) by adopting the formula (14).

$$RLH = \frac{\text{Maximum height at which lesion appears}}{\text{Plant height}} \times 100$$

(Eqn.1.)

The rice sheath blight rating scale (15) was employed to document the lesion height and the progression of the disease. Isolates have been categorized into four categories according to the severity of the disease as highly virulent (8-9), moderately virulent (4-7.9), less virulent (1-3.9) and avirulent (0).

Molecular characterization

The DNA of all *Rhizoctonia* isolates were extracted using the modified CTAB method (16). Molecular identification of *Rhizoctonia* isolates was confirmed through PCR assays. Universal primers ITS1 (forward) and ITS4 (reverse) were used to amplify the ITS region for general fungal identification. Additionally, AG1-IA specific primers were used for the identification of *R. solani* (17) and species-specific primers designed by Johanson (18) were employed for species-level identification of *Rhizoctonia*. The PCR product was resolved by agarose gel electrophoresis and sequenced using the Sanger technique. The DNA sequences were analyzed for similarity by comparing them with the sequences in the NCBI database using the BLAST program.

Host range studies

A pot culture assay was conducted to determine the host range of thirteen non-host plants (wheat, maize, ragi, cowpea, sorghum, green gram, soybean, swollen finger grass, jungle rice, nut grass, crowfoot grass, pigweed and khaki weed) with the virulent isolates of *R. solani* and *R. oryzae-sativae*. The seeds of each species were surface sterilized and 2-3 seeds were planted in pre-sterilized soil-filled earthen pots.

Inoculation methods varied depending on the plant species and family. For the *Graminaceae* and *Cyperaceae* families, a mycelial disc was inoculated to the leaf sheath near water line. In contrast, for *Amaranthaceae* and *Fabaceae* families, mature sclerotia prepared by culturing *Rhizoctonia* on PDA for 10-12 days were harvested and positioned in the root region using the sterilized forceps. The inoculated sheaths were covered with moist cotton and regularly moistened with sterile distilled water to ensure humidity. Pots were watered once daily to maintain adequate moisture and the assay was conducted until the disease symptoms were observed.

The plant height and lesion height were recorded after the sheath blight symptoms appeared on each host plant. The RLH was computed adopting the formula as mentioned in Eqn. 1 (14).

Results

Morphological characterization

A total of 37 different isolates of *Rhizoctonia* species were isolated from sheath blight infected samples. Among the 37 isolates, 30 isolates exhibited moderate and rapid mycelial growth with hyaline mycelium, whereas 7 isolates showed slow mycelial growth with hyaline mycelium that eventually turned brown. The isolates formed sclerotial bodies in three patterns: central, peripheral and scattered. Scattered sclerotia were exhibited by 14 isolates, while 9 showed central sclerotia. Peripheral sclerotia were displayed by 7 isolates and 7 isolates had both scattered and central patterns. The duration required for sclerotia formation ranged from 7 to 9 days. The smooth texture of sclerotia was observed in 23 isolates and 14 with a rough texture. The highest number of sclerotia was observed in R8 (268 No.), whereas the lowest count was recorded in the R20 isolate (12). The results of the morphological characteristics of the *Rhizoctonia* isolates are summarized in Table 1 and shown in Fig. 1.

Virulence spectrum of *Rhizoctonia* isolates

Virulence analysis of all the *Rhizoctonia* isolates induced sheath blight symptoms on rice and variation was observed among the isolates concerning RLH ranging from 32.13 to 68.73 %. The results indicated that isolate R29 exhibited maximum virulence with a RLH of 68.74 % followed by isolate R7 with 67.99 %. Out of the 37 isolates, 8 were classified as highly virulent, 26 as moderately virulent and 3 showed a less virulent reaction which are presented in Table 2.

Table 1. Mycelial and sclerotial characteristics of Rhizoctonia spp. isolates

Isolate name	Variety name	Place of collection	Mycelial morphology	Growth pattern	Sclerotial characters					
					Pattern	Days to form sclerotia	Sclerotial count/ plate	Colour	Texture	Diameter (mm)
R1	CR 1009	Ariyalur	Initially hyaline, later turn to brown	Scarce	Scattered	7	206	Olive Brown	Smooth	2.00
R2	IW Ponni	Bhavanisagar	Hyaline	Moderate	Scattered	7	86	Brown	Smooth	5.22
R3	ADT 45	Bhavanisagar	Hyaline	Moderate	Scattered + Central	8	64	Olive Brown	Smooth	1.91
R4	ADT 37	Bhavanisagar	Hyaline	Moderate	Scattered	7	56	Brown	Smooth	2.73
R5	BPT 5204	Virudachalam	Hyaline	Moderate	Scattered + central	7	16	Brown	Smooth	4.06
R6	CR 1009	Virudachalam	Hyaline	Rapid	Peripheral	8	48	Brown	Smooth	1.47
R7	ASD 16	Sivapuri	Initially hyaline, later turn to brown	Scarce	Central	7	16	Olive brown	Rough	0.56
R8	ADT 36	Panruti	Initially hyaline, later turn to brown	Scarce	Scattered	7	268	Olive brown	Smooth	1.24
R9	CO 52	Aduthurai	Hyaline	Moderate	Scattered	8	94	Olive Brown	Rough	2.21
R10	ADT 19	Aduthurai	Initially hyaline, later light brown	Scarce	Scattered	9	56	Brown	Smooth	1.62
R11	BPT5204	Aduthurai	Hyaline	Moderate	Scattered	7	28	Olive brown	Rough	4.45
R12	ADT53	Aduthurai	Initially colourless later yellow and turn brown	Scarce	Scattered	8	166	Olive Brown	Smooth	0.92
R13	IR20	Palayiur	Hyaline	Moderate	Scattered + central	7	56	Brown	Smooth	4.58
R14	White ponni	Thirupazhanam	Intially colourless, later brown	Scarce	Central	7	48	Brown	Smooth	1.24
R15	CO52	Mayiladuthurai	Hyaline	Rapid	Peripheral	7	96	Brown	Smooth	1.92
R16	CO43	Balarajpuram	Hyaline	Moderate	Peripheral	9	76	Brown	Rough	2.73
R17	CO43	Kuzhumuni	Hyaline	Moderate	Central	7	27	Brown	Smooth	1.78
R18	ASD16	Orayur	Hyaline	Moderate	Peripheral	7	22	Brown	Rough	1.63
R19	White ponni	Kannanur	Hyaline	Moderate	Scattered + Central	8	58	Brown	Rough	1.74
R20	White ponni	Kulithalai	Hyaline	Rapid	Scattered	8	12	Brown	Rough	1.92
R21	CO43	k. sathanur	Hyaline	Rapid	Central	7	64	Brown	Rough	2.14
R22	CORH2	Purathakudi	Hyaline	Rapid	Scattered + central	7	96	Olive brown	Smooth	2.45
R23	ADT43	Emappur	Hyaline	Moderate	peripheral	7	74	Brown	Smooth	1.57
R24	Ponmani	Saravanapakam	Hyaline	Moderate	Central	9	46	Brown	Rough	2.50
R25	ADT37	Someshwarapuram	Hyaline	Rapid	Scattered	7	248	Olive brown	Rough	1.80
R26	ADT51	Thiruvennainallur	Hyaline	Moderate	Central	7	78	brown	Smooth	2.46
R27	ADT51	Vasanthakrishnapuram	Hyaline	Moderate	Scattered + Central	8	102	Olive brown	Smooth	1.96
R28	Co50	Aviyur	Intially colourless, later turn to brown	Scarce	Scattered	8	106	Olive brown	Rough	1.42
R29	ADT43	Ulundurpet	Hyaline	Moderate	Scattered + Central	7	167	brown	Rough	4.80
R30	ADT43	Nemeli	Hyaline	Moderate	Scattered	7	88	Olive brown	Smooth	4.90
R31	Ponmani	Parikkal	Hyaline	Moderate	Central	8	14	brown	Smooth	1.93
R32	Ponmani	Thirukovilur	Hyaline	Moderate	Peripheral	9	96	brown	Smooth	2.60
R33	RNR15024	Thiruvannamalai	Hyaline	Moderate	Scattered	8	64	Olive brown	Rough	1.73
R34	RNR15024	Vettavalam	Hyaline	Moderate	Peripheral	7	56	brown	Smooth	2.40
R35	CO42	Chellapatti	Hyaline	Rapid	Central	8	24	Olive brown	Rough	1.98
R36	CO43	Kodikulam	Hyaline	Moderate	Central	8	18	brown	Smooth	3.50
R37	White ponni	Othakadai	Hyaline	Rapid	Scattered	7	68	brown	Smooth	2.36

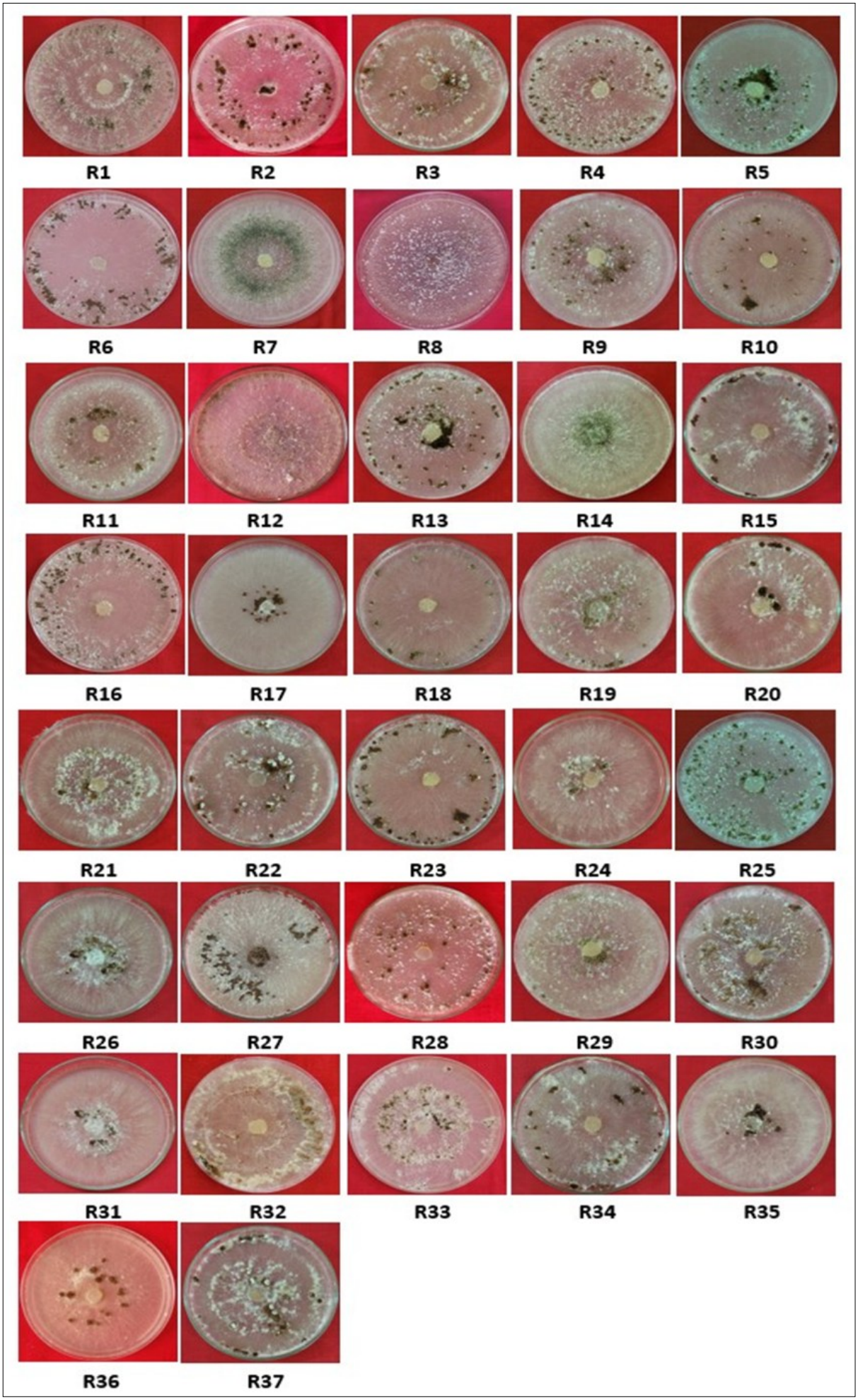

Fig. 1. Cultural and sclerotial characteristics of different *Rhizoctonia* spp. isolates.

Table 2. Virulence analysis of *Rhizoctonia* isolates causing sheath blight disease in rice

Isolate code	Lesion height (cm)	Plant height (cm)	Relative lesion height (%)	Average rating scale*	Category of virulence **
R1	66.4^{n}	99.7^{abc}	66.55^{j}	9	HV
R2	48.6^{jkl}	112.4^{hij}	42.96^{fgh}	5	MV
R3	68.9^{n}	104.7^{de}	65.87^{j}	9	HV
R4	52.0^{lm}	110.2^{ghi}	47.15^{hi}	7	MV
R5	38.3^{bcde}	106.2^{cde}	36.08^{cde}	5	MV
R6	65.9^{n}	98.7^{ab}	66.75^{j}	9	HV
R7	66.6^{n}	98.0	67.99^{j}	9	HV
R8	45.3^{fghijk}	124.7^{mn}	36.31^{cde}	5	MV
R9	37.3^{bcd}	112.8^{ij}	33.08^{bc}	7	MV
R10	49.3^{jklm}	124.1^{lmn}	39.71^{def}	5	MV
R11	68.6^{n}	105.0^{def}	65.33^{j}	9	HV
R12	45.0^{fghijk}	110.3^{ghi}	40.83^{efg}	5	MV
R13	40.6^{bcdefg}	117.4^{jk}	34.59^{bcd}	5	MV
R14	30.3	125.7^{n}	24.09	3	LV
R15	44.3^{efghij}	103.4^{bcde}	42.84^{fgh}	5	MV
R16	65.0^{n}	99.8^{abc}	65.17^{j}	9	HV
R17	68.7^{n}	102.5^{abcd}	67.07^{j}	9	HV
R18	49.6^{jklm}	125.8^{n}	39.44^{def}	5	MV
R19	40.6^{bcdefg}	116.6^{jk}	34.79^{cd}	5	MV
R20	51.0^{klm}	109.7^{fghi}	46.52^{ghi}	7	MV
R21	46.0^{ghijkl}	126.6^{n}	36.32^{cde}	5	MV
R22	41.6^{cdefgh}	114.6^{ij}	36.27^{cde}	5	MV
R23	51.0^{klm}	120.7^{klm}	42.25^{fgh}	5	MV
R24	36.6^{bcd}	113.4^{bcd}	32.26^{bc}	5	MV
R25	48.9^{jklm}	119.8^{kl}	40.82^{efg}	5	MV
R26	35.3^{ab}	109.7^{fghi}	32.16^{bc}	5	MV
R27	52.0^{lm}	112.4^{hij}	46.26^{ghi}	7	MV
R28	37.4^{bcd}	116.3^{jk}	32.15^{bc}	5	MV
R29	67.5^{n}	98.3^{a}	68.74^{j}	9	HV
R30	35.7^{abc}	107.7^{abc}	33.12^{bc}	5	MV
R31	55.0^{m}	109.7^{m}	50.18^{i}	7	MV
R32	34.8^{ab}	120.1^{klm}	28.98^{b}	3	LV
R33	39.7^{bcdef}	129.7^{n}	30.62^{bc}	3	LV
R34	47.2^{hijkl}	103.8^{cde}	45.51^{ghi}	5	MV
R35	48.2^{ijkl}	113.3^{ij}	42.53^{fgh}	5	MV
R36	49.9^{jklm}	98.8^{ab}	50.59^{i}	7	MV
R37	42.4^{defghi}	99.6^{abc}	42.59^{fgh}	5	MV

Molecular characterization of *Rhizoctonia* isolates

In our study, ITS1/ITS4 primers successfully identified all the *Rhizoctonia* isolates with an amplicon size of ~650bp (Fig. 2A). Molecular analysis of the specific AG1-1A gene of *Rhizoctonia solani* showed variability among the isolates. Out of the 37 isolates tested, 30 isolates exhibited specific amplification of the 265 bp fragment (Fig. 2B) and proved their belonging to the AG-1A group.

Detection using species-specific primers

PCR amplification using the species-specific primers identified 30 isolates as *R. solani* and 7 isolates as *R. oryzae-sativae*. The *R. solani*-specific primer ITS-1F and GMRS-3R successfully identified 30 isolates (R2-R6, R9, R11, R13, R15-R27, R29-R37) which was represented by an amplicon size of 550 bp (Fig. 2C). Other primer pair GMROS-6F and R635R showing specificity to *R. oryzae-sativae* produced a band size of ~1.2 kb (R1, R7, R8, R10, R12, R14 and R28 isolates) confirming that these 7 isolates belonged to the species *R. oryzae-sativae* (Fig. 2D). None of the isolates were amplified by the *R. oryzae* specific primer, suggesting that the species was not present in Tamil Nadu. The ITS1/ITS4 sequences of the virulent isolates of *R. solani* (R29) and *R. oryzae-sativae* (R7) isolates were submitted to NCBI and provided with the accession numbers, OQ940459 for *R. solani* isolate and OR352416 for *R. oryzae-sativae* isolate.

Host range studies

The experimental findings showed that both *R. solani* and *R. oryzae sativae* successfully infected all the crop plants including wheat, maize, ragi, sorghum, cowpea, green gram, soybean upon artificial inoculation. Among the weed hosts, swollen fingergrass, jungle rice, nutgrass, crowfoot grass showed symptoms except pigweed and khaki weed. Symptom expression varied among all non host plants tested, maize plant showed the highest lesion length (67 and 73 mm) after 7 days of being inoculated with both *R. solani* and *R. oryzae-sativae*. The fungus produced damping-off symptoms in green gram and root rot and web blight symptoms in soybean which are presented in Table 3 and Fig. 3. Re-isolation of the fungus from all the infected plant species exhibited typical *Rhizoctonia* characters on PDA.

Discussion

Sheath blight complex disease of rice poses a challenging biotic stress, resulting in substantial global yield losses ranging from 10 % to 50 %, depending on the severity of infection and environmental conditions. Our results indicate that *R. solani* AG1-1A was the predominant subgroup causing rice sheath blight disease in Tamil Nadu, India. Two species of *Rhizoctonia i.e.*, *R. solani* and *R. oryzae-sativae* were recorded in surveyed locations which documented *R. solani* (sheath blight) as the

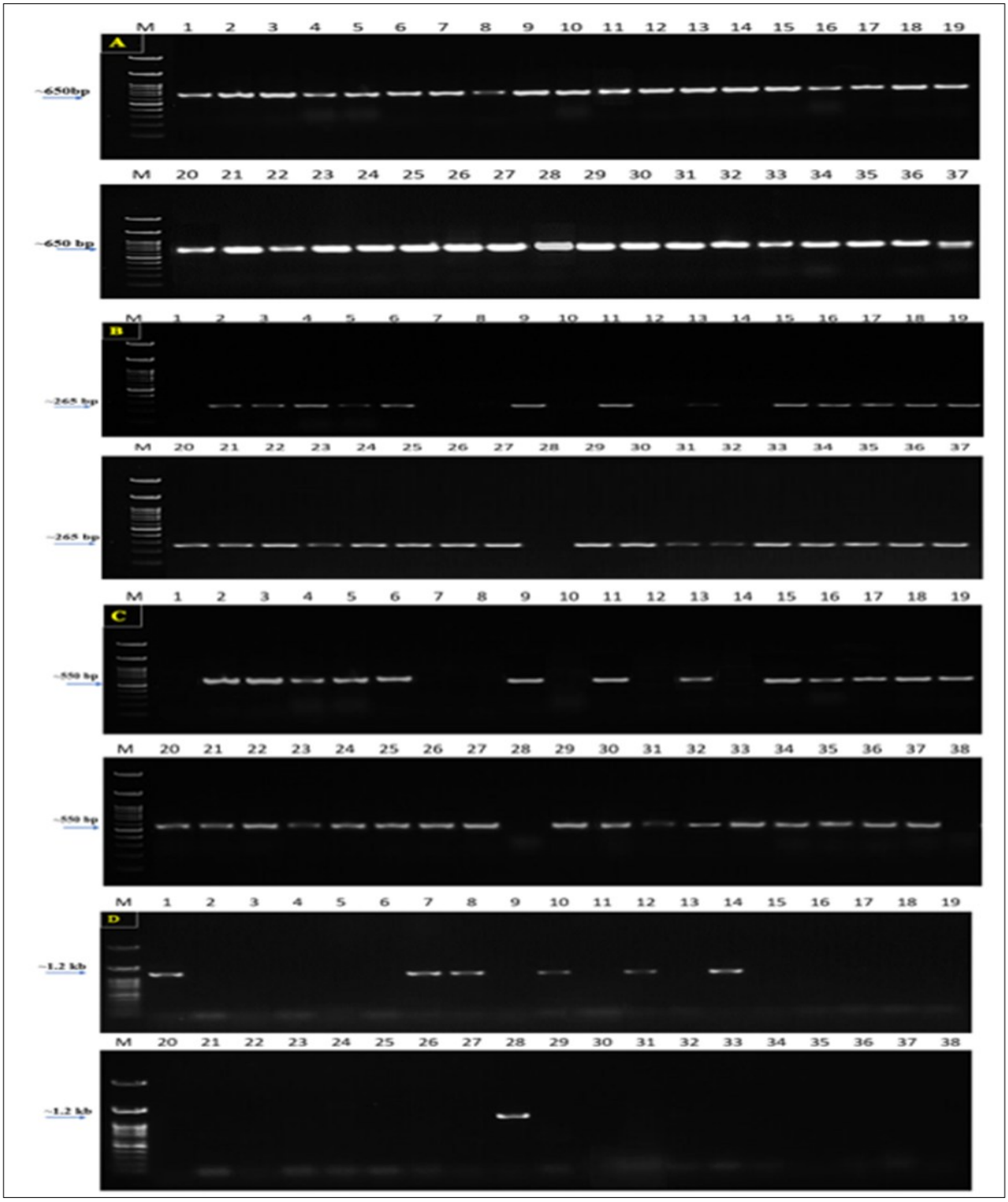

Fig. 2. PCR amplification of 18Sr RNA region of *Rhizoctonia* isolates (A). DNA amplification of *R. solani* isolates with AG1-1A specific primer (B). PCR amplification of *Rhizoctonia* spp. using species-specific primer pairs: species-specific amplification of *R. solani* isolates amplified at ~550bp (C). *R. oryzae-sative* isolates produced a band size of ~1.2kb (D). M: 100 bp ladder; L1 - 37: *Rhizoctonia spp*. R1– 37 isolates; L 38: Nuclease free water.

Table 3. Host range study of *Rhizoctonia* spp

Host plant	*Rhizoctonia solani*			*Rhizoctonia oryzae-sativae*		
	Infection rate (days)	Lesion height (mm)	Infection (%)	Infection rate (days)	Lesion height (mm)	Infection (%)
Wheat	5	19	24.3	5	15	17.4
Maize	7	67	58.2	7	73	60.8
Ragi	4	25	34.2	4	18	20.6
Cowpea	5	15	22	5	24	34.7
Sorghum	5	56	52.8	5	32	43.2
Green gram	5	-	-	5	-	-
Soybean	7	-	-	7	-	-
Swollen fingergrass	4	19	36.2	4	19	38.6
Jungle rice	3	23	45.8	4	24	45.0
Nut grass	5	13	30.3	5	18	37.6
Crowfoot grass	4	14	36.6	4	16	35.9
Pig weed	-	-	-	-	-	-
Khaki weed		-	-	-	-	-

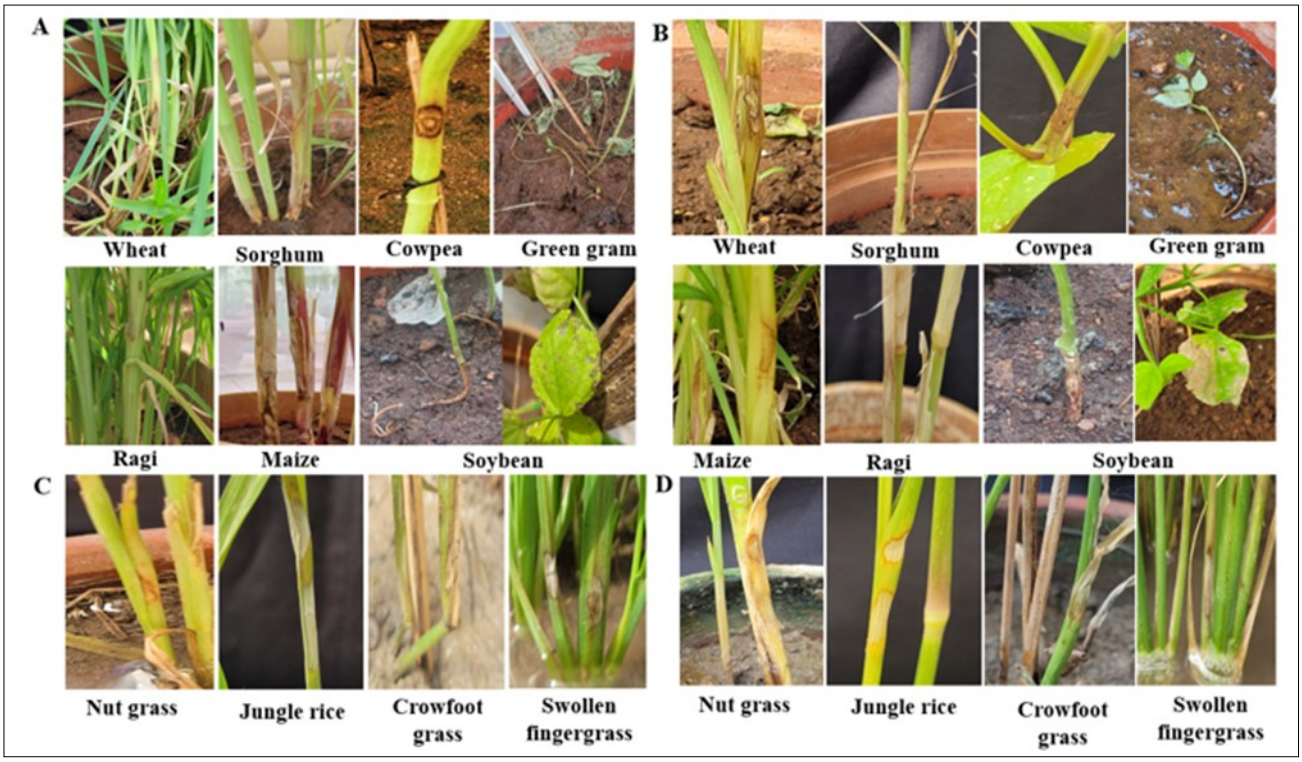

Fig. 3. Host range studies of *R. solani* and *R. oryzae sativae* on different crops (A and B) and weed species (C and D).

prevalent pathogen and notably documented the occurrence of *R. oryzae-sativae* (aggregate sheath spot).

In our present study, majority of the isolates produced cultures with a dense, hyaline mycelium and seven isolates showed initial presence of hyaline mycelium which turned to pale brown colour upon maturity and displayed sparse mycelial growth and were considered tentatively as *R. oryzae-sativae*. *R. solani* isolates produced brown to dark brown sclerotia which were relatively large, irregular shaped, measuring about 1–5mm in diameter whereas, *R. oryzae-sativae* isolates produced globose, white sclerotia that later turned to brown with diameter ranging from 0.56-2 mm. All isolates showed variations with respect to their sclerotial number, diameter, sclerotial colour and texture. These significant morphological variations have been documented previously as well (12, 19, 20).

Virulence spectrum of all the 37 *Rhizoctonia* isolates were carried out and found variation in the degree of virulence. Based on the disease reaction, the isolates were categorized as highly virulent, moderately virulent and less virulent. Variation in the pathogenicity of *Rhizoctonia* isolates on rice has been studied by many workers (9, 21, 22).

In our present investigation, PCR amplification of the ITS region produced an amplicon of 650 bp and shared over 99 % similarity with the isolates of *Rhizoctonia* found in the NCBI database. Similar amplicon size of 661bp have been documented for *Rhizoctonia* isolates with specific ITS1/ITS4 primer pairs (23). Based on the molecular analysis of anastomosis group of *R. solani*, 30 isolates were identified as *R. solani* AG1-IA which has been previously reported (24).

A PCR-based technique described previously (17) allowed precise and accurate identification of the *Rhizoctonia* species accountable for rice sheath diseases. The current study identified thirty isolates as *R. solani* and seven isolates as belonging to *R. oryzae-sativae* based on the species-specific primers. These findings align with several studies who have reported the *Rhizoctonia solani* as the predominant species causing rice sheath blight (25-27). As per the available information, documentation of the presence and distribution of *Rhizoctonia oryzae-sativae*, the causal agent of rice sheath disease serves as the first report in Tamil Nadu.

Host range study of *R. solani* and *R. oryzae-sativae was conducted on several different plant species and found* that they were pathogenic on several crop plants such as wheat, maize, ragi, sorghum, cowpea, green gram and soybean as well as weed hosts like swollen fingergrass, nutgrass, jungle rice and crow foot grass. Several researchers have reported that *Rhizoctonia* species have a broad host range and symptomatology *viz*., sheath blight, damping-off of seedling, root rot or collar rot, stem canker, crown rot and aerial blight on different crops (28-31).

Conclusion

Sheath blight disease complex poses a significant threat to rice crops, affecting the sheath tissue and leading to detrimental impacts on plant health and yield. Gaining insights into the characteristics and virulence of each pathogen is crucial to formulate efficient strategies for disease management and safeguarding crop productivity. By comprehensively understanding these aspects, effective measures can be devised to mitigate the impact of the disease and protect rice crops from potential damage.

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Authors' contributions

GC devised the experiments. NS planned and carried out the experiments, collected the data and wrote the initial draft of the manuscript. The manuscript's initial draft was revised by LR and GC. NS, KR, PR, UD & RM updated and refined the draft. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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