J.Mycopathol.Res. 64(2) : 385-397, 2026, ISSN : 0971-3719 (Print), 2583-6315 (Online) doi.10.57023/JMycR.64.2.2026.385

Morpho-Genetic Diversity of *Rhizoctonia* species Associated with Rice Sheath Blight in Northwestern Region of India

ASHISH SINGH BISHT[1], BIJENDER KUMAR[1*], DEEPAK SINGH BISHT[2], RAJSHREE VERMA[1], NIMISHA MAURYA[1], YOGENDRA SINGH[1], SHAILBALA SHARMA[1], AJAY KUMAR PANDEY[3] AND DINESH PANDEY[4]

[1]Department of Plant Pathology, [3]Department of Entomology and [4]Department of Molecular Biology & Genetic Engineering, G. B. Pant University of Agriculture and Technology, Pantnagar, 263145 Uttarakhand

[2] ICAR-National Institute for Plant Biotechnology, LBS Centre, Pusa Campus, New Delhi – 110012

Received : 15.04.2026 Accepted : 25.05.2026 Published : 29.06.2026

Sheath blight of rice, caused by *Rhizoctonia solani* Kuhn, is one of the most significant yield-limiting diseases affecting rice cultivation in India. This pathogen exhibits significant genetic diversity, with multiple species contributing to sheath blight incidence. In the present study, 38 isolates of *Rhizoctonia* spp. associated with rice sheath blight were collected from diverse agro-ecological zones of northwestern India. The isolates were cultured on potato dextrose agar medium, notable variations were observed in colony morphology, texture, growth rate, and hyphal width of the isolates. Further variation was evident in the sclerotial characteristics, including color, texture, arrangement, diameter, and the number of sclerotia per plate. The taxonomic identity of the isolates was confirmed through sequencing of the Internal Transcribed Spacer (ITS) region. The ITS sequences of all isolates were analyzed to evaluate nucleotide and haplotype diversity, as well as phylogenetic relationships. Phylogenetic analysis demonstrated distinct clustering patterns, while haplotype analysis identified 11, 12, and 11 unique haplotypes. Additionally, Tajima's D test indicated the presence of multiple rare alleles within the ITS regions.

Keywords : BLAST, characterization, haplotype analysis,nucleotide diversity, *Rhizoctonia*

INTRODUCTION

Rhizoctonia solani Kuhn [Teleomorph *Thanatephorus cucumeris* (Frank) Donk] is the most prominent species within its genus, recognized for its wide host range, which includes over 250 plant species, including crops with substantial economic value (Anderson, 1982; Chahal *et al.* 2003).

This pathogen is responsible for causing sheath blight disease in rice, which is regarded as the second most destructive disease of this crop after blast (Adhipathi *et al.* 2013; Verma *et al*. 2025; Kumar *et al*. 2025; Maurya *et al*. 2025). *R. solani* first causes lesions on rice sheaths, which can occasionally spread to the upper leaves and sometime even up to panicle. The premature senescence of infected leaves and sheaths reduces the leaf area, thereby impairing photosynthesis and ultimately leading to significant yield losses (IRRI, 2015). In India, Butler reported sheath blight disease in 1918. Its symptoms were similar to banded sclerotial disease of sugarcane. It was later reported to have occurred in the Gurdaspur district of Punjab. In nations of Asian continent like Thailand and India, annual yield losses from rice sheath blight have been reported to range from 10% to 20% (Gianessi, 2014). Bisht and Kumar (2024) have also reported severe incidence (64.49%) of sheath blight during *Kharif* 2022-23 in the Udham Singh Nagar district of Uttarakhand, India.

Rhizoctonia solani Kuhn [Teleomorph*Thanatephorus cucumeris* (Frank) Donk], *R. oryzae* Ryker and Gooch [Teleomorph *Waitea circinata*], and *R. oryzae-sativae* (Sawada) Mordue [Teleomorph *Ceratorhiza oryzae-sativae*] are the

*Correspondence: bij2810@gmail.com

main *Rhizoctonia* species that cause rice sheath blight complex (Johanson *et al*. 1998; Sandoval and Cumagun, 2019); however, *R. solani* is the most prevalent of these species. The visual diagnosis of the sheath blight disease complex is made more difficult by the development of lesions in the early stages of infection. Both genetic and morphological variation have been observed in population structure studies of the pathogen (Singh *et al*. 2018; Kumar *et al*. 2021; Moni *et al*. 2016). Breeding efforts targeting into create sheath blight-resistant rice varieties and efficient disease management depend on correctly identifying species and studying variability among *Rhizoctonia* isolates. Significant genetic diversity among *Rhizoctonia* species has been revealed by ITS sequencing (Lore *et al*. 2015; Sandoval and Cumagun, 2019), which has made it easier to identify closely related species linked to the sheath blight complex (Johanson *et al*. 1998; Sandoval and Cumagun, 2019).

The aim of the current study was to: (i) examine the morphological variability among *Rhizoctonia* isolates; (ii) to accurately identify closely related species within the sheath blight complex, using BLAST analysis; and (iii) evaluate the genetic variability of the isolates using haplotype analysis and nucleotide diversity. The isolates of *Rhizoctonia* were collected from different agro-ecological zones of northwestern rice growing region of India.

MATERIALS AND METHODS

Collection of Disease Samples and Isolation of Pathogen

In northwestern rice growing region of India, sheath blight is one of the important diseases of rice, spatial distribution of disease significantly varied across the rice growing regions of the state (Bisht and Kumar, 2024; Bisht *et al*. 2022). Plants exhibiting particular characteristics of rice sheath blight were carefully collected and labeled from different rice growing areas of north-western India (Table1). Potato Dextrose Agar (PDA) medium was used for isolation of pathogens following standard protocol for isolation of fungus given by Tuite (1969). After cutting the diseased tissues (5 × 5 mm) pieces, they were surface sterilized for one minute using 1% sodium hypochlorite and then rinsed three times with sterile distilled water. To avoid contamination these cut pieces were pressed in between sterilized blotter paper for removing excess moisture and placed on PDA media aseptically incubated at (27 ± 2°C). Two days after inoculation mycelia of pathogen was picked from the periphery and transferred into new PDA plate for obtaining pure culture of pathogens.

Pathogenicity Test

The pathogenicity test was conducted under controlled glasshouse conditions using seedlings of a susceptible rice variety Pant Dhan 4, the seedlings were transplanted into pots containing sterilized soil. At 50 days after transplanting, the rice plants were inoculated with bits of fungal culture from each isolate, which were placed at the center of the tillers just above the water level, following the methodology outlined by Singh *et al.* (2010). Control plants were maintained without pathogen inoculation to serve as a baseline for comparison.The glasshouse environment was maintained under optimal conditions, characterized by high humidity i.e. 95% and a temperature of 28 ± 2 °C, to facilitate the development of the disease. After a two-week incubation period, the inoculated plants began to exhibit symptoms characteristic of rice sheath blight. Leaves displaying the symptoms of rice sheath blight were subsequently collected and brought to the laboratory for further pathogen re-isolation.

Morphological characterization

The published protocol were used to examine the morphological traits of *Rhizoctonia* species (Gopireddy *et al*. 2017; Singh *et al*. 2018). In order to determine the color of the colony and the sclerotia as well as their texture, pattern, and location, the isolates of *Rhizoctonia* species were cultured in Petri plates using PDA medium. The size of hyphae was measured using an Olympus CX43 microscope, with 10 hyphae randomly selected per isolate. The growth rate of mycelial were recorded in three replicates (at 24, 48, 72, 96 and 120 hours) after incubation. After ten days of incubation, the number and size of sclerotia on each plate were counted.

Pathogenic variability

The pathogenic variability of all isolates was assessed through artificial inoculation under glasshouse conditions. The highly susceptible rice variety, Pant Dhan 4, was selected as the host for the quantitative measurement of disease severity caused by the various isolates. Three rice seedlings were transplanted into plastic pots, with the pots arranged randomly in a completely randomized design with three replications. The inoculum for each fungal isolate was propagated on a sterilized mixture of barley grains and Typha (*Typha angustata*) pieces in a 1:1 (v/v) ratio, and incubated at 28±2°C for 15 days. Inoculation of the rice plants was made 50 days after transplanting, with the inoculum placed in the center of the tillers just above the water level (Singh *et al*. 2010). The inoculated plants were gently tied with twine to ensure proper contact between the tillers. The relative lesion height,was measured 21 days post-inoculation. The average percentage of vertical disease spread was converted to a 0-9 scale using the Standard Evaluation System (SES) for rice sheath blight (IRRI, 2015).

0 = No infection
1 = lesion limited to the lower 20% of plant height
3 = lesion limited to the lower 20-30% of the plant height
5 = lesion limited to the lower 31- 45% of the plant height
7 = lesion limited to the lower 46- 65% of the plant height
9 = lesion more than 65% of the plant height

$$PDI = \frac{\text{Sum of all disease rating}}{\text{total number of hills x maximum disease grade}} \times 100$$

Molecular characterization of pathogen DNA isolation

To facilitate molecular characterization of the pathogen, all isolates were cultured in flasks containing potato dextrose broth at a temperature of 28 ± 2 °C for 7 days. After the incubation period, HiPurA® Fungal DNA Purification Kit (MB543) was used to extract fungal genomic DNA. The DNA concentration was then determined using a Thermo Scientific™ NanoDrop™ One and confirmed by gel electrophoresis on a 0.9% agarose gel.

PCR amplification and molecular characterization

The internal transcribed spacer (ITS) region was amplified using the universal primers ITS-1 and ITS-4 in order to perform molecular identification of the isolates that were collected. An initial denaturation at 95°C for five minutes was the first step in optimizing the amplification conditions. Thirty-five cycles followed, each consisting of one minute of denaturation at 95°C, one minute of primer annealing at 55°C, and one minute of extension at 72°C. For seven minutes, a last extension was carried out at 72°C.The polymerase chain reaction (PCR) amplification process was conducted using a SimpliAmp thermal cycler (Applied Biosystems). The resulting PCR products were analyzed via gel electrophoresis on a 2% agarose gel, and the banding patterns were documented using the Bio-Rad Gel Doc XR+ imaging system. The amplified DNA fragments were then sent to Barcode BioSciences Pvt. Ltd. in Bengaluru, India, for Sanger sequencing to determine their nucleotide sequences. The obtained ITS sequences were subsequently uploaded to the GenBank database (https://www.ncbi.nlm.nih.gov /genbank/), and a BLAST analysis was conducted through the NCBI database tool to identify the closest taxonomic affiliations.

Phylogenetic analysis

In order to investigate the evolutionary relationships between and among species, the internal transcribed spacer (ITS) sequences of 38 isolates of *Rhizoctonia* species were analyzed. MEGA 11 software was utilized for phylogenetic analysis, and then neighbor-joining method was used to infer evolutionary history (Tamura *et al*., 2021). Using BioEdit software (Version 7.2.5), the sequences were assembled together and aligned to produce a consensus sequence identity matrix (Hall, 1999). Subsequently, a comprehensive phylogenetic tree was created to represent all three pathogens examined in this study.

Nucleotide diversity and haplotype analysis

The study of nucleotide diversity and haplotype characteristics was performed using 38 isolates obtained in this research, along with ITS sequences of reference strains sourced from the NCBI GenBank database. The DNA sequences from all isolates were aligned using MEGA 11 software for multiple sequence alignment. Subsequently, DNA polymorphism analysis was performed using DnaSP v6.12.03 to evaluate various genetic characteristics, including the number of polymorphic sites, nucleotide diversity, Theta values (per site and sequence), the mean nucleotide differences, and Tajima's D statistic. Additionally, the study identified haplotype counts and estimated haplotype diversity.

The haplotype data obtained from DnaSP v6.12.03 were further processed to construct a haplotype network tree using the median-joining algorithm in PopART v1.7 software.

RESULTS AND DISCUSSION

Isolation and Pathogenicity Test

Surveys were conducted during the *Kharif* seasons of 2022 and 2023 to collect samples of rice sheath blight for the isolation of the associated pathogen. Fungal colonies began to grow on potato dextrose agar (PDA) plates two days post-isolation. Colonies exhibiting the distinctive morphological characteristics of *Rhizoctonia* spp. were selected and sub-cultured onto new PDA plates for purification. A total of thirty-eight isolates were prepared for studying morphological and genetic diversity (Table 1).Within two weeks post-inoculation, all treated plants exhibited characteristic symptoms of rice sheath blight disease. The symptoms manifested as circular, oval, or irregular greenish-gray patches on the leaf sheaths. As these patches expanded, their centers turned greyish-white, bordered by irregular blackish-brown or purple-brown margins. In contrast, control plants exhibited no symptoms.Re-isolation of the pathogen from the sheaths and leaves of inoculated plants revealed fungal colonies exhibiting characteristics consistent with *Rhizoctonia* species.

Morphological characteristics Colony characteristics

Among the 38 isolates examined, 5 exhibited a slow rate of colony growth (<30.0 mm/day), 12 showed a moderate growth rate (30–35 mm/day), and 21 showed a rapid rate of colony growth (>35 mm/day).The predominant characteristic observed across the isolates was a light brown coloration accompanied by aerial mycelial growth. Three distinct types of colony texture were identified: appressed, aerial, and fluffy, with the aerial texture being the most frequently observed among the isolates (Fig. 1). Notably, six isolates (RSASB8, RSASB17, RSASB18, RSASB21, RSASB39, and RSASB40) exhibited larger hyphal widths, ranging from 7.25 to 7.60 ìm (Table 2).

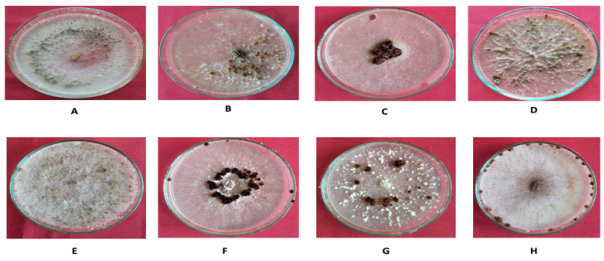

Fig 1 : Cultural and morphological diversity between the isolates of rice sheath blight associatedpathogens

Sclerotial characteristics

A total of 22 isolates (57.89%) exhibited sclerotia with a dark brown coloration. Among these, sclerotia with a rough surface were also observed in 22 (57.89%) isolates of *Rhizoctonia* (Table 2). The time required for sclerotia formation, ranged between 3 to 5 days (Table S1). The position and arrangement of sclerotial bodies were found to be either embedded or aerial, scattered, distributed in central, or peripheral ring patterns (Fig. 2). Variations in sclerotial morphology included sclerotia with and without aggregation, depressed sclerotia, and sclerotia with fluid on the surface (Fig. 2). Based on production of sclerotia per plate, 21 isolates were grouped as excellent (>80 sclerotia per plate), 5 as very good (41–80 sclerotia), and 12 as good (1–40 sclerotia) (Table 2). Additionally, variations in sclerotial size were recorded, with 26.31% of isolates producing smaller sclerotia (<1 mm), 34.21% producing medium-sized sclerotia (1–2 mm), and 39.47% producing larger sclerotia (>2 mm).

Pathogenic variability

The average relative lesion height (RLH) induced by various isolates of *Rhizoctonia* on the Pant Dhan-4 rice cultivar ranged from 15.65% to 52.4%. Among the 40 fungal isolates tested for

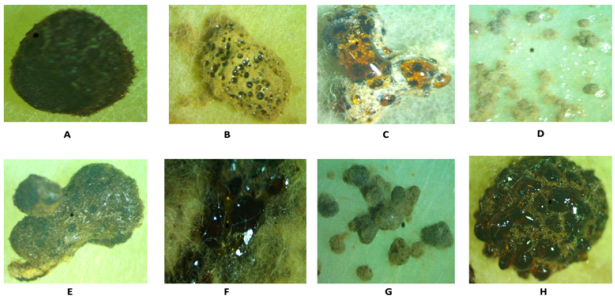

Fig 2 : Variability in sclerotial characters between the isolates causing rice sheath blight

their pathogenicity, isolate RSASB32 produced largest lesions with 52.4% relative lesion height while, RSASB17 exhibited the lowest relative lesion height of 15.65%. Seven isolates, namely RSASB11, RSASB19, RSASB23, RSASB32, RSASB34,RSASB36, and, RSASB37, produced more than 45% relative lesion height. In contrast, isolates RSASB1, RSASB4, RSASB8, RSASB17, RSASB26, RSASB27, RSASB21, and RSASB22 exhibited minimal pathogenicity, with relative lesion height values below 30%. The remaining 21 isolates were categorized as moderately pathogenic, with relative lesion height values ranging from 30% to 45% (Table1).

Molecular Characterization of Pathogen

PCR amplification was carried out using DNA obtained from the fungal isolates in order to verify the morphological identification of *Rhizoctonia* isolates. Using the ITS1 and ITS4 primers, PCR products ranging from 600 to 800 base pairs were successfully amplified for each isolate. The amplified ITS regions were sequenced, a BLAST search was performed against the NCBI GenBank database to examine the nucleotide sequences that were obtained. The BLAST analysis showed a maximum identity between 80-100% of 29 isolates (76.31%) with *Rhizoctonia solani,* seven (18.42%) with *Ceratorhiza oryzae-sativae*(anamorph *R. oryzae-sativae*) and two (5.26%) with *Waitea circinata* (anamorph *R. oryzae*). The obtained sequences were registered in NCBI GenBank, and respective accession numbers were assigned (Table1).

Phylogenetic analysis

Phylogenetic relationship was inferred using consensus sequences obtained from diverse set of fungal isolates, consisting of 29 *Rhizoctonia solani*, 7 *Ceratorhiza oryzae sativae* and 2 *Waitea circinata***,** reference isolate sequences retrieved from the NCBI GeneBank database were included in the analysis alongside the isolate sequences. Total 35 strains of *R. solani*, 34 strains of *C. oryzae sativae* and 34 strain of *W. circinata* used for constructing neighbor-joining tree (Fig. 3). Phylogenetic analysis revealed, the isolates of *R. solani* were making distinct clustering pattern. All twenty-nine isolates from this study grouped into a single cluster along with *R. solani* strains from China, Hyderabad, Andhra Pradesh, and New Delhi. Notably, one isolate, RSASB 36, formed a separate clade from the other strains, suggesting an independent evolutionary trajectory (Fig. 3). This finding indicates that the *R. solani* strains causing sheath blight in northwestern region on India share a common ancestral lineage with the other strains analyzed in the study (Fig. 3). Further analysis of ITS sequences of seven isolates of *Ceratorhiza oryzae-sativae* from this study, combined with 34 additional *Ceratorhiza* strains, revealed a major cluster distinct from *Rhizoctonia* and *Waitea* (Fig. 3). Similarly, phylogenetic analysis of two *Waitea circinata* isolates from this study, along with 34 geographically diverse *Waitea* strains, showed distinct clustering patterns within a neighbor-joining tree (Fig. 3).

Nucleotide diversity and haplotyping analysis

A total of 493 nucleotide sites, excluding those with missing or incomplete data, were examined across 34 sequences of *Ceratorhiza oryzae-sativae*. Within these sequences, 78 sites were identified as polymorphic (segregating). The mean nucleotide differences (kt) and nucleotide diversity (PiT) were determined to be 8.5918 and 0.01743, respectively. Tajima's D test was conducted to evaluate the neutrality of nucleotide variations, yielding a statistically significant negative value ($D = -2.24344$, $P < 0.001$), indicating potential deviations from neutrality.

Among the 34 isolates, 12 haplotypes (h) were detected, with a haplotype diversity (hd) of 0.58645. The genetic differences among isolates were visualized using a haplotype tree based on nucleotide sequences (Fig. 4A).

For *Waitea circinata*, a total of 479 nucleotide sites, excluding gaps and missing data, were analyzed across 21 sequences. The analysis revealed 144 polymorphic sites. The estimated average nucleotide difference (kt) and nucleotide diversity (PiT) were 28.43810 and 0.05937, respectively. Tajima's D test, used to assess the neutrality of mutations, produced a negative value

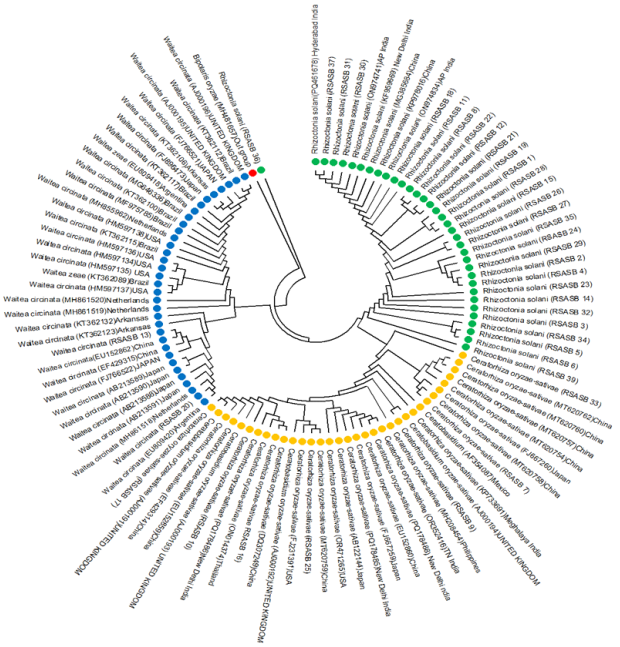

Fig 3: The evolutionary relationships between geographically different isolates of *Waitea circinata*, *Ceratorhiza oryzae-sativae*, and *Rhizoctonia solani* are depicted in a phylogenetic tree. The inferred evolutionary history of the examined species is represented by the bootstrap consensus tree, which is generated from 1000 replicates. *Bipolaris oryzae* was determined as the outgroup in the evolutionary analyses, which were carried out using MEGA 11 software.

(D = -1.26487), though it was not statistically significant (P > 0.10). Among the 21 isolates, 11 haplotypes (h) were identified, with a haplotype diversity (hd) of 0.90476. To depict genetic differentiation among isolates, a haplotype tree based on nucleotide sequences was constructed (Fig. 4B).

Similarly, for *Rhizoctonia solani*, 234 nucleotide sites, excluding gaps and missing data, were analyzed across 35 sequences. Among these, 189 sites were identified as polymorphic. The estimated average nucleotide difference (kt) and nucleotide diversity (PiT) were 20.76975 and 0.08876, respectively. Tajima's D test produced a statistically significant negative value (D = -2.6071, P < 0.001), indicating the influence of potential non-neutral evolutionary forces. A total of 11 haplotypes (h) were detected among the 35

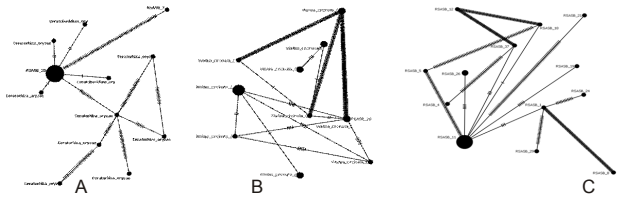

Fig 4: Based on nucleotide polymorphisms, the haplotype tree reveals distinct haplotype groups among the various rice sheath blight pathogens. (A) for the 34 isolates of *C. oryzae-sativae*, 12 haplotype groups were found; (B) eleven haplotypes were found for 21 isolates of *W. circinata*, (C) Eleven haplotype groupings were found in the 35 *R. solani* isolates

isolates, with a haplotype diversity (hd) of 0.113. To illustrate the genetic differentiation among isolates, a haplotype tree based on nucleotide sequences was generated (Fig. 4C).

Rice sheath blight poses a significant challenge to rice cultivation worldwide. Several factors contribute to the increasing prevalence of the disease, including favorable environmental conditions, the absence of genetic resistance, continuous rice monocropping, and limited awareness among farmers regarding effective disease management practices.*R. solani* Kuhn, *R.oryzae* Ryker and Gooch [Teleomorph *Waitea circinata*], and *R. oryzae-sativae* (Swada) Mordue [Teleomorph *Ceratorhiza oryzae-sativae*] are recognized as the causative agents of the disease (Johanson *et al*. 1998; Sandoval and Cumagun, 2019).The plant-pathogenic species of *Rhizoctonia* constitute a globally distributed group of fungi with complex taxonomy.

Despite the importance of *Rhizoctonia* genetic diversity as a constraint in northwestern region of India, limited research has been conducted to understand its variability. To bridge this knowledge gap, our study collected and examined 38 isolates of the pathogen from different rice-growing regions of northwestern region of India to identify and characterize *Rhizoctonia* species

Table 1 : Details of *Rhizoctonia* isolates collected during *Kharif* season 2022 and 2023 from different geographical region of northwestern India

S. No.	Isolate name	Location	Latitude	Longitude	District	*Rhizoctonia solani*	*Rhizoctonia oryzae-sativae*	*Rhizoctonia oryzae*	Accession No.	Relative lesion height (%)
1	RSASB 1	Saikot	30.3679470	79.3039812	Chamoli	+	-	-	PP769608	25.76
2	RSASB 2	Pipalkoti	30.4337317	79.4294906	Chamoli	+	-	-	PP770718	38.34
3	RSASB 3	Rudraprayag	30.2843950	78.9779625	Rudraprayag	+	-	-	PQ842613	37.45
4	RSASB 4	Augustyamuni	30.3921495	79.0312697	Rudraprayag	+	-	-	PP770719	27.67
5	RSASB 5	Ghansali	30.4255176	78.6657759	Tehri Garhwal	+	-	-	PQ844694	38.87
6	RSASB 6	Narendranagar	30.1359445	78.3083273	Tehri Garhwal	+	-	-	PQ844697	40.78
7	RSASB 7	Kotdwar	29.7699707	78.4097327	Pauri Garhwal	-	+	-	PQ774239	32.54
8	RSASB 8	Satpuli	29.9393166	78.7053644	Pauri Garhwal	+	-	-	PQ826495	20.45
9	RSASB 9	Uttarkashi	30.7108600	78.3536036	Uttarkashi	-	+	-	PQ842726	38.66
10	RSASB 10	Chinyalisaur	30.5854889	78.3187814	Uttarkashi	-	+	-	PQ850634	39.65
11	RSASB 11	Rishikesh	30.0807515	78.1878850	Dehradun	+	-	-	PQ844701	45.76
12	RSASB 12	Thano	30.2376089	78.2068602	Dehradun	+	-	-	PP808709	32.62
13	RSASB 13	Banjarawala	30.2836753	78.0065445	Dehradun	-	-	+	PQ850633	42.87
14	RSASB 14	Naya gaon	30.3651282	77.8066278	Dehradun	+	-	-	PQ844703	35.87
15	RSASB 15	Vikash-Nagar	30.4765421	77.7569009	Dehradun	+	-	-	PP770724	41.46
16	RSASB 16	Raiwala	30.017609	78.219901	Haridwar	-	+	-	PQ826333	31.78

17	RSASB 17	Bageshwar	29.8376660	79.7661689	Bageshwar	-	+	-	PQ826330	19.45
18	RSASB 18	Khanpur	29.6575793	78.0160897	Haridwar	+	-	-	PP770725	32.76
19	RSASB 19	Narsan	29.70117731	77.8445313	Haridwar	+	-	-	PP770730	45.57
20	RSASB 20	Roorkee	29.8763158	77.8338019	Haridwar	-	-	+	PQ858621	29.65
21	RSASB 21	Garur	29.8959321	79.6099376	Bageshwar	+	-	-	PP808712	15.65
22	RSASB 22	Laksar	29.7529315	78.01142968	Haridwar	+	-	-	PP808710	28.11
23	RSASB 23	Bajrikot	29.3155257	80.1018598	Champawat	+	-	-	PQ842654	48.36
24	RSASB 24	Pithoragarh	29.5885402	80.1915462	Pithoragarh	+	-	-	PP770731	35.43
25	RSASB 25	Didihat	29.7975322	80.2515701	Pithoragarh	-	+	-	PQ826328	37.31
26	RSASB 26	Hawalbagh	29.6422330	79.63113067	Almora	+	-	-	PP770732	25.76
27	RSASB 27	Kwarab	29.55118398	79.6092263	Almora	+	-	-	PP776798	23.65
28	RSASB 28	Haldwani	29.2190131	79.5480677	Nainital	+	-	-	PP770735	32.45
29	RSASB 29	Ranibagh	29.2877627	79.5464532	Nainital	+	-	-	PP770736	30.65
30	RSASB 30	Kotabagh	29.3960156	79.2982643	Nainital	+	-	-	PQ826487	41.25
31	RSASB 31	Bailparao	29.3089715	79.1941421	Nainital	+	-	-	PQ826488	39.36
32	RSASB 32	Dineshpur	29.0464152	79.3127425	Udham Singh Nagar	+	-	-	PQ843071	52.4
33	RSASB 33	Jaspur	29.2812830	78.8065724	Udham Singh Nagar	-	+	-	PQ843105	38.43
34	RSASB 34	Sitarganjh	28.9260864	79.6978394	Udham Singh Nagar	+	-	-	PQ844705	46.43

35	RSASB 35	Kashipur	29.2181015	78.9128917	Udham Singh Nagar	+	-	-	PQ844750	44.15
36	RSASB 36	Khatima	28.9128655	79.9815641	Udham Singh Nagar	+	-	-	PQ842648	31.11
37	RSASB 37	Ramnagar	29.3920805	79.11224974	Nainital	+	-	-	PQ826319	48.32
38	RSASB 38	Pantnagar	29.0184430	79.4821636	Udham Singh Nagar	+	-	-	PQ844691	35.23

associated to sheath blight of rice.The genetic diversity of rice sheath blight has been studied for the first time in these locations, and notably, the presence of *Waitea circinata* and *Ceratorhiza oryzae-sativae* in the sheath blight complex has also been reported for the first time in these regions. The fungus exhibits considerable morphological diversity. Several studies have documented variability among geographically distinct isolates of the pathogen in terms of the abundance, size, and color of sclerotia, as well as the color of the mycelium (Parmeter and Whitney, 1970; Manian, 1982).

In the present investigation, a substantial variation was observed among different isolates with respect to the size and number of sclerotia. The findings indicated that *Rhizoctonia* isolates producing a greater number of sclerotia exhibited smaller sclerotial diameters, whereas isolates with fewer sclerotia showed larger diameters. Additionally, faster-growing isolates were characterized by narrower hyphal widths compared to slower-growing ones. Previous studies (Singh *et al.,* 2014, 2018; Budiarti *et al.,* 2019; Gopireddy*et al.* 2017) have also documented variations in colony and sclerotial characteristics among *Rhizoctonia* isolates from different geographic regions. In our study, notable variations were observed in colony growth rate (22.0–40 mm/day), hyphal width (5.25–7.60 µm), sclerotial diameter (0.67–2.80 mm), and sclerotia count (14–192). However, no distinct correlation was found between these traits and the agro-ecological zones.These findings correlate with the results reported by Goswami *et al*. (2010), Kuiry *et al*. (2014), and Budiarti *et al*. (2019).

Understanding the variability of this pathogen is crucial for epidemiology and effective disease management. Furthermore, this information helps researchers in developing rice cultivars with improved disease resistance. Numerous studies have highlighted the substantial genetic diversity of the sheath blight pathogen (Liu and Sinclair, 1993; Vilgalys and Hester, 1990). The PCR based molecular methods have been used to evaluate genetic diversity in *R. solani* because there is no uniform host-differential system for characterizing isolates of this fungus. In this study, ITS sequence-based approach, previously applied to other rice fungal pathogens (Pramesh *et al*. 2024; Amoghavarsha *et al.* 2022; Sharanabasav*et al.,* 2021), was utilized to evaluate genetic diversity among different isolates. The findings suggest that rice cultivars in northwestern India exhibit differential responses to *Rhizoctonia* populations collected from various locations, which are characterized by significant inter- and intra-specific genetic diversity.The phylogenetic analysis revealed that isolates from different rice-growing regions of northwestern India clustered with isolates from other Indian states and other countries.

The genetic diversity inferred from phylogenetic analysis was further supported by nucleotide diversity in ITS sequences and haplotype analysis. The haplotype analysis revealed twelve distinct haplotypes among geographically diverse *C. oryzae-sativae* isolates.However, some differences were observed between phylogenetic clusters and haplotype groupings. For instance, isolates RSASB 7 and RSASB 10 formed separate haplotypes but were part of the same phylogenetic cluster (Fig. 3). Similar patterns have been documented for *Magnaporthe oryzae* (Amoghavarsha *et al*. 2022) and *Bipolaris* spp. (Pramesh *et al*.2024) isolates from India. In

Table 2 : Grouping of different *Rhizoctonia solani* isolates of rice on the basis of colony and sclerotial characteristics

Characteristics	Group#	Description	Isolate	Frequency of isolate (%)
Colony characteristics				
Colour	CC-1	White (Wh)	RSASBS 1, RSASB 5, RSASB 11, RSASB 12, RSASB 16, RSASB 17, RSASB 21, RSASB 22, RSASB 26, RSASB 27, RSASB 33, RSASB 35, RSASB 36, RSASB 39, RSASB 40,	37.5
	CC-2	Light brown (LB)	RSASB 2, RSASB 3, RSASB 7, RSASB 23, RSASB 24, RSASB 25, RSASB 32, RSASB 38	20
	CC-3	Mid brown (MB)	RSASB 4, RSASB 6, RSASB 8, RSASB 9, RSASB 10, RSASB 14, RSASB 15, RSASB 18, RSASB 19, RSASB 20, RSASB 28, RSASB 34	30
	CC-4	Dark brown (DB)	RSASB 13, RSASB 29, RSASB 30, RSASB 31, RSASB 37	12.5
Texture	CT-1	Appressed	RSASB 2, RSASB 3, RSASB 10, RSASB 11, RSASB 13, RSASB 19, RSASB 20, RSASB 23, RSASB 28, RSASB 29, RSASB 30, RSASB 32, RSASB 38	32.5
	CT-2	Aerial	RSASB 4, RSASB 5, RSASB 6, RSASB 8, RSASB 9, RSASB 14, RSASB 15, RSASB 16, RSASB 18, RSASB 24, RSASB 26, RSASB 31, RSASB 34, RSASB 36, RSASB 40	37.5
	CT-3	Fluffy	RSASB 1, RSASB 7, RSASB 12, RSASB 17, RSASB 21, RSASB 22, RSASB 25, RSASB 27, RSASB 33, RSASB 35, RSASB 37, RSASB 39	30
Growth rate (mm) per day	CG-1	< 30.0 (Slow)	RSASB 8, RSASB 17, RSASB 18, RSASB 21, RSASB 39, RSASB 40	15
	CG-2	30.0 - 35.0 (Medium)	RSASB 1, RSASB 4, RSASB 7, RSASB 12, RSASB16, RSASB 20, RSASB 22, RSASB 28, RSASB 29, RSASB 26, RSASB 27, RSASB 36	30
	CG-4	> 35.0 (Fast)	RSASB 2, RSASB 3, RSASB 5, RSASB 6, RSASB 9, RSASB 10, RSASB 11, RSASB 13, RSASB 14, RSASB 15, RSASB 19, RSASB 23, RSASB 24, RSASB 25, RSASB 30, RSASB 31, RSASB 32, RSASB 33, RSASB 34, RSASB 35, RSASB 37, RSASB 38	55
Hyphal width (µm)	HW-1	7.03 – 7.60	RSASB 8, RSASB 17, RSASB 18, RSASB 21, RSASB 39, RSASB 40	15
	HW-2	6.15 – 6.90	RSASB 1, RSASB 4, RSASB 7, RSASB 12, RSASB16, RSASB 20, RSASB 22, RSASB 28, RSASB 29, RSASB 26, RSASB 27, RSASB 36	30
	HW-3	5.16 – 5.94	RSASB 2, RSASB 3, RSASB 5, RSASB 6, RSASB 9, RSASB 10, RSASB 11, RSASB 13, RSASB 14, RSASB 15, RSASB 19, RSASB 23, RSASB 24, RSASB 25, RSASB 30, RSASB 31, RSASB 32, RSASB 33, RSASB 34, RSASB 35, RSASB 37, RSASB 38	55
Sclerotial characteristics				
Time taken to form (days)	SF-1	3	RSASB 2, RSASB 3, RSASB 5, RSASB 6, RSASB 9, RSASB 10, RSASB 11, RSASB 13, RSASB 14, RSASB 15, RSASB 19	27.5
	SF-2	4	RSASB 1, RSASB 8, RSASB 17, RSASB 40, RSASB 22, RSASB 25, RSASB 32, RSASB 33, RSASB 29, RSASB 26	25
	SF-3	5	RSASB 12, RSASB 4, RSASB 7, RSASB 21, RSASB 18, RSASB 37, RSASB 35, RSASB 34, RSASB 31, RSASB 30, RSASB 24, RSASB 23, RSASB 16, RSASB 20, RSASB 28, RSASB 27, RSASB 36, RSASB 38, RSASB 39	47.5
Colour	SC-1	Light brown (LB)	RSASB 1, RSASB 6, RSASB 10, RSASB 12, RSASB 13, RSASB 15, RSASB 17, RSASB 20, RSASB 21, RSASB 22, RSASB 23, RSASB 24, RSASB 25, RSASB 26, RSASB 27, RSASB 28, RSASB 29, RSASB 30, RSASB 31, RSASB 33, RSASB 36, RSASB 40	55

	SC-2	Dark brown (DB)	RSASB 2, RSASB 3, RSASB 4, RSASB 5, RSASB 7, RSASB 8, RSASB 9, RSASB 11, RSASB 14, RSASB 16, RSASB 18, RSASB 19, RSASB 32, RSASB 34, RSASB 35, RSASB 38, RSASB 39	45
Texture	ST-1	Rough	RSASB 2, RSASB 3, RSASB 4, RSASB 5, RSASB 6, RSASB 7, RSASB 8, RSASB 10, RSASB 11, RSASB 12, RSASB 13, RSASB 14, RSASB 15, RSASB 18, RSASB 19, RSASB 23, RSASB 32, RSASB 33, RSASB 34, RSASB 35, RSASB 37, RSASB 40	60
	ST-2	Smooth	RSASB 1, RSASB 9, RSASB 16, RSASB 17, RSASB 20, RSASB 21, RSASB 22, RSASB 24, RSASB 25, RSASB 26, RSASB 27, RSASB 28, RSASB 29, RSASB 30, RSASB 31, RSASB 36	40
Position and pattern	SPP-1a	Aerial evenly scattered	RSASB 1, RSASB 6, RSASB 21	7.5
	SPP-1b	Embedded evenly scattered	RSASB 5, RSASB 32, RSASB 38	7.5
	SPP-2a	Aerial centrally clustered		0
	SPP-2b	Embedded centrally clustered	RSASB 2, RSASB 3, RSASB 9, RSASB 11, RSASB 16	12.5
	SPP-3a	Aerial unevenly scattered	RSASB 7, RSASB 13, RSASB 14, RSASB 24, RSASB 25, RSASB 26, RSASB 27, RSASB 35, RSASB 36, RSASB 37, RSASB 39, RSASB 40	30
	SPP-3b	Embedded unevenly scattered	RSASB 10, RSASB 15, RSASB 18, RSASB 20, RSASB 23, RSASB 29, RSASB 30, RSASB 31, RSASB 34	22.5
	SPP-4	Embedded peripheral ring	RSASB 4, RSASB 8, RSASB 12, RSASB 19, RSASB 33	12.5
	SPP-5	Embedded concentric ring	RSASB 17, RSASB 22, RSASB 28	7.5
Diameter (mm)	SD-1	<1.0	RSASB 1, RSASB 6, RSASB 17, RSASB 21, RSASB 22, RSASB 24, RSASB 25, RSASB 28, RSASB 31, RSASB 36	25
	SD-2	1.0 - 2.0	RSASB 7, RSASB 8,RSASB 12, RSASB 13, RSASB 16, RSASB 18, RSASB 19, RSASB 26, RSASB 27, RSASB 30, RSASB 32, RSASB 33, RSASB 38, RSASB 40	35
	SD-3	>2.0	RSASB 2, RSASB 3, RSASB 4, RSASB 5, RSASB 9, RSASB 10, RSASB 11, RSASB 14, RSASB 15, RSASB 20, RSASB 23, RSASB 29, RSASB 34, RSASB 35, RSASB 37, RSASB 39	40
Number per plate	SN-1	1 – 40 (Good)	RSASB 2, RSASB 3, RSASB 7, RSASB 9, RSASB 10, RSASB 11, RSASB 12, RSASB 13, RSASB 14, RSASB 23, RSASB 35, RSASB 37	30
	SN-2	41 -80 (Very good)	RSASB 4, RSASB 8, RSASB 27, RSASB 31, RSASB 39, RSASB 40	15
	SN-3	>80 (Excellent)	RSASB 1, RSASB 5, RSASB 6, RSASB 15, RSASB 16, RSASB 17, RSASB 18, RSASB 19, RSASB 20, RSASB 21, RSASB 22, RSASB 24, RSASB 25, RSASB 26, RSASB 28, RSASB 29, RSASB 30, RSASB 32, RSASB 33, RSASB 34, RSASB 36, RSASB 38	55

position and pattern, SD- Sclerotia diameter, SN- Sclerotia number

contrast, *W. circinata* isolates exhibited eleven haplotypes but only five phylogenetic sub-clusters. Certain isolates, such as RSASB 20, formed distinct haplogroups, reflecting their genetic divergence. Among *R. solani* isolates, eleven haplotypes were identified, with isolates such as RSASB12, 18, 37, 26, 5, 4, 11, 21, 19, 1, 24, 29, and RSASB 8 forming distinct haplotypes. This indicates a high degree of genetic diversity within these populations.

ACKNOWLEDGEMENTS

The authors want to thank Department of Plant Pathology G. B. Pant University of Agriculture and Technology and ICAR-National Institute for Plant Biotechnology, LBS Centre, Pusa Campus, New Delhi for providing lab facility for research.

DECLARATION

Conflict of Interest.Authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in NCBI GenBank. The accession numbers of all the sequences are listed in Table 1.

REFERENCES

Adhipathi, P., Singh, V., Meena, S.C. 2013. Virulence diversity of *Rhizoctonia solani* causing sheath blight disease in rice and its host pathogen interaction. *Bioscan***8**: 949-952.

Amoghavarsha, C., Pramesh, D., Naik, G.R., Naik, M.K., Yadav, M.K., Ngangkham, U., Chidanandappa, E., Raghunandana, A., Sharanabasav, H., E. Manjunatha, S. 2022. Morpho molecular diversity and avirulence genes distribution among the diverse isolates of *Magnaporthe oryzae* from Southern India. *J. Appl. Microbiol.***132**: 1275-1290.

Bisht, A.S., Maurya, N., Kumar, B. 2022. Prevalence and distribution of rice sheath blight disease in different rice growing (ecosystem) areas of Uttarakhand. *Plant Dis. Res.***37**: 226.

Bisht, A.S., Kumar, B. 2024. Geospatial survey of rice sheath blight in Uttarakhand. *Pantnagar J. Res.***22**:526–531.DOI: 10.13140/RG.2.2.34825.56166

Budiarti, S.W., Lukman, R., Wibowo, A., Sumardiyono, C. and Priyatmojo, A., 2020. The cultural and morphological variability among *Rhizoctonia solani* isolates causing banded leaf and sheath blight of maize in Indonesia. *Arch. Phytopathol. Plant Protect.***53**: 17-36.

Butler, S.E.J. 1918. Fungi and Disease in Plants. An Introduction to the Diseases of Field and Plantation Crops, Especially Those of India and the East.[With Plates.]. Thacker, Spink & Company.

Chahal, K.S., Sokhi, S.S., Rattan, G.S. 2003. Investigations on sheath blight of rice in Punjab. *Indian Phytopathol.***56**: 22-26.

Gopireddy, B.M., Devi, G.U., Kumar, K.V., Babu, T.R., Naidu, T. 2017. Cultural and morphological characterization of *Rhizoctonia solani* f. sp. *sasakii* isolates collected from different districts of Andhra Pradesh. International *J. Curr. Microbiol. Appl. Sci.* **6**: 3457-3469.

Goswami, B.K., Bhuiyan, K.A., Mian, I.H. 2010. Morphological and pathogenic variations in the isolates of *Rhizoctonia solani* in Bangladesh. *Bangladesh J. Agricult. Res.* **35**: 375-380.

Gianessi, L.P. 2014. Importance of pesticides for growing rice in South and South East Asia. *Inter.Pesticide Benefit Case Study* **108**: 30-33.

Hall, T.A. 1999. January. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: *Nucleic acids symposium series* **41**: 95-98.

IRRI Rice Knowledge Bank (2015) Sheath Blight. Available online: http://www.knowledgebank.irri.org/training/factsheets/pestmanagement/diseases/item/sheath-blight.

Johanson, A., Turner, H.C., McKay, G.J., Brown, A.E. 1998. A PCR-based method to distinguish fungi of the rice sheath-blight complex, *Rhizoctonia solani, R. oryzae* and *R. oryzae-sativae*. *FEMS Microbiol. Lett.* **162**: 289-294.

Kuiry, S.P., Mondal, A., Banerjee, S., Dutta, S. 2014. Morphological variability in Rhizoctonia solani isolates from different agro-ecological zones of West Bengal, India. *Arch. Phytopathol. Plant Protect.* **47**: 728-736.

Kumar, B., Bhatt, S., Bisht A. S. 2025. Evaluation of Azoxystrobin and Thifluzamide-Based Treatments forEffective Management of Sheath Blight in Rice. *J.Mycopathol.Res.* **63**: 405-411

Kumar, S., Kaur, H., Hunjan, M.S. 2021. Genetic diversity and virulence spectrum of Rhizoctonia solani, the incitant of banded leaf and sheath blight of maize. *J.Phytopathol.* **169**: 486-499.

Liu, Z.L., Sinclair, J.B. 1993. Differentiation of intraspecific groups within anastomosis group 1 of *Rhizoctonia solani* using ribosomal DNA internal transcribed spacer and isozyme comparisons. *Can. J. Plant Pathol.* **15**: 272-280.

Lore, J.S., Jain, J., Hunjan, M.S., Gargas, G., Mangat, G.S., Sandhu, J.S. 2015. Virulence spectrum and genetic structure of Rhizoctonia isolates associated with rice sheath blight in the northern region of India. *Eur. J. Plant Pathol.* **143**: 847-860.

Manian, S. 1982. *Studies on the sheath blight disease of rice* (Doctoral dissertation, Ph. D. Thesis, University of Madras, Madras).

Moni, Z.R., Ali, M.A., Alam, M.S., Rahman, M.A., Bhuiyan, M.R., Mian, M.S., Iftekharuddaula, K.M., Latif, M.A., Khan, M.A.I. 2016. Morphological and genetical variability among *Rhizoctonia solani* isolates causing sheath blight disease of rice. *Rice Sci.* **23**: 42-50.

Maurya, N., Kumar, B., Singh, R. P., Tewari, R., Bisht, A. S. 2025. Efficacy of fungicides and biocontrol agents against *Bipolaris oryzae*, the causal agent of brown spot of rice. *Ind. J. Agricult. Sci.* **95**: 129-134.

Parmeter, J.R. Jr, Whitney, H.S.1970. Taxonomy and nomenclature of the imperfect state. In: *Rhizoctonia solaniBiology and Pathology* (Ed. J.R. Parmeter Jr.). Berkeley, University of California Press, pp 7–20.

Pramesh, D., Prasannakumar, M.K., Raghunandana, A., Kumar, M.K., Ngangkham, U., Pushpa, H.D., Buella, P.P., Kunduru, B., Manjunatha, C. 2024. Identification and characterization of multiple fungal pathogens associated with brown spot disease of rice in India. *3 Biotech*. **14**:187.

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sánchez-Gracia, A., 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol.Biol.Evol.* **34**: 3299-3302.

Sandoval, R.F.C., Cumagun, C.J.R. 2019. Phenotypic and molecular analyses of Rhizoctonia spp. associated with rice and other hosts. *Microorganisms* **7**: 88.

Sharanabasav, H., Pramesh, D., Prasannakumar, M.K., Chidanandappa, E., Yadav, M.K., Ngangkham, U., Parivallal, B., Raghavendra, B.T., Manjunatha, C., Sharma, S.K., Karthik, N. 2021. Morpho molecular and mating type locus diversity of Ustilaginoidea virens: an incitant of false smut of rice from Southern parts of India. *J. Appl. Microbiol.* **131**:2372-2386.

Singh, R., Sunder, S. and Dodan, D.S., 2010. Standardization of inoculation method in nursery beds and management of sheath blight of rice through host resistance, chemicals and botanicals. *Ind. Phytopathol.* **63**: 286.

Singh, V., Amaradasa, B.S., Karjagi, C.G., Lakshman, D.K., Hooda, K.S., Kumar, A. 2018. Morphological and molecular variability among Indian isolates of Rhizoctonia solani causing banded leaf and sheath blight in maize. *Eur. J. Plant Pathol.* **152**: 45-60.

Singh, V., Kumar, S., Lal, M., Hooda, K.S., 2014. Cultural and morphological variability among Rhizoctonia solani isolates from trans-gangetic plains of India. *Res. Crops* **15:**644-650.

Tamura, K., Stecher, G., Kumar, S.2021. MEGA11: molecular evolutionary genetics analysis version 11. *Mol.Biol.Evol.* **38**: 3022-3027.

Tuite, J. 1969. Plant pathological methods: fungi and bacteria. Burgess Publishing Company, Minneosta.

Verma, R., Bisht, A. S., Roy, B., Kumar, S.,Anjali 2025. Unveiling the Dynamics of Rice Blast: Insights into Pathogenesis, Epidemiology, and Management. In: *Detection, Diagnosis and Management of Air-Borne Diseases in Agricultural Crops* (pp. 143-159). Singapore: Springer Nature Singapore.

Vilgalys, R., Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J.Bacteriol.***172**:4238-4246.