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RESEARCH PAPER

Deciphering the signature of seedborne fungi linked to rice sheath rot disease: insights from ITS rDNA sequencing analysis

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ABSTRACT

Sarocladium oryzae and *Fusarium* spp. are the causal agents of sheath rot, a re-emerging rice disease that has recently gained importance in Indonesia and can cause yield losses of up to 85%. Both pathogens are seedborne, making their accurate identification and management essential. Conventional morphological identification is time-consuming and often inaccurate due to overlapping symptoms among fungal species. In this study, we demonstrated the seedborne transmission of sheath rot pathogens and provided novel insights by highlighting the predominance of *F. equiseti* and the detection of infections in asymptomatic seeds. A total of 75 fungal isolates were obtained from rice leaf sheaths, seeds, and harvested grains across CMS, inbred, and hybrid rice varieties. ITS rDNA sequencing identified 42 isolates as *S. oryzae* and 33 as *Fusarium* spp., including *F. equiseti* (29), *F. incarnatum* (1), and *F. proliferatum* (3). The detection of these pathogens in both pre-planting seed samples and post-harvest grains demonstrates their ability to spread through seeds. Importantly, their presence in asymptomatic seeds and grains indicates that routine visual inspection is insufficient for seed health monitoring.

Keywords: fungal identification, *Fusarium* spp, *Sarocladium oryzae*, seed health, seed transmission

INTRODUCTION

Rice sheath rot is a widespread disease in rice-growing regions and poses a serious threat to global rice production. The main pathogens associated with the disease are *Sarocladium oryzae*, species within the *Fusarium fujikuroi* complex, and *Pseudomonas fuscovaginae*. These pathogens produce phytotoxins that cause necrosis and are responsible for grain discoloration, sterility, and yield reduction. Importantly, all three pathogens are transmitted through seeds (Bigirimana et al., 2015).

Seedborne pathogens significantly contribute to yield and quality losses and play a key role in the spread of rice diseases (Tiwari, 2016). Among the 52 fungal pathogens reported to infect rice, 41 are seedborne (Reddy & Sathyanarayana, 2001; Mew & Gonzales, 2002). This highlights the importance of maintaining high-quality rice seeds to secure food production (Reddy & Sathyanarayana, 2001; Dossou & Silue, 2017). Preventing long-distance dissemination of seedborne fungi through seed exchange and minimizing financial losses require rapid and accurate pathogen detection (Mancini et al., 2016).

Traditional identification methods based on morphology are time-consuming, require specialized expertise, and often fail to distinguish closely related fungi due to overlapping symptoms (Ward et al., 2005; Mancini et al., 2016). As a result, molecular techniques have become indispensable for accurate detection. Sequencing of regions such as the translation elongation factor 1-alpha (TEF1-α) and the internal transcribed spacer (ITS) has been widely applied to identify sheath rot pathogens (Pramunadipta et al., 2022). The ITS rDNA region is particularly valuable because it combines both conserved and highly variable regions, allowing species-level resolution and differentiation among closely related isolates (Hibbett, 1992; Bruns et al., 1991; Yao et al., 1992; Schoch et al., 2012).

Despite these advances, few studies have examined the diversity and transmission of sheath rot pathogens across different rice types (CMS, hybrid, and inbred) and growth stages. In this study, pathogenic fungi were isolated from leaf sheaths,

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seeds, and harvested grains and identified using ITS rDNA sequencing. This approach provides new insights into the seedborne nature of *S. oryzae* and *Fusarium* spp. and highlights their potential role in disease transmission from seed to plant and back to grain.

MATERIALS AND METHODS

Samples. Rice leaf sheaths and infected seeds were collected from mature rice plants in four seed production areas in East Java, Indonesia (Kediri, Blitar, and Ngawi districts). Seeds were categorized into symptomatic and asymptomatic groups. They were planted and maintained until harvest, after which grains were collected. In total, 75 fungal isolates were obtained from rice leaf sheaths, parent seeds, and harvested grains of CMS, hybrid, and inbred rice (Table 1).

Table 1. Fungal isolate samples and their origins

No.	Isolate code	Source	Sample Group	
1	B.B_ 2_031022	Seed	CMS	
2	AH_F2_(5)_1	Grain	CMS	
3	PDH_08_S2_280922	Sheath	Hybrid	
4	S2_PDH_08_280922	Sheath	Hybrid	
5	PDH_08_280922	Seed	Hybrid	
6	B.B_280922	Seed	Hybrid	
7	BH_F1_(1)	Grain	Hybrid	
8	BH_F1_(1)_2	Grain	Hybrid	
9	B2H_F2_(1)_2	Grain	Hybrid	
10	B4_B2H5_F2	Grain	Hybrid	
11	D-3_D3H3F2	Seed	Inbred	
12	G_S5_1_1_011022	Seed	Inbred	
13	C_11_19-10	Seed	Inbred	
14	C_168_21-10-22	Seed	Inbred	
15	P_C_75_19-10-22	Seed	Inbred	Symptomatic
16	C_19_18-10-22)	Seed	Inbred	
17	C-3_C2H5_CBU	Seed	Inbred	
18	D-4_D3H4	Seed	Inbred	
19	D-5_D3H4	Seed	Inbred	
20	D-3_D3H3F2	Grain	Inbred	
21	C-5_C2H3	Grain	Inbred	
22	D-2_D3H2_F2	Grain	Inbred	
23	D-6_D3H5_F2	Grain	Inbred	
24	D7_H5F2	Grain	Inbred	
25	C-4_C2H4_F2	Grain	Inbred	
26	C-7_C2H2F2	Grain	Inbred	
27	C-2_C2H5_F2	Grain	Inbred	
28	CH_F2_(1)_1	Grain	Inbred	
29	DH_F2_(3)_1	Grain	Inbred2	
30	BN_F2_(4)_3	Grain	Hybrid	Asymptomatic
31	BN_F2_(5)_1	Grain	Hybrid	
32	B-3_B2N-F2	Grain	Hybrid	
33	C.69_19-10-22	Seed	Inbred	

Table 1. Continued. Fungal isolate samples and their origins

No.	Isolate code	Source	Sample Group	
34	C75_19-10-22	Seed	Inbred	
35	PC_72_19-10-22	Seed	Inbred	
36	D3N_F2_D-1	Grain	Inbred	
37	C1_C2N_F2	Grain	Inbred	
38	CN_F2_(3)_2	Grain	Inbred	
39	CN_F2_(3)_1B	Grain	Inbred	
40	CH_F2_(4)_2	Grain	Inbred	
41	DN_F2_(4)_1	Grain	Inbred2	
42	9_P_BPH_10-5_1		Soil	
43	G4_U2_ 52_300922	Seed	CMS	
44	G4_U1_ 53_290922	Seed	CMS	
45	G5_U2_ 66_300922	Seed	CMS	
46	G3_U1_ 34_300922	Seed	CMS	
47	G3_U1_ 33_290922	Seed	CMS	
48	BH_(3)_8)	Seed	Hybrid	
49	B5_B2H(2)_24/6_F2	Grain	Hybrid	
50	B-1_B2H(1)_F2-2	Grain	Hybrid	
51	BH_F2_(3)_3	Grain	Hybrid	
52	G_U1_ CHR_26-10-22	Sheath	Inbred	
53	P5U_2_ CHR_26-10-22	Sheath	Inbred	
54	G1_U1_ 14_011022	Seed	Inbred	
55	G1_U2_ 14_290922	Seed	Inbred	Symptomatic
56	G3_U2_ 35_300922	Seed	Inbred	
57	G1_U1_ 10_290922	Seed	Inbred	
58	U4_ 051022	Seed	Inbred	
59	C_133_21-22	Seed	Inbred	
60	C_67_19-10-22	Seed	Inbred	
61	C_70_19-10-22	Seed	Inbred	
62	C-4_C2H_F2_2	Grain	Inbred	
63	C-6_C2H_F2	Grain	Inbred	
64	CH_F2_(3)_2	Grain	Inbred	
65	DH_F2_(1)_1	Grain	Inbred2	
66	S3_U2_ 32_290922	Seed	CMS	Asymptomatic
67	G1_U1_ 9_300922	Seed	CMS	
68	GU5_ CHR_26-10	Sheath	Inbred	
69	S_4_U1_ 45_290922	Seed	Inbred	
70	S5_U1_ 57_290922	Seed	Inbred	
71	DN_(3)_1	Seed	Inbred2	
72	DN_(2)_1	Seed	Inbred2	
73	DN_F2_(3)_1	Grain	Inbred2	
74	CN_F2_(2)_2	Grain	Inbred	
75	CN_F2_(5)_4)	Grain	Inbred	

Isolation of Fungal Associated with Rice Sheath Rot. Fungi were isolated from symptomatic and asymptomatic tissues following the method of Chowdhury et al. (2015) with slight modifications. Small tissue sections (~5 mm²) were excised from the margins of infected and healthy areas, surface-sterilized with 1% sodium hypochlorite, rinsed with sterile water, dried on sterile filter paper, and plated on potato dextrose agar (PDA). Plates were incubated at 25 ºC for 5–7 days, and emerging fungal colonies were purified by sub-culturing. For seed isolation, 150 rice seeds (10 seeds per Petri dish, five sampling points, and three replicates) were plated and incubated under the same conditions.

DNA Extraction. Genomic DNA was extracted from fresh mycelium using a CTAB-based method adapted from Abd-Elsalam et al. (2003). Mycelial tissues were ground in liquid nitrogen, mixed with CTAB extraction buffer, and purified with chloroform:isoamyl alcohol (24:1). DNA was precipitated with isopropanol, pelleted by centrifugation, washed with 70% ethanol, air-dried, and resuspended in 1× TE buffer.

PCR Amplification and rDNA Sequencing. The 5.8S rRNA gene and internal transcribed spacers regions (ITS1 and ITS2) were amplified using the primer pair ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), following White et al. (1990). PCR reactions were performed using a SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific, USA) under the following conditions: initial denaturation at 94 ºC for 2 min 30 s, 30 cycles of denaturation at 94 ºC for 30 s, annealing at 58 ºC for 30 s, and extension at 72 ºC for 30 s, followed by a final extension at 72 ºC for 8 min.

PCR products were visualized on a 1.5% agarose gel stained with FluoroVue™ Nucleic Acid Gel Stain (Smobio, Taiwan) and documented using a Gel Doc System (Quantum CX-5, Vilber, France). PCR products were purified using a GenePHlow™ Gel/PCR Kit (Geneaid, Taiwan) according to the manufacturer's instructions.

Cycle sequencing was performed using the BigDye Terminator™ v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA). Sequencing products were purified using a magnetic bead-based purification kit (MCLAB, USA) following the manufacturer's protocol and analyzed using an Applied Biosystems 3500xl Genetic Analyzer (Thermo Fisher Scientific, USA).

Phylogenetic Analysis. Sequences were edited using BioEdit Sequence Alignment Editor v. 7.0.5.3 to remove ambiguous regions (Hall, 1999). The edited sequences were used as queries in the Basic Local Alignment Search Tool (BLAST) to confirm amplicon identities (Altschul et al., 1990). All sample sequences, together with reference sequences (Table 2), were aligned using the ClustalW plug-in in MEGA 11 (Tamura et al., 2021). Reference ITS rDNA sequences retrieved from GenBank were used for phylogenetic analysis.

Rhizoctonia solani, a well-established fungal pathogen of *Oryza sativa,* was selected as the outgroup (Ou, 1985; Lee & Rush, 1983; Molla et al., 2020; Nayoyani & Kasiamdari, 2022). Bootstrap analysis with 1000 replicates was conducted to assess branch support. Evolutionary distances were calculated using the Maximum Composite Likelihood method (Tamura et al., 2004).

RESULTS AND DISCUSSION

Isolation of Fungal Associated with Rice Sheath Rot Disease. Seedborne pathogens play a crucial role in the spread of rice diseases, reducing both yield and grain quality. Previous studies have identified *Sarocladium oryzae* and *Fusarium* spp. as the major causal agents of sheath rot in Indonesia (Pramunadipta et al., 2020; Bigirimana et al., 2015). Typical symptoms include brown to reddish lesions on the leaf sheaths enclosing the panicles, which may result in poor grain filling or sterility (Nair, 1976; Ou, 1985).

In this study, sheath rot symptoms were observed in CMS, hybrid, and inbred rice grown in four production areas of East Java. A total of 470 isolates were initially obtained, from which 110 were subcultured and 75 representative isolates were selected for molecular identification. In some cases, multiple colonies were recovered from a single plant part or seed, particularly from tissues exhibiting severe symptoms. Representative colony morphotypes are shown in Figure 1A.

ITS rDNA Sequencing and Phylogenetic Analysis. ITS rDNA sequencing confirmed the identity of 42 isolates as *S. oryzae* and 33 isolates as *Fusarium* spp., including *F. equiseti* (29 isolates), *F. incarnatum* (1 isolate), and *F. proliferatum* (3 isolates). *S. oryzae* was consistently detected across all rice types and sample sources, confirming its primary role in sheath rot disease. Among the *Fusarium* isolates, *F. equiseti* was predominant, particularly in inbred rice samples,

Table 2. GenBank reference sequences of *Sarocladium* sp. and *Fusarium* spp.

No	Isolate	Species name	Accession No.	Origin
1	ShR10	*Fusarium equiseti*	MN544890.1	Sheath rot infected rice, India
2	ELTX21	*Fusarium equiseti*	OL344049.1	*Oryza sativa,* USA
3	ZJ09	*Fusarium equiseti*	MT560634.1	*Oryza sativa*, China
4	MA-1	*Fusarium fujikuroi*	OR244135.1	*Oryza sativa*, India
5	FT-R1	*Fusarium fujikuroi*	OP346575.1	Rice root, Iran
6	Pure culture	*Fusarium fujikuroi*	MK424837.1	Roots of rice, China
7	7SHP	*Fusarium incarnatum*	OR342110.1	Rice stalks, Uzbekistan
8	1SHD	*Fusarium incarnatum*	OR342109.1	Rice seeds, Uzbekistan
9	JS3	*Fusarium incarnatum*	MT889972.1	Rice spikelet, China
10	VSL314	*Fusarium oxysporum*	MH370295.1	Tomato stem base, Mexico
11	W848A	*Fusarium oxysporum*	AF440566.1	Tomato root, USA
12	SMC21	*Fusarium oxysporum*	AF440565.1	Tomato root, USA
13	F2	*Fusarium proliferatum*	MT394055.1	Rice sheath, India
14	FproStRIN2	*Fusarium proliferatum*	KJ466114.1	Rice grain, India
15	HF1415	*Fusarium proliferatum*	KP097732.1	*Oryza sativa*, South Korea
16	CBS 212.79	*Sarocladium bacillisporum*	HG965002.1	*Sarocladium bacillisporum*
17	CBS 388.67	*Sarocladium bacillisporum*	HG965003.1	Insect, Romania
18	CBS 749.69T	*Sarocladium bacillisporum*	HG965006.1	Soil, Netherlands
19	CBS 383.73	*Sarocladium bifurcatum*	HG965008.1	*Ustilago* sp., Canada
20	CBS 425.73	*Sarocladium gamsii*	HG965014.1	Dead stem of bamboo, India
21	CBS 382.73	*Sarocladium glaucum*	HG965018.1	Dead petiole of Pandanus lerum, Sri Lanka
22	CBS 100350	*Sarocladium glaucum*	HG965020.1	Dead stem of bamboo, India
23	UTHSC 02-2564	*Sarocladium hominis*	HG965011.1	Dead stem of bamboo, Japan
24	CBS 397.70A	*Sarocladium implicatum*	HG965021.1	*Homo sapiensleg*, USA
25	CBS 959.72^{NT}	*Sarocladium implicatum*	HG965023.1	*Saccharum officinarum*, Jamaica
26	CBS 428.67 ^{T}	*Sarocladium ochraceum*	HG965025.1	Dessert soil, Egypt
27	CBS 180.74 ^{ET}	*Sarocladium oryzae*	HG965026.1	*Zea mays*, Kenya
28	CBS 399.73	*Sarocladium oryzae*	HG965027.1	*Oryza sativa*, India
29	CBS 414.81	*Sarocladium oryzae*	HG965028.1	*Oryza sativa*, India
30	CBS 361.75	*Sarocladium oryzae*	AY566993.1	*Oryza sativa*, Nigeria
31	SO 2	*Sarocladium oryzae*	MT012231.1	*Oryza sativa*, Kenya
32	SO 3	*Sarocladium oryzae*	MT012232.1	Rice leaf sheath, Indonesia
33	SO 5	*Sarocladium oryzae*	MT012234.1	Rice leaf sheath, Indonesia
34	SO 8	*Sarocladium oryzae*	MT012236.1	Rice leaf sheath, Indonesia
35	SO 11	*Sarocladium oryzae*	MT012233.1	Rice leaf sheath, Indonesia
36	SO 13	*Sarocladium oryzae*	MT012235.1	Rice leaf sheath, Indonesia
37	UTHSC 02-1892T	*Sarocladium pseudostrictum*	HG965029.1	Rice leaf sheath, Indonesia
38	CBS 346.70T	*Sarocladium strictum*	FN691453.1	*Homo sapiens* sputum, USA

Table 2. Continued. GenBank reference sequences of *Sarocladium* sp. and *Fusarium* spp.

No	Isolate	Species name	Accession No.	Origin
39	MUCL 9939T	*Sarocladium subulatum*	HG965031.1	*Triticum aestivum*, Germany
40	CBS 200.84	*Sarocladium summerbellii*	HG965033.1	Soil, Egypt
41	CBS 797.69	*Sarocladium summerbellii*	HG965035.1	Water in air moistener, Netherlands. Decaying leaf of *Canna indica*, Netherlands
42	CBS 951.72	*Sarocladium summerbellii*	HG965037.1	Agricultural soil, Netherlands
43	MUCL 12011	*Sarocladium terricola*	HG965039.1	Decaying leaf of *Milletalaurentii*, Congo
44	CBS 800.69^{T}	*Sarocladium zeae*	FN691451.1	*Zea mays* stalk, USA
45	SB5	*Rhizoctonia solani*	MH600071.1	Infected rice sheath, India

T = Type strain; NT = Neotype strain; ET = Epitype strain.

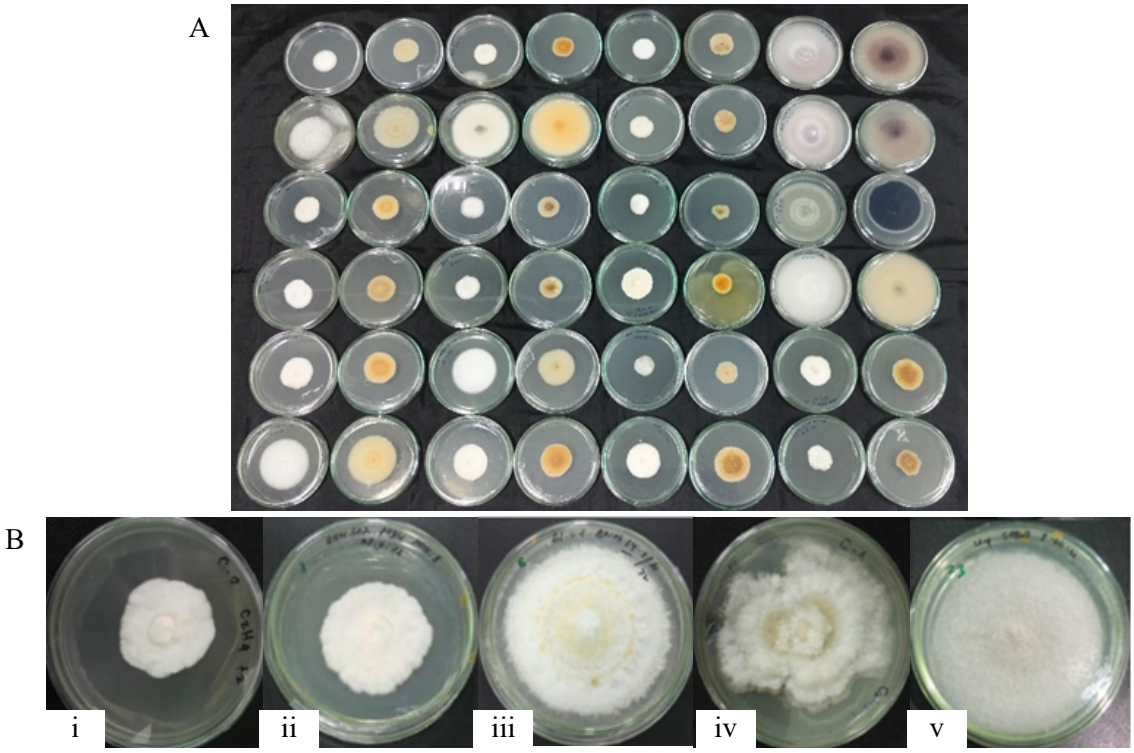

Figure 1. Morphotypes and morphological characteristics of seed-borne fungal isolates from rice. A. Morphotypes of isolates obtained from leaf sheaths, parent seeds, and harvested grains; B. Morphological characteristics of the identified isolates: (i) *Sarocladium oryzae* (isolated from leaf sheath); (ii) *S. oryzae* (isolated from seed); (iii) *Fusarium equiseti* (isolated from harvested grains); (iv) *F. incarnatum* (isolated from harvested grains); (v) *F. proliferatum* (isolated from leaf sheath).

suggesting its significant contribution to the disease complex.

ITS rDNA sequencing is a widely used and powerful method used for identifying and characterizing fungal pathogens, including those causing sheath rot disease in rice. This approach involves amplifying and sequencing the internal transcribed spacer regions of ribosomal RNA genes, which are highly variable among fungal species but conserved within species, enabling reliable identification and differentiation (Schoch et al., 2012). BLAST analysis of the ITS rDNA sequences revealed that all isolates showed high sequence identity (96.58–100%) with reference sequences deposited in GenBank. The *S. oryzae* isolates exhibited 96.58–100% sequence identity, while isolates identified as *F. equiseti* (99.63–100%), *F. proliferatum* (100%), and *F. incarnatum* showed similarly high identity values with their respective references (Table 3). These results

Table 3. Identification of 75 pathogenic fungal isolates

No	Isolate code	Source		Sample Group	Percentage identity (%)	Hit.
1	B.B_ 2_031022	Seed	CMS		98.01–99.34	*Sarocladium oryzae*
2	AH_F2_(5)_1	Grain	CMS		99.60–99.80	*Sarocladium oryzae*
3	PDH_08_S2_280922	Sheath	Hybrid		99.72–100	*Sarocladium oryzae*
4	S2_PDH_08_280922	Sheath	Hybrid		98.40–100	*Sarocladium oryzae*
5	PDH_08_280922	Seed	Hybrid		98.05–100	*Sarocladium oryzae*
6	B.B_280922	Seed	Hybrid		98.05–100	*Sarocladium oryzae*
7	BH_F1_(1)	Grain	Hybrid		98.45–99.81	*Sarocladium oryzae*
8	BH_F1_(1)_2	Grain	Hybrid		97.93–100	*Sarocladium oryzae*
9	B2H_F2_(1)_2	Grain	Hybrid		97.14–100	*Sarocladium oryzae*
10	B4_B2H5_F2	Grain	Hybrid		97.02–100	*Sarocladium oryzae*
11	D-3_D3H3F2	Seed	Inbred		96.91–99.78	*Sarocladium oryzae*
12	G_S5_1_1_011022	Seed	Inbred		98.06–100	*Sarocladium oryzae*
13	C_11_19-10	Seed	Inbred	Symptomatic	98.05–100	*Sarocladium oryzae*
14	C_168_21-10-22	Seed	Inbred		98.58–100	*Sarocladium oryzae*
15	P_C_75_19-10-22	Seed	Inbred		97.91–99.58	*Sarocladium oryzae*
16	C_19_18-10-22)	Seed	Inbred		99.38–100	*Sarocladium oryzae*
17	C-3_C2H5_CBU	Seed	Inbred		97.67–100	*Sarocladium oryzae*
18	D-4_D3H4	Seed	Inbred		97.48–100	*Sarocladium oryzae*
19	D-5_D3H4	Seed	Inbred		97.54–99.43	*Sarocladium oryzae*
20	D-3_D3H3F2	Grain	Inbred		96.91–99.78	*Sarocladium oryzae*
21	C-5_C2H3	Grain	Inbred		98.11–100	*Sarocladium oryzae*
22	D-2_D3H2_F2	Grain	Inbred		98.25–100	*Sarocladium oryzae*
23	D-6_D3H5_F2	Grain	Inbred		97.86–99.81	*Sarocladium oryzae*
24	D7_H5F2	Grain	Inbred		97.79–99.78	*Sarocladium oryzae*
25	C-4_C2H4_F2	Grain	Inbred		98.25–100	*Sarocladium oryzae*
26	C-7_C2H2F2	Grain	Inbred		98.11–100	*Sarocladium oryzae*
27	C-2_C2H5_F2	Grain	Inbred		96.58–100	*Sarocladium oryzae*
28	CH_F2_(1)_1	Grain	Inbred		100	*Sarocladium oryzae*
29	DH_F2_(3)_1	Grain	Inbred2		100	*Sarocladium oryzae*
30	BN_F2_(4)_3	Grain	Hybrid		99.32–100	*Sarocladium oryzae*
31	BN_F2_(5)_1	Grain	Hybrid		98.90–100	*Sarocladium oryzae*
32	B-3_B2N-F2	Grain	Hybrid		97.87–99.62	*Sarocladium oryzae*
33	C.69_19-10-22	Seed	Inbred		97.74–99.81	*Sarocladium oryzae*
34	C75_19-10-22	Seed	Inbred		98.05–100	*Sarocladium oryzae*
35	PC_72_19-10-22	Seed	Inbred		98.56–100	*Sarocladium oryzae*
36	D3N_F2_D-1	Grain	Inbred	Asymptomatic	98.25–100	*Sarocladium oryzae*
37	C1_C2N_F2	Grain	Inbred		99.21–100	*Sarocladium oryzae*
38	CN_F2_(3)_2	Grain	Inbred		99.15–100	*Sarocladium oryzae*
39	CN_F2_(3)_1B	Grain	Inbred		98.09–100	*Sarocladium oryzae*
40	CH_F2_(4)_2	Grain	Inbred		98.09–100	*Sarocladium oryzae*

Table 3. Continued. Identification of 75 pathogenic fungal isolates

No	Isolate code	Source		Sample group	Percentage identity (%)	Hit.
41	DN_F2_(4)_1	Grain	Inbred2		99.28–99.64	*Sarocladium oryzae*
42	9_P_BPH_10-5_1		Soil		96.90–99.43	*Sarocladium oryzae*
43	G4_U2_ 52_300922	Seed	CMS		100	*Fusarium equiseti*
44	G4_U1_ 53_290922	Seed	CMS		99.80–100	*Fusarium equiseti*
45	G5_U2_ 66_300922	Seed	CMS		99.80–100	*Fusarium equiseti*
46	G3_U1_ 34_300922	Seed	CMS		100	*Fusarium equiseti*
47	G3_U1_ 33_290922	Seed	CMS		100	*Fusarium equiseti*
48	BH_(3)_8)	Seed	Hybrid		99.14–100	*Fusarium equiseti*
49	B5_B2H(2)_24/6_F2	Grain	Hybrid		99.42–99.81	*Fusarium equiseti*
50	B-1_B2H(1)_F2-2	Grain	Hybrid		100	*Fusarium equiseti*
51	BH_F2_(3)_3	Grain	Hybrid		100	*Fusarium equiseti*
52	G_U1_ CHR_26-10-22	Sheath	Inbred		100	*Fusarium equiseti*
53	P5U_2_ CHR_26-10-22	Sheath	Inbred		100	*Fusarium equiseti*
54	G1_U1_ 14_011022	Seed	Inbred		100	*Fusarium equiseti*
55	G1_U2_ 14_290922	Seed	Inbred	Symptomatic	99.53–99.77	*Fusarium equiseti*
56	G3_U2_ 35_300922	Seed	Inbred		99.80–100	*Fusarium equiseti*
57	G1_U1_ 10_290922	Seed	Inbred		100	*Fusarium equiseti*
58	U4_ 051022	Seed	Inbred		100	*Fusarium proliferatum*
59	C_133_21-22	Seed	Inbred		100	*Fusarium equiseti*
60	C_67_19-10-22	Seed	Inbred		99.79	*Fusarium equiseti*
61	C_70_19-10-22	Seed	Inbred		100	*Fusarium equiseti*
62	C-4_C2H_F2_2	Grain	Inbred		100	*Fusarium incarnatum*
63	C-6_C2H_F2	Grain	Inbred		100	*Fusarium equiseti*
64	CH_F2_(3)_2	Grain	Inbred		100	*Fusarium proliferatum*
65	DH_F2_(1)_1	Grain	Inbred2		100	*Fusarium equiseti*
66	S3_U2_ 32_290922	Seed	CMS		100	*Fusarium equiseti*
67	G1_U1_ 9_300922	Seed	CMS		99.60	*Fusarium equiseti*
68	GU5_ CHR_26-10	Sheath	Inbred		99.79–100	*Fusarium equiseti*
69	S_4_U1_ 45_290922	Seed	Inbred		99.80–100	*Fusarium equiseti*
70	S5_U1_ 57_290922	Seed	Inbred		99.79	*Fusarium equiseti*
71	DN_(3)_1	Seed	Inbred2	Asymptomatic	100	*Fusarium equiseti*
72	DN_(2)_1	Seed	Inbred2		99.44–100	*Fusarium equiseti*
73	DN_F2_(3)_1	Grain	Inbred2		100	*Fusarium equiseti*
74	CN_F2_(2)_2	Grain	Inbred		100	*Fusarium equiseti*
75	CN_F2_(5)_4)	Grain	Inbred		99.63–100	*Fusarium proliferatum*

confirm the accuracy of the molecular identification.

Sarocladium oryzae was the only *Sarocladium* species detected across all sample sources, including rice leaf sheaths, seeds, and harvested grains, further confirming its established role in sheath rot disease. In addition, three *Fusarium* species were identified from all sample sources, indicating their potential involvement in synergistic interactions with *S. oryzae*. Among them, *F. equiseti* was more frequently isolated than *F. incarnatum* and *F. proliferatum* from seed and harvested grain of inbred rice samples.

Maximum-likelihood (ML) phylogenetic

analysis showed that sample isolates and their reference sequences clustered within the same branches of the phylogenetic tree. Closely related samples grouped together, indicating high genetic similarity, while organisms sharing similar characteristics clustered within the same branches (BSCI, 2020). This pattern suggests strong phylogenetic relationships among pathogens isolated from different plant developmental stages, supporting the traceability of pathogens transmitted "to and from" seeds.

Phylogenetic analysis revealed that *S. oryzae* isolates formed a single cluster with reference sequences, indicating genetic homogeneity compared with other *Sarocladium* species (Figure 2). In contrast, *Fusarium* isolates exhibited greater diversity. The *Fusarium* phylogeny was divided into two main clusters: cluster 1 comprised *F. equiseti* and *F. incarnatum*, while cluster 2 included *F. proliferatum* grouped with *F. fujikuroi* (Figure 3). The combined phylogenetic tree further confirmed the clear separation between the two pathogenic fungal groups, *Sarocladium* and *Fusarium*, within a single ingroup that was distinctly separated from *Rhizoctonia solani*, which served as the outgroup (Figure 4).

Fusarium equiseti, a member of the *Fusarium incarnatum-equiseti* species complex (Aoki et al., 2014), is predominantly known as a pathogen for barley (Marín et al., 2012) and wheat (Castellá & Cabañes, 2014) and has also been isolated from rice stem tissues (Fisher & Petrini, 1992). *F. proliferatum*, a member of the *Fusarium fujikuroi* species complex (FFSC), has been reported as a pathogen causing sheath rot disease (Abbas et al., 1998; Prabhukarthikeyan et al., 2020). This diversity suggests that different *Fusarium* species or strains may contribute to sheath rot disease to varying degrees, potentially influencing disease severity and symptom expression.

An important finding of this study is the predominance of *F. equiseti* in inbred rice samples. This is noteworthy because most previous reports on rice sheath rot have emphasized *S. oryzae* as the primary pathogen (Reddy & Sathyanarayana, 2001; Bigirimana et al., 2015), while the role of *F. equiseti* has been less frequently documented. The dominance of *F. equiseti* observed here suggests a broader pathogen diversity contributing to sheath rot disease, potentially influenced by varietal genetic backgrounds or local agroecological conditions. Earlier studies primarily associated *F. equiseti* with diseases in wheat and barley (Marín et al., 2012; Castellá & Cabañes, 2014); therefore, its detection as a dominant pathogen in inbred rice provides novel evidence of an expanded host range. This finding highlights the importance of considering *F. equiseti* in sheath rot management strategies, particularly in inbred rice production systems.

Significance of Seedborne Transmission and Implications for Disease Management. *S. oryzae* isolates clustered together with reference strains, indicating genetic homogeneity within the species (Figure 2). In contrast, *Fusarium* isolates exhibited higher genetic diversity and formed two distinct clusters: *F. equiseti* and *F. incarnatum* grouped in cluster 1, while *F. proliferatum* clustered with *F. fujikuroi* in cluster 2 (Figure 3). Combined phylogenetic analysis of all isolates confirmed the clear separation between the genera *Sarocladium* and *Fusarium*.

An in-depth analysis of molecular identification revealed the successful detection of *S. oryzae* in F1 hybrid rice derived from leaf sheaths (PDH_08_S2_280922 and S2_PDH_08_280922), planted seeds (PDH_08_280922 and B.B_280922), and harvested grains (BH_F1_(1), BH_F1_(1)_2, B2H_F2_(1)_2, and B4_B2H5_F2). Similarly, *F. equiseti* was identified in inbred rice lines isolated from leaf sheaths (G_U1_CHR_26-10-22 and P5U_2_CHR_26-10-22), seeds (G1_U1_14_011022, G1_U2_14_290922), and harvested grains (C_133_21-22 and C_67_19-10-22). All of these isolates were obtained from symptomatic samples.

Further observations demonstrated that both *S. oryzae* and *F. equiseti* were also transmitted from asymptomatic materials of inbred rice line. In contrast, *F. incarnatum* and *F. proliferatum* were exclusively associated with harvested grains and seed or leaf sheath samples, respectively. Notably, *S. oryzae* was isolated from visually healthy rice seeds and grains represented by isolates C_69_19-10-22 and C1_C2N_F2, respectively. In addition, the transmission of *F. equiseti* was detected from GU5_ CHR_26-10 leaf sheath to S_4_U1_ 45_290922 seeds and subsequently to infected CN_F2_(2)_2 harvested grain samples. The evidence strongly indicates that these pathogens can be transferred to and from seeds, suggesting that seeds play a critical role in pathogen persistence throughout the rice life cycle.

Previous studies have consistently reported *S. oryzae* as a seedborne pathogen responsible for sheath rot disease in rice. The pathogen has been isolated from rice seeds, leaf sheaths, and grains, and its seedborne transmission has been well documented (Mew & Gonzales, 2002; Phookamsak et al., 2019). Our findings align with these reports, as *S. oryzae* was

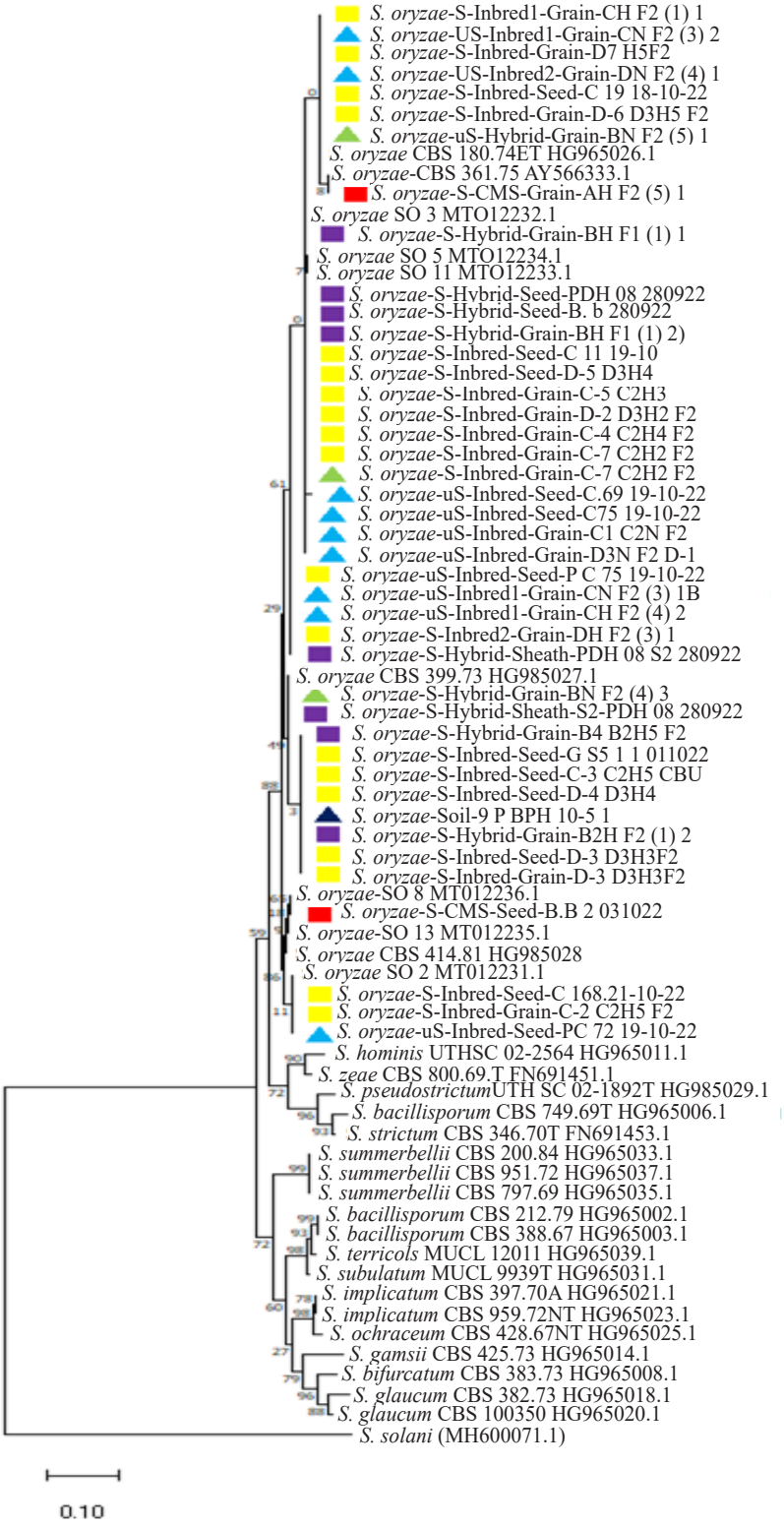

Figure 2. Phylogenetic tree based on comparative ITS rDNA gene sequence analysis of *Sarocladium oryzae* samples and references sequences. Different shapes and colors represent samples isolated from different source materials: Symptomatic (S) inbred, symptomatic hybrid, symptomatic (S) CMS, asymptomatic (As) inbred, asymptomatic (As)-hybrid, and soil *Rhizoctonia solani* as the outgroup.

recovered from both symptomatic and asymptomatic seeds, leaf sheaths, and harvested grains, further confirming its key role in sheath rot epidemiology.

Earlier studies have shown that Fusarium spesies, including *F. equiseti, F. proliferatum*, and *F. incarnatum* can colonize rice seeds and grains

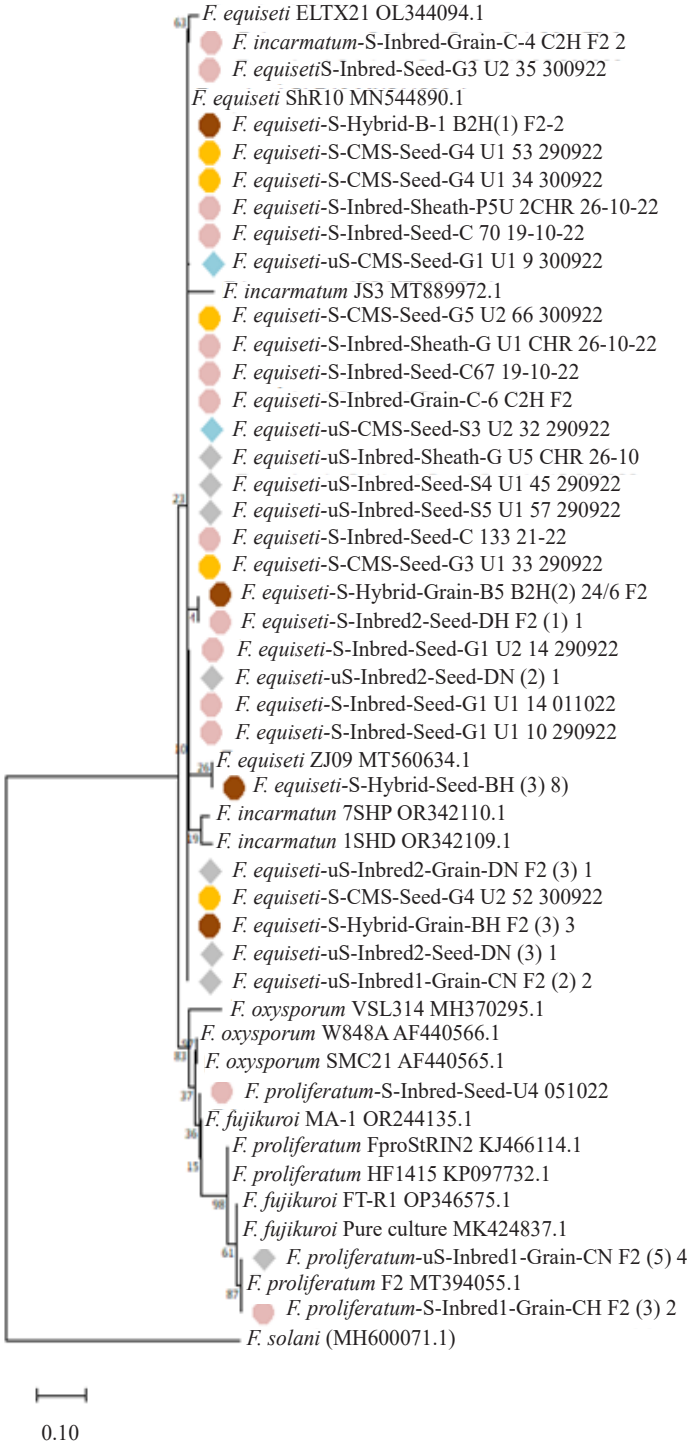

Figure 3. Maximum-likelihood phylogenetic tree based on ITS rDNA gene sequence analysis of *Fusarium* spp. and reference sequences. Different shapes and colors represent samples isolated from different source materials: Symptomatic (S) inbred, symptomatic (S) hybrid, symptomatic (S) CMS, asymptomatic (As) inbred, asymptomatic (As) CMS, and *Rhizoctonia solani* as the outgroup.

and persist as seed associated fungi (Desjardins et al., 2000). Some studies have suggested synergistic interactions between *Fusarium* spp. and *S. oryzae*, potentially leading to increased disease severity (Reddy et al., 2000). Our results support these observations, particularly the frequent detection of *F. equiseti* in both symptomatic and asymptomatic rice tissues.

Importantly, this study provides new evidence that *F. equiseti* can be transmitted from asymptomatic seeds to harvested grains, highlighting the risk posed by latent infections and emphasizing the importance of seed health in disease management strategies.

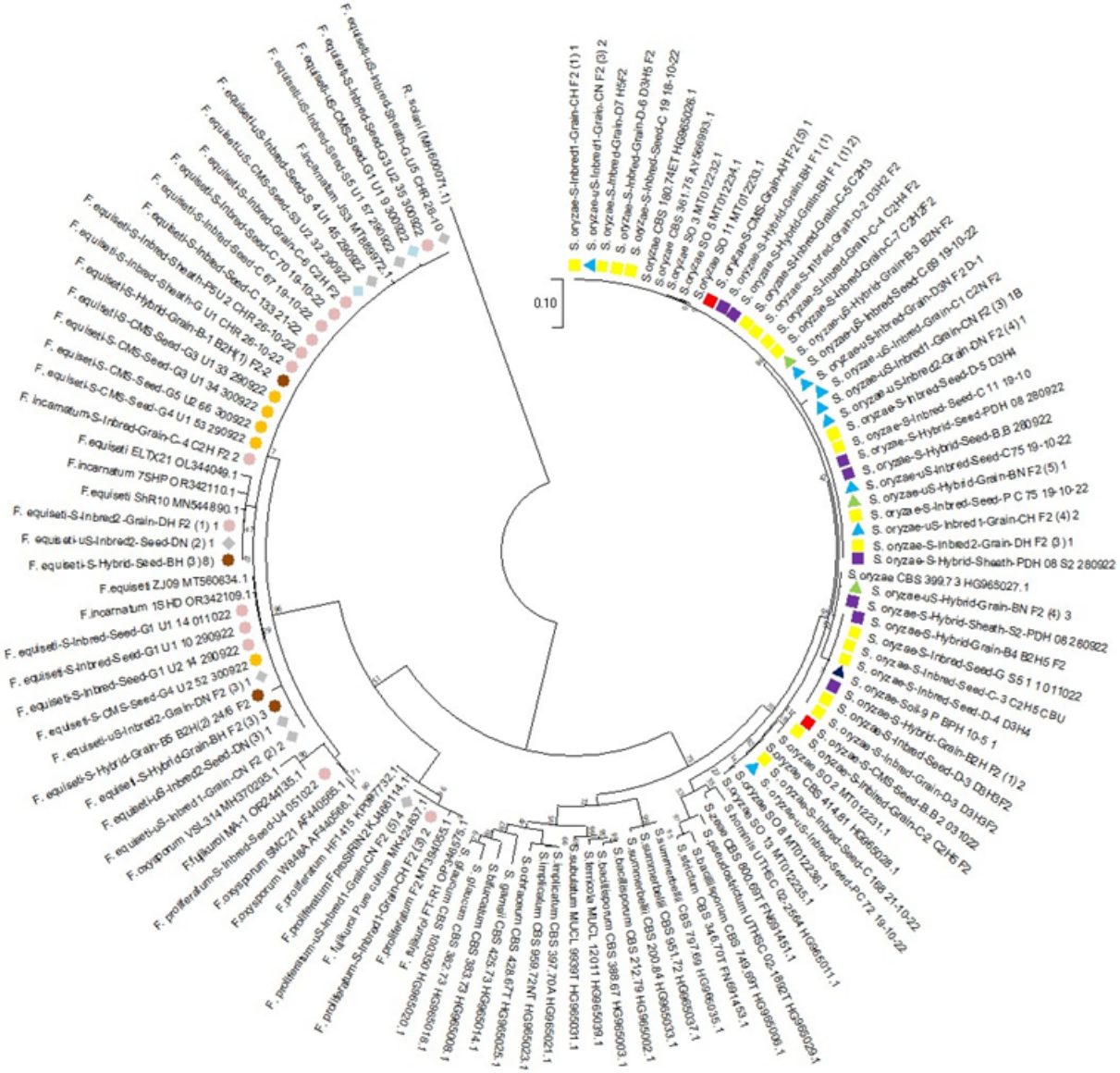

Figure 4. Combined phylogenetic tree of all isolates and reference sequences. *Rhizoctonia solani* (MH600071.1) was used as the outgroup.

The detection of *S. oryzae* and *F. equiseti* in visually healthy (asymptomatic) rice seeds represents a major and novel contribution to understanding the epidemiology of sheath rot disease. The presence of latent infections in asymptomatic seeds demonstrates the limitations of relying solely on visual inspection for seed health assessment. This finding is consistent with reports by Singh & Vishunavat (2015). who emphasized that asymptomatic seeds often serve as hidden carriers of seedborne pathogens. Therefore, our results strongly support the integration of molecular-based detection methods into seed certification systems to prevent pathogen dissemination through seed distribution.

Overall, the results reinforce the concept that *S. oryzae* and *Fusarium* spp. are important seedborne pathogens in rice. Infected seeds can act as carriers, facilitating long-distance pathogen spread, reducing crop yield, and compromising grain quality. Early detection and control of seedborne pathogens remain the most effective strategies for disease prevention. The presence of *S. oryzae* and *Fusarium* spp. in rice seeds underscores the critical role of seedborne transmission in sheath rot epidemiology and highlights the need for stringent seed health testing and certification to ensure that only pathogen-free seeds are distributed to farmers.

As a future perspective, further studies should explore the potential use of silica nanoparticles as seed treatments to enhance rice resistance against

sheath rot pathogens. Although this approach was not directly evaluated in the present study, previous research has demonstrated the effectiveness of silica nanoparticles in enhancing plant disease resistance (Kumar et al., 2020). In addition, integrated disease management remains essential to reduce the risk of seedborne pathogen dissemination. Such strategies include the use of healthy seeds through appropriate seed treatments, crop rotation, deployment of resistant varieties, and the implementation of good agricultural practices. Integrating conventional management approaches with emerging technologies may provide a more comprehensive framework for effective sheath rot management.

CONCLUSION

This study demonstrates that sheath rot pathogens are transmitted through rice seeds, with infection potentially initiating as early as the seedling stage. ITS rDNA sequencing proved to be a reliable tool for identifying *Sarocladium oryzae* and diverse *Fusarium* species associated with sheath rot disease. The results reaffirm the primary role of *S. oryzae* in sheath rot and reveal the significant contribution of *Fusarium equiseti*, particularly its occurrence in asymptomatic seeds and grains. These findings provide novel evidence that asymptomatic seed lots may act as hidden carriers, underscoring the critical role of seedborne transmission in the epidemiology of sheath rot. Consequently, the implementation of rigorous seed health testing and certification, combined with integrated disease management strategies, is essential to prevent pathogen dissemination and to safeguard rice yield and grain quality.

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AUTHORS' CONTRIBUTIONS

H, ETSP, AW, and AP conceived and designed the experiment. H collected samples and conducted fungal isolation, rice plant cultivation, and fungal re-isolation. H, AA, and YL performed the molecular analyses. H, AA, and YL also carried out data analysis and interpretation. H drafted the manuscript. All authors contributed to feedback on the research design, data analysis, interpretation, and overall manuscript structure. All authors have read and approved the final manuscript.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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