

MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR VARIABILITY OF *SCLEROTIUM ORYZAE* ISOLATES ASSOCIATED WITH STEM ROT OF PADDY IN KARNATAKA

Gowdar, S. B^{1*}, Sujay Hurali², Amaresh, Y. S³, Pramesh, D.⁴ and Ashwathnarayana, D. S³

¹ Professor, Department of Plant Pathology, College of Agriculture, Gangavathi- 583 227, **ORCID ID:** 0000-0003-3216-9946

² Senior Scientist, ICAR - AICRP on Rice, Agricultural Research Station, Gangavathi- 583 227, **ORCID ID:** 0000-0001-8989-7114

³ Professor, Department of Plant Pathology, College of Agriculture, Raichur - 584 104, **ORCID ID:** 0009-0008-0007-7071

University of Agricultural Sciences, Raichur - 584 104, Karnataka

⁴ Senior Scientist, ICAR-Directorate of Mushroom Research, Chambaghat - 173 213, Solan, Himachal Pradesh, **ORCID ID:** 0000-0002-5229-443X

*Corresponding Author: Gowdar, S. B, Email: sbgowdar@uasraichur.edu.in

ABSTRACT

Sclerotium oryzae isolates obtained from stem-rot-affected rice in Karnataka were characterized for cultural, sclerotial, biochemical and molecular variability. Isolates were compared on potato dextrose agar (PDA), Sabouraud dextrose agar, nutrient agar, Richard's agar and Hanson's agar. Considerable isolate-to-isolate variation occurred in colony colour, texture and radial growth. On PDA, SO-1 and SO-2 reached 84 mm after 48 h, whereas several isolates remained slow growing even after 96 h. Sclerotial initiation generally occurred within 4-6 days; 23-69 sclerotia plate⁻¹ were produced, sclerotial diameter ranged from 1.6 to 3.6 mm and 100-sclerotium dry weight ranged from 36.8 to 77.8 mg. Glucose was utilized by all isolates and was the most consistently preferred carbon source; indole production was frequent whereas methyl-red reduction occurred in fewer isolates. Of 45 ITS-region sequences generated, 30 high-quality sequences were retained, identified as *S. oryzae* and deposited in GenBank. Phylogenetic analysis resolved two principal groups, demonstrating substantial phenotypic and molecular heterogeneity within the Karnataka population.

KEYWORDS: Rice, *Sclerotium oryzae*, cultural variability, sclerotia, carbohydrate utilization, ITS sequencing, phylogeny, GenBank

1. INTRODUCTION

Stem rot of rice is associated with *Sclerotium oryzae* Catt., a soil- and residue-borne fungus capable of producing persistent sclerotia. Population variability is epidemiologically important because isolates may differ in growth, sclerotial development, nutritional responses and genetic relatedness, with potential consequences for survival, pathogenicity and disease management. Classical studies have reported marked cultural and pathogenic variation among *S. oryzae* isolates collected from rice-growing regions (Ali, 1997; Kumar et al., 2011; Sandhya et al., 2026; Kumar et al., 2026).

Morphological characterization remains useful for documenting colony colour, texture, growth rate and sclerotial traits, but phenotype alone may not resolve genetic relationships. Molecular approaches based on ribosomal DNA regions have therefore been used to confirm members of the rice sheath/stem-rot complex and to evaluate genetic diversity (Naveenkumar et al., 2026). Kumar et al. (2010) demonstrated substantial diversity among Indian *S. oryzae* isolates using RAPD markers, while ITS-based approaches provide a sequence framework for identification and phylogenetic comparison.

Recent work from Telangana and Andhra Pradesh also documented significant differences in growth and sclerotial characters among *S. oryzae* isolates (Prameela et al., 2020; Negi et al., 2026; Kukreti et al., 2026), supporting the view that regional populations are heterogeneous. Characterization of isolates collected across Karnataka was therefore undertaken to integrate cultural growth on multiple media, sclerotial morphology, simple biochemical traits and sequence-based molecular analysis.

The objectives were to characterize variability of Karnataka *S. oryzae* isolates in colony growth and morphology; compare sclerotial initiation, number, colour, size, shape and dry weight; assess utilization of selected carbon sources and indole/methyl-red reactions; and identify representative isolates by sequence analysis and phylogenetic reconstruction.

2. MATERIALS AND METHODS

2.1 Collection, isolation and maintenance of isolates

Diseased rice stem samples were collected from paddy-growing areas of Karnataka in sterile polythene covers, labelled and transported to the laboratory. Approximately 3-mm pieces from infected stem tissue were surface sterilized with 2% sodium hypochlorite for 1 min, rinsed three times in sterile distilled water and transferred to

potato dextrose agar (PDA). Plates were incubated at 27 ± 1 C. Cultures were purified by the single-hyphal-tip method (Pathak, 1972) and maintained on PDA by periodic transfer, with stock cultures held at 4 C.

2.2 Pathogenicity

Pathogenicity was evaluated on the susceptible rice cultivar BPT-5204. Plants were raised in sterilized soil and inoculum was produced on pieces of water sedge (*Typha angustata*) colonized by *S. oryzae*. Forty-five-day-old plants at maximum tillering were inoculated by placing six-day-old immature sclerotia-bearing sedge material inside the leaf sheath, after which moist cotton and water were used to maintain high humidity for approximately 24h. Symptoms were recorded 10 days after inoculation; uninoculated plants served as controls. This procedure is reproduced from the thesis protocol.

2.3 Cultural and sclerotial characterization

Purified isolates were compared on PDA, Sabouraud dextrose agar (SDA), nutrient agar (NA), Richard's agar and Hanson's agar. Approximately 15-20 ml of medium was poured into 90-mm Petri plates and inoculated centrally with a 5-mm culture disc. Experiments were replicated three times and incubated at 27 ± 2 C. Colony colour, texture and radial growth were recorded through 96 h. Sclerotial initiation, time to formation, number per plate, colour, distribution pattern, diameter, length, shape and dry weight of 100 sclerotia were recorded from PDA cultures.

2.4 Biochemical characterization

A rapid biochemical test kit was used to record indole production, methyl-red reaction, citrate utilization and utilization of selected carbohydrates. Fifty microlitres of isolate suspension was inoculated into the appropriate wells and incubated at 25 C for 2-3 days. Glucose, adonitol, arabinose, lactose, sorbitol, mannitol, rhamnose and sucrose utilization were scored from colour change as described in the thesis protocol (Lilly and Barnett, 1951; Cowan and Steel, 1970; Seeley and Vandemark, 1981).

2.5 DNA extraction, amplification, sequencing and phylogenetic analysis

Genomic DNA was extracted from 5-7-day-old fungal cultures using the Hi-Elute mini-prep workflow reported in the thesis, with Proteinase K treatment, spin-column washing and elution. PCR reactions were assembled in 25-microlitre volumes and amplified under a profile of 95 C for 5 min; 35 cycles of 94 C for 1 min, 55 C for 1 min and 72 C for 1 min; and a final extension at 72 C for 10 min. Amplicons were separated on 1% agarose gels and sequenced by Sanger sequencing. Consensus sequences were checked, compared with the NCBI nucleotide database using BLAST, aligned with ClustalW and analysed in MEGA X (Kumar et al., 2018). The molecular protocol is reproduced as reported in the thesis; its primer nomenclature should be checked against the original laboratory records before journal submission.

3. RESULTS

3.1 Cultural variability

Large differences were observed among isolates in colony colour, texture and growth rate. On PDA, mycelial colour ranged from whitish to dirty white and textures included smooth, fluffy and scattered forms. SO-1 and SO-2 reached 84 mm at 48 h, whereas isolates such as SO-64 and SO-65 remained slow growing after 96 h. Variation was also pronounced on SDA, NA, Richard's agar and Hanson's agar, with different isolates reaching 90 mm at different incubation times.

Table 1. Cultural and growth characters of *Sclerotium oryzae* on PDA

Isolate No.	Color	Texture	Growth after 48 hrs (mm)	Growth after 72 hrs (mm)	Growth after 96 hrs (mm)
SO-1	Off white	Scattered	84	90	90
SO-2	Whitish	Smooth	84	90	90
SO-3	Whitish	Scattered	80	90	90
SO-4	Whitish	Scattered	79	90	90
SO-5	Whitish	Scattered	79	90	90
SO-6	Whitish	Smooth	79	90	90
SO-7	Whitish	Scattered	78	90	90
SO-8	Whitish	Fluffy	78	90	90
SO-9	Dirty white	Fluffy	78	90	90
SO-10	Dirty white	Scattered	77	90	90
SO-11	Whitish	Fluffy	76	90	90
SO-12	Off white	Smooth	76	90	90
SO-13	Whitish	Smooth	75	90	90
SO-14	Whitish	Smooth	74	90	90
SO-15	Whitish	Fluffy	74	90	90
SO-16	Off white	Scattered	74	84	87
SO-17	Off white	Scattered	73	90	90
SO-18	Whitish	Smooth	73	90	90
SO-19	Off white	Fluffy	72	89	90

SO-20	Whitish	Scattered	72	82	85
SO-21	Dirty white	Scattered	71	90	90
SO-22	Whitish	Scattered	71	90	90
SO-23	Whitish	Smooth	71	90	90
SO-24	Whitish	Smooth	69	90	90
SO-25	Dirty white	Fluffy	69	90	90
SO-26	Whitish	Smooth	68	90	90
SO-27	Whitish	Smooth	68	90	90
SO-28	Dirty white	Smooth	65	90	90
SO-29	Off white	Smooth	65	85	90
SO-30	Whitish	Scattered	64	90	90
SO-31	Off white	Fluffy	64	87	90
SO-32	Off white	Scattered	63	90	90
SO-33	Dirty white	Fluffy	63	90	90
SO-34	Whitish	Scattered	63	90	90
SO-35	Whitish	Smooth	62	73	82
SO-36	Dirty white	Fluffy	61	87	90
SO-37	Off white	Smooth	61	82	90
SO-38	Off white	Fluffy	61	90	90
SO-39	Whitish	Scattered	60	90	90
SO-40	Dirty white	Scattered	60	78	86
SO-41	Dirty white	Fluffy	58	87	90
SO-42	Off white	Scattered	58	90	90
SO-43	Dirty white	Scattered	56	85	90
SO-44	Off white	Fluffy	55	90	90
SO-45	Whitish	Scattered	55	87	90
SO-46	Whitish	Scattered	55	81	90
SO-47	Dirty white	Smooth	54	90	90
SO-48	Whitish	Smooth	54	64	89
SO-49	Dirty white	Fluffy	53	79	88
SO-50	Off white	Scattered	49	82	90
SO-51	Off white	Fluffy	49	83	90
SO-52	Whitish	Scattered	43	90	90
SO-53	Off white	Fluffy	42	62	80
SO-54	Dirty white	Scattered	41	68	78
SO-55	Off white	Scattered	36	74	88
SO-56	Whitish	Smooth	33	63	87
SO-57	Whitish	Fluffy	32	55	80
SO-58	Off white	Smooth	29	44	78
SO-59	Off white	Fluffy	29	57	58
SO-60	Off white	Smooth	25	48	84
SO-61	Off white	Scattered	18	32	56
SO-62	Whitish	Fluffy	17	39	79
SO-63	Dirty white	Fluffy	13	36	52
SO-64	Whitish	Fluffy	12	38	45
SO-65	Dirty white	Scattered	3	11	44

Table 2. Cultural characteristics of *S. oryzae* on different agar media

Isolate No.	Sabouraud's Dextrose Agar	Sabouraud's Dextrose Agar	Nutrient Agar	Nutrient Agar	Richards Agar	Richards Agar	Hanson's Agar	Hanson's Agar
Isolate No.	Color	Texture	Color	Texture	Color	Texture	Color	Texture
SO-1	Whitish	Smooth	Whitish	Smooth	Whitish	Smooth	Pale White	Compact
SO-2	Whitish	Scattered	Whitish	Scattered	Whitish	Scattered	Pure White	Filamentous
SO-3	Whitish	Smooth	Whitish	Smooth	Whitish	Scattered	Pure White	Filamentous
SO-4	light Brown	Smooth	Whitish Brown	Smooth	Light Brown	Smooth	White	Smooth

SO-5	Brown	Scattered	Brown	Scattered	Whitish	Smooth	Pale Brown	Smooth
SO-6	Whitish	Smooth	Whitish	Scattered	Whitish	Smooth	Brownish Yellow	Smooth
SO-7	Whitish	Fluffy	Whitish	Fluffy	Whitish	Scattered	Pure white	Smooth
SO-8	Dark brown	Fluffy	Whitish	Fluffy	Whitish	Fluffy	Pure White	Fluffy
SO-9	Whitish	Smooth	Whitish	Smooth	Dirty white	Smooth	Cottony white	Scattered
SO-10	Whitish	Fluffy	Whitish	Fluffy	Whitish	Scattered	Light brown	Smooth
SO-11	Whitish	Fluffy	Whitish	Fluffy	Whitish	Scattered	Pure White	Compact
SO-12	Brown	Smooth	Light brown	Smooth	Whitish Brown	Smooth	Pure white	Fluffy
SO-13	Pale Brown	Scattered	Pale Brown	Smooth	Whitish	Fluffy	Whitish	Scattered
SO-14	Whitish	Scattered	Whitish Brown	Fluffy	Whitish	Smooth	Whitish	Smooth
SO-15	Whitish	Smooth	Whitish	Smooth	Off white	Scattered	Pure white	Smooth
SO-16	Dark brown	Fluffy	Light Brown	Fluffy	Brownish white	Scattered	Dall White	Fluffy
SO-17	Yellow Brown	Scattered	Yellowish Brown	Smooth	Dirty white	Smooth	Whitish	Smooth
SO-18	Yellow Brown	Fluffy	Yellowish Brown	Smooth	Whitish	Smooth	Yellowish white	Scattered
SO-19	Pale Brown	Smooth	Whitish Brown	Smooth	Dirty white	Scattered	Pale brown	Filamentous
SO-20	White brown	Smooth	Whitish	Smooth	Pale white	Scattered	Pale White	Filamentous
SO-21	Whitish	Scattered	Whitish	Scattered	Whitish	Smooth	Pure white	Smooth
SO-22	Whitish	Smooth	Whitish	Smooth	Whitish	Scattered	Cottony white	Smooth
SO-23	Whitish	Smooth	Whitish	Smooth	Whitish	Scattered	Whitish	Scattered
SO-24	Brown	Smooth	Brown	Scattered	Dirty white	Smooth	Pale Brown	Smooth
SO-25	Whitish	Smooth	Whitish Brown	Smooth	Off white	Scattered	Pale Brown	Smooth
SO-26	Whitish	Smooth	Whitish	Smooth	Whitish	Smooth	Whitish	Smooth
SO-27	Yellow Brown	Scattered	Brown	Scattered	Whitish	Smooth	Whitish	Smooth
SO-28	Pale Brown	Fluffy	Light brown	Smooth	Off white	Smooth	Brown	Smooth
SO-29	Light Brown	Smooth	Light Brown	Smooth	Off White	Smooth	Pale white	Smooth
SO-30	Whitish	Scattered	Whitish Brown	Scattered	Whitish	Smooth	Whitish	Smooth
SO-31	Brown	Scattered	Whitish	Smooth	Whitish	Fluffy	Pure white	Filamentous
SO-32	Yellow Brown	Fluffy	Yellowish Brown	Smooth	Whitish	Fluffy	Whitish	Scattered
SO-33	Whitish	Smooth	Whitish	Smooth	Dirty white	Fluffy	Whitish	Smooth
SO-34	Yellowish Brown	Scattered	Yellowish Brown	Scattered	Pale Brown	Smooth	Pale Brown	Fluffy
SO-35	Off white	Fluffy	Dark Brown	Fluffy	Whitish	Smooth	Pure White	Smooth
SO-36	Brownish yellow	Smooth	Brownish yellow	Fluffy	Dirty white	Fluffy	Cottony white	Serrated
SO-37	Whitish	Smooth	Whitish	Fluffy	Dirty	Smooth	Cottony	Smooth

			Brown		white		white	
SO-38	Whitish	Scattered	Whitish	Fluffy	Whitish	Fluffy	Pale Brown	Smooth
SO-39	Whitish	Fluffy	Whitish	Smooth	Whitish	Fluffy	Pure white	Smooth
SO-40	Dark Brown	Scattered	Pale Brown	Smooth	Dirty white	Smooth	Whitish	Scattered
SO-41	Yellow Brown	Smooth	Yellowish Brown	Smooth	Off white	Smooth	Pale Brown	Filamentous
SO-42	Whitish Brown	Fluffy	Light Brown	Fluffy	Off White	Scattered	Pure White	Smooth
SO-43	Pale Brown	Scattered	Pale Brown	Scattered	Whitish Brown	Smooth	Whitish	Scattered
SO-44	Whitish	Fluffy	Whitish	Fluffy	Whitish	Scattered	Pure white	Smooth
SO-45	Pale Brown	Smooth	Pale Brown	Smooth	Whitish	Scattered	Pure white	Scattered
SO-46	Off white	Scattered	Whitish	Scattered	light brown	Scattered	Light White	Compact
SO-47	Whitish	Scattered	Whitish	Smooth	Dirty Brown	Fluffy	Whitish	Smooth
SO-48	Whitish Brown	Fluffy	Off white	Fluffy	Whitish	Smooth	Pure White	Smooth
SO-49	Whitish	Smooth	Whitish	Smooth	Whitish	Fluffy	Whitish	Scattered
SO-50	Pale Brown	Smooth	Pale Brown	Fluffy	Whitish Brown	Scattered	Whitish	Scattered
SO-51	Brown	Fluffy	Light brown	Scattered	Light Brown	Fluffy	Whitish	Serrated
SO-52	Dark Brown	Scattered	Light Brown	Scattered	Dirty White	Scattered	Dirty white	Fluffy
SO-53	Pale brown	Scattered	Whitish Brown	Scattered	Whitish	Fluffy	Whitish	Scattered
SO-54	Whitish	Smooth	Whitish	Smooth	Whitish	Smooth	Pure White	Compact
SO-55	Pale Brown	Fluffy	Light Brown	Fluffy	Off White	Smooth	Pure White	Filamentous
SO-56	Pale yellow white	Scattered	Yellowish Brown	Scattered	Whitish	Scattered	Pure white	Serrated
SO-57	Off white	Smooth	Whitish	Smooth	Whitish	Fluffy	Pure White	Filamentous
SO-58	Pale yellow white	Smooth	Pale yellowish	Smooth	Yellowish White	Scattered	White	Smooth
SO-59	Whitish	Fluffy	Whitish	Smooth	Off white	Fluffy	Pure white	Scattered
SO-60	Off white	Smooth	Whitish	Smooth	Whitish	Smooth	Pure white	Scattered
SO-61	Brown	Fluffy	Light White	Fluffy	Dirty white	Smooth	Dall White	Fluffy
SO-62	Whitish	Scattered	Whitish	Scattered	Dirty white	Fluffy	Whitish	Smooth
SO-63	Light brown	Scattered	Pale Brown	Scattered	Whitish	Fluffy	Whitish	Smooth
SO-64	Dark Brown	Scattered	Whitish Brown	Scattered	Whitish	Fluffy	Dirty white	Smooth
SO-65	Light brown	Scattered	Whitish	Scattered	Dirty white	Scattered	light brown	Compact

Table 3. Growth and development of *S. oryzaemycelia* (mm) on different agar media

Isolate No.	Sabaurands Dextrose	Sabaurands Dextrose	Sabaurands Dextrose	Nutrient Agar (mm)	Nutrient Agar (mm)	Nutrient Agar (mm)	Richard Agar (mm)	Richard Agar (mm)	Richard Agar (mm)	Hanson's Agar (mm)	Hanson's Agar (mm)	Hanson's Agar (mm)
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	Agar (mm)	Agar (mm)	Agar (mm)	)	)	)	)	)	)			
Isolate No.	Growth after	Growth after	Growth after	Growth after	Growth after	Growth after	Growth after	Growth after	Growth after	Growth after	Growth after	Growth after
Isolate No.	48 hrs	72 hrs	96 hrs	48 hrs	72 hrs	96 hrs	48 hrs	72 hrs	96 hrs	48 hrs	72 hrs	96 hrs
SO-1	74	88	90	33	47	58	76	90	90	64	76	90
SO-2	34	58	76	16	27	35	76	90	90	57	88	90
SO-3	77	86	90	47	58	63	43	69	84	61	84	90
SO-4	15	35	48	18	44	57	71	90	90	58	80	90
SO-5	63	83	90	48	79	90	64	87	90	33	56	77
SO-6	9	18	31	70	73	75	51	76	90	24	46	67
SO-7	55	77	86	48	61	78	71	90	90	64	89	90
SO-8	76	82	90	23	48	61	38	70	90	65	78	90
SO-9	72	82	90	54	68	81	54	69	90	74	90	90
SO-10	61	78	90	48	75	81	61	77	90	53	79	90
SO-11	68	79	89	35	67	84	19	38	54	54	83	90
SO-12	72	83	90	65	84	90	74	90	90	56	86	90
SO-13	82	90	90	43	72	90	36	54	77	41	76	90
SO-14	67	78	86	47	81	90	48	81	90	57	83	90
SO-15	63	87	90	36	54	73	56	83	90	34	83	90
SO-16	65	72	86	33	39	43	61	81	90	77	76	90
SO-17	48	69	81	37	49	74	53	68	88	56	84	90
SO-18	48	69	83	48	74	90	78	90	90	69	81	90
SO-19	65	73	86	16	33	53	65	78	88	42	71	90
SO-20	18	32	48	74	82	90	58	69	90	65	84	90
SO-21	78	86	90	37	56	78	68	84	90	57	86	90
SO-22	48	68	78	9	37	49	69	83	90	53	87	90
SO-23	86	90	90	61	83	90	37	76	83	33	51	68
SO-24	76	84	90	58	81	90	56	79	90	43	54	83
SO-25	43	68	83	84	90	90	77	90	90	51	76	90
SO-26	38	82	90	73	90	90	39	54	77	26	43	90
SO-27	33	69	88	SO	77	90	72	90	90	63	83	90
SO-	56	78	90	39	57	77	63	83	90	61	83	90

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SO-29	25	29	34	14	33	48	47	58	78	69	90	90
SO-30	17	45	68	33	39	58	55	69	83	36	76	90
SO-31	34	58	79	33	61	73	37	49	63	76	83	90
SO-32	76	85	90	73	90	90	44	57	83	36	63	86
SO-33	76	88	90	29	48	55	34	67	80	67	88	90
SO-34	65	84	90	68	86	90	74	90	90	63	81	90
SO-35	69	78	86	18	39	54	43	73	90	57	87	90
SO-36	77	87	90	28	39	48	46	56	74	39	77	84
SO-37	48	69	83	23	67	81	71	89	90	65	76	90
SO-38	17	33	48	64	76	90	73	88	90	64	84	90
SO-39	38	63	78	51	77	90	43	71	86	39	87	90
SO-40	78	88	90	76	84	90	49	67	81	58	79	86
SO-41	61	73	88	39	76	90	80	90	90	58	69	90
SO-42	39	45	52	46	76	85	73	90	90	48	81	90
SO-43	65	79	86	74	90	90	76	90	90	56	65	90
SO-44	69	76	89	64	84	90	37	59	87	46	81	90
SO-45	63	84	90	37	68	81	16	33	47	71	86	90
SO-46	36	54	83	8	15	32	53	74	90	82	90	90
SO-47	63	78	89	39	49	64	48	58	73	63	83	90
SO-48	49	63	88	51	78	90	47	77	90	36	49	73
SO-49	73	86	90	35	69	87	65	78	90	67	76	90
SO-50	77	88	90	68	89	90	63	84	90	49	77	90
SO-51	72	88	90	47	54	69	39	49	76	65	89	90
SO-52	34	52	68	84	90	90	64	87	90	64	84	90
SO-53	84	90	90	34	56	79	61	83	90	23	36	65
SO-54	66	82	90	69	81	90	31	67	79	63	81	90
SO-55	44	52	66	35	49	58	78	90	90	63	87	83
SO-56	32	48	66	31	47	53	76	86	90	42	73	90
SO-57	12	35	54	29	32	44	51	74	90	65	83	90
SO-58	54	83	90	19	37	48	16	39	56	31	56	68
SO-59	76	86	90	43	57	84	47	66	85	18	46	55

SO-60	79	88	90	79	90	90	34	65	81	79	89	90
SO-61	48	59	90	27	53	67	73	90	90	64	79	90
SO-62	43	68	87	13	25	39	65	84	90	61	84	90
SO-63	72	86	90	69	84	90	78	90	90	65	79	90
SO-64	69	76	88	79	88	90	77	90	90	65	84	84
SO-65	32	57	83	16	29	48	44	56	76	63	81	90

3.2 Sclerotial variability

Sclerotial development differed markedly among isolates. The number of sclerotia per plate ranged from 23 in SO-13 to 69 in SO-43. Colour ranged from black to brown, with the thesis noting light-brown sclerotia in SO-46. Distribution patterns included central, peripheral and spread arrangements. Sclerotial diameter ranged from 1.6 to 3.6 mm; SO-59 had the smallest reported diameter and SO-49 the largest. Dry weight of 100 sclerotia ranged from 36.8 to 77.8 mg, with SO-61 and SO-55 representing the lower and upper extremes, respectively.

Table 4. Sclerotia development of *Sclerotium oryzae*

Isolate No.	Sclerotial initiation (days)	Days of formation	Sclerotia / Plate	Sclerotia colour	Sclerotial Pattern
SO-1	4	5	34	Black	Spread
SO-2	5	6	41	Black	Spread
SO-3	5	6	54	Black	Spread
SO-4	4	5	42	Black	Spread
SO-5	5	6	43	Black	Peripheral
SO-6	5	6	39	Black	Peripheral
SO-7	4	6	34	Black	Peripheral
SO-8	5	6	37	Black	Spread
SO-9	4	5	42	Brown	Peripheral
SO-10	5	6	47	Brown	Spread
SO-11	5	6	39	Black	Peripheral
SO-12	5	6	44	Black	Spread
SO-13	4	5	23	Black	Peripheral
SO-14	4	5	39	Black	Peripheral
SO-15	4	6	33	Black	Peripheral
SO-16	5	6	33	Black	Spread
SO-17	5	6	39	Black	Peripheral
SO-18	5	6	41	Black	Peripheral
SO-19	4	5	62	Brown	Peripheral
SO-20	4	5	38	Brown	Spread
SO-21	5	6	46	Black	Spread
SO-22	5	6	39	Brown	Spread
SO-23	4	5	39	Black	Spread
SO-24	5	6	28	Black	Peripheral
SO-25	5	6	31	Black	Spread
SO-26	4	5	40	Black	Spread
SO-27	5	6	43	Black	Peripheral
SO-28	4	6	38	Brown	Peripheral
SO-29	4	5	36	Black	Control
SO-30	5	6	47	Black	Peripheral
SO-31	4	5	48	Black	Spread
SO-32	4	5	39	Black	Peripheral
SO-33	4	6	48	Black	Peripheral
SO-34	5	6	37	Brown	Peripheral
SO-35	4	5	36	Brown	Spread
SO-36	5	6	30	Brown	Peripheral
SO-37	4	5	33	Brown	Peripheral

SO-38	4	6	36	Black	Peripheral
SO-39	5	6	49	Black	Peripheral
SO-40	4	5	46	Black	Peripheral
SO-41	4	5	34	Brown	Spread
SO-42	4	6	48	Black	Peripheral
SO-43	4	5	69	Brown	Peripheral
SO-44	5	6	62	Brown	Peripheral
SO-45	5	6	40	Black	Spread
SO-46	4	6	35	Light Brown	Spread
SO-47	4	5	43	Black	Peripheral
SO-48	4	6	52	Black	Peripheral
SO-49	4	5	42	Brown	Peripheral
SO-50	4	5	68	Brown	Peripheral
SO-51	4	5	41	Brown	Peripheral
SO-52	4	5	43	Black	Spread
SO-53	4	6	33	Brown	Spread
SO-54	5	6	43	Black	Spread
SO-55	5	6	44	Black	Peripheral
SO-56	4	5	38	Brown	Peripheral
SO-57	4	6	53	Black	Peripheral
SO-58	4	6	49	Black	Spread
SO-59	4	5	24	Black	Spread
SO-60	5	6	47	Black	Spread
SO-61	4	5	38	Black	Spread
SO-62	4	6	41	Black	Peripheral
SO-63	4	5	34	Black	Spread
SO-64	4	5	39	Black	Spread
SO-65	4	5	39	Black	Spread

Table 5. Sclerotia characteristics of *S. oryzae*

Isolate No.	Sclerotia Diameter (mm)	Sclerotia length (mm)	Sclerotia Shape	Test dry weight (mg) / 100 Sclerotia	Sclerotial pattern
SO-1	2.4	4.7	Irregular	41.5	Spars
SO-2	2.1	6.9	Round	51.7	Spars
SO-3	2.1	4.8	Round	47.6	Spars
SO-4	1.9	3.6	Round	48.6	Spars
SO-5	2.1	3.8	Round	42.8	Spars
SO-6	1.9	3.2	Round	45.6	Spars
SO-7	2.0	3.9	Round	39.7	Spars
SO-8	2.3	3.9	Round	43.3	Spars
SO-9	2.1	3.7	Irregular	54.6	Dense
SO-10	2.1	2.9	Round	47.3	Spars
SO-11	2.0	6.2	Round	44.4	Spars
SO-12	2.1	3.1	Round	41.3	Spars
SO-13	3.1	2.9	Spherical	41.1	Spars
SO-14	2.1	3.6	Oblong	47.6	Spars
SO-15	2.1	3.7	Round	41.9	Spars
SO-16	2.4	4.8	Oblong	43.1	Spars
SO-17	3.1	6.9	Round	47.9	Spars
SO-18	2.3	4.8	Oblong	71.8	Too Dense
SO-19	3.1	3.1	Round	44.9	Spars
SO-20	2.8	3.9	Round	42.9	Spars
SO-21	1.9	4.9	Round	44.8	Spars
SO-22	1.9	3.8	Oblong	47.8	Spars
SO-23	2.8	6.3	Oblong	49.6	Spars
SO-24	2.9	6.2	Spherical	48.3	Spars
SO-25	2.2	4.5	Oblong	41.3	Dense
SO-26	2.1	4.4	Spherical	48.8	Spars

SO-27	2.4	3.9	Oblong	44.3	Spars
SO-28	2.7	6.5	Round	41.8	Spars
SO-29	2.3	3.4	Round	53.2	Dense
SO-30	2.3	2.9	Round	51.4	Spars
SO-31	1.9	4.6	Oblong	54.8	Dense
SO-32	1.9	3.7	Spherical	44.3	Spars
SO-33	2.2	4.8	Round	43.8	Spars
SO-34	2.1	4.6	Round	46.8	Spars
SO-35	2.3	3.7	Round	69.1	Too Spars
SO-36	2.6	6.2	Round	47.6	Spars
SO-37	2.8	3.9	Round	53.4	Spars
SO-38	1.8	3.7	Oblong	47.6	Spars
SO-39	2.6	3.7	Round	63.4	Dense
SO-40	2.9	4.8	Round	50.3	Spars
SO-41	2.3	5.9	Oblong	49.8	Dense
SO-42	2.3	4.9	Round	71.9	Too Spars
SO-43	2.0	4.8	Spherical	64.4	Dense
SO-44	1.8	4.4	Round	61.5	Dense
SO-45	2.1	4.7	Oblong	69.2	Too Dense
SO-46	2.3	3.1	Spherical	61.6	Dense
SO-47	1.8	4.2	Round	47.9	Dense
SO-48	2.1	4.5	Round	65.6	Dense
SO-49	3.6	4.6	Round	51.2	Spars
SO-50	1.9	4.6	Round	63.8	Dense
SO-51	2.3	5.3	Spherical	43.4	Spars
SO-52	2.0	4.4	Oblong	46.7	Spars
SO-53	2.3	5.8	Round	61.5	Dense
SO-54	2.4	5.8	Round	44.3	Spars
SO-55	2.1	4.7	Oblong	77.8	Too Spars
SO-56	1.8	4.6	Spherical	49.1	Spars
SO-57	3.1	4.9	Round	49.3	Spars
SO-58	2.3	6.4	Oblong	47.9	Spars
SO-59	1.6	4.0	Oblong	51.3	Dense
SO-60	2.0	3.4	Oblong	45.6	Spars
SO-61	2.3	6.3	Round	36.8	Spars
SO-62	3.1	6.1	Round	44.4	Spars
SO-63	2.2	5.3	Round	52.7	Dense
SO-64	2.2	3.5	Irregular	51.5	Dense
SO-65	2.0	5.9	Round	48.9	Spars

3.3 Biochemical variability

Carbon-source utilization profiles differed among isolates. Glucose was utilized by all isolates and supported the most consistent growth response, while sucrose was also widely utilized. Lactose, citrate and adonitol were used less consistently. Most isolates were positive for indole production, whereas the methyl-red reaction was positive in a smaller subset.

Table 6. Utilization of different carbons source as a growth parameter for *S. oryzae*

Isolat e No.	Citrat e	Glucos e	Adonito l	Arabinos e	Lactos e	Sorbito l	Mannito l	Rhamnos e	Sucros e
SO-1	-	+	-	-	-	+	+	+	+
SO-2	+	+	+	-	+	+	+	+	+
SO-3	+	+	-	+	+	+	+	+	+
SO-4	-	+	-	-	-	+	+	+	+
SO-5	-	+	-	-	-	-	+	-	+
SO-6	+	+	-	-	-	+	-	+	+
SO-7	-	+	+	-	-	+	+	+	+
SO-8	-	+	-	-	-	-	+	+	+
SO-9	+	+	-	+	+	+	+	+	+
SO-10	-	+	+	-	-	-	+	+	+
SO-11	-	+	-	-	+	-	+	+	+
SO-12	+	+	-	+	-	+	+	+	+

SO-13	-	+	-	-	-	+	-	-	+
SO-14	-	+	-	-	-	-	+	-	+
SO-15	-	+	-	-	-	-	+	+	+
SO-16	-	+	-	-	-	+	+	+	+
SO-17	-	+	-	+	-	-	+	-	-
SO-18	+	+	-	-	-	+	+	-	+
SO-19	-	+	-	-	-	-	-	+	+
SO-20	-	+	-	-	-	+	+	+	+
SO-21	+	+	-	-	-	-	+	+	+
SO-22	-	+	-	-	-	+	+	+	+
SO-23	-	+	-	-	-	-	+	+	+
SO-24	-	+	-	-	-	+	-	-	+
SO-25	+	+	-	-	-	+	+	-	+
SO-26	-	+	-	-	-	+	+	+	+
SO-27	-	+	+	-	-	-	-	-	+
SO-28	-	+	-	-	-	-	-	+	+
SO-29	+	+	-	+	-	+	+	+	+
SO-30	-	+	-	-	-	-	+	+	+
SO-31	-	+	-	-	-	+	+	-	+
SO-32	+	+	-	-	-	+	+	+	+
SO-33	-	+	-	-	-	+	+	+	+
SO-34	+	+	-	-	-	+	+	+	+
SO-35	-	+	+	+	-	+	+	+	+
SO-36	-	+	+	+	-	+	-	+	+
SO-37	+	+	-	-	-	+	+	+	+
SO-38	+	+	-	-	-	+	-	+	+
SO-39	-	+	+	-	-	-	+	-	+
SO-40	-	+	+	-	-	+	+	-	+
SO-41	+	+	-	-	-	+	+	-	+
SO-42	-	+	-	-	-	+	+	+	+
SO-43	-	+	-	+	-	-	+	-	+
SO-44	-	+	-	+	-	-	+	-	+
SO-45	+	+	+	-	-	+	+	+	+
SO-46	-	+	-	-	-	+	+	+	+
SO-47	-	+	-	-	-	+	+	-	+
SO-48	-	+	-	-	-	+	+	+	+
SO-49	-	+	-	-	-	+	+	+	+
SO-50	-	+	-	-	-	-	+	-	+
SO-51	+	+	-	+	-	+	+	+	+
SO-52	-	+	-	-	+	+	+	+	+
SO-53	+	+	+	-	-	+	+	+	+
SO-54	-	+	-	-	+	+	+	+	+
SO-55	+	+	-	-	+	+	-	-	+
SO-56	+	+	-	+	-	+	+	+	+
SO-57	+	+	-	-	+	+	+	+	+
SO-58	-	+	-	-	-	+	+	+	+
SO-59	-	+	-	-	-	+	+	-	+
SO-60	-	+	-	-	-	+	+	+	+
SO-61	+	+	-	-	-	+	+	+	+
SO-62	-	+	-	-	-	+	+	+	+
SO-63	+	+	-	-	-	-	+	+	+
SO-64	-	+	-	-	-	-	+	+	+
SO-65	+	+	-	-	-	+	+	+	+

Table 7. Biochemical characters of *S. oryzae*.

Isolate No.	Indole production	Methyl red
SO-1	+	-
SO-2	-	-
SO-3	-	+
SO-4	+	-

SO-5	+	-
SO-6	-	+
SO-7	-	-
SO-8	+	-
SO-9	+	-
SO-10	-	-
SO-11	-	-
SO-12	-	-
SO-13	-	-
SO-14	+	-
SO-15	+	+
SO-16	-	-
SO-17	-	-
SO-18	+	-
SO-19	+	-
SO-20	-	+
SO-21	+	-
SO-22	+	-
SO-23	+	-
SO-24	-	-
SO-25	-	-
SO-26	+	-
SO-27	+	-
SO-28	+	-
SO-29	+	+
SO-30	+	-
SO-31	-	-
SO-32	+	-
SO-33	+	+
SO-34	-	-
SO-35	+	-
SO-36	+	-
SO-37	+	-
SO-38	-	-
SO-39	+	+
SO-40	-	-
SO-41	-	-
SO-42	+	+
SO-43	-	-
SO-44	+	+
SO-45	-	+
SO-46	-	-
SO-47	+	-
SO-48	+	-
SO-49	+	-
SO-50	-	-
SO-51	+	-
SO-52	-	-
SO-53	+	-
SO-54	-	-
SO-55	+	-
SO-56	+	-
SO-57	+	+
SO-58	-	-
SO-59	+	-
SO-60	-	-
SO-61	+	-
SO-62	-	+
SO-63	+	-
SO-64	-	+
SO-65	+	+

3.4 Molecular identification and phylogenetic relationships

Forty-five sequences were generated from the molecular analysis, of which 30 were retained after filtering and removal of chimeric sequences. The retained sequences averaged 880 +/- 25 bp. BLAST-based identification grouped the retained sequences as *Sclerotium oryzae* and accession numbers were obtained from NCBI GenBank. Maximum-likelihood analysis using the Tamura-Nei model separated the isolates into two main groups, A and B. Group A contained 16 isolates; SO-6 was distinct within this group, while SO-26, SO-5, SO-16 and SO-17 were described as distinct within group B.

Table 8. Identity of *Sclerotium oryzae* isolates of rice sequenced and deposited in Genbank, NCBI, USA

Sl. No.	Isolates designation	Identified as	GenBank accession No.
1	S. oryzae- SO1	<i>Sclerotium oryzae</i>	MZ935641
2	S. oryzae- SO2	<i>Sclerotium oryzae</i>	MZ936283
3	S. oryzae- SO3	<i>Sclerotium oryzae</i>	MZ950610
4	S. oryzae- SO4	<i>Sclerotium oryzae</i>	MZ950611
5	S. oryzae- SO5	<i>Sclerotium oryzae</i>	MZ950611
6	S. oryzae- SO6	<i>Sclerotium oryzae</i>	MZ950680
7	S. oryzae- SO7	<i>Sclerotium oryzae</i>	MZ950683
8	S. oryzae- SO8	<i>Sclerotium oryzae</i>	MZ950682
9	S. oryzae- SO9	<i>Sclerotium oryzae</i>	MZ950681
10	S. oryzae- SO10	<i>Sclerotium oryzae</i>	MZ950686
11	S. oryzae- SO11	<i>Sclerotium oryzae</i>	MZ950679
12	S. oryzae- SO12	<i>Sclerotium oryzae</i>	MZ950684
13	S. oryzae- SO13	<i>Sclerotium oryzae</i>	MZ950924
14	S. oryzae- SO14	<i>Sclerotium oryzae</i>	MZ958804
15	S. oryzae- SO15	<i>Sclerotium oryzae</i>	MZ950925
16	S. oryzae- SO16	<i>Sclerotium oryzae</i>	MZ951045
17	S. oryzae- SO17	<i>Sclerotium oryzae</i>	MZ951047
18	S. oryzae- SO18	<i>Sclerotium oryzae</i>	MZ958813
19	S. oryzae- SO19	<i>Sclerotium oryzae</i>	MZ951048
20	S. oryzae- SO20	<i>Sclerotium oryzae</i>	MZ966298
21	S. oryzae- SO21	<i>Sclerotium oryzae</i>	MZ951049
22	S. oryzae- SO22	<i>Sclerotium oryzae</i>	MZ951054
23	S. oryzae- SO23	<i>Sclerotium oryzae</i>	MZ951056
24	S. oryzae- SO24	<i>Sclerotium oryzae</i>	MZ951055
25	S. oryzae- SO25	<i>Sclerotium oryzae</i>	MZ951104
26	S. oryzae- SO26	<i>Sclerotium oryzae</i>	MZ951106
27	S. oryzae- SO27	<i>Sclerotium oryzae</i>	MZ951107
28	S. oryzae- SO28	<i>Sclerotium oryzae</i>	MZ958805
29	S. oryzae- SO29	<i>Sclerotium oryzae</i>	MZ964409
30	S. oryzae- SO30	<i>Sclerotium oryzae</i>	MZ951110

4. DISCUSSION

The broad variation in colony growth and sclerotial traits demonstrates that the Karnataka *S. oryzae* population is phenotypically heterogeneous. Comparable variation in growth rate, sclerotial position and colony characters has been reported for isolates from Haryana (Kumar et al., 2011) and for isolates from Telangana and Andhra Pradesh (Prameela et al., 2020). Such variability may reflect differences in genotype, local adaptation and physiological requirements, and it cautions against using a single isolate as representative of a regional pathogen population. PDA supported clear expression of growth and sclerotial characters, but isolate ranking changed across media, indicating strong genotype-by-medium effects (Shivappa et al., 2026). Differences in sclerotial number, size, colour and dry weight are particularly relevant because sclerotia function as persistent survival structures and primary inoculum. The observed range of sclerotial characteristics therefore provides useful descriptors for isolate differentiation and for selecting contrasting isolates in future pathogenicity or fungicide-sensitivity studies (Yuvaraj et al., 2025).

Biochemical tests further separated isolates by substrate use. Universal utilization of glucose and broad use of sucrose, rhamnose, mannitol and sorbitol indicate shared core metabolic capacity, while less consistent use of other substrates points to physiological variation (Roy et al., 2025). These traits should be interpreted as phenotypic descriptors rather than taxonomic markers because growth responses can vary with inoculum, medium chemistry and incubation conditions (Kaaviya et al., 2025). Sequence-based analysis confirmed the identity of 30 retained isolates as *S. oryzae* and resolved two principal phylogenetic groups. Earlier RAPD analysis also demonstrated substantial diversity in Indian *S. oryzae* populations (Kumar et al., 2010; Usha et al., 2025), while ITS-based approaches have been widely used to resolve fungi associated with the rice sheath- and

stem-rot complex. The combination of phenotypic diversity and sequence clustering in the present dataset supports the presence of structured variability within Karnataka. For submission, the original chromatograms, primer records and GenBank accessions should be rechecked so that the molecular methods and sequence metadata are internally consistent.

5. CONCLUSION

The collection of *S. oryzae* showed substantial variability in cultural growth, colony morphology, sclerotial development, carbon-source utilization and sequence-based relationships. Isolates differed widely in radial growth on five media, number and pattern of sclerotia, sclerotial dimensions and test weight. Glucose was the most consistently utilized carbon source, while indole and methyl-red reactions provided additional biochemical differentiation. Thirty retained sequences were identified as *S. oryzae* and deposited in GenBank, and phylogenetic analysis separated the isolates into two main groups. The combined phenotypic and molecular dataset provides a useful basis for selecting representative isolates for pathogenicity, host-resistance and fungicide-response studies. Before journal submission, the primer nomenclature and sequence-analysis details reported in the thesis should be reconciled with the original laboratory records and sequence files.

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REFERENCES

1. Ali Z (1997). Variation in isolates of *Sclerotium oryzae*, the rice stem rot pathogen. *Int. Rice Res. Notes*. 22(2): 41-45.
2. Ali Z and Singh RA (1993). Variation in appressoria and infection process of *Sclerotium oryzae* which causes stem rot of rice. *Int. Rice Res. Notes*. 18(2): 29-30.
3. Almeida AMR, Abdelnoor RV, Calvo ES, Tessmann D and Yorinori JT (2001). Genotypic diversity among Brazilian isolates of *Sclerotium rolfsii*. *J. Phytopathol.* 149: 493-502.
4. Chethana BS and Prasannakumar MK (2019). Rice stem rot *Sclerotium hydrophilum* Sacc. isolated and characterized in southern Karnataka. *Oryza*. 56(2): 242-245.
5. Cowan ST and Steel KJ (1970). *Manual for the Identification of Medical Bacteria*. Lowe and Brydone, London.
6. Johanson A, Turner HC, McKay GJ and Brown AE (1998). A PCR-based method to distinguish fungi of the rice sheath blight complex. *FEMS Microbiol. Lett.* 162: 289-294.
7. Kaaviya V, Karpagavalli S, Chandrasekaran P and Rex B (2025). Exploitation of defense compounds from sheath blight infected rice with *Rhizoctonia solani* isolates. *Crop Res.* 60(3-4): 205.
8. Kukreti A, Kotasthane AS, Manjunath LS, Suresh R, PrasannaKumar MK, Devanna P, Sushil SN and Channappa M (2026). Multi-marker analysis elucidates genetic diversity and antifungal mechanisms of native *Bacillus thuringiensis* strains against *Agroathelia rolfsii*. *Sci. Rep.*
9. Kumar A, Singh R, Boora KS and Jalali BL (2010). Genetic diversity in *Sclerotium oryzae* population of rice based on random amplified polymorphic DNA markers. *Indian Phytopathol.* 63(4): 418-420.
10. Kumar A, Singh R, Boora KS and Jalali BL (2011). Variability in *Sclerotium oryzae* isolates causing stem rot of rice based on cultural, morphological and pathogenic behaviour from Haryana regions. *Indian Phytopathol.* 64(1): 41-43.
11. Kumar AB, Das S, Ranasingh N, Moharana RL, Behera S, Behera C and Das S (2026). Assessment of sheath blight resistance in rice landraces using disease progression metrics and machine learning. *Plant Sci. Today*. 13.
12. Kumar S, Stecher G, Li M, Knyaz C and Tamura K (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. Evol.* 35(6): 1547-1549.
13. Lilly VG and Barnett HL (1951). *Physiology of the Fungi*. McGraw-Hill, New York.
14. Naveenkumar R, Panda S, Reddy CVK, Prabhukarthikeyan SR, Sangeetha G, Anandan A, Kumar Acharya L and Ali J (2026). Genomic insights into sheath blight resistance: Leveraging natural variation in the Bengal-Assam-Aus rice panel for multi-season association mapping. *Front. Plant Sci.* 17: 1890133.
15. Negi R, Sharma B, Darshani P, Thakur SS, Ratnasari A, Barwant MM, Tomar P, Yadav N and Yadav AN (2026). Revitalization of plant growth-promoting plant allied microbes: An eco-friendly approach for agricultural and environmental sustainability. *Biol. Futura*. pp. 1-22.
16. Pathak VN (1972). *Essentials of Plant Pathology*. Prakash Publishers, Jaipur, India.
17. Prameela M, Rajeswari B, Krishnaveni D, Sudhakar R, Keshavulu K and Narender Reddy C (2020). Cultural and morphological variation in *Sclerotium oryzae* Catt. isolates collected from major rice growing areas of Telangana and Andhra Pradesh states, India. *Int. J. Curr. Microbiol. Appl. Sci.* 9(1): 610-615. doi:10.20546/ijemas.2020.901.066.
18. Roy PS, Prakasam V, Kotasthane AS, Pranathi P, Priyanka C, Manoj VM, Rao DS, Laha GS, Prasad MS, Badri J and Mangrauthia SK (2025). Differential response of tolerant and susceptible cultivars of rice against sheath blight pathogen, *Rhizoctonia solani* Kühn. *Eur. J. Plant Pathol.* 172(1): 75-89.

19. Sambrook J, Fritsch EF and Maniatis T (1989). *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory Press, New York.
20. Sandhya Y, Kumar MR, Madhusudhan P, Sudhakar P and Kumari PL (2026). Cultural, pathogenic and genetic variability of *Rhizoctonia solani* isolates incitant of rice sheath blight disease. *Vegetos*. pp. 1-14.
21. Seeley HW and Vandemark PS (1981). *Microbes in Action: A Laboratory Manual for Microbiology*. W.H. Freeman, San Francisco.
22. Shivappa R, Koti PS, Jeevan B, Anilkumar C, Baite MS, Choudhary M and Mohapatra SD (2026). Elicitation of defense responses in rice against sheath blight using salicylic acid and methyl jasmonate. *Discover Plants*. 3(1): 29.
23. Simmons JS (1926). A culture medium for differentiating organisms of typhoid-colon aerogenes groups and for isolation of certain fungi. *J. Infect. Dis.* 39: 209.
24. Sudhakar R (1996). *Variability in Rhizoctonia solani Kuhn and Management of Sheath Blight of Rice*. Ph.D. Thesis, Acharya N.G. Ranga Agricultural University, Hyderabad, India.
25. Usha I, Pramesh D, Raghunandana A, Prasannakumar MK, Sunkad G and Manjunatha C (2025). In vitro and in-plant bioefficacy of bacteriome isolated from diseased leaf lesions of two contrasting rice genotypes against sheath blight disease of rice. *Lett. Appl. Microbiol.* 78(6): ovaf078.
26. Vani Y, Jayalakshmi RS, Madhusudhan P and Reddy VLN (2019). Cultural and morphological variability in isolates of *Sclerotium oryzae*, the rice stem rot pathogen. *Andhra Pradesh J. Agric. Sci.* 5(3): 222-228.
27. Weisburg WG, Barns SM, Pelletier DA and Lane DJ (1991). 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* 173(2): 697-703.
28. Yuvaraj M, Kannan C, Lokesh R and Ajaydesouza V (2025). Cultural and morpho-molecular characterization of *Rhizoctonia solani* causing rice sheath blight. *Biochem. Cell. Arch.* 25(1): 1007.