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Research Article

Foot Rot of *Salvia hispanica* Caused by *Agroathelia rolfsii* (= *Athelia rolfsii*) in Southern India

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Abstract

Chia (*Salvia hispanica* L.) is an economically important medicinal crop cultivated in Karnataka, India. During field surveys of 114 chia fields spanning approximately 130 hectares in southern Karnataka, foot rot disease incidence ranged from 10.6% to 23.54%. A fungal pathogen was consistently isolated from symptomatic tissues and preliminarily identified as *Agroathelia rolfsii* based on cultural and morphological characteristics. The identity of nine representative isolates was confirmed by ITS-rDNA sequencing. BLASTn analysis showed high similarity to authenticated *A. rolfsii* isolates, and nrITS-based phylogenetic analysis grouped the chia isolates with reference strains of *A. rolfsii*. Pathogenicity assays with isolate SrSh12 reproduced typical foot rot symptoms within 5–7 days after inoculation, and the pathogen was successfully re-isolated from symptomatic plants, thereby fulfilling Koch's postulates. These results confirm *A. rolfsii* as the causal agent of foot rot in chia and provide a foundation for developing integrated disease management strategies.

Keywords: *Agroathelia*, Chia, ITS-rDNA, Molecular phylogeny, Pathogenicity

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1. Introduction

Plant diseases pose a significant threat to global agriculture, causing estimated crop yield losses of up to 12% worldwide (Reeleder, 2003). Among the various causal agents, pathogenic fungi cause numerous destructive diseases affecting economically important crops. Fungal isolates can be differentiated based on their morphological characteristics, host specificity, pathogenicity, and genetic variability, including their ability to form stable vegetative heterokaryons through genetic recombination (Capote *et al*., 2012). Accurate detection and identification of plant pathogens constitute the first and most critical steps in developing effective disease prevention, management, and control strategies (Mahadevakumar and Sridhar, 2020a, 2020b).

Over the past two decades, there has been a marked increase in the emergence of new plant diseases and severe epidemics caused by fungal pathogens. Advances in molecular biology have greatly facilitated the identification of novel fungal species, new host–pathogen associations, and cryptic pathogens that are difficult to distinguish using conventional methods (Mahadevakumar and Sridhar, 2021, 2022). Consequently, rapid, reliable, and accurate identification of fungal pathogens has become indispensable for informed decision-making in plant disease management. Although traditional cultural and morphological approaches remain fundamental to fungal identification, they are increasingly complemented by molecular barcoding and phylogenetic analyses to enhance the accuracy and reliability of species identification (Swamy *et al*., 2020a, 2020b; Tejaswini *et al*., 2023a,b).

Salvia hispanica L., commonly known as chia, is an emerging crop cultivated in both southern and northern regions of Karnataka, India. Native to Central America and belonging to the family Lamiaceae, chia has gained worldwide recognition as a “superfood” for its exceptional nutritional profile and potential health benefits (Kulczyński *et al.*, 2019). Chia seeds are rich in essential fatty acids, dietary fibre, antioxidants, and minerals such as calcium, phosphorus, and iron. Their consumption has been associated with improved cardiovascular health, better glycaemic control, and enhanced digestive function, making chia an important nutraceutical component of modern diets (Rabail *et al.*, 2022).

Historically, chia was an important pseudocereal and staple food of ancient Mesoamerican civilizations, particularly the Mayans and Aztecs. Chia seeds were valued as a source of energy and endurance, and the word “chia” is believed to be associated with “strength.” Today, chia is cultivated commercially in several countries, with major production occurring in Mexico, Guatemala, Argentina, Bolivia, Ecuador, and Australia (Valdivia-López and Tecante, 2015). In recent years, its cultivation has expanded considerably in response to increasing consumer demand and commercial value. However, the expansion of chia cultivation has also increased the crop's exposure to various biotic stresses, particularly fungal diseases and insect pests, which can adversely affect seed yield and quality.

Given the growing importance of chia as an emerging nutraceutical crop, understanding the biotic constraints that affect its cultivation is essential to sustaining productivity and crop quality. In India, chia cultivation is still in its early stages of expansion, and information on

disease incidence, pathogen diversity, epidemiology, and effective management strategies remains limited. Moreover, reports of pathogens associated with chia in India are largely confined to Karnataka, underscoring the need for systematic investigations into fungal diseases across different stages of crop growth.

During field surveys conducted in southern Karnataka as part of the present investigation, typical symptoms of foot rot were observed in chia fields, indicating the potential emergence of the disease as a constraint to chia cultivation. Considering the limited information available on the etiology of foot rot in chia, the present study was undertaken to isolate, identify, and characterize the associated fungal pathogen using cultural and micromorphological characteristics, molecular sequencing, and phylogenetic analyses. Furthermore, pathogenicity assays were conducted in accordance with Koch's postulates to confirm the causal association between the identified pathogen and foot rot disease in chia.

2. Materials and methods

2.1 Field investigation and disease incidence

A roving field survey was conducted to assess the occurrence of foot rot disease in chia across different locations in the Mysore and Chamarajanagar districts from October 2022 to January 2023. A total of 114 chia fields, covering approximately 130 hectares, were surveyed across 12 locations (Table 1). In each field, 250 plants were assessed by examining 50 plants from each of the four corners and 50 plants from the central portion of the field. The number of plants exhibiting typical foot rot symptoms was recorded, and disease incidence (%) was calculated using the following formula (Mahadevakumar *et al.*, 2017; Tejaswini *et al*., 2023a).

$$\text{Disease Incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants assessed}} \times 100$$

2.2 Isolation and cultural morphology

A total of 20 diseased chia plants exhibiting typical foot rot symptoms were collected from different surveyed locations for pathogen identification. Symptomatic plant tissues were cut into small segments (approximately 0.5 cm), surface-sterilized with 2% sodium hypochlorite (NaOCl) for 1 min, rinsed three times with sterile distilled water, and blot-dried under aseptic conditions. The surface-sterilized tissue segments were aseptically placed on potato dextrose agar (PDA; Hi-Media, Mumbai, India) supplemented with chloramphenicol (50 mg/L) to minimize bacterial contamination (Sampathkumar *et al.*, 2026). The inoculated plates were incubated at 28 ± 2°C for 4–6 days under alternating 12-h light and dark photoperiods. Mycelial growth emerging from the infected tissue segments was transferred to fresh PDA plates and repeatedly sub-cultured, as required, to obtain pure fungal cultures. Cultural characteristics, including colony morphology, colour, texture, and radial growth, were

recorded after 7–8 days of incubation. Micromorphological characteristics, including hyphal morphology and sclerotial characteristics such as formation, number, size, and texture, were examined using a light microscope. Representative pure cultures were subsequently preserved in the culture collection of the Department of Studies in Microbiology, University of Mysore, Mysuru, Karnataka, India, with the isolate codes SrSh3, SrSh2, SrSh5, SrSh6, SrSh8, SrSh9, SrSh10, SrSh11, and SrSh12, respectively.

2.3 Molecular identification

To substantiate the morphological identification, nine isolates representing one isolate from each location were selected for molecular characterization based on their geographical origin, cultural and morphological characteristics, and host-associated variability. Genomic DNA (gDNA) was extracted from 12–15-day-old fungal cultures using the CTAB method (Zhang *et al.*, 1998). The quality and concentration of the extracted DNA were assessed using an ND Lite 1000 spectrophotometer (Thermo Scientific). The internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) was amplified using the universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al.*, 1990). PCR amplification was carried out in a Bio-Rad T100 thermal cycler in a total reaction volume of 25 µl containing 1.0 µl of template DNA (~50 ng), 12.5 µl of *Taq* DNA polymerase master mix (GeNei, Bangalore, India), 1.0 µl each of forward and reverse primers (10 pmol; Eurofins, Bangalore, India), and 9.5 µl of nuclease-free water. The PCR cycling conditions consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 45 s, and extension at 72°C for 3 min, with a final extension at 72°C for 10 min. The amplified PCR products were separated by electrophoresis on a 1.5% agarose gel, purified, and sequenced bidirectionally using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The resulting forward and reverse sequences were assembled into consensus sequences using BioEdit software. The consensus sequences were compared with reference sequences in the NCBI nucleotide database using the BLASTn algorithm to identify the closest sequence matches and facilitate molecular identification of the isolates. The validated consensus sequences were subsequently deposited in the NCBI GenBank database, and accession numbers were obtained.

2.4 Phylogenetic analysis

Consensus ITS-rDNA sequences of *Agroathelia rolfsii* and allied species were retrieved from the NCBI GenBank database and compiled for phylogenetic analysis. Reference sequences showing high similarity in preliminary BLASTn searches were selected and included in the final dataset along with the sequences generated in the present study. The ITS-rDNA sequences were aligned using MEGA X software (Kumar *et al*., 2018), and phylogenetic relationships were inferred using the Maximum Likelihood (ML) method. The robustness of the resulting tree topology was evaluated using 1,000 bootstrap replications, with bootstrap support values indicated at the corresponding nodes. The phylogenetic tree includes isolate or taxon information and GenBank accession numbers for both the reference sequences and

those generated in the present study. *Chloroscypha enterochroma* and *Sclerotium cepivorum* (NKI286) were included as outgroup taxa to root the phylogenetic tree.

2.5 Pathogenicity assay

Pathogenicity assays were conducted on two-week-old chia seedlings using the polycup method (Tejaswini *et al.,* 2023a). A representative isolate, SrSh12, selected based on its typical cultural and morphological characteristics, was used for inoculation to confirm its pathogenicity and fulfil Koch's postulates. The experiment comprised of 10 plants per replicate, with independent replications and repeated twice totalling 60 inoculated plants. Each seedling was inoculated with a PDA plug containing actively growing mycelium whereas control seedlings received sterile PDA plugs near soil surface. Following inoculation, the plants were maintained under high-humidity conditions (>80% relative humidity; 28 ± 2 °C) for 48 h and subsequently transferred to a greenhouse maintained at 28±2°C and 60% RH. Disease development was monitored periodically, and disease incidence was calculated as the percentage of inoculated plants exhibiting symptoms. The pathogen was re-isolated from symptomatic tissues, and its identity was confirmed through cultural and morphological characterization, thereby fulfilling Koch's postulates.

3. Results

3.1 Disease symptoms and incidence

A roving field survey conducted from October 2022 to January 2023 revealed widespread foot rot in chia. The disease was characterized by dark necrotic lesions at the collar region, root decay, white mycelial growth, formation of small globoid sclerotia, and eventual plant collapse (Fig. 1).

The survey covered approximately 130 ha, comprising 114 fields across 12 locations in southern Karnataka (Table 1). Foot rot was recorded at all locations, with disease incidence ranging from 10.60 to 23.54% and an overall mean of 15.77 ± 3.65%. The highest incidence was recorded at Hommaragalli (23.54 ± 2.57%), followed by DMG Halli (19.86 ± 3.02%), Devalapura (18.40 ± 2.51%), and Karimuddanahalli (17.10 ± 1.97%). The lowest incidence occurred at Kallegowdanahalli (10.60 ± 2.41%), Santhe Saragur (11.73 ± 1.95%), and Gaddige (12.42 ± 2.68%). The number of fields surveyed per location ranged from 5 to 18, while the number of isolates recovered ranged from 1 to 3. Beechanahalli yielded the maximum number of isolates (3), although its disease incidence was moderate (16.28 ± 3.29%). No clear relationship was observed between disease incidence and the number of isolates recovered. Overall, the survey showed considerable spatial variation in the incidence of chia foot rot. Hommaragalli, DMG Halli, Devalapura, and Karimuddanahalli recorded comparatively higher disease levels. Differences in incidence may be associated with soil, moisture, cropping history, cultivar, agronomic practices, and environmental conditions, although these factors require further investigation. The survey confirms the occurrence and

distribution of foot rot in chia and provides a basis for characterizing and identifying the associated pathogen(s).



Figure1. Field symptoms of foot rot on chia. (A) Healthy chia crop under field conditions. (B) Diseased plants exhibiting wilting, yellowing, and drying are associated with foot rot infection. (C) Infected plant showing basal stem and root rot with adhering soil due to fungal mycelial growth. (D) Close-up of the collar and root region showing characteristic white mycelial growth and sclerotial formation on infected tissues.

Table 1. The incidence of foot rot associated with chia plants was recorded from Southern Karnataka, India.

Sl. No.	Location	GPS Coordinates	N*	Disease Incidence	No. Isolates
1	Karimuddanahalli	12°12'08.8"N, 76°22'35.8"E	10	17.1±1.97	2
2	Vaddaragudi	12°06'44.0"N, 76°20'09.1"E	8	15.25±2.92	1
3	Gaddige	12°11'20.0"N, 76°22'23.4"E	12	12.42±2.68	2
4	Santhe Saragur	12°00'25.8"N, 76°23'22.2"E	10	11.73±1.95	2
5	Beechanahalli	11°59'17.8"N, 76°20'34.3"E	18	16.28±3.29	3
6	Thumbasoge	12°01'36.6"N, 76°23'03.5"E	7	13.43±2.44	1
7	Devalapura	12°13'42.4"N, 76°27'53.5"E	5	18.4±2.51	1
8	DMG Halli	12°17'32.2"N, 76°30'40.8"E	7	19.86±3.02	1
9	Kallegowdanahalli	12º05′39.5″N, 76º34′04.7″E	5	10.6±2.41	1
10	Ankanathapura	12°45'19.0"N, 77°00'50.3"E	9	14.88±2.03	1
11	Doddakereyur	12º13′82.8″N, 76º39′27.8″E	10	15.9±2.51	1
12	Hommaragalli	12°07'19.8"N, 76°28'04.2"E	13	23.54±2.57	2
Mean ± SE			114	15.77±3.65	18

Note: *Number of fields evaluated for incidence.

3.2 Cultural and morphological identification

Overall, 20 diseased samples associated with foot rot were sampled from the surveyed areas and they were subjected to phytopathological analysis. The frequency of isolation of the fungal pathogen varied among the samples; however, all infected samples consistently yielded uniform single colony, resulting in the establishment of 18 pure cultures. The identification was further supported by detailed morphological examination. All 18 isolates were subsequently used for cultural and morphological characterization. The colonies grown on PDA medium exhibited typical aerial, dense, cottony, whitish mycelial growth, with globoid sclerotia developing within 10–12 days of incubation (Fig. 2).

The mycelium consisted of hyaline, septate hyphae with prominent clamp connections, forming an interconnected network that ultimately differentiated into sclerotial bodies (Fig. 3). No significant variation was observed among the isolates in growth rate, colony coloration, or the time required for sclerotial formation (10–15 days). The initial formation of small white sclerotial bodies was observed within 7–8 days, and they gradually turned from light tan to brown upon maturation (10–12 days). The sclerotia measured 0.6–2.2 mm in diameter, with the number per plate ranging between 240 and 410 (mean: 382 ± 21; n = 18). Based on the cultural and morphological observations, the isolated fungal pathogen was

identified as *Agroathelia rolfsii* (Sacc.). Redhead & Mullineux (syn. *Sclerotium rolfsii* Sacc.), (Sampathkumar *et al.*, 2026; Mullen, 2001; Punja *et al.*, 1985).

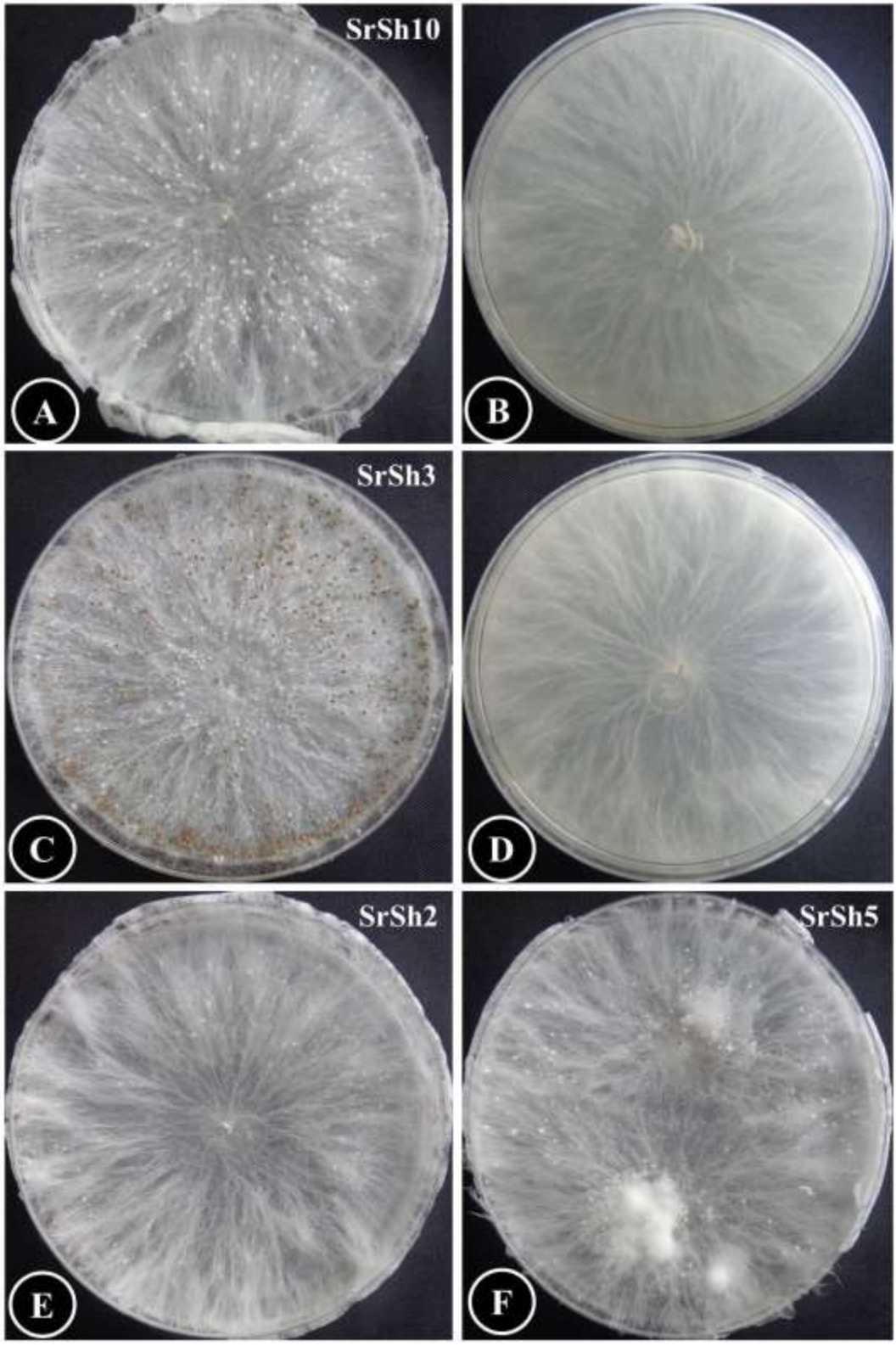

Figure 2. Cultural characteristics of four representative *Agroathelia rolfsii* isolates grown on potato dextrose agar (PDA) after 7 days of incubation at 28 ± 2°C, showing abundant, fast-growing white, cottony mycelial growth with the initiation of sclerotial formation.

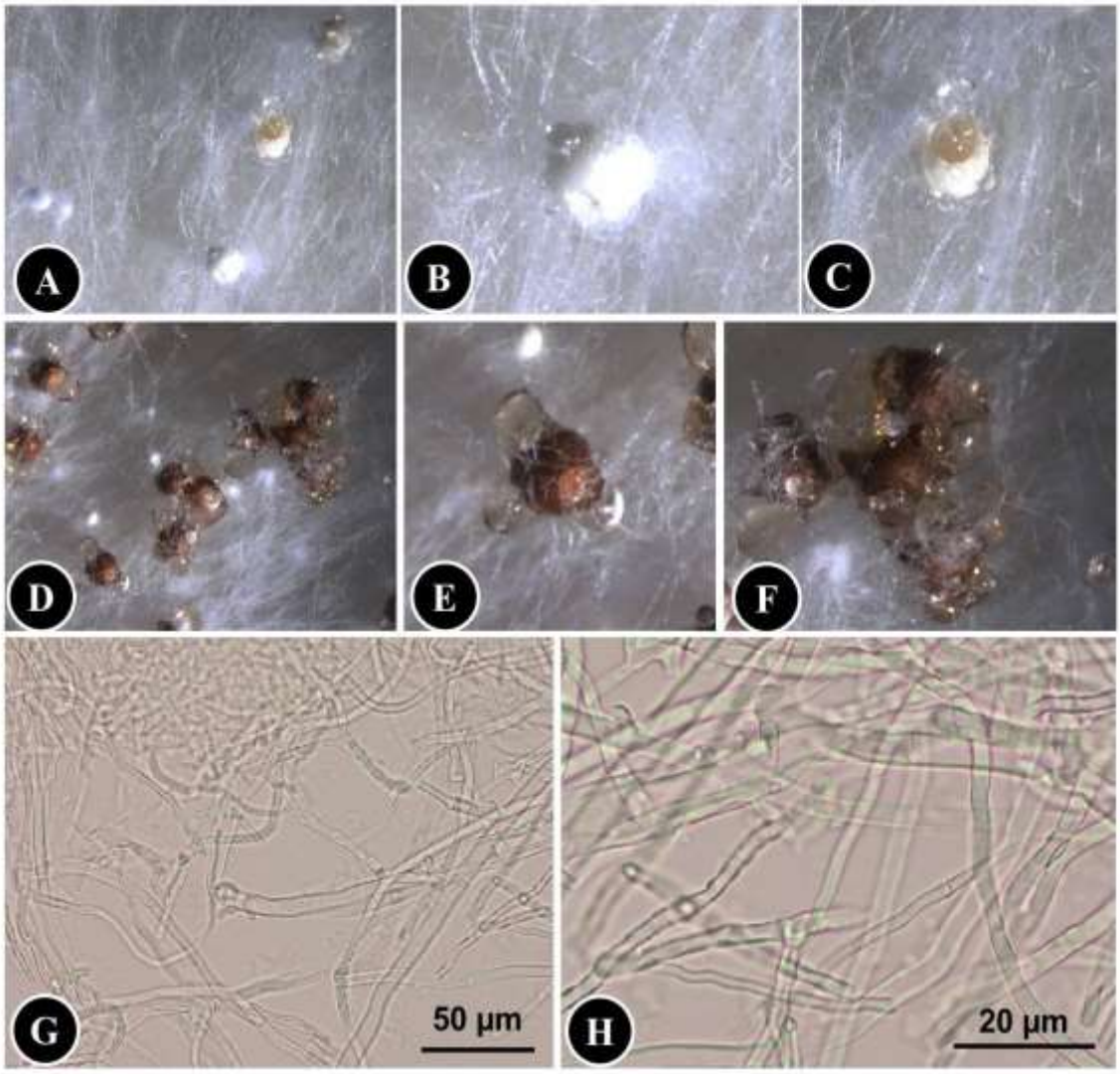

Fig.3. Cultural and microscopic characteristics of *Agroathelia rolfsii* isolated from diseased chia plants. (A–C) Initiation of immature, white to cream-colored sclerotia on potato dextrose agar. (D–F) Development and maturation of abundant, globose to irregular, dark brown sclerotia after incubation. (G–H) Hyaline, septate, branched hyphae with characteristic clamp connections were observed under a light microscope.

3.3. Molecular identification and phylogenetic analysis

A total of nine isolates of *Agroathelia rolfsii* were used for molecular identification by ITS-rDNA sequencing. PCR amplification of the ITS-rDNA region from the selected isolates yielded products of the expected size. The amplified fragments were sequenced in both directions, and the resulting consensus sequences were analyzed using the BLASTn tool. Sequence similarity analysis revealed that all nine isolates showed 98–100% identity with

Agroathelia rolfsii reference sequences (LHBJ2-4: MZ956758; Sr3: KC292637; CPC:23947: MK411221; GKVK-beetroot: MW350729; GKVK-Raagi: MW349663). The representative sequences of *Agroathelia rolfsii* isolates generated in the present study were deposited in GenBank and assigned accession numbers. (SrSh3-OR295433, SrSh2-OR295432, SrSh5-OR295434, SrSh6-OR295435, SrSh8-OR295436, SrSh9-OR295437, SrSh10-OR295438, SrSh11-OR295439, SrSh12-OR295440).

Furthermore, phylogenetic analysis based on the maximum likelihood (ML) method demonstrated that the nine *Agroathelia rolfsii* isolates from the present study clustered within a common clade with reference isolates, including *A. rolfsii* SrGg07 and CBS:132553, as well as additional *A. rolfsii* sequences retrieved from GenBank (Fig. 4).

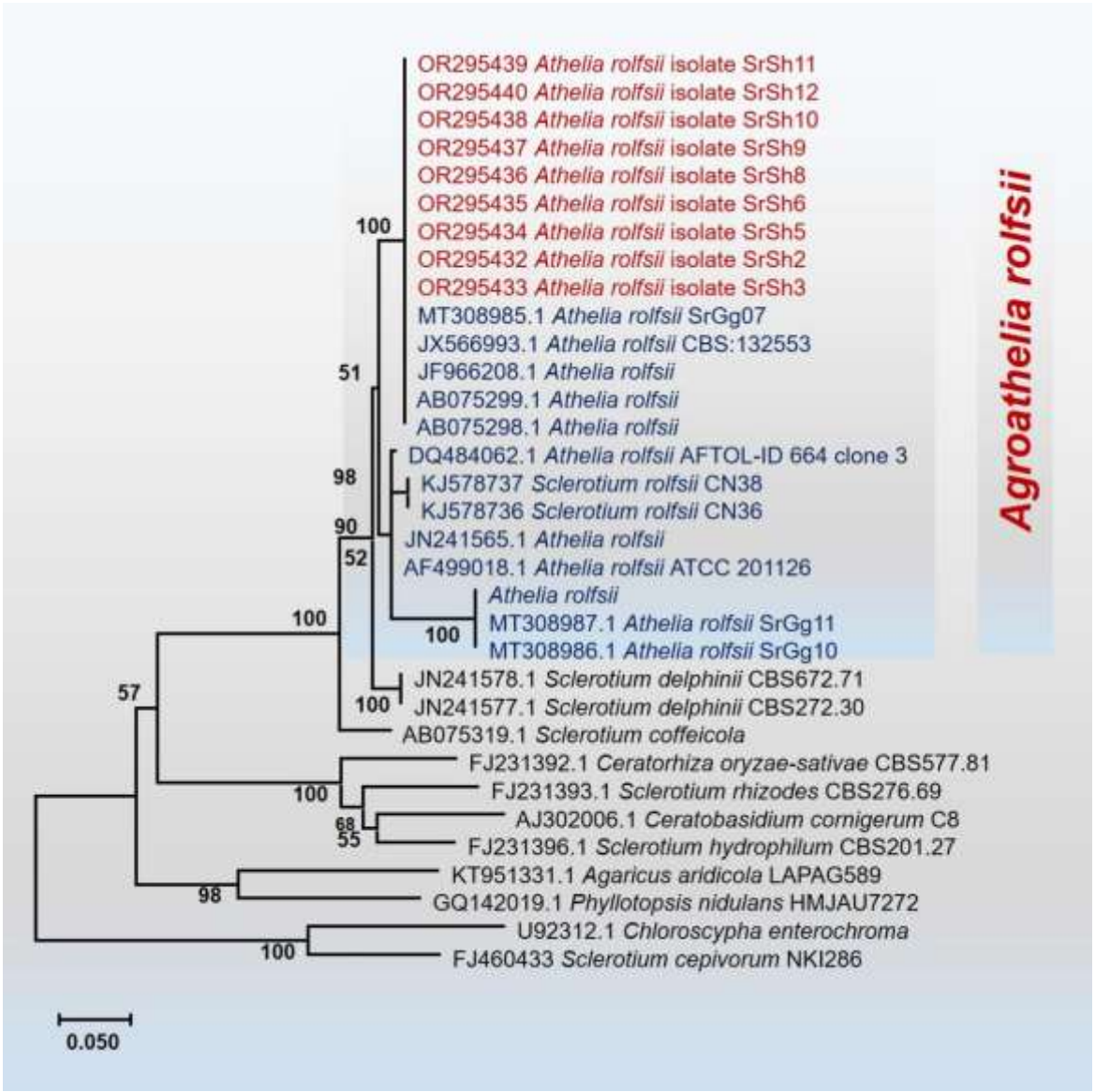

Figure 4. Phylogenetic tree built based on ITS-rDNA of *Agroathelia rolfsii* by Maximum Likelihood (ML) method showed that the sequence generated in the present study (red font) shared a common clade with the reference sequence, thus confirming the identity. The tree is rooted to *Chloroscypha enterochroma* and *Sclerotium cepivorum* (NK1286).

3.4 Pathogenicity Assay

Pathogenicity tests were conducted using the *Agroathelia rolfsii* isolate SrSh12 to confirm its ability to cause disease in chia plants. Including all replicates, a total of 60 plants were inoculated and among them, 52 plants developed characteristic foot rot symptoms within 4–5 days after inoculation, resulting in a disease incidence of 86.6%. In contrast, the control plants remained symptom-free throughout the experimental period (Fig. 5). The pathogen was successfully re-isolated from symptomatic tissues, and its identity was confirmed by its cultural and morphological characteristics, thereby fulfilling Koch's postulates.



Figure 5. Pathogenicity assay of *Agroathelia rolfsii* on chia under greenhouse conditions. (A) Healthy, non-inoculated control plants showing normal growth. (B–C) Chia plants inoculated by soil drenching with *A. rolfsii*, exhibiting severe wilting, collar rot, and plant death following infection.

4. Discussion

Agroathelia rolfsii is a destructive soil-borne pathogen with a broad host range and has been reported from numerous economically important crops worldwide (Aycock 1966; Mullen 2001; Punja 1985; Maharachchikumbura *et al.*, 2026; Mahadevakumar *et al.*, 2026). In the present study, foot rot was consistently observed in all surveyed chia-growing areas of southern Karnataka, and the pathogen was isolated from more than 90% of infected plants, indicating its widespread occurrence and strong association with the disease. The ability of *A. rolfsii* to survive as persistent sclerotia in soil enables long-term survival and serves as a primary source of inoculum, explaining its high prevalence under field conditions.

The cultural, morphological, pathogenicity, and molecular characterisation of the isolates obtained in the present study were consistent with previous reports of *Agroathelia rolfsii* infecting diverse host plants in India (Mahadevakumar and Janardhana, 2016; Tejaswini *et al.*, 2022; Aljabali *et al.*, 2025; Sampathkumar *et al.*, 2026). Previous reports of southern blight of common bean (*Phaseolus vulgaris* L.), and fruit rot of pumpkin (*Cucurbita maxima* Duchesne) reported similar morphological features (Mahadevakumar *et al.*, 2015a, b). In addition, *A. rolfsii* has been reported as the causal agent of southern blight in wild coffee

(*Psychotria nervosa* Sw.), cabbage (*Brassica oleracea* var. *capitata*), and tomato (*Solanum lycopersicum* L.), as well as foot rot of muskmelon (*Cucumis melo* L.) (Mahadevakumar and Janardhana, 2016; Tejaswini *et al.*, 2022; Aljabali *et al*., 2025; Sampathkumar *et al.*, 2026). The consistency of the present findings with previous studies provides further evidence for identifying the pathogen as *A. rolfsii* and demonstrates its extensive host range and ecological adaptability. Further, the ITS-rDNA sequence data showed the greatest similarity to the reference sequences. Several sequences reported on *Gomphrena globosa* (SrGg07, SrGg10 & SrGg11) showed similarity, and they shared a common clade.

The high disease prevalence observed during the survey has important implications for chia cultivation. As chia cultivation continues to expand due to its nutritional and economic value, southern blight may become a major production constraint, reducing plant stand, seed yield, and ultimately farmers' income if appropriate management practices are not implemented. Regular field surveillance and accurate pathogen identification are therefore essential for minimizing disease spread and economic losses.

The present study provides updated information on the occurrence and characterization of *Agroathelia rolfsii* associated with foot rot of chia in southern Karnataka. Chia is an emerging high-value crop because of its nutritional and medicinal properties, but its expanding cultivation has been accompanied by the emergence of several diseases, including southern blight, phytoplasma-associated phyllody and virescence, charcoal rot, begomovirus infection, and seed-borne fungal pathogens (Celli *et al.*, 2014; Fidelis *et al.*, 2020; Joy *et al.*, 2022, 2024a, b). As cultivation continues to expand, diseases caused by *A. rolfsii* could result in significant economic losses by reducing plant stand and seed yield. Therefore, the findings of the present study provide a foundation for developing integrated disease management strategies, including early diagnosis, regular field monitoring, field sanitation, crop rotation, the use of disease-free planting material, evaluation of resistant germplasm, and effective biological and chemical control measures, which will be essential for minimizing losses and ensuring the sustainable cultivation of chia in India.

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