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Morpho-molecular Characterization of *Agroathelia rolfsii* Causing Basal Stem Rot Disease of Cowpea (*Vigna unguiculata*) in the Semi-deciduous Forest and Transitional Zone of Ghana

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ABSTRACT

Background: Stem rot disease, caused by *Agroathelia rolfsii* (soil-borne) has increasingly become a threat to cowpea cultivation. This study, carried out in the 2023 and 2024 farming seasons, aimed to isolate and identify causal pathogen(s) of Basal stem rot from cowpea-growing districts in nine farmers' fields within Ejisu-Juaben, Ejura-Sekyedumase and kwadaso municipalities in the semi-deciduous forest zone of Ghana.

Methods: Fungal pathogens were isolated from infected cowpea plants exhibiting symptoms of Basal stem rot. The pathogen was identified as *A. rolfsii* using morphological and phylogenetic studies of the sequenced internal transcribed spacer (ITS) region.

Result: All isolates from kwadaso and ejura municipalities exhibited a pure white mycelia colour, compared to isolates from Ejisu municipality that had a dull white mycelia colouration with clamp connections. All isolates formed fluffy textured mycelia in culture. No significant variations (P= 0.736) were observed in the isolate's radial growth after the first 48 hours and 72 hours (P= 0.736) and the number of days it took to form sclerotia. The isolates differed significantly (P<0.001) in the number of sclerotia produced per plate, with a mean of 183.11. The sequenced amplicon of the PCR identified the pathogen as *Agroathelia rolfsii*.

Key words: *Agroathelia rolfsii*, Cowpea, ITS, Morphology, Phylogeny.

INTRODUCTION

Cowpea (*Vigna unguiculata*) serves as a multipurpose dietary staple across several Sahelian nations, supplying sustenance for humans and livestock while serving as a vital source of revenue for farmers and grain merchants, particularly in tropical regions. The crop is primarily cultivated for its dried grains; approximately one-quarter of the harvest is either consumed directly by farming households or sold as fresh green pods in local markets (Kebede, 2020).

The grain makes up over 80% of Ghana's cowpea production, mostly in the savannah regions (Egbadzor *et al*., 2013). According to FAOSTAT (2021), the total cultivated area of cowpea was about 149,102 (ha), total production of almost 202,735 tons with a yield (hg/ha) of 13,597 in Ghana. Although cowpea seed yields vary greatly, they are the lowest in the world, averaging 310 kg/ha (Ofosu-Budu *et al*., 2008). Despite its significance, cowpea production is limited by several factors. In Ghana, pests and diseases, inadequate soil fertility, as well as drought, are the main obstacles to cowpea production (ICRISAT, 2013). Diseases can infect cowpea at several stages, including emergence, vegetative and reproductive phases, resulting in significant plant damage and thus leading to yield loss or total production failure.

Agroathelia rolfsii (= *Sclerotium rolfsii*) has been recognized as a significant soil-borne fungus responsible for basal stem rot disease in cowpea globally. The pathogen is recognized as a cosmopolitan parasite residing in soil and is extensively distributed, especially in warm, humid climates (Adandonon *et al*., 2004). It is reported to infect tomato plants and many other crops, including common bean, soybean, potato, lentil, sesame, chili, brinjal and widely distributed in tropics, sub tropics and warm regions (Paparu *et al*., 2020; Babu and Deepika, 2022). The pathogen survives the winter and persists as sclerotia

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in soil and on infected plants or residues for an extended duration.

Historically, morphological and cultural characteristics have been used to identify *Sclerotium* at the genus level. Nevertheless, the delimitation of *Sclerotium* to the species level is the extent of these identification methods. *A. rolfsii* is separated from two of the most related species, *A. delphinii* and *A. coffeicola*, by sclerotial morphology and growth, occasionally developing morphology that is inseparable at high temperatures (Paul *et al*., 2023). Furthermore, when cultivated in the medium, isolates of *A. rolfsii* from various hosts and geographic regions usually display varying morphological traits (Paparu *et al*., 2020; Paul *et al*., 2017). However, morphology-based species delimitation is still insufficient for species-level *Sclerotium* resolution. *Agroathelia* identification problems have been successfully resolved by sequencing conserved sections or genes, such as the ITS-region, using the sequences to inform phylogenetic analysis (Paul *et al*., 2017; Xu *et al*., 2010). The development of successful management techniques to control the disease depends on the accurate identification of pathogens through both morphological and molecular investigation (Paul *et al*., 2017). Therefore, the aim of this study was to isolate and identify, *via* morphology and molecular analysis, the pathogen(s) associated with cowpea basal stem rot disease in the semi-deciduous forest zone of Ghana.

MATERIALS AND METHODS

Study area

This research was carried out in the Ejisu-Juaben, Ejura-Sekyedumase and Kwadaso Municipalities within the semi-deciduous forest zone of Ghana from 2023 to 2024. Ejisu-Juaben Municipality lies within Latitudes 1°15′N and 1°45′N and Longitude 6°15′W and 7°00′W. Ejura-Sekyedumase Municipality is located within Longitudes 1°5W and 1°39 W and Latitudes 7°9N and 7°36N. Kwadaso municipality lies within Latitude 6°41′41″N and Longitude 1°39′16″W. All the study areas experience a bimodal rainfall pattern. A non-probability convenience sampling strategy was utilized in selecting 9 farmers' fields in each study area (Ejisu-Juaben municipality, Ejura-Sekyedumase municipality and kwadaso municipality). A simple random sampling technique was applied to obtain a total of 45 infected cowpea plants exhibiting symptoms of basal stem rot.

Sample collection

A non-probability convenience sampling technique was employed to select farmers' fields and a simple random method was employed to sample infected cowpea plants with the typical symptoms of basal stem rot (leaves, stems and roots) in the selected municipalities during the 2023-2024 farming seasons (Fig 1). The disease samples were sent to the crop research institute (CSIR-CRI), Plant Pathology Laboratory in Fumesua, Ghana, where the fungus was subsequently cultured and morphologically identified.

Inoculum preparation and incubation

A modified procedure by Tanimu *et al*. (2018) was used to prepare potato dextrose agar (PDA) as the culturing medium. Sclerotia and mycelia were obtained from the infected cowpea tissues sampled in Ejusu, Kwadaso and Ejura municipalities. The sclerotia were dried and stored in Petri dishes incubated at 30°C. A sterilized scalpel was used to cut tissues of about 5 mm from infected stems and roots. The tissues were surface sterilized in 5% NaOCl for 3 minutes, rinsed in 3 exchanges of sterile distilled water, dried on sterile filter paper and placed on PDA. The plates were incubated at room temperature (28±2°C) for 4 to 7 days depending on the appearance of fruiting bodies.

Plates were incubated under Near Ultra Violet (NUV) light provided by a 4ft fluorescent tube (Philips, TLD 36 W/80). To maximize induction of sporulation and promote equal distribution of light radiation, shelves in the incubation room were supplied with two 4ft fluorescent tubes hanging horizontally, 20 cm from each other and the plates/dishes placed 40 cm from the tubes. All media plates were sealed with parafilm before incubation.

Morphological identification of fungus

The stereo binocular microscope (Leica MS5) and the compound microscope (Leica D/M 14/97) were used for fungal identification. Different magnifications of the microscopes were used for the identification of fungal colonies based on the description by Mathur and Kongsdal (2004).

Morphological characters studied included mycelial growth rates, colony morphology and pigmentation, presence or absence of septa and conidia dimensions. From one-week-old cultures, 9 mm diameter discs were



Fig 1: Signs and symptoms of stem rot disease observed on cowpea.

taken and plated on PDA and incubated at 28°C (Summerell, 2006). The mycelial radial growth was measured daily along perpendicular lines beneath the Petri dish for 7 days (Miyashira *et al.*, 2010). Cultural characteristics (colours, margin, form, elevation presence or absence of septa) were determined visually following Summerell (2006) and Watanabe (2010). The length and width of conidia produced by 5 isolates each were measured using AmScope 3.7.

Molecular characterization of fungus

Genomic DNA extraction and polymerase chain reaction and gel electrophoresis

Seven-day-old pure cultures of the isolate were obtained and genomic gDNA was extracted using the CTAB method (Doyle and Doyle, 1987). The *A. rolfsii* DNA sample was resuspended in 20 µl of Nanopure water in a 0.2 ml PCR tube plus Hotstart Taq polymerase. The nuclear rDNA ITS sections of all isolates were amplified using a pair of universal primer, namely ITS1 (5′-TCCGTAGGT GAACCT GCGGC-3′) and ITS4 (5′-TCCTCCGCT TATTGA TATGC-3′) (White *et al.*, 1990). Prime PCR machine with a temperature profile of denaturation at 94°C for 3 min (1 cycle), then annealed at 56°C and 58°C for 30 s for ITS primer. Extension at 72°C for 1 min and ended with a final extension at 72°C for 7 min (1 cycle) and 35 cycles of denaturation for 30 s. The amplicons were held at 4°C until ready to load onto the gel. The amplified PCR products were subjected to gel electrophoresis alongside a 100 bp DNA marker on 1.5% agarose gel stained with SYBR II (Garbeva *et al.*, 2001). The generated DNA bands were observed under UV light and bands were captured with a camera and cleaned up using Qiagen MinElute 96 UF PCR purification with DNA pellet dissolved in 40 µl TE buffer. The isolates were subsequently identified by sequencing (Functional Biosciences Inc, Madison WI, USA).

Sequencing of the isolates

The purified PCR products were run and analyzed on an AB13730XL DNA analyzer device. Amplified products were sequenced and nucleotides were compared with those in NCBI GenBank. Plate and sequencing procedures were carried out using the Sanger dideoxy chain termination method (Du *et al.*, 2021). Sequence alignment was conducted by comparing the ITS1 and ITS4 sequences with the NCBI sequence database using the basic local alignment search tool (BLAST) algorithm and corresponding searches were carried out in order to guarantee proper identification. Hits corresponding to *A. rolfsii* with a per cent identity greater than or equal to 93% were selected and exported as FASTA files (Du *et al.*, 2021). The resulting FASTA file was imported into R and aligned using MSA ClustalW. The *A. rolfsii* sequences of the isolates were deposited at the GenBank of NCBI, accession numbers were assigned and published on the NCBI website.

Phylogenetic tree

Phylogenetic analyses were performed using MEGA 11. The generated tree file was then imported into FigTree version 1.4.4 for visualization and final editing of the phylogenetic tree.

RESULTS AND DISCUSSION

Morphological characterization

***A. rolfsii* isolates growth rate**

The morphological identification of the isolated pathogens provides valuable information about the variability among the cowpea stem rot disease isolates from the different locations. Conventional techniques were utilized to study the morphological characteristics of 27 *A. rolfsii* isolates from Ejisu-Juaben Municipality, Ejura-Sekyedumase Municipality and *Kwadaso Municipality* based on growth rate, colony colour and texture, hyphal diameter, sclerotial shape, colour and distribution (Table 1). Phenotypically, all isolates from Kwadaso and Ejura Municipalities exhibited a pure white mycelia colour, compared to isolates from Ejisu municipality that had a dull white mycelia colouration with clamp connections (Fig 2). The pure white mycelia observed in isolates from Kwadaso and Ejura, compared to the dull white appearance of Ejisu isolates, could indicate genetic or environmental influences on pigment expression, as previously reported in fungal isolates of *A. rolfsii*

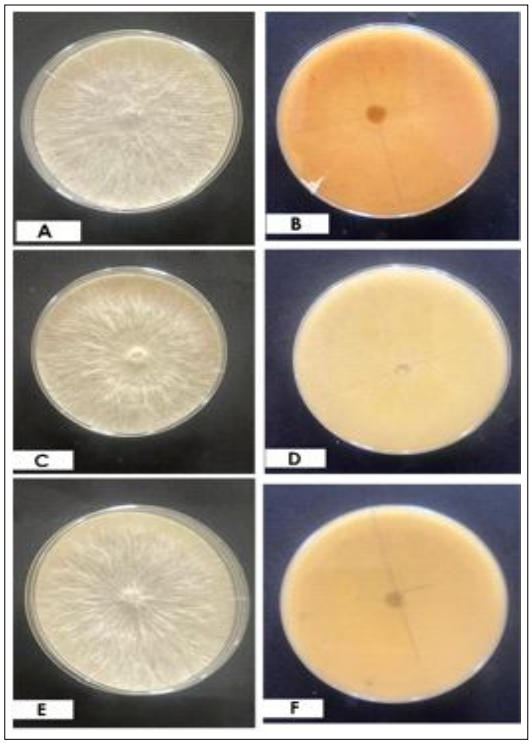


Fig 2: Morphological characteristics of *A. rolfsii* on PDA 10th DAI
Notes: Ejisu (A, B); Ejura (C, D); Kwadaso (E, F) - obverse and reverse of colonies.

(Sarma *et al.*, 2002, Ayyandurai *et al.*, 2022). All isolates formed fluffy textured mycelia in culture within the study locations, typical of basidiomycetes fungi, specifically, *A. rolfsii* (Paparu *et al.*, 2020; Ayyandurai *et al.*, 2022). Microscopic examination revealed that the mycelium was hyaline and branching, consisting of septate hyphae.

In Divyashree *et al*. (2024) considerable variation in total growth and growth rate was observed among isolates that achieved maximum radial growth (93.80 mm) by the 4th DAI. Among them, 4 isolates achieved maximum radial growth (90.00 mm) by the 3rd DAI similar to Srividya *et al.* (2020), while the remaining isolates reached this growth by the 4th DAI. The remaining isolates were categorized as slow-multiplying isolates. The least growth was observed in the isolate with (51 mm) and considered as the slowest growing isolate among the 10 isolates. Relatively in this study Regarding radial growth, no significant variations (p= 0.736) were observed in the isolates after the first 48 hours of inoculation. The mean radial growth was 58.83 mm, ranging from 56.28 mm to 57.67 mm (Table 2). Moreover, after 72 hours, the isolates did not differ significantly (P = 0.736) in their radial growth and the mean growth rose to 74.83 mm. Isolate Ejura recorded the fastest growth of 75.69 mm, which was 1.15% faster than isolate Ejisu (74.54) and 1.42% faster than isolate Kwadaso (74.27 mm). Radial growth differences observed at 72 hours further support the presence of distinct traits among the isolates. This variation in growth rate aligns with studies by Sarma *et al.* (2002) and Yaşar and Mert (2019), which demonstrated that *A. rolfsii* isolates from different environments exhibit differences in colony expansion, often linked to temperature adaptation and substrate utilization efficiency. Similarly, the significantly larger hyphal diameter in Ejisu isolates suggests a more aggressive mycelial expansion, which could influence pathogenicity and host colonization. Also, Vleugels *et al*. (2013) found a positive correlation between mycelial growth rate and isolate aggressiveness but Lehner *et al*. (2016) reported no such association. These contradictory findings indicate that mycelial growth rate may not be a reliable marker for aggressiveness of *A. rolfsii* isolates.

Similarly, significant variations (P<0.001) were observed in hyphal diameter among the isolates. The average hyphal diameter was 5.211 mm with a fold change of 0.688. Ejisu isolates (5.689 mm) were the largest, followed by Kwadaso (5.289 mm) and Ejura (4.656 mm) isolates, respectively.

Table 1: Morphological parameters of *A. rolfsii* isolates in Ghana.

Municipality	*A. rolfsii* isolates	Location	Colony colour	Colony texture	Sclerotial distribution	Sclerotial shape	Sclerotial colour
Kwadaso	Kstr 1	Kwadaso	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Kstr 2	Kwadaso	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Kstr 3	Kwadaso	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Kstr 4	Kwadaso	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Kstr 5	Kwadaso	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Kstr 6	Kwadaso	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Kstr 7	Kwadaso	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Kstr 8	Kwadaso	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Kstr 9	Kwadaso	Pure white	Fluffy	Periphery	Irregular	Dark brown
Ejisu-Juaben	Ejsu 1	Ejisu	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Ejsu 2	Ejisu	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Ejsu 3	Ejisu	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Ejsu 4	Ejisu	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Ejsu 5	Ejisu	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Ejsu 6	Ejisu	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Ejsu 7	Ejisu	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Ejsu 8	Ejisu	Pure white	Fluffy	Periphery	Irregular	Dark brown
	Ejsu 9	Ejisu	Pure white	Fluffy	Periphery	Irregular	Dark brown
Ejura-Sekyedumase	Ejra 1	Ejura	Dull white	Fluffy	All over	Irregular	Dark brown
	Ejra 2	Ejura	Dull white	Fluffy	All over	Irregular	Dark brown
	Ejra 3	Ejura	Dull white	Fluffy	All over	Irregular	Dark brown
	Ejra 4	Ejura	Dull white	Fluffy	All over	Irregular	Dark brown
	Ejra 5	Ejura	Dull white	Fluffy	All over	Irregular	Dark brown
	Ejra 6	Ejura	Dull white	Fluffy	All over	Irregular	Dark brown
	Ejra 7	Ejura	Dull white	Fluffy	All over	Irregular	Dark brown
	Ejra 8	Ejura	Dull white	Fluffy	All over	Irregular	Dark brown
	Ejra 9	Ejura	Dull white	Fluffy	All over	Irregular	Dark brown

Characteristics of sclerotia

The production of sclerotia is crucial for the survival of pathogens during winter in soil or plant waste (Paul *et al*., 2023). Variations in the quantity and dimensions of sclerotia across isolates of *A. rolfsii* have been documented (Le *et al*., 2012; Narayan *et al*., 2017; Xie *et al*., 2014). But in this study, there was no variation with coloration of the sclerotial bodies produced by all isolates. Dark brown, irregularly shaped sclerotia with a smooth, shiny surface were observed. However, in terms of sclerotia distribution, significant variations were observed in the isolates. All isolates in Ejura produced sclerotia all over the Petri dish, while isolates in Kwadaso had sclerotia at the periphery and centre of the mycelia. Isolates in Ejisu exhibited sclerotia only at the periphery of the mycelia. Kumar *et al*. (2014) indicated in his study cultural and morphological variability *A. rolfsii* Isolates infecting groundnut that sclerotia were scattered all over the plate singly or joined together, preferably at the periphery and/or centre of the petri plate. Manu *et al*. (2018) also asserted that, the peripheral region of Petri dish was mostly preferred by most of the *A. rolfsii* isolates but, isolates like Hiriyur chickpea, Bangalore groundnut, Mandya wheat and Dharwad soybean, sclerotia production was uniformly distributed all over the plate. However, the mandya ragi isolate produced its sclerotia at peripheral and also at the centre of the Petri dish.

Adhikari *et al*. (2022) observed that S. rolfsii isolates which require a longer duration to form sclerotia had a slow mycelial growth rate and had larger sclerotial size than the fast growing isolates. Kokub *et al*. (2007) and Manu *et al*. (2018) reported similar results. They found some isolates were comparatively fast growing and produced the higher number of sclerotia than the others. In the present study, no significant variations (P= 0.145) were observed among the isolates in the number of days it took to form sclerotia. On average, isolates took 4.11 days to commence sclerotia formation. Ejura isolates began sclerotia formation at 4.67 DAI, while Ejisu and Kwadaso began at 4.33 and 3.33 days, respectively (Table 3). This results agrees with Manu *et al*. (2018) when he said that the time required for sclerotial initiation was 4th day onwards in majority of isolates. Again *et al*. (2025) also reported sclerotia formation within a week of inoculation. Since no significant differences were observed in the time taken to initiate sclerotia formation, Ejura isolates displayed a significantly higher count compared to Ejisu and Kwadaso. A higher sclerotia count has been associated with increased pathogen persistence in soil, enhancing its ability to cause reinfections across cropping seasons (Xie and Vallad, 2010; Remesal *et al*., 2012). Moreover, the unique patterns of sclerotia formation suggest inherent genetic variability or responses to localised environmental conditions.

Conversely, the isolates differed significantly (P<0.001) in the number of sclerotia produced per plate. The mean number of sclerotia produced was 183.11 sclerotia. Ejura isolates produced the highest number of sclerotia (204), higher than isolates from ejisu and kwadaso by 15.7 and 47 sclerotia, respectively.

Sclerotia diameter did not differ significantly (P= 0.063) among the isolates. The average sclerotia diameter was 1.433 mm, while the highest and lowest diameters were 1.53 mm and 1.33 mm, respectively. Although no significant differences were recorded in sclerotia diameter, the overall mean diameter is consistent with reports by Kokub *et al.* (2007) and Yaşar and Mert (2019) for *A. rolfsii* isolates from cowpea and other legumes. Manu *et al*. (2018) said Bangalore groundnut isolate produced bigger sclerotial bodies (2.10 mm) followed by Dharwad soybean isolate (1.90 mm) and Hiriyur onion isolate (1.85 mm), but there was no significant difference between these isolates in his work on Morphological and cultural variability among the *A. rolfsii* isolates. Continuing, ragi isolates produce different sclerotial diameter with respect to the regions *i.e*., Bangalore isolate produce bigger sclerotia (1.40 mm) whereas, Tumkur isolate produced smaller sclerotia (1.10 mm). Chickpea isolates of Hiriyur and Bangalore and Hiriyur Cyperus isolate produced similar sized sclerotia

Table 2: Growth rate after inoculation.

Location	Radial growth at 48 hrs (mm)	Radial growth at 72 hrs (mm)	Hyphal diameter
Ejura	57.67^{a}	75.69^{a}	4.656^{a}
Ejisu	56.60^{a}	74.54^{a}	5.689^{b}
Kwadaso	56.28^{a}	74.27^{a}	5.289^{b}
LSD	3.810	4.053a	0.4387
P-value	0.736	0.736	<.001
Cv %	6.9	5.6	8.7

Table 3: Sclerotia characteristics.

Isolate	Days to sclerotia initiation	Number of sclerotia/plate	Sclerotium diameter (mm)
Ejura	4.67±0.33a	204.0±3.51c	1.33±0.03a
Ejisu	4.33±0.33a	188.3±2.33b	1.53±0.03a
Kwadaso	3.33±0.33a	157.0±1.53a	1.43±0.03a

(1.10 mm) whereas, Mandya wheat and Dharwad field bean isolates produced 1.20 and 1.30 mm sclerotia respectively. Sulladmath *et al*. (1977) and Sarma *et al*. (2002) reported the variation among the isolates of *Agroatheli rolfsii,* suggesting that, the variation among isolates depends on soil type, host crop and the environmental factors.

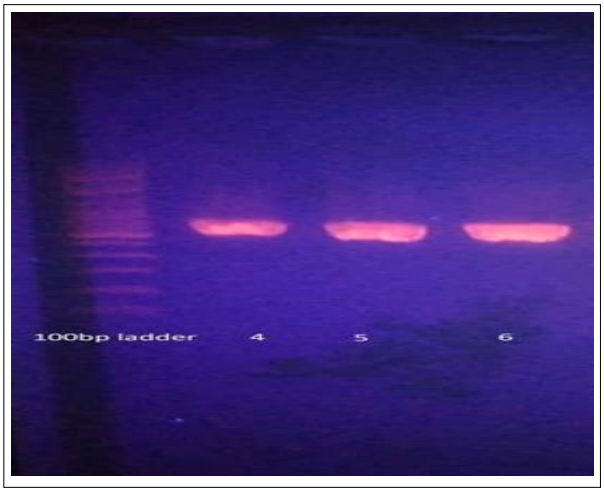


Fig 3: Gel showing PCR composite amplified product of *A. rolfsii*: 4- Kwadaso sample, 5- Ejura sample and 6-Ejisu sample, using ITS1/1TS4 primer, DNA ladder (100 bp).

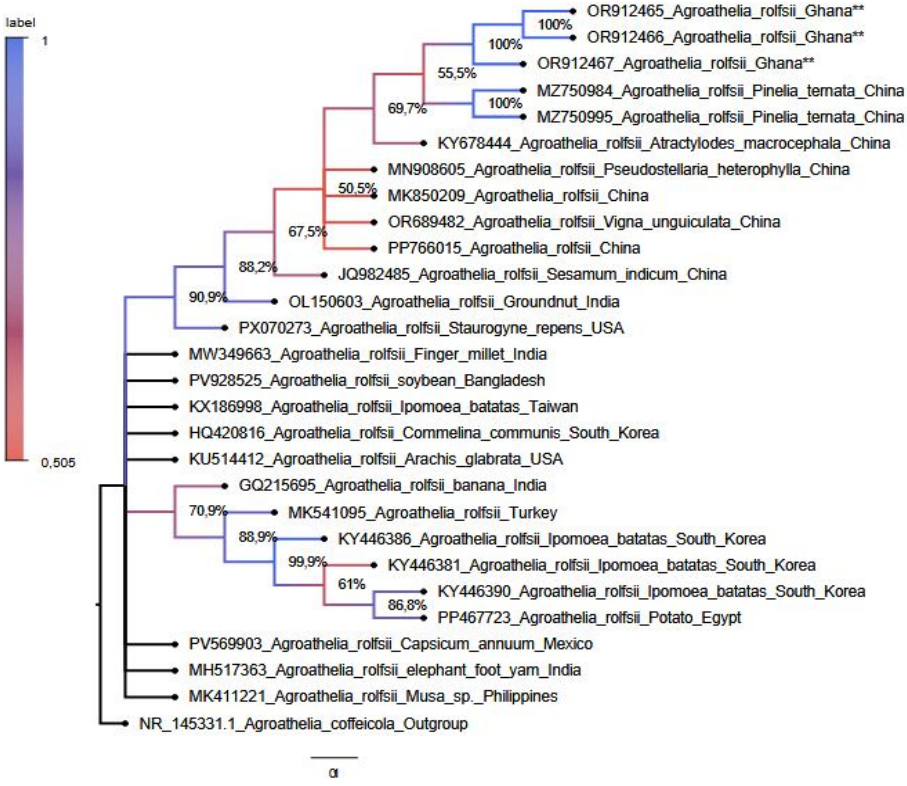
Given the observed morphological differences, it is likely that the isolates represent different strains variability of *A. rolfsii*. This hypothesis can be further validated through molecular characterisation. The variations may have direct implications for disease management, as some strains exhibit greater virulence and fungicide resistance than others.

Based on the combined morphological characteristics of white fluffy mycelia, the presence of clamp connections and the production of dark brown sclerotia, these isolates share features consistent with basidiomycete fungi in the order Athelia, particularly *Agroatheli rolfsii*, a well-documented pathogen associated with stem rot diseases in leguminous crops (Paul *et al.*, 2017; Paparu *et al.*, 2020). This morphological characterisation provides a foundation for molecular identification, which would be essential to confirm the taxonomic identity of these isolates.

Molecular characterization

Molecular identification of isolates

The molecular identification using PCR with the universal ITS primer pair successfully amplified the composite DNA



Bootstrap values are shown at the nodes. *Agroathelia coffeicola* was used as an outgroup while *Agroathelia rolfsii* isolates were grouped in the tree. Newly described isolates were indicated with the symbol (**) in the phylogenetic tree.

Fig 4: Phylogenetic tree constructed using ITS sequences from *A. rolfsii* isolates.

of all the isolates from each of the municipalities (Ejura, ejisu and kwadaso), by producing 600 bp on the gel, thereby confirming the identity of *A. rolfsii* as the main causal agent of stem end rot of cowpea in the study areas (Fig 3). This is supported by earlier research which has found that when using ITS-1 and ITS-4 primers for *S. rolfsii*, the ITS region of rDNA amplification typically yields amplicons between 600 and 700 bp (Meena *et al.*, 2023). A phylogenetic study revealed that *A. rolfsii* isolates identified in this study based on ITS sequences were closely related to a number of isolates, including *Agroathelia coffeicola*, known to infect other hosts.

Phylogenetic analysis

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987) (Fig 4). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. This analysis involved 29 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 591 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.*, 2021).

CONCLUSION

The findings in this research offer vital information into the causative agent based on detailed morphological and molecular characterizations, *A. rolfsii* was consistently identified as the primary causal agent of cowpea stem rot across all the study locations. This study is significant due to the novel finding and accurate identification of the *S. rolfsii* species associated with basal stem rot disease in cowpea in Ghana. Consequently, the findings of this investigation, given the employed methodologies, should be considered in the formulation of disease prevention and control strategies. Further research is necessary to ascertain the epidemiology of these fungal diseases and the effects of basal stem rot disease on cowpea productivity and farmers' income levels in Ghana.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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