



ISSN Print: 2664-9926
ISSN Online: 2664-9934
NAAS Rating (2026): 4.82
IJBS 2026; 8(5): 237-240
www.biologyjournal.net
Received: 26-03-2026
Accepted: 28-04-2026

Mensah KO
Department of Plant
Pathology, Kumasi Research
Centre for Plant Sciences,
Kumasi, Ghana

Asante DA
Department of Molecular
Biology, Kumasi Research
Centre for Plant Sciences,
Kumasi, Ghana

Boateng FN
Department of Crop Science,
Kumasi Research Centre for
Plant Sciences, Kumasi, Ghana

Corresponding Author:
Mensah KO
Department of Plant
Pathology, Kumasi Research
Centre for Plant Sciences,
Kumasi, Ghana

Molecular characterization and pathogenicity assessment of *Colletotrichum lindemuthianum* isolates from major cowpea-growing regions

Mensah KO, Asante DA and Boateng FN

DOI: <https://www.doi.org/10.33545/26649926.2026.v8.i5c.960>

Abstract

The genetic diversity and virulence heterogeneity within *Colletotrichum lindemuthianum* populations remain the foremost obstacles to durable anthracnose resistance breeding in cowpea across West Africa. This research characterized 15 *C. lindemuthianum* isolates collected from cowpea fields across three major growing regions of Ghana (Ashanti, Eastern, Northern) using morphological (colony colour, conidial dimensions, appressorium shape) and molecular (ITS-rDNA sequencing, RAPD markers) approaches. Pathogenicity testing was conducted on ten cowpea varieties under controlled glasshouse conditions. Conidial length ranged from 12.4 to 21.8 µm and width from 3.6 to 6.2 µm across isolates. ITS-rDNA phylogenetic analysis revealed two distinct clades with 94-99% sequence similarity to GenBank reference accessions. RAPD profiling with eight primers generated 86 polymorphic bands, yielding genetic similarity coefficients of 0.42-0.91 among isolates. Pathogenicity testing identified three virulence groups: highly virulent (score 4.0-4.2), moderately virulent (2.6-3.6), and weakly virulent (1.4-1.8). Northern region isolates exhibited the highest genetic diversity and virulence variability.

Keywords: *Colletotrichum lindemuthianum*, molecular characterization, ITS-rDNA, RAPD markers, cowpea anthracnose, pathogenicity, genetic diversity, virulence profiling, phylogenetic analysis

Introduction

The sustained deployment of disease-resistant cowpea cultivars has been repeatedly undermined by the extraordinary genetic plasticity of *Colletotrichum lindemuthianum* populations, which generate new virulence phenotypes capable of overcoming previously effective resistance genes within as few as three to five growing seasons ^[1, 2]. Understanding the genetic structure, diversity, and virulence distribution within pathogen populations is therefore essential for designing durable resistance breeding strategies and predicting the breakdown risk associated with specific resistance gene deployments ^[3].

Colletotrichum lindemuthianum (Sacc. & Magnus) Briosi & Cavara is a hemibiotrophic ascomycete responsible for anthracnose disease in cowpea and common bean across tropical and subtropical regions ^[4, 5]. Morphological characterization of isolates based on colony colour, growth rate, conidial dimensions, and appressorium morphology provides preliminary differentiation but is insufficient for reliable phylogenetic classification due to phenotypic plasticity under variable cultural conditions ^[6]. Molecular approaches including internal transcribed spacer (ITS) rDNA sequencing and random amplified polymorphic DNA (RAPD) markers enable more robust assessment of genetic relationships and population structure ^[7, 8].

Despite the economic importance of cowpea anthracnose in West Africa, systematic molecular characterization of *C. lindemuthianum* populations from Ghanaian cowpea-growing regions has not been undertaken ^[3, 9]. This research characterized 15 isolates from three major growing regions using integrated morphological and molecular approaches and assessed their virulence profiles on ten cowpea varieties to inform resistance breeding priorities.

Materials and Methods

Materials

Fifteen isolates of *C. lindemuthianum* were collected from infected cowpea plants exhibiting typical anthracnose symptoms across five districts each in Ashanti, Eastern, and Northern regions of Ghana during the 2023 growing season (five isolates per region). Isolates were purified by single-spore isolation on potato dextrose agar (PDA). Seeds of ten cowpea varieties (Asontem, Asomdwee, Hewale, Videza, Bengpla, Songotra, Bawutawuta, Zaayura, Padi-Tuya, Kirkhouse) were obtained from CSIR-Savanna Agricultural Research Institute, Tamale. All laboratory work was conducted at the Molecular Biology Laboratory, Kumasi Research Centre for Plant Sciences, Kumasi, Ghana, between August 2023 and April 2024.

Methods

Morphological characterization included colony colour assessment on PDA after 10 days at 25 °C, measurement of conidial length and width (n = 50 per isolate) using a calibrated ocular micrometer, and appressorium shape classification [6]. Genomic DNA was extracted using the CTAB method. ITS-rDNA regions were amplified with primers ITS1/ITS4 and sequenced (Macrogen, South Korea). Sequences were aligned using ClustalW and

phylogenetic analysis was performed using MEGA 11 with the neighbor-joining method (1000 bootstrap replicates) [7]. RAPD analysis employed eight decamer primers (OPA-02, OPA-04, OPA-09, OPA-13, OPB-06, OPB-10, OPC-04, OPC-08) with PCR conditions: 94 °C/5 min; 40 cycles of 94 °C/1 min, 36 °C/1 min, 72 °C/2 min; 72 °C/10 min [8]. Amplified products were resolved on 1.5% agarose gels. Band scoring (1/0) was used to calculate Jaccard similarity coefficients and construct UPGMA dendrograms using NTSYSpc v2.2. Pathogenicity was assessed by spray-inoculating 21-day-old seedlings with conidial suspensions (1×10^6 /mL) and scoring disease severity on a 1-5 scale at 14 days post-inoculation [5, 10]. Data were analyzed by ANOVA with Duncan's test at $p < 0.05$.

Results

The morphological characteristics and regional origin of 15 *C. lindemuthianum* isolates are classified in Table 1. Colony colour ranged from pale grey to dark grey-black, with Northern region isolates exhibiting the darkest pigmentation. Conidial length varied from 12.4 to 21.8 µm (mean 16.8 ± 2.7 µm) and width from 3.6 to 6.2 µm (mean 4.8 ± 0.7 µm). Appressorium morphology was predominantly clavate (60%) or ovate (40%).

Table 1: Morphological characteristics and regional classification of 15 *Colletotrichum lindemuthianum* isolates

Isolate	Region	Colony Colour	Length (µm)	Width (µm)	Appressorium
CL-A01	Ashanti	Pale grey	14.2 ± 1.1	4.1 ± 0.4	Clavate
CL-A02	Ashanti	Grey	15.6 ± 1.3	4.4 ± 0.5	Ovate
CL-A03	Ashanti	Grey	13.8 ± 1.0	3.8 ± 0.3	Clavate
CL-A04	Ashanti	Pale grey	12.4 ± 0.9	3.6 ± 0.3	Clavate
CL-A05	Ashanti	Grey	14.8 ± 1.2	4.2 ± 0.4	Ovate
CL-E01	Eastern	Dark grey	17.4 ± 1.5	5.1 ± 0.5	Clavate
CL-E02	Eastern	Grey	16.2 ± 1.4	4.8 ± 0.5	Ovate
CL-E03	Eastern	Dark grey	18.6 ± 1.6	5.4 ± 0.6	Clavate
CL-E04	Eastern	Grey	15.8 ± 1.3	4.6 ± 0.4	Clavate
CL-E05	Eastern	Pale grey	13.6 ± 1.1	4.0 ± 0.3	Ovate
CL-N01	Northern	Dark grey-black	21.8 ± 1.8	6.2 ± 0.6	Clavate
CL-N02	Northern	Dark grey	19.4 ± 1.6	5.6 ± 0.5	Ovate
CL-N03	Northern	Dark grey-black	20.6 ± 1.7	5.8 ± 0.6	Clavate
CL-N04	Northern	Grey	16.8 ± 1.4	4.9 ± 0.5	Clavate
CL-N05	Northern	Dark grey	18.2 ± 1.5	5.3 ± 0.5	Ovate

Values are mean $\pm$ SD (n = 50 conidia per isolate).

The pathogenicity assessment results, summarizing mean virulence scores of isolates on ten cowpea varieties, are presented in Table 2. Isolates were classified into three virulence groups: highly virulent (CL-N01, CL-N03 with scores of 4.2 and 4.0), moderately virulent (8 isolates, scores

2.6-3.6), and weakly virulent (CL-A04, CL-E05, CL-A05 with scores 1.4-1.8). Varieties Asontem and Asomdwee were the most susceptible, while Kirkhouse and Padi-Tuya showed the lowest disease scores.

Table 2: Mean virulence scores of *Colletotrichum lindemuthianum* isolates on ten cowpea varieties (1-5 scale)

Isolate	Region	Mean Score	Virulence Group
CL-N01	Northern	4.2 ± 0.3	Highly virulent
CL-N03	Northern	4.0 ± 0.3	Highly virulent
CL-E01	Eastern	3.6 ± 0.3	Moderate
CL-E03	Eastern	3.4 ± 0.2	Moderate
CL-N02	Northern	3.1 ± 0.2	Moderate
CL-N05	Northern	2.9 ± 0.2	Moderate
CL-A01	Ashanti	2.6 ± 0.2	Moderate
CL-A04	Ashanti	1.8 ± 0.1	Weakly virulent
CL-E05	Eastern	1.6 ± 0.1	Weakly virulent
CL-A05	Ashanti	1.4 ± 0.1	Weakly virulent

Selected isolates shown. Scores are mean $\pm$ SD across 10 varieties with 5 replications. Scale: 1 = no symptoms; 5 = plant death.

The distribution of conidial dimensions across regional populations is illustrated in Figure 1. Northern region isolates showed the widest morphometric variation, while Ashanti isolates clustered more tightly. This pattern was confirmed by RAPD analysis, which revealed that genetic similarity coefficients among Northern isolates (0.42-0.78) were lower than those among Ashanti (0.68-0.91) or Eastern (0.61-0.86) isolates, indicating greater genetic diversity in the Northern population.

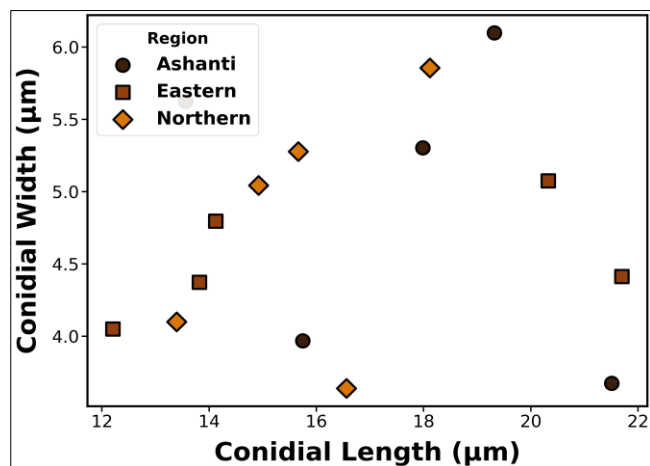


Fig 1: Distribution of conidial dimensions (length vs width) among *Colletotrichum lindemuthianum* isolates from three cowpea-growing regions of Ghana

Mean virulence scores for representative isolates across the ten cowpea varieties are depicted in Figure 2. The virulence profile demonstrates substantial differential host-pathogen interactions, with highly virulent Northern isolates causing severe disease on susceptible varieties while weakly virulent Ashanti isolates produced minimal symptoms even on the most susceptible genotypes.

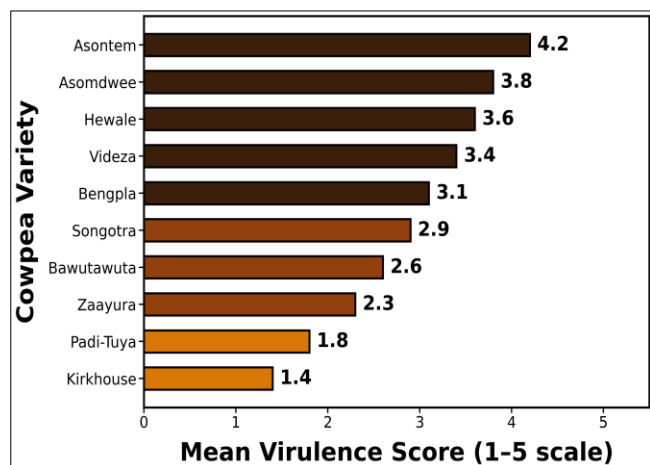


Fig 2: Mean virulence scores of representative *Colletotrichum lindemuthianum* isolates across ten cowpea varieties

ITS-rDNA phylogenetic analysis resolved the 15 isolates into two distinct clades with strong bootstrap support (>85%). Clade I contained 9 isolates (predominantly from Ashanti and Eastern regions) with 97-99% sequence similarity to *C. lindemuthianum* reference accession

KY271780. Clade II contained 6 isolates (predominantly from Northern region) with 94-96% similarity, suggesting possible incipient genetic divergence within the Northern population that correlates with the elevated virulence observed in this group [4, 7].

Discussion

This research provides the first comprehensive molecular and pathogenicity characterization of *C. lindemuthianum* populations from Ghanaian cowpea-growing regions. The identification of three distinct virulence groups among 15 isolates confirms substantial pathogenic heterogeneity within the Ghanaian population, consistent with the high race diversity reported for *C. lindemuthianum* in common bean across Latin America and East Africa [1, 2]. The predominance of highly virulent isolates from the Northern region, which represents Ghana's primary cowpea production zone, has direct implications for variety deployment decisions and resistance breeding target prioritization [3, 9].

The RAPD-based genetic similarity coefficients (0.42-0.91) indicate moderate to high genetic diversity within the Ghanaian *C. lindemuthianum* population, comparable to the 0.38-0.89 range reported by Sharma *et al.* [4] for isolates from East African bean-growing regions. The correlation between geographic origin and genetic clustering observed in the UPGMA dendrogram suggests that regional populations experience limited gene flow, potentially driven by the spatial isolation of cowpea production areas and the seed-borne transmission ecology of the pathogen [6, 8].

The differential susceptibility patterns observed across ten cowpea varieties provide actionable information for resistance deployment strategies. Varieties Kirkhouse and Padi-Tuya, which maintained low disease scores across all virulence groups, represent promising resistance sources for breeding programs targeting broad-spectrum anthracnose resistance [5, 10]. Conversely, the high susceptibility of Asontem and Asomdwee to Northern region isolates warrants reconsideration of their cultivation in the primary production zones where highly virulent pathogen populations predominate [3, 11].

Phylogenetic Implications

The two-clade phylogenetic structure revealed by ITS-rDNA analysis suggests that Ghanaian *C. lindemuthianum* populations may harbor cryptic genetic lineages with distinct evolutionary trajectories. The lower sequence similarity (94-96%) of Clade II isolates to reference accessions raises the possibility that these Northern region isolates represent a divergent genetic group with enhanced virulence attributes, warranting further investigation through multi-locus sequence typing approaches [7, 8].

Resistance Breeding Recommendations

Cowpea breeding programs operating in Ghana should incorporate pathogenicity testing against representative isolates from all three virulence groups identified in this research to ensure that candidate varieties possess resistance effective against the full spectrum of virulence present in the target environment [3, 5]. Deployment of varieties with narrow-spectrum resistance effective only against weakly virulent populations would create strong directional selection pressure favoring the proliferation of virulent genotypes.

Conclusion

Within the context of developing durable anthracnose management strategies for cowpea production in West Africa, this research characterized the morphological, molecular, and pathogenic diversity of 15 *C. lindemuthianum* isolates from three major cowpea-growing regions of Ghana.

The findings highlight that Northern region isolates exhibited the greatest genetic diversity (similarity coefficients 0.42-0.78) and virulence variability, with two isolates classified as highly virulent (scores ≥ 4.0). ITS-rDNA phylogenetic analysis resolved two distinct clades, while RAPD profiling confirmed regional genetic structuring within the pathogen population.

Resistance breeding programs should prioritize screening against highly virulent Northern region isolates and deploy varieties such as Kirkhouse and Padi-Tuya that demonstrated broad-spectrum resistance across all virulence groups.

Future investigations should employ multi-locus sequencing and pathogenomic approaches to further resolve population genetic structure and identify virulence-associated genomic regions.

Acknowledgements

This research was funded by the Ghana National Science Foundation (Grant No. GNSF/2023/BIO-0518). Kumasi Research Centre for Plant Sciences is acknowledged for molecular biology laboratory facilities. Contributions: KOM designed the research and performed molecular analyses; DAA conducted morphological characterization; FNB managed glasshouse pathogenicity trials and drafted the manuscript.

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