



Molecular and Morpho-cultural Characterization of *Colletotrichum gloeosporioides* Causing Grape Anthracnose

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Grape (*Vitis vinifera* L.) is an economically important fruit crop, and anthracnose can adversely affect vine growth, berry yield, and fruit quality. The present study characterised eleven isolates of *Colletotrichum gloeosporioides* collected from grape-growing districts of Northern Karnataka using morpho-cultural and molecular approaches. On potato dextrose agar, the isolates showed variation in colony colour, pigmentation, growth pattern, sectoring, conidial dimensions, and sporulation intensity. Colony colour ranged from white-grey to grey-white, and pigmentation varied from light orange to dark brown. Most isolates exhibited fluffy growth with regular margins, while sectoring was observed in Cg-1, Cg-3, Cg-4, Cg-5, and Cg-6. The isolates produced septate, hyaline mycelium and oblong, unicellular conidia with a single oil globule. Mean conidial dimensions ranged from $11.44 \times 5.65 \mu\text{m}$ to $15.81 \times 5.81 \mu\text{m}$, with sporulation ranging from moderate to excellent. PCR amplification of the internal transcribed spacer

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region and species-specific markers produced amplicons of approximately 550 bp and 450 bp, respectively. Sequence comparison showed high similarity with reference sequences of *C. gloeosporioides*. Phylogenetic analysis of three representative isolates placed them within the *C. gloeosporioides* clade, consistent with the morphological observations and species-specific PCR results. These findings document morpho-cultural variability among the isolates and support their molecular identification as *C. gloeosporioides*.

Keywords: Grape anthracnose; *Colletotrichum gloeosporioides*; morpho-cultural characterization; molecular characterization; internal transcribed spacer.

1. Introduction

Grape (*Vitis vinifera* L.) is an economically important fruit crop traditionally associated with temperate climates but successfully adapted to subtropical and tropical regions. Grapes are nutritionally rich, containing sugars, organic acids (tartaric and malic), vitamins (B₁ and B₂), minerals (calcium, phosphorus and iron), and anthocyanin pigments. Grapes are consumed fresh and processed into raisins, juice and wine, contributing substantially to the global fruit and beverage industries (Radha & Mathew, 2007). Globally, grape cultivation covers about 7.1 million hectares, producing approximately 77.7 million tonnes with an average productivity of 10.9 t ha⁻¹ in 2024. The leading producers include China, Italy, France, Spain, the USA, Turkey, and India (International Organisation of Vine and Wine, 2025). In India, table grape production is dominated by varieties such as ‘Thompson Seedless’ and ‘Sharad Seedless’. Approximately 85% of the country's grape-growing area is concentrated in tropical regions, with a total area of 179.63 thousand hectares, production of 3,904.29 thousand tonnes, and average productivity of 21.74 t ha⁻¹. Major grape-growing states include Maharashtra, Karnataka, Telangana and Tamil Nadu. Among these, Karnataka stands out with a production of 1,224.67 thousand tonnes from 47.12 thousand hectares, at a productivity of 25.99 t ha⁻¹ (IndiaStat, 2024).

Historically, the disease has been associated with *Elsinoe ampelina* (de Bary) Shear, with its anamorph *Sphaceloma ampelinum* (de Bary) and the synonym *Gloeosporium ampelophagum* (Pass.) Sacc. (Poolsawat et al., 2009, 2010). However, in warm, tropical and subtropical environments, anthracnose disease has also been associated with *Colletotrichum gloeosporioides* (Sawant et al., 2012). However, the sexual stage has not been consistently observed in many countries, which is possibly due to the absence of compatible mating types or the lack of harsh winter conditions that can promote ascocarp formation in grape tissues as an overwintering survival strategy (Magarey et al., 1993a; Magarey et al., 1993b).

Grape anthracnose, popularly referred to as “bird’s eye spot”, is characterised by lesions on leaves, shoots, tendrils and berries, which can result in premature defoliation, shoot damage, reduced vine growth and deterioration of berry yield and quality (Thind et al., 2004; Sompong et al., 2012). In India, anthracnose was first recorded near Pune in 1903. Later, the disease spread to Madras (Ramakrishnan, 1955), Mysore (Gandhi, 1956), and Uttar Pradesh (Prasad & Nirvan, 1965). Chatta (1992) reported anthracnose in the vineyards of Shalimar, Kashmir, noting that the disease was prevalent from May to August. Native to Europe, grape anthracnose hinders vine development and has spread globally, causing severe epidemics, particularly during warm and humid seasons (Agrios, 2005; Thind et al., 2004). Although these historical records establish the widespread occurrence of the disease, increasing grape cultivation in warm and humid environments highlights the need for accurate characterisation of the pathogens associated with anthracnose. Overlapping symptoms and close morphological similarities among *Colletotrichum* species often limit reliable identification based on morphology alone. Therefore, the study combined morpho-cultural and molecular approaches to characterise the *Colletotrichum* isolates. Such characterisation provides a reliable basis for accurate disease diagnosis and effective disease management strategies. Recent studies reinforce the value of integrating morpho-cultural observations with molecular data for *Colletotrichum* identification. Regional surveillance has documented grape anthracnose across Northern Karnataka (Mohammad Gouse et al., 2025), while phenotypic and molecular characterisation of *Colletotrichum* isolates in India has demonstrated the complementary use of culture, morphology, ITS sequencing, and phylogenetic analysis (Patil et al., 2026). Recent studies of the *C. gloeosporioides* species complex and grape-associated *Colletotrichum* likewise support combined phenotypic and molecular approaches for resolving closely related taxa (Thao et al., 2024; Amulothu & Sriram, 2026; Yin et al., 2026).

Despite this progress, integrated information on morpho-cultural variability and molecular confirmation of grape-associated *C. gloeosporioides* isolates from multiple grape-growing locations in Northern Karnataka remains limited.

Objective: The objective of the present study was to characterise eleven *Colletotrichum gloeosporioides* isolates collected from grape-growing districts of Northern Karnataka using morpho-cultural and molecular approaches and to assess the phylogenetic placement of representative isolates based on ITS sequences.

2. Materials and Methods

2.1 Collection and Designation of *Colletotrichum gloeosporioides* Isolates

A total of eleven *C. gloeosporioides* isolates were collected from different grape-growing districts of Northern Karnataka and designated as shown in Table 1.

2.2 Morpho-cultural Characterisation of *Colletotrichum gloeosporioides*

2.2.1 Isolation of the Pathogen

Diseased grape samples exhibiting typical anthracnose symptoms were collected for pathogen isolation using the tissue segment technique. Small tissue segments (~5 mm²) were excised from the margins of symptomatic lesions and surface-sterilised with 1% sodium hypochlorite for 1 min. The segments were subsequently rinsed three times with sterile distilled water, blotted dry on sterile paper towels, and aseptically transferred onto potato dextrose agar (PDA) plates. The plates were incubated at 25 ± 1°C in a BOD

incubator and monitored periodically for fungal growth and sporulation. Emerging fungal colonies were aseptically transferred to fresh PDA plates to obtain pure cultures and incubated at room temperature for 10 days.

Table 1. Details of *C. gloeosporioides* isolates collected from different grape-growing areas of Karnataka

District	Locality	Isolate code
Raichur Koppal	MARS Horticulture Garden	Cg-1
	Mataldinni	Cg-2
	Mayadhunsi	Cg-3
	Ryavanki	Cg-4
Kalaburgi	Mashal	Cg-5
	Madanhippargi	Cg-6
Bagalkot	Anaganawadi	Cg-7
	Lokpur	Cg-8
Vijayapura	Hittanahalli	Cg-9
	Babanagar	Cg-10
	Rugi	Cg-11

2.2.2 Identification of the Pathogens

The pathogen was initially characterised based on cultural and morphological features, including colony growth pattern, colour, texture, mycelial morphology, conidial characteristics, and the formation of reproductive and resting structures. Morphological observations were made using 10-day-old cultures, and conidial dimensions were measured using an image-capturing microscope. Molecular characterisation was subsequently employed as the criterion for species-level identification. Sporulation intensity was evaluated using the grading scale described in Table 2 by Vandana *et al.* (2022).

Table 2. Scoring scale used for assessing sporulation intensity of *Colletotrichum gloeosporioides* isolates

Sl. No.	Score	Grade	Conidia/microscopic field
1	++++	Excellent	>75
2	+++	Good	50 – 75
3	++	Moderate	25 – 50
4	+	Poor	1 – 25
5	-	No sporulation	-

2.3 Molecular Characterisation of *C. gloeosporioides*

Molecular characterisation of 11 *C. gloeosporioides* isolates obtained from the surveyed areas was performed using PCR-based amplification and nucleotide sequence analysis. The internal transcribed spacer (ITS) region of the ribosomal DNA was amplified using the universal primers ITS1 and ITS4, while species-specific amplification was performed using the primer pair CgInt-F and ITS4-R.

2.3.1 DNA Extraction

For genomic DNA extraction, fungal mycelia were cultured in 100 mL potato dextrose broth (PDB), inoculated with three agar plugs from 15-day-old cultures, and incubated at $25 \pm 1^\circ\text{C}$ for 10 days. The mycelia were harvested by filtration through double-layered sterile filter paper and dried between sterile filter papers under aseptic conditions in a laminar airflow cabinet. Total genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method described by Möller *et al.* (1992).

Briefly, the dried mycelium was ground to a fine powder in liquid nitrogen using a sterilised mortar and pestle. Approximately 700 μL of extraction buffer (pH 8.0; 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% PVP, and 0.5% β -mercaptoethanol) was added, and the homogenate was incubated at 60°C for 1 h in a water bath. An equal volume (700 μL) of chilled chloroform: isoamyl alcohol (24:1, v/v) was added, mixed thoroughly, and centrifuged at 10,000 rpm for 15 min at 4°C . The aqueous phase was carefully transferred to a fresh 1.5-mL microcentrifuge tube and mixed with an equal volume of pre-chilled isopropanol. The mixture was incubated at -20°C overnight to precipitate the DNA.

The DNA pellet was recovered by centrifugation at 10,000 rpm for 10 min, washed three times with 70% ethanol, air-dried, and dissolved in 50 μL TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). RNase was added (1 μL of a 20 mg/mL stock solution), and the mixture was incubated at 37°C for 45 min. The extracted genomic DNA was subsequently stored at -20°C until further analysis.

2.3.2 PCR Amplification Using ITS and Species-Specific Primers

The ITS region was amplified using the universal primers ITS1 and ITS4 in a 25- μL PCR reaction mixture (Table 3). PCR amplification was performed according to the conditions described by Blanco *et al.* (2001): initial denaturation at 94°C for 4 min, followed by 36 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1.5 min, with a final extension at 72°C for 5 min.

Species-specific amplification was performed using CgInt-F and ITS4-R in a 25- μ L reaction mixture (Table 3). The thermal cycling conditions comprised an initial denaturation at 94°C for 5 min, followed by 34 cycles of denaturation at 94°C for 60 s, annealing at 55°C for 60 s, and extension at 72°C for 1.5 min, followed by a final extension at 72°C for 5 min, as described by Chowdappa *et al.* (2009).

The PCR products were separated by electrophoresis on 1.5% agarose gels and visualised using a gel documentation system. Amplification profiles were recorded for further molecular identification and sequence analysis.

Table 3. List of primers and their sequences used for analysis of *C. gloeosporioides*

Organism	Primers	Sequences
Universal fungal ITS	ITS-1 - F	5'-AACGTTACCAAACCTGTTG-3'
	ITS-4 - R	5'-AAGTTCAGCGGTATTCCT-3'
<i>Colletotrichum gloeosporioides</i>	CgInt -F	5'-GGCCTCCCGCCTCCGGGCGG-3'
	ITS-4 - R	5'- TCCTCCGCTTATTGATATGC-3'

2.3.3 Sequencing and *in silico* Analysis

The amplified PCR products of three representative isolates were purified by gel elution and subjected to bidirectional Sanger sequencing at Medauxin Pvt. Ltd., Bengaluru, India. The forward and reverse sequences were assembled and edited using BioEdit software to generate consensus sequences. The consensus sequences were subjected to homology searches using the BLASTn algorithm available through the National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov/>). The sequences were compared with previously published sequences available in the NCBI GenBank database, and the validated sequences were submitted to GenBank for accession numbers. Multiple sequence alignment was performed using the ClustalW algorithm, and phylogenetic analysis was conducted using MEGA 11 (Raja *et al.*, 2017).

3. Results and Discussion

3.1 Morpho-cultural Characteristics of *Colletotrichum gloeosporioides*

Eleven isolates (Cg-1 to Cg-11) exhibited considerable morphological variability in colony colour, pigmentation, growth pattern, sectoring, mean conidial dimensions, and sporulation intensity. The mycelium was septate and hyaline, producing oblong, unicellular, hyaline conidia with a single oil globule. Dark brown to black, hair-like setae arose from the acervuli (Fig. 1a). The mean conidial dimensions ranged from $11.44 \times 5.65 \mu\text{m}$ in Cg-4 to $15.81 \times 5.81 \mu\text{m}$ in Cg-1. Sporulation intensity varied among the isolates, with most exhibiting good to excellent sporulation (+++ to ++++), whereas Cg-8 and Cg-11 showed moderate sporulation (++). Colony colour varied from white-grey and grey to white, while pigmentation ranged from black and white, light orange, light brown, whitish brown, and brownish white to dark brown. Most isolates exhibited fluffy growth with regular margins, whereas a few showed flat growth with regular margins. Sectoring was observed in isolates Cg-1, Cg-3, Cg-4, Cg-5, and Cg-6, whereas it was absent in the remaining isolates (Fig. 1b). These observations demonstrate considerable morphological variability among the *C. gloeosporioides* isolates examined in the present study (Table 4).

Similar morphological characteristics have been reported previously. Rite (2017) observed that *C. gloeosporioides* exhibited its highest growth rate on potato dextrose agar among the eight media evaluated, with an average growth rate of 12.15 mm/day. Bandgar *et al.* (2018) described the conidia as hyaline, unicellular, and cylindrical, measuring $10.00\text{--}12.33 \times 3.00\text{--}4.33 \mu\text{m}$. Similarly, Reecha *et al.* (2022) reported that the pathogen produces unicellular, hyaline conidia that are cylindrical to dumbbell-shaped with rounded ends.

3.2 Molecular Characterisation

Based on morphological characteristics, all 11 isolates were initially identified as *Colletotrichum gloeosporioides*. Molecular analysis further supported their identification. Amplification of the ITS region produced an amplicon of approximately 550 bp in all 11 isolates (Fig. 2a). Furthermore, all isolates produced a species-specific amplicon of approximately 450 bp with the CgInt/ITS4 primer combination (Fig. 2b).

Sequence analysis of three representative isolates showed high similarity to reference sequences of *C. gloeosporioides* available in the NCBI database (Table 5). The ITS sequences clustered closely with reference sequences of *C. gloeosporioides*, supporting the morphological and species-specific PCR-based identification of the isolates.

The present findings were consistent with previous reports. Chowdappa *et al.* (2009) and Sawant *et al.* (2012) reported an approximately 450-bp amplicon for *C. gloeosporioides* using the CgInt/ITS4 primer combination. Similarly, Bincader *et al.* (2022) identified 32 of 37 *Colletotrichum* isolates associated with mango anthracnose as *C. gloeosporioides* using the CgInt/ITS4 and CaInt2/ITS4 primer combinations, while the remaining five isolates were identified as *C. acutatum*.

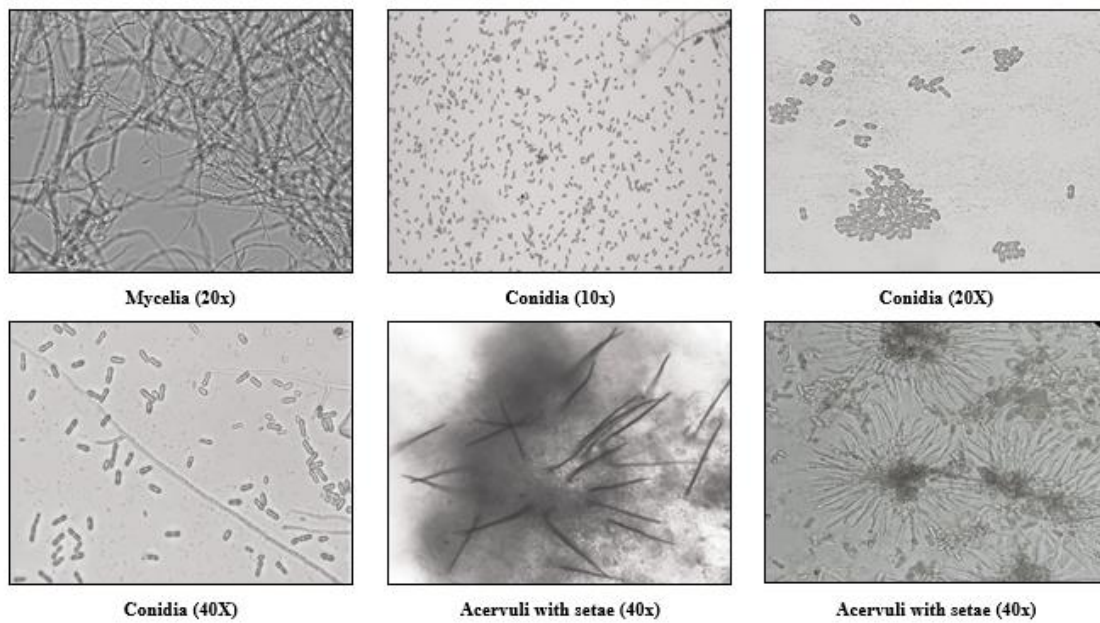


Fig. 1a. Morphological characteristics of *C. gloeosporioides*

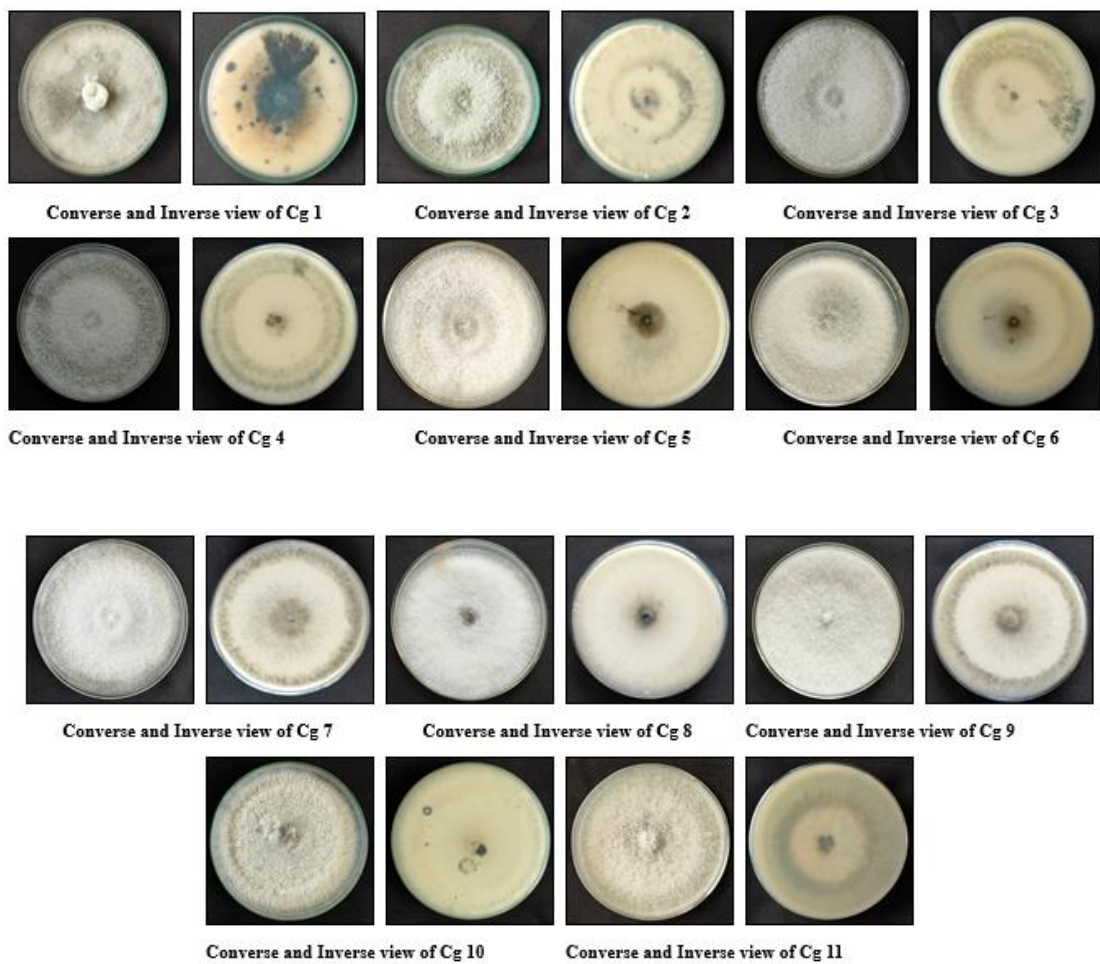


Fig. 1b. Cultural characteristics of *C. gloeosporioides* isolates on potato dextrose agar

Table 4. Morpho-cultural characteristics of *Colletotrichum gloeosporioides* isolates on potato dextrose agar

Sl. No.	Isolate	Colony colour	Pigmentation	Growth pattern	Sectoring	Mean conidial size (μm)	Sporulation*
1	Cg-1	White-grey	Black and white	Flat with regular margin	Present	15.81×5.81	++++
2	Cg-2	Grey-white	Light orange	Fluffy with regular margin	Absent	12.49×4.50	++++
3	Cg-3	Grey	Light brown	Fluffy with regular margin	Present	12.29×4.37	+++
4	Cg-4	Grey	Light brown	Fluffy with regular margin	Present	11.44×5.65	++++
5	Cg-5	White-grey	Light brown	Fluffy with regular margin	Present	13.30×4.92	+++
6	Cg-6	Grey	Light brown	Fluffy with regular margin	Present	12.92×5.13	++++
7	Cg-7	White	Whitish brown	Fluffy with regular margin	Absent	13.15×4.32	++++
8	Cg-8	White	White	Fluffy with regular margin	Absent	12.86×4.63	++
9	Cg-9	White	Brownish white	Flat with regular margin	Absent	13.63×4.64	++++
10	Cg-10	Grey-white	Light brown	Flat with regular margin	Absent	12.45×4.12	++++
11	Cg-11	Grey-white	Dark brown	Flat with regular margin	Absent	12.57×4.44	++

* +++++ Excellent, +++ Good, ++ Moderate, + Poor; (Magnification 20x)

3.3 Phylogenetic Analysis of Representative *C. gloeosporioides* Isolates Based on ITS Sequences

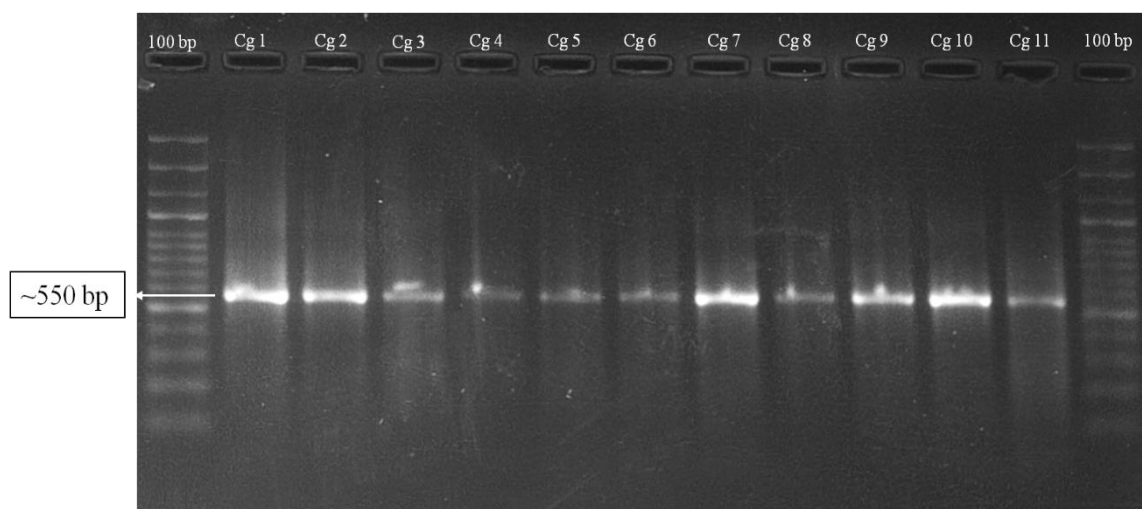
To further validate the identity of the representative isolates, we performed a phylogenetic analysis in MEGA 12 using ITS sequences and corresponding reference sequences retrieved from the NCBI database. The phylogenetic tree was constructed using the Neighbour-Joining (NJ) method based on a multiple sequence alignment generated using ClustalW.

The three representative isolates, Cg-1 (PX247503), Cg-2 (PX247504), and Cg-10 (PX247505), clustered closely with the reference isolates of *Colletotrichum gloeosporioides*, with similarity ranging from 99.35% to 100%, forming a distinct clade (Table 5, Fig. 3). The clustering pattern was consistent with the BLASTn results and further supported the identification of the three isolates as *C. gloeosporioides*. The major branches were supported by high bootstrap values (81-100%). The isolates were clearly separated from the outgroup, *Pythium insidiosum*, further supporting their phylogenetic placement within the genus *Colletotrichum*.

The phylogenetic placement observed in the present study is consistent with previous studies in which sequence-based phylogenetic analyses have been successfully used to establish the taxonomic relationships of *Colletotrichum* isolates. For instance, Santos *et al.* (2018) characterised *Elsinoë ampelina* isolates from Brazil and Australia using morphological, pathogenicity, and multilocus phylogenetic analyses based on ITS, TEF, and HIS3 sequences, with the isolates clustering into a well-supported clade. The concordance between molecular characterisation, BLAST results, and phylogenetic clustering in the present study provides additional evidence supporting the identification of the representative isolates as *C. gloeosporioides*.

Table 5. Molecular characterization and GenBank accession details of *Colletotrichum gloeosporioides* isolates causing grape anthracnose

Sl. No.	Isolate	NCBI BLAST similarity search results	Query cover (%)	ITS identity (%)	Origin	Accession number
1	Cg-1	<i>Colletotrichum gloeosporioides</i> isolate AV-184	99	100	India	PX247503
2	Cg-2	<i>Colletotrichum gloeosporioides</i> isolate AV-133	100	99.57	India	PX247504
3	Cg-10	<i>Colletotrichum gloeosporioides</i> isolate AV-143	100	99.35	India	PX247505

**Fig. 2a. PCR-amplified product of the ITS region of *C. gloeosporioides* isolates using universal primers**

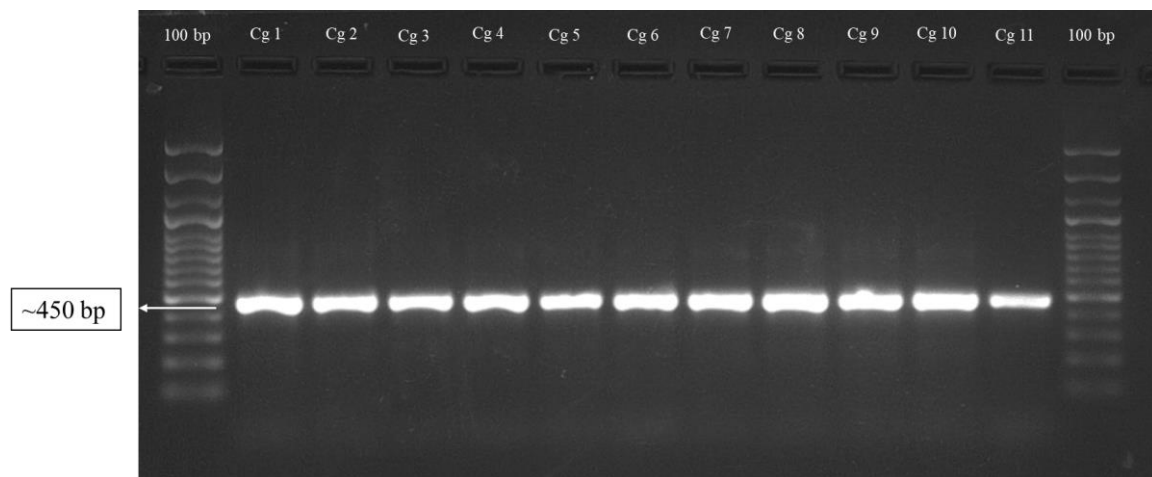


Fig. 2b. PCR-amplified product of *C. gloeosporioides* isolates using the CgInt-specific primer

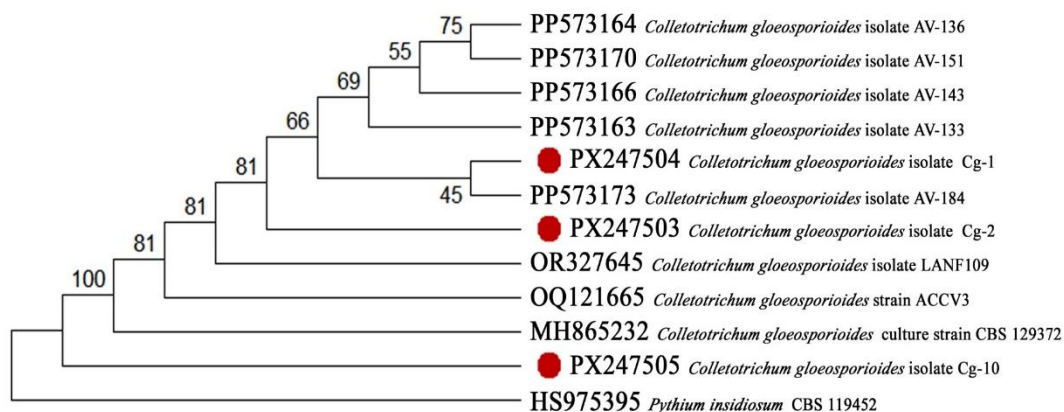


Fig. 3. Phylogenetic tree analysis of *C. gloeosporioides* isolates causing grape anthracnose with NCBI reference strains (ITS)

4. Conclusion

The study characterised eleven grape-associated *Colletotrichum gloeosporioides* isolates from Northern Karnataka through morpho-cultural assessment and molecular analysis. Considerable phenotypic variation was observed in colony colour, pigmentation, growth pattern, sectoring, conidial dimensions, and sporulation. Despite this variability, the isolates shared key morphological features, including septate, hyaline mycelium and oblong, unicellular, hyaline conidia. Molecular amplification of the ITS region produced an approximately 550-bp product, while species-specific PCR generated an approximately 450-bp amplicon across the isolates. Sequence comparison of three representative isolates showed high similarity with *C. gloeosporioides* reference sequences, and ITS-based phylogenetic analysis placed these isolates within the corresponding clade. The combined morpho-cultural and molecular results therefore support the identification of the examined isolates as *C. gloeosporioides*. The observed phenotypic variability also indicates heterogeneity among isolates from different grape-growing locations. Further investigations of pathogenicity, genetic diversity, and epidemiological significance would help clarify the biological importance of this variability and strengthen future disease-management studies.

5. Limitation

The study examined eleven isolates from selected grape-growing districts of Northern Karnataka, and molecular sequencing was conducted for only three representative isolates. Species identification relied primarily on ITS sequencing and species-specific PCR, while additional protein-coding loci were not analysed. Pathogenicity, broader population-level genetic diversity, and epidemiological behaviour were not evaluated. Accordingly, the findings describe the morpho-cultural variability and molecular placement of the isolates examined but should not be interpreted as a complete representation of the wider *C. gloeosporioides* population associated with grape anthracnose.

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Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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