

# Genomic and pathogenic insights of *Colletotrichum viniferum*, an emerging ripe rot pathogen in grapes

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## Research Article

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# Abstract

Grape ripe rot caused by *Colletotrichum* species is a significant limitation on fruit quality and yield in viticulture and requires integrative pathogen characterisation for precise diagnosis and management. Symptomatic bunches harvested during the 2025 growing season from Maharashtra (Sangli) and Karnataka (Athni) showed typical anthracnose lesions of high field severity (PDI = 50–70%). Both isolates formed circular colonies on potato dextrose agar with regular radial expansion (~ 1.01 to 1.1 cm/day), grey-pigmented aerial mycelium, conidiomata, and hyaline, aseptate, cylindrical conidia measuring 12–16 × 4.6–6.0 µm. Pathogenicity assays revealed deep necrotic lesions at seven days post-inoculation (PDI = 30–46%), and re-isolation confirmed Koch's hypothesis. ITS amplification and neighbour-joining phylogeny resolved GRR1 and GRR2 within a strongly supported *Colletotrichum viniferum* clade—whole-genome sequencing of GRR1 assembled into a 69.88 Mb draft genome with 5,670 contigs. Annotation predicted 5,158 protein-coding genes, with a focus on amino acid (184 genes), carbohydrate (89 genes), energy (50 genes) and secondary metabolic (50 genes) pathways, while ANI analysis validated species identity. Comparative orthology classified 16,151 proteins into clusters, including 1,405 lineage-specific groups and identified 50 virulence-associated proteins, 29.8% of which are potential effectors, as well as several CAZyme families (GH5, GH13, GH43) involved in host cell wall breakdown. Secretome prediction identified signal peptides in 7.85% of proteins, highlighting their potential for host interactions. This study represents the first report of *Colletotrichum viniferum* infecting grapevine in India. These combined phenotypic, phylogenetic and genomic datasets identified both isolates as *C. viniferum* and provide a quantitative framework for studying pathogenic specialisation, evolution and disease management in grapevine pathosystems.

## 1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the world's most commercially valuable perennial fruit crops, supporting extensive wine table-grape and processing industries in diverse production settings [1]. Expansion into diverse habitats demonstrates continued market demand and acknowledgement of grape polyphenols' cardioprotective, anticancer, antioxidant and antidiabetic effects [2]. However, cultivation under warm, humid conditions promotes fungal infections, which reduce yield, degrade postharvest quality and disrupt berry physiology, ultimately compromising sensory and industrial qualities [3].

Fruit rots and anthracnose remain severe constraints in viticultural regions across Asia, the Americas and Australasia, with losses often exceeding 30% and occasionally reaching 50–60% under favourable conditions [4]. Necrosis, cracking and tissue degeneration reduce both marketability and processing appropriateness [5]. Molecular analyses indicate that *Colletotrichum* species are the primary cause of ripe rot and anthracnose-like symptoms, rather than *Elsinoë ampelina*, as previously reported [6]. This genus is globally distributed, infects more than 197 plant species and has broad tissue-colonisation potential, underscoring its agricultural importance [7, 8]. Further phylogenetic refinement has revealed significant taxonomic and pathogenic diversity, underlining its role in agricultural disease systems [9].

Several *Colletotrichum* taxa, including *C. gloeosporioides*, *C. acutatum*, *C. truncatum* and *C. viniferum*, have been identified on grapevine, with species composition varying by viticultural location [4, 10]. *C. viniferum* is distinguished by vigorous berry colonisation, characterised by deep necrotic lesions and concentric acervuli [11] and subsequent research has demonstrated its pathogenicity [12]. Phenotypic plasticity, morphological convergence, and culture-dependent variability all complicate species identification in *Colletotrichum*, leading to past misdiagnoses [13]. Multilocus phylogenetic analysis has thus become critical for the reliable delimitation and detection of cryptic diversity [14], and subsequent research has reaffirmed its taxonomic importance [15]. Despite global recognition of *Colletotrichum* diversity, species-level research within Indian viticultural systems remains minimal, leaving the regional pathogen composition unclear. Notably, *Colletotrichum viniferum* has not previously been reported from India. Although breakthroughs in high-throughput sequencing have increased our understanding of evolutionary links and virulence factors, genetic resources for *C. viniferum* remain scarce. The present study characterised an anthracnose-associated isolate from symptomatic grape berries through integrated morphological, whole-genome and pathogenicity analyses. This work establishes species identity and pathogenicity of *C. viniferum* in Indian vineyards, refining pathogen inventories.

## 2. Material and Methods

### Sample collection and isolation of the associated pathogen

Grape samples were collected from a commercial vineyard in Sangli, Maharashtra, India (16°51'11"N 74°34'59"E) and Athni, Karnataka, India (16° 43' 26.70" N 75° 3' 49.83" E) during 2025. Twenty symptomatic clusters were randomly selected from each vineyard for subsequent analyses. Disease severity was determined using a 0–9 grading scale, and the per cent disease index (PDI) was calculated according to established phytopathometric protocols [16, 17]. Samples were collected in butter paper bags and carried to the laboratory without damage for further study.

Infected berries were segmented (~ 5 × 5 mm) and cut from advancing lesion borders, including adjacent healthy tissue and surface-sterilised with 1% sodium hypochlorite for one minute, then rinsed three times with sterile distilled water. The segments were blotted dry and placed on

4% Potato Dextrose Agar (PDA; HiMedia Laboratories, India). Later, plates were incubated at  $27 \pm 1^\circ\text{C}$  with a 12 h light/12 h dark photoperiod and examined daily for fungal growth. Purification of the fungal culture was performed by single-spore isolation [18, 19] and the isolates were designated as GRR1 and GRR2, respectively.

### Cultural and morphological characterisation

The isolates: colony properties (colour, pigmentation, texture and radial development) were measured from cultures grown on PDA under similar incubation conditions, with three biological replicates. A modified slide culture and the blotter paper method were used to study conidial morphology [20, 21]. Microscopic features and conidial morphometry were studied under a fluorescent microscope (EVOS M700, Invitrogen, Thermo Fisher Scientific, USA) at 60× magnification. Measurements were taken from at least 30 conidia per replicate and analysed using morphological criteria to differentiate *Colletotrichum* species [22].

### Pathogenicity assessment

Isolates were maintained on PDA for 7–10 days at  $27^\circ\text{C}$  under alternating 12 h light and 12 h dark conditions, following established *Colletotrichum* culturing protocols [23]. Conidial suspension was prepared by flooding plates with sterile distilled water, gently scraping the colony surface, filtering through sterile muslin cloth and adjusting the conidial concentration to  $10^6$  conidia/mL using a hemocytometer, following standard pathogenicity protocols [22]. To test pathogenicity, physiologically mature, healthy grape berries were surface-sterilised, then inoculated with 30  $\mu\text{L}$  of a conidial suspension using a sterile syringe. Control was injected with sterile distilled water [24]. Inoculated berries were incubated in moist chambers at  $27^\circ\text{C}$  and 90% relative humidity to facilitate infection. Ten days post-inoculation (dpi), symptom development was documented, and lesion diameters were measured to calculate the percentage disease index (PDI). The pathogen was re-isolated from symptomatic tissues and identified based on morphological characteristics and nuc rDNA ITS1-5.8S-ITS4 sequence analysis [25], thereby fulfilling Koch's postulates.

### DNA extraction, ITS amplification and phylogenetic analysis

Genomic DNA was isolated from the actively growing mycelium of both the isolates using the CTAB method as described [26]. A sterile mortar and pestle to macerate 100 mg of fresh mycelium in 1000  $\mu\text{L}$  CTAB extraction buffer (2.5% CTAB, 4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, and 0.2%  $\beta$ -mercaptoethanol; pH 8.0). DNA quality and concentration were assessed by agarose gel electrophoresis (0.8%) and a NanoDrop spectrophotometer (DeNovix, Wilmington, DE, USA). The internal transcribed spacer (ITS) region of rDNA was amplified with universal primers ITS1/ITS4 [27]. The PCR amplification procedure included an initial denaturation at  $94^\circ\text{C}$  for 5 minutes, followed by 35 cycles of  $94^\circ\text{C}$  for 60 seconds,  $55^\circ\text{C}$  for 60 seconds, and  $72^\circ\text{C}$  for 60 seconds, and a final extension at  $72^\circ\text{C}$  for 7 minutes. Amplified products were purified and Sanger sequenced at Eurofins Genomics, Bangalore, Karnataka, India.

Raw sequences obtained from the nuclear rDNA ITS1-5.8S-ITS4 region were edited and assembled into consensus sequences using MEGA 11 [28]. Preliminary identification was performed using BLASTn against the NCBI GenBank database. Taxonomic identity was further verified by cross-checking with curated fungal databases. The validated sequences were submitted to GenBank, and accession numbers were obtained. Reference sequences representing closely related *Colletotrichum* species were retrieved from GenBank based on recent taxonomic studies. Multiple sequence alignment was performed using the MUSCLE algorithm implemented in MEGA 11, followed by manual refinement to remove ambiguously aligned regions. Phylogenetic relationships were determined using the Maximum Likelihood (ML) method in MEGA 11 with the Tamura–Nei nucleotide substitution model. Bootstrap support was assessed using 1000 replicates, with values of 70% or higher indicating significant support. *Fusarium verticillioides* isolate PMER1 served as the outgroup. Species delimitation relied on multilocus phylogenetic placement, genealogical concordance across loci, and clustering with verified and ex-type reference strains.

### Whole-genome sequencing

Genomic DNA from isolate GRR1 was utilised to generate a paired-end sequencing library using Illumina protocols, including fragmentation, end repair, A-tailing and indexed adapter ligation. The library concentration and size distribution were assessed using a Qubit 4.0 fluorometer (Invitrogen, Thermo Fisher Scientific, USA) and an Agilent Bioanalyzer. To obtain raw reads, the Illumina NovaSeq 6000 platform was used with a  $2 \times 150$ -bp paired-end protocol. Raw FASTQ readings were analysed with FastQC v0.11.9 [29] and trimmed with Fastp v0.23.4 (-trim\_poly\_g -cut\_front -cut\_tail -average\_qual 30) [30]. Reads shorter than 50 bp were deleted, while filtered reads were re-evaluated using FastQC. K-mer distributions of filtered reads were created using KMC v3.0 [31] with the parameters -k21 -m64 -ci1 and the genome size was estimated using GenomeScope v2 [32]. *De novo* assembly was performed using the AATF pipeline [33] followed by polishing with ntEdit v2.0.3 [34]. Repetitive sections were detected and masked with RedMask.

QUAST v5.0.2 [35] was used to compute assembly statistics such as contig distribution, N50, GC content and assembly size, while BUSCO v5 [36] was used to evaluate genome completeness. The results were shown using BlobToolKit [37]. The ITS region was extracted using ITSx v1.1.2 [38] and species identification was validated using MegaBLAST/BLASTN [39].

The GALBA pipeline [40] was used to predict genes from protein evidence for *Colletotrichum* species obtained from NCBI. Protein alignments were performed with Miniprot, proteins were classified with Miniprothint and gene models were predicted with AUGUSTUS [41]. DIAMOND BLASTx v2.0.6 [42] was used to annotate predicted coding sequences against the *Colletotrichum* NR database and the results were processed using Blast2GO [43]. The domain definition was revised using InterProScan v5 [44] and the KEGG orthology assignments were obtained using KAAS/KofamScan [45]. GAEV was used to visualise integrated annotations.

The average nucleotide identity between the GRR1 genome and related reference genomes was determined using pyANI [46]. OrthoVenn3 to perform orthologous clustering of GRR1 and representative *Colletotrichum* species. Comparative assembly statistics were calculated using publicly accessible genomes. DIAMOND BLASTx searches against the Database for Fungal Virulence Factors (DFVF) were used to identify virulence-associated genes and Circos plots generated in TBtools were used to display important scaffolds [47]. Effector candidates were predicted from the annotated proteome with EffectorP v3.0 [48].

#### Statistical analysis:

PDI values obtained from pathogenicity studies were analysed using analysis of variance (ANOVA) with a completely randomised design. Mean comparisons were performed at  $P < 0.05$  [49]. Bootstrap resampling (1000 replicates) was used to assess the reliability of the phylogenetic tree. Bootstrap values  $\geq 70\%$  indicated significant clade support.

### 3. Results

#### Symptomatology

Typical anthracnose (ripe rot) symptoms were observed on grape berries collected during the 2025 growing season from Sangli, Maharashtra, and Athni, Karnataka (Fig. 1), where initial infection appeared as irregular brown-to-black lesions with distinct dark margins on the berry surface; as the disease progressed, lesions enlarged and coalesced, leading to tissue maceration and eventual collapse of infected berries, and in advanced stages, subepidermal acervuli developed that ruptured the epidermis to release characteristic salmon conidial masses, confirming typical anthracnose symptomatology; field severity assessment revealed a per cent disease index (PDI) ranging from 50–70% and two representative isolates were obtained, namely GRR1 with a PDI of 58–70% and GRR2 with a comparatively lower PDI range of 50–56%, selected for further characterization.

#### Morphological and cultural characterisation

The isolates cultured on potato dextrose agar (PDA) produced colonies with consistent circular growth, regular margins, and uniform radial expansion (Fig. 2A and B), with mean growth rates of  $1.10 \pm 0.04$  cm/day for isolate GRR1 and  $1.03 \pm 0.03$  cm/day for isolate GRR2; the mycelium was initially white and cottony with profuse aerial growth, later turning grey. Conidiomata were observed on the colony surface (Fig. 2C). Microscopic examination revealed hyaline, aseptate conidia that were cylindrical with rounded ends, thin-walled and guttulate, measuring  $12\text{--}16 \times 4.6\text{--}6.0$   $\mu\text{m}$  (Fig. 2F). Conidiogenous cells were present within the conidiomata (Fig. 2D and E). In contrast, setae were absent. These cultural and morphological characteristics are consistent with published descriptions of the genus *Colletotrichum*.

#### Pathogenicity assessment

The isolate GRR1 exhibited initial symptom development on grape berries four days after inoculation, characterised by localised browning at the inoculation site, which progressed by 7 dpi into irregular, sunken, necrotic lesions with pronounced tissue deterioration, closely resembling field symptoms (Fig. 3B); under identical conditions, GRR2 followed a similar infection and symptom progression (Fig. 3C). Control berries inoculated with sterile distilled water remained symptom-free (Fig. 3A). The experiment, conducted with three independent replications, consistently reproduced symptoms across treatments. Disease severity assessment revealed a per cent disease index (PDI) of 35–46% for GRR1, while GRR2 exhibited a comparatively lower PDI range of 30–37%, indicating moderate infection levels. The pathogen was successfully re-isolated from symptomatic tissues, and its identity was confirmed based on concordant cultural, morphological, and ITS rDNA sequence characteristics with the original isolates, thereby fulfilling Koch's postulates and confirming both GRR1 and GRR2 as the causal agents of anthracnose in grape berries.

#### Molecular characterisation

The ribosomal Internal Transcribed Spacer (ITS) region from both isolates was successfully amplified, yielding approximately 550 bp amplicons, and the sequences were deposited in GenBank under accession numbers PX676537 and PZ326989, respectively, ensuring data accessibility and reproducibility. A neighbour-joining analysis in MEGA11 was performed to infer phylogenetic relationships based on aligned ITS sequences of representative taxa from the *Colletotrichum gloeosporioides* species complex (CGSC), with 1,000 bootstrap replicates to assess clade robustness. Phylogenetic analysis based on sequence comparison placed both grape-derived isolates, GRR1 and GRR2, within the *Colletotrichum* clade, clearly distinguishing them from the outgroup taxon, *Fusarium verticillioides*, and confirming the appropriate rooting of the

tree. The topology revealed that both isolates clustered within a well-supported monophyletic lineage corresponding to *Colletotrichum viniferum*, grouping with reference isolates WK2-1, WK1-1, MMT1, CNU171001, JF2, and col-1 (Table 1), with strong bootstrap support values of 97% for GRR1 and 96% for GRR2. The short branch lengths within this cluster indicated low genetic diversity and close evolutionary relatedness among members. The *C. viniferum* clade was clearly distinct from neighbouring clades comprising closely related taxa such as *C. siamense*, *C. gloeosporioides*, and *C. tropicale*, highlighting its evolutionary separation within the CGSC (Fig. 4). More distantly related taxa, including *C. truncatum* and other *Colletotrichum* species, formed separate, well-supported lineages, further supporting the overall tree topology and confirming the placement of GRR1 and GRR2 within *Colletotrichum viniferum*.

Table 1  
Sequences of *Colletotrichum* species complex used in the phylogenetic analysis and GenBank accession numbers of respective strains.

| Sl. No. | Organism                                                 | Accession number |
|---------|----------------------------------------------------------|------------------|
| 1       | <i>Colletotrichum theobromicola</i> isolate K3           | LC800443         |
| 2       | <i>Colletotrichum kahawae</i> isolate CBS:125394         | MH863503         |
| 3       | <i>Colletotrichum grevilleae</i> isolate PGCo1           | ON849040         |
| 4       | <i>Colletotrichum fragariae</i> isolate 6846             | HQ188923         |
| 5       | <i>Glomerella cingulata</i> isolate L66                  | DQ185443         |
| 6       | <i>Colletotrichum grossum</i> isolate CAU G7             | NR_152283        |
| 7       | <i>Colletotrichum gloeosporioides</i> isolate CBS 112999 | LC383994         |
| 8       | <i>Colletotrichum gloeosporioides</i> isolate BM6        | MT476841         |
| 9       | <i>Colletotrichum siamense</i> isolate CDH214            | PX765640         |
| 10      | <i>Colletotrichum siamense</i> isolate CBS125379         | PQ169446         |
| 11      | <i>Colletotrichum viniferum</i> isolate WK2-1            | PQ217135         |
| 12      | <i>Colletotrichum viniferum</i> isolate WK1-1            | PQ217131         |
| 13      | <i>Colletotrichum viniferum</i> isolate CNU171001        | MG182340         |
| 14      | <i>Colletotrichum viniferum</i> isolate JF2              | PQ217123         |
| 15      | <i>Colletotrichum viniferum</i> isolate col-1            | OM638721         |
| 16      | <i>Colletotrichum tropicale</i> isolate CBS 124949       | MW492604         |
| 17      | <i>Colletotrichum truncatum</i> isolate BJY-1            | ON102792         |
| 18      | <i>Colletotrichum truncatum</i> isolate CBS 125469       | MH863694         |
| 19      | <i>Fusarium verticillioides</i> isolate PMER1            | PX567842         |

### Whole genome sequencing

Whole-genome sequencing of *C. viniferum* isolate GRR1 (Bioproject-PRJNA1402739 and Biosample ID- SAMN54603521) yielded 21.73 million paired-end reads (3.26 Gb), with 19.65 million high-quality reads (2.36 Gb; Q30) retained after preprocessing and used for *de novo* assembly. The polished assembly comprised 5,670 contigs totalling 69.88 Mb, with a maximum contig length of 318,670 bp. The GC content was 46.36%, consistent with filamentous ascomycetes. Assembly continuity metrics revealed N50 = 57,474 bp, L50 = 350 bp and N90 = 6,682 bp (L90 = 1,563 bp), indicating moderate fragmentation but adequate genome coverage. Genome completeness assessment using BUSCO (fungi\_odb10) yielded 99.9% complete orthologs, with low fragmentation (0.1%) and duplications (0.4%), indicating a nearly complete haploid assembly suitable for downstream studies (Table 3).

Table 3  
Assembly statistics of the reference-based  
scaffolded genomes

| Parameter                             | GRR1       |
|---------------------------------------|------------|
| Contigs                               | 5670       |
| Number of contigs ( $\geq 0$ bp)      | 18,557     |
| Number of contigs ( $\geq 1,000$ bp)  | 3,725      |
| Number of contigs ( $\geq 5,000$ bp)  | 1,785      |
| Number of contigs ( $\geq 10,000$ bp) | 1,300      |
| Number of contigs ( $\geq 25,000$ bp) | 797        |
| Number of contigs ( $\geq 50,000$ bp) | 417        |
| Largest contig (bp)                   | 3,18,670   |
| Total length (bp)                     | 69,881,983 |
| Total length ( $\geq 0$ bp)           | 73,606,962 |
| Total length ( $\geq 1,000$ bp)       | 68,563,124 |
| Total length ( $\geq 5,000$ bp)       | 64,172,710 |
| Total length ( $\geq 10,000$ bp)      | 60,728,700 |
| Total length ( $\geq 25,000$ bp)      | 52,520,504 |
| Total length ( $\geq 50,000$ bp)      | 38,532,419 |
| GC (%)                                | 45.89      |
| N50 (bp)                              | 57,474     |
| N90 (bp)                              | 6,682      |
| L50 (bp)                              | 350        |
| L90 (bp)                              | 1,563      |
| # N's per 100 kbp                     | 9.12       |
| Complete BUSCOs (C)                   | 99.9%      |

The GALBA pipeline, which uses evidence-based structural annotation, predicted 5,158 protein-coding genes. Functional classification using KEGG mapping found enrichment in basic metabolic categories, specifically amino acid metabolism (184 genes), carbohydrate metabolism (89), energy metabolism (50) and secondary metabolite pathways (50), indicating a physiologically flexible genome (Fig. 6). Average Nucleotide Identity (ANI) comparisons further resolved GRR1 within *C. viniferum*, showing strong genomic coherence with reference strains and clear separation from related taxa such as *C. truncatum* and *C. graminicola*, confirming species identity at whole-genome resolution (Fig. 5).

A comparative orthology analysis of five related *Colletotrichum* genomes identified 16,151 predicted proteins in orthologous clusters, including a conserved core of 8,720 clusters shared across all taxa and 1,405 GRR1-specific clusters, representing lineage-specific gene content (Fig. 7). Cluster-sharing patterns reveal close evolutionary affinity within the *C. gloeosporioides* complex, suggesting adaptive specialisation related to host contact or environmental persistence. Despite assembly fragmentation, the GRR1 genome size (69.88 Mb) is larger than that of most congeners ( $\approx 50$ –63 Mb) (Table 2), indicating a relative increase while preserving outstanding gene-space completeness.

Table 2  
Comparative genome statistics of *Colletotrichum viniferum* GRR1 and related *Colletotrichum* Species

| Features               | <i>C. viniferum</i> (GRR1) | <i>C. viniferum</i> | <i>C. siamense</i> | <i>C. gleosporioides</i> | <i>C. higginsianum</i> | <i>C. fructicola</i> | <i>C. aenigma</i> | <i>C. scoville</i> | <i>C. spaethianum</i> |
|------------------------|----------------------------|---------------------|--------------------|--------------------------|------------------------|----------------------|-------------------|--------------------|-----------------------|
| Genome size (Mb)       | 69.88                      | 73.41               | 55.96              | 62.78                    | 53.40                  | 56.80                | 59.20             | 52.03              | 50.91                 |
| GC (%)                 | 46.36                      | 40.07               | 52.67              | 50.12                    | 54.6                   | 53.45                | -                 | 51.8               | 51.1                  |
| # Contigs              | 5669                       | 70                  | 235                | 28                       | 28                     | 510                  | 79                | 16                 | 84                    |
| Largest contigs (bp)   | 3,18,670                   | 6,450,000           | 2,573,469          | 7,950,879                | 4,980,000              | 12,84,201            | -                 | 6,315,016          | 33,03,556             |
| N50 contig length (bp) | 57,474                     | 2,100,000           | 9,27,217           | 5,444,682                | 5,060,000              | 2,85,441             | 51,81,253         | 4,871,440          | 11,20,470             |
| BUSCO (%)              | 99.9                       | -                   | 99.6               | 99.3                     | 99.0                   | 99.6                 | 99.7              | 98.6               | 92.6                  |
| Reference              | This study                 | [63]                | [64]               | [65]                     | [66]                   | [67]                 | [68]              | [69]               | [70]                  |

DFVF homology searches yielded 50 candidate virulence-associated proteins, including polyketide synthases, chitin synthases, chitinases, ABC transporters, regulatory signalling components (adenylate cyclase, Ste12-like TF, histidine kinase) and secondary-metabolism enzymes such as NRPS. These characteristics support conserved pathogenicity processes, including toxin production, cell wall remodelling, host signalling regulation and stress adaptation (Fig. 8). Effector prediction identified 29.8% of the examined secreted proteins (n = 477) as potential effectors, most of which were cytoplasmic, suggesting that intracellular host targeting is the preferred strategy.

Consistent with phytopathogenic adaptation, the genome encodes a diverse CAZyme repertoire dominated by glycoside hydrolases (GH5, GH13, GH43), auxiliary activity enzymes and carbohydrate esterases, as well as AA3 oxidoreductases and AA9 lytic polysaccharide monooxygenases involved in oxidative cellulose degradation. These enzymatic additions demonstrate strong potential for plant cell wall breakdown and nutrient uptake. Secretome investigations projected signal peptides in 7.85% of proteins, with cleavage sites common to eukaryotic secretion pathways. At the same time, complementary modelling revealed significant subsets of transmembrane and secretory proteins, emphasising complex host-interface and transport capabilities.

Overall, genome architecture, orthology patterns, functional annotations, and virulence-associated gene inventories indicate that GRR1 is a genomically complete and metabolically competent member of *C. viniferum*, with molecular characteristics consistent with pathogenic specialisation and host interaction within the CGSC.

## 4. Discussion

*Colletotrichum* species are significant phytopathogens affecting a wide range of crops, including grapevine, owing to their broad host range and tissue-colonisation potential [18]. Their epidemiological success contributes to substantial economic losses from ripe rot and anthracnose in viticulture [50]. Several species, including *C. acutatum*, *C. aenigma*, *C. fructicola*, *C. karstii*, and *C. viniferum*, have been linked to grape ripe rot worldwide [22], and the discovery of *C. viniferum* here broadens its known geographic range.

The cultural phenotype of both isolates is consistent with the diagnostic growth dynamics of *Colletotrichum*, in which circular colonies with uniform radial expansion on PDA indicate vegetative stability within the *C. gleosporioides* complex [18]. Pigmentation ontogeny from white aerial mycelium to grey tones with reverse zonation represents sporulation-linked developmental changes, whereas surface conidiomata suggest active asexual differentiation employed in morphological systematics [51]. Cylindrical, hyaline, aseptate conidia with rounded termini and appropriate morphometrics are consistent with grape-associated *Colletotrichum* species, while guttulation with thin walls indicates dispersal-adaptive propagule [12].

Conidiogenous placement inside conidiomata and the absence of setae are compatible with the conventional reproductive architecture and intra-complex [18]. These morphocultural traits support placement within the *C. gleosporioides* complex; however, pronounced morphological plasticity necessitates validation of pathogenicity and molecular inference, with symptom reproduction and pathogen re-isolation fulfilling Koch's postulates and reflecting hemibiotrophic infection dynamics [52]. Reproducible symptom induction and disease-free controls confirm pathogenic competence, consistent with the hemibiotrophic infection behaviour described for *Colletotrichum* spp. [53]. The moderate-severity

range represents the typical aggressiveness of grape ripe-rot pathogens under controlled inoculation regimens [13]. Re-isolation that meets Koch's postulates etiologically verifies GRR1 as the causal agent, according to conventional pathogen verification frameworks in plant pathology [52]. As a result, phenotypic concordance provides significant genus-level corroboration while emphasising the importance of phylogenetic resolution for definitive species delimitation in conserved *Colletotrichum* lineages [5]. ITS-based phylogenetic placement within a supported *C. viniferum* clade confirms species identity and aligns with established taxonomic frameworks [22].

Whole-genome sequencing provided functional and evolutionary context. The assembly quality and ortholog completeness were comparable to those of previously reported *Colletotrichum* genomes, indicating analytical reliability [36, 54]. Genome size and composition suggest lineage-specific plasticity linked to ecological adaptation rather than host range contraction [55]. Functional pathway enrichment indicates metabolic diversity, a characteristic of hemibiotrophic infections [18], whereas ANI coherence reinforces species placement within *C. viniferum* [56, 57]. Ortholog patterns indicating conserved core architecture and lineage-specific gene complements are consistent with concepts of adaptive evolution in filamentous pathogens [54].

Virulence-associated genetic markers provide further context for pathogenicity [58]. Detection of PKS/NRPS clusters, transporters and regulatory elements shows common infection architectures that mediate secondary metabolite synthesis and stress tolerance [59]. Secretome enrichment and effector prediction indicate potential immune-modulation mechanisms in *Colletotrichum* that are consistent with host-pathogen interaction models [57, 60]. In contrast, CAZyme repertoires support plant cell wall degradation and nutrient acquisition [61]. Signal peptide-associated and membrane-linked proteins reveal additional host-interface communication and transport pathways unique to filamentous phytopathogens [54].

GRR1 and GRR2 are the pathogenic representatives of *C. viniferum*, according to integrated phenotypic, phylogenetic, and genomic evidence, providing mechanistic insight into the aetiology of grape ripe rot and a genomic framework for investigating virulence evolution and host adaptation within the *Colletotrichum* lineage [62].

## 5. Conclusion

This study identifies *C. viniferum* as the causative agent of grape ripe rot. It provides an integrative phenotypic-to-genome-scale characterisation of *C. viniferum* found in grape berries in this region. Congruent symptomatological, morpho-cultural, pathogenicity, phylogenetic and genomic evidence provide a robust multi-tier diagnostic framework for resolving taxa within the *C. gloeosporioides* complex, thereby increasing taxonomic confidence in morphologically conserved lineages. Functional genomic insights reveal virulence-associated repertoires and metabolic plasticity compatible with pathogenic specialisation, thereby broadening our biological and genomic understanding of this species. This study improves comparative and epidemiological frameworks for investigating pathogen evolution and infection dynamics by expanding genomic data for agriculturally relevant fungi. Overall, these findings advance monitoring, resistance breeding and precision disease management strategies, underscoring the importance of integrative genomic plant pathology for long-term crop security.

## Declarations

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### Declaration of Competing Interest

The authors declare that there is no conflict of interest

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### Author contributions

**K N Pallavi**- Investigation, Methodology, Conceptualisation, Data curation, Formal analysis, **M K PrasannaKumar**- Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, **R Karan, J Harish, Keerti Sharma, Kiran B M**- Data curation and Formal analysis, **C Manjunatha, Rame Gowda H V, Pramesh D, Nithish, Ankit, Swathi S Patil**– Formal analysis.

### Data availability

Data will be made available on request.

### Statement of ethical approval

This study did not require ethical approval as it did not involve human participants, animals, or sensitive data.

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## Figures

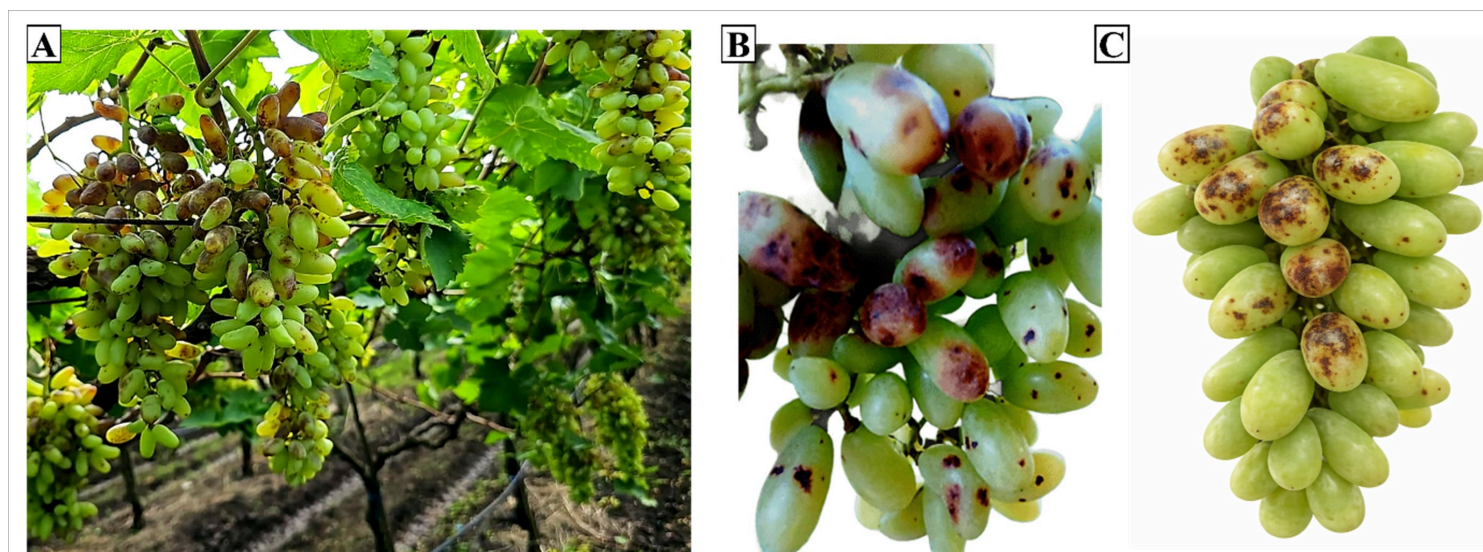


**Figure 1**

(A) Sample collected for pathogen isolation; close view of ripe rot symptoms of the sample from (B) Maharashtra and (C) Karnataka.

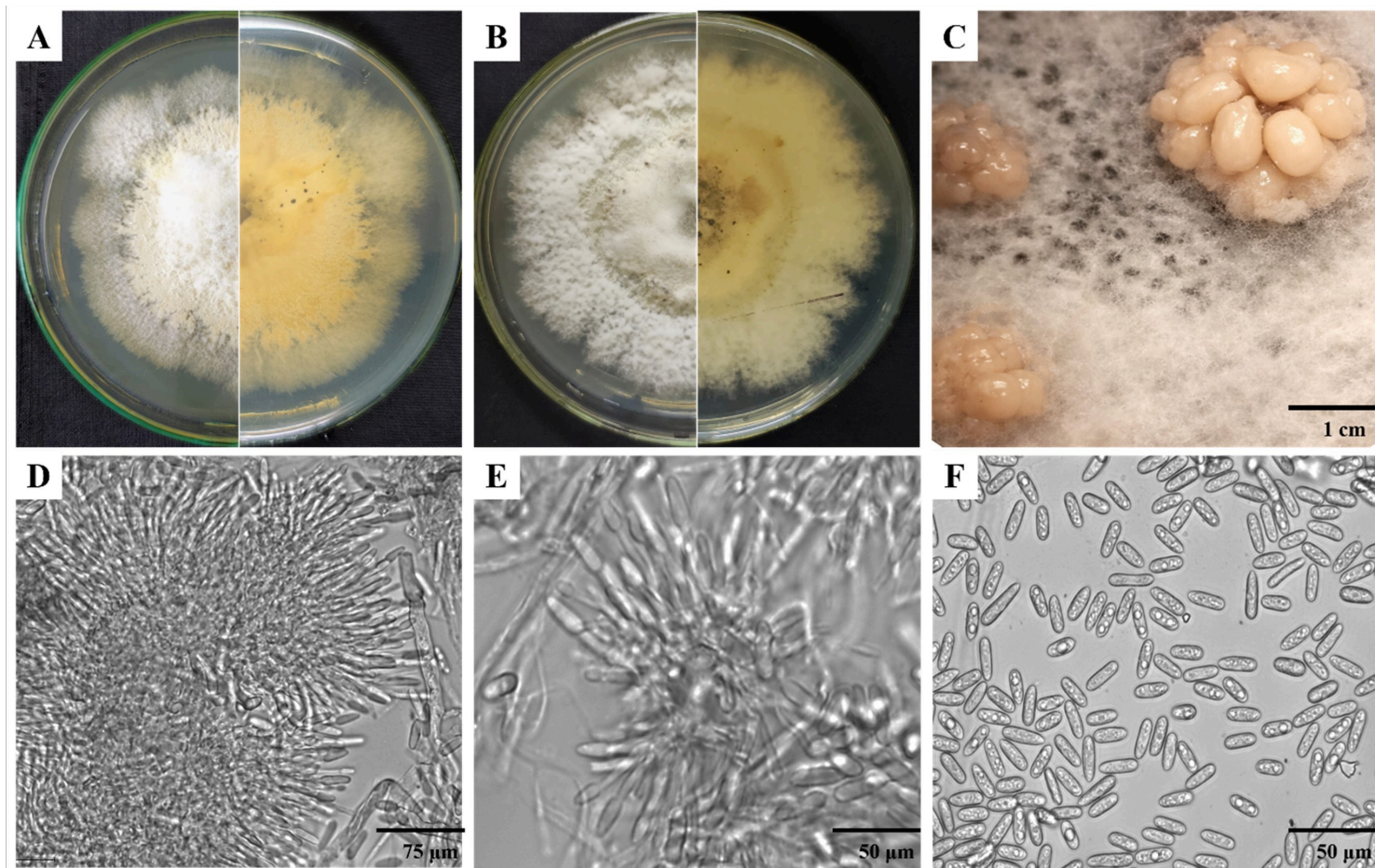


**Figure 2**

Morphology of the isolated fungus: Colony obverse and reverse (7 days old) of (A) GRR1 isolate (B) GRR2 isolate, (C) Conidiomata, (D) Acervulus, Closer view (E) and (F) conidia.

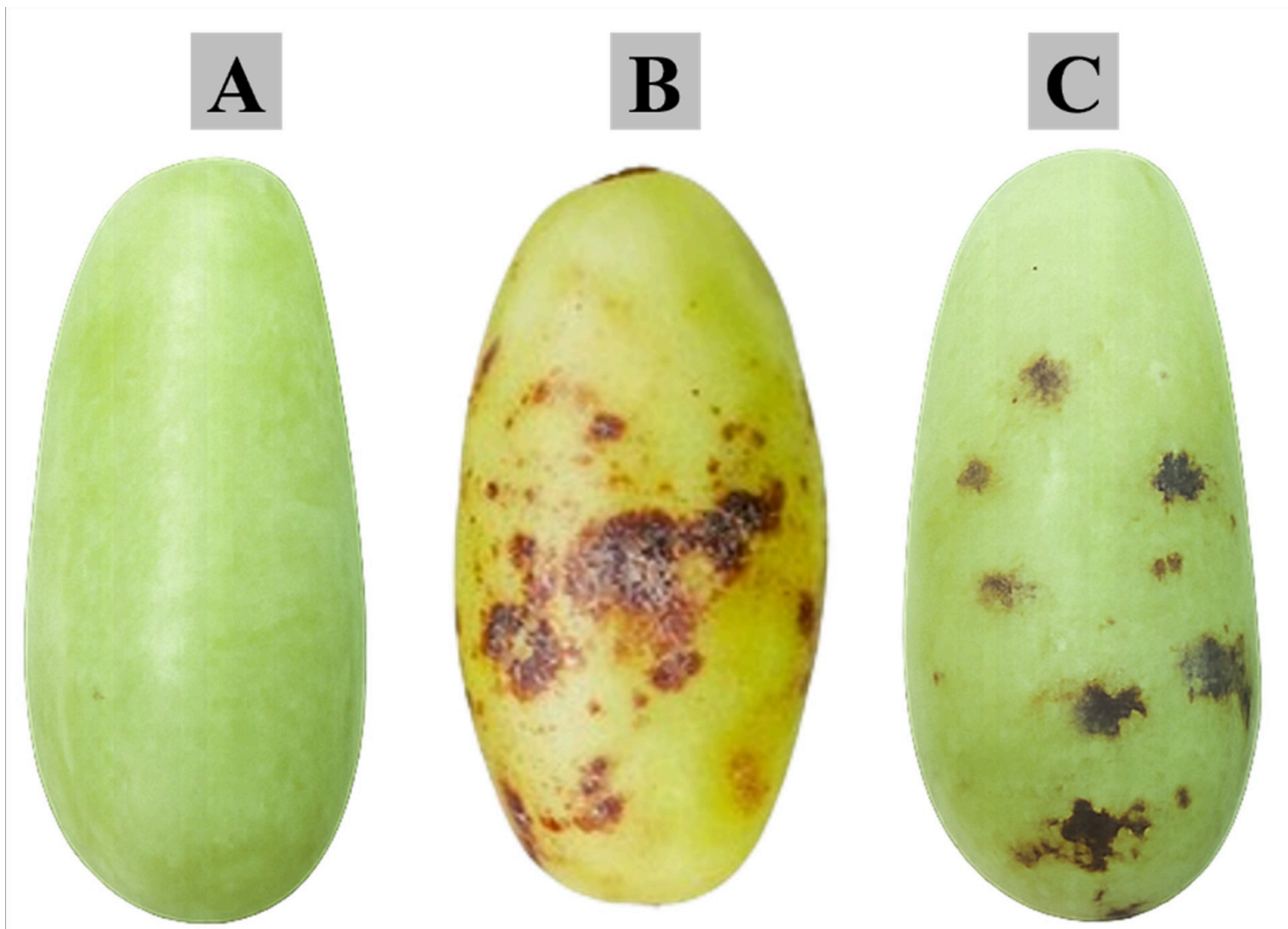


**Figure 3**

Pathogenicity assay: (A) Control; (B) Pathogenicity of GRR1 (7 dpi) and (C) Pathogenicity of GRR2 (7 dpi)

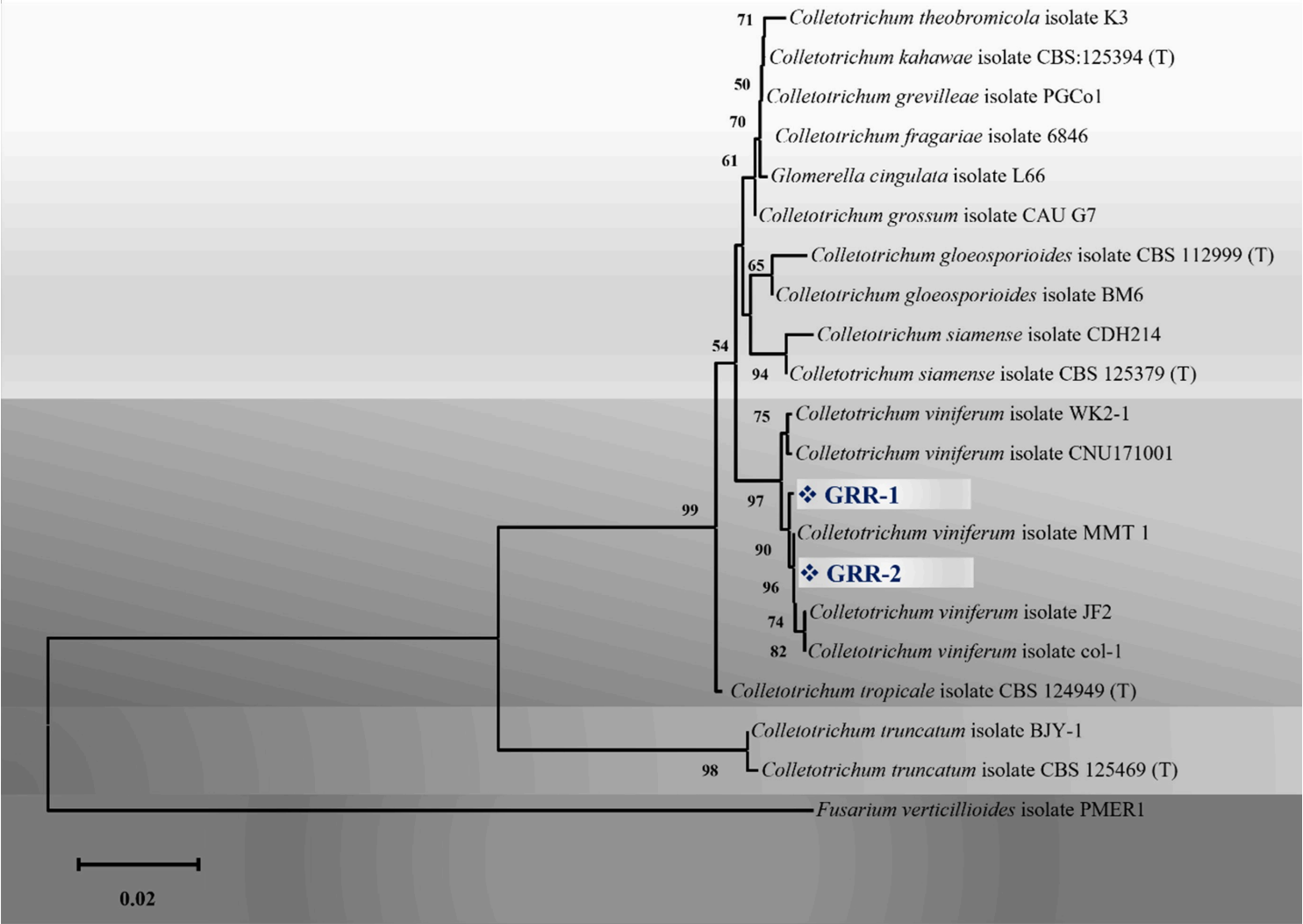


**Figure 4**

Phylogenetic relationships inferred from ITS gene sequences using the Maximum Likelihood method with 1000 bootstrap replicates. (T)-ex type strains

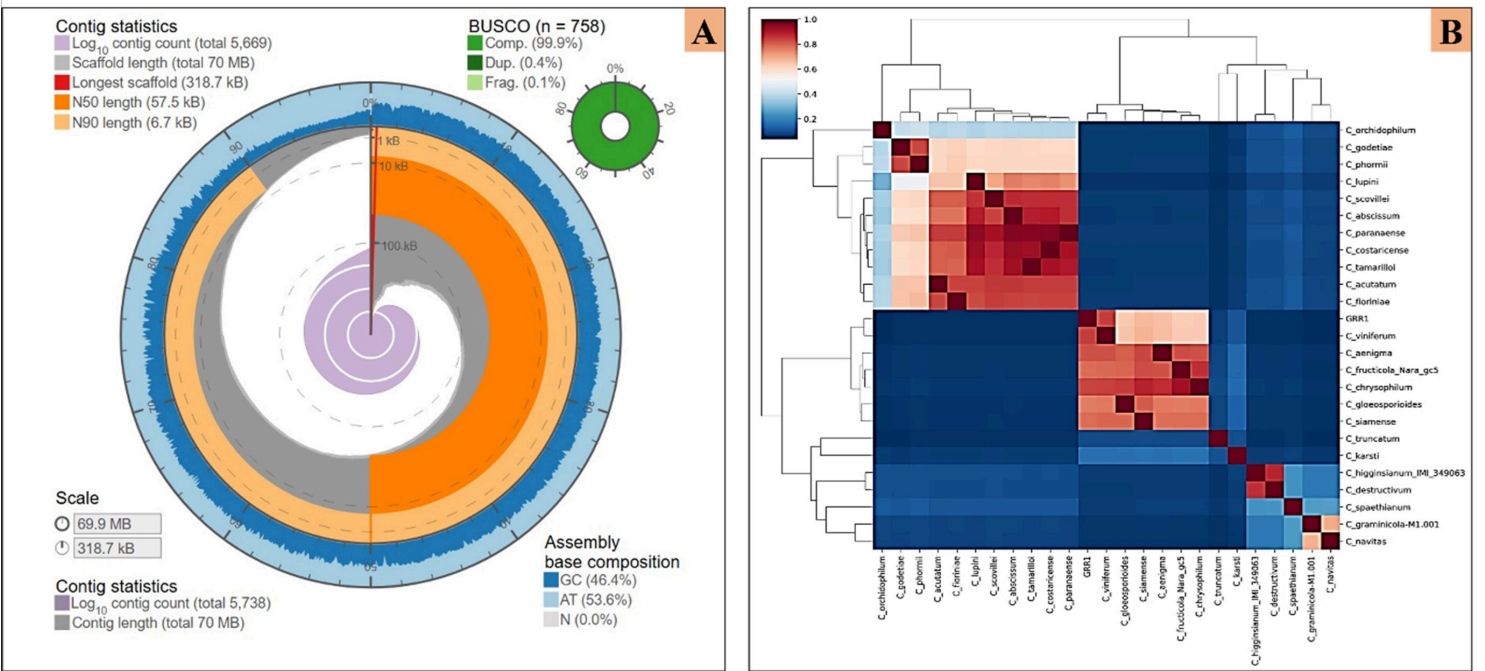


Figure 5

(A) Snail plot of *Colletotrichum viniferum* GRR1 strain genome assembly and (B), Average nucleotide identity between *C. viniferum* GRR1 and reference genomes.

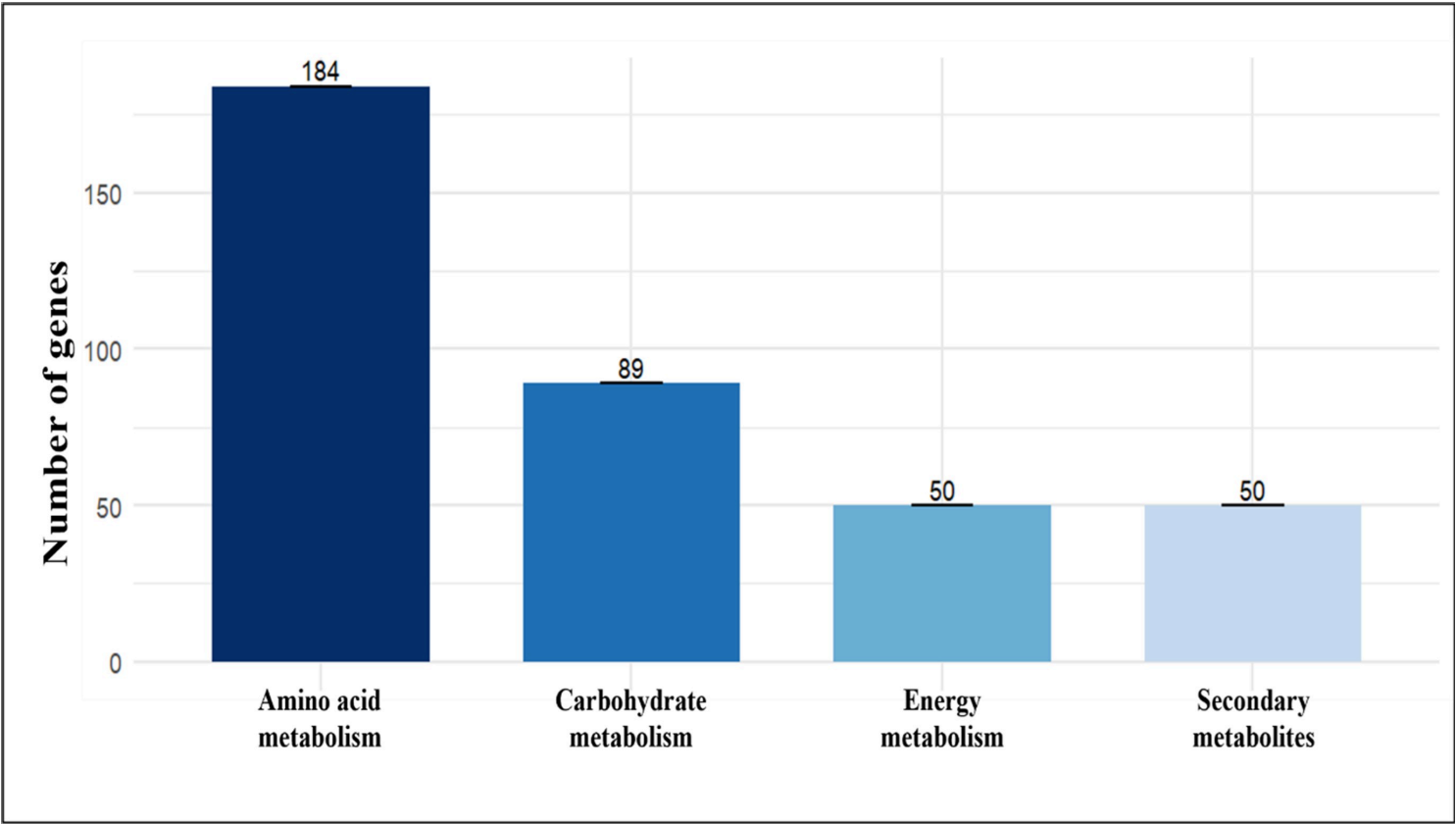


Figure 6

KEGG pathway distribution of *Colletotrichum viniferum* GRR1

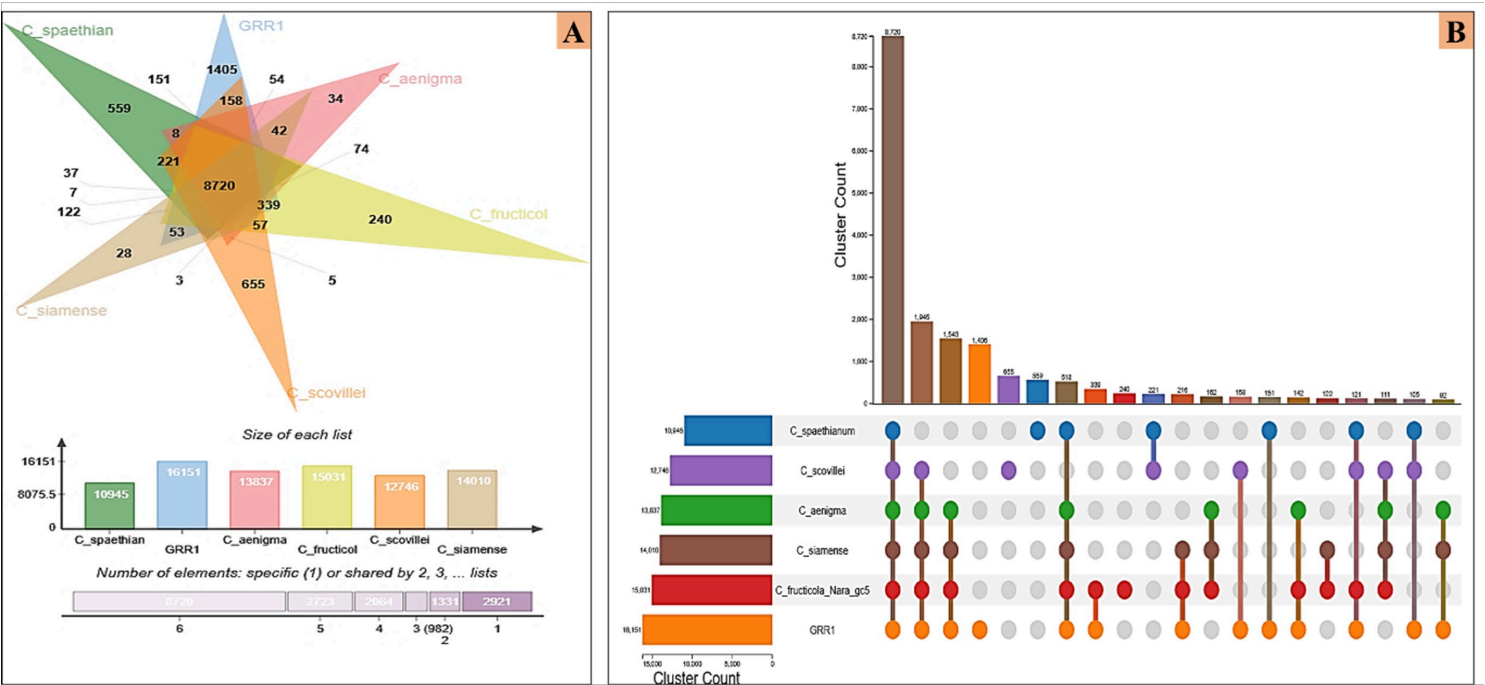


Figure 7

