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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do programa de Pós-graduação em Biotecnologia Vegetal, área de concentração em Biotecnologia Vegetal, para a obtenção do título de Doutor.

Dr. Mário Lúcio Vilela de Resende
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FERNANDA RODRIGUES SILVA

FERRAMENTAS BIOTECNOLÓGICAS NA INTERAÇÃO PLANTA-PATÓGENO: FILOGENIA MULTILOCUS E GENÔMICA FUNCIONAL NA CARACTERIZAÇÃO DE *Colletotrichum falcatum* EM *Saccharum* spp. E DE CHITINASES EM *Coffea* spp.

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APROVADA em 10 de julho de 2025

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-Eclesiastes 9:10

RESUMO

As doenças fúngicas causam perdas significativas na agricultura, impactando diretamente culturas de alta relevância econômica, como a cana-de-açúcar (*Saccharum* spp.) e o café (*Coffea* spp.). A falta de uma identificação precisa dos patógenos e o conhecimento limitado acerca dos mecanismos de defesa das plantas dificultam a implementação de estratégias de manejo mais eficazes. Esta tese teve como objetivo investigar os aspectos moleculares da interação planta-patógeno, por meio da integração de ferramentas da biotecnologia vegetal, aplicadas a dois patossistemas distintos. No primeiro estudo, isolados de *Colletotrichum falcatum* associados à podridão vermelha da cana-de-açúcar no sul da Flórida foram caracterizados através de uma análise filogenética multilocus, microscopia e testes de patogenicidade. Esta abordagem possibilitou a confirmação da identidade do patógeno, a descrição de eventos do processo infeccioso e a avaliação da virulência dos isolados em variedades comerciais. No segundo estudo, foi realizada uma caracterização genômica abrangente dos genes e proteínas de quitinases em *Coffea arabica* e seus progenitores (*C. canephora* e *C. eugenioides*). Análises *in silico* foram empregadas, incluindo análises filogenéticas, predição de domínios funcionais, estrutura gênica, localização subcelular e identificação de ortólogos, permitindo a identificação de genes candidatos potencialmente envolvidos na resistência a doenças fúngicas. Os resultados evidenciam o potencial das abordagens moleculares e genômicas para aprofundar o conhecimento sobre patogenicidade fúngica e mecanismos de defesa vegetal. Esta tese demonstra como a biotecnologia pode contribuir para fundamentar estratégias de manejo mais direcionadas e apoiar programas de melhoramento genético em culturas de interesse agrícola.

Palavras-chave: cana-de-açúcar; café; *Colletotrichum falcatum*; quitinases; interação planta-patógeno; biotecnologia vegetal.

ABSTRACT

Fungal diseases cause significant agricultural losses, directly impacting economically important crops such as sugarcane (*Saccharum* spp.) and coffee (*Coffea* spp.). Inadequate pathogen identification and limited understanding of plant defense mechanisms represent major obstacles to the implementation of more effective disease management strategies. This thesis aimed to investigate the molecular aspects of the plant-pathogen interaction by integrating plant biotechnology tools applied to two distinct pathosystems. In the first study, isolates of *Colletotrichum falcatum* associated with red rot of sugarcane in southern Florida were characterized through multilocus phylogenetic analysis, microscopy, and pathogenicity tests. This approach allowed for the confirmation of the pathogen's identity, the description of events in the infectious process, and the assessment of the virulence of the isolates in commercial varieties. The second study conducted a comprehensive genomic characterization of chitinase genes and proteins in *Coffea arabica* and its progenitors (*C. canephora* and *C. eugenioides*). *In silico* analyses were employed, including phylogenetic analyses, functional domain prediction, gene structure, subcellular localization, and identification of orthologs, allowing for the identification of candidate genes potentially involved in resistance to fungal diseases. The results highlight the potential of molecular and genomic approaches to enhance the understanding of fungal pathogenicity and plant defense mechanisms. This thesis demonstrates how biotechnology can support the development of more targeted disease management strategies and inform breeding programs for crops of agricultural importance.

Keywords: sugarcane; coffee; *Colletotrichum falcatum*; chitinases; plant–pathogen interaction; plant biotechnology.

INDICADORES DE IMPACTO

Este trabalho apresenta impactos sociais, tecnológicos, econômicos e ambientais ao integrar a caracterização de um patógeno de grande importância agrícola e o estudo de mecanismos de defesa de plantas hospedeiras. No primeiro enfoque, a caracterização molecular de isolados de *Colletotrichum falcatum*, agente causal da podridão vermelha da cana-de-açúcar, permitiu o diagnóstico mais preciso de um patógeno ainda pouco explorado no sul da Flórida. Essas informações contribuem para orientar viveiristas, produtores e técnicos na adoção de estratégias de manejo mais eficazes, reduzindo o uso indiscriminado de fungicidas e favorecendo um cultivo mais sustentável. O impacto econômico é relevante, pois a doença pode ocasionar perdas de até 35% na produtividade, comprometendo a rentabilidade de produtores e a regularidade do fornecimento de matéria-prima para a indústria açucareira regional. No segundo enfoque, a caracterização genômica de genes e proteínas de quitinases em *Coffea arabica* e em seus progenitores *C. canephora* e *C. eugenioides* representa um avanço científico e tecnológico ao fornecer pela primeira vez um panorama completo dessa família gênica em café. Esses resultados oferecem bases concretas para programas de melhoramento e para a seleção de cultivares com maior potencial de resistência a patógenos, apoiando a sustentabilidade da cafeicultura, que é uma das principais atividades agrícolas e econômicas do Brasil. Do ponto de vista social, este trabalho contribui para a segurança alimentar e para a continuidade da produção de café e cana, culturas essenciais para a economia agrícola. Os impactos se alinham às áreas temáticas de Meio ambiente, Saúde, Tecnologia e Produção e Trabalho, além de dialogarem diretamente com os Objetivos de Desenvolvimento Sustentável da ONU, em especial Fome Zero e Agricultura Sustentável, Saúde e Bem-estar, Trabalho Decente e Crescimento Econômico, Indústria, Inovação e Infraestrutura e Vida Terrestre, demonstrando como a biotecnologia pode gerar impactos práticos para culturas essenciais da agricultura mundial.

IMPACT INDICATORS

This work presents social, technological, economic, and environmental impacts by integrating the characterization of a pathogen of great agricultural importance with the study of host plant defense mechanisms. The first focus involved the molecular characterization of *Colletotrichum falcatum* isolates, the causal agent of sugarcane red rot, which enabled a more accurate diagnosis of a pathogen that has still been studied little in southern Florida. This information helps guide nurseries, growers, and technicians in adopting more effective management strategies, reducing indiscriminate fungicide use, and promoting more sustainable cultivation. The economic impact is significant, as the disease can cause yield losses of up to 35%, compromising farm profitability and the consistent supply of raw material to the regional sugar industry. The second focus comprised the genomic characterization of chitinase genes and proteins in *Coffea arabica* and its progenitor species *C. canephora* and *C. eugenioides*, representing a scientific and technological advance by providing, for the first time, a comprehensive overview of this gene family in coffee. These results provide a concrete basis for breeding programs and the selection of cultivars with greater resistance potential to pathogens, supporting the sustainability of coffee production, which is one of the main agricultural and economic activities in Brazil. From a social perspective, this work contributes to food security and to the continuity of coffee and sugarcane production, crops that are essential to agricultural economies. The impacts align with the thematic areas of Environment, Health, Technology and Production, and Labor, and are directly connected to the United Nations Sustainable Development Goals, particularly Zero Hunger and Sustainable Agriculture, Good Health and Well-being, Decent Work and Economic Growth, Industry, Innovation and Infrastructure, and Life on Land, demonstrating how biotechnology can generate practical impacts for essential crops in global agriculture.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

Doenças de plantas são responsáveis por grande parte das perdas na produção agrícola, especialmente em regiões tropicais e subtropicais em que as condições climáticas de alta umidade e temperatura favorecem significativamente o desenvolvimento de patógenos (Ploetz, 2007, Drenth e Guest, 2016; Hossain et al., 2024). Culturas tropicais, como o café (*Coffea* spp.) e a cana-de-açúcar (*Saccharum* spp.), fundamentais na economia de diversos países, são constantemente afetadas por doenças fúngicas com perdas de até 20 e 35%, respectivamente (Talhinhas et al., 2017; Silva et al., 2023). A cana-de-açúcar é a principal fonte de açúcar e é essencial na produção de biocombustíveis renováveis (OECD-FAO, 2023), enquanto o café está entre as bebidas mundialmente mais consumidas (ICO, 2023). Além das perdas diretas na produção, as doenças também afetam a qualidade do produto final, reduzindo seu aproveitamento industrial e comercial, o que impacta o valor de mercado dessas culturas e a eficiência de seus sistemas produtivos (Lazebnik, Rosenfeld e Shami, 2024; Mehdi et al., 2024; Peck e Boa, 2024). O uso de defensivos químicos é, em geral, eficaz no manejo de doenças fúngicas (Capucho et al., 2013; Bhuiyan, Croft e Tucker, 2015; Chaulagain, Raid e Rott, 2019; Zhang et al., 2025). No entanto, a falta de uma identificação precisa do patógeno resulta no uso indiscriminado de fungicidas, aumentando os custos de produção e contribuindo para a poluição ambiental (Pathak et al., 2022). Além disso, como planta e patógeno estão em constante coevolução, a aplicação repetida dos mesmos princípios ativos favorece a seleção de isolados resistentes, resultando na redução da eficácia dos fungicidas ao longo do tempo (Viswanathan e Selvakumar, 2020; Saavedra et al., 2023). Para que o manejo de doenças de plantas seja eficaz e tecnologias sustentáveis possam ser desenvolvidas, é fundamental realizar um estudo abrangente tanto do patógeno quanto da planta hospedeira, assim como da dinâmica da interação entre eles.

A biotecnologia vegetal tem se destacado como um conjunto de tecnologias que possibilita aprofundar o entendimento das interações planta-patógeno e aprimorar as práticas de manejo de doenças (Ijaz et al., 2024). Técnicas como a filogenia multilocus, análises genômicas, predição de domínios funcionais e mapeamento de genes de defesa viabilizam a caracterização genética detalhada tanto de patógenos quanto de plantas hospedeiras (Malathi et al., 2011; Qiao et al., 2021; Haxim et al., 2022; Lv et al., 2022). Essa abordagem integrada possibilita a identificação de patógenos, bem como de genes associados à virulência, resistência

e resposta imune, contribuindo para o desenvolvimento de cultivares mais tolerantes e sistemas de produção mais sustentáveis (Manzoor et al., 2024).

A identificação precisa dos patógenos é um pré-requisito fundamental para a implementação de medidas eficazes de controle (Hossain et al., 2020). No entanto, muitos fungos fitopatogênicos, como as espécies do gênero *Colletotrichum*, apresentam elevada plasticidade morfológica, dificultando o diagnóstico com base apenas em características fenotípicas (Phoulivong et al., 2010). Nesse contexto, técnicas moleculares baseadas no sequenciamento de múltiplos loci, como ITS, *TUB2*, *GAPDH*, *CHS-1* e *ACT*, têm se consolidado como abordagens eficazes na identificação de espécies e no estudo da variabilidade genética de isolados (Cannon et al., 2012; Weir, Johnston e Damm, 2012; Alhudaib, Ismail e Magistà, 2023). Além disso, a integração dessas abordagens moleculares com análises microscópicas contribui para o entendimento do desenvolvimento do patógeno e sua interação com o hospedeiro em nível celular, fornecendo informações relevantes que auxiliam no manejo da doença. No primeiro capítulo deste trabalho, essas abordagens biotecnológicas foram empregadas para caracterizar molecularmente isolados de *Colletotrichum falcatum* associados à podridão vermelha da cana-de-açúcar no sul da Flórida, um patossistema ainda pouco estudado nesta região. Além disso, foi realizado o monitoramento do desenvolvimento de *C. falcatum* em nível celular por meio de microscopia de luz e testes de patogenicidade em três variedades comerciais da região a fim de compreender a capacidade de infecção do patógeno e as possíveis variações em sua virulência.

O entendimento dos mecanismos de defesa da planta hospedeira é tão importante quanto a caracterização do patógeno para o desenvolvimento de estratégias de manejo mais sustentáveis. Avanços em biotecnologia têm possibilitado a investigação detalhada de genes envolvidos na imunidade vegetal, permitindo a identificação e caracterização de famílias gênicas complexas associadas à resposta contra doenças fúngicas (Santos et al., 2022; Xuan et al., 2024). Esses genes podem ser explorados em programas de melhoramento genético, seja por superexpressão ou introdução em culturas transgênicas, visando o desenvolvimento de plantas mais tolerantes ou resistentes a patógenos (Khan et al., 2017; Sharma et al., 2023; Chouhan, Ahmed e Gandhi, 2023; Manzoor et al., 2024). Dentre esses genes, destacam-se aqueles que codificam quitinases, enzimas produzidas em resposta à infecção fúngica, capazes de degradar a parede celular dos fungos e ativar mecanismos de defesa na planta hospedeira (Oliveira et al., 2020). No segundo capítulo deste estudo, foram caracterizados genes e proteínas de quitinases em *Coffea arabica* e seus progenitores, *C. canephora* e *C. eugenioides*.

Foram conduzidas análises genômicas e abordagens *in silico*, incluindo as análises filogenéticas, mapeamento cromossômico, predição de domínios funcionais, estrutura gênica, localização subcelular e análise de genes ortólogos, com o objetivo de identificar genes com potencial envolvimento na resistência a patógenos.

Apesar de abordarem patossistemas diferentes, os dois estudos aqui apresentados se complementam por compartilharem o mesmo objetivo central: gerar conhecimento sobre os aspectos moleculares envolvidos na interação entre plantas e patógenos fúngicos. Enquanto a caracterização de *C. falcatum* fornece dados essenciais sobre a identificação e virulência do patógeno, a caracterização das quitinases em *Coffea* spp. oferece uma base para a compreensão dos mecanismos de defesa ativados pelas plantas. Ambos os estudos se beneficiam da integração de ferramentas biotecnológicas avançadas para preencher lacunas de conhecimento em fitopatologia, alternando o enfoque entre a caracterização do patógeno e a análise de genes e proteínas relacionados à resposta da planta hospedeira.

Essa integração de diferentes técnicas que abrangem desde o PCR, da biologia molecular clássica, até a genômica e bioinformática, permite uma abordagem mais completa das relações entre plantas e seus patógenos. Portanto, esta tese apresenta dois enfoques distintos, sustentados pela biotecnologia: (i) o uso de técnicas moleculares para a identificação e caracterização de *C. falcatum* em cana-de-açúcar, e (ii) a aplicação de ferramentas genômicas e de bioinformática para a análise de quitinases em café. Em conjunto, esses estudos exemplificam como a biotecnologia vegetal pode ser aplicada para compreender, monitorar e explorar os mecanismos envolvidos na dinâmica planta-patógeno, contribuindo para a ampliação do conhecimento em fitopatologia para embasar futuras aplicações em manejo de doenças e melhoramento genético.

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ARTIGO 1–Molecular characterization and pathogenicity of *Colletotrichum falcatum* causing red rot on sugarcane in southern Florida

Article

Molecular characterization and pathogenicity of *Colletotrichum falcatum* causing red rot on sugarcane in southern Florida

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Abstract: Red rot disease reduces sugarcane yield and impacts the sugar quality, posing an important threat to the sugarcane industry in Florida. Although *Colletotrichum falcatum*, the causal agent of red rot in Florida, was first reported in 1984 based on morphology, molecular and pathological data have remained limited, highlighting the critical need for comprehensive characterization. Thirteen isolates were obtained from three local sugarcane varieties in Belle Glade, Florida. Phylogenetic analyses of five genetic markers (ITS, *ACT*, *TUB2*, *GAPDH*, and *CHS-1*) confirmed all strains as *C. falcatum*. In addition, the study documented the disease progression at the cellular level and assessed the pathogenicity of representative strains using the leaf sheath and whole seeding inoculation methods. The varieties CP96-1252 and CP89-2143 showed greater host resistance. These findings represent the first report of *C. falcatum* causing red rot in southern Florida, offer valuable insights for/into red rot management, and provide a basis for future breeding programs to enhance sugarcane resistance to red rot disease.

Keywords: molecular identification 1; leaf sheath assay 2; sugarcane 3; red rot 4; Everglades Agriculture Area 5; Florida 6; *Colletotrichum falcatum* disease cycle 7.

1. Introduction

Sugarcane (*Saccharum* ssp.) is a global cash tropical grass crop cultivated across 26.3 million hectares, with a total production of approximately 1.9 billion tons [1]. In the 2022 and 2023 seasons, the United States (U.S.) ranked as the sixth largest sugarcane producer in the world [2]. Florida alone is responsible for more than 50% of the value of the sugar derived from sugarcane nationwide, generating approximately $712 million U.S. dollars in 2020 [3]. Plant diseases significantly impact sugarcane production, especially in tropical and subtropical regions

where warm temperatures and high humidity are conducive to disease development. Fungal pathogens can directly infect the interior of the sugarcane stalks, affecting yield, juice quality, and sugar content [4–6]. Among these, red rot stands out as one of the most important fungal diseases affecting sugarcane worldwide [7–9].

The foliar phase of this disease is characterized by reddish lesions that extend along the midrib and leaf blade, with a pale color at the center where the pathogen sporulates, producing conidia within acervuli [10,11]. The pathogen subsequently infects cane stalks, which is known as the stalk phase [10], moving downwards through the vascular tissues, resulting in a reddish discoloration that can be observed by splitting the stalks longitudinally [12]. This infection leads to the repression of sucrose transporters [13] and the inversion of sucrose, compromising juice quality and reducing cane weight by 29 to 83% [14,15].

Three species of *Colletotrichum* (*C. falcatum*, *C. siamense* and *C. plurivorum*) have been reported to be associated with sugarcane red rot in Brazil, [9,16], although *Colletotrichum falcatum* is the predominant etiological agent in regions such as Bangladesh, Brazil, India, and the U.S. [8,9,16–19]. Despite its global importance, this disease remains poorly characterized, with critical knowledge gaps regarding the causative agent in the U.S. and its development in sugarcane living tissue.

Red rot was first observed in the U.S. in 1909 in Louisiana [20]. Since then, it has caused significant losses in sugarcane production in the southern U.S. [21]. In Florida, the first report of *C. falcatum* on sugarcane was performed based on morphological characteristics in 1984 [17]. Accurate pathogen identification is essential for developing effective management strategies [22]. However, morphological and physiological characteristics alone are often insufficient to differentiate between *Colletotrichum* species due to significant overlap [23]. For example, in 2009, six new species of *Colletotrichum* were identified causing disease on grasses, yet they showed no significant morphological similarities to other grass-associated *Colletotrichum* species, highlighting the necessity for molecular techniques in an accurate identification [24].

Colletotrichum falcatum has exhibited high variability in virulence, leading to the emergence of new pathotypes [5,8,25,26]. These new pathotypes pose a challenge to red rot management and breeding efforts, as they can overcome the resistance of existing varieties [25–27]. In Florida, the limited information on the resistance levels of local sugarcane varieties to red rot further complicates disease control.

Although extensive research has been conducted on the symptomatology and overall life cycle of *C. falcatum* in sugarcane, the majority of studies have focused on the stalk phase [28–31]. Consequently, our knowledge of the pathogen development within sugarcane leaves remains limited. A comprehensive understanding of the foliar phase is essential for elucidating the host-pathogen interaction during leaf infection and ultimately developing more effective management strategies against red rot [32,33].

Given the limited understanding of *Colletotrichum* species and the gaps in knowledge regarding their pathogenicity in Florida, there is a critical need to perform the molecular characterization and assess the pathogenicity of the *Colletotrichum* species associated with sugarcane in southern Florida. Therefore, the aims of this study were to: (i) conduct the multilocus molecular phylogenetic analysis of *Colletotrichum* strains from southern Florida, (ii) investigate the foliar phase of *C. falcatum* at the

cellular level, (iii) assess the pathogenicity of representative isolates on three commercial sugarcane varieties grown in the region. These findings are vital for improving red rot management strategies and enhancing sugarcane resistance to this devastating disease.

2. Materials and Methods

2.1. Fungal isolation

Sugarcane leaf samples were collected in 2022 and 2023 from field sugarcane plants from varieties CL85-1040, CP96-1252, and CP89-2143 exhibiting symptoms of red rot at the University of Florida Everglades Research and Education Center (EREC), Belle Glade, Florida (26°40'03"N 80°38'06" W). One symptomatic leaf was collected per plant, resulting in a total of 13 leaves for fungal isolation. Triangular fragments of healthy and symptomatic tissue were surface sterilized in 70% ethanol for 30 s, 1.2% of sodium hypochlorite for 3 min, washed three times in sterile distilled water (SDW), and dried in a sterile paper towel. Four fragments of each leaf were plated in potato dextrose agar (PDA) (BD Difco™, New York, NY, USA) amended with 1% (v/v) neomycin-penicillin-streptomycin (Sigma-Aldrich St Louis, MO, USA) at 23°C for seven to ten days [34].

Single spores were transferred to new PDA plates to establish pure cultures and stored in 50% glycerol (v/v) at -80°C. A negative control was obtained from anthracnose lesions from the crown of celery, subjected to the same isolation process, and included in the analyses.

2.2. DNA extraction, PCR, and sequencing

Genomic DNA of the 14 strains collected from the three sugarcane varieties CL85-1040 (n= 3), CP96-1252 (n= 6), CP89-2143 (n= 4), and from celery (n=1) was extracted using 50 mg of fungal mycelia grown on PDA for two weeks at 23°C under continuous fluorescent light. DNA was extracted using the SYNERGY™ 2.0 DNA Extraction Kit (OPS Diagnostics, Lebanon, NJ, USA), following the manufacturer's instructions. Total DNA obtained was eluted in 50 µL of Monarch® DNA Elution Buffer (New England Biolabs, MA, USA). The final DNA concentration was measured using a NanoDropTM2000/2000c Spectrophotometers (Thermo Fisher Scientific, Waltham, MA, USA). Primers used in the study were synthesized by Integrated DNA Technologies (IDT®, Coralville, IA, USA)(Table 1). Polymerase chain reaction (PCR) was performed in a 25 µL reaction volume containing 1 µL of DNA (25 ng/µL), 12.5 µL of GoTaq® Green Master Mix Kit (PROMEGA, Madison, WI, USA), and 11.5 µL of water. The cycling conditions were initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 51°C, 59°C, 56°C, 58°C, and 59°C for ITS, *ACT*, *TUB2*, *GAPDH*, and *CHS-1*, respectively, for 45 s, extension at 72°C for 59 s and a final extension at 72°C for 10 min. Following PCR amplification, the resulting DNA products were visualized on a 1% agarose gel stained with SYBR safe (Thermo Fisher Scientific, Waltham, MA, USA). The amplified DNA was purified before Sanger sequencing on the ABI 3730XL sequencer at Molecular Cloning Laboratories (MCLAB, South San Franciso, CA, USA).

Table 1. Sequences, annealing temperature, and references of primers used to amplify ITS region and *ACT*, *TUB2*, *GAPDH*, and *CHS-1* genes.

Locus[1]	Primer	Sequence (5'-3')	Ta(°C)[2]	PS[3]	References
ITS	ITS1F	CTTGGTCATTTAGAGGAAGTAA	49.7	429 bp	[35]
	ITS4	TCCTCCGCTTATTATATGC	52.1		[36]
ACT	ACT-512	ATGTGCAAGGCCGGTTTCGC	61.7	252 bp	[37]
	ACT-783R	TACGAGTCCTTCTGGCCCAT	57.9		[37]
TUB2	Bt1	AACATGCGTGAGATTGTAAGT	52.3	750 bp	[38]
	Bt2	ACCCTCAGTGTAGT GACCCTTGGC	62.5		[38]
GAPDH	GDF1	GCCGTCAACGACCCCTTCATTGA	61.7	150 bp	[39]
	GDR1	GGGTGGAGTCGTACTTGAGCATGT	60.6		[39]
CHS-1	CHS79F	TGGGGCAAGGATGCTTGGAAGAAG	61.7	300 bp	[37]
	CHS354R	TGGAAGAACCATCTGTGAGAGTTG	56.6		[37]

[1]ITS, internal transcribed spacer; *ACT*, actin; *TUB2*, β-tubulin; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *CHS-1*, Chitin synthase
[2]Ta, annealing temperature
[3]PS, product size; bp, base pair

2.3. Phylogenetic analyses

In addition to the 14 strains obtained in this study, 18 reference sequences were retrieved from NCBI GenBank, totaling 32 strains used in the phylogenetic analyses (Table 2). Raw DNA sequences from the 14 strains were manually annotated for each of the five gene regions (ITS, *ACT*, *TUB2*, *GAPDH*, and *CHS-1*) to ensure the accuracy and high quality of the final consensus sequences before performing a BLAST search and aligning them using Geneious Prime 6.0.6. Sequence alignment was performed with MAFFT v. 7.407_1, and the most conserved regions were filtered by Gblocks v.0.91.1 integrated within NGPhylogeny.fr [40–42]. Phylogenetic trees were constructed using both Maximum Likelihood (ML), estimated with MEGA v.11 [43] and the Bayesian inference (BI) performed with MrBayes v. 3.2.6. For the ML analysis, the best-fit model K2+G+I (ITS), K2+I (*ACT*), K2+G (*TUB2*, *GAPDH* and *CHS-1*) and TN93+G (concatenated tree) were selected based on the Bayesian Information Criterion (BIC), with 1,000 bootstrap replicates [44]. The BI analysis employed SYM+I+G (ITS), HKY+I (*ACT*), HKY+G (*TUB2*, *GAPDH*), and GTR+G (*CHS-1*) substitution models based on Akaike information criterion (AIC) and utilized a Markov Chain Monte Carlo (MCMC) search with 5,000,000 generations, sampling trees every 100 generations [45]. The phylogenetic trees of the individual and concatenated genes were developed using iTOL (https://itol.embl.de/). *Colletotrichum boninense* was used as the outgroup. All sequences resulting from this study of *Colletotrichum* spp. from sugarcane (n= 13) and one from celery (n= 1) were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under the following accession numbers: PP782092 to PP782105 (ITS), PP855401 to PP855414 (*ACT*), PQ349366 to PQ349379 (*TUB2*), PQ349380 to PQ349393 (*GAPDH*), and PQ349352 to PQ349365 (*CHS-1*) (Table 2).

Table 2. Strains used in the molecular phylogenetic analyses.

					GenBank Accession Numbers					
Strain[1]	**Species**	**Host**	**Collection date[2]**	**Country**	**ITS**	***ACT***	***TUB2***	***GAPDH***	***CHS-1***	**References**
CML 3855	*C. falcatum*	*Sarccharum officinarum* sp.	N/A	Brazil	MW471107	MW455488				[16]
CML 4075	*C. falcatum*	*Sarccharum officinarum* sp.	N/A	Brazil	MW471109	MW455490				[16]
Cf2	*C. falcatum*	*Sarccharum officinarum* sp.	August-2022	China	PP217423	OR542861	OR542897			[46]
LC885	*C. falcatum*	*Sarccharum officinarum* sp.	N/A	China	HM171677	HM171665	HM171680	HM171671		[47]
Cf64-8	*C. falcatum*	*Sarccharum officinarum* sp.	N/A	India	FJ002093	FJ008099		FJ002020		[8]
CBS 147945*	*C. falcatum*	*Sarccharum officinarum* sp.	N/A	Indonesia	JQ005772	JQ005835	JQ005856		JQ005793	[47]
CBS 130836*	*C. gaminicola*	*Zea mays*	N/A	USA	DQ003110	JQ005830	JQ005851	MW740221	JQ005788	[48–50]
CBS 125086*	*C. navitas*	*Panicum virgatum*	N/A	USA	GQ919067	JQ005832	JQ005853		JQ005790	[49,51]

MAFF 511115*	*C. nicholsonii*	*Paspalum dilatatum*	N/A	Japan	EU554126	JQ005833	JQ005854	MW740226	JQ005791	[49,50,52]
CBS 131301*	*C. sublineola*	*Sorghum bicolor*	N/A	*Burkina Faso*	DQ003114	JQ005834	JQ005855	MW740232	JQ005792	[33,48,50]
MAFF 305403*	*C. paspali*	*Paspalum notatum*	N/A	Japan	EU554100	JX519235	JX519244	MW740210	JX519227	[50,52,53]
CBS 129661*	*C. eremochloae*	*Eremochloa ophiuroides*	N/A	USA	JQ478447	JX519236	JX519245		JX519228	[53]
MAFF 510857*	*C. miscanthi*	*Miscanthus sinensis*	N/A	Japan	EU554121	JX519237	JX519246	MW740225	JX519229	[50,52,53]
MAFF 511155*	*C. eleusines*	*Eleusine indica*	N/A	Japan	EU554131	JX519234	JX519243	MW740217	JX519226	[50,52,53]
GZC46	*C. cereale*	*Lolium multiflorum*	June-2022	China	OR976053	OR995786	OR995792	OR995768	OR995774	[54]
MAFF 305460*	*C. jacksonii*	*Echinochloa esculenta*	N/A	Japan	EU554108	JX519233	JX519241	MW740224	JX519224	[50,52,53]

CBS 124945*	*C. theobromicola*	*Theobroma cacao*	N/A	Panama	JX010294	JX009444	JX010447	JX010006	JX009869.1	[55]
CBS 123755*	*C. boninense*	*Crinum asiaticum* var.	N/A	Japan	JQ005153	JQ005501	JQ005588	JQ005240	JQ005327	[56]
KX144	*C. falcatum*	*Sarccharum officinarum* sp./ CL85-1040	August-2022	Florida, USA	PP782093	PP855401	PQ349366	PQ349380	PQ349352	First report in this study
KX652	*C. falcatum*	*Sarccharum officinarum* sp./ CL85-1040	July-2023	Florida, USA	PP782096	PP855404	PQ349369	PQ349383	PQ349355	First report in this study
KX653	*C. falcatum*	*Sarccharum officinarum* sp./ CL85-1040	July-2023	Florida, USA	PP782097	PP855405	PQ349370	PQ349384	PQ349356	First report in this study

KX145	*C. falcatum*	*Sarccharum officinarum* sp./ CP96-1252	September-2022	Florida, USA	PP782094	PP855402	PQ349367	PQ349381	PQ349353	First report in this study
KX657	*C. falcatum*	*Sarccharum officinarum* sp./ CP96-1252	July-2023	Florida, USA	PP782101	PP855408	PQ349374	PQ349388	PQ349360	First report in this study
KX658	*C. falcatum*	*Sarccharum officinarum* sp./ CP96-1252	July-2023	Florida, USA	PP782102	PP855409	PQ349375	PQ349389	PQ349361	First report in this study
KX659	*C. falcatum*	*Sarccharum officinarum* sp./ CP96-1252	July-2023	Florida, USA	PP782103	PP855410	PQ349376	PQ349390	PQ349362	First report in this study
KX661	*C. falcatum*	*Sarccharum officinarum* sp./ CP96-1252	July-2023	Florida, USA	PP782104	PP855411	PQ349377	PQ349391	PQ349363	First report in this study

KX662	*C. falcatum*	*Sarccharum officinarum* sp./ CP96-1252	July-2023	Florida, USA	PP782105	PP855412	PQ349378	PQ349392	PQ349364	First report in this study
KX146	*C. falcatum*	*Sarccharum officinarum* sp./ CP89-2143	September-2022	Florida, USA	PP782095	PP855403	PQ349368	PQ349382	PQ349354	First report in this study
KX654	*C. falcatum*	*Sarccharum officinarum* sp./ CP89-2143	July-2023	Florida, USA	PP782098	PP855406	PQ349371	PQ349385	PQ349357	First report in this study
KX655	*C. falcatum*	*Sarccharum officinarum* sp./ CP89-2143	July-2023	Florida, USA	PP782099	PP855407	PQ349372	PQ349386	PQ349358	First report in this study

KX656	*C. falcatum*	*Sarccharum officinarum* sp./ CP89-2143	July-2023	Florida, USA	PP782100	PP855413	PQ349373	PQ349387	PQ349359	First report in this study
KX164	*C. theobromicola*	*Apium graveolens*	December-2022	Florida, USA	PP782092	PP855414	PQ349379	PQ349393	PQ349365	First report in this study

[1]*, ex-type cultures
[2]N/A, not available

2.4. Fungal inoculum

Colletotrichum strains were grown on PDA at 23°C under continuous fluorescent for two weeks. The spores were scraped using a sterilized mini pestle in SDW and filtered in a double layer of cotton gauze [57,58]. The spore suspension was centrifuged three times at 3500 g, 25°C for 5 min, and the SDW was changed between the centrifugation cycles [57,58]. A spore concentration was adjusted to 10^5 spores/ml and immediately used for both leaf sheath assays and seedling spray inoculations [57,58].

2.5. Sugarcane plants

Single-eye pieces were obtained from sugarcane stalks (CL85-1040, CP96-1252, and CP89-2143) and planted 2 cm apart in a rectangular tray (40 x 50 cm) filled with a potting mixture (Professional Growing mix, Sun Gro Horticulture, Agawam, MA, USA). One-week-old seedlings were transferred to pots (21 x 22 cm) with a potting mixture for the whole seedling spray inoculation experiment. The variety CL85-1040 used in the leaf sheaf assay was kept in the trays, and one-month-old seedlings were cut at the soil line and brought to the laboratory for the leaf sheath assay. Osmocote fertilizer (14-14-14), (ICL growing solutions, Tel Aviv, Israel) was added one week after planting the seed eyes and seedlings. The plants were kept in the greenhouse at ambient temperature, under 1 min irrigation twice a day, and then they were used to set up the whole plant or the leaf sheath experiments.

2.6. Leaf sheath colonization assay

The colonization of *C. falcatum* on the cellular level was investigated using the most susceptible variety, CL85-1040, and the most virulent strain, KX144, in a time-course experiment. The choice of this variety and strain was based on preliminary results from greenhouse experiments (data not shown). Detached sugarcane leaf sheaths were collected and

prepared according to Belisário et al. [58] with modifications. Briefly, the leaf sheaths were inoculated with 100 µL of KX144 spore suspension (10^5 spores/mL) and incubated at 21°C ± 1°C under continuous fluorescent light in 100% humidity. For negative controls, we used two treatments: 1) inoculation with the non-pathogenic *Colletotrichum theobromicola* isolate KX164, originally isolated from celery, and 2) with SDW. Treatments were arranged in a completely randomized design with four replicates. The evaluation was performed at 12, 24, 36, 48, 60, 72, and 146 hours after inoculation (hai). A total of 50 infection sites per repetition were evaluated randomly using the Leica ICC50 E microscope (Leica Microsystems, Leica, Wetzlar, Germany) and visualized with Leica Application Suite LAS EZ v. 3.4.0 software, following the diagrammatic scale developed by Xavier et al. [57].

2.7. Pathogenicity tests

2.7.1. Leaf sheath pathogenicity assay

One fungal strain KX144, KX145, and KX146 from each variety CL85-1040, CP96-1252, and CP89-2143, respectively, were selected for the leaf sheath pathogenicity assay to inoculate on the 'CL85-1040'. Additionally, KX164 (negative control) was included. All plants were inoculated with a spore suspension of 10^5 spores/ml. Inoculation with SDW was used as a control. The evaluation was performed at 60 hai based on 50 infection sites per repetition under the light microscope based on the scale developed by Xavier et al. [57] with modifications. Briefly, it was performed in two different ways: (i) how the pathogen was colonizing the plant cells, and (ii) how the plant was responding against the pathogen by restricting its growth to one cell or by the presence of red vesicles at the infection sites.

2.7.2. Whole seedling spray inoculation

The pathogenicity of strains (KX144, KX145, and KX146) from local sugarcane varieties (CL85-1040, CP96-1252, and CP89-2143, respectively) was assessed by spray-inoculating each variety with a spore suspension of 10^5 spores/mL in 1% (v/v) Tween 20. The strain KX164 from celery and Tween 20 were used as the negative control. After spray inoculation, the plants were bagged with a clear bag and maintained in a 100% humidity chamber for 48 hours. After that, the bags were removed, and the plants were kept on the greenhouse benches, where they were irrigated twice a day at 7 am and 7 pm for one minute each time. Treatments were arranged in a completely randomized block design with four replicates and two plants per pot per replicate. Disease severity was evaluated weekly after disease symptoms were observed, and re-isolation was performed after the last assessment to fulfill Koch's postulate. The experiment was performed three times (October and November/2023 and February/2024).

2.7.3 Statistical analysis

Statistical analyses were performed in R version 4.3.2 [59]. The two repetitions of the leaf sheath virulence experiment were analyzed through a cumulative logistic mixed model (CLMM) using the *clmm* function of the 'ordinal' package v.4.1 [60]. This approach appropriately models ordinal data [61,62]. Experiments, fungi, and their interaction were treated as fixed effects, and replicates within experiments were treated as random effects. In both pathogenicity tests statistical analyses (spray inoculation and leaf sheath virulence), the means and 95% confidence intervals of each group were estimated using the *emmeans* function of the 'emmeans' package v.1.10.4 [63]. Statistically significant pairwise differences ($p < 0.05$

following Tukey's adjustment) were visualized with a compact letter display via the *cld* function of the 'multcomp' package [64]. All graphs were created using functions from the 'tidyverse' [65] and 'ggplot2' packages [66].

The AUDPC resultant from the three repetitions of the whole seedlings spray inoculation experiment were analyzed through generalized linear mixed models (GLMMs) using the function *glmmTMB* of the 'glmmTMB' package v.1.1.9 [67]. A Tweedie distribution with a log link function was employed to account for zero-inflated data due to negative controls. Variety, fungal strain, and their interaction were included as fixed effects, while experiment and experimental unit were included as random effects. Model fit was assessed via plotting the simulated quantile residuals using the *simulateResiduals* function from the 'DHARMa' package [68]. Those figures and comparisons by Akaike's information criteria (AIC) subsequently confirmed that model fit was improved by modeling dispersion as varying among the different strains. A Wald $\chi 2$ test was then performed, followed by the calculation of estimated marginal means, 95% confidence intervals, and pairwise comparisons (using Tukey's adjustment) among fungal strains within each variety, and for each variety averaged across all strains.

3. Results

3.1. Colletotrichum spp. in southern Florida

Red rot was observed on local sugarcane varieties at the Everglades Research and Education Center, University of Florida, in Belle Glade, Florida. The average disease incidence in the field was approximately 60–80% during the summers of 2022 and 2023. These varieties had not been treated with any pesticides. Symptoms of red rot included reddish lesions on the midrib, with a pale center where the pathogen sporulated, producing black dots corresponding to acervuli when visualized at higher magnification. Thirteen isolates collected from three sugarcane varieties [CL85-1040 (n= 3), CP96-1252 (n= 6), and CP89-2143 (n= 4)] were identified as *Colletotrichum* spp. based on morphological traits such as the presence of acervuli with setae, banana-shaped spores, and gray mycelium with orange sporulation on PDA media.

3.2. Molecular Characterization

The final alignment of the concatenated gene sequences contained a total of 1568 bp (Figure 1) (ITS: 412 bp; *ACT*: 277 bp, *TUB2*: 497 bp, *GAPDH*: 121 bp, *CHS-1*: 261 bp), which were utilized in the phylogenetic analyses, considering alignment gaps and missing data from incomplete genes. Representing 507 phylogenetically informative site patterns (ITS: 76, *ACT*: 121, *TUB2*: 200, *GAPDH*: 56, *CHS-1*: 54) (Figures S1-S5). Based on the concatenated alignment, our sugarcane strains from Florida had 99.8% similarity with the *C. falcatum* isolate from sugarcane CML4075 in Brazil [16]. Furthermore, the concatenated phylogenetic tree revealed that all 13 sugarcane strains from Florida clustered within the *Colletotrichum falcatum* clade alongside reference isolates of this species, confirming their species identification. All 13 strains had identical ITS, *ACT, TUB2, GAPDH*, and *CHS-1* sequences, except for KX145, KX146, KX652, and KX661, which exhibited a single nucleotide polymorphism at the same position in the *GAPDH* gene.

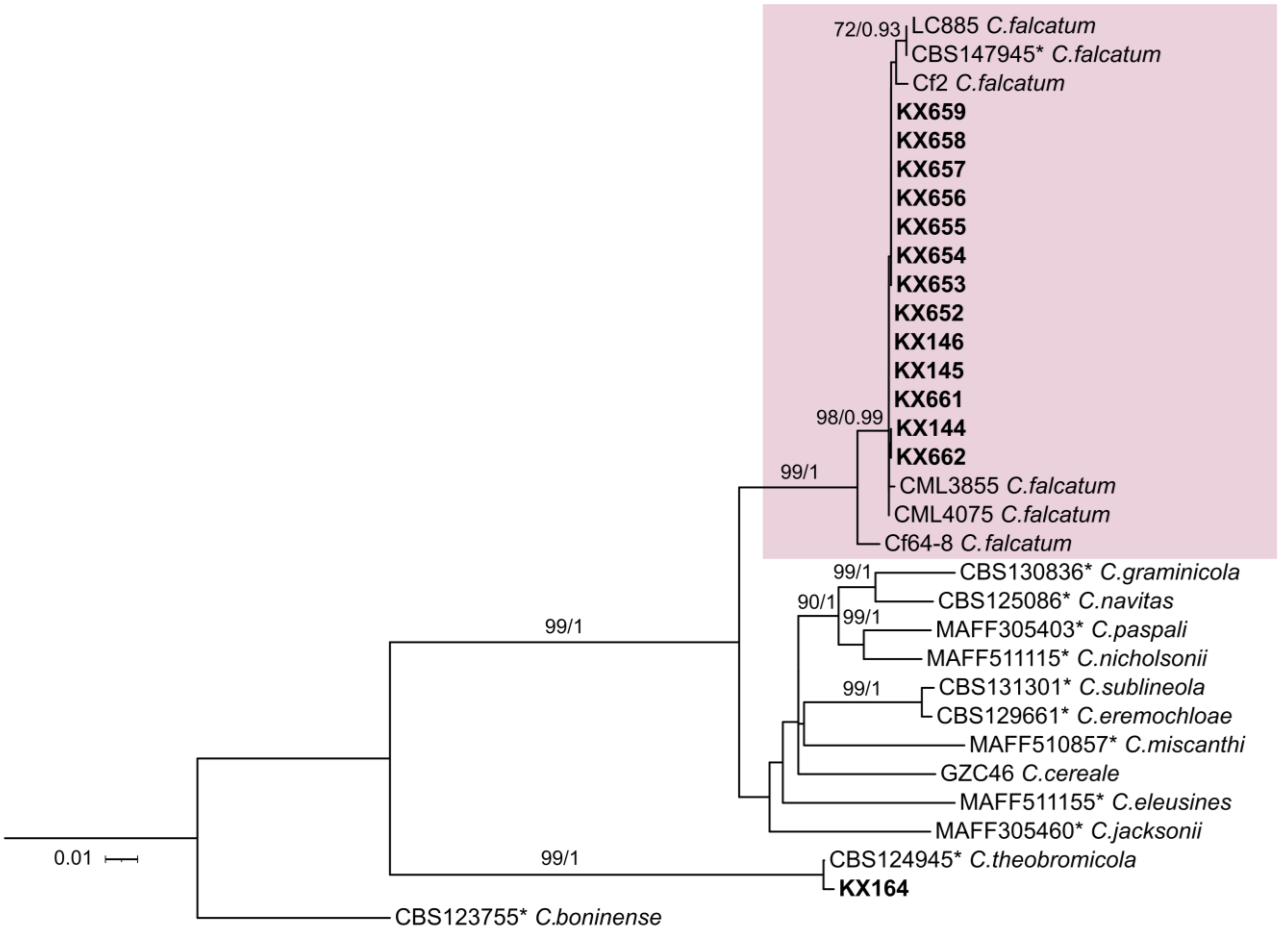

Figure 1. The consensus phylogenetic tree of 32 *Colletotrichum* species strains was generated based on maximum likelihood (ML) and Bayesian inference (BI) analyses of combined ITS, *ACT, TUB2*, *GAPDH*, and *CHS-1* gene sequences. Bootstrap support values and Bayesian posterior probabilities (only values ≥ 70% and ≥ 0.9, respectively) are shown at the nodes. Isolates from this study are highlighted in bold, and ex-type cultures are marked with an asterisk. *Colletotrichum boninense* was used as the outgroup.

3.3. Leaf sheath development assay

At the earliest time point (12 hai), pathogen spores germinated, formed appressoria (Figure 2a, Figure 3a), and infected the host cells. In some instances, primary thick hyphae were already forming (Figure 2b, Figure 3a), indicating the start of biotrophic infection and colonization. By 24 hai, the pathogen had extended its colonization to two cells (Figure 2c, Figure 3b), and at 36 hai, it had spread to three or more cells (Figure 2d, Figure 3c). The formation of the thin secondary hyphae developing from primary thick hyphae was observed at 36 hai (Figure 2e-f, Figure 3c), marking the transition from biotrophic to necrotrophic infection. From 48 to 72 hai, extensive colonization by both thick and thin hyphae was evident across multiple host cells (Figure 2g, Figure 3d). Remarkably, by 146 hai (or 6 days after inoculation), the pathogen had completed its life cycle, with the formation of acervulus, from which conidia were formed and released (Figure 2h, Figure 3 e-f).

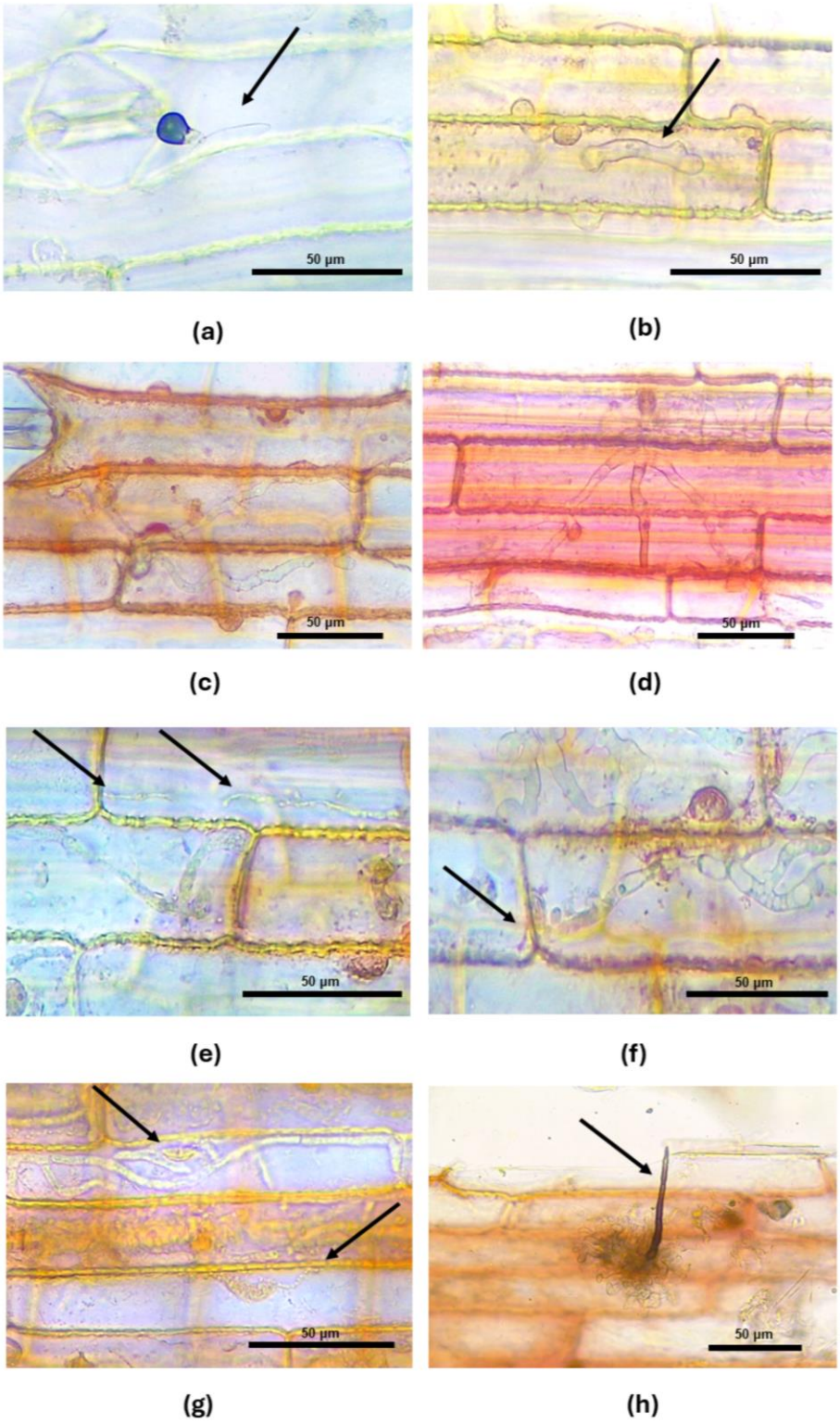

Figure 2. Time course of *C. falcatum* KX144 development in sugarcane leaf sheaths (CL85-1040). **(a)** Germinated spores with appressorium (black arrow) at 12 hai. **(b)** Primary hyphae (black arrow) in one cell at 12 hai. **(c)** Primary hyphae (black arrow) in two cells at 24 hai. **(d)** Primary hyphae (black arrow) in three or more cells at 36 hai. **(e-g)** Secondary hyphae (black arrows) and shifting from biotrophic to necrotrophic phase at 36-48 hai. **(h)** Acervulus with setae (black arrow) at 146 hai.

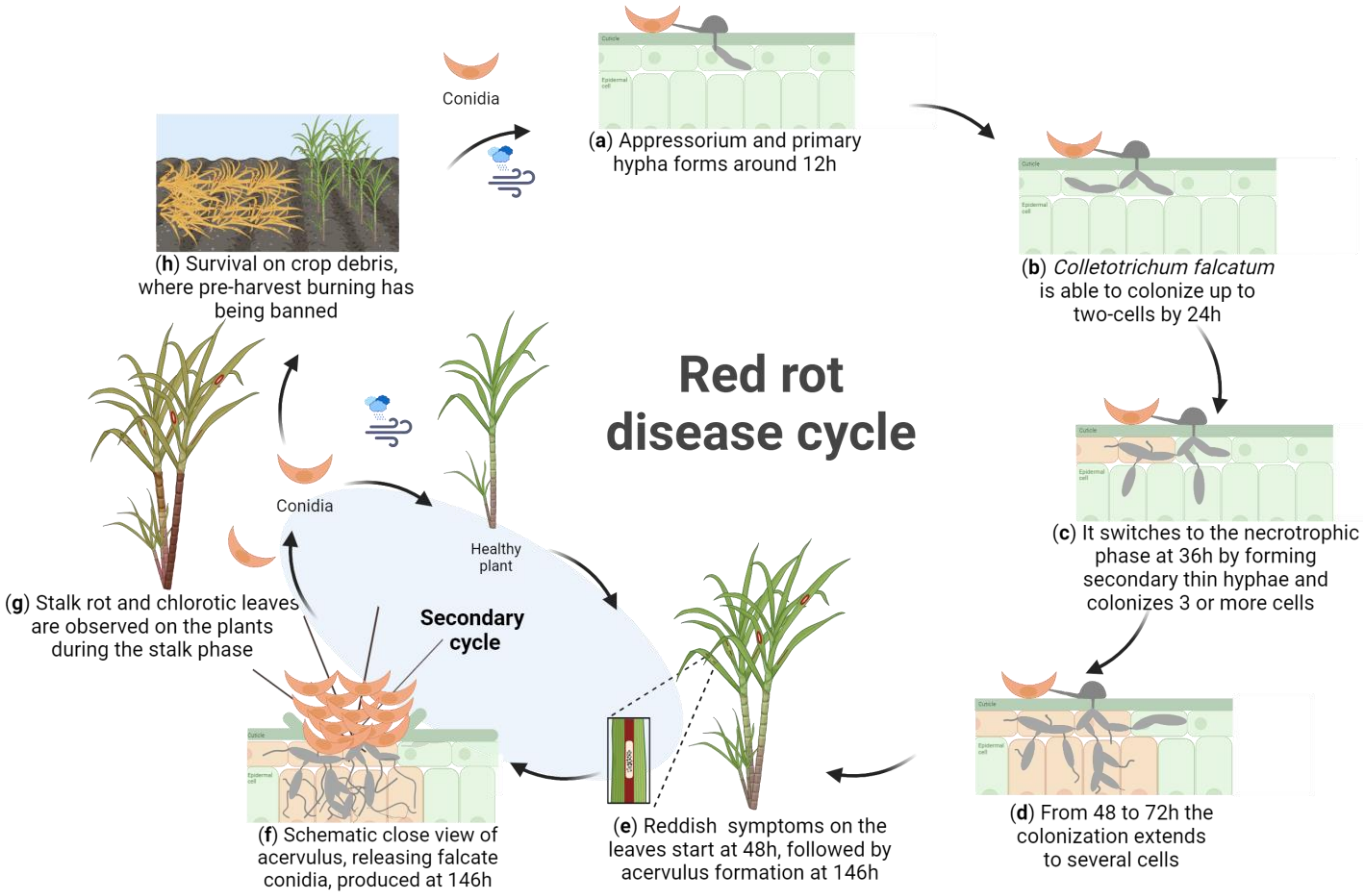

Figure 3. Scheme of red rot disease cycle on sugarcane: **(a-f) Foliar phase**: **(a)** *Colletotrichum falcatum* spore germinates, forms appressorium, and primary hyphae by 12 hai, **(b)** primary hyphae colonizes up to two cells by 24 hai, **(c)** thin secondary hyphae forms by 36 hai, **(d)** colonization of thick and thin hyphae continues to several cells from 48 to 72 hai, **(e)** first reddish lesions visible on the leaves at 48 hai, acervuli are produced around 146 hai. **(e-h) The stalk phase** [28,69]**: (e)** *Colletotrichum falcatum* spores infect the stalk, initiating the colonization of internal tissues. **(f)** The symptoms of red rot in the stalks become visible, and **(g)** may lead to stalk collapse. During the secondary cycle, spores can be released by rain splashes and disseminated by wind to healthy plants in the same season or **(h)** survive in crop debris [69], becoming a source of inoculum in subsequent seasons [28]. Created with BioRender.com.

3.4. Pathogenicity tests

3.4.1. Leaf sheath pathogenicity assay

The leaf sheath time course assay above revealed that colonization beyond two host cells would lead to a compatible interaction. Furthermore, no significant differences were observed between 48, 60, and 72 hai. Therefore, the virulence of strains KX144, KX145, and KX146 on the variety CL85-1040 was assessed at 60 hai. In addition, the assessment of plant responses revealed distinct infection sites characterized by hyphae constricted within a single cell (Figure 4a). Additionally, some sites were observed to be surrounded by red vesicles, indicating active defense mechanisms at play (Figure 4b).

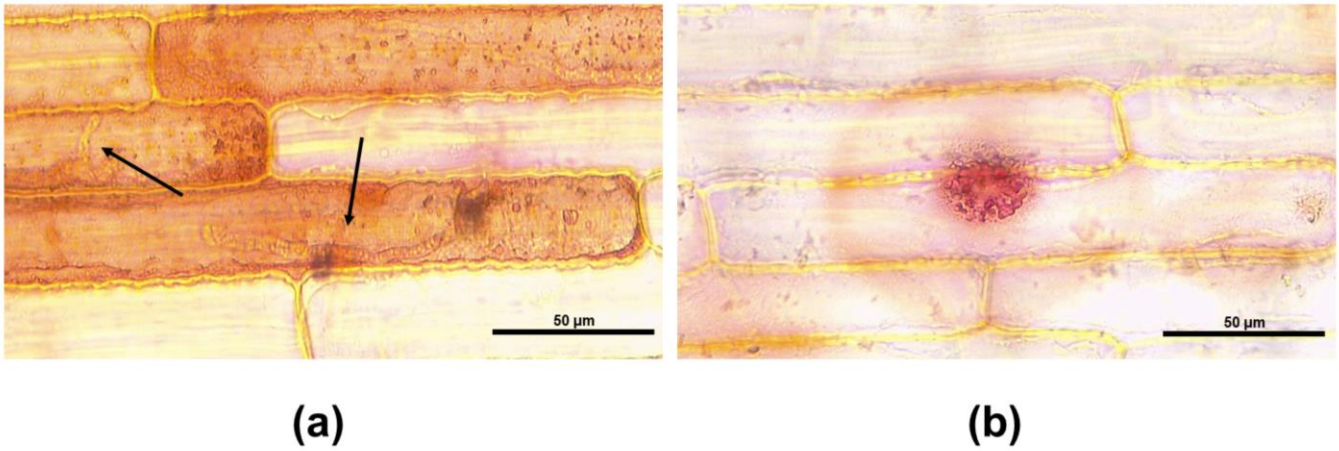

Figure 4. Plant responses to *Colletotrichum* infection in detached sugarcane leaf sheaths (CL85-1040) inoculated with strain KX164 from celery. **(a)** Primary hyphae (black arrows) in one cell. **(b)** Red vesicles around the infection site.

There were no significant differences between the two experimental replications (p = 0.7247), allowing the data to be combined for further analysis. Our results indicated that strain KX144 exhibited the greatest virulence, with a higher capacity to colonize more than two cells (p < 0.0001) (Figure 5). Correspondingly, this strain triggered the lowest observed plant response. Conversely, the negative control (KX164) caused the strongest plant response. Strains KX145 and KX146 demonstrated similar behavior and were not statistically different from each other, suggesting a lower level of virulence compared to KX144 (Figure 5).

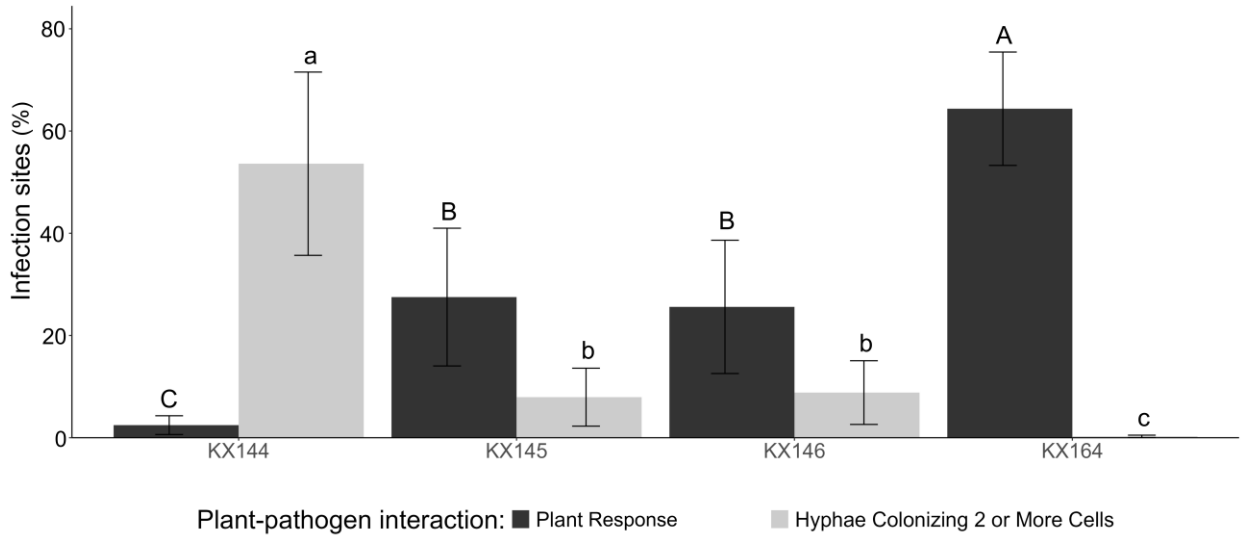

Figure 5. Plant response and pathogen colonization in sugarcane leaf sheaths (CL85-1040) at 60 hai. Different letters indicate significant differences (p < 0.05) based on the CLMM: uppercase letters for plant responses and lowercase letters for pathogen colonization.

3.4.2. Whole seedling spray inoculation

The pathogenicity of Florida *Colletotrichum falcatum* strains was evaluated in sugarcane whole seedlings in the greenhouse. The first

symptoms of red rot, characterized as small reddish lesions, were observed on the midrib and leaf blade at 48 hai, when the plants were removed from the humidity chambers. Overall, the symptoms gradually increase in size, forming pale necrotic lesions in the center, where black dots (acervuli) first appeared at 4, 5, and 7 days in experiments 1, 2, and 3, respectively, indicating that the pathogen can complete its life cycle as early as 4 days after inoculation (Figure 6). However, there were some specific symptoms that were characteristic among the isolates regardless of the sugarcane variety inoculated. Isolate KX144 caused oval-shaped lesions with a light pale center surrounded by dark red to brown borders on lesions found both on the leaf blade and midrib (Figure 6a). Plants inoculated with isolate KX146 displayed round lesions, red to orange, with a pale center primarily on the midrib (Figure 6c). Whereas isolate KX145 induced the formation of fine, elongated reddish lesions mainly on the leaf blade of newly developed leaves (Figure 6b).

In summary, all three strains, KX144 from CL85-1040, KX145 from CP96-1252, and KX146 from CP89-2143, exhibited pathogenicity on all sugarcane varieties tested in this study. Re-isolations from symptomatic leaves confirmed that red rot was caused by the inoculated strains. No pathogen was recovered from the control treatments. The presence of banana-shaped conidia (Figure 6d-f) in the reisolated cultures fulfilled Koch's postulate.

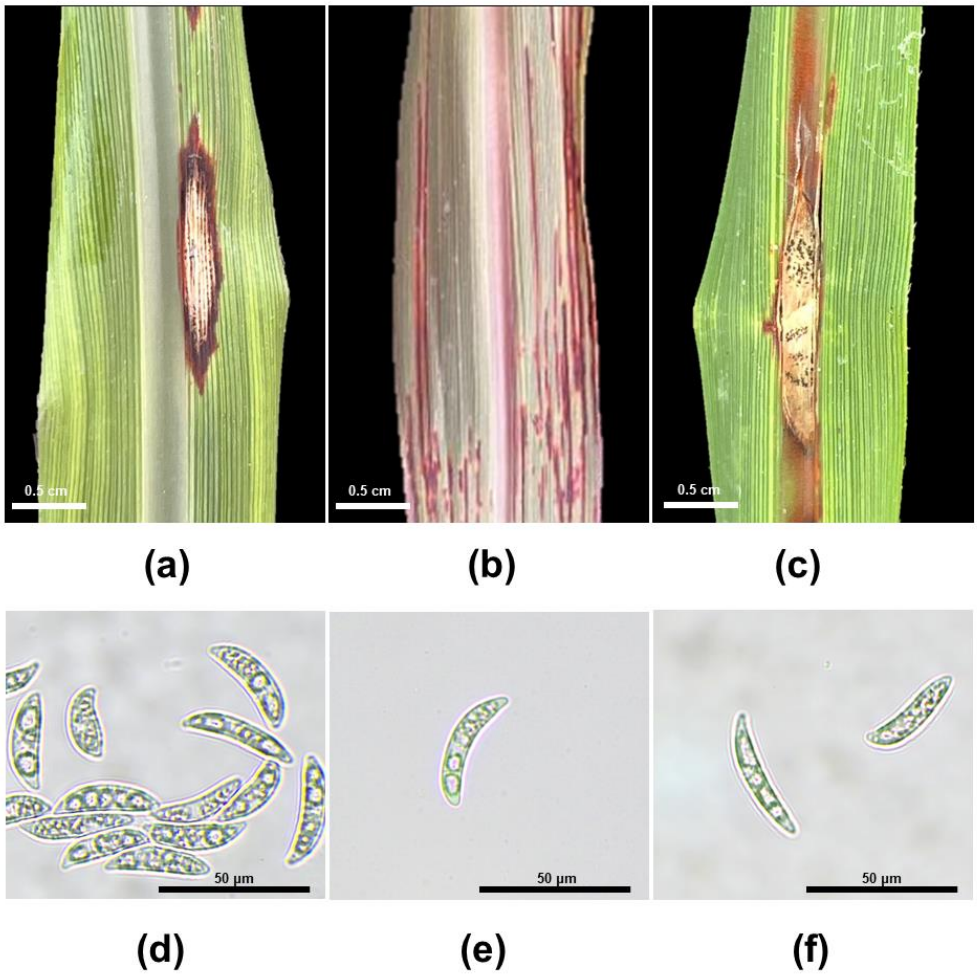

Figure 6. Symptoms of red rot sugarcane varieties inoculated with their respective *Colletotrichum falcatum* strains: (a) KX144 on CL85-1040, (b) KX145 on CL96-1252, (c) KX146 on CL89-2143. Re-isolated banana-shaped conidia of each strain: (d) KX144, (e) KX145, (f) KX146.

The GLMM model indicated that the Area Under Disease Progress Curve (AUDPC) was significantly affected by fungi ($p < 0.0001$) and fungi-variety interaction ($p = 0.0014$). Based on the AUDPC, strain KX144 isolated from variety CL85-1040 was the most virulent among the fungi tested. The exception was on the variety CP89-2143, in which there was no statistical difference among the evaluated strains. The strains KX145 and KX146 showed similar virulence among the varieties. The negative control KX164 was not significantly different from plants sprayed with water (Tween 1%), which is not shown in the graph (Figure 7).

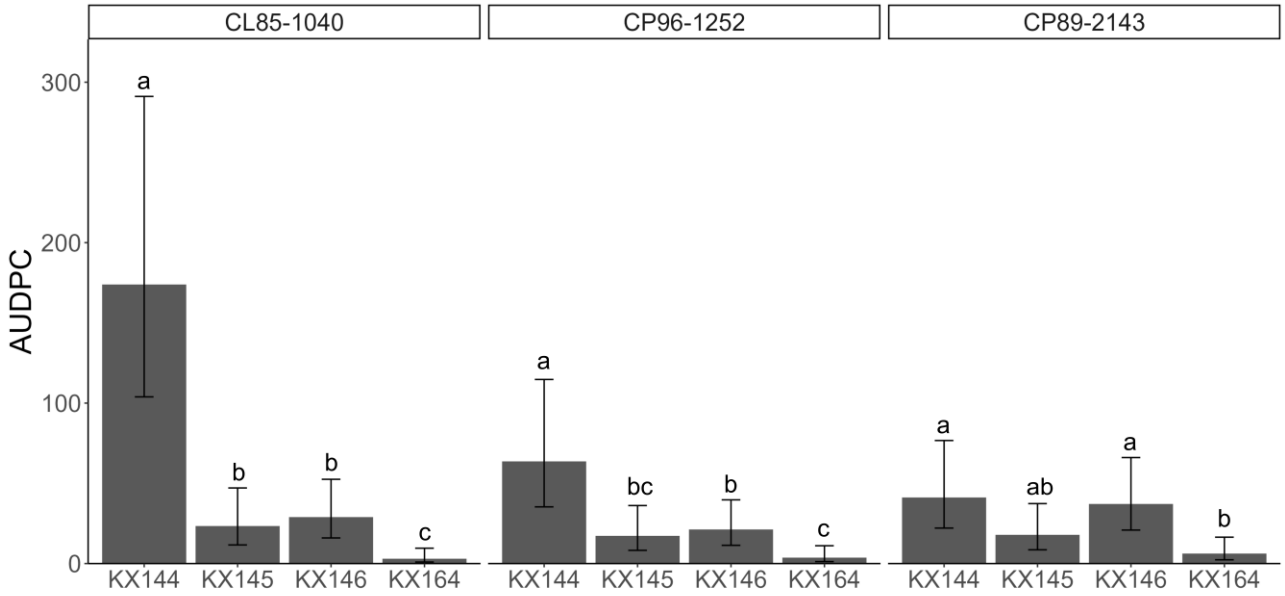

Figure 7. Area Under Disease Progress Curve (AUDPC) of three combined experiments. Different letters represent statistically different ($p < 0.05$) calculated by the generalized linear-mixed models (GLMMs).

To assess resistance levels among the three sugarcane varieties, AUDPC values were calculated for each variety based on the combined disease severity caused by all three strains (KX144, KX145 and KX146). Varieties CP96-1252 and CP89-2143 exhibited significantly lower AUDPC values compared to variety CL85-1040, indicating greater resistance to red rot (Figure 8).

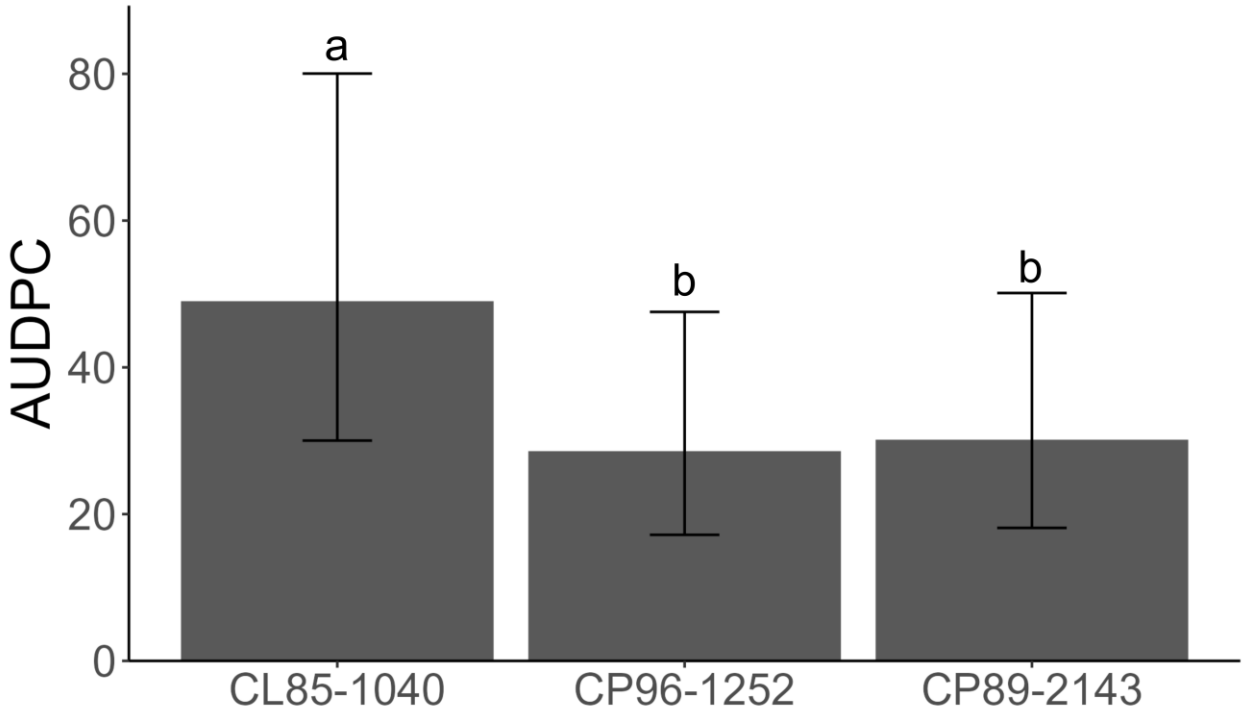

Figure 8. Area Under Disease Progress Curve (AUDPC) comparing sugarcane varieties, based on combined ratings for three *Colletotrichum falcatum* strains (KX144, KX145, KX146). Different letters represent statistically different ($p < 0.05$) determined by the generalized linear-mixed models (GLMMs).

4. Discussion

This study presents the first comprehensive characterization of *Colletotrichum falcatum* as the causative agent of red rot on sugarcane in southern Florida. Recent observations at the Everglades Research and Education Center in Belle Glade, Florida, have revealed a concerning incidence of red rot, with infection rates ranging from 60% to 80% during the summer months across local sugarcane varieties. Similar red rot outbreaks have also been reported in other major sugarcane-producing regions, such as India [31] suggesting the global relevance of this disease. Although red rot has been present in Florida for several years, its economic impact on sugarcane yields has remained minimal, especially when compared to other tropical regions like Brazil, where the disease has caused yield losses of up to 24.2% [69]. A key factor that may play a role in mitigating the impact of red rot in Florida is the pre-harvest practice of burning fields, which likely reduces pathogen inoculum by eliminating crop debris between seasons.

This study collected 13 *Colletotrichum* isolates for molecular and pathological characterization, addressing the lack of detailed data on *Colletotrichum* in the region. Multilocus molecular phylogenetic analysis revealed that all 13 isolates from symptomatic sugarcane leaves clustered within the *Colletotrichum falcatum* clade. This identification was supported by using key genetic markers— ITS, *TUB2*, *ACT*, *GAPDH*, and *CHS-1*—based on the established methodologies from earlier studies in the *Colletotrichum graminicola* complex [70,71]. Notably, at least two of these markers have also been employed in recent studies of *C. falcatum* from Pakistan, Bangladesh, and Brazil [72,8,16]. These findings highlight the reliability of these genetic markers in accurately identifying, assessing genetic diversity, and determining evolutionary relationships within *C. falcatum* populations across different regions.

While most previous studies have focused on the stalk phase of red rot [28–30], the present study is the first to target the foliar phase of the fungal life cycle.

The leaf sheath assays provide a unique view into the cellular interaction between pathogens and hosts, as seen in *Colletotrichum sublineola*-sweet sorghum [57] and *Colletotrichum graminicola*-maize [58] pathosystems. We monitored the *C. falcatum* life cycle within sugarcane living tissue at seven time points and developed the red rot disease cycle from spore germination to pathogen sporulation. Our observations on the strain KX144 align with the established hemibiotrophic nature seen in other *Colletotrichum* species [33]. Initially, this fungus colonizes as a biotroph, acquiring nutrients from living host cells before switching to a necrotrophic phase, where it feeds on dead tissues [33]. Spores of *C. falcatum* germinate and form primary hyphae at 12 hai (Figure 2b, Figure 3a). This primary hypha is similar to haustoria in biotrophic pathogens [73], likely facilitating nutrient acquisition and secretion of effector molecules into the host cell cytoplasm, promoting infection and/or inducing a plant response during the initial biotrophic phase [73,74]. By 24 hai, we observed colonization of up to two plant cells (Figure 2c, Figure 3b). This finding aligns with Xavier et al. [57] that proposed this threshold to separate compatible (≥2 colonized cells) from incompatible interactions (restricted to one cell) studying *C. sublineola* on sorghum plants. The transition from biotrophy to necrotrophy for strain KX144 was observed at 36 hai, marked by the development of secondary hyphae (Figure 2e-f, Figure 3c). Notably, the complete *C. falcatum* life cycle, including acervuli formation (conidia-bearing structures), was achieved within 146 hai (6 days) (Figure 2h, Figure 3e-f). These findings from the leaf sheath assay mirrored those observed from whole seedling inoculations, where the life cycle was completed within 4 to 7 days. *Colletotrichum falcatum* manifests a significant impact on the sugarcane stalks in what is known as the stalk phase. Previous research indicates that *C. falcatum* spores can infect the stalk through wounds or runoff from the midrib, initiating colonization of the internal tissues (Figure 3e) [28]. As symptoms of red rot become visible in the stalks (Figure 3f), the disease may ultimately lead to stalk collapse (Figure 3g). Spores produced on the leaves, midrib, and stalks can overwinter on crop debris, contributing to the disease cycle by allowing falcate spores to be released by rain splashes and disseminated by wind to healthy plants in the same season or by surviving in crop debris to become a source of inoculum in subsequent seasons (Figure 3h) [28,69]. Understanding the complete disease is crucial for developing a management strategy that addresses both foliar and stalk impacts on sugarcane.

We reconstructed the key events of the *C. falcatum* foliar life cycle in sugarcane by systematically analyzing 50 infection sites per repetition. It is crucial to note that multiple infection processes often occur simultaneously, sometimes within the same leaf sheath. In some instances, plant cells exhibited strong responses to pathogen infection, while in others, the pathogen colonized several plant cells, as demonstrated in the virulence assay. This study significantly advances our understanding of *C. falcatum*'s foliar life cycle and pathogenicity in sugarcane. By employing the leaf sheath assay, we were able to identify key stages of infection in real time, capturing details of both the biotrophic and necrotrophic phases. This approach offers advantages over previous studies using GFP-tagged *C. falcatum* to explore host-pathogen interactions [75] as our method uses intact leaf sheaths, which minimizes biases introduced by artificial injuries required in GFP assays. Furthermore, our study allowed us to observe the natural behavior of *C. falcatum* and host cells, providing a clearer view of their interactions.

To better understand the responses of sugarcane plants against *Colletotrichum,* we used a *C. theobromicola* isolate (KX164) from celery, which is non-pathogenic to sugarcane. These responses included hyphae confinement or red vesicle formation (Figure 4), serving as a marker for virulence assessment at the cellular level. Microscopic observations from the leaf sheath assay and macroscopic symptom development in the greenhouse experiments consistently demonstrated that strain KX144 exhibited the highest virulence (Figure 5, Figure 7). It successfully colonized multiple cells and caused the most severe symptoms (oval lesions with dark borders) on the susceptible sugarcane variety CL85-1040. Conversely, stains KX145 and KX146 showed intermediate virulence, reflected in both their colonization ability and the disease they induced (Figure 5). Notably,

all three strains exhibited distinct disease symptom profiles when inoculated on their original hosts or alternative sugarcane varieties (Figure 6), highlighting potential strain-specific interactions and the influence of host genotype on disease development. This specificity in *C. falcatum* has been associated with the breakdown of varieties due to the emergence of new pathotypes [25,26,76]. It could be attributed to many factors, such as the host of origin, adaptation to the host, environmental conditions [25], and different production of hydrolytic enzymes [8,77]. Genetic diversity in the *C. falcatum* population manifests as distinct pathotypes [8,25], which can complicate disease management and breeding for resistance. While the limited scope of this study (sampling and variety testing) restricts definitive conclusions, the observed variation in virulence among the three *C. falcatum* strains suggests the potential presence of different races in Florida. Further investigation is required to definitively identify which resistant genes are involved that could potentially be different races in this pathogen population. Additionally, the varieties CP96-1252 and CP89-2143 demonstrated greater resistance compared to CL85-1040 (Figure 8). Given that red rot resistance is one of the criteria for varietal resistance for sugarcane commercial cultivation in other regions [78], future studies on the plant-host interaction mechanisms underlying this resistance could provide valuable insights for breeding if red rot becomes an epidemic in southern Florida in the future.

In conclusion, only one species of *Colletotrichum, C. falcatum*, was found associated with sugarcane in southern Florida. Therefore, it is essential to increase the survey area to represent the entire sugarcane growing area in the state, as well as other states. Additionally, it is important to investigate if there are different races within this pathogen population in Florida, as we observed a high degree of pathological diversity among the three evaluated strains when causing red rot under greenhouse conditions on local sugarcane varieties. This diversity can have significant implications for breeding programs. Furthermore, screening a wider range of sugarcane varieties for resistance is crucial to developing effective management strategies. Future research should also focus on fungicide resistance, increasing sample sizes for more comprehensive analysis, and exploring further resistance mechanisms in sugarcane varieties.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: Maximum likelihood (ML) tree of 32 *Colletotrichum* spp. strains based on ITS region. The tree generated by Bayesian inference (BI) had a similar topology. Figure S2: Maximum likelihood (ML) tree of 32 *Colletotrichum* spp. strains based on *ACT* gene. The tree generated by Bayesian inference (BI) had a similar topology. Figure S3: Maximum likelihood (ML) tree of 29 *Colletotrichum* spp. strains based on *TUB2* gene. The tree generated by Bayesian inference (BI) had a similar topology. Figure S4: Maximum likelihood (ML) tree of 26 *Colletotrichum* spp. strains based on *GAPDH* gene. The tree generated by Bayesian inference (BI) had a similar topology. Figure S5: Maximum likelihood (ML) tree of 27 *Colletotrichum* spp. strains based on *CHS-1* gene. The tree generated by Bayesian inference (BI) had a similar topology.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Supplementary material

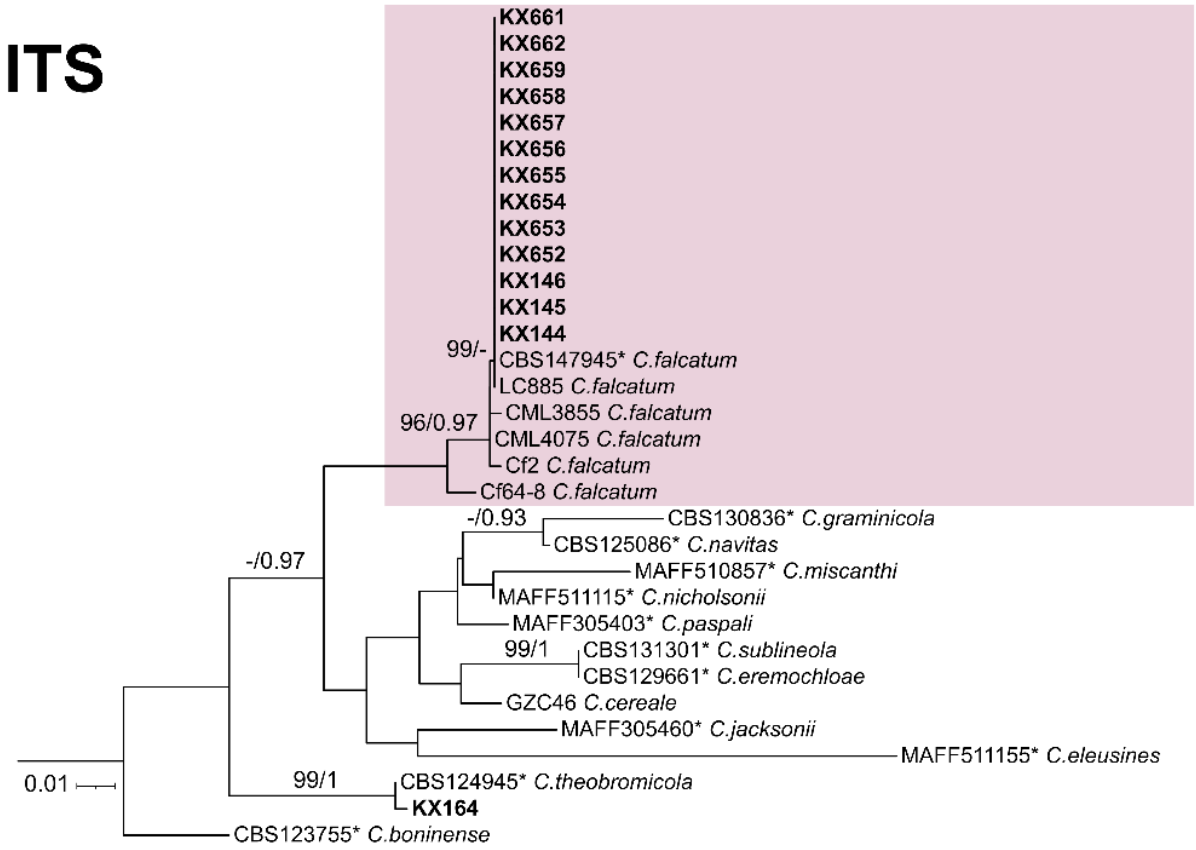

Figure S1. Maximum likelihood (ML) tree of 32 *Colletotrichum* spp. strains based on ITS region. The tree generated by Bayesian inference (BI) had a similar topology.

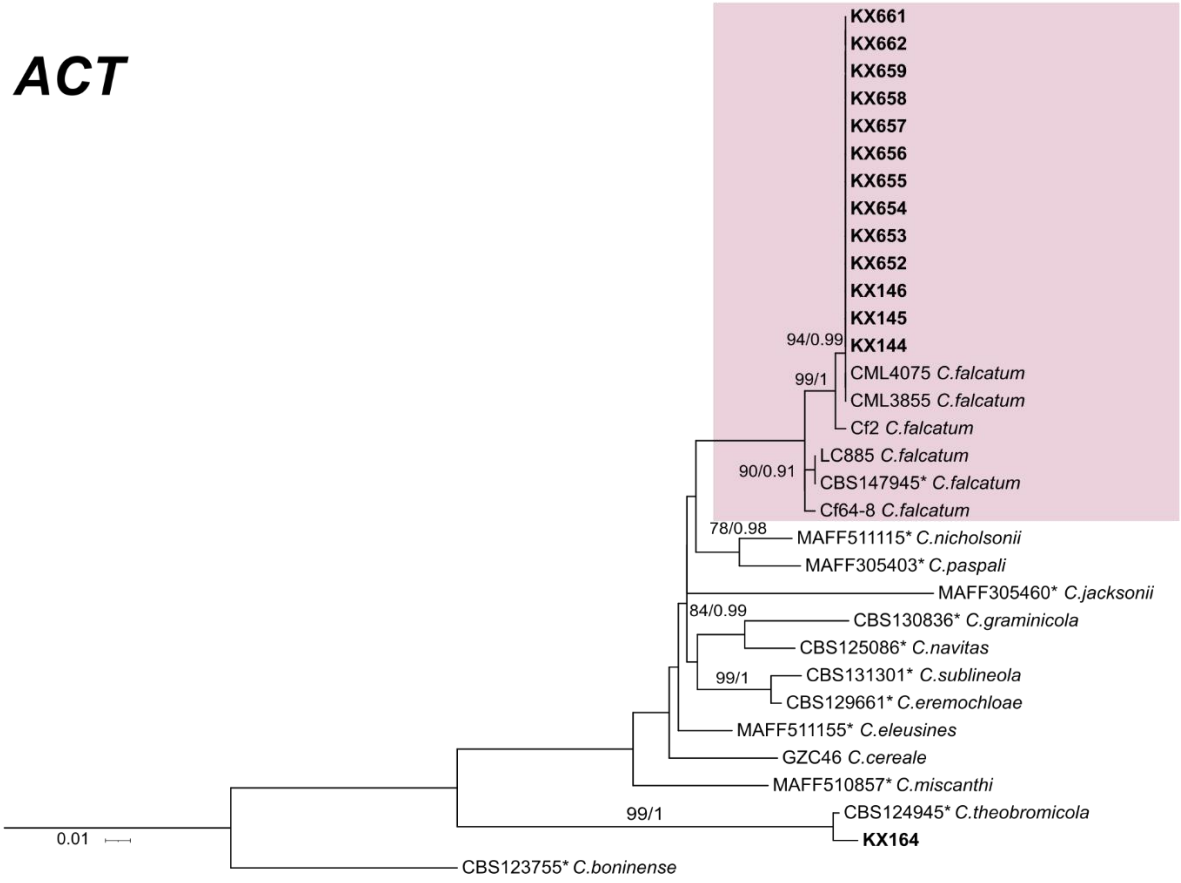

Figure S2. Maximum likelihood (ML) tree of 32 *Colletotrichum* spp. strains based on *ACT* gene. The tree generated by Bayesian inference (BI) had a similar topology.

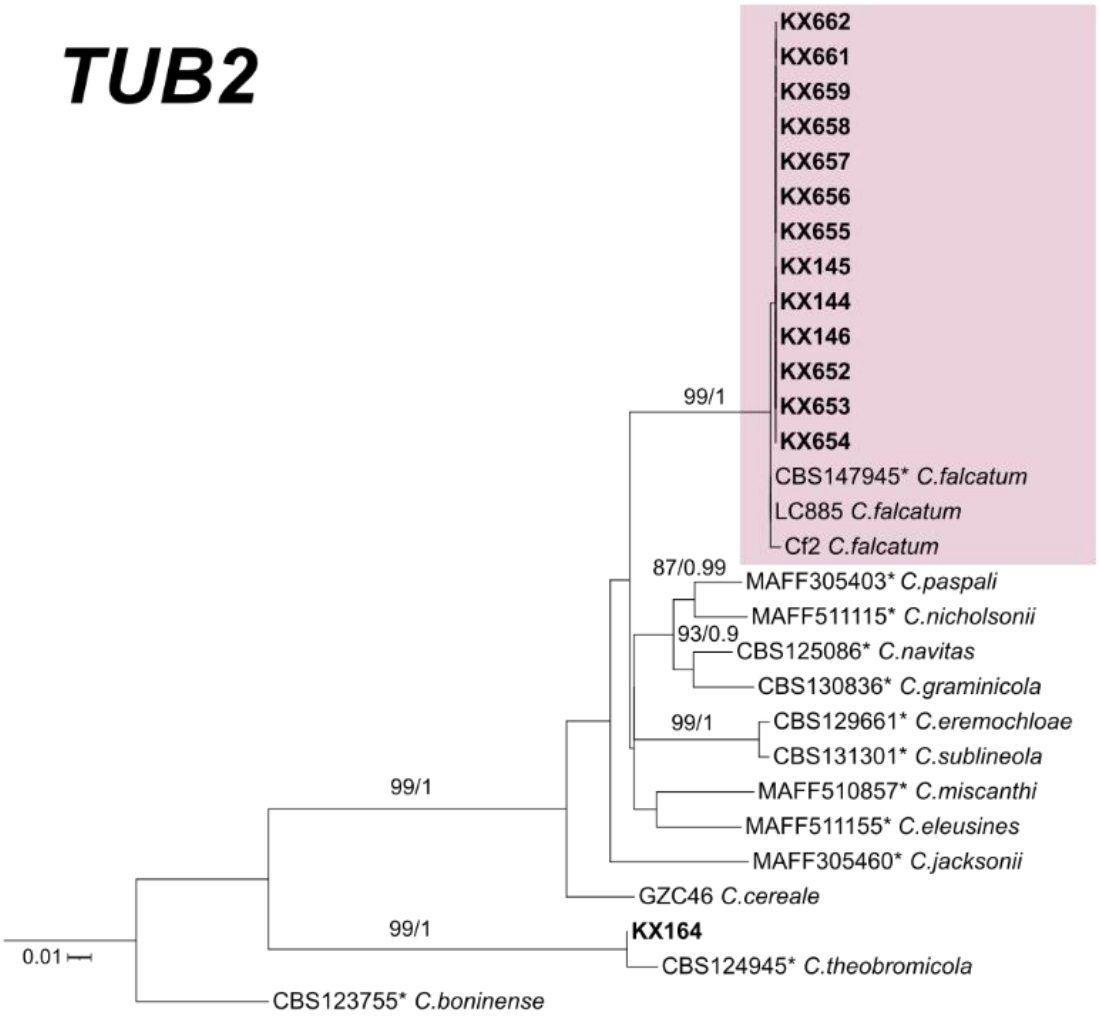

Figure S3. Maximum likelihood (ML) tree of 29 *Colletotrichum* spp. strains based on *TUB2* gene. The tree generated by Bayesian inference (BI) had a similar topology.

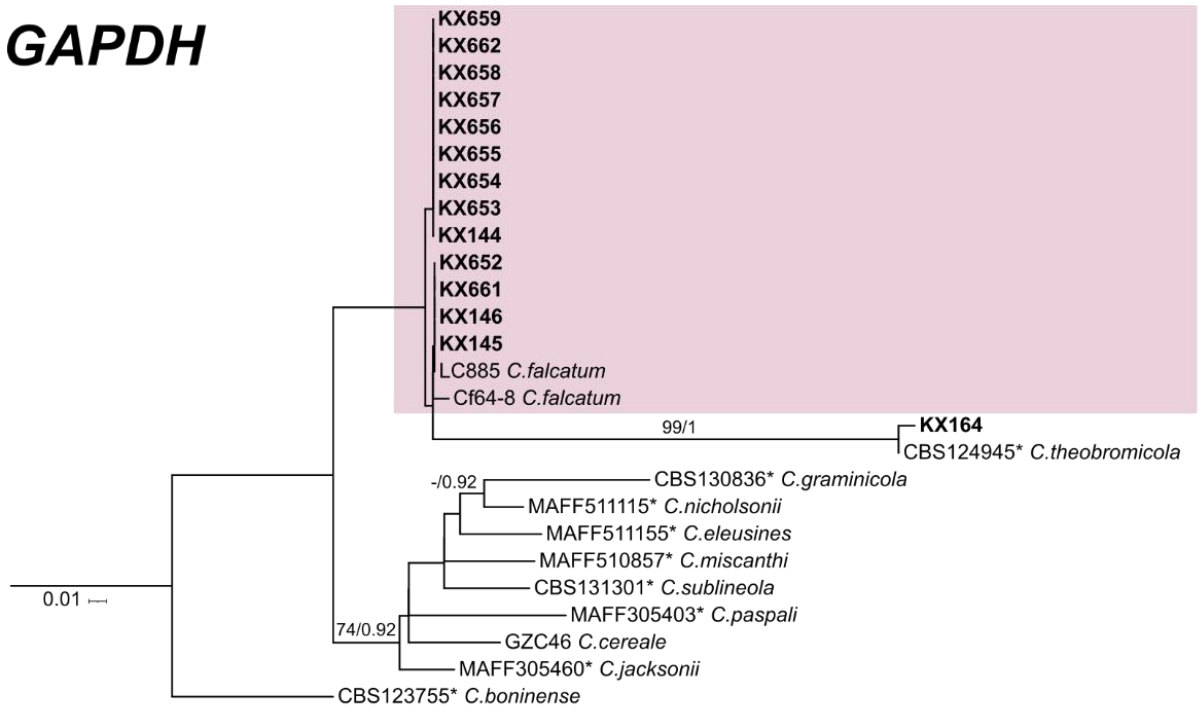

Figure S4. Maximum likelihood (ML) tree of 26 *Colletotrichum* spp. strains based on *GAPDH* gene. The tree generated by Bayesian inference (BI) had a similar topology.

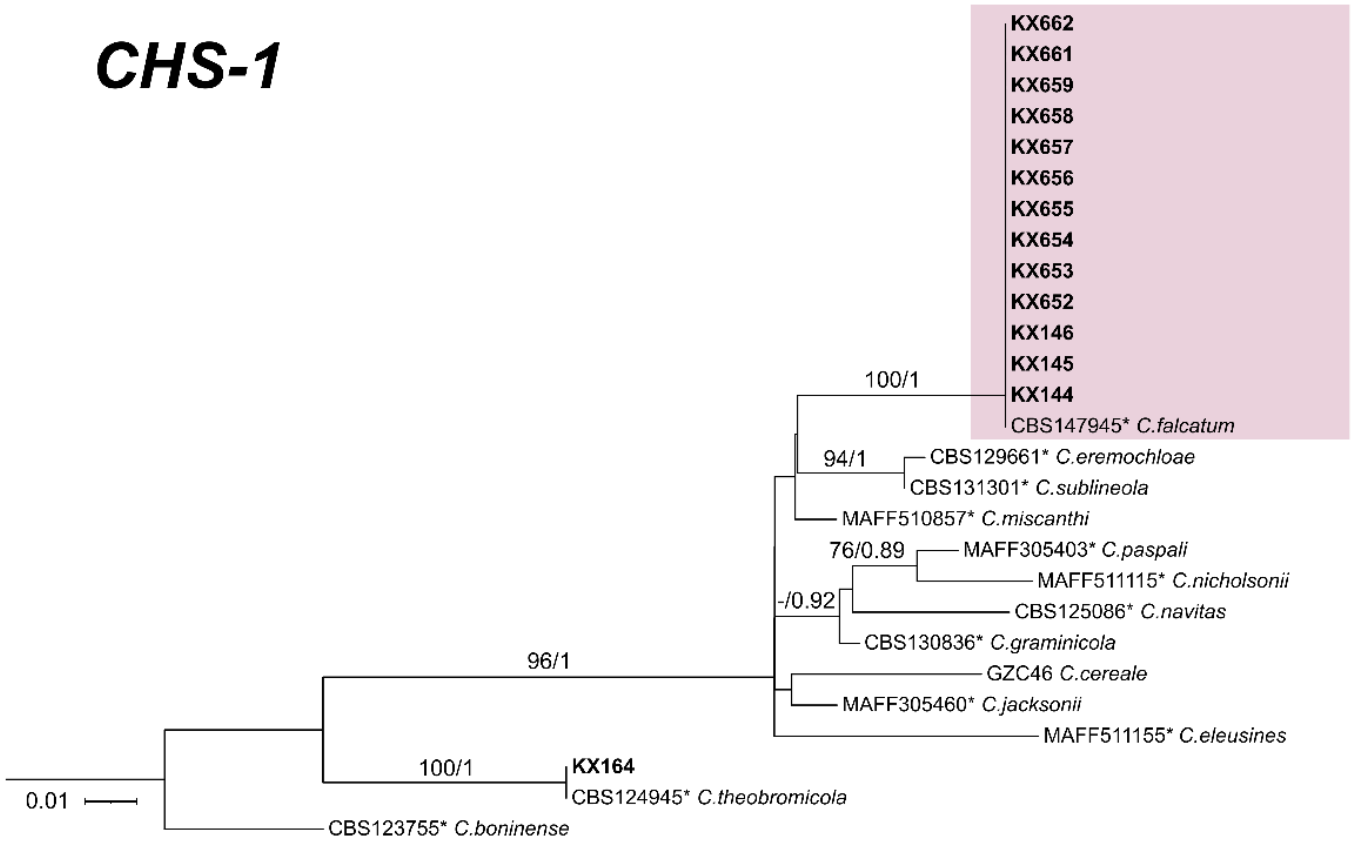

Figure S5. Maximum likelihood (ML) tree of 27 *Colletotrichum* spp. strains based on *CHS-1* gene. The tree generated by Bayesian inference (BI) had a similar topology.

Artigo 2- Redigido conforme a norma do periódico científico- Versão preliminar	
Título do artigo:	Genome-wide analysis reveals chitinases as putative defense-related proteins against fungi in the genomes of *Coffea arabica* and its progenitors
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ARTIGO 2– Genome-wide analysis reveals chitinases as putative defense-related proteins against fungi in the genomes of *Coffea arabica* and its progenitors

Article

Genome-wide analysis reveals chitinases as putative defense-related proteins against fungi in the genomes of *Coffea arabica* and its progenitors

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Abstract: Chitinases have been demonstrated to enhance plant resistance to fungi in various pathosystems. Although there is evidence of the effectiveness of these proteins in coffee-fungus interactions, no genome-wide identification or characterization of coffee chitinases has been performed. In this study, we employed phylogenetic analysis, domain architecture, gene structure analysis, and subcellular localization to identify and characterize putative genes and proteins in the genomes of *Coffea arabica* and its progenitors, *Coffea canephora* and *Coffea eugenioides*. A total of 113, 47, and 69 putative chitinase proteins were identified in *C. arabica*, *C. canephora*, and *C. eugenioides*, respectively. These chitinases were classified according to their catalytic domains, GH18 and GH19, and into Classes I, II, III, IV, and V, as determined through phylogenetic analysis based on the *Arabidopsis thaliana* classification. Furthermore, based on orthologous analysis, we identified ten, six, and seven putative chitinases associated with fungal defense responses in *C. arabica*, *C. chanephora*, and *C. eugenioides*, respectively. These findings are valuable for future studies focusing on coffee chitinases, particularly on genetic programs involved in plant pathogen resistance.

Keywords: Fungal resistance 1; *Coffea arabica* 2; *Coffea canephora* 3; *Coffea eugenioides* 4; molecular characterization 5

1. Introduction

Coffee is one of the most economically significant commodities globally. In the 2023/2024 season, total production reached 178.0 million bags [1]. Among *Coffea* species, *C. arabica* (Arabica coffee) and *C. canephora* are noteworthy, comprising 57.4% and 42.6% of global commercial coffee production, respectively [1]. *Coffea arabica* is allotetraploid (2n = 4x = 44), distinguishing it from other *Coffea* species that are diploid (2n = 2x = 22) [2]. It originated from a single natural hybridization event between *C. canephora* and *C. eugenioides* [3]. Despite being allotetraploid, this species has diploid meiotic behavior and follows disomic inheritance [4,5].

Overall, *C. arabica* is considered to have superior drinking quality compared to *C. canephora* due to its lower caffeine and higher sucrose content [6,7]. *Coffea arabica* is cultivated in tropical and subtropical regions across Africa, the Caribbean, Central

America, Mexico, South America, Asia, and Oceania. Brazil is the largest producer of Arabica coffee, accounting for 56.27% of global production [8]. The cultivation of coffee is highly sensitive to climate conditions, posing significant challenges in tropical regions where climate change contributes to production losses, reduced yields, compromised quality, and an increased incidence of pests and diseases [9,10]. Various fungal diseases significantly impact coffee production worldwide [11]. Although coffee producers have adopted resistant cultivars to manage fungal diseases such as Coffee leaf rust (*Hemileia vastatrix*) [12], resistant cultivars for other diseases, such as brown eye spot (*Cercospora coffeicola*), have yet to be developed [13]. In this context, studying plant defense mechanisms in coffee is particularly relevant, since fungal pathogens remain a major challenge to coffee production.

Understanding plant defense mechanisms is crucial in the pursuit of disease resistance. Currently, the plant's immune system is described through two main lines of defense [14,15]. In the first, the recognition of molecules common to microorganisms (microbe- or pathogen-associated molecular patterns, MAMPs/ PAMPs), such as fungal chitin, initiates PAMP-triggered immunity (PTI) [16]. The second line of plant defense is triggered by the recognition of effector molecules and is known as effector-triggered immunity (ETI). Effectors are typically molecules that enhance the virulence of pathogens [14]. Despite involving different ligands, PTI and ETI activate signaling pathways that are frequently overlapping and interconnected, forming a continuum of signaling [17,18]. These signaling events result in plants activating defense responses, including the production of reactive oxygen species (ROS), antimicrobial compounds, and pathogenesis-related proteins (PRs) such as antioxidant and hydrolytic enzymes [18,19].

Hydrolytic PRs, such as chitinases, exhibit constitutive activity and demonstrate heightened activity in response to biotic and abiotic stresses [20]. These proteins display high diversity and functionality, playing critical roles in various cellular processes such as growth, development, and stress responses, particularly in defense against phytopathogens [21]. Chitinases catalyze the hydrolysis of β-1,4 bonds in chitin [22], inhibiting pathogen growth and establishment. Moreover, by breaking down chitin, these enzymes release PAMPs, which are subsequently recognized by the plant's immune system, thereby activating the defense response [19].

Chitinases are distributed across various plant chromosomes, and their classification is based on amino acid sequence similarity, three-dimensional structures, and catalytic mechanisms [23–25]. They are initially divided into two groups according to their catalytic domains: GH18 (Glyco_hydro_18) and GH19 (Glyco_hydro_19). Subsequently, they are categorized into five Classes (I, II, III, IV, and V) based on amino acid sequences, conserved motifs, and the presence of a chitin-binding domain (CBD) [26,27]. Most plant chitinases belong to Classes I, II, and IV, which are part of the GH19 family [20,22].

Class I proteins contain a CBD, a GH19 catalytic domain, and a C-terminal extension. Class II proteins are homologous to Class I but lack the CBD [28], whereas Class IV proteins possess a shorter CBD and catalytic region due to deletions [29]. Throughout evolution, several chitinases have lost their ability to hydrolyze or bind chitin [21]. The GH18 group exhibits lower antifungal activity compared to GH19 [30,31] and includes endochitinases that lack a CBD and contain a GH18 catalytic domain. These chitinases are classified into Classes III and V. Class III chitinases feature a C-terminal region and function similarly to lysozymes [26].

Protein chitinases vary in number and mode of action across different species [27,32,33]. Duplication events can alter the genetic composition of chromosomal segments and modify the functions of their gene products [34]. Chitinase families have evolved and expanded through tandem and segmental duplication events, contributing to their high functional diversity [27,33,35]. This diversity is closely related to resistance to biotic stresses and to coevolutionary dynamics between plants and pathogens [36].

Chitinases have been described as a promising source of plant resistance to fungal pathogens through overexpression and genetic transformation [37–40]. In coffee, fungal diseases such as leaf rust and brown eye spot remain among the main threats to

production. Chitinases are directly involved in antifungal defense by degrading the chitin present in fungal cell walls, which makes them particularly relevant for coffee pathosystems. Proteomic analyses have revealed differences in chitinase expression between *C. arabica* cultivars resistant and susceptible to *H. vastatrix* [37,38]. However, the functional characterization and genomic annotation of many of these genes/proteins remain limited. Characterizing gene families, such as chitinases, provides insights into their evolutionary and functional relationships, which can inform genetic improvement programs aimed at enhancing resistance to fungal diseases in coffee. In this context, the current study aimed to characterize the chitinase genes and proteins in the genomes of *C. arabica* and its diploid progenitors, *C. canephora* and *C. eugenioides*.

2. Results

2.1. Identification of Putative Chitinase

To characterize the chitinase gene family in coffee, searches were performed using genome-wide HMM profiles of the conserved chitinase domains Glyco_hydro_18 (PF00704) and Glyco_hydro_19 (PF00182), from the Pfam database [39]. A total of 113, 47, and 69 putative chitinase proteins were identified in *C. arabica* (Ca), *C. canephora* (Cc), and *C. eugenioides* (Ce), respectively, with predicted protein lengths ranging from 84 to 788 amino acids (aa). These proteins are encoded by 110 genes in *C. arabica*, 47 in *C. canephora*, and 66 genes in *C. eugenioides*, with three isoforms in *C. arabica* and three in *C. eugenioides*. Domain-based searches revealed that most proteins contained the GH18 catalytic domain (82 in *C. arabica*, 35 in *C. canephora*, and 48 in *C. eugenioides),* whereas a smaller proportion contained the GH19 domain (28, 12, and 18, respectively) (Table 1).

Table 1. Putative chitinase proteins: number of proteins, protein size range, number of coding genes, and number of coding genes classified into GH18 and GH19 groups.

Coffee species	Proteins	Proteins size	Genes	GH18 genes	GH19 genes
Coffea arabica	113	141-782	110	82	28
Coffea canephora	47	84-788	47	35	12
Coffea eugenioides	69	187-862	66	48	18

2.2. Phylogenetic Analysis

To classify the identified chitinase proteins, a maximum likelihood phylogenetic analysis was performed, which separated the proteins from *C. arabica* (n = 113), *C. canephora* (n = 47), and *C. eugenioides* (n = 69) into three main branches. These branches were further categorized into two major groups (GH18 and GH19) and five classes (I, II, III, IV, and V), following the classification established for *A. thaliana* [40]. In the GH19 group, a total of 58 proteins were assigned to three classes: Class I, with nine proteins (Ca = 4; Cc = 4; Ce = 1); Class II, with 10 proteins (Ca = 5; Cc = 3; Ce = 2); and Class IV, with 39 proteins (Ca = 19; Cc = 5; Ce = 15) (Figure 1, Figure 4).

The GH18 group contained 171 proteins divided into two classes: Class III, with 115 chitinases (Ca = 57; Cc = 23; Ce = 35) and Class V, with 56 proteins (Ca = 28; Cc = 12; Ce = 16) (Figure 1, Figure 5).

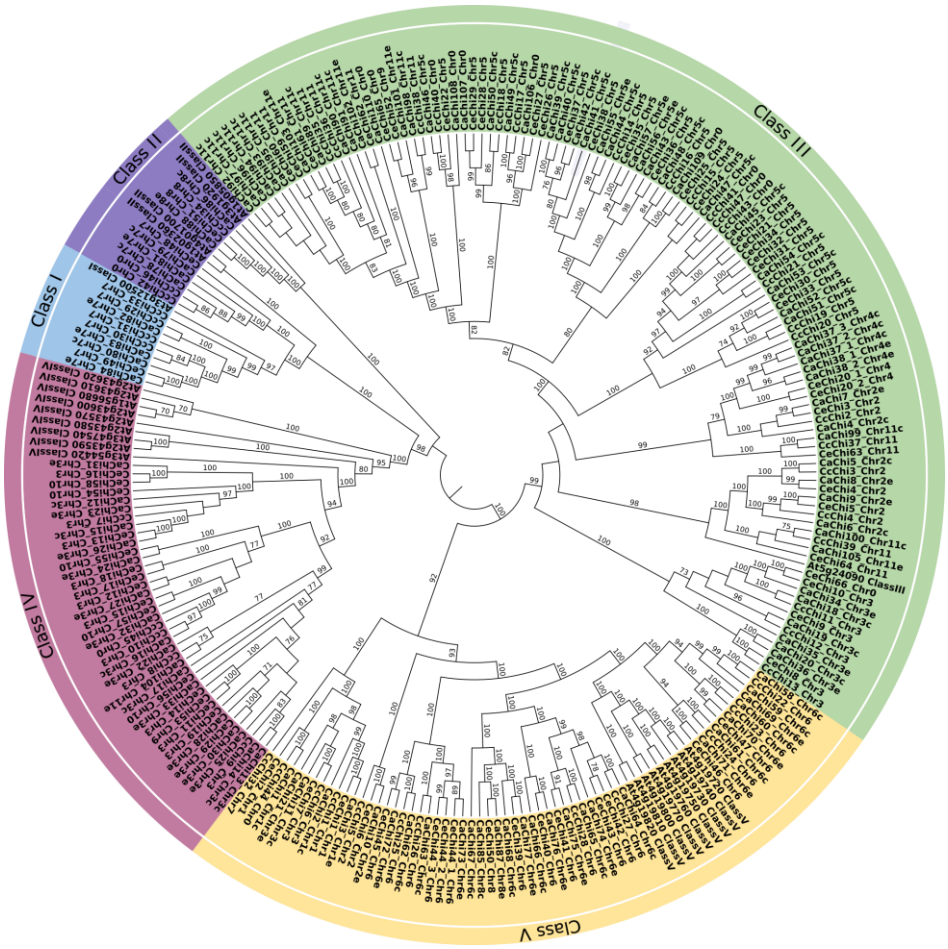

Figure 1. Maximum likelihood phylogenetic tree constructed with putative chitinase proteins from *Arabidopsis thaliana, Coffea arabica, Coffea canephora,* and *Coffea eugenioides*. Proteins were classified into five major classes according to their glycosyl hydrolase families: GH19 (Classes I, II, and IV) and GH18 (Classes III and V), represented in different colors. Bootstrap support values ≥ 70% are shown at the nodes. The *A. thaliana* protein At1g02360 was used as the outgroup.

2.3. Chromosomal Location and Distribution

To investigate the genomic organization of the chitinase family, chromosomal maps were generated for each *Coffea* genome using the start and end positions of the coding genes. For consistency, both genes and their corresponding proteins were named according to the species and chromosomal position: *C. arabica* (CaChi1 to CaChi110); *C. canephora* (CcChi1 to CcChi47) and *C. eugenioides* (CeChi1 to CeChi66). Isoforms were numbered within the same codes (e.g., CaChi37_1 to CaChi37_3; CeChi44_1 to CeChi44_3).

Although *C. arabica* is an allotetraploid species (2n = 4x = 44), its reference genome is represented by 22 chromosomes due to the high similarity of homoeologous pairs derived from its diploid progenitors, *C. canephora* and *C. eugenioides*, each contributing 11 chromosomes (2n = 2x = 22). In *C. arabica*, 105 genes were mapped across 18 chromosomes (Chr) of the two subgenomes: 60 genes in the *C. canephora* subgenome and 45 genes in the *C. eugenioides*. Notably, no genes were detected on chromosomes nine and ten of either subgenome, and five genes (CaChi106 to CaChi110) were not annotated as being anchored to any specific chromosome. Genes from the same chitinase classes were found on homologous chromosomes of both subgenomes: Class I (Chr7), Class II (Chr7 and Chr8), Class III (Chr2-5, and Chr11), Class IV (Chr3), and Class V (Chr1, Chr3, Chr6, Chr8) (Figure 2).

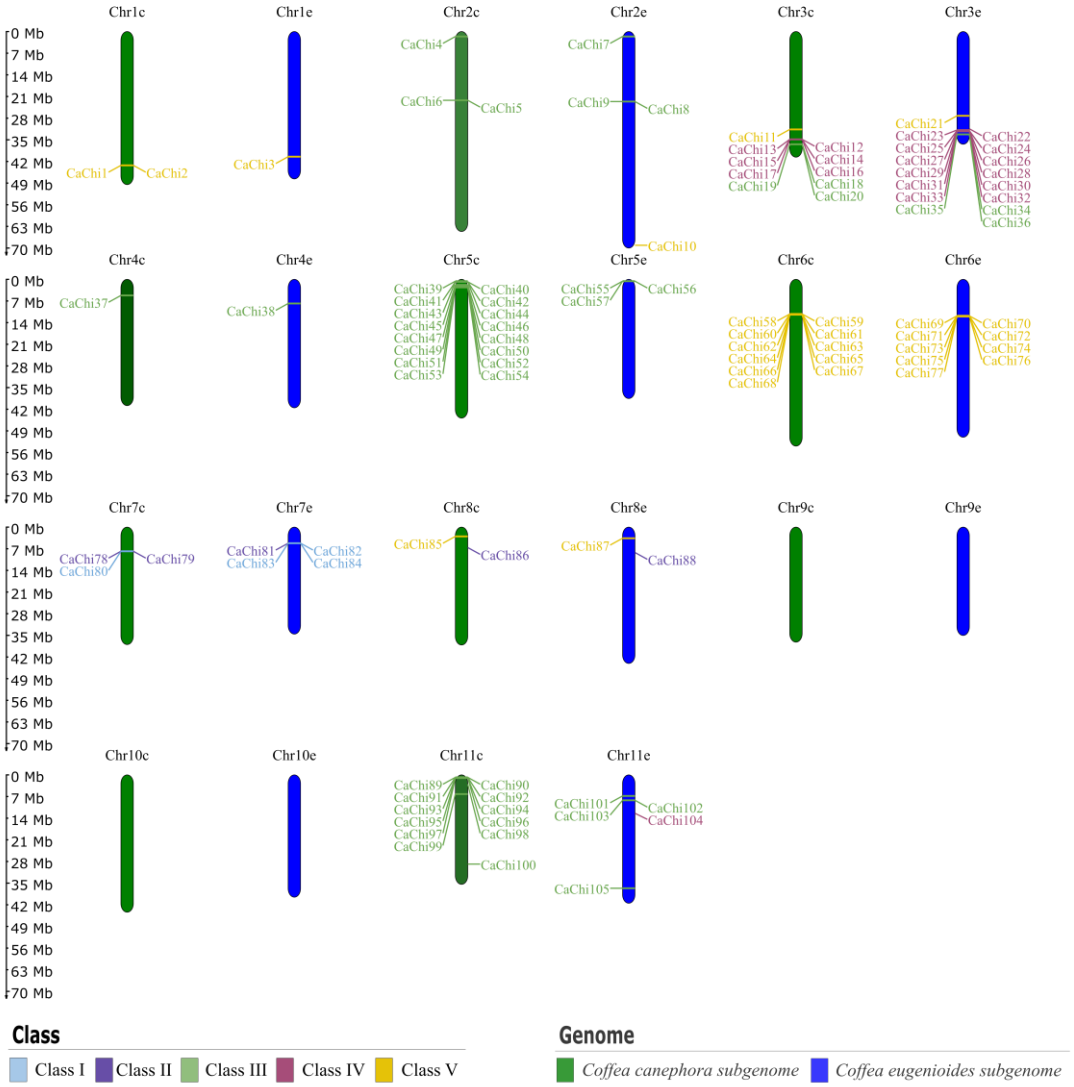

Figure 2. Chromosomal location of chitinase genes in *Coffea arabica*. The complete set of 22 chromosomes (2n = 44) is shown, representing both parental subgenomes. The prefix "Chr" indicates chromosome. Chromosomes labeled with "c" (e.g, Chr1c) refer to the *C. canephora* subgenome, and those with "e" (e.g, Chr1e) to the *C. eugenioides* subgenome. Individual chitinase genes are indicated on each chromosome, with gene classes distinguished by color coding.

In *C. canephora*, 39 genes were distributed across eight of the eleven chromosomes, while eight others were not assigned to any specific chromosome (Figure 3A). In *C. eugenioides*, 64 genes were mapped to all chromosomes except for two that could not be assigned (Figure 3B). In both diploid genomes, chitinase gene classes were predominantly located on homologous chromosomes, although some chromosomes contained genes from more than one class, a pattern also observed in *C. arabica*.

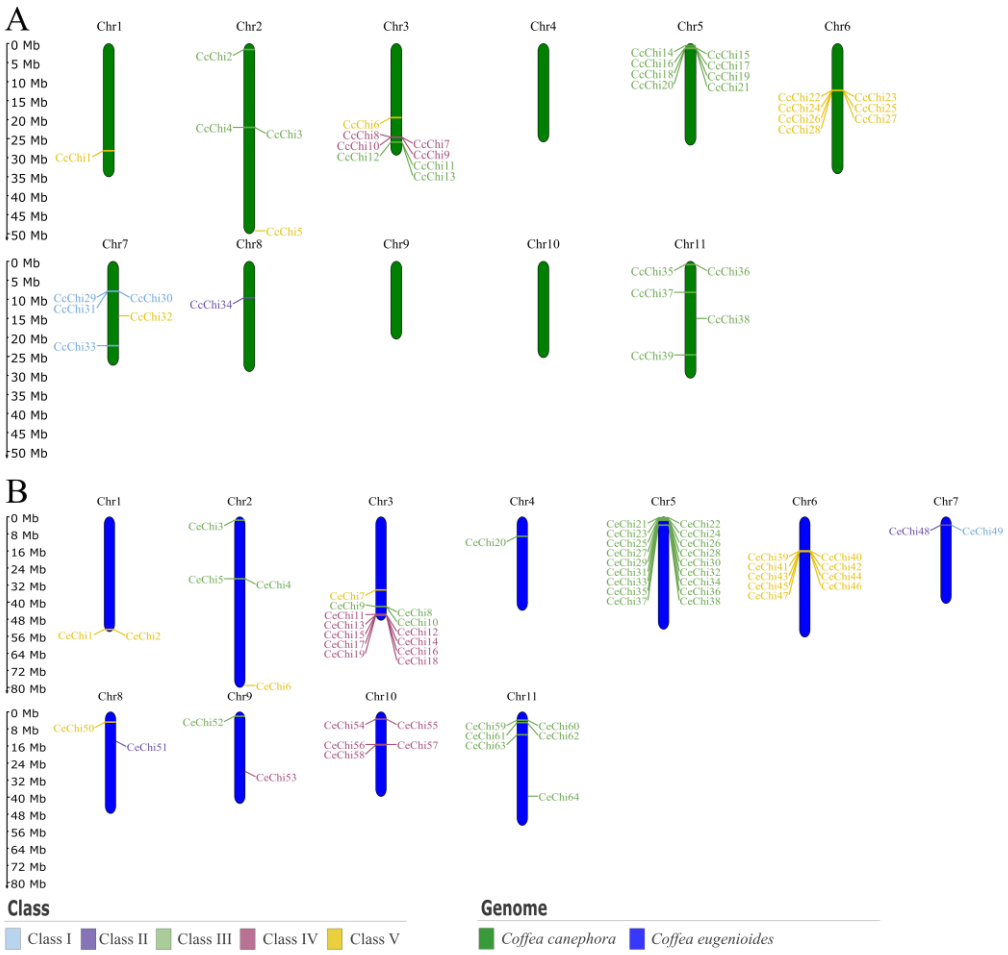

Figure 3. Chromosomal location of chitinase genes in *Coffea* spp. (**A**) *Coffea canephora* and (**B**) *Coffea eugenioides*. Each panel shows the complete chromosome set of the respective diploid species (2n = 22), with chitinase genes mapped to their physical positions. Chromosomes are labeled with the prefix "Chr" followed by their number. Gene classes are color-coded, allowing the visualization of how different chitinase groups are distributed across chromosomes.

2.4. Domain architecture analysis and subcellular localization

Signal peptides, transmembrane domains, conserved domains, and subcellular localization were analyzed to provide functional insights into the identified proteins. Most sequences exhibited the characteristic chitinase domains GH18 or GH19 when analyzed against the Pfam 35.0 database. However, 14 proteins from *C. arabica* (Table S1), two from *C. canephora* (Table S2), and five from *C. eugenioides* (Table S3) did not show these domains in Pfam. These sequences were reanalyzed in the CDD v.3.21 database and confirmed as members of the GH18_chitinase-like superfamily (cl10447), clustering with Class III chitinases in the phylogenetic analysis.

Proteins containing the Glyco_hydro_19 catalytic domain were grouped into Classes I, II, and IV (Figure 4). Class I proteins generally carried only the GH19 domain, although some (CaChi82, CcChi29, and CeChi49) also contained a chitin-binding domain (CBD) and a signal peptide (SP), resembling the reference protein At3g12500. CeChi49 also carried an extra GH19 domain and a transmembrane domain (TMD), overlapping with the SP, while CaChi84 had a TMD but no CBD. Class II proteins typically contained GH19 and SP domains, except for CcChi42 and CcChi34, which lacked SP. A few Class II proteins (CaChi78, CcChi46, CaChi79, CaChi81, and CeChi48) also showed a TMD overlapping with the SP (Figure 4C).

Most Class IV proteins contained GH19, CBD, and SP domains. Exceptions included CaChi15 (no SP), CeChi54 and CeChi58 (no CBD), CaChi29 and CeChi55 (lacking both).

CaChi29 also had an additional GH19 catalytic domain (Figure 4C). Subcellular localization predictions indicated that most GH19 proteins were extracellular, although a few exceptions were detected, such as CaChi15 and CcChi33 (plastids). The subcellular location of CaChi80 and CcChi30 could not be determined (Figure 4B).

Proteins containing the Glyco_hydro_18 catalytic domain were grouped into Classes III and V (Figure 5A). Most GH18 proteins carried GH18 and SP domains, but several exceptions were observed. Some Class III proteins lacked SP, while others carried additional domains such as Reverse Transcriptase-like 3 domain (RVT-3) (CaChi57), extra GH18 domains (CcChi20, CaChi46, CcChi39), or PKR-like kinase (PKR) (CaChi37_1, CaChi37_3, CaChi38_1, and CeChi20_1) (Figure 5C).

In Class V, many proteins displayed SP overlapping with TMD, only TMD, or only SP. A subset also carried the PKR domain, with variable combinations of SP and/or TMD. Predicted subcellular localization of GH18 chitinases was more diverse than that of GH19, including nucleus, vacuole, extracellular space, plastids, cell membrane, cytoplasm, or Golgi complex. Approximately 58% were predicted to be extracellular, while ~18% had undetermined locations (Figure 5B).

2.5. Gene structure analysis

The exon-intron structures of *Coffea* spp. chitinases genes were analyzed to assess their structural diversity. Most Class I and II chitinase genes contained three exons, with a few exceptions such as CeChi49 (*n*=7), CcChi30 (*n*=1), CcChi31/CcChi33 (*n*=4). In Class IV, the majority had two or three exons, except for CeChi13 (*n*=4) and CeChi55 (*n*=1). In *Coffea arabica*, GH19 chitinase genes typically exhibited the standard structure of two or three exons (Figure 4D).

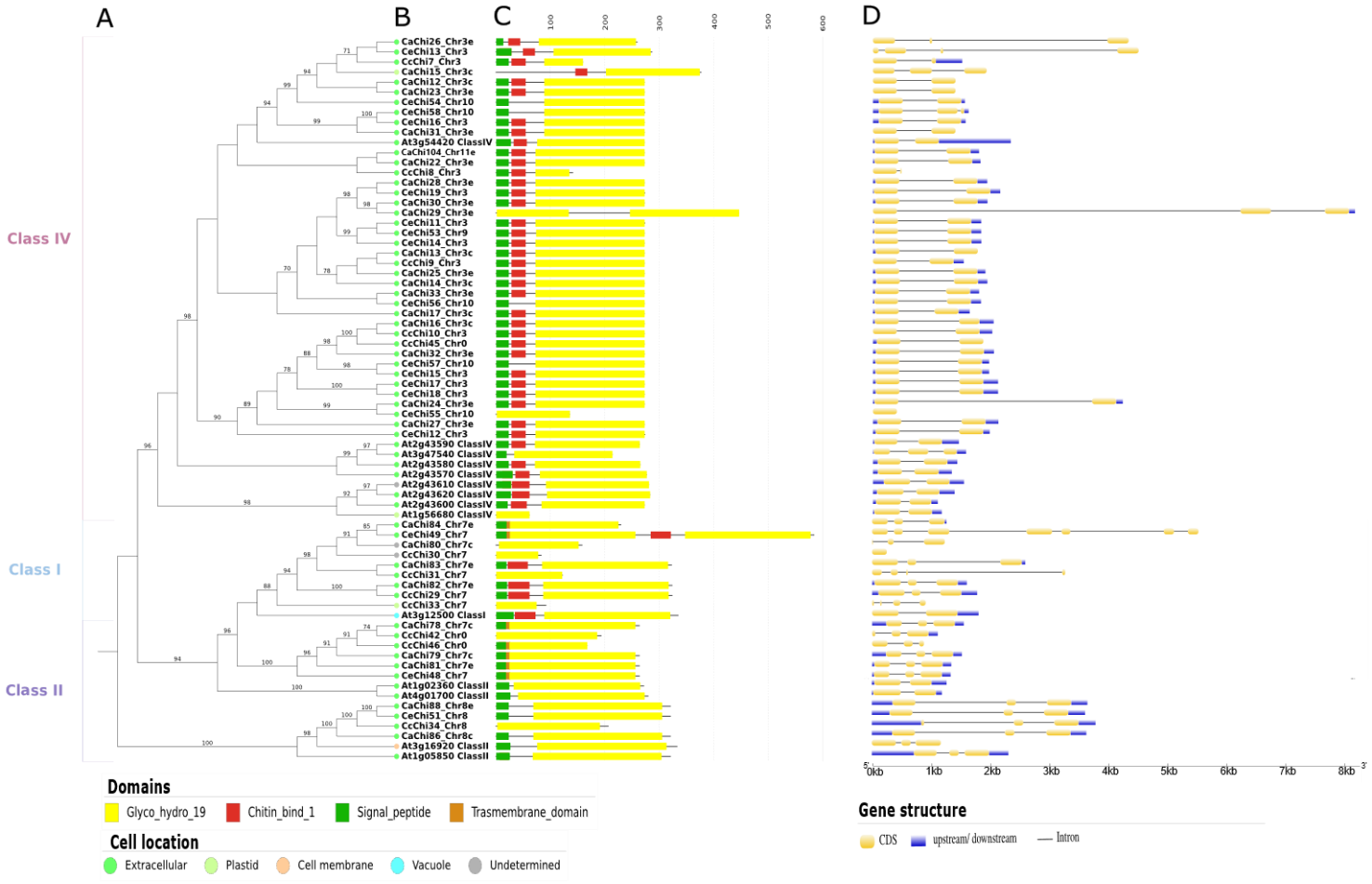

Figure 4. Characterization of putative GH19 chitinases from *Arabidopsis thaliana, Coffea arabica, Coffea canephora,* and *Coffea eugenioides.* (A) Maximum likelihood phylogenetic tree with bootstrap support values ≥ 70% shown at the nodes, using *A. thaliana* At1g02360 as the outgroup; (B) Predicted subcellular localization, with different colors representing distinct cellular compartments; (C) Conserved domain structure of the chitinase proteins; (D) exon-intron structure of the genes, with exons shown in yellow and introns represented by black lines.

Class III GH18 chitinase genes were more variable, ranging from one to three exons, with some showing higher counts, such as CaChi55 (*n*=4), CcChi20 (*n*=6), and CcChi39 (*n*=6). Isoform-coding genes, including CaChi37 (*n*=6), CaChi38 (*n*=4), and CeChi20 (*n*=4), also showed exon variability. Similarly, Class V genes usually contained one to three exons, with exceptions such as CeChi40 (*n*=4) and a cluster in the final clade containing six to eight exons. Many GH18 genes were intronless, in contrast to GH19, where only two intronless genes were detected (Figure 4D, Figure 5D).

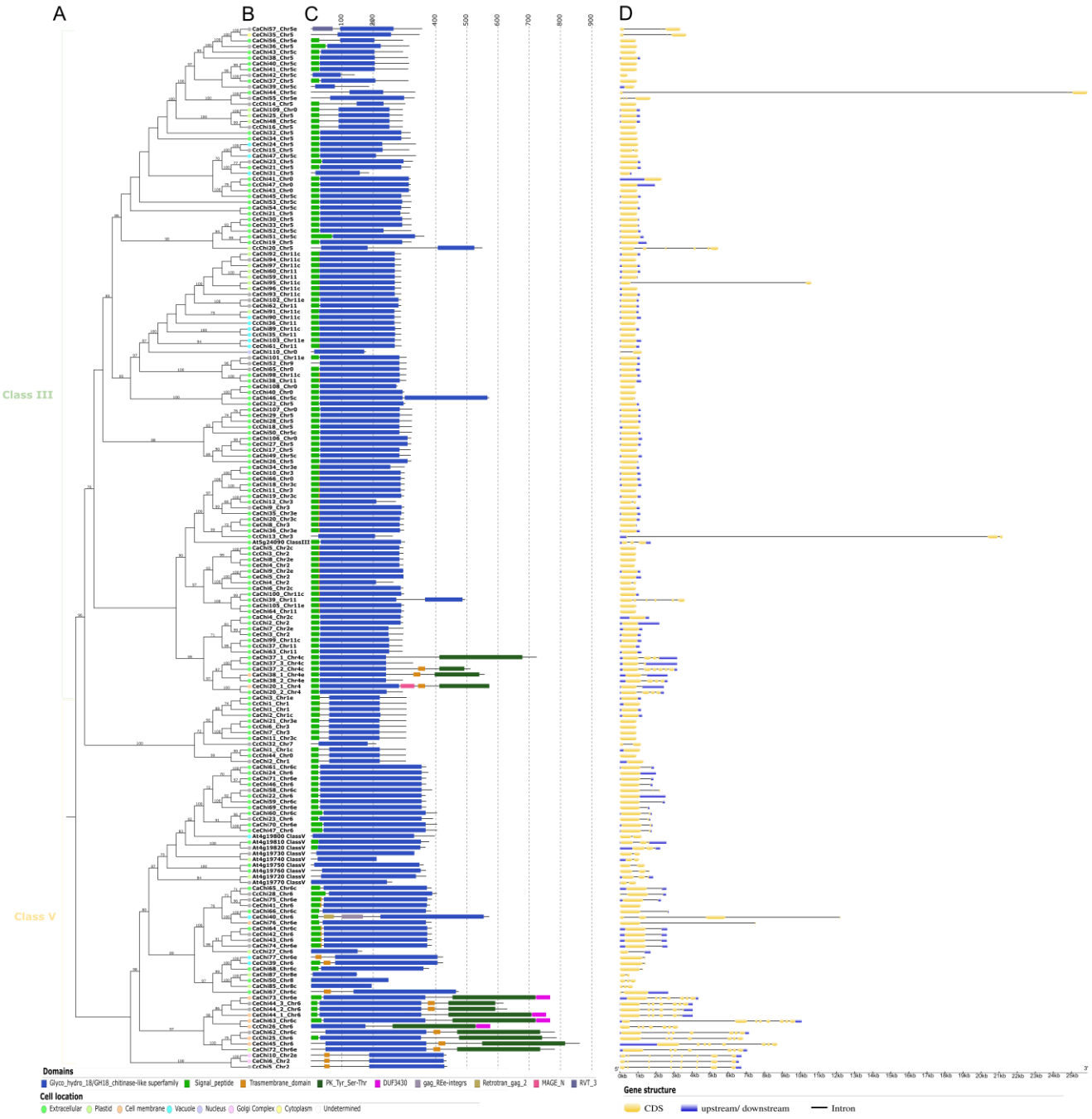

Figure 5. Characterization of putative GH18 chitinases from *Arabidopsis thaliana, Coffea arabica, Coffea canephora,* and *Coffea eugenioides*. (A) Maximum likelihood phylogenetic tree with bootstrap support values ≥ 70% shown at the nodes, using *A. thaliana* At4g19720 as the outgroup; (B) Predicted subcellular localization, with different colors representing distinct cellular compartments; (C) Conserved domain structure of the chitinase proteins; (D) exon-intron structure of the genes, with exons shown in yellow and introns represented by black lines.

2.6. Orthologs analysis

To explore the functional conservation of *Coffea* chitinases, we performed an ortholog analysis including all proteins identified in this study *C. arabica* (*n*=113), *C. canephora* (*n*=47), and *C. eugenioides* (*n*=69), together with 27 reference proteins (QRef) previously described as responsive to fungal pathogen infections. A total of 58 ortholog groups (orthogroups) were identified (Table S4). Among these, 52 included chitinases from *C. arabica*, 33 from *C. canephora*, 50 from *C. eugenioides,* and nine from reference proteins. Patterns of conservation varied among species. Twenty-four orthogroups contained genes from all three coffee species, while 17 were shared between *C. arabica* and *C. eugenioides*. In contrast, only three orthogroups comprised *C. arabica* and *C. canephora*. Species-specific groups were also found, with three exclusives to *C. eugenioides* and two to *C. arabica*, whereas no groups were exclusive to *C. canephora* or shared only between the two diploid species (Figure 6).

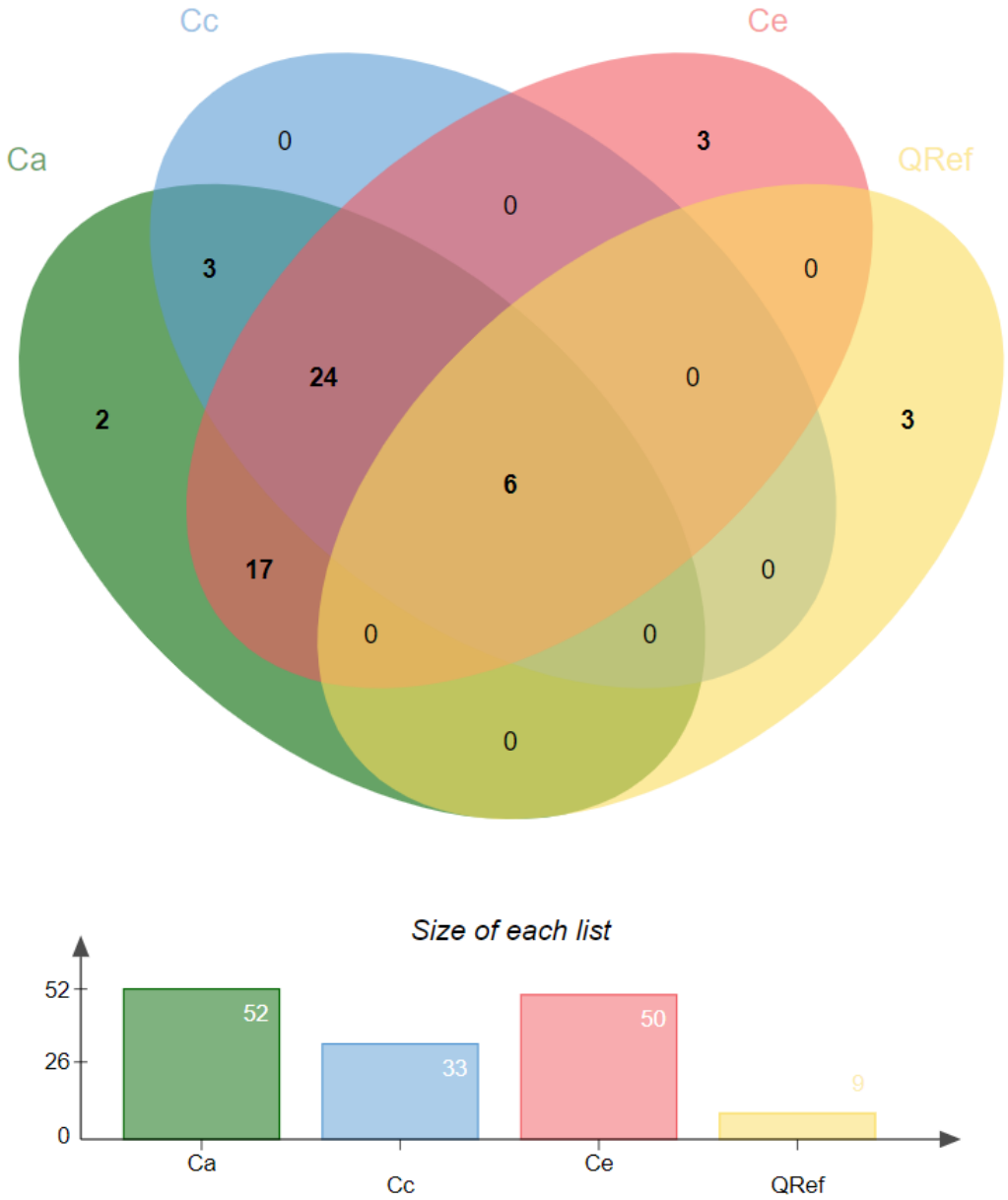

Figure 6. Orthologous groups of chitinases (orthogroups) among the three coffee species, *Coffea arabica* (Ca), *Coffea canephora* (Cc), *Coffea eugenioides* (Ce), and reference chitinases (QRef). The Venn diagram displays shared and unique orthogroups across species. The "Size of each list" indicates the number of orthogroups assigned to each species or reference sequence.

Six orthogroups included chitinases from all three coffee species together with reference proteins, including McChit1, MdCHI1, CaChitIV, IbChiA, FvChi-14, FnCHIT2, and CHI2 (Figure 6, Figure 7).

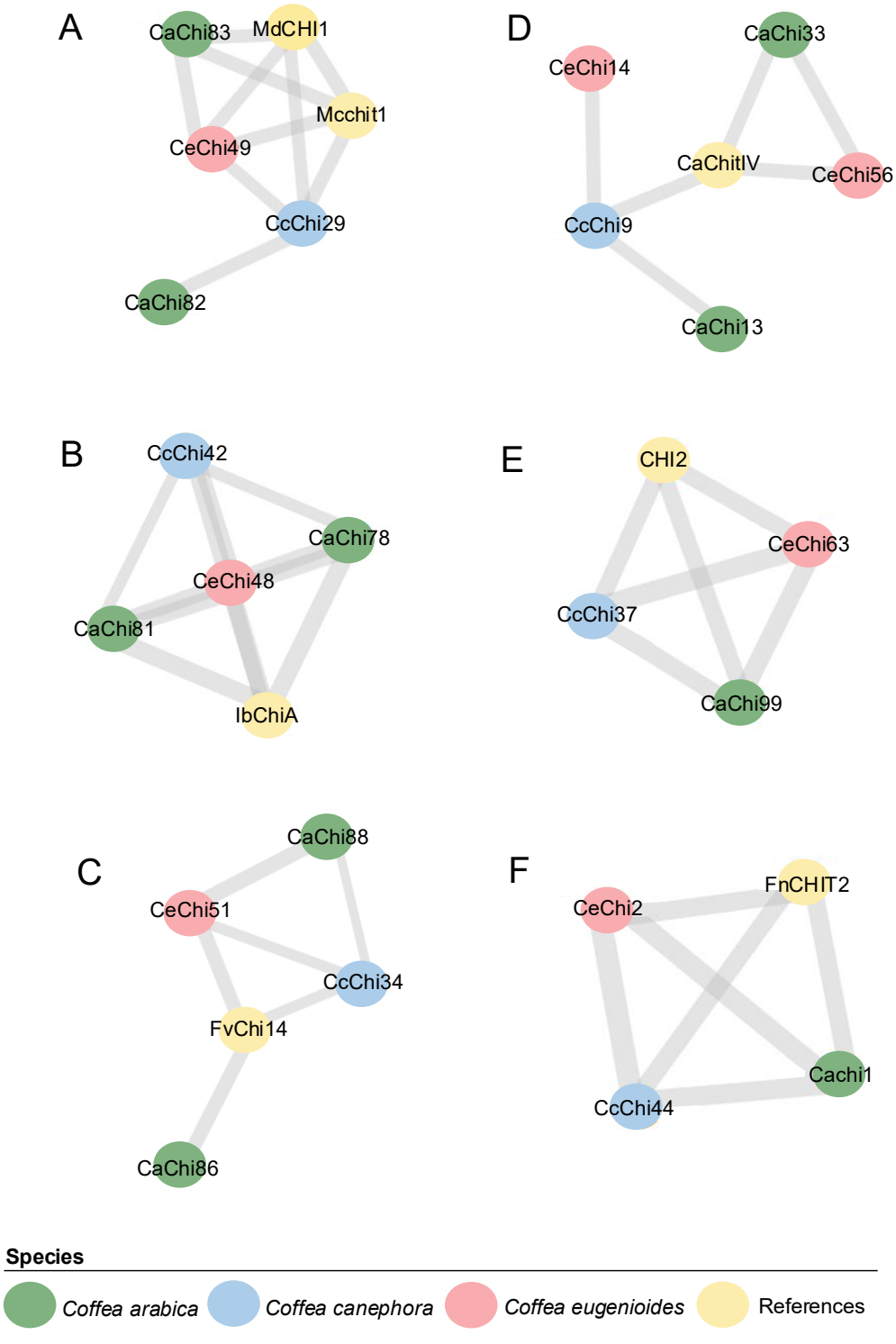

Figure 7. Orthologous Clusters grouping putative chitinases from *Coffea arabica* (CaChi), *Coffea canephora* (CcChi), and *Coffea eugenioides* (CeChi) to chitinase references. (A) Chitinases Class I clustered with references MdCHI1 and Mcchit1; (B) Chitinases Class II clustered with reference IbCHIA; (C) Chitinases Class II clustered with reference FvChi-14; (D) Chitinases Class IV clustered with reference CaChitIV; (E) Chitinases Class III clustered with reference CHI2; (F) Chitinases Class V clustered with reference FnCHIT2. Node colors represent different species. Line thickness indicates the degree of sequence similarity between proteins.

2.7. Putative Fungal Defense Response Chitinase in Coffea spp.

Based on orthology with reference proteins known to respond to fungal infection, six clusters of *Coffea* chitinases were identified as candidates for defense-related functions. All proteins for which localization could be predicted were assigned to the extracellular region.

Four clusters belonged to the GH19 family. Cluster 1 comprised Class I chitinases located on chromosome 7, ranging from 323 to 584 amino acids (aa). These proteins typically contained SP and CBD domains and three exons; however, CeChi49 was exceptional, with seven exons, a TMD, and an additional GH19 domain. Clusters two and three contained Class II chitinases, characterized by the absence of a CBD domain and the presence of three exons. Most proteins also possessed an SP domain. Cluster two genes, located on chromosome 7 (except for CcChi42, which was not annotated as anchored on any chromosome), ranged from 194 to 264 aa, and the three proteins of 264 aa carried a TMD. Cluster three genes were located on chromosome 8, mostly encoding proteins of 321 aa with an SP; the exception was CcChi34, with 207 aa and no SP. Cluster IV included Class IV chitinases of 273 aa with two exons, most of which carried SP and CBD. These were chromosome 3, except CeChi56, which was mapped to chromosome 10 and lacked the CBD (Table 2).

Two clusters belonged to the GH18 family. Cluster five comprised Class III chitinases of 294 aa, encoded by single-exon genes and carrying SP. Cluster six included Class V chitinases of 305 aa, also with a single exon and SP (Table 2).

Table 2. Characterization summary of putative chitinases associated with defense response to fungi in *Coffea arabica*, *Coffea canephora*, and *Coffea eugenioides*.

Cluster	Reference	Protein	Class	Size	Chr	Cell localization	Domains[1]				Exons
1	Mcchit1 [41][42] MdCHI1 [43]	CcChi29	I	324	7	Extracellular	SP	.	CBD	GH19	3
1	Mcchit1 [41][42] MdCHI1 [43]	CeChi49	I	584	7	Extracellular	SP	TMD	CBD	2 GH19	7
1	Mcchit1 [41][42] MdCHI1 [43]	CaChi83	I	323	7e	Extracellular	SP	.	CBD	GH19	3
1	Mcchit1 [41][42] MdCHI1 [43]	CaChi82	I	324	7e	Extracellular	SP	.	CBD	GH19	3
2	IbChiA [44]	CaChi81	II	264	7e	Extracellular	SP	TMD	.	GH19	3
2	IbChiA [44]	CaChi78	II	264	7c	Extracellular	SP	TMD	.	GH19	3
2	IbChiA [44]	CcChi42	II	194	0	Extracellular	.	.	.	GH19	3
2	IbChiA [44]	CeChi48	II	264	7	Extracellular	SP	TMD	.	GH19	3
3	FvChi-14 [45]	CcChi34	II	207	8	Extracellular	.	.	.	GH19	3
3	FvChi-14 [45]	CeChi51	II	321	8	Extracellular	SP	.	.	GH19	3
3	FvChi-14 [45]	CaChi86	II	321	8c	Extracellular	SP	.	.	GH19	3
3	FvChi-14 [45]	CaChi88	II	321	8e	Extracellular	SP	.	.	GH19	3
4	CaChitIV [46]	CcChi9	IV	273	3	Extracellular	SP	.	CBD	GH19	2
4	CaChitIV [46]	CeChi56	IV	273	10	Extracellular	SP	.	.	GH19	2
4	CaChitIV [46]	CaChi33	IV	273	3e	Extracellular	SP	.	CBD	GH19	2
4	CaChitIV [46]	CeChi14	IV	273	3	Extracellular	SP	.	CBD	GH19	2
4	CaChitIV [46]	CaChi13	IV	273	3c	Extracellular	SP	.	CBD	GH19	2
5	CHI2[50]	CcChi37	III	294	11	Extracellular	SP	.	.	GH18	1
5	CHI2[50]	CeChi63	III	294	11	Extracellular	SP	.	.	GH18	1
5	CHI2 [50]	CaChi99	III	294	11c	Extracellular	SP	.	.	GH18	1
6	FnCHIT2 [48]	CcChi44	V	305	0	Extracellular	SP	.	.	GH18	1
6	FnCHIT2 [48]	CeChi2	V	305	1	Undeteminet ed	SP	.	.	GH18	1
6	FnCHIT2 [48]	CaChi1	V	305	1c	Extracellular	SP	.	.	GH18	1

[1]SP: Signal Peptide; TMD: Transmembrane domain; GH19: Glyco_hydro19; GH18: Glyco_hydro18; CBD: Chitin-binding domain.

3. Discussion

Chitinases are proteins encoded by large multigene families, with individual genes associated with diverse stress-related functions [49]. Numerous studies have focused on their role as pathogenesis-related proteins in different pathosystems, emphasizing expression profiles [26,33,45,50], over-expression studies [51], and the transformation of plants with chitinase genes [32,52] to enhance plant resistance to pathogens. Proteomic analyses revealed a potential role of chitinases in the coffee-fungus interaction [37]. However, no comprehensive characterization of coffee chitinases has been performed. This is the first study to characterize putative chitinase proteins and genes in the genomes of *C. arabica* and its progenitors. Integrating genome-wide analysis, we identified a total of 229 putative chitinase proteins in the three coffee species. Furthermore, based on orthologous analyses, we selected 10, six, and seven putative chitinase genes associated with plant resistance to pathogen infection in *C. arabica*, *C. canephora*, and *C. eugenioides*, respectively.[37]

We obtained a higher number of putative chitinase proteins in *C. arabica* (*n*=113) compared with its progenitors, *C. canephora* (*n*=47) and *C. eugenioides* (*n*=69). This finding is consistent with its allotetraploid status [2], which results in approximately double the genome size of its progenitors [53]. Plant chitinases are generally classified according to two catalytic domains, GH18 and GH19 [21]. Based on the pfam 35.0 database, nearly all chitinases contained one of these domains, with the exception of 14 proteins from *C. arabica*, two from *C. canephora*, and five from *C. eugenioides*. These proteins instead presented the GH18_chitinase-like superfamily (cl10447). Because of the high functional and structural diversity of chitinases, these proteins were retained as candidates for the analyses. Beyond the conserved catalytic domains, several coffee chitinases also displayed features such as signal peptides, transmembrane domains, or additional domains (e.g., kinase or reverse transcriptase-like), indicating structural diversity that may contribute to functional specialization. Phylogenetic analysis further divided the *Coffea* chitinases into two main families (GH18 and GH19) and, in a secondary classification, into five classes (I, II, III, IV, and V), following the previous classification of *Arabidopsis* chitinases [40]. Although the majority of plant chitinases have been reported in classes I, II, and IV within the GH19 family [20,22], our results revealed a higher representation of GH18 chitinases in the three coffee genomes.

Chitinase genes in *Coffea* spp. are distributed across various chromosomes and generally occur in tandem, suggesting that tandem duplication has played an important role in their expansion, as observed in other crops such as grape, soybean, tomato, and apple [27,33,50,54]. In *C. arabica*, the subgenome derived from *C. canephora* contained more chitinase genes than the subgenome derived from *C. eugenioides*. Notably, 60 chitinase genes were identified in the *C. canephora*-derived subgenome of *C. arabica*, compared with 47 in the *C. canephora* reference genome. This increase may reflect tandem duplications that occurred after polyploidization or from differences between the *C. canephora* accession used as the reference genome and the one that contributed to the origin of *C. arabica*, or copy number variation already present in the ancestral *C. canephora* donor. This asymmetry supports previously reported subgenome dominance and biased homoeologous exchange favoring the *C. canephora*-derived subgenome, which may be linked to adaptive traits inherited from *C. canephora*, such as its broader ecological range and greater genetic diversity [53]. Consistent with this, in the orthology analysis we observed that *C. eugenioides* often contributed multiple paralogs to orthogroups containing reference chitinases, suggesting lineage-specific duplication or retention within this progenitor.

As expected for *C. arabica*, which is known for its high structural conservation with its progenitors in terms of chromosome number and gene content per chromosome [53], the distribution of chitinase genes was also largely conserved among *C. arabica*, *C. canephora*, and *C. eugenioides*. Most chitinase genes from the same class were located on homologous chromosomes across the three genomes, indicating a conserved gene

organization. However, some chromosomes in *C. arabica* contained additional or divergent classes, suggesting post-polyploidization diversification. Furthermore, no chitinase genes were found on chromosomes 9 and 10 of *C. arabica* and *C. canephora*, while *C. eugenioides* showed genes on all chromosomes. This suggests that *C. arabica* may have lost certain chitinases or that these genes were relocated to other chromosomes. Interestingly, most chitinases located on chromosomes 9 and 10 of *C. eugenioides* belonged to Class IV, while *C. arabica* contained more Class IV genes on chromosomes 3c and 3e, pointing to potential relocation events. If the genes were simply unannotated, we would expect to find more unanchored Class IV chitinases. However, most unanchored chitinases in all three genomes belong to Class III. Combined with the high genome similarity among the species, this suggests that the absence of some genes is not due to annotation errors. The results indicate that *C. arabica* may have undergone gene loss or gene relocation events. Chitinase genes present in the progenitors and missing in *C. arabica* may be a valuable target for functional studies and genetic improvement, including the introgression of genes associated with enhanced disease resistance in coffee.

In this study, orthologous analysis with previously characterized chitinases associated with enhanced resistance to fungal pathogens enabled the identification of 10, six, and seven candidate genes in *C. arabica*, *C. canephora*, and *C. eugenioides*, respectively. Six orthogroups were identified, each comprising chitinases from all three coffee species and at least one reference gene with known antifungal function. Among these, four genes belonged to the GH19 family and two to GH18. Within GH19, one orthogroup included Class I chitinases that possessed the characteristic chitin-binding domain (CBD) of this class [28]. These chitinases correspond to Mcchit1 and MdCHI1, whose overexpression has been shown to enhance resistance to fungal pathogens in rice and apple, respectively [41,42].

Over evolutionary time, some chitinases have lost the ability to hydrolyze or bind to chitin [21], such as the two groups of Class II chitinase proteins, homologous to Class I but lacking the CBD [28]. However, the absence of this domain does not necessarily lead to a loss of enzymatic activity. For example, one of these Class II groups is orthologous to IbChiA, which is highly expressed in resistant sweet potato and exhibits antifungal activity against *Ceratocystis fimbriata* [44]. The other is orthologous to FvChi-14, whose overexpression has been shown to increase *Arabidopsis thaliana* resistance to *Colletotrichum higginsianum* [45].

A fourth orthogroup included Class IV chitinases associated with a defense-related gene regulator, CaChitIV, from *Capsicum annuum* [46]. Most Class IV chitinases from this orthogroup contained the CBD, except CeChi56. Due to deletions, Class IV chitinases generally have shorter CBD and catalytic regions [29]. In contrast, the coffee GH18 chitinases lacked a CBD, as Classes III and V consist of endochitinases that function similarly to lysozymes, acting through their catalytic domain [26]. Chitinases from these classes were orthologous to CHI2 and FnCHIT2, respectively, both of which have demonstrated critical roles in fungal resistance when overexpressed in transgenic plants [43,55].

All putative chitinases in coffee associated with resistance to fungal pathogens were predicted to be extracellularly localized, except for CeChi2, whose subcellular location could not be determined. This general pattern suggests that these chitinases may function via the secretory pathway, through which many proteins involved in plant-pathogen interactions are released [56]. In addition, most of the corresponding genes contained two or fewer introns, consistent with the typical structure of stress-responsive genes that require rapid transcription [50,57]. An exception is CeChi49, which displayed a more complex exon-intron structure due to a duplicated GH19 domain. Overall, our results support the identification of chitinases associated with fungal defense response in *C. arabica* and its progenitors. These findings provide valuable insights into future research focusing on plant resistance sources to fungal pathogens. The candidate genes identified are orthologous to genes previously used in successful overexpression and transformation studies, supporting their relevance as promising resources for coffee improvement. These

candidates can be prioritized for functional studies and, once validated, applied in breeding strategies, such as marker-assisted selection and transgenic approaches, to enhance resistance in coffee.

4. Materials and Methods

4.1. Coffea spp. Genomes

Analysis of chitinase genes and proteins across the genomes of *C. arabica* and its progenitors, *C. canephora* and *C. eugenioides*, involved downloading the FASTA files containing predicted genes and proteins, along with GFF (General Feature Format) annotation files. Genomes used were as follows: *C. arabica* (Caturra red-Cara_1.0, GenBank assembly accession: GCA_003713225.1), *C. eugenioides* (Ceug_1.0, GenBank assembly accession: GCA_003713205.1) from NCBI (National Center for Biotechnology Information), and *C. canephora*, available at NCBI and the Coffee Genome Hub (https://coffee-genome-hub.southgreen.fr/).

4.2. Identification of Putative Chitinase

The HMMER (http://hmmer.org/) software package v. 3.2.1 was used to identify candidate sequences for chitinases in the *C. arabica*, *C. canephora,* and *C. eugenioides* genomes. The Pfam-A.hmm file containing the Hidden Markov Model (HMM) profiles of representative protein families was downloaded from the Pfam database [39]. The function 'hmmfetch' was used to retrieve the HMM profiles for the characteristic domains of chitinases, Glyco_hydro_18 (PF00704.31) and Glyco_hydro_19 (PF00182.22). These HMM profiles were subsequently used to search for proteins with the GH18 and GH19 domains in the predicted protein files from *C. arabica*, *C. canephora,* and *C. eugenioides* genomes, using 'hmmsearch'. Sequences within the threshold (e-value $<10^{-4}$) were selected for further analysis.

4.3. Phylogenetic analyses

Protein sequences from *Coffea* spp. genomes identified as putative chitinases were aligned with the chitinase proteins of *A. thaliana* described by Passarinho and Vries, 2002 [40]. Three phylogenetic trees were constructed: one including all sequences identified as belonging to the GH18 and GH19 groups, and two with sequences from each group analyzed separately. Amino acid sequences were aligned using MAFFT v. 6.903 [58] with standard parameters. Due to the lack of similarity between the GH18 and GH19 groups, only the separate alignments were filtered using Gblocks 0.91b [59] to detect the most conserved regions. MAFFT and Gblocks were executed on NGPhylogeny.fr [60]. Trees were inferred using IQ-TREE [61] with the Maximum Likelihood (ML) method and 1,000 bootstrap replicates. The best-fit model, WAG+G4, was selected for all phylogenetic trees based on the Bayesian information criterion (BIC). The phylogenetic trees were visualized and edited using the Interactive Tree of Life (iTOL) [62]. Chitinase proteins from *A. thaliana* were used as outgroups in the phylogenetic trees: At1g02360 for the GH18+GH19 concatenated and individual GH19 trees, and At4g19720 for the individual GH18 tree.

4.4. Chromosomal Location and Distribution

Chromosomal location data for chitinase-coding genes (GFF annotation file) and chromosome sizes in Mb for the *C. arabica*, *C. canephora*, and *C. eugenioides* genomes were collected from the NCBI database and the Coffee Genome Hub. Chromosomal mapping and schematic visualization of chitinase genes were performed using MapGene2Chromosome v.2.1 (MG2C, http://mg2c.iask.in/mg2c_v2.1/). Gene position data (start and end coordinates), chromosome numbers, chromosome lengths, and specific colors were provided in the format required by MG2C.

4.5. Domain architecture analysis and subcellular location

Protein sequences identified as chitinases based on GH18 and GH19 HMM profiles and *Arabidopsis* references [40] were validated for domain presence using NCBI's Conserved Domain Database (CDD) [63] with Pfam v.35.0. Sequences that lacked domain matches in Pfam were reanalyzed against the CDD v.3.21 database using the same default parameters, including an E-value threshold of 0.01, composition-based statistics adjustment, and a maximum of 500 hits, with result mode set to "Concise". Signal peptides were predicted with SignalP 6.0 [64], and transmembrane domains with TMHMM 2.0 [65]. The subcellular localization was predicted using Plant-mSubP [66] (http://bioinfo.usu.edu/Plant-mSubP/) with the four available prediction methodologies (AAC, Dipep, PseAACNCCDipep, and NCCDipepCTDCCTDTQSO). The most frequent prediction was considered (Table S5, Table S6, Table S7). The localization was classified as undetermined when each method yielded a different outcome. All tools were used with their default confidence score thresholds.

4.6. Gene structure analysis

Gene structure was examined using the Gene Structure Display Server (GSDS) [67], based on CDS and corresponding genomic DNA sequences provided in FASTA format through the "Sequence FASTA" option. CDS sequences were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/) by searching for protein IDs, from which the associated gene IDs and genomic sequences were obtained. For *Coffea canephora*, genomic sequences were not available in NCBI. Thus, protein sequences were aligned against the Coffee Genome Hub (https://coffee-genome-hub.southgreen.fr/) using BLAST, and the corresponding genomic regions were identified based on matching gene IDs. Domain architecture, gene structure, and subcellular localization were annotated and mapped onto the phylogenetic tree using the Interactive Tree of Life (iTOL), with visual features customized through the iTOL annotation editor [62]. Final figures were edited using Inkscape v.1.3.2 (https://inkscape.org).

4.7. Orthogroups Analysis

The protein sequences of candidate chitinases from the genomes of three coffee species were analyzed alongside functionally characterized plant chitinases, which are known to respond to fungal diseases based on a literature review of Chen et al, 2024 [68] and are here referred to as reference chitinases (QRef) (Table S8). These reference sequences correspond to chitinases used in genetic transformation or overexpression experiments that improved plant resistance to fungal pathogens. The FASTA sequences of QRef were retrieved from the NCBI database or from other databases specified in the consulted articles. We then performed the orthologous analysis using OrthoVenn3 [69] with the default OrthoMCL algorithm parameters, including an E-value of 10^{-2} and an inflation value of 1.5. Annotation, protein similarity, and cluster relationship network options were enabled.

5. Conclusions

Plant chitinases exhibit high diversity and play key roles in growth, development, and responses to biotic and abiotic stress. Identifying and characterizing these genes/proteins is essential for their application in biotechnology. This study provides the first comprehensive characterization of chitinase genes and proteins in the genome of *Coffea arabica* and its diploid progenitors, *C. canephora* and *C. eugenioides*, based on phylogenetic relationships, domain architecture, gene structure, and subcellular localization. Characterizing the progenitor genomes expands the available gene pool and helps overcome the complexity of the allotetraploid *C. arabica* genome. We found that chitinases are highly conserved across the three genomes, supporting the potential use of genes from the progenitor species in *C. arabica* through genetic transformation. Furthermore, ortholog analysis enabled the identification of putative chitinases involved in plant-pathogen interactions, which could be explored not only in coffee but also in other

crops through gene overexpression, the development of biobased fungicides, and the generation of transgenic plants. Altogether, these findings provide a valuable foundation for future breeding and biotechnological strategies aimed at improving fungal disease resistance.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Putative Chitinases Identified in the *Coffea arabica* Genome: Accession Number, Annotation, Protein Size, Gene Locus, Chromosomal Location, Start and End Positions, Number of Exons, Glycoside Hydrolase Domain, and Chitinase Class; Table S2: Putative Chitinases Identified in the *Coffea canephora* Genome: Accession Number, Annotation, Protein Size, Gene Locus, Chromosomal Location, Start and End Positions, Number of Exons, Glycoside Hydrolase Domain, and Chitinase Class; Table S3: Putative Chitinases Identified in the *Coffea eugenioides* Genome: Accession Number, Annotation, Protein Size, Gene Locus, Chromosomal Location, Start and End Positions, Number of Exons, Glycoside Hydrolase Domain, and Chitinase Class; Table S4: Orthogroups of chitinase genes identified across the genomes of *Coffea arabica*, *C. canephora*, *C. eugenioides*, and reference sequences (Qref) previously associated with responses to fungal pathogens. Orthologous groups shared between *Coffea* spp. and QRef sequences are highlighted in bold; Table S5: Subcellular localization prediction of *Coffea arabica* putative chitinases; Table S6: Subcellular localization prediction of *Coffea canephora* putative chitinases; Table S7: Subcellular localization prediction of *Coffea eugenioides* putative chitinases; Table S8: Functionally characterized plant chitinase genes/proteins involved in resistance to fungal diseases in plants, based on a literature review of Chen et al, 2024 [68].

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Supplementary material

Chitinase	Accession number	Annotation	Protein Size	Gene Locus	Chr	Start	End	Chr	GH	Class
CaChi1	XP_027107412.1	chitinase 2-like [*Coffea arabica*]	305 aa	LOC113727420	Chr1c	44245226	44246346	1	GH 18	V
CaChi2	XP_027108691.1	chitinase 2-like [*Coffea arabica*]	307 aa	LOC113728487	Chr1c	44309006	44310238	1	GH 18	V
CaChi3	XP_027088143.1	chitinase 2-like [*Coffea arabica*]	307 aa	LOC113709552	Chr1e	41446511	41447678	1	GH 18	V
CaChi4	XP_027103649.1	acidic endochitinase SE2-like [*Coffea arabica*]	296 aa	LOC113724934	Chr2c	1692276	1693897	1	GH 18	III
CaChi5	XP_027101941.1	acidic endochitinase-like [*Coffea arabica*]	297 aa	LOC113722907	Chr2c	22795729	22796625	1	GH 18	III
CaChi6	XP_027102185.1	acidic endochitinase-like [*Coffea arabica*]	297 aa	LOC113723146	Chr2c	22799481	22800374	1	GH 18	III
CaChi7	XP_027110162.1	acidic endochitinase SE2-like [*Coffea arabica*]	297 aa	LOC113729974	Chr2e	1631745	1632987	1	GH 18	III
CaChi8	XP_027109748.1	acidic endochitinase-like [*Coffea arabica*]	297 aa	LOC113729695	Chr2e	23076345	23077238	1	GH 18	III
CaChi9	XP_027109615.1	acidic endochitinase-like [*Coffea arabica*]	297 aa	LOC113729525	Chr2e	23080668	23081798	1	GH 18	III
CaChi10	XP_027115398.1	chitinase domain-containing protein 1 [*Coffea arabica*]	435 aa	LOC113733292	Chr2e	70751952	70758686	8	GH 18	V
CaChi11	XP_027118123.1	chitinase 2-like [*Coffea arabica*]	306 aa	LOC113735298	Chr3c	32374788	32375708	1	GH 18	V
CaChi12	XP_027117692.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113735016	Chr3c	35696937	35698353	2	GH 19	IV
CaChi13	XP_027117690.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113735014	Chr3c	35708853	35710786	2	GH 19	IV
CaChi14	XP_027117691.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113735015	Chr3c	35726899	35728859	2	GH 19	IV
CaChi15	XP_027118672.1	endochitinase EP3-like [*Coffea arabica*]	377 aa	LOC113735903	Chr3c	35737045	35738994	3	GH 19	IV
CaChi16	XP_027117688.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113735012	Chr3c	35742846	35744912	2	GH 19	IV
CaChi17	XP_027117689.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113735013	Chr3c	35752430	35754087	2	GH 19	IV
CaChi18	XP_027117780.1	basic endochitinase-like [*Coffea arabica*]	301 aa	LOC113735055	Chr3c	37192068	37193256	1	GH 18	III
CaChi19	XP_027117782.1	basic endochitinase-like [*Coffea arabica*]	299 aa	LOC113735057	Chr3c	37265989	37267143	1	GH 18	III
CaChi20	XP_027117783.1	basic endochitinase-like [*Coffea arabica*]	299 aa	LOC113735058	Chr3c	37278315	37279409	1	GH 18	III
CaChi21	XP_027120795.1	chitinase 2-like [*Coffea arabica*]	306 aa	LOC113737837	Chr3e	27807163	27808083	1	GH 18	V
CaChi22	XP_027119819.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113736845	Chr3e	32249792	32251635	2	GH 19	IV
CaChi23	XP_027119830.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113736854	Chr3e	32692542	32693957	2	GH 19	IV
CaChi24	XP_027119831.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113736856	Chr3e	32714798	32719078	2	GH 19	IV
CaChi25	XP_027119828.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113736852	Chr3e	32725163	32727090	2	GH 19	IV
CaChi26	XP_027120657.1	endochitinase EP3-like, partial [*Coffea arabica*]	260 aa	LOC113737661	Chr3e	32732162	32736543	3	GH 19	IV
CaChi27	XP_027119827.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113736851	Chr3e	32738839	32740988	2	GH 19	IV
CaChi28	XP_027119829.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113736853	Chr3e	32748209	32750167	2	GH 19	IV

CaChi29	XP_027120663.1	endochitinase EP3-like [*Coffea arabica*]	446 aa	LOC113737668	Chr3e	32956266	32964591	3	GH 19	IV
CaChi30	XP_027119836.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113736860	Chr3e	32971345	32973307	2	GH 19	IV
CaChi31	XP_027119838.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113736862	Chr3e	32979258	32980673	2	GH 19	IV
CaChi32	XP_027119837.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113736861	Chr3e	32982041	32984114	2	GH 19	IV
CaChi33	XP_027119835.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113736859	Chr3e	32989383	32991202	2	GH 19	IV
CaChi34	XP_027120271.1	basic endochitinase-like [*Coffea arabica*]	301 aa	LOC113737208	Chr3e	34009749	34010819	1	GH 18	III
CaChi35	XP_027119883.1	basic endochitinase-like [*Coffea arabica*]	299 aa	LOC113736886	Chr3e	34054300	34055430	1	GH 18	III
CaChi36	XP_027120242.1	hevamine-A-like [*Coffea arabica*]	299 aa	LOC113737178	Chr3e	34064465	34065560	1	GH 18	III
CaChi37_1	XP_027122875.1	cysteine-rich receptor-like protein kinase 15 isoform X1 [*Coffea arabica*]	724 aa	LOC113739755	Chr4c	5362405	5365575	4	GH 18	III
CaChi37_2	XP_027122876.1	acidic endochitinase SE2-like isoform X2 [*Coffea arabica*]	512 aa	LOC113739755	Chr4c	5362405	5365575	2	GH 18	III
CaChi37_3	XP_027122877.1	acidic endochitinase SE2-like isoform X3 [*Coffea arabica*]	328 aa	LOC113739755	Chr4c	5362405	5365575	6	GH 18	III
CaChi38_1	XP_027125521.1	acidic endochitinase SE2-like isoform X1 [*Coffea arabica*]	557 aa	LOC113742037	Chr4e	7979965	7982602	1	GH 18	III
CaChi38_2	XP_027125522.1	acidic endochitinase SE2-like isoform X2 [*Coffea arabica*]	295 aa	LOC113742037	Chr4e	7979965	7982602	4	GH 18	III
CaChi39	XP_027064422.1	hevamine-A-like [*Coffea arabica*]	187 aa	LOC113690630	Chr5c	525183	525986	1	GH 18	III
CaChi40	XP_027064432.1	acidic endochitinase-like [*Coffea arabica*]	315 aa	LOC113690638	Chr5c	720149	721096	1	GH 18	III
CaChi41	XP_027064433.1	acidic endochitinase-like [*Coffea arabica*]	312 aa	LOC113690639	Chr5c	769008	769946	1	GH 18	III
CaChi42	XP_027064434.1	hevamine-A-like [*Coffea arabica*]	141 aa	LOC113690641	Chr5c	787099	787524	1	GH 18	III
CaChi43	XP_027064447.1	acidic endochitinase-like [*Coffea arabica*]	296 aa	LOC113690650	Chr5c	831500	832390	1	GH 18	III
CaChi44	XP_027064449.1	acidic endochitinase-like [*Coffea arabica*]	335 aa	LOC113690652	Chr5c	855522	881406	3	GH 18	III
CaChi45	XP_027063467.1	acidic endochitinase-like [*Coffea arabica*]	320 aa	LOC113689871	Chr5c	1865455	1866599	1	GH 18	III
CaChi46	XP_027063803.1	chitinase 3-like [*Coffea arabica*]	572 aa	LOC113690174	Chr5c	1891495	1893359	1	GH 18	III
CaChi47	XP_027062675.1	hevamine-A-like [*Coffea arabica*]	337 aa	LOC113689037	Chr5c	2359958	2360971	1	GH 18	III
CaChi48	XP_027063686.1	acidic endochitinase-like [*Coffea arabica*]	295 aa	LOC113690068	Chr5c	2369526	2370632	1	GH 18	III
CaChi49	XP_027063906.1	acidic endochitinase-like [*Coffea arabica*]	320 aa	LOC113690267	Chr5c	2469521	2470739	1	GH 18	III
CaChi50	XP_027063379.1	acidic endochitinase-like [*Coffea arabica*]	324 aa	LOC113689794	Chr5c	2493592	2494728	1	GH 18	III
CaChi51	XP_027063994.1	acidic endochitinase-like [*Coffea arabica*]	364 aa	LOC113690342	Chr5c	2518687	2519998	1	GH 18	III
CaChi52	XP_027064790.1	acidic endochitinase-like [*Coffea arabica*]	322 aa	LOC113690880	Chr5c	2589399	2590540	1	GH 18	III

CaChi53	XP_027064936.1	acidic endochitinase-like [*Coffea arabica*]	323 aa	LOC113690972	Chr5c	2642331	2643358	1	GH 18	III
CaChi54	XP_027063723.1	hevamine-A-like [*Coffea arabica*]	320 aa	LOC113690103	Chr5c	2661864	2662968	1	GH 18	III
CaChi55	XP_027061985.1	acidic endochitinase-like [*Coffea arabica*]	333 aa	LOC113688378	Chr5e	632162	633859	4	GH 18_like	III
CaChi56	XP_027061993.1	acidic endochitinase-like [*Coffea arabica*]	296 aa	LOC113688383	Chr5e	663464	664354	1	GH 18	III
CaChi57	XP_027061995.1	acidic endochitinase-like [*Coffea arabica*]	357 aa	LOC113688385	Chr5e	686162	689509	3	GH 18	III
CaChi58	XP_027067386.1	class V chitinase-like [*Coffea arabica*]	389 aa	LOC113692994	Chr6c	11485707	11487928	3	GH 18	V
CaChi59	XP_027067391.1	class V chitinase-like [*Coffea arabica*]	370 aa	LOC113692998	Chr6c	11563093	11565583	2	GH 18	V
CaChi60	XP_027068281.1	class V chitinase-like [*Coffea arabica*]	405 aa	LOC113693790	Chr6c	11572285	11574052	2	GH 18	V
CaChi61	XP_027066687.1	class V chitinase-like [*Coffea arabica*]	370 aa	LOC113692472	Chr6c	11578024	11579912	2	GH 18	V
CaChi62	XP_027066318.1	cysteine-rich receptor-like protein kinase 4 [*Coffea arabica*]	782 aa	LOC113692149	Chr6c	11585825	11592969	7	GH 18	V
CaChi63	XP_027066547.1	cysteine-rich receptor-like protein kinase 10 [*Coffea arabica*]	767 aa	LOC113692349	Chr6c	11610034	11620097	8	GH 18	V
CaChi64	XP_027068696.1	class V chitinase-like [*Coffea arabica*]	388 aa	LOC113694043	Chr6c	11630043	11632669	2	GH 18	V
CaChi65	XP_027066590.1	class V chitinase-like [*Coffea arabica*]	387 aa	LOC113692387	Chr6c	11637025	11639604	2	GH 18	V
CaChi66	XP_027067392.1	class V chitinase-like [*Coffea arabica*]	386 aa	LOC113692999	Chr6c	11641552	11644257	2	GH 18	V
CaChi67	XP_027067393.1	class V chitinase-like [*Coffea arabica*]	474 aa	LOC113693000	Chr6c	11644940	11647617	2	GH 18	V
CaChi68	XP_027067394.1	class V chitinase-like [*Coffea arabica*]	379 aa	LOC113693002	Chr6c	11650941	11652215	2	GH 18	V
CaChi69	XP_027071630.1	class V chitinase-like [*Coffea arabica*]	370 aa	LOC113696408	Chr6e	12103381	12105023	2	GH 18	V
CaChi70	XP_027070850.1	class V chitinase-like [*Coffea arabica*]	405 aa	LOC113695870	Chr6e	12120457	12122259	2	GH 18	V
CaChi71	XP_027070851.1	class V chitinase-like [*Coffea arabica*]	370 aa	LOC113695871	Chr6e	12127012	12128865	2	GH 18	V
CaChi72	XP_027070848.1	G-type lectin S-receptor-like serine/threonine-protein kinase At1g11410 [*Coffea arabica*]	782 aa	LOC113695868	Chr6e	12132160	12139205	7	GH 18	V
CaChi73	XP_027070849.1	cysteine-rich receptor-like protein kinase 10 [*Coffea arabica*]	767 aa	LOC113695869	Chr6e	12150120	12154464	7	GH 18	V
CaChi74	XP_027069248.1	class V chitinase-like [*Coffea arabica*]	388 aa	LOC113694603	Chr6e	12169508	12172139	2	GH 18	V
CaChi75	XP_027072825.1	class V chitinase-like [*Coffea arabica*]	388 aa	LOC113697422	Chr6e	12183504	12185843	2	GH 18	V
CaChi76	XP_027071631.1	class V chitinase-like [*Coffea arabica*]	387 aa	LOC113696409	Chr6e	12186681	12194206	2	GH 18	V
CaChi77	XP_027071632.1	class V chitinase-like [*Coffea arabica*]	424 aa	LOC113696410	Chr6e	12197485	12198893	2	GH 18	V
CaChi78	XP_027075439.1	basic endochitinase-like [*Coffea arabica*]	264 aa	LOC113699341	Chr7c	8043261	8044853	3	GH 19	II
CaChi79	XP_027075438.1	basic endochitinase-like [*Coffea arabica*]	264 aa	LOC113699340	Chr7c	8067888	8069447	3	GH 19	II

CaChi80	XP_027075648.1	LOW QUALITY PROTEIN: endochitinase A2-like [*Coffea arabica*]	159 aa	LOC113699477	Chr7c	8087743	8089005	3	GH 19	I
CaChi81	XP_027079248.1	basic endochitinase-like [*Coffea arabica*]	264 aa	LOC113702310	Chr7e	5234464	5235839	3	GH 19	II
CaChi82	XP_027079245.1	endochitinase-like [*Coffea arabica*]	324 aa	LOC113702307	Chr7e	5248748	5250392	3	GH 19	I
CaChi83	XP_027079246.1	LOW QUALITY PROTEIN: basic endochitinase A-like [*Coffea arabica*]	323 aa	LOC113702308	Chr7e	5252189	5254849	3	GH 19	I
CaChi84	XP_027079250.1	endochitinase-like [*Coffea arabica*]	230 aa	LOC113702311	Chr7e	5258908	5260199	3	GH 19	I
CaChi85	XP_027085066.1	class V chitinase-like [*Coffea arabica*]	195 aa	LOC113707104	Chr8c	3092930	3093639	3	GH 18	V
CaChi86	XP_027084699.1	chitinase-like protein 2 [*Coffea arabica*]	321 aa	LOC113706865	Chr8c	6823940	6827606	3	GH 19	II
CaChi87	XP_027081129.1	class V chitinase-like [*Coffea arabica*]	149 aa	LOC113703845	Chr8e	3715623	3716156	2	GH 18	V
CaChi88	XP_027080527.1	chitinase-like protein 2 [*Coffea arabica*]	321 aa	LOC113703384	Chr8e	8301214	8304898	3	GH 19	II
CaChi89	XP_027098000.1	acidic endochitinase-like [*Coffea arabica*]	290 aa	LOC113717379	Chr11c	764767	765820	1	GH 18_like	III
CaChi90	XP_027097402.1	acidic endochitinase-like [*Coffea arabica*]	289 aa	LOC113716991	Chr11c	814948	816114	1	GH 18_like	III
CaChi91	XP_027097312.1	acidic endochitinase-like [*Coffea arabica*]	289 aa	LOC113716947	Chr11c	873018	874053	1	GH 18_like	III
CaChi92	XP_027095365.1	acidic endochitinase-like [*Coffea arabica*]	290 aa	LOC113715380	Chr11c	893644	894767	1	GH 18_like	III
CaChi93	XP_027095434.1	acidic endochitinase-like [*Coffea arabica*]	290 aa	LOC113715434	Chr11c	948283	949384	1	GH 18_like	III
CaChi94	XP_027097507.1	acidic endochitinase-like [*Coffea arabica*]	290 aa	LOC113717057	Chr11c	987257	988160	1	GH 18_like	III
CaChi95	XP_027097508.1	acidic endochitinase-like [*Coffea arabica*]	290 aa	LOC113717058	Chr11c	993952	1004557	2	GH 18_like	III
CaChi96	XP_027097513.1	acidic endochitinase-like [*Coffea arabica*]	290 aa	LOC113717064	Chr11c	1023759	1024734	1	GH 18_like	III
CaChi97	XP_027095393.1	acidic endochitinase-like [*Coffea arabica*]	290 aa	LOC113715408	Chr11c	1039024	1040124	1	GH 18_like	III
CaChi98	XP_027096545.1	acidic endochitinase-like [*Coffea arabica*]	306 aa	LOC113716416	Chr11c	3046237	3047346	1	GH 18_like	III
CaChi99	XP_027096458.1	acidic endochitinase-like [*Coffea arabica*]	294 aa	LOC113716360	Chr11c	6390901	6392087	1	GH 18	III
CaChi100	XP_027095332.1	acidic endochitinase-like [*Coffea arabica*]	299 aa	LOC113715345	Chr11c	29475666	29476706	1	GH 18	III
CaChi101	XP_027098572.1	acidic endochitinase-like [*Coffea arabica*]	307 aa	LOC113717922	Chr11e	6967217	6968320	1	GH 18_like	III
CaChi102	XP_027100040.1	acidic endochitinase-like [*Coffea arabica*]	290 aa	LOC113719150	Chr11e	8357204	8358244	1	GH 18	III

CaChi103	XP_027100511.1	acidic endochitinase-like [*Coffea arabica*]	290 aa	LOC113719497	Chr11e	8506691	8507868	1	GH 18_like	III
CaChi104	XP_027098235.1	acidic endochitinase-like [*Coffea arabica*]	299 aa	LOC113717659	Chr11e	37530668	37531573	1	GH 18	III
CaChi105	XP_027098536.1	endochitinase EP3-like [*Coffea arabica*]	273 aa	LOC113717890	Chr11e	12797454	12799272	2	GH 19	IV
CaChi106	XP_027101769.1	acidic endochitinase-like [*Coffea arabica*]	322 aa	LOC113722718	Chr0	2785680	2786907	1	GH 18	III
CaChi107	XP_027101733.1	acidic endochitinase-like [*Coffea arabica*]	325 aa	LOC113722674	Chr0	2763820	2764963	1	GH 18	III
CaChi108	XP_027101739.1	acidic endochitinase-like [*Coffea arabica*]	278 aa	LOC113722683	Chr0	3242929	3243765	1	GH 18	III
CaChi109	XP_027101723.1	hevamine-A-like [*Coffea arabica*]	295 aa	LOC113722649	Chr0	2924297	2925402	1	GH 18	III
CaChi110	XP_027101737.1	acidic endochitinase-like [*Coffea arabica*]	177 aa	LOC113722681	Chr0	3134975	3136188	2	GH 18_like	III

Table S1: Putative Chitinases Identified in the *Coffea arabica* Genome: Accession Number, Annotation, Protein Size, Gene Locus, Chromosomal Location, Start and End Positions, Number of Exons, Glycoside Hydrolase Domain, and Chitinase Class.

Chitinase	Accession number	Annotation	Protein Size	Gene Locus	Chr	Start	End	Exons	GH	Class
CcChi1	CDP08970.1	Glyco_hydro_18 domain-containing protein	307 aa	Cc01t12020.1 /GSCOCT000 28122001	Chr1	30734610	30735733	1	GH18	V
CcChi2	CDP05113.1	Glyco_hydro_18 domain-containing protein	296 aa	Cc02t02070.1 /GSCOCT000 20051001	Chr2	1647724	1649909	1	GH18	III
CcChi3	CDP09311.1	Glyco_hydro_18 domain-containing protein	297 aa	Cc02t26320.1 /GSCOCT000 28621001	Chr2	23952478	23953371	1	GH18	III
CcChi4	CDP09309.1	Glyco_hydro_18 domain-containing protein	265 aa	Cc02t26340.1 /GSCOCT000 28619001	Chr2	23956554	23957447	2	GH18	III
CcChi5	CDP08698.1	Glyco_18 domain-containing protein	437 aa	Cc02t39160.1 /GSCOCT000 27755001	Chr2	53735461	53742189	8	GH18	V
CcChi6	CDP03456.1	Glyco_hydro_18 domain-containing protein	306 aa	Cc03t12410.1 /GSCOCT000 15223001	Chr3	21275123	21276043	1	GH18	V
CcChi7	CDP10826.1	Chitin-binding type-1 domain-containing protein	160 aa	Cc03t13690.1 /GSCOCT000 31721001	Chr3	26765803	26767333	2	GH19	IV
CcChi8	CDP10827.1	Chitin-binding type-1 domain-containing protein	142 aa	Cc03t13700.1 /GSCOCT000 31722001	Chr3	26779852	26780341	2	GH19	IV
CcChi9	CDP10828.1	Chitin-binding type-1 domain-containing protein	273 aa	Cc03t13710.1 /GSCOCT000 31723001	Chr3	26796208	26797763	2	GH19	IV
CcChi10	CDP10829.1	Chitin-binding type-1 domain-containing protein	273 aa	Cc03t13720.1 /GSCOCT000 31724001	Chr3	26812845	26814889	2	GH19	IV
CcChi11	CDP10876.1	Glyco_hydro_18 domain-containing protein	301 aa	Cc03t14190.1 /GSCOCT000 31813001	Chr3	28218212	28219117	1	GH18	III
CcChi12	CDP10878.1	Glyco_hydro_18 domain-containing protein	273 aa	Cc03t14210.1 /GSCOCT000 31815001	Chr3	28263078	28263976	2	GH18	III
CcChi13	CDP10880.1	Glyco_hydro_18 domain-containing protein	263 aa	Cc03t14230.1 /GSCOCT000 31817001	Chr3	28276947	28298139	3	GH18	III
CcChi14	CDP16059.1	Glyco_hydro_18 domain-containing protein	303 aa	Cc05t00350.1 /GSCOCT000 17064001	Chr5	546649	547560	1	GH18	III
CcChi15	CDP17849.1	Glyco_hydro_18 domain-containing protein	316 aa	Cc05t00740.1 /GSCOCT000 13163001	Chr5	1293221	1294234	2	GH18	III
CcChi16	CDP17850.1	Glyco_hydro_18 domain-containing protein	295 aa	Cc05t00750.1 /GSCOCT000 13165001	Chr5	1307838	1308725	1	GH18	III
CcChi17	CDP17851.1	Glyco_hydro_18 domain-containing protein	320 aa	Cc05t00760.1 /GSCOCT000 13166001	Chr5	1371039	1372001	1	GH18	III
CcChi18	CDP17852.1	Glyco_hydro_18 domain-containing protein	324 aa	Cc05t00770.1 /GSCOCT000 13168001	Chr5	1393880	1394987	1	GH18	III

CcChi19	CDP17853.1	Glyco_hydro_18 domain-containing protein	323 aa	Cc05t00780.1 /GSCOCT000 13169001	Chr5	1422512	1423991	1	GH18	III
CcChi20	CDP17855.1	Glyco_hydro_18 domain-containing protein	550 aa	Cc05t00800.1 /GSCOCT000 13171001	Chr5	1437287	1442731	7	GH18	III
CcChi21	CDP17856.1	Glyco_hydro_18 domain-containing protein	317 aa	Cc05t00810.1 /GSCOCT000 13173001	Chr5	1484489	1485442	1	GH18	III
CcChi22	CDP10191.1	Glyco_18 domain-containing protein	368 aa	Cc06t15410.1 /GSCOCT000 30831001	Chr6	13383027	13385553	1	GH18	V
CcChi23	CDP10190.1	Glyco_18 domain-containing protein	392 aa	Cc06t15420.1 /GSCOCT000 30830001	Chr6	13388642	13390321	2	GH18	V
CcChi24	CDP10189.1	Glyco_18 domain-containing protein	377 aa	Cc06t15430.1 /GSCOCT000 30829001	Chr6	13394345	13396342	1	GH18	V
CcChi25	CDP10188.1	Protein kinase domain-containing protein	788 aa	Cc06t15440.1 /GSCOCT000 30827001	Chr6	13401420	13408241	7	GH18	V
CcChi26	CDP10187.1	Protein kinase domain-containing protein	575 aa	Cc06t15450.1 /GSCOCT000 30823001	Chr6	13433816	13437032	7	GH18	V
CcChi27	CDP10186.1	Glyco_hydro_18 domain-containing protein	165 aa	Cc06t15460.1 /GSCOCT000 30820001	Chr6	13448425	13450124	2	GH18	V
CcChi28	CDP10185.1	Glyco_18 domain-containing protein	404 aa	Cc06t15470.1 /GSCOCT000 30819001	Chr6	613454185	13456742	3	GH18	V
CcChi29	CDP01819.1	Chitin-binding type-1 domain-containing protein	324 aa	Cc07t11550.1 /GSCOCT000 36989001	Chr7	8501168	8502992	3	GH19	I
CcChi30	CDP01818.1	Glyco_hydro_19_cat domain-containing protein	84 aa	Cc07t11560.1 /GSCOCT000 36988001	Chr7	8503948	78504202	1	GH19	I
CcChi31	CDP01817.1	Glyco_hydro_19_cat domain-containing protein	123 aa	Cc07t11570.1 /GSCOCT000 36987001	Chr7	8504265	8507618	4	GH19	I
CcChi32	CDP04708.1	Glyco_hydro_18 domain-containing protein	210 aa	Cc07t18290.1 /GSCOCT000 18772001	Chr7	15612984	15614149	2	GH18	V
CcChi33	CDP17958.1	Glyco_hydro_19_cat domain-containing protein	93 aa	Cc07t20630.1 /GSCOCT000 07212001	Chr7	24205735	24206664	4	GH19	I
CcChi34	CDP07195.1	Glyco_hydro_19_cat domain-containing protein	207 aa	Cc08t05740.1 /GSCOCT000 24363001	Chr8	10470183	10474006	3	GH19	II
CcChi35	CDP16892.1	Class III chitinase	290 aa	Cc11t00400.1 /GSCOCT000 19478001	Chr11	863891	864763	1	GH18_like	III
CcChi36	CDP16893.1	Acidic endochitinase	289 aa	Cc11t00410.1 /GSCOCT000 19481001	Chr11	927744	928613	1	GH18_like	III

CcChi37	CDP16516.1	Glyco_hydro_18 domain-containing protein	294 aa	Cc11t02530.1 /GSCOCT00018513001	Chr11	8915164	8916258	1	GH18	III
CcChi38	CDP11638.1	Glyco_hydro_18 domain-containing protein	306 aa	Cc11t04040.1 /GSCOCT00034028001	Chr11	16348297	16349474	1	GH18	III
CcChi39	CDP00697.1	Glyco_hydro_18 domain-containing protein	495 aa	Cc11t09370.1 /GSCOCT00032743001	Chr11	26786345	26789938	6	GH18	III
CcChi40	CDP18755.1	Glyco_hydro_18 domain-containing protein	300 aa	Cc00t05840.1 /GSCOCT00012078001	Chr0	46714752	46715654	1	GH18	III
CcChi41	CDP18756.1	Glyco_hydro_18 domain-containing protein	320 aa	Cc00t05850.1 /GSCOCT00012079001	Chr0	46740216	46742534	1	GH18	III
CcChi42	CDP19988.1	Glyco_hydro_19_cat domain-containing protein	194 aa	Cc00t14300.1 /GSCOCT00010613001	Chr0	103197479	103198619	3	GH19	II
CcChi43	CDP21087.1	Glyco_hydro_18 domain-containing protein	320 aa	Cc00t24350.1 /GSCOCT00007449001	Chr0	153640826	153641788	1	GH18	III
CcChi44	CDP21287.1	Glyco_hydro_18 domain-containing protein	305 aa	Cc00t26220.1 /GSCOCT00004642001	Chr0	164355624	164356541	1	GH18	V
CcChi45	CDP21309.1	Chitin-binding type-1 domain-containing protein	273 aa	Cc00t26430.1 /GSCOCT00001144001	Chr0	166029763	166031655	2	GH19	IV
CcChi46	CDP21993.1	Glyco_hydro_19_cat domain-containing protein	168 aa	Cc00t33180.1 /GSCOCT00003246001	Chr0	194144747	194145646	3	GH19	II
CcChi47	CDP22251.1	Glyco_hydro_18 domain-containing protein	320 aa	Cc00t35710.1 /GSCOCT00010453001	Chr0	204588727	204590666	1	GH18	III

Table S2: Putative Chitinases Identified in the *Coffea canephora* Genome: Accession Number, Annotation, Protein Size, Gene Locus, Chromosomal Location, Start and End Positions, Number of Exons, Glycoside Hydrolase Domain, and Chitinase Class

Chitinase	Accession number	Annotation	Protein Size	Gene Locus	Chr	Start	End	Exons	Domain	Class
CeChi1	XP_027159407.1	chitinase 2-like [*Coffea eugenioides*]	307 aa	LOC113760866	Chr1	52771756	52772925	1	GH18	V
CeChi2	XP_027150045.1	chitinase 2-like [*Coffea eugenioides*]	305 aa	LOC113750245	Chr1	52829431	52830736	1	GH18	V
	-									
CeChi3	XP_027163861.1	acidic endochitinase SE2-like [*Coffea eugenioides*]	297 aa	LOC113764027	Chr2	1638766	1639921	1	GH18	III
CeChi4	XP_027163981.1	acidic endochitinase-like [*Coffea eugenioides*]	297 aa	LOC113764118	Chr2	28965015	28965908	1	GH18	III
CeChi5	XP_027163068.1	acidic endochitinase-like [*Coffea eugenioides*]	297 aa	LOC113763445	Chr2	28969497	28970670	1	GH18	III
CeChi6	XP_027161345.1	chitinase domain-containing protein 1 [*Coffea eugenioides*]	435 aa	LOC113762207	Chr2	78924029	78930611	8	GH18	V
	-									
CeChi7	XP_027164444.1	chitinase 2-like [*Coffea eugenioides*]	306 aa	LOC113764674	Chr3	34098972	34099892	1	GH18	V
CeChi8	XP_027166848.1	hevamine-A-like [*Coffea eugenioides*]	298 aa	LOC113766882	Chr3	41955720	41956651	1	GH18	III
CeChi9	XP_027165382.1	LOW QUALITY PROTEIN: basic endochitinase-like [*Coffea eugenioides*]	300 aa	LOC113765412	Chr3	41976828	41977920	1	GH18	III
CeChi10	XP_027165073.1	basic endochitinase-like [*Coffea eugenioides*]	301 aa	LOC113765185	Chr3	42029779	42030914	1	GH18	III
CeChi11	XP_027164462.1	endochitinase EP3-like [*Coffea eugenioides*]	274 aa	LOC113764694	Chr3	45711822	45713676	2	GH19	IV
CeChi12	XP_027164461.1	endochitinase EP3-like [*Coffea eugenioides*]	274 aa	LOC113764692	Chr3	45719395	45721395	2	GH19	IV
CeChi13	XP_027166706.1	endochitinase EP3-like [*Coffea eugenioides*]	287 aa	LOC113766750	Chr3	45723638	45728189	4	GH19	IV
CeChi14	XP_027164463.1	endochitinase EP3-like [*Coffea eugenioides*]	273 aa	LOC113764695	Chr3	45732896	45734753	2	GH19	IV
CeChi15	XP_027164460.1	endochitinase EP3-like [*Coffea eugenioides*]	273 aa	LOC113764691	Chr3	45739586	45741576	2	GH19	IV
CeChi16	XP_027164464.1	endochitinase EP3-like [*Coffea eugenioides*]	273 aa	LOC113764696	Chr3	45742882	45744469	2	GH19	IV
CeChi17	XP_027164637.1	endochitinase EP3-like [*Coffea eugenioides*]	273 aa	LOC113764825	Chr3	45817847	45819990	2	GH19	IV
CeChi18	XP_027164662.1	endochitinase EP3-like [*Coffea eugenioides*]	273 aa	LOC113764843	Chr3	45935906	45938049	2	GH19	IV
CeChi19	XP_027165648.1	endochitinase EP3-like [*Coffea eugenioides*]	274 aa	LOC113765615	Chr3	46145515	46147696	2	GH19	IV
	-									
CeChi20_1	XP_027169845.1	chitinase 1-like isoform X1 [*Coffea eugenioides*]	572 aa	LOC113769563	Chr4	9012190	9014626	2	GH18	III
CeChi20_2	XP_027169846.1	acidic endochitinase SE2-like isoform X2 [*Coffea eugenioides*]	295 aa	LOC113769563	Chr4	9012190	9014626	4	GH18	III
CeChi21	XP_027170822.1	acidic endochitinase SE2-like [*Coffea eugenioides*]	320 aa	LOC113770539	Chr5	430237	431391	1	GH18	III
CeChi22	XP_027171156.1	acidic endochitinase-like [*Coffea eugenioides*]	303 aa	LOC113770775	Chr5	467848	468902	1	GH18	III
CeChi23	XP_027170790.1	acidic endochitinase SE2-like [*Coffea eugenioides*]	327 aa	LOC113770517	Chr5	522543	523669	1	GH18	III

CeChi24	XP_027170476.1	hevamine-A-like [*Coffea eugenioides*]	337 aa	LOC113770259	Chr5	1157729	1158742	1	GH18	III
CeChi25	XP_027173031.1	hevamine-A-like [*Coffea eugenioides*]	295 aa	LOC113772594	Chr5	1166529	1167639	1	GH18	III
CeChi26	XP_027171280.1	acidic endochitinase-like [*Coffea eugenioides*]	322 aa	LOC113770867	Chr5	1281753	1282761	1	GH18	III
CeChi27	XP_027171348.1	acidic endochitinase-like [*Coffea eugenioides*]	322 aa	LOC113770922	Chr5	1316610	1317758	1	GH18	III
CeChi28	XP_027170866.1	acidic endochitinase-like [*Coffea eugenioides*]	324 aa	LOC113770567	Chr5	1339125	1340257	1	GH18	III
CeChi29	XP_027170949.1	acidic endochitinase-like [*Coffea eugenioides*]	325 aa	LOC113770626	Chr5	1377013	1378148	1	GH18	III
CeChi30	XP_027170816.1	acidic endochitinase-like [*Coffea eugenioides*]	323 aa	LOC113770535	Chr5	1401406	1402461	1	GH18	III
CeChi31	XP_027170490.1	hevamine-A-like, partial [*Coffea eugenioides*]	187 aa	LOC113770269	Chr5	1441780	1442423	1	GH18	III
CeChi32	XP_027172807.1	acidic endochitinase-like [*Coffea eugenioides*]	320 aa	LOC113772415	Chr5	1459983	1460945	1	GH18	III
CeChi33	XP_027172850.1	acidic endochitinase-like [*Coffea eugenioides*]	323 aa	LOC113772457	Chr5	1590150	1591205	1	GH18	III
CeChi34	XP_027172721.1	acidic endochitinase-like [*Coffea eugenioides*]	320 aa	LOC113772327	Chr5	1694950	1695934	1	GH18	III
CeChi35	XP_027171439.1	acidic endochitinase-like [*Coffea eugenioides*]	349 aa	LOC113771007	Chr5	3843201	3846879	3	GH18	III
CeChi36	XP_027171441.1	acidic endochitinase-like [*Coffea eugenioides*]	316 aa	LOC113771009	Chr5	3902151	3903106	1	GH18	III
CeChi37	XP_027171442.1	acidic endochitinase-like [*Coffea eugenioides*]	313 aa	LOC113771010	Chr5	3918261	3919202	1	GH18	III
CeChi38	XP_027171157.1	acidic endochitinase-like [*Coffea eugenioides*]	312 aa	LOC113770776	Chr5	3962193	3963309	1	GH18	III
CeChi39	XP_027174109.1	class V chitinase-like [*Coffea eugenioides*]	424 aa	LOC113773684	Chr6	16045934	16047342	2	GH18	V
CeChi40	XP_027174110.1	class V chitinase-like [*Coffea eugenioides*]	572 aa	LOC113773685	Chr6	16050554	16062755	4	GH18	V
CeChi41	XP_027174111.1	class V chitinase-like [*Coffea eugenioides*]	382 aa	LOC113773686	Chr6	16065348	16066496	1	GH18	V
CeChi42	XP_027175900.1	class V chitinase-like [*Coffea eugenioides*]	388 aa	LOC113775287	Chr6	16071060	16073658	2	GH18	V
CeChi43	XP_027175910.1	class V chitinase-like [*Coffea eugenioides*]	388 aa	LOC113775295	Chr6	16122185	16124783	2	GH18	V
CeChi44	XP_027177136.1	cysteine-rich receptor-like protein kinase 10 isoform X1 [Coffea	754 aa	LOC113776222	Chr6	16134388	16138421	7	GH18	V
CeChi44_1	XP_027177138.1	class V chitinase-like isoform X3 [*Coffea eugenioides*]	618 aa	LOC113776222	Chr6	16134388	16138421	7	GH18	V
CeChi44_2	XP_027177137.1	class V chitinase-like isoform X2 [*Coffea eugenioides*]	630 aa	LOC113776222	Chr6	16134388	16138421	7	GH18	V
CeChi45	XP_027174115.1	uncharacterized protein LOC113773691 [*Coffea eugenioides*]	862 aa	LOC113773691	Chr6	16162971	16171693	8	GH18	V
CeChi46	XP_027174117.1	class V chitinase-like [*Coffea eugenioides*]	370 aa	LOC113773692	Chr6	16173745	16175553	2	GH18	V
CeChi47	XP_027176704.1	class V chitinase-like [*Coffea eugenioides*]	405 aa	LOC113775863	Chr6	16180379	16182139	2	GH18	V
CeChi48	XP_027178929.1	basic endochitinase-like [*Coffea eugenioides*]	264 aa	LOC113777898	Chr7	3897450	3898813	3	GH19	II

CeChi49	XP_027179416.1	26 kDa endochitinase 1-like [*Coffea eugenioides*]	584 aa	LOC113778272	Chr7	3910391	3916056	7	GH19	I
CeChi50	XP_027181648.1	class V chitinase-like [*Coffea eugenioides*]	249 aa	LOC113780025	Chr8	4759404	4760280	3	GH18	V
CeChi51	XP_027183302.1	chitinase-like protein 2 [*Coffea eugenioides*]	321 aa	LOC113781544	Chr8	13677804	13681448	3	GH19	II
CeChi52	XP_027184542.1	acidic endochitinase-like [*Coffea eugenioides*]	307 aa	LOC113782816	Chr9	2037798	2038902	1	GH18_like	III
CeChi53	XP_027185483.1	endochitinase EP3-like [*Coffea eugenioides*]	273 aa	LOC113783522	Chr9	27814324	27816178	2	GH19	IV
CeChi54	XP_027149256.1	endochitinase EP3-like [*Coffea eugenioides*]	273 aa	LOC113749641	Chr10	3315412	3316987	2	GH19	IV
CeChi55	XP_027149257.1	chitinase 6-like [*Coffea eugenioides*]	136 aa	LOC113749642	Chr10	3321899	3322309	1	GH19	IV
CeChi56	XP_027149795.1	endochitinase EP3-like [*Coffea eugenioides*]	273 aa	LOC113750075	Chr10	15381209	15383060	2	GH19	IV
CeChi57	XP_027149794.1	endochitinase EP3-like [*Coffea eugenioides*]	273 aa	LOC113750074	Chr10	15388211	15390201	2	GH19	IV
CeChi58	XP_027149796.1	endochitinase EP3-like [*Coffea eugenioides*]	274 aa	LOC113750076	Chr10	15391507	15393146	3	GH19	IV
CeChi59	XP_027154252.1	acidic endochitinase-like [*Coffea eugenioides*]	290 aa	LOC113754110	Chr11	3872908	3873897	1	GH18_like	III
CeChi60	XP_027154178.1	acidic endochitinase-like [*Coffea eugenioides*]	290 aa	LOC113754058	Chr11	3887906	3889031	1	GH18_like	III
CeChi61	XP_027154475.1	acidic endochitinase-like [*Coffea eugenioides*]	291 aa	LOC113754294	Chr11	5067608	5068684	1	GH18_like	III
CeChi62	XP_027153149.1	acidic endochitinase-like [*Coffea eugenioides*]	290 aa	LOC113753245	Chr11	5240858	5241908	1	GH18	III
CeChi63	XP_027150928.1	acidic endochitinase-like [*Coffea eugenioides*]	294 aa	LOC113751201	Chr11	10802464	10803653	1	GH18	III
CeChi64	XP_027154739.1	acidic endochitinase-like [*Coffea eugenioides*]	299 aa	LOC113754492	Chr11	39453700	39454599	1	GH18	III
CeChi65	XP_027155718.1	acidic endochitinase-like [*Coffea eugenioides*]	307 aa	LOC113756123	Chr0	3581	4683	1	GH18_like	III
CeChi66	XP_027155649.1	basic endochitinase-like [*Coffea eugenioides*]	301 aa	LOC113756027	Chr0	40967	42102	1	GH18	III

Table S3: Putative Chitinases Identified in the *Coffea eugenioides* Genome: Accession Number, Annotation, Protein Size, Gene Locus, Chromosomal Location, Start and End Positions, Number of Exons, Glycoside Hydrolase Domain, and Chitinase Class.

Cluster1	QRef|RCH10 QRef|Chit2 QRef|RCC11 QRef|_Chitinase QRef|TcChi1 QRef|Chi_CH5B QRef|EuCHIT2 QRef|Tob_CHI QRef|DrChit
Cluster2	Ce|CeChi59_Chr11 Ce|CeChi60_Chr11 Ca|CaChi96_Chr11c Ca|CaChi92_Chr11c Ca|CaChi93_Chr11c Ca|CaChi94_Chr11c Ca|CaChi97_Chr11c
Cluster3	**QRef|Mcchit1 QRef|MdCHI1 Cc|CcChi29_Chr7 Ce|CeChi49_Chr7 Ca|CaChi83_Chr7e Ca|CaChi82_Chr7e**
Cluster4	Ce|CeChi12_Chr3 Ce|CeChi15_Chr3 Ce|CeChi57_Chr10 Cc|CcChi10_Chr3 Ca|CaChi16_Chr3c Ca|CaChi27_Chr3e
Cluster5	Ca|CaChi41_Chr5c Ca|CaChi40_Chr5c Cc|CcChi14_Chr5 Ce|CeChi37_Chr5 Ca|CaChi44_Chr5c Ce|CeChi38_Chr5
Cluster6	**QRef|CaChitIV Cc|CcChi9_Chr3 Ce|CeChi56_Chr10 Ca|CaChi33_Chr3e Ce|CeChi14_Chr3 Ca|CaChi13_Chr3c**
Cluster7	Ce|CeChi52_Chr9 Ce|CeChi65_Chr0 Cc|CcChi38_Chr11 Ca|CaChi98_Chr11c Ca|CaChi101_Chr11e
Cluster8	Ce|CeChi10_Chr3 Ce|CeChi66_Chr0 Cc|CcChi11_Chr3 Ca|CaChi18_Chr3c Ca|CaChi34_Chr3e
Cluster9	**Ca|CaChi81_Chr7e Ca|CaChi78_Chr7c QRef|IbChiA Cc|CcChi42_Chr0 Ce|CeChi48_Chr7**
Cluster10	**QRef|FvChi14 Cc|CcChi34_Chr8 Ce|CeChi51_Chr8 Ca|CaChi86_Chr8c Ca|CaChi88_Chr8e**
Cluster11	Cc|CcChi47_Chr0 Cc|CcChi41_Chr0 Cc|CcChi43_Chr0 Ca|CaChi45_Chr5c
Cluster12	Ce|CeChi34_Chr5 Ce|CeChi32_Chr5 Cc|CcChi21_Chr5 Ca|CaChi54_Chr5c
Cluster13	Ce|CeChi30_Chr5 Ce|CeChi33_Chr5 Cc|CcChi19_Chr5 Ca|CaChi51_Chr5c
Cluster14	Ca|CaChi62_Chr6c Ca|CaChi72_Chr6e Cc|CcChi25_Chr6 Ce|CeChi45_Chr6
Cluster15	Ca|CaChi63_Chr6c Ca|CaChi73_Chr6e Cc|CcChi26_Chr6 Ce|CeChi44_Chr6
Cluster16	**QRef|FnCHIT2 Cc|CcChi44_Chr0 Ce|CeChi2_Chr1Ca|CaChi1_Chr1c**
Cluster17	**QRef|CHI2 Cc|CcChi37_Chr11 Ce|CeChi63_Chr11 Ca|CaChi99_Chr11c**
Cluster18	Cc|CcChi39_Chr11 Ce|CeChi64_Chr11 Ca|CaChi100_Chr11c Ca|CaChi104_Chr11e
Cluster19	Cc|CcChi16_Chr5 Ce|CeChi25_Chr5 Ca|CaChi48_Chr5c Ca|CaChi109_Chr0
Cluster20	Cc|CcChi3_Chr2 Ce|CeChi4_Chr2 Ca|CaChi5_Chr2c Ca|CaChi8_Chr2e
Cluster21	Cc|CcChi36_Chr11 Ce|CeChi61_Chr11 Ca|CaChi90_Chr11c Ca|CaChi103_Chr11e
Cluster22	Cc|CcChi24_Chr6 Ce|CeChi46_Chr6 Ca|CaChi61_Chr6c Ca|CaChi71_Chr6e
Cluster23	Cc|CcChi2_Chr2 Ce|CeChi3_Chr2 Ca|CaChi4_Chr2c Ca|CaChi7_Chr2e
Cluster24	Cc|CcChi23_Chr6 Ce|CeChi47_Chr6 Ca|CaChi60_Chr6c Ca|CaChi70_Chr6e
Cluster25	Cc|CcChi28_Chr6 Ce|CeChi41_Chr6 Ca|CaChi65_Chr6c Ca|CaChi75_Chr6e
Cluster26	QRef|Chi2 QRef|CEMB-chiII QRef|pcht28
Cluster27	Ce|CeChi16_Chr3 Ce|CeChi58_Chr10 Ca|CaChi31_Chr3e
Cluster28	Ce|CeChi42_Chr6 Ce|CeChi43_Chr6 Ca|CaChi64_Chr6c
Cluster29	Ca|CaChi38_Chr4e Ca|CaChi37_Chr4c Ce|CeChi20_Chr4
Cluster30	Ca|CaChi23_Chr3e Ca|CaChi12_Chr3c Ce|CeChi54_Chr10
Cluster31	Ca|CaChi76_Chr6e Ca|CaChi66_Chr6c Ce|CeChi40_Chr6
Cluster32	Ca|CaChi36_Chr3e Ca|CaChi20_Chr3c Ce|CeChi8_Chr3
Cluster33	Cc|CcChi18_Chr5 Ce|CeChi28_Chr5 Ca|CaChi50_Chr5c
Cluster34	Cc|CcChi1_Chr1 Ce|CeChi1_Chr1 Ca|CaChi3_Chr1e
Cluster35	Cc|CcChi40_Chr0 Ce|CeChi22_Chr5 Ca|CaChi46_Chr5c
Cluster36	Cc|CcChi15_Chr5 Ce|CeChi24_Chr5 Ca|CaChi47_Chr5c
Cluster37	Cc|CcChi6_Chr3 Ce|CeChi7_Chr3 Ca|CaChi21_Chr3e
Cluster38	Cc|CcChi5_Chr2 Ce|CeChi6_Chr2 Ca|CaChi10_Chr2e

Cluster39	Cc|CcChi12_Chr3 Ce|CeChi9_Chr3 Ca|CaChi19_Chr3c
Cluster40	Cc|CcChi17_Chr5 Ce|CeChi26_Chr5 Ca|CaChi49_Chr5c
Cluster41	QRef|Chi26 QRef|CHI
Cluster42	Ce|CeChi11_Chr3 Ce|CeChi53_Chr9
Cluster43	Ce|CeChi18_Chr3 Ce|CeChi17_Chr3
Cluster44	Ce|CeChi21_Chr5 Ce|CeChi23_Chr5
Cluster45	Ca|CaChi59_Chr6c Ca|CaChi69_Chr6e
Cluster46	Ca|CaChi22_Chr3e Ca|CaChi105_Chr11e
Cluster47	Cc|CcChi35_Chr11 Ca|CaChi89_Chr11c
Cluster48	Cc|CcChi22_Chr6 Ca|CaChi58_Chr6c
Cluster49	Ce|CeChi36_Chr5 Ca|CaChi43_Chr5c
Cluster50	Ce|CeChi39_Chr6 Ca|CaChi77_Chr6e
Cluster51	Ce|CeChi50_Chr8 Ca|CaChi85_Chr8c
Cluster52	Ce|CeChi13_Chr3 Ca|CaChi26_Chr3e
Cluster53	Ce|CeChi19_Chr3 Ca|CaChi30_Chr3e
Cluster54	Ce|CeChi62_Chr11 Ca|CaChi102_Chr11e
Cluster55	Ce|CeChi27_Chr5 Ca|CaChi106_Chr0
Cluster56	Ce|CeChi35_Chr5 Ca|CaChi57_Chr5e
Cluster57	Ce|CeChi5_Chr2 Ca|CaChi9_Chr2e
Cluster58	Ce|CeChi29_Chr5 Ca|CaChi107_Chr0

Table S4: Orthogroups of chitinase genes identified across the genomes of *Coffea arabica*, *C. canephora*, *C. eugenioides*, and reference sequences (Qref) previously associated with responses to fungal pathogens. Orthologous groups shared between *Coffea* spp. and QRef sequences are highlighted in bold.

Source species	Gene/Protein ID	GH	Class	Annotation	Recipient species	Target fungus	References
Bitter melon	DQ407723.1 ABD66068.1	19	I	Mcchit1	Rice	*Magnaporthe grisea* *Rhizoctonia solani*	[1]
					Cotton	*Verticillium dahliae*	[2]
Barley	AJ276226.1 CAB99486.1	19	II	Chi2	Potato	*Alternaria solani*	[3]
	AAA56786.1	19	II	CHI	Blackgram	*Corynespora cassiicola*	[4]
					Tobacco	*Rhizoctonia solani*	[5]
	M62904.1 AAA32941.1	19	II	Chi26	Wheat	*Fusarium graminearum*	[6]
						Puccinia recondite *Puccinia striiformis* f. sp. tritici *Blumeria graminis*	[7]
	KC899774.1 AGS38341.1	19	II	CEMB-chiII	Sugarcane	*Colletotrichum falcatum*	[8]
Wheat	AAD28730	19	VII	Chi194	Tomato	*Fusarium oxysporum* f. sp. Lycopersici	[9]
Bean	S43926.1 AAB23263.1			Chi CH5B	Cotton	*Verticillium dahlia*	[10]
					Canola	*Rhizoctonia solani*	[11]
					Tobacco	*Rhizoctonia solani*	[12]
					Strawberry	*Botrytis cinerea*	[13]
Chinese wild strawberry	MN709779 QLY89005.1	18	V	FnCHIT2	*Arabidopsis*	*Colletotrichum higginsianum*	[14]

Cacao	U30324 AAA80656.1	19	I	TcChi1	Cacao	*Colletotrichum gloeosporioides*	[15]
Cucumber	NM_001308904.2 NP_001295833.1	18	III	CHI2	Cucumber	*Botrytis cinerea*	[16]
Eucommia ulmoides	KJ413009.1 AHX74093.1	19	I	EuCHIT2	Tobacco	*Erysiphe cichoracearum*	[17]
Apple	LOC103401024 NP_001280823.1	19	II	MdCHI1	Apple	*Colletotrichum gloeosporioides* *Alternaria alternata*	[18]
Brassica juncea	EF586206 ABQ57389.1	19	IV	Bj chitinase IV	*Brassica juncea*	*Alternaria brassica*	[19]
Mulberry	EXB55192.1	19	IV	MnChi18	*Arabidopsis*	*Botrytis cinerea*	[20]
Maize	MG017374.1 AYK28286.1	19	I	Chit2	Corn	Fusarium graminearum	[21]
Capsicum annuum	KJ649334.1 AJF11981.1	19	IV	CaChitIV	Arabidopsis	*Hyaloperonospora arabidopsidis*	[22]
Rice	LOC_Os03g30470 XP_015629397.1			RCH10	Rose	*Diplocarpon rosae*	[23]
					Lilium	*Botrytis cinérea*	[24]
	LOC_Os05g33130 XP_015640432.1	19	I	Chitinase2 Cht-2 RCC2 RCG3 RC7 ChtBD1 RC24	Banana	*Mycosphaerella fijiensis*	[25]
					Chrysanthemum	*Botrytis cinerea*	[26]

					Cucumber	*Botrytis cinerea*	[27]
					Cucumber	*Botrytis cinerea*	[28]
					Grapevine	Uncinula necator	[29]
					Italian ryegrass	*Puccinia coronata*	[30]
					Indica Rice	*Rhizoctonia solani*	[31]
					Peanut	*Cercospora arachidicola*	[32]
					Rice	*Magnaporthe grisea*	[33]
					Strawberry	*Sphaerotheca humuli*	[34]
					Tomato	*Alternaria solani* *Fusarium oxysporum* f. sp. Lycopersici	[35]
					Wheat	*Puccinia striiformis* f. sp. Tritici	[36]
	X54367.1 CAA38249.1	19	I	Chil1 RCC11 Rchit	Finger millet	*Pyricularia grisea*	[37]
					Grapevine	*Uncinula necator*	[38]
					Litchi	*Phomopsis* sp.	[39]

					Peanut	*Aspergillus flavus*	[40]
					Rice	*Rhizoctonia solani*	[41]
	LOC_Os11g47510 ABA95474.1	18			Rice	*Rhizoctonia solani*	[42]
Roundleaved Sundew	KU516826.1 AMM76171.1	19	I	DrChit	Tobacco	*Trichoderma viride*	[43]
Strawberry	OQ211094.1 WGF83129.1	19	II	FvChi-14	*Arabidopsis*	*Colletotrichum higginsianum*	[44]
Sweet potato	MN971588.1 QOD94995.1	19	II	IbChiA	Sweet potato	*Ceratocystis fimbriata*	[45]
Sugarbeet	A23392.1 CAA01677.1	19	IV	Chitinase IV	Silver birch	*Melampsoridium botulinum*	[46]
						Pyrenopeziza betulicola	[47]
Tobacco	X16938.1 CAA34812.1	19	I	Tob CHI	Tobacco	*Rhizoctonia solani*	[48]
					Peanut	*Cercospora arachidicola*	[49]
Wild rice	EU850802.1 ACJ24349.1	19	IV	OgChitIVa	*Arabidopsis*	*Botrytis cinerea*	[50]
Wild tomato	LOC107008831 XP_015063508.1			pcht28	Strawberry	*Verticillium dahliae*	[51]

					Tomato	*Verticillium dahliae* race 2	[52]

Table S5. Functionally characterized plant chitinase genes/proteins involved in resistance to fungal diseases in plants, based on a literature review of Chen et al, 2024 [53].

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TERCEIRA PARTE

2 CONSIDERAÇÕES FINAIS

Esta tese reuniu dois estudos distintos que, embora abordem diferentes patossistemas e culturas, compartilham o objetivo comum de aprofundar o conhecimento sobre aspectos moleculares relacionados a doenças fúngicas em plantas. No primeiro capítulo, foi realizada a identificação, caracterização molecular e de virulência de isolados de *C. falcatum* associados à podridão vermelha da cana-de-açúcar no sul da Flórida. Os resultados permitiram não apenas confirmar a identidade dos isolados como *C. falcatum* por meio de análises filogenéticas, mas também identificar e descrever eventos-chave do ciclo de infecção foliar, possibilitando a reconstrução do ciclo da doença e contribuindo significativamente para o entendimento da biologia do patógeno. Além disso, a avaliação da virulência em diferentes variedades comerciais revelou uma alta diversidade entre os isolados e indicou duas variedades com aparente maior tolerância à doença, fornecendo informações relevantes para os programas de melhoramento genético.

No segundo capítulo, foi conduzida a primeira caracterização genômica abrangente de genes e proteínas de quitinases nos genomas de *C. arabica* (tetraploide) e de seus progenitores diploides, *C. canephora* e *C. eugenioides*. A análise identificou famílias gênicas de quitinases conservadas, domínios funcionais e genes candidatos à defesa contra doenças fúngicas. Esses genes, por sua homologia e potencial papel funcional, podem ser explorados em programas de melhoramento, especialmente em *C. arabica*, por meio da superexpressão ou introdução de genes dos seus progenitores. A caracterização dos progenitores desta importante espécie de café expandiu o repertório genético disponível e oferece bases para futuras estratégias de melhoramento genético e biotecnologia voltadas ao aumento da resistência a doenças fúngicas.

Em conjunto, os dois artigos desta tese evidenciam o potencial da biotecnologia vegetal na geração de conhecimento aplicado à fitopatologia. Ao abordar tanto a caracterização de um patógeno relevante quanto a identificação de genes associados à defesa vegetal, os estudos apresentados contribuem com informações fundamentais para a compreensão das relações planta-patógeno e fornecem uma base sólida para futuras aplicações em programas de manejo e melhoramento de culturas de importância econômica.