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Research Article

Genetic analysis and resistance mechanisms in *Anthurium* against anthracnose caused by *Colletotrichum gloeosporioides*

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Abstract - This study aimed to elucidate the genetic mechanisms conferring resistance to anthracnose in *Anthurium* cultivars, caused by the fungal pathogen *Colletotrichum gloeosporioides*. Anthracnose represents a major constraint in *Anthurium* cultivation, adversely affecting both yield and ornamental value. To address the limited understanding of molecular resistance mechanisms, this research focused on two key defense-related enzymes: chitinase and β-1,3-glucanase. Ten cultivars were artificially inoculated with *C. gloeosporioides*, and subsequent analyses included DNA extraction, PCR amplification, sequencing, and enzymatic assays to evaluate physiological responses. Genetic similarity assessments were performed to determine cultivar relationships. The results indicated that 'Plew Tien Phuket' and 'Plew Tien Lampang' exhibited higher resistance, characterized by elevated enzyme activities and reduced lesion formation, compared to susceptible cultivars such as 'Merengue'. Increased activities of chitinase and β-1,3-glucanase in inoculated plants suggest their critical role in defense responses. Genetic analyses revealed significant diversity among cultivars, with resistant varieties displaying high genetic similarity, underscoring their potential as breeding resources. Collectively, these findings enhance our understanding of anthracnose resistance in *Anthurium* and propose chitinase and β-1,3-glucanase as promising markers for future breeding programs.

Keywords: Anthracnose, *Anthurium*, *Colletotrichum gloeosporioides*, chitinase, glucanase

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1. Introduction

Anthurium (*Anthurium andraeanum*), a member of the Araceae family, ranks among the top-selling tropical ornamentals worldwide due to its vibrant spathes, long vase life, and year-round flowering capacity (Teixeira da Silva et al., 2015). Globally, *Anthurium* holds substantial commercial value, particularly in the cut-flower trade and potted plant markets across Europe, Asia, and the Americas. Countries such as the Netherlands, Colombia, and Thailand play vital roles in international *Anthurium* supply chains (Pizano, 2003; Faust & Dole, 2021; Devrani et al., 2023). Despite its economic significance, the cultivation of *Anthurium* is challenged by various production issues, including susceptibility to fungal pathogens and postharvest deterioration. These vulnerabilities have prompted reliance on agrochemicals, raising sustainability and environmental concerns, especially in commercial-scale operations (Darras, 2020). As a result, there is growing momentum toward adopting environmentally friendly cultivation methods, such as integrated pest management, biological control strategies, and the development of genetically resistant cultivars (Darras, 2020).

Among biotic stresses, one of the most critical threats to *Anthurium* cultivation is anthracnose, a disease primarily caused by *Colletotrichum gloeosporioides*, which significantly reduces ornamental quality and market value (Winkelmann et al., 2020; Daughtrey & Buitenhuis, 2020; Teixeira da Silva et al., 2015), not only compromising ornamental quality but also imposing severe economic and ecological costs. In commercial settings, the disease can lead to flower deformities, necrotic lesions on leaves and spathes, and premature plant decline, resulting in significant revenue losses due to reduced marketability and export rejection (Winkelmann et al., 2020; Daughtrey & Buitenhuis, 2020). Yield losses can range from 20% to over 50% in severely affected batches, especially under high-humidity conditions favorable to pathogen spread. To manage outbreaks, growers often resort to frequent fungicide applications, which increase production costs and contribute to the emergence of fungicide-resistant strains of *Colletotrichum* (Dean et al., 2012). This overreliance on chemical control also poses ecological risks, including contamination of soil and water resources and adverse effects on beneficial microorganisms (Darras, 2020). Given the rising demand for sustainable ornamental production, addressing anthracnose with environmentally responsible strategies has become an urgent priority in the global floriculture industry.

Numerous studies have established that chitinase and β-1,3-glucanase play central roles in plant defense against fungal pathogens, functioning as pathogenesis-related (PR) proteins that degrade critical components of fungal cell walls. In *Solanum lycopersicum* (tomato), chitinase expression has been linked to enhanced resistance against *Fusarium oxysporum*, while in *Capsicum annuum* (chili), β-1,3-glucanase contributes to defense against *Phytophthora capsici* (Fernandez-Gutierrez & Gutierrez-Gonzalez, 2021). Similar defense responses were reported in *Oryza sativa* (rice), where upregulation of these enzymes correlates with resistance to *Magnaporthe oryzae*, the causal agent of rice blast. In garlic (*Allium sativum*), cultivar-specific increases in chitinase and glucanase activities were observed upon exposure to chitosan, further supporting their role in stress response signaling (Filyushin et al., 2023). The conserved function of these enzymes across species underlines their importance in general plant immunity and highlights their utility as biochemical markers for disease resistance. However, their contribution to anthracnose resistance in *Anthurium* has not been thoroughly investigated, underscoring the novelty and relevance of the current study.

Despite some advancements in understanding plant-pathogen interactions in model crops, research specifically focused on *Anthurium* remains limited. Most previous studies have relied heavily on phenotypic evaluations such as lesion scoring, symptom severity, and field-based screening to assess disease resistance. For instance, Teixeira da Silva et al. (2015) reviewed breeding efforts that selected cultivars based on visible disease symptoms and horticultural performance, while Srisamoot and Padsri (2018) evaluated genetic diversity using ISSR markers but did not correlate molecular traits with pathogen resistance. Although these studies provided valuable insights into cultivar variation, they lacked direct biochemical or molecular investigation into resistance mechanisms. As a result, the underlying molecular pathways contributing to anthracnose resistance remain poorly understood.

The novelty of this study lies in its integrated approach that combines biochemical assays of defense-related enzymes with molecular characterization of their corresponding genes, specifically within the context of *Anthurium*. This genus has received limited molecular investigation. While similar methods have been used in food and field crops, their application in *Anthurium* is rare and under-documented. To our knowledge, this is among the first studies to directly link chitinase and β-1,3-glucanase activity levels with their gene sequence variation in *Anthurium* cultivars exhibiting different responses to anthracnose. This integrated approach provides a comprehensive understanding of resistance mechanisms at both functional and genetic levels, but also provides foundational data for future use in molecular breeding and marker-assisted selection programs in ornamental crops.

2. Materials and methods

2.1 Sample collection and initial diagnosis

The ten *Anthurium* cultivars used in this study were selected to represent a broad spectrum of genetic diversity, commercial relevance, and variability in anthracnose resistance traits. These cultivars were sourced from both commercial breeding programs and regional collections within Thailand, a recognized hub for *Anthurium* diversity and cultivation. 'Plew Tien Phuket' and 'Plew Tien Lampang' are locally bred cultivars developed through controlled crossbreeding of resistant parent lines, historically exhibiting low incidence of anthracnose in germplasm plots and selected for both ornamental qualities and disease resilience. In contrast, commercially popular cultivars such as 'Merengue', 'Red Hot', and 'Cherry Pink' were primarily bred for aesthetic traits, including spathe coloration and shape, with less emphasis on disease resistance, making them valuable for contrasting disease response profiles. The remaining cultivars comprised a mixture of locally adapted lines and imported hybrids, contributing to the genetic heterogeneity necessary for meaningful comparative analysis. Although the sample size is relatively modest, it reflects a balanced representation of resistance profiles and commercial importance, similar to previous genetic diversity and enzyme-response studies in ornamental and tropical crops (Teixeira da Silva et al., 2015; Chanaeng et al., 2017). This stratified selection enabled the investigation of molecular and biochemical differences among genetically distinct cultivars, aiming to elucidate associations between gene variation, defense enzyme activity, and phenotypic resistance to anthracnose. While this study provides valuable insights, we acknowledge that expanding the cultivar set in future research would further enhance the resolution and generalizability of our findings.

2.2 Inoculation and experimental design

The pathogenicity of *C. gloeosporioides* was confirmed through a modified application of Koch's postulates (Ross & Woodward, 2016; Wang et al., 2020). For each of the ten *Anthurium* cultivars tested, five healthy plants were selected as biological replicates. From each plant, one fully expanded mature leaf was randomly chosen, and ten wound sites were created per leaf using a sterile needle to mimic natural pathogen invasion and ensure uniform infection. Each wound was inoculated with either 10 µL of *C. gloeosporioides* spore suspension (1×10^6 spores/mL) or sterile distilled water for the control group. Plants were arranged in a randomized complete block design within the greenhouse to minimize positional effects, and treatments were assigned randomly to ensure unbiased results. Following inoculation, plants were incubated under controlled environmental conditions of 28–30°C and 80–90% relative humidity to simulate conditions favorable for anthracnose development. Disease severity was assessed at eight days post-inoculation by counting the number of lesions and measuring their lengths. The disease severity index (DSI) was calculated by multiplying the number of lesions per leaf by the average lesion length and dividing by the total number of wounds tested.

For enzymatic activity assays, leaf samples were collected from each of the five biological replicates per treatment, and enzyme extractions and activity measurements were performed in triplicate (technical replicates) to ensure reproducibility. Molecular analyses were conducted using independently extracted DNA from the same biological replicates. All experiments were repeated twice to confirm the consistency of results.

Statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Before analysis, data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test to validate the assumptions for parametric testing. As the raw data for DSI and enzymatic activity conformed to these assumptions, no data transformations were necessary. Differences in DSI and enzyme activity among *Anthurium* cultivars were assessed using one-way analysis of variance (ANOVA). When significant differences were detected ($P < 0.05$), Duncan's Multiple Range Test (DMRT) was applied as a post hoc test to identify pairwise differences among cultivars. Results are reported as mean ± standard deviation (SD) of five biological replicates, with technical replicates averaged before statistical analysis to avoid pseudo-replication.

2.3 Assessment of physiological changes after inoculation

To investigate the biochemical defense response, three cultivars were selected from the panel of ten *Anthurium* cultivars. The selection included 'Plew Tien Phuket' and 'Plew Tien Lampang', representing highly resistant cultivars with low disease severity and high genetic similarity in resistance-related genes, and 'Merengue', a highly susceptible cultivar with the highest disease severity index and lowest enzymatic activity levels. This selection was based on contrasting disease response profiles (Table 1), genetic divergence revealed by sequence analysis (Table 4 and Table 5), and distinct phylogenetic positions (Figure 3). By choosing cultivars from both ends of the resistance spectrum, the study aimed to provide clear insights into the relationship between enzyme activity and anthracnose resistance.

Leaf samples from each cultivar were collected under both control and pathogen-inoculated conditions. Enzyme activities for chitinase and β-1,3-glucanase were determined using spectrophotometric assays as described by Filyushin et al.

(2023). The assays were conducted in triplicate, and enzyme activity was expressed as µg of reducing sugar per mg of fresh tissue per minute. Statistical significance between the control and inoculated samples was determined through comparative analysis.

2.4 Molecular characterization of resistance-related genes

The genetic basis of anthracnose resistance in *Anthurium* was investigated by analyzing the nucleotide sequence of chitinase and β-1,3-glucanase genes. In this study, the primers for chitinase and β-1,3-glucanase were designed to amplify partial coding regions of each gene, specifically targeting conserved domains known to be involved in anti-fungal activity (Figure 1).

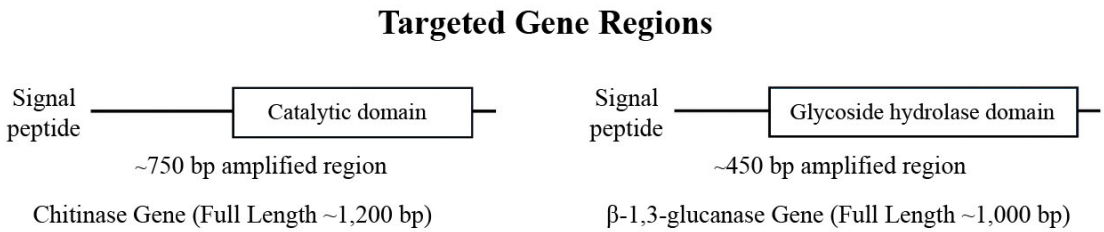

Figure 1. Schematic representation of the targeted gene regions in the chitinase and β-1,3-glucanase genes of *Anthurium*.

For the chitinase gene, amplification focused on the catalytic domain that encodes the active site responsible for hydrolyzing chitin in fungal cell walls. The β-1,3-glucanase primers were designed to amplify a region containing part of the glycoside hydrolase family domain, which plays a central role in degrading β-glucan in fungal pathogens. These regions were selected due to their functional relevance in plant-pathogen interactions and their moderate sequence variability, which is advantageous for detecting genetic polymorphisms among cultivars. Primer design was guided by multiple sequence alignment of related sequences from GenBank and validated through in silico PCR and BLAST analysis to ensure specificity and amplification efficiency. DNA was extracted from young leaves and spathes using a modified cetyltrimethylammonium bromide (CTAB) method, and the quality of DNA was assessed through agarose gel electrophoresis. Polymerase chain reaction (PCR) was used to amplify specific regions of the chitinase and β-1,3-glucanase genes. The primers used were the same as those reported by Srisamoot et al. (2020). The chitinase forward primer was 5′-GTC TCG GAG GAG GTT TTC CT-3′ and the reverse primer was 5′-TGA GTC ACC AAC CCA CAC AG-3′. For the β-1,3-glucanase gene, the forward primer was 5′-TCA ACG CCA GAG GAC TAC AA-3′ and the reverse primer was 5′-GCC CAG AAC CAA TAC TGC AT-3′. The PCR reaction mixture consisted of 25 µL total volume, including 1X PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 µM of each primer, 1.25 units of *Taq* DNA polymerase, and 20 ng of template DNA. Thermal cycling conditions were as follows: initial denaturation at 94°C for 3 minutes; followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds (optimized through gradient PCR), and

extension at 72°C for 45 seconds; with a final extension step at 72°C for 5 minutes. During amplification of the β-1,3-glucanase gene, multiple non-specific DNA bands were observed. To address this issue, optimization steps were taken, including gradient PCR to determine the optimal annealing temperature and the redesign of primers to improve binding specificity. Final primer sets were selected based on their ability to yield single, clearly defined bands. These optimized conditions were used in subsequent PCR amplifications to ensure the accuracy of downstream nucleotide sequencing and genetic similarity analyses. Amplification products were purified using the QIAquick Gel Extraction Kit and sequenced through a commercial sequencing service. The nucleotide sequences were analyzed using MEGA11 software to compare genetic similarity between anthracnose-resistant and susceptible *Anthurium* cultivars. Evolutionary distances were calculated using the Kimura 2-parameter method, and a dendrogram was constructed using the UPGMA method to visualize genetic relationships.

2.5 Data analysis

Statistical analyses of lesion size, DSI, and enzyme activities were performed using ANOVA followed by DMRT to determine significant differences between varieties. Genetic similarity and nucleotide diversity were calculated for the chitinase and β-1,3-glucanase genes, with average genetic similarity between varieties calculated to assess relatedness. Evolutionary distances and a dendrogram were constructed to interpret the genetic clustering patterns and provide insights into the genetic mechanisms underlying anthracnose resistance.

3. Results and discussion

3.1 Confirmation of anthracnose infection in *Anthurium* cultivars

Initial diagnosis confirmed that symptomatic leaves of *Anthurium* exhibited characteristic anthracnose symptoms, including brown lesions with defined margins and dark centers, consistent with fungal infection (Photita et al., 2005; Sakinah et al., 2014). These visible symptoms are typical of necrotrophic fungal pathogens, which cause tissue necrosis as part of their infection strategy (Dean et al., 2012). Pathogen isolation yielded *C. gloeosporioides* colonies displaying creamy-white mycelia with orange spore masses, aligning with previous descriptions by Fu et al. (2019), thus supporting accurate identification of the pathogen.

The development of conidial masses with a distinctive orange hue is a hallmark of active sporulation, indicating the pathogen's high virulence and reproductive potential under favorable conditions (Cannon et al., 2012). Pathogenicity tests through artificial inoculation and application of Koch's postulates validated *C. gloeosporioides* as the causal agent (Agrios, 2005; Photita et al., 2005). Lesions developed rapidly on inoculated plants within eight days, confirming successful infection and establishing a reliable foundation for subsequent resistance assessments.

The relatively short incubation period observed in this study highlights the aggressive nature of *C. gloeosporioides* under conducive environmental conditions, particularly in humid environments favorable to spore germination and infection (Sakinah et al., 2014; Cannon et al., 2012). The consistency of symptom expression across multiple cultivars further supports the robustness of the experimental design and the pathogen's ability to uniformly infect *Anthurium* (Photita et al., 2005). This standardized infection system ensures that differences

observed in disease severity and defense responses across cultivars can be attributed to intrinsic plant factors, rather than variability in pathogen exposure. These results provide critical validation of the experimental framework, allowing for reliable downstream analyses of biochemical and genetic resistance mechanisms.

3.2 Disease severity index (DSI) highlights cultivar resistance variation

Disease severity assessment across the ten *Anthurium* cultivars revealed significant variation in susceptibility to anthracnose, highlighting distinct resistance profiles (Rex et al., 2019). The cultivar 'Merengue' exhibited the highest DSI value (3.949), reflecting extensive lesion development, with an average lesion diameter of 5.06 mm and 39 lesions per leaf. Such high susceptibility suggests limited innate defense capacity, allowing rapid pathogen colonization and tissue degradation (Alkan et al., 2015). Conversely, resistant cultivars like 'Plew Tien Phuket' and 'Plew Tien Lampang' displayed markedly lower DSI values of 0.868 and 0.960, respectively, with reduced lesion sizes (2.41 mm and 2.53 mm) and fewer lesions per leaf (18 and 19, respectively) (Table 1).

Table 1. Evaluation of anthracnose severity in *Anthurium* cultivars.

No.	Cultivars	Average Lesion Diameter (mm)	Std. Deviation	Std. Error	Total Number of Disease Spots	DSI
1	Cherry Pink	3.72^{c}	0.00707	0.00316	30	2.232
2	Chompoo Noppon	2.87^{e}	0.02864	0.01281	26	1.494
3	Merengue	5.06^{a}	0.01789	0.00800	39	3.949
4	Plew Tien Lampang	2.53^{f}	0.17672	0.07903	19	0.960
5	Plew Tien Phuket	2.41^{f}	0.25371	0.11346	18	0.868
6	Red Bar	3.35^{d}	0.07950	0.03555	29	1.942
7	Red Hot	4.31^{b}	0.03937	0.01761	34	2.930
8	Red Strong	3.73^{c}	0.00548	0.00245	31	2.310
9	Sang Tien	3.38^{d}	0.05683	0.02542	29	1.962
10	Sun Red	4.34^{b}	0.12498	0.05589	34	2.951

Note: Different letters in the same column indicate statistically significant differences at the 95% confidence level, based on Duncan's New Multiple Range Test (DMRT).

The clear stratification of disease responses among cultivars underscores the genetic diversity inherent in *Anthurium* germplasm concerning anthracnose resistance (Teixeira da Silva et al., 2015). Notably, moderately susceptible cultivars, such as 'Red Hot' and 'Cherry Pink,' exhibited intermediate DSI values, suggesting partial resistance mechanisms that may involve delayed or less effective defense responses (Simko et al., 2021).

These phenotypic differences are crucial, as they reflect not only visible disease progression but also underlying biochemical and molecular processes that govern host-pathogen interactions (Dean et al., 2012). The observable disease severity provides essential baseline data that supports subsequent interpretations of enzymatic activity and genetic analysis, reinforcing the hypothesis that cultivar-specific resistance involves both constitutive and inducible defense strategies (Dehgahi et al., 2015).

3.3 Enhanced enzymatic activity in resistant cultivars

For enzymatic activity analysis, three cultivars were selected to represent the full spectrum of anthracnose resistance

observed in the broader panel of ten cultivars. These included 'Plew Tien Phuket' and 'Plew Tien Lampang', which consistently demonstrated strong resistance to *C. gloeosporioides* in both phenotypic and genetic analyses, and 'Merengue', which exhibited the highest disease severity index and lowest enzyme activity levels, indicating high susceptibility. These selections were based on their distinct disease response profiles (Table 1), genetic divergence (Table 4 and 5), and positions in the phylogenetic tree (Figure 3), thereby capturing both ends of the resistance spectrum. By focusing on these contrasting cultivars, the study aimed to clearly elucidate the relationship between defense enzyme activity and anthracnose resistance, while ensuring that the findings are broadly applicable to the diversity within *Anthurium* germplasm.

The activities of chitinase and β-1,3-glucanase enzymes were quantitatively analyzed to investigate their involvement in defense responses against *C. gloeosporioides* (Khan et al., 2018). Resistant cultivars such as 'Plew Tien Phuket' and 'Plew Tien Lampang' demonstrated higher baseline chitinase activity under control conditions compared to the susceptible 'Merengue' (Cletus et al., 2013). Following pathogen inoculation, these resistant cultivars showed a notable increase in chitinase activity, illustrating a strong inducible defense response (Van Loon et al., 2006).

β-1,3-glucanase activity mirrored this pattern, with resistant cultivars exhibiting significant increases upon infection, whereas 'Merengue' showed only a marginal rise. These statistically significant differences ($P < 0.05$), as shown in Table 2, confirm that enzymatic response intensity correlates strongly with observed phenotypic resistance (Figueiredo et al., 2018).

Table 2. Enzymatic activity of chitinase and β-1,3-glucanase in the leaves of selected *Anthurium* cultivars under control and *C. gloeosporioides* inoculated conditions.

Sample	Variety	Chitinase Activity (μg/mL)	β-1,3-glucanase Activity (μg/mL)
Control	Merengue	0.526 ± 0.018^{b}	0.398 ± 0.012^{d}
	Plew Tien Lampang	0.674 ± 0.034^{a}	0.636 ± 0.020^{c}
	Plew Tien Phuket	0.701 ± 0.032^{a}	0.662 ± 0.037^{c}
Inoculated	Merengue	0.585 ± 0.039^{b}	0.423 ± 0.029^{d}
	Plew Tien Lampang	0.743 ± 0.063^{a}	0.900 ± 0.003^{b}
	Plew Tien Phuket	0.756 ± 0.072^{a}	0.980 ± 0.014^{a}

Note: Values represent mean ± standard deviation (n = 3). Enzyme activity is expressed as μg of reducing sugar per mg of fresh tissue per minute. Superscript letters indicate statistically significant differences within the same column at $P < 0.05$, determined by Duncan's New Multiple Range Test (DMRT). Higher enzyme activity indicates stronger defense response following inoculation. For specific cultivar details and resistance profiles, refer to Table 1.

The elevated enzyme activities in resistant cultivars suggest an effective and rapid recognition of pathogen attack, followed by activation of defense pathways (Sels et al., 2008). Chitinase degrades chitin, a key structural component of fungal cell walls, thereby directly inhibiting fungal proliferation (Grover, 2012). Similarly, β-1,3-glucanase targets β-glucans, contributing to pathogen cell wall disintegration (Balasubramanian et al., 2012). The dual role of constitutive and inducible enzyme activities highlights the complexity of *Anthurium* defense strategies (Dehgahi et al., 2015).

3.4 Genetic analysis of chitinase and β-1,3-glucanase genes reveals diversity

The study's results demonstrated successful DNA amplification in all ten *Anthurium* cultivars examined. Gel electrophoresis revealed distinct DNA bands, as shown in Figure 2.

The agarose gel electrophoresis displayed DNA fragments of approximately 750 base pairs for the chitinase gene and 450 base pairs for the β-1,3-glucanase gene. However, the amplification of the β-1,3-glucanase gene resulted in the formation of multiple nonspecific DNA bands. This phenomenon often occurs when primers bind to DNA sequences that share similarity with the target gene, leading to unintended amplifications (Garafutdinov et al., 2020; Sakhabutdinova et al., 2020). One possible cause is primer specificity; if primers are not highly specific to the target sequence, they may anneal to homologous regions within the genome, particularly if these regions have similar nucleotide compositions. Additionally, nonspecific amplification can be influenced by the annealing temperature during PCR. Lower annealing temperatures increase the likelihood of primers binding to nonspecific sites, as they reduce the stringency required for primer-DNA binding (Sipos et al., 2007; Lorenz, 2012). Optimizing primer design to enhance specificity, and carefully adjusting the annealing temperature, are critical steps to minimize nonspecific amplifications and ensure accurate PCR results (Lorenz, 2012).

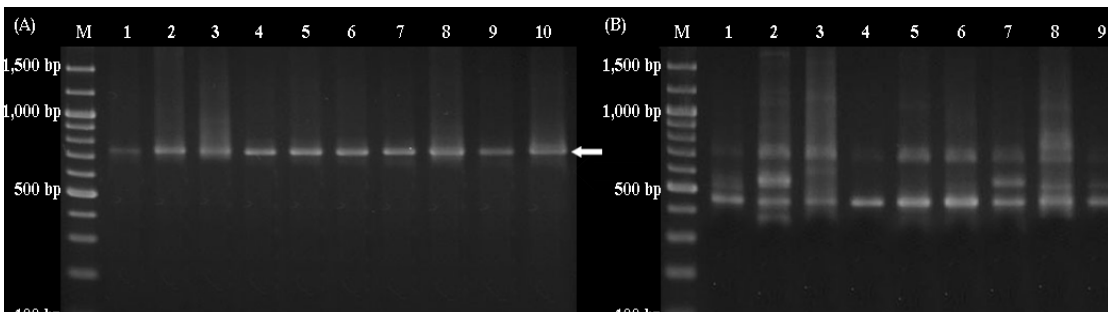

Figure 2. The amplification products of chitinase (A) and β-1,3-glucanase (B) gene of various *Anthurium* cultivars. M was 1kb DNA ladder marker, 1-10 number of *Anthurium* cultivars was shown in Table 1. The white arrow indicates the desired DNA band from the PCR reaction.

PCR amplification and sequencing of targeted regions within the chitinase and β-1,3-glucanase genes revealed meaningful genetic diversity among the *Anthurium* cultivars (Srisamoot et al., 2020). Resistant cultivars such as 'Plew Tien Phuket' and 'Plew Tien Lampang' shared high sequence similarity, implying conserved functional alleles associated with disease resistance (Zheng et al., 2018). In contrast, the susceptible cultivar 'Merengue' exhibited notable sequence divergence, suggesting alterations that may compromise gene function or expression (Bhadauria et al., 2017). Higher GC content observed in resistant cultivars is associated with greater gene stability and potentially higher expression levels (Šmarda et al., 2014) (Table 3). The variations identified across cultivars in these resistance-associated genes provide valuable molecular markers for distinguishing resistant and susceptible types (Guimarães, 2007; De Mori & Cipriani, 2023). These findings complement the biochemical data, reinforcing the hypothesis that sequence polymorphism in functional domains contributes to cultivar-specific defense capacity.

The observed variation in GC content of the β-1,3-glucanase gene sequences, ranging from 43.8% to 52.7%, holds significance for both gene stability and amplification efficiency. GC-rich regions contribute to greater thermal stability of the DNA double helix due to the triple hydrogen bonding between guanine and cytosine nucleotides (Šmarda et al., 2014). This increased stability can enhance the structural integrity of functionally important gene regions, potentially supporting higher levels of gene expression (Vinogradov, 2003). Notably, the resistant cultivars 'Plew Tien Phuket' and 'Plew Tien Lampang' exhibited higher GC contents (above 50%), which may be associated with more stable β-1,3-glucanase gene configurations, aligning with their stronger defense responses.

From a technical perspective, GC content influences PCR amplification dynamics. Higher GC regions generally require elevated annealing temperatures to ensure primer specificity and reduce secondary structure formation, which can otherwise hinder efficient amplification (McDowell et al., 1998). During our optimization, we observed that primers targeting higher GC content sequences performed optimally at slightly elevated annealing temperatures, which facilitated successful amplification and sequencing. These findings underline the biological and technical relevance of GC content variation in our study, supporting both the robustness of our genetic analysis and the potential functional significance of the β-1,3-glucanase gene in anthracnose resistance.

Table 3. Comparative nucleotide analysis of chitinase and β-1,3-glucanase genes in *Anthurium* cultivars.

Variety	Chitinase						β-1,3-Glucanase					
	Length (bp)	A	T	G	C	CG Content (%)	Length (bp)	A	T	G	C	CG Content (%)
Cherry Pink	655	143	159	158	195	53.9	490	110	122	135	123	52.7
Chompoo Noppon	667	161	174	163	169	49.8	489	100	141	141	107	50.7
Merengue	722	146	201	202	173	51.9	478	124	126	147	81	47.7
Plew Tien Lampang	717	160	167	199	191	54.4	442	122	108	108	104	48
Plew Tien Phuket	683	212	153	180	138	46	472	105	140	91	136	48.1
Red Bar	684	214	188	127	155	41.2	470	101	162	81	126	44
Red Hot	715	157	172	175	211	54	460	113	118	134	95	49.8
Red Strong	690	170	193	161	166	47.7	473	104	131	132	106	50.3
Sang Tien	675	163	156	175	181	52.7	476	117	108	113	138	52.7
Sun Red	699	222	208	122	147	38.5	482	106	165	85	126	43.8
Average	**690.7**	**174.8**	**177.1**	**166.2**	**172.6**	**49.01**	**473.2**	**110.2**	**132.1**	**116.7**	**114.2**	**48.78**

The average genetic closeness for the β-1,3-glucanase gene among the tested *Anthurium* cultivars was 55.00%, reflecting a moderate degree of genetic diversity within the group. However, certain cultivars exhibited higher similarity levels, suggesting shared genetic characteristics that could influence their defense capabilities. Notably, the Red Bar and Sun Red cultivars demonstrated the highest genetic closeness to the Plew Tien Phuket variety, with a similarity coefficient of 79.70%. This high genetic similarity suggests that these cultivars may possess similar genetic configurations within the β-1,3-glucanase gene, potentially contributing to parallel levels of pathogen resistance. In contrast, the Merengue

variety showed the lowest genetic closeness to Plew Tien Phuket, with a similarity coefficient of only 34.00% (Table 5).

This low level of genetic similarity indicates significant divergence in the β-1,3-glucanase gene sequence, suggesting that Merengue may have unique genetic traits that differentiate its response to pathogen challenges compared to other cultivars. Such genetic variability could influence breeding strategies, as identifying genetically distant cultivars may allow for the combination of diverse resistance traits in future breeding programs. These findings underscore the importance of genetic diversity in developing *Anthurium* cultivars with enhanced disease resistance and adaptability.

These findings reveal significant genetic diversity among the *Anthurium* cultivars, particularly in genes related to chitinase and β-1,3-glucanase, both of which are crucial for plant defense against fungal pathogens. Chitinase degrades the chitin in fungal cell walls, while β-1,3-glucanase breaks down β-glucans, another major component of fungal cell walls. Previous research has demonstrated that these enzymes play a critical role in the plant's ability to resist fungal infections by strengthening their cell walls and degrading pathogen structures (Dean et al., 2012; Xie & Duan, 2023).

The high genetic similarity observed between Pliew Tien Lampang and Pliew Tien Phuket indicates that these cultivars likely share genetic traits associated with increased anthracnose resistance. This is consistent with previous studies that have found that certain genetic similarities can be linked to enhanced disease resistance (Teixeira da Silva et al., 2015). On the other hand, the lower similarity of Merringue to other resistant cultivars suggests that genetic divergence in these key resistance-related genes may make it more susceptible to anthracnose. Genetic variation within the same cultivars, particularly in defense-related genes, is known to influence disease resistance and susceptibility (Fernandez-Gutierrez & Gutierrez-Gonzalez, 2021). This genetic diversity has important implications for *Anthurium* breeding programs, as understanding the sequence variability in these defense-related genes could help in selecting cultivars with enhanced resistance to diseases. Further studies could explore the functional consequences of these sequence variations to establish correlations between gene composition and pathogen resistance in *Anthurium*.

3.5 Phylogenetic clustering of cultivars based on resistance-related genes

Phylogenetic analysis using UPGMA clustering based on sequence data from the chitinase and β-1,3-glucanase genes revealed three major subgroups corresponding to disease resistance profiles (Tamura et al., 2013). Resistant cultivars clustered closely together, reflecting their genetic similarity and shared defense mechanisms (Bhadauria et al., 2017). Susceptible cultivars, including 'Merengue,' were placed in distinct clades, correlating with their lower enzymatic activity and higher disease severity (Figueiredo et al., 2018) (Figure 3).

Table 4. The similarity coefficient from the nucleotide sequences of the chitinase genes of different *Anthurium* cultivars.

No. 1		Similarity coefficient (%)									
		2	3	4	5	6	7	8	9	10	
1	Sang Tien	100									
2	Plew Tien Lampang	88.20	100								
3	Plew Tien Phuket	87.60	98.80	100							
4	Red Bar	63.60	63.30	64.20	100						
5	Sun Red	37.60	37.30	38.20	69.70	100					
6	Red Strong	36.70	37.00	37.30	44.20	47.10	100				
7	Chompoo Noppon	36.70	37.00	37.30	43.90	46.80	99.70	100			
8	Red Hot	35.00	37.30	36.70	36.40	35.50	40.20	40.50	100		
9	Cherry Pink	35.00	37.30	36.70	36.40	35.50	40.20	40.50	100.00	100	
10	Merengue	34.40	36.70	36.10	39.00	35.80	42.50	42.20	54.90	51.50	100
Average		47.80									

Table 5. The similarity coefficient from the nucleotide sequences of the β-1,3-glucanase genes of different *Anthurium* cultivars.

No. 1		Similarity coefficient (%)									
		2	3	4	5	6	7	8	9	10	
1	Sang Tien	100									
2	Plew Tien Lampang	82.70	100								
3	Plew Tien Phuket	68.20	70.10	100							
4	Red Bar	47.90	49.90	79.70	100						
5	Sun Red	47.90	49.90	79.70	100.00	100					
6	Red Strong	43.80	45.20	45.20	65.50	65.50	100				
7	Chompoo Noppon	39.50	40.50	40.50	60.80	60.80	95.30	100			
8	Red Hot	36.70	36.70	37.50	46.60	46.60	65.80	64.70	100		
9	Cherry Pink	40.50	42.20	40.30	57.30	57.30	80.00	80.00	63.30	100	
10	Merengue	33.40	34.20	34.00	37.50	37.50	52.30	52.60	67.10	51.50	
Average		47.80									

This clustering not only visualizes genetic relatedness but also provides practical insights for breeding programs. Cultivars within the resistant cluster represent valuable genetic resources for developing anthracnose-resistant hybrids (Teixeira da Silva et al., 2015). The integration of phylogenetic, biochemical, and phenotypic data enhances the predictive power of resistance screening in *Anthurium* germplasm (Guimarães, 2007; De Mori & Cipriani, 2023).

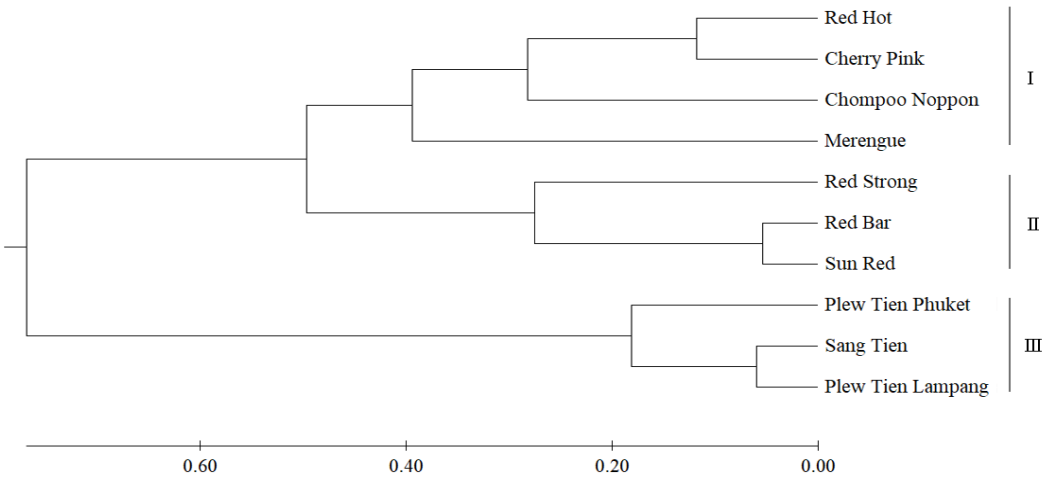

Figure 3. Phylogenetic relationships among *Anthurium* cultivars based on amino acid sequences of the chitinase and β-1,3-glucanase genes. The tree was constructed using the neighbor-joining method with bootstrap analysis (1,000 replicates). Cultivars exhibiting high anthracnose resistance clustered closely, indicating conservation of functional domains despite nucleotide-level variation.

In addition to nucleotide-based phylogenetic analysis, amino acid sequences of the chitinase and β-1,3-glucanase genes were analyzed to evaluate functional conservation among cultivars. Despite the high level of nucleotide sequence divergence observed (Table 4 and Table 5), the amino acid sequences demonstrated greater similarity across cultivars, particularly within conserved catalytic domains (Figure 3). Resistant cultivars such as 'Plew Tien Phuket' and 'Plew Tien Lampang' clustered closely together, reflecting not only genetic proximity but also conservation of functionally critical regions of the enzyme. This suggests that, while synonymous mutations may account for much of the nucleotide variation, the biochemical function of these enzymes is likely maintained among cultivars exhibiting resistance to *C. gloeosporioides*. Conversely, susceptible cultivars like 'Merengue' displayed minor amino acid differences, potentially affecting enzyme efficiency and contributing to lower defense responses. The amino acid-based phylogenetic tree thus provides a clearer view of the evolutionary and functional relationships among cultivars, reinforcing the relevance of these enzymes in anthracnose resistance.

3.6 Integrated insights: Linking enzymatic responses and genetic similarity

Synthesizing findings across phenotypic, enzymatic, and molecular levels reveals a clear correlation between disease resistance and defense gene profiles in *Anthurium* (Cletus et al., 2013; Khan et al., 2018). Resistant cultivars such as 'Plew Tien Phuket' and 'Plew Tien Lampang' consistently demonstrated superior resistance across all parameters, including low disease severity, elevated defense enzyme activity, and strong genetic similarity in resistance-related genes. Mechanistically, the observed elevation of chitinase and β-1,3-glucanase activities in these cultivars can be explained by their critical roles in degrading fungal cell wall components. Chitinase hydrolyzes chitin, a major structural polysaccharide in fungal cell walls, leading to hyphal degradation and inhibition of spore germination (Grover, 2012), while β-1,3-glucanase targets β-glucans, compromising fungal cell integrity

and impeding colonization (Balasubramanian et al., 2012). The heightened enzyme activities in resistant cultivars suggest more effective recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), triggering accelerated defense signaling pathways involving salicylic acid and jasmonic acid (Pusztahelyi et al., 2015). These enzymatic responses likely operate in concert with reactive oxygen species (ROS) production and cell wall reinforcement, contributing to localized hypersensitive responses that restrict pathogen spread (Sels et al., 2008). Furthermore, amino acid sequence analysis confirmed that, despite significant nucleotide polymorphisms, many variations were synonymous mutations that did not alter the enzyme's amino acid composition. The conservation of functional domains across resistant cultivars reinforces the functional significance of these genes in maintaining enzyme activity against *C. gloeosporioides*. The strong concordance between enzymatic activity, gene sequence variation, and phenotypic resistance supports the potential application of marker-assisted selection (MAS) in *Anthurium* breeding programs to efficiently identify resistant genotypes (Bhadauria et al., 2017; Guimarães, 2007; De Mori & Cipriani, 2023). Expanding the cultivar pool and targeting additional defense-related genes in future research will further refine breeding strategies and contribute to the sustainable management of anthracnose in commercial *Anthurium* production (Teixeira da Silva et al., 2015).

While this study focused on chitinase and β-1,3-glucanase enzymes due to their well-established roles in hydrolyzing fungal cell wall components, future research should expand to include a broader spectrum of defense-related molecular mechanisms. Profiling additional pathogenesis-related (PR) proteins such as PR-4 and PR-5, as well as enzymes involved in the phenylpropanoid pathway like phenylalanine ammonia-lyase (PAL) and peroxidases, could provide a more comprehensive understanding of the multilayered immune responses in *Anthurium*. Moreover, integrating transcriptomic approaches would enable the identification of novel defense-associated genes and regulatory networks activated during *C. gloeosporioides* infection. Such systems-level analyses will refine our understanding of host–pathogen interactions and support the development of more effective breeding strategies for durable anthracnose resistance.

4. Conclusions

This research addresses the critical issue of anthracnose disease in *Anthurium*, caused by *C. gloeosporioides*, which significantly impacts the ornamental plant industry. Traditional control methods, such as chemical fungicides, are becoming less effective due to increasing pathogen resistance and environmental concerns, underscoring the urgent need for alternative strategies. Although some progress has been made in understanding plant-pathogen interactions, the genetic mechanisms underlying anthracnose resistance in *Anthurium* remain poorly characterized. To bridge this gap, this study investigated the role of defence-related enzymes, specifically chitinase and β-1,3-glucanase, which are vital in plant defences against fungal infections. Sequence analysis of these genes across ten *Anthurium* cultivars, combined with enzymatic activity assays following inoculation with *C. gloeosporioides*, provided insights into the cultivars' defence responses. The genetic diversity analysis revealed an average similarity of 47.80% for the chitinase gene and 55.00% for the β-1,3-glucanase gene, with high genetic closeness observed between resistant cultivars such as 'Plew Tien Phuket' and 'Plew Tien Lampang'. This suggests that similar defence mechanisms may contribute to their enhanced resistance, whereas cultivars like 'Merengue', which displayed lower genetic similarity, may

harbor unique variations associated with higher susceptibility. These findings highlight the significance of chitinase and β-1,3-glucanase as potential biochemical markers for breeding programs aimed at developing disease-resistant cultivars. Future research should focus on identifying specific genetic markers linked to these enzymes to facilitate marker-assisted selection (MAS) for improved anthracnose resistance in *Anthurium*. Moreover, expanding the analysis to include additional defence-related genes, such as PR proteins and enzymes from the phenylpropanoid pathway, alongside comprehensive transcriptomic profiling, will deepen our understanding of the complex molecular defence networks in *Anthurium*. Such integrative approaches will strengthen breeding strategies and contribute to the sustainable management of anthracnose in the global floriculture industry.

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