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Assessment of genetic diversity in *Colletotrichum* sp. causing cucumber anthracnose using random amplified polymorphic DNA and inter-simple sequence repeat markers

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ABSTRACT

Anthracnose caused by Colletotrichum sp. is an important disease causing severe damage to the quality and yield of cucumbers in the Mekong Delta of Viet Nam. This study was conducted to assess the genetic diversity of Colletotrichum isolates collected from the Mekong Delta using 13 random amplified polymorphic DNA (RAPD) and 10 inter-simple sequence repeat (ISSR) primers. In addition, associations between these molecular markers and pathogenicity were explored using stepwise multiple regression analysis (MRA). The molecular markers showed high percentages of polymorphic bands (71-100%). Similarity coefficients among the isolates (74-82%) derived from the combined RAPD-ISSR data indicated a moderate to high level of genetic similarity. Two isolates, DT and CT, had the highest similarity coefficients. A total of 13 significant alleles were found to be associated with disease severity and AUDPC indices. S1189-458 and ISSR848-675 were the most significant alleles associated with both disease severity and AUDPC indices. Overall, RAPD and ISSR markers are useful for assessing genetic diversity, while their association with pathogenicity requires further validation.

1. INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most important crops in the Cucurbitaceae family. Cucumbers are used in daily meals and also applied in the cosmetic and health industries because of their richness of vitamins (Samba et al., 2023). According to the Food and Agriculture Organization of the United Nations (FAO, 2023), the estimated total cucumber production worldwide in 2023 was approximately 97,814,093 tons. In Viet Nam, they are commonly grown in the Mekong Delta. Cucumber plants are susceptible to many pathogens, especially anthracnose.

Anthracnose, caused by *Colletotrichum* sp., causes severe damage in cucumber cultivation, especially under high-humidity conditions (Matsuo et al., 2022). *Colletotrichum* sp. causes symptoms on various plant parts from the seedling stage, with dark brown lesions on cotyledons, leaves, stems, and fruits. In general, leaf lesions initially appear as water-soaked areas, then become tan or dark brown circular or angular spots. Severe infection can lead to coalescence of lesions, resulting in larger blighted areas on the leaves, which can significantly affect the plant's health and productivity (Roberts et al., 2023).

Understanding the genetic diversity of *Colletotrichum* in specific areas is important in developing appropriate disease control strategies (Patel et al., 2018). Several molecular approaches have been used to assess the genetic diversity of *Colletotrichum* sp. (Patel et al., 2018). RAPD techniques are widely used in genetic studies because of their simplicity and low cost (Kumar et al., 2011). RAPD markers amplify random DNA segments with a single primer of arbitrary nucleotide sequence (Williams et al., 1990). Additionally, the ISSR technique is a PCR-based method using a single primer targeting common microsatellites. The ISSR technique offers high reproducibility and sensitivity. Therefore, ISSR markers have been used in many studies on the genetic diversity of plant pathogen populations (Patel et al., 2018; Palacioglu et al., 2020).

Therefore, this study aimed at assessing the genetic diversity and pathogenicity of *Colletotrichum* sp. causing anthracnose on cucumber in the Mekong Delta of Viet Nam. Furthermore, molecular markers associated with pathogenicity were addressed to provide a basis for further studies on quantitative trait loci (QTLs) associated with pathogenic genes in order to develop effective disease control measures.

2. MATERIALS AND METHOD

2.1. Materials

Eleven fungal isolates of *Colletotrichum* sp. were isolated from infected cucumber leaves in the Mekong Delta of Viet Nam. The characteristics of these pathogens are described in Table 1 and Figure 1. The RAPD (Ratanacherdchai et al., 2007) and ISSR (Patel et al., 2018) primers used in this study are listed in Table 2.

Table 1. Fungal isolates of *Colletotrichum* sp. used in this study

Isolate	Conidia shape	Conidia length x width (µm)	Mycelia color	Sampling origin
AG1	Straight, cylindrical, one end slightly acute, septate and one-celled	3.75-7.5 x 1.25-2.5	White	Long Xuyen, An Giang
AG2	Straight, cylindrical, guttulate and one-celled	2.5-3.75 x 1.25-2.5	White	Binh Hoa, An Giang
BT	Short, cylindrical, non-septate and one-celled	4.75-7.25 x 1.75-6	White	Binh Dai, Vinh Long
CT	Straight, cylindrical, non-septate and one-celled	5.0-8.75 x 2.5-3.75	Grey	Binh Thuy, Can Tho
DT	Straight, cylindrical, septate or non-septate and one-celled	3.5-8.5 x 1.25-2.5	Grey	Lai Vung, Dong Thap
HG1	Straight, cylindrical, guttulate and one-celled	7.5-8.5 x 2.5-3.75	White	Chau Thanh, Can Tho
HG2	Straight, cylindrical, septate, one-celled	5-8.5 x 2.5-3.75	White	Chau Thanh, Can Tho
LA	Straight, cylindrical, non-septate, one-celled	5-11.25 x 1.25-2.5	White	Thanh Hoa, Tay Ninh
ST	Straight, cylindrical, non-septate and one-celled	3.75-11.25 x 2.5-5	Grey	My Thuan, Can Tho
TV	Straight, cylindrical, non-septate and one-celled	3.75-11.3 x 2.5-3.75	White	Chau Thanh, Tra Vinh
VL	Crescent-shaped, straight, septate, one-celled	3.75-12.5 x 2.5-3.75	White	Tam Binh, Vinh Long

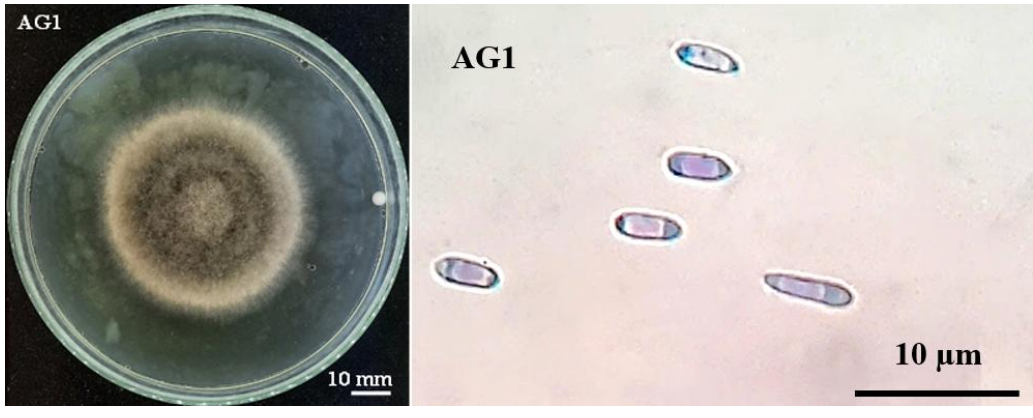

Figure 1. Colony morphology (a) and conidial characteristics (b) of *Colletotrichum* sp. isolate AG1

Table 2. RAPD and ISSR primer sequences in this research

RAPD primers		ISSR primers	
Name	**Sequence (5'-3')**	**Name**	**Sequence (5'-3')**
S1027	ACGAGCATGG	UBC809	AGAGAGAGAGAGAGAGG
S1063	GGTCCTACCA	ISSR830	TGTGTGTGTGTGTGTGG
S1089	CAGCGAGTAG	ISSR845	CTCTCTCTCTCTCTCTRG
S1136	GTGTCGAGTC	ISSR4	AGCAGCAGCAGCAGCGC
S1155	GAAGGCTCCC	ISSR860	TGTGTGTGTGTGTGTGRA
S1184	GACGGCTATC	ISSR848	CACACACACACACACARG
S1189	AGTCCCCCTC	ISSR823	TCTCTCTCTCTCTCTCC
S1234	TCGCAGCGTT	ISSR12	GTGTGTGTGTGTGTGT
S1239	TGACAGCCCC	ISSR857	ACACACACACACACACYG
S1265	GAGCTACCGT	ISSR859	TGTGTGTGTGTGTGTGRC
S1313	CTACGATGCC		
S1320	TGTCCTAGCC		
S1358	ACCCCAACCA		

2.2. Methods

2.2.1. Pathogenicity assessment of 11 isolates of Colletotrichum sp.

The cultivated cucumber variety in the Mekong Delta, Trang Nong 123, was used to assess the pathogenicity of 11 Colletotrichum sp. isolates. The experiment was conducted in a completely randomized design, with three replicates per treatment (1 plant/replicate).

Cucumber plants were grown in 25×30 cm pots with about 5 kg of soil/pot. Cucumber seeds were soaked in warm water for 2 hours to break dormancy before sowing. The plants were watered twice a day. At 7 days after sowing, Dau Trau N-P-K fertilizer (16-16-8) (Binh Dien Fertilizer Joint Stock Company, Viet Nam) was applied for basal fertilization using 0.02-0.025 kg in 10 litres of water.

Eleven *Colletotrichum* isolates were cultured on PDA media and incubated at room temperature for two weeks. Spore density was adjusted to 4×10^5 spores/mL. *Colletotrichum* sp. suspension was sprayed on the leaves when the cucumber plants had 3 true leaves (Nga et al., 2011).

The percentage of diseased leaf area was measured at 4, 5, and 6 days after inoculation using the ImageJ software. Disease severity was recorded as the percentage of diseased leaf area at 6 days after inoculation. AUDPC index was calculated as described by Simko and Piepho (2012):

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

Where y_i is the percentage of diseased leaf area at the i^{th} observation, t_i is the time at the i^{th} observation, and n is the total number of observations.

2.2.2. DNA extraction and amplification by RAPD and ISSR markers

DNA extraction was done according to Sambrook et al. (1989). DNA samples were amplified using RAPD and ISSR primers listed in Table 2. A 25 µL PCR reaction contained 16.75 µL sterile distilled water, 5 µL buffer, 1 µL RAPD or ISSR primer, 0.25 µL Taq DNA polymerase and 2 µL of DNA sample. The PCR program included 5 steps. The initial denaturation step was performed at 94°C for 3 minutes, followed by 35 cycles: denaturation at 94°C for 30 seconds, primer annealing at 35°C for 30 seconds, DNA elongation at 72°C for 1 minute, and a final extension of 10 minutes at 72°C. The optimal annealing temperature for ISSR markers varied as indicated in Table 2. PCR products were visualized using 2% agarose gel electrophoresis.

2.2.3. Phylogenetic analysis

The sizes of the PCR bands obtained from electrophoresis were detected by Gelanalyzer v2019 (Istvan & Istvan, 2019). The data was represented in a binominal form where 1 represented the presence of RAPD or ISSR bands and 0 represented no band. Dendrograms from cluster analyses were generated using the Unweighted Pair – Group Method for Arithmetic Average (UPGMA) with the SAHN module of NTSYSpc version 2.1 of Applied Biostatics Inc., US (Rohlf, 2000). The bootstrapping analysis was performed with 2,000 replicates using FREETREE software (Pavlícek et al., 1999). The similarity coefficient was calculated based on the

Simple Matching coefficient (Rohlf, 2000) as follows:

$$S = \frac{m}{n} = \frac{a+b}{a+b+c+d}$$

Where a is the total number of attributes where both of first and second DNA profiles have a value of 1; b is the total number of attributes where both of first and second DNA profiles have a value of 0; c is the total number of attributes where first DNA profile has value 0 and second DNA profile has value 1; d is the total number of attributes where first DNA profile has value 1 and first DNA profile has value 0; m is number of matching attributes; n is total number of attributes.

For dominant markers (RAPD and ISSR), the polymorphic information content (PIC) of each marker was calculated according to the formula (Powell et al., 1996):

$$\text{PIC} = 1 - \sum p_i^2$$

where p_i is the frequency of the presence of the *i*th band across all isolates. Band presence was scored as "1" and absence as "0", and allele frequencies were estimated based on the proportion of isolates showing band presence.

2.2.4. Association analysis

Association between the molecular markers (RAPD and ISSR) and pathogenicity (disease severity and AUDPC indices) was assessed using stepwise multiple regression analysis (MRA) in SPSS version 25. Disease severity and AUDPC indices were treated as dependent variables, while RAPD or ISSR molecular markers were treated as independent variables. The selection of independent variables for the regression analysis was based on F values with probabilities of 0.045 and 0.099 for entry and removal, respectively (Omri et al., 2021).

3. RESULTS AND DISCUSSION

3.1. Pathogenicity assessment of *Colletotrichum* sp. isolates on cucumber

Disease severity of eleven fungal isolates on cucumber cultivar Trang Nong 123 ranged from 39.70% to 84.66% at 6 days after inoculation (Table 3 and Figure 2). Under net house conditions, the fungal isolates AG1 and AG2 from cucumber leaves collected in An Giang province displayed the highest virulence with disease severities of 84.66% and 72.52%, respectively. The isolate AG1 exhibited the highest AUDPC index (AUDPC = 117). This value significantly differed from the AUDPC indices observed in the other isolates. The isolates with much lower virulence were CT, DT, ST, TV and VL, exhibiting a disease severity of approximately 40%. Also, the AUDPC indices showed no statistically significant differences among these isolates.

Table 3. Disease severity and AUDPC indices of *Colletotrichum* sp. isolates on cucumber Trang Nong 123

Fungal isolates	Disease severity	AUDPC index
AG1	84.7^{a}	117^{a}
AG2	72.5^{ab}	92.6^{b}
BT	58.5^{bcd}	82.4^{b-d}
CT	39.7^{d}	55.8^{f}
DT	42.8^{cd}	60.5^{ef}
HG1	51.1^{cd}	84.9^{bc}
HG2	52.4^{cd}	78.1^{b-e}
LA	60.3^{bc}	78.1^{b-e}
ST	41.1^{d}	65.8^{d-f}
TV	40.1^{d}	54.5^{f}
VL	39.9^{d}	68.3^{c-f}
P-value	< 0.001	< 0.001
CV (%)	13.1	8.1

Values followed by the same letter within a column are not significantly different. Data are presented as the mean of three replicates.

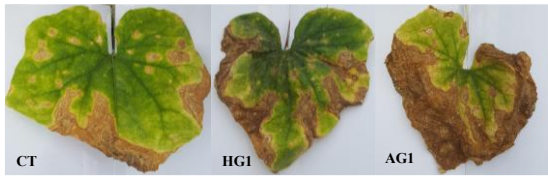

Figure 2. Disease symptoms caused by three different *Colletotrichum* isolates on cucumber leaves at 6 days after inoculation

Such variability in pathogenicity is consistent with previous reports describing significant differences in virulence among *Colletotrichum* isolates associated with anthracnose on diverse host plants. Studies on *Colletotrichum* populations infecting Chinese fir, mango, and tea-oil tree similarly demonstrated substantial variation in disease development, even among isolates belonging to the same species or originating from similar agroecological regions (Mo et al., 2018; Tovar-Pedraza et al., 2020; Wang et al., 2020; He et al., 2022). These observations supported the notion that pathogenicity in *Colletotrichum* is a highly variable trait.

The observed differences in pathogenicity among isolates may be explained by the hemibiotrophic lifestyle of *Colletotrichum*, which involves a biotrophic phase followed by a necrotrophic phase (Villa-Rivera et al., 2017). During infection, conidial germination and appressorium formation enable penetration of the pathogen into host cells, followed by the secretion of cell wall–degrading enzymes and other virulence factors that induce host tissue necrosis (Palenchar et al., 2009; Kodama et al., 2023). The identification of distinct pathogenicity levels among isolates in this study provides an important basis for further investigations into the genetic determinants of pathogenicity in *Colletotrichum* sp. infecting cucumber.

3.2. Genetic diversity of *Colletotrichum* sp. isolates using RAPD markers

The genetic diversity analysis of 11 isolates of *Colletotrichum* sp. by 13 RAPD molecular markers was presented in Table 4. The total number of amplified bands was 263. The average number of amplified bands for the RAPD primer was 20.2, ranging from 5 (S1063) to 30 bands (S1239).

Analysis results of 13 RAPD primers were used to construct the dendrogram using NTSYSpc software to reflect genetic diversity between 11 fungal isolates (Figures 3 and 4). The similarity coefficients among the 11 fungal isolates ranged from 68% to 86%, indicating a moderate to high level of genetic relatedness. The PIC values varied among primers, ranging from 0.201 to 0.318, with an average of 0.268. Primer S1089 showed the highest PIC value (0.318), followed by S1027 (0.306) and S1155 (0.303), whereas primer S1320 exhibited the lowest PIC value (0.201). Based on the cluster analysis, the isolates were classified into three main groups. Group I was further subdivided into subgroup Ia, comprising isolates AG1, LA, ST, and HG2, and subgroup Ib, represented by isolate HG1, reflecting variation in similarity coefficients within this group. Group II included isolates TV and VL, which shared a similarity coefficient of 75%. Group III was divided into two subgroups: subgroup IIIa consisted of isolates AG2, DT, and CT, where AG2 showed a 77% similarity coefficient with DT and CT, while DT and CT exhibited a higher similarity of 80%; subgroup IIIb was represented by isolate BT, which shared a 73% similarity coefficient with subgroup Ia. At the group level, Group I and Group II exhibited a similarity coefficient of 70% and showed a lower similarity (68%) with Group III, suggesting a clear genetic differentiation among the three major clusters.

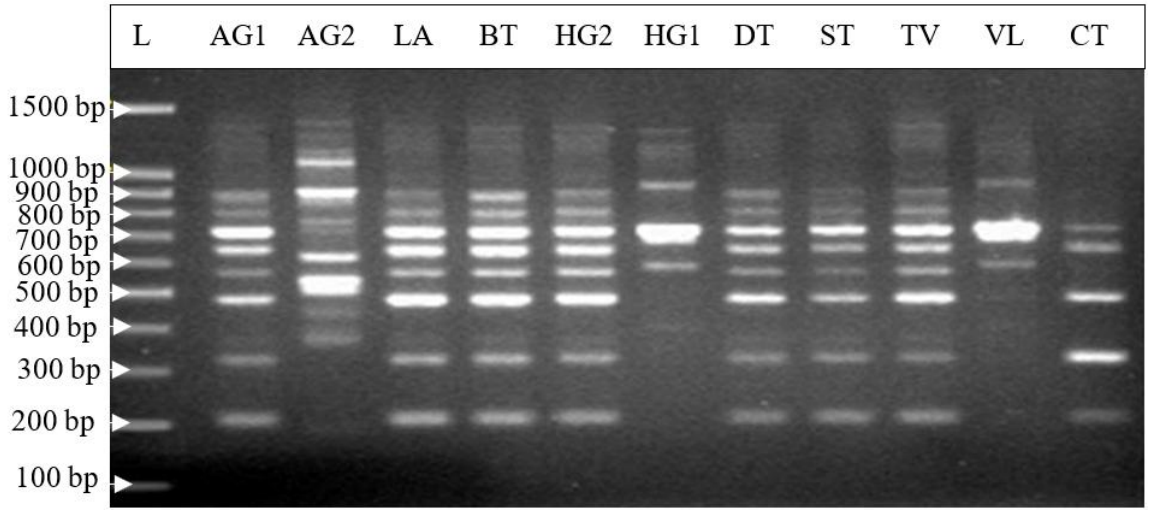

Figure 3. RAPD banding pattern of eleven *Colletotrichum* isolates generated using primer S1089

L: 100 bp DNA ladder RTU (Bio-Helix)

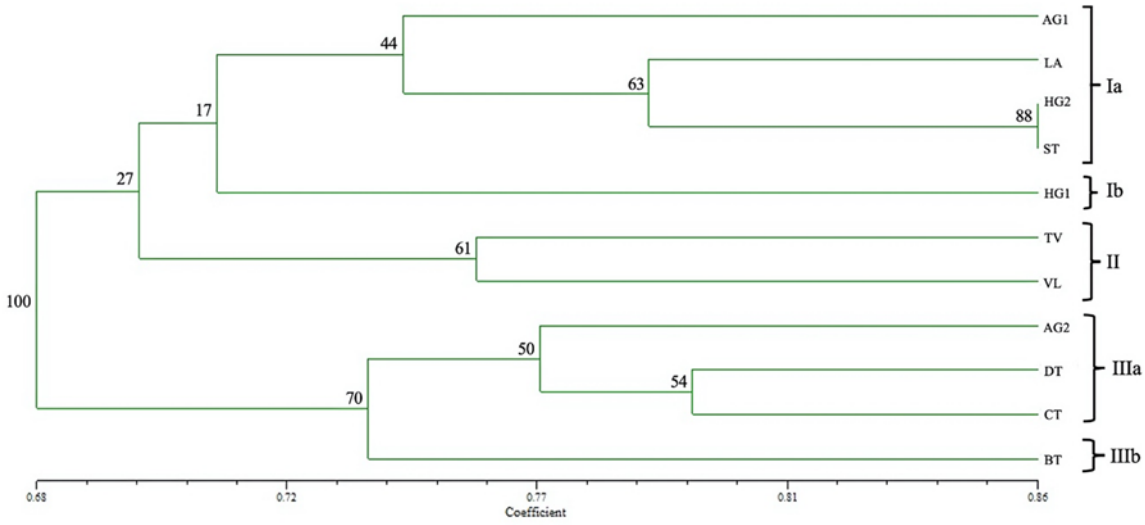

Figure 4. Dendrogram showing clusters of eleven *Colletotrichum* isolates using thirteen RAPD markers

Coefficient represents similarity values.

Comparable levels of RAPD polymorphism have been widely reported in previous studies on *Colletotrichum* spp. infecting various crops. For example, Sangdee et al. (2011) reported 31.81% polymorphism among 10 isolates of *C. capsici* causing chili anthracnose in Thailand using RAPD markers. In contrast, Mahmodi et al. (2014a) observed a much higher level of polymorphism (96%) among *C. truncatum*, *C. gloeosporioides*, and *C. dematium* infecting legumes. Similarly, Patel et al. (2018) reported 80.6% polymorphism in *C. falcatum* using 35 RAPD primers. These findings collectively highlight the effectiveness of RAPD markers in detecting genetic variation within *Colletotrichum* populations.

In the present study, although a relatively high level of genetic diversity was detected, no clear relationship was observed between RAPD-based clustering and pathogenicity traits, including disease severity and AUDPC. This observation is consistent with several previous studies in which RAPD clustering was more closely associated with geographic origin than with pathogenicity. Moreover, the relationship between RAPD-based grouping and fungal virulence has often been weak or inconsistent (Sangdee et al., 2011; Jebaraj et al., 2017; Esfahani, 2018). However, Munaut et al. (1998) reported a partial association between RAPD clustering, geographic origin, and pathogenicity, suggesting that this relationship may be context-dependent.

Further studies involving a larger number of *Colletotrichum* isolates collected from different geographical regions are required to better understand genetic variation and the potential dispersal patterns of this pathogen in the Mekong Delta.

Table 4. The number of polymorphic bands of 13 RAPD primers in the analysis of 11 fungal isolates

Marker	Total band	Polymorphic band	PIC
S1027	27	27	0.306
S1063	5	5	0.258
S1089	13	13	0.318
S1136	17	17	0.251
S1155	18	18	0.303
S1184	27	27	0.267
S1189	23	23	0.287
S1234	22	22	0.285
S1239	30	30	0.255
S1265	21	21	0.268
S1313	18	18	0.266
S1320	21	21	0.201
S1358	21	21	0.220
Total	263	263	-
Average	20.2	20.2	0.268

3.3. Genetic diversity of *Colletotrichum* sp. isolates using ISSR markers

Results of the genetic diversity analysis of 11 isolates of *Colletotrichum* sp. by 10 ISSR molecular

markers are presented in Table 5. The total number of amplified bands was 339 bands, and polymorphic bands accounted for 89% of total bands, ranging from 71% (UBC809) to 100% (ISSR4). The average number of polymorphic bands for ISSR primers was 30.1. The ISSR845 marker had the highest number of polymorphic bands (36), while the ISSR4 marker had the highest percentage of polymorphic bands (100%). This polymorphism is usually due to genetic variants, as indicated by the number of alleles at a locus and their frequency distribution in a population.

Analysis results from 10 ISSR primers were used to construct a dendrogram using the NTSYSpc software to reflect genetic diversity among 11 fungal isolates (Figures 5 and 6). The genetic similarity coefficients among the 11 fungal isolates ranged from 0.77 to 0.87, indicating a relatively high level of genetic diversity within the studied fungal population. The PIC values varied among primers, ranging from 0.165 to 0.216, with an average of 0.183. Primer UBC809 showed the highest PIC value (0.216), whereas primer ISSR4 exhibited the lowest PIC value (0.165). Cluster analysis based on ISSR profiles grouped the isolates into two main clusters. Group II comprised a single isolate, HG2, whereas Group I included the remaining ten isolates, with similarity coefficients ranging from 0.78 to 0.87. Group I was further subdivided into two subgroups. Subgroup Ib consisted solely of isolate AG1, which was genetically distinct from the isolates in subgroup Ia, including TV, HG1, CT, DT, VL, LA, ST, BT, and AG2. The isolates within subgroup Ia exhibited similarity coefficients ranging from 0.786 to 0.87. Notably, isolates HG1 and CT showed the highest genetic similarity (87%), and both shared a similarity coefficient of 85.2% with isolate TV.

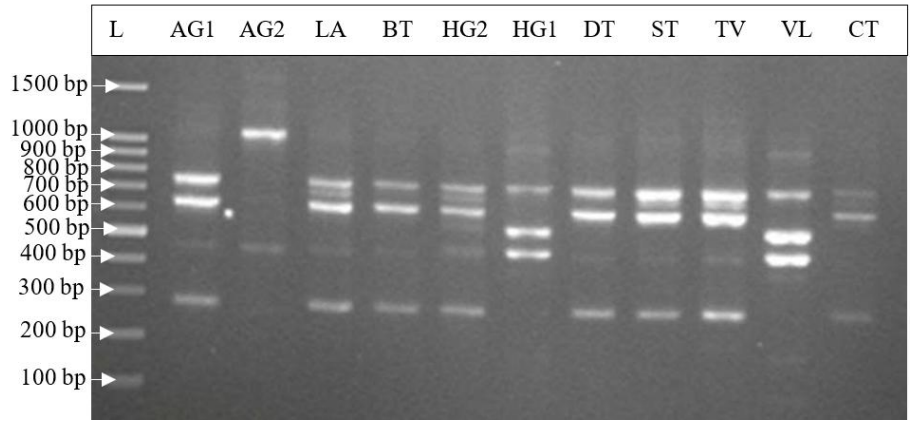

Figure 5. ISSR banding pattern of eleven *Colletotrichum* isolates generated using primer ISSR848

L: 100 bp DNA ladder RTU (Bio-Helix)

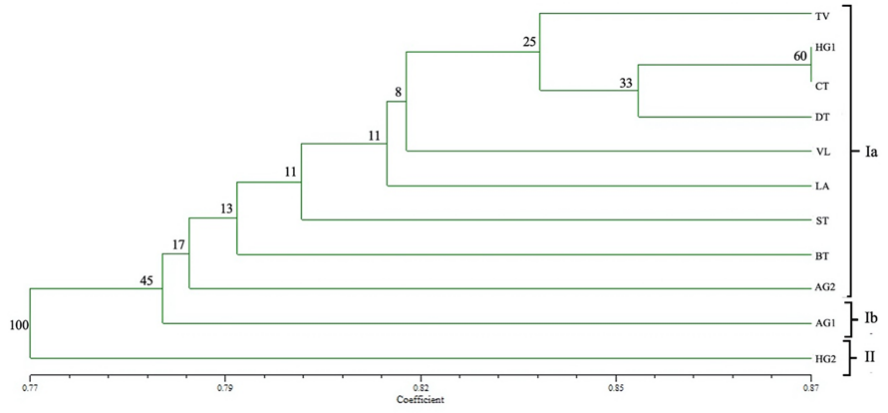

Figure 6. Dendrogram showing clusters of eleven *Colletotrichum* isolates using ten ISSR markers

Coefficient represents similarity values.

Similar findings have been reported in previous studies using ISSR markers to characterize genetic diversity in *Colletotrichum* spp. For instance, Patel et al. (2018) identified 21 out of 35 ISSR primers as polymorphic, corresponding to 68.07% polymorphism among 11 isolates of *C. falcatum* infecting sugarcane. Likewise, Mahmodi et al. (2014a) reported 100% polymorphism among *Colletotrichum* isolates from legumes using ISSR markers. More recently, Pineda-Vaca et al. (2025) observed a 58.87% polymorphism rate among 62 isolates of *C. siamensis* collected from mango orchards in Mexico. Collectively, these studies demonstrate that ISSR markers are a robust and reliable tool for assessing genetic structure in *Colletotrichum* populations.

Table 5. The number of polymorphic bands of 10 ISSR primers in the analysis of 11 isolates of *Colletotrichum* sp.

ISSR markers	Total bands	Polymorphic bands n (%)	PIC
UBC809	48	34 (71)	0.216
ISSR830	23	22 (96)	0.184
ISSR845	40	36 (90)	0.180
ISSR4	34	34 (100)	0.165
ISSR860	32	29 (91)	0.184
ISSR848	39	33 (85)	0.195
ISSR823	34	30 (88)	0.183
ISSR12	31	30 (97)	0.170
ISSR857	30	28 (93)	0.175
ISSR859	28	25 (89)	0.181
Total	339	301 (89)	-
Average	33.9	30.1	0.183

In the present study, despite the relatively high level of ISSR polymorphism detected, no clear relationship was observed between ISSR-based clustering and pathogenicity traits, including disease severity and AUDPC. This finding is consistent with previous reports on *Colletotrichum* isolates infecting mango and chili, where ISSR-derived genetic groupings were not correlated with virulence levels (Mahmodi et al., 2014b; Neto et al., 2022). Nevertheless, ISSR polymorphism remains useful for grouping *Colletotrichum* isolates according to species identity and geographic origin (Mahmodi et al., 2014a; Pineda-Vaca et al., 2025), although its ability to predict pathogenicity appears limited. It should be noted that ISSR markers reflect genome-wide variation and do not directly target genes associated with virulence.

Further studies involving a larger number of *Colletotrichum* isolates from diverse geographical regions are needed to better elucidate genetic variation and potential dispersal patterns of this pathogen in the Mekong Delta.

3.4. Genetic diversity of *Colletotrichum* sp. isolates using a combination of RAPD and ISSR markers

RAPD and ISSR marker data were combined to construct the dendrogram in Figure 3. The similarity coefficient of 11 fungal isolates ranged from 0.74 to 0.82, and 11 fungal isolates were classified into 3 groups (I, II and III). Group I included 4 isolates AG2, DT, CT and BT, which were divided into 2 subgroups Ia and Ib. Subgroup Ia comprised three isolates (AG2, DT, and CT), which exhibited approximately 78% similarity among themselves and shared a similarity coefficient of 76% with subgroup Ib, represented by isolate BT. Within subgroup Ia, isolates DT and CT showed the highest similarity coefficient, reaching 82%. Group II included six isolates (HG1, ST, VL, HG2, LA, and TV) and was further divided into three subgroups at a similarity coefficient threshold of 0.76. Subgroup IIa consisted of isolates HG1, ST, and VL, among which HG1 and ST exhibited the highest similarity coefficient (80%). Subgroup IIb comprised isolates HG2 and LA, showing a similarity coefficient of approximately 0.78. Subgroup IIc included isolate TV, which shared a similarity coefficient of 75% with both subgroups IIa and IIb. Group III was represented by a single isolate (AG1) and exhibited the lowest similarity coefficient (0.74) when compared with the other isolates, indicating a distinct genetic divergence.

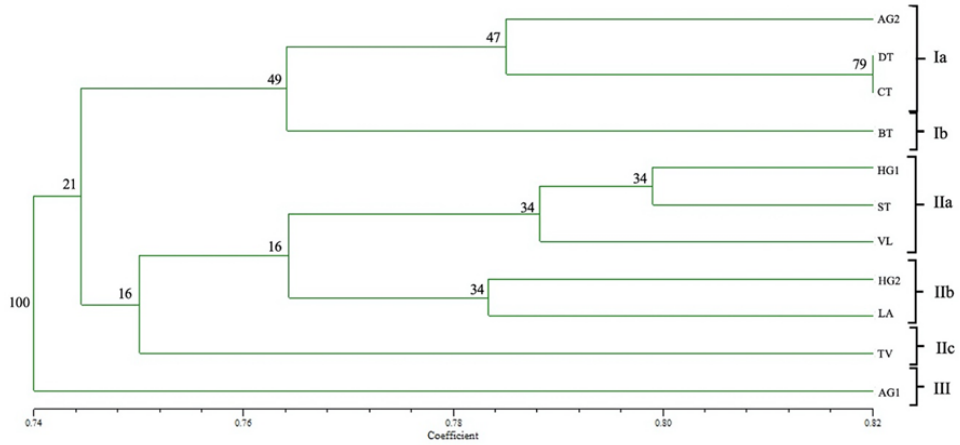

Figure 7. Dendrogram showing clusters of eleven *Colletotrichum* isolates using a combination of thirteen RAPD and ten ISSR markers

Coefficient represents similarity values.

Similar patterns have been reported in studies of *Colletotrichum gloeosporioides* infecting mango, in which highly virulent isolates were genetically distinct from less virulent ones based on combined molecular marker analyses (Rojas-Martínez et al., 2008). However, other studies have demonstrated weak or inconsistent associations between genetic similarity and virulence (Neto et al., 2022), suggesting that such relationships may be dependent on the pathogen–host system and the type of molecular markers used.

In addition, the combined use of molecular markers has been shown to improve the resolution of genetic differentiation among isolates, particularly in relation to their geographic origin (Patel et al., 2018). In the present study, isolate AG1 exhibited the highest AUDPC value ($p < 0.05$) and was separated into a distinct cluster in the combined RAPD–ISSR dendrogram, indicating a relatively high level of genetic divergence compared to the other isolates.

Nevertheless, this observation should be interpreted with caution, as marker-based clustering does not necessarily reflect functional differences in pathogenicity. The distinct genetic profile of isolate AG1 highlights its potential biological significance and warrants further investigation using more robust molecular approaches, such as sequence-based phylogenetic analysis or genome-wide studies.

3.5. Association analysis between molecular markers and pathogenicity of the pathogen

As shown in Table 6, the variation in AUDPC indices could be explained by 10 different RAPD alleles with $R^2 = 1.0$ in which allele S1184-458 and allele S1027-204 were two significantly associated alleles (beta = 1.029 and -0.677) and were the highest contributors with 77.9% of the variation. Allele S1189-458 had a strong positive association, while allele S1027-204 was negatively associated with the AUDPC indices. For disease severity, only 2 alleles were associated with disease severity, contributing to the explanation of 91.6% variation, namely S1189-458 and S1063-867. Both alleles were positively associated with disease severity where the S1189-458 allele showing the strongest association (beta = 0.736; $R^2 = 0.717$).

As far as the ISSR markers are concerned, in the AUDPC indices, only the ISSR848-675 allele showed an association with beta = 0.767; $p < 0.05$ and $R^2 = 0.588$. Disease severity was explained by 2 alleles with the strongest contribution from marker ISSR848-675, which showed the strongest positive association (beta = 0.953, $p < 0.05$).

Table 6. Association of RAPD and ISSR markers with pathogenicity of *Colletotrichum* sp. by stepwise MRA

Markers	Pathogenicity	Alleles	R	R^2	R^2 change	Standardized beta coefficient	P value
RAPD	AUDPC	S1189-458	0.772	0.596	0.596	1.029	< 0.05
		+ S1027-204	0.883	0.779	0.183	-0.677	< 0.05
		+ S1239-552	0.977	0.954	0.175	0.350	< 0.05
		+ S1027-579	0.993	0.985	0.031	-0.257	< 0.05
		+ S1027-322	0.997	0.995	0.009	-0.055	< 0.05
		+ S1358-992	0.999	0.999	0.004	0.119	< 0.05
		+ S1155-206	1.000	1.000	0.001	0.061	< 0.05
		+ S1136-658	1.000	1.000	0.000	0.008	< 0.05
		+ S1265-265	1.000	1.000	0.000	-0.001	< 0.05
		+ S1184-1123	1.000	1.000	0.000	0.000	< 0.05
	Disease severity	S1189-458	0.847	0.717	0.717	0.736	0.000
		+ S1063-867	0.957	0.916	0.199	0.460	0.002
ISSR	AUDPC	ISSR848-675	0.767	0.588	0.588	0.767	0.010
	Disease severity	ISSR848-675	0.849	0.721	0.721	0.953	0.000
		+ ISSR859-2030	0.940	0.883	0.162	0.416	0.017

The results of this study suggest that several RAPD and ISSR alleles may serve as preliminary indicators of potential genetic associations with pathogenicity-related traits in *Colletotrichum* causing anthracnose in cucumber. However, these associations should be interpreted with caution, as they do not provide direct evidence of linkage to functional genes involved in pathogenicity.

Further studies are required to validate these findings using larger pathogen populations and more robust statistical approaches. In addition, integrating advanced molecular techniques, such as sequence-based analyses and functional genomics, will be essential for elucidating the genetic basis of pathogenicity in *Colletotrichum*.

4. CONCLUSION

RAPD and ISSR markers used in this study revealed high levels of polymorphism among *Colletotrichum* isolates, demonstrating their effectiveness in assessing genetic diversity. RAPD markers showed slightly higher discriminatory power than ISSR markers under the conditions tested. Cluster analysis based on both marker systems did not show a clear relationship with pathogenicity traits, including disease severity and AUDPC. Association analysis identified several RAPD and ISSR alleles that were statistically related to pathogenicity parameters, with S1189-458 and ISSR848-675 consistently detected across analyses. However, these alleles should be considered as preliminary indicators rather than definitive markers of pathogenicity. Further studies using larger populations and sequence-based approaches are required to validate these associations and to better understand the genetic basis of pathogenicity in *Colletotrichum*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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