Molecular identification and characterization of *Colletotrichum* sp. isolates from Tahiti lime, tamarillo, and mango

Identificación y caracterización molecular de *Colletotrichum* sp. aislados de lima Tahití, tomate de árbol y mango

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ABSTRACT

Anthracnose is a very limiting disease affecting production, as well as postharvest quality of numerous fruit crops in Colombia. The current management practices for this disease are partially effective due to limited information about the etiology, the inoculum sources, population structure and variation of the pathogen. A total of 293 *Colletotrichum* isolates were obtained from symptomatic tissues collected from Tahiti lime, tamarillo and mango orchards. To determine the *Colletotrichum* species causing the symptoms, amplification, and PCR product analysis for intergenic regions of the ribosomal DNA were conducted. Genetic diversity of the fungal population was assessed with Random Amplified Microsatellites (RAMS). Results of this study indicated that anthracnose in Tahiti lime and tamarillo are caused by *Colletotrichun acutatum* whereas symptoms on mango were induced by the species *Colletotrichum gloeosporioides*, which was also fund in few citrus samples. RAMS data analysis indicated the existence of two distinct species groups, with a low similarity index (35%). RAM profiles also showed a clear host differentiation of isolates. The *C. acutatum* population originated from tamarillo exhibited a narrow and homogeneous genetic base, while the *C. acutatum* population from Tahiti lime was more heterogeneous and genetically complex, as determined by the analysis of molecular variance (AMOVA) and of Ni-Li coefficient. The *C. gloeosporioides* population originated from mango and Tahiti lime was heterogeneous and highly diverse, with clear host differentiation according to RAM profiles. Collectively, the results from this study provide new insight into the general characteristics of *Colletotrichum* populations on various hosts; this type of knowledge will prove useful in designing more effective management practices.

Key words: anthracnose, specific primers, genetic variability, RAMS, *C. acutatum, C. gloeosporioides.*

RESUMEN

La antracnosis es una enfermedad limitante para la producción y comercialización de diversos frutales cultivados en Colombia, y su manejo es deficiente, en parte por el desconocimiento de las especies implicadas, sus fuentes de inoculo y su estructura poblacional, y los niveles de variación del patógeno. Fueron utilizados 293 aislamientos obtenidos de tejidos con síntomas de antracnosis en cultivos de lima Tahití, tomate de árbol y mango para identificar las especies de *Colletotrichum* asociadas a la enfermedad, mediante la amplificación y el análisis de las regiones intergénicas del ADN ribosomal. Posteriormente, se evaluó la diversidad genética de la población mediante el uso de datos moleculares generados con marcadores tipo RAMS. Se identificó la especie *C. acutatum* en lima Tahití y tomate de árbol y *C. gloeosporioides* en mango y lima Tahití. En el análisis de variabilidad se detectaron dos grupos correspondientes a las especies *C. acutatum* y *C. gloeosporioides* (similitud de 35%). En general, ambos grupos de *Colletotrichum* se caracterizaron por presentar diferenciación por hospedero. En la población de *C. acutatum* de tomate de árbol se encontró una base genética estrecha y homogénea, mientras que la población de lima Tahití fue medianamente heterogénea y compleja de acuerdo con el análisis de varianza molecular y el coeficiente Ni-Li. La población de *C. gloeosporioides* de lima Tahití y mango se encontró heterogénea y altamente diversa. En general, para toda la población analizada se observó una agrupación acorde con el hospedero de origen. Los resultados obtenidos en este estudio proporcionan un primer acercamiento en la caracterización del patógeno en estos hospedantes contribuyendo al diseño de estrategias de manejo más efectivas.

Palabras clave: antracnosis, cebadores específicos, variabilidad genética, RAMS, *C. acutatum, C. gloeosporioides.*

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Introduction

Anthracnose is one of the most severely limiting diseases in the production of different fruit crops cultivated in Colombia. This disease is caused by *Colletotrichum* sp. species, which cause different kinds of symptoms in leaf tissues, young stems, flowers and fruits, producing great economic loss in various species (Osorio, 2000). Loses estimated in national fruit production are above 50% in crops of tamarillo, mango, lulo, blackberry, passion fruit, soursop, Mexican lime, avocado, papaya, and Tahiti lime, among others. This situation is more evident in humid production areas, during very wet periods (Páez, 1995; Freeman and Katan, 1997; Arauz, 2000; Afanador *et al*., 2003; Osorio *et al*., 2005), or under improper storage conditions. Control of the disease in Colombia focuses on combining cultural practices (removal of infected plant parts) with intensive use of fungicides of low or unstable efficacy, which generates rapid adaptation and appearance of resistant populations of the pathogen.

The causal agent of anthracnose presents broad variability in morphological, genetic, and pathogenic characteristics, which have been the object of numerous studies. Historically, morphological attributes (size and conidial shape, color and colony appearance, growth habit) have been used to separate species of *Colletotrichum*; nevertheless, some of these characteristics do not allow solving taxonomic relationships with certainty due to its notable plasticity (Freeman *et al.,* 2000; Peres et al., 2005; McKay et al., 2009). *Colletotrichum* sp. also shows great physiological versatility (biotrophic, hemibitrophic, necrotrophic behavior) that enables it to associate with various hosts and plant organs; this fact hinders clearly establishing taxonomic groups regarding origin, symptom type, pathogenicity, or host range (Freeman et al., 1998; Peres et al., 2005; MacKenzie *et al.,* 2007, 2009). Polymorphisms in some regions of the genome (GepR1, mitochondrial DNA, ribosomal DNA, etc.) used in multiple studies have been useful in differentiating species or sub-species groups (Freeman *et al.,* 1998; MacKenzie *et al.,* 2009), which exhibit a certain specificity to source hosts, or geographic origin, common in some cases. The genetic variation present in the population reflects the ability of the pathogen to evolve and adapt, providing key information when designing strategies of more effective control, such as the search for disease-resistant cultivars (Burdon and Silk, 1997; Mahuku *et al*., 2002; Ospina and Osorio, 2005; Abang *et al*., 2005).

Several studies have focused on more effective strategies for prevention of anthracnose, but their success has been limited by scarce epidemiological knowledge and the genetic variability of the pathogen. Hence, we need to reach higher understanding of the genetic structure of the pathogen population by identifying the *Colletotrichum* species directly involved, along with the genetic and pathogenic structure of the population.

The main objective of this study was to establish the genetic diversity in *Colletotrichum* sp. isolates that infect tamarillo, Tahiti lime, and mango based on morphological and pathogenic characteristics, as well as molecular analysis based on the amplification of an intergenic region of the ribosomal DNA (Liyanage *et al*., 1992; Freeman *et al.,* 2000; Afanador *et al.,* 2003) and the determination of genetic variability levels within the species, using RAM-type markers. This knowledge contributes in designing comprehensive strategies of preventive management of anthracnose.

Materials and methods

Biological material

Bearing in mind the lack of prior knowledge on the structure of pathogen population, a hierarchical sampling system was applied (crop, region, variety, farm, orchard) in areas of citrus, tamarillo and mango production, from where only organs with early symptoms of anthracnose were sampled. In total, 83 farms in 25 municipalities were visited, yielding 300 isolates. In this study, we evaluated 293 isolates from the *Colletotrichum* collection of the Plant Pathology Laboratory of the Tibaitatá Research Center (Corpoica, Mosquera/Cundinamarca). Of these isolates, 96 originated from Tahiti lime (Quindio, Risaralda, Caldas, Santander, and Meta), 98 from tamarillo (Antioquia and Cundinamarca), and 99 from mango (Tolima and Cundinamarca) (Tab. 1). A sample of 60 isolates (20 from each crop) was used to study morphological traits of conidia and length of hyphae terminals. The data obtained thus were subjected to cluster analysis by using the Ward algorithm. We also determined growth of the colony in fungicide-amended medium, optimum growth temperature, and presence or absence of setae in the 293 isolates; the trials were replicated three times.

Activation and culture of isolates

Each strain of the fungus was reactivated in oatmeal agar medium (“Difco. 0052”, Difeo Laboratories, Detroit, MI), at a temperature of 28°C during 8 d. From these cultures, a suspension of spores was prepared in sterile distilled water from which 2 μL were taken and added to flasks containing 125 mL of V8 liquid medium (200 mL L^{-1} *V8 Juice*) and 50 μg mL^{-1} of streptomycin sulfate. The inoculated flasks were

TABLE 1. Description of origin for 293 colombian isolates of *Colletotrichum* sp. used in this study.

Species[1]	City/Town of origin[2]	Host	Organ
C. acutatum	Armenia (7), Pereira (9), Caicedonia (11), Manizales (3), Pompeya (4), Villavicencio (8), Cumaral (10), Restrepo (2), Lebrija (14), Girón (7), Rionegro (1)	Tahiti lime	Flower
C. acutatum	Andalucía (2), Ciénaga (5)	Mexican lime	Flower
C. acutatum	Santa Rosa de Osos (20), Entrerríos (2), Pandi (9), San Bernardo (2), Silvania (37)	Tamarillo 'Red'	Fruit
C. acutatum	Rionegro (13)	Tamarillo 'Red'	Fruit
C. acutatum	Silvania (15)	Tamarillo 'Yellow'	Fruit
C. gloeosporioides	Coello (9), La Mesa (6), Anapoima (56)	Mango 'Tommy Atkins'	Fruit
C. gloeosporioides	Coello (9), Guamo (4), Espinal (13), Anapoima (1)	Mango 'Hilacha'	Fruit
C. gloeosporioides	Coello (1)	Mango 'Irwin'	Fruit
C. gloeosporioides	Montenegro (1)	Orange	Flower
C. gloeosporioides	Ciénaga (2), Andalucía (2)	Mexican Lime	Leaf
C. gloeosporioides	Villavicencio (4), Armenia (1), Caicedonia (2), Manizales (1)	Tahiti lime	Flower

1 Based on three tests for species identification: benomyl sensitivity, colony growth on streptomycin-copper hydroxide medium and ribosomal DNA (rDNA) amplification with taxon-specific primers.
2 Numbers in parenthesis represent total number of isolates collected at a given location.

incubated at 28°C during 8 d (Mahuku, 2004). After this period, the resulting mycelium was collected via vacuum filtration, dehydrated and macerated with liquid nitrogen in sterile mortar until obtaining a fine powder for DNA extraction. For DNA extraction, we followed the protocol described by Kelemu *et al.* (1997, 1999). The concentration of DNA extracted from all isolates was quantified with a fluorometer (Hoefer DNA QUANT 200®, Golden Valley, MN), and then the sample was fitted to a final concentration of 20 ng μL^{-1} to identify species with taxon-specific primers and 5 ng μL^{-1} for the RAM amplification.

Molecular determination of *Colletotrichum* sp. species

The molecular identification of *Colletotrichum* species involved in the infection of Tahiti lime, tamarillo, and mango was conducted through polymerase chain reaction amplification (PCR) of sequences from the intergenic region of ribosomal DNA. For this, specific primers were used derived from *C. gloeosporioides CgInt* (5´-GGCCTCC-CGCCTCCGGGCGG-3´) and from *C. acutatum CaInt2* (5´-GGGGAAGCCTCTCGCGG-3´) combined with the ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) universal primer by following the amplification conditions suggested by Freeman *et al.* (2000) and Afanador *et al.* (2003).

For the *C. acutatum* isolates, the amplification was carried out in a final volume of 20 µL containing the Taq Polymerase Promega® buffer (1X) (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 0.1% Triton® X-100); 1.5 mM de MgCl2; 200 µM from each dinucleotide (dATP, dCTP, dGTP, and dTTP (Promega®), 0.3 µM of each primer; one unit from the Taq Polymerase Promega® enzyme, and 40 ng from the DNA sample. The amplification profile used consisted of an initial 5-min cycle at 95°C, followed by 40 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min, and a final extension cycle at 72°C for 7 min.

To determine the *C. gloeosporioides* species, the ITS4 and *Cg*Int primers were used, performing amplifications under the same conditions described previously for *C. acutatum*. The amplifications were carried out in a thermocycler (PT-100 MJ Research Inc., Watertown, MA) programmed with an initial five-minute denaturing at 95°C, followed by 40 cycles of amplification (denaturing for 30 s at 95°C, 65°C of banding for 30 s, and extension at 72°C for 1 min), and the final extension cycle for 7 min at 72°C. The amplification products were visualized through electrophoresis in 1.5% agarose gels treated with ethidium bromide. As positive controls for the size of the amplification products, we included DNA from the TOM 021 isolate belonging to the *C. acutatum* species (Afanador *et al.,* 2003) and from reference isolate 16134 of *C. gloeosporioides* (Kelemu *et al.,* 1997, 1999). In addition, we included the DNA from the 120-COL isolate of *C. lindemuthianum,* a pathogen that causes anthracnose in the common bean, as negative control for this species.

Amplification of DNA by RAM markers

Eight RAM primers (Hantula *et al.,* 1996) were used to amplify microsatellites (Tab. 2) using amplification conditions described by Ganley and Bradshaw (2001) (Tab. 3). The reactions of amplification consisted in a final 12.5 µL volume composed of: 1.25 µL of Taq Polymerase Promega® buffer (10X) (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 0.1%

Triton® X-100); 1 µL of $MgCl_2$ 25 mM; 0.75 µL of the RAM primer 10 µM, 2 µL of dNTP 1.25 mM (dATP, dCTP, dGTP, and dTTP Promega®), 0.5 µL of the Taq polymerase enzyme Promega®, and 1 µL of the DNA sample at a 5-ng concentration, and carried out in a thermocycler programmed with different cycles, specific for each of the RAM primers (Tab. 2). The amplification products were visualized through electrophoresis in 1.5% agarose gels stained with ethidium bromide from a 1 mg mL^{-1} solution at 90 V/5 h for 30 min and visualized in an Eagle eye II from Strategene. To estimate the size of the amplified product we used two molecular weight patterns: 1 kb DNA Ladder Promega® with a reading range between 10,000 and 250 pb, and a molecular weight pattern of 100 bp DNA Ladder Promega® with a reading range between 1,500 and 100 pb.

For product analysis, each DNA fragment generated was independently analyzed assuming that same size DNA fragments represented the same genetic locus. By reading the bands obtained with each RAM primer, a binary matrix was constructed for the 293 *Colletotrichum* sp. isolates with which a similarity analysis was performed by using the NTSYS program version 2.1 (Rohlf, 2000) and the Simqual subprogram for molecular markers. The estimations of the similarity analysis were calculated with the Dice coefficient, also known as the Nei-Li coefficient. The resulting similarity matrices were then analyzed by the SAHN program to construct dendrograms using the Unweighted Pair Group Method with Arithmetic Mean "UPGMA".

Complementarily, a multiple correspondence analysis (MCA) was performed via the SAS statistical program (SAS Institute Inc., 2000) to visualize the multidimensional representation of individuals. The analysis of molecular variance (AMOVA) was also done to establish the relationship among the isolates collected from the three fruit species (Tahiti lime, mango, and tamarillo), examine the genetic distances among the corresponding isolates to each host, and determine the relationship among the isolates belonging to each species involved. This statistical program was also used to determine the overall diversity and diversity within and among populations.

TABLE 2. Primers used in this study.

Primer	Sequence (5`→3`)[1]
TG	HBH TGT GTG TGT GTG TGT
CGA	DHB CGA CGA CGA CGA CGA
CT	DYD CTC TCT CTC TCT CTC
CA	DBD ACA CAC ACA CAC ACA
GT	VHV GTG TGT GTG TGT GTG
AG	HBH AGA GAG AGA GAG AGA
CCA	DDB CCA CCA CCA CCA CCA
ACA	BDD ACA ACA ACA ACA ACA

1 Letter designation for degenerate primer sites are: H (A, T ór C); B (G, T or C); V (G, A or C) and D (G, A or T).
Source: Gandley and Bradshaw (2001).

Results and discussion

The sampling scheme used in this study represented the geographic diversity of fruit production, which is high for citrus, medium for tamarillo, and low for mango. The varietal composition, however, was very narrow both for acid limes and tamarillo (two genotypes per species sampled), and narrow for mango (three varieties). A total of 300 isolates resulted from this sampling, which were morphologically typified for their preliminary assignment to the *C. acutatum* or *C. gloeosporioides* species; of which 293 were selected for this study.

Molecular identification of species

Isolates amplified with specific primers revealed a 490-pb DNA fragment with the *Ca*Int2/ITS4 primer combination for the *C. acutatum* species, and 450 pb for *C. gloeosporioides* using *Cg*Int/ITS4 primers (Fig. 1). The morphological tests made for the same isolates permitted

TABLE 3. Amplification conditions of *Colletotrichum* sp. microsatellites with selected RAM primers.

RAM primer	Amplification phases					Number of cycles
	Initial denaturation	Denaturation	Annealing	Extension	Final extension	
AG	95°C por 5 min	95°C por 40 s	50°C por 45 s	72°C por 2 min	72°C por 10 min	35
TG	95°C por 5 min	95°C por 40 s	55°C por 45 s	72°C por 2 min	72°C por 10 min	35
ACA	95°C por 5 min	95°C por 1 min	49°C por 45 s	72°C por 2 min	72°C por 10 min	35
CT	95°C por 5 min	95°C por 30 s	41°C por 45 s	72°C por 2 min	72°C por 10 min	35
CA	95°C por 5 min	95°C por 40 s	50°C por 45 s	72°C por 2 min	72°C por 10 min	35
CGA	95°C por 5 min	95°C por 40 s	61°C por 45 s	72°C por 2 min	72°C por 10 min	37
CCA	95°C por 5 min	95°C por 40 s	55°C por 45 s	72°C por 2 min	72°C por 10 min	35
GT	95°C por 5 min	95°C por 30 s	58°C por 45 s	72°C por 2 min	72°C por 10 min	35

Source: Gandley and Bradshaw (2001).

unequivocally assigning each isolate to the corresponding species (data not shown).

According to these tests, 62% of the isolates were identified as *C. acutatum* and 38% as *C. gloeosporioides*. All the isolates collected from tamarillo belonged to the *C. acutatum* species*;* those from mango to *C. gloeosporioides;* while from the 93 isolates from Tahiti lime, 83 were *C. acutatum* and 10 were *C. gloeosporioides*. The coincidence of results of molecular and morphological characterization suggests that through standardized tests, some morpholo-gical attributes (colony color, growth habit) are reliable to separate these two species.

C. acutatum was previously reported as causing anthracnose in tamarillo and Tahiti lime in Colombia (Afanador *et al*., 2003; Ospina and Osorio, 2005; Reyes *et al.,* 2007), while other studies indicate the ability of this species to infect a broad range of crops (Freeman *et al.,* 1998; Talhinhas *et al*., 2002). Also, studies on citrus species show that *C. gloeosporioides* is frequently isolated from senescent tissues (Ospina and Osorio, 2005; Peres *et al.,* 2005); but its role in the disease has not been demonstrated. Some isolates used in this study were included in cross-infection tests in other hosts. The results indicate that *C. acutatum* isolates exhibit greater specificity to the source host and inability to infect mango fruits; nevertheless, cross-infections were observed in inoculations to petals of Tahiti lime, with mango or tamarillo isolates. These results agree with those reported by Freeman *et al.* (1998) under artificial inoculation conditions, and suggest the existence of physiological versatility in some isolates.

Structure of the *Colletotrichum* sp. populations

Eight RAM primers used in this study generated specific band patterns for each host, and in total 203 bands with molecular weights between 200 and 2,500 pb were evaluated in the whole population (Fig. 2). The study found different haplotypes for *C. acutatum* isolates from Tahiti lime and tamarillo, and for *C. gloeosporioides* isolates from mango and Tahiti lime. The similarity analysis performed established six significant groups in the population, with high levels of similarity (91.5-96.7%) in the *C. acutatum* species, and low (63.3-88.3%) in *C. gloeosporioides* groups. Isolates belonging to the *C. acutatum* species from the same host were, in general, very similar; while in the *C. gloeosporioides* species from mango and Tahiti lime greater variation was found (Fig. 3). Similar results were obtained with the multiple-correspondence analysis (Fig. 4). In all, these results indicate that the two species implicated in anthracnose of the three hosts exhibit notable genetic distance; likewise, the two pathogen species show genetic differentiation according to the host, confirming reports from studies based on mitochondrial DNA variation (Freeman *et al.,* 2000) or on data of ribosomal DNA sequence (MacKenzie *et al.,* 2009).

Genetic diversity of *C. acutatum*

A total of 116 polymorphic loci were identified among the 181 *C. acutatum* isolates studied. The analysis of molecular variance (AMOVA) revealed a clear distribution of the total variation observed in *C. acutatum,* of which 78% was attributed to differences among isolates of different hosts (tamarillo *vs.* Tahiti lime); while 22% was attributed to differences among isolates from the same host. These results were corroborated by the high genetic differentiation coefficient in this population (Fst = 0.78), which revealed great differences between *C. acutatum* isolates from Tahiti lime and tamarillo and suggest specialization in *C. acutatum* isolates for their host of origin.

The level of similarity among isolates was estimated with the Nei-Li coefficient, according to which genetic groups associated to the source host were found. A first group was formed by tamarillo isolates, with a high average level of similarity (92%), and the second group by lime isolates, with a mean similarity of 82%, resulting from two different subgroups whose similarity coefficients were 91.5 and 73%. Additionally, the biological and morphological tests, as well as the data from RAM markers indicated that isolates from tamarillo are more homogeneous than those from Tahiti lime; this is probably related to the greater geographic diversity of the isolates from the citrus species. Specialization of *C. acutatum* genetic groups for a particular host was previously described (Peres *et al.,* 2005; MacKenzie *et al.,* 2009); likewise, McKay *et al.* (2009) found geographic subdivision in this species associated to almond anthracnose in Australia. The results from the present study indicate specialization of *C. acutatum* isolates in two hosts (Tahiti lime and tamarillo), but no geographic subdivision of the population; this aspect, however, may be of importance in Colombia due to the high biophysical heterogeneity in production areas of the fruit species studied and should be examined in greater detail. On the other hand, the high homogeneity within the two groups suggests a clonal structure of the *C. acutatum* population; future studies would be necessary to elucidate this aspect, given that the existence of the teleomorph *Glomerella acutata* has been reported in studies in New Zealand and the United States, which describe its contribution to the genetic variability of the pathogen in other hosts like apples (Johnston and Jones, 1997).

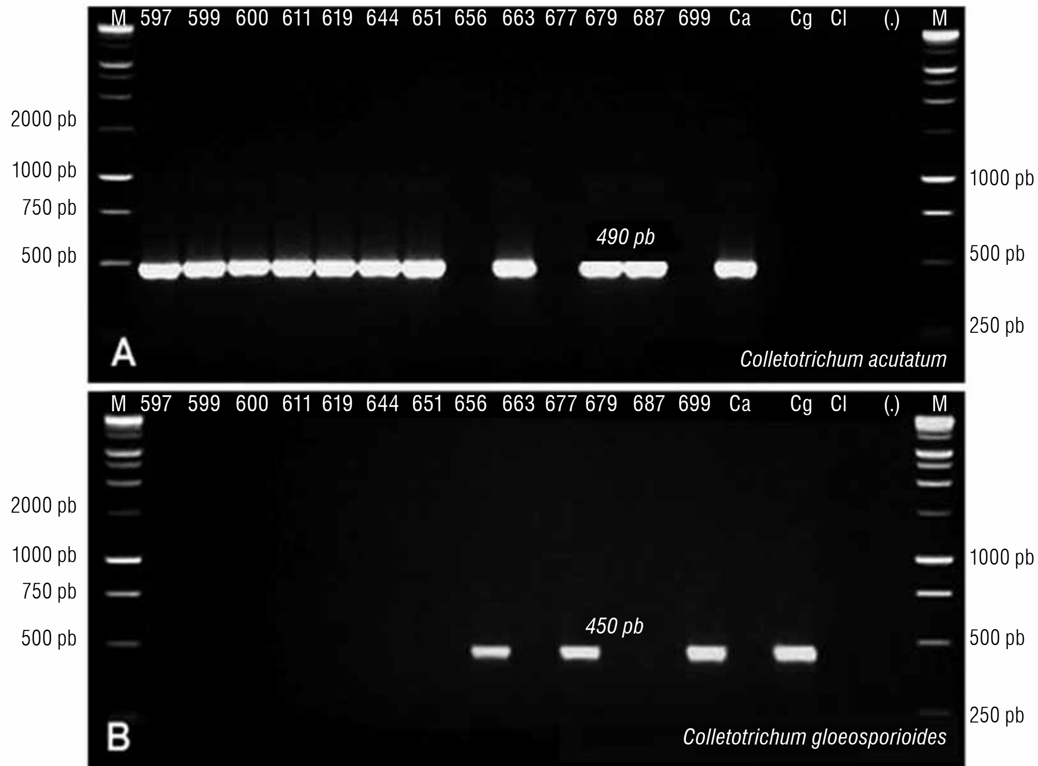

FIGURE 1. Taxonomic identification of *Colletotrichum* sp. isolates from Tahiti lime with species-specific primers. A, *C. acutatum* (primers *Ca*Int2/ITS4); B, *C. gloeosporioides* (primers *Cg*Int/ITS4). (Ca) Positive control for *C. acutatum;* (Cg) Positive control for *C. gloeosporioides*; (Cl) Negative control from *C. lindemuthianum.* (-) Negative control (no DNA).

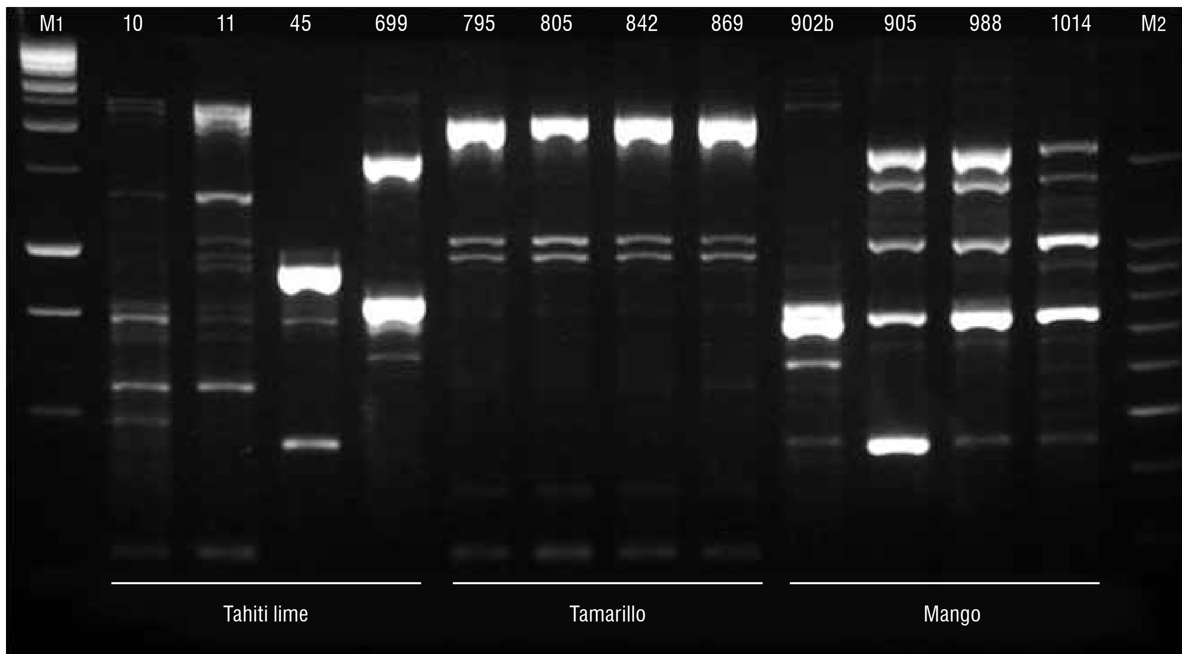

FIGURE 2. Amplification patterns generated with RAM primer CCA and DNA of *Colletotrichum* sp. isolates obtained from anthracnose lesions on three fruit hosts. (M1) DNA marker 1 kb; (M2) DNA marker 100 bp. *C. acutatum* isolates:10, 11 from Tahiti lime, and 795, 805, 842, 869 from tamarillo. *C*. *gloeosporioides* isolates: 45, 699 from Tahiti lime, and 902b, 905, 988, 1014 from mango.

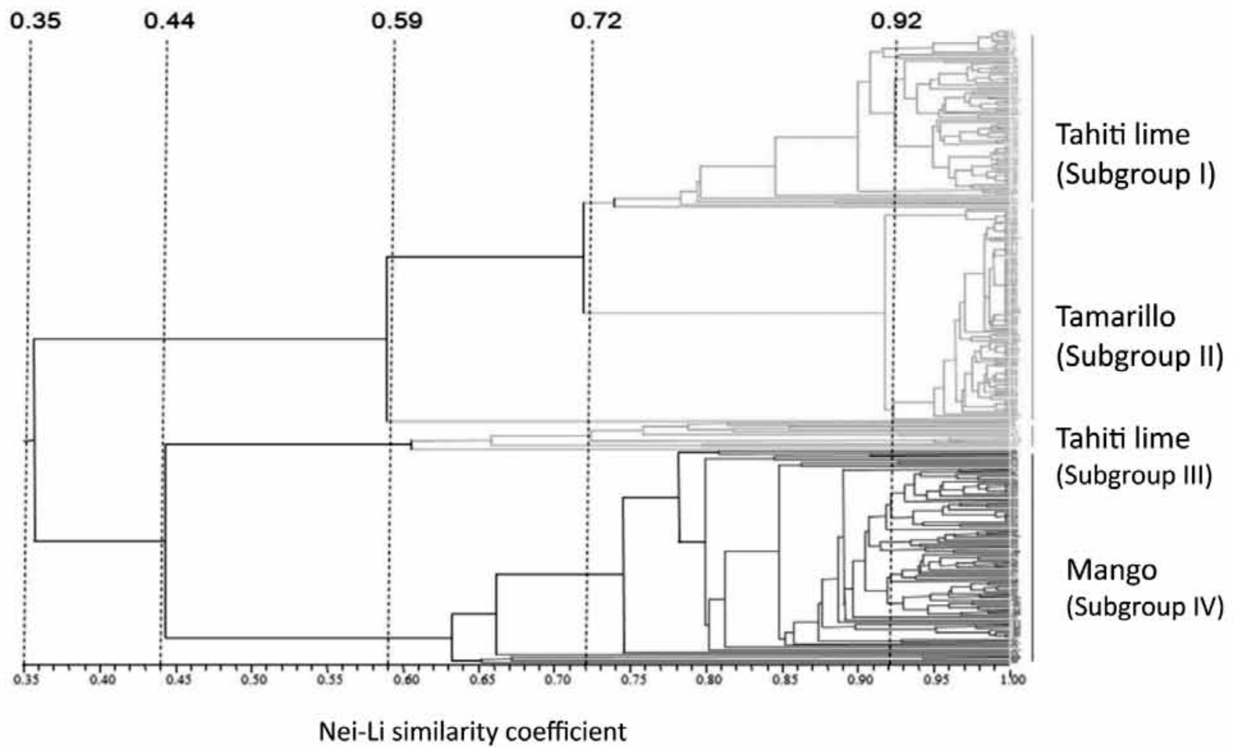

FIGURE 3. UPGMA dendrogram showing similarity values (Nei-Li) after analysis of RAM data from a population of 293 isolates of *Colletotrichum* sp. causing anthracnose in Tahiti lime, tamarillo and mango.

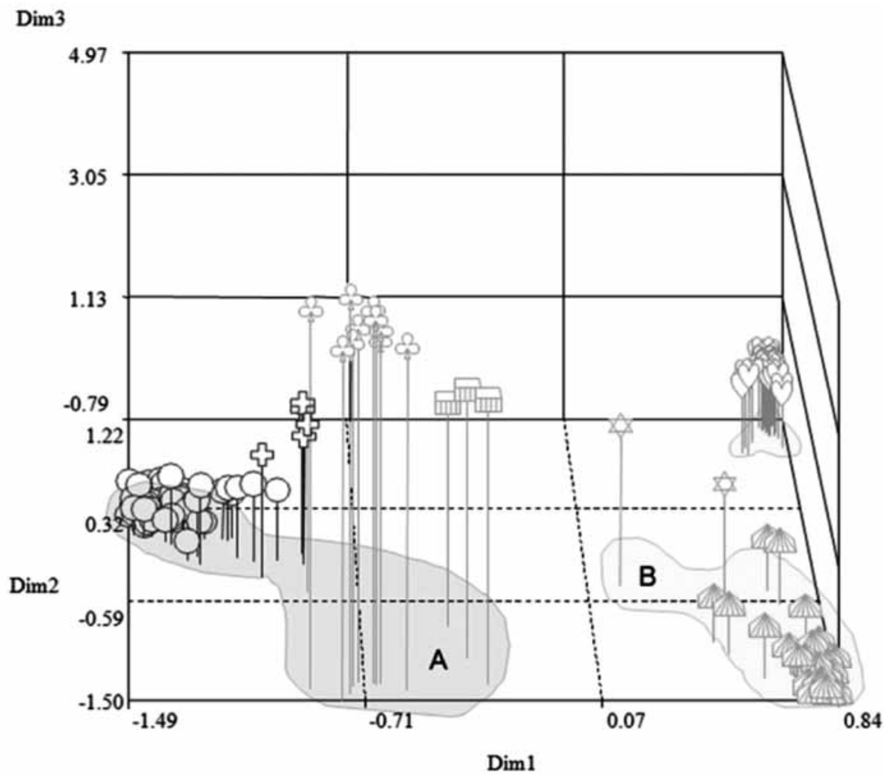

FIGURE 4. Three-dimensional representation derived from the genetic structure of 293 *Colletotrichum* sp. isolates obtained from anthracnose lesions on Tahiti lime, tamarillo and mango. A. *C. gloeosporioides;* B. *C. acutatum*.

Genetic diversity of *C. gloeosporioides*

A total of 106 polymorphic loci were identified among the 112 *C. gloeosporioides* isolates from mango and Tahiti lime. The analysis of molecular variance (AMOVA) of the RAM data for this population showed high levels of variation in this species, explained mainly by differences among isolates from different hosts (Hst = 68.5%). These results were corroborated by a high genetic differentiation coefficient in this population (Fst = 0.69). The remaining 31.5% of the total variation is attributed to differences among isolates within each host; this level of intra-group variation is higher than that observed for the *C. acutatum* species and suggests that the *C. gloeosporioides* species could have a greater potential for genetic change and adaptation.

The cluster analysis and the dendrogram constructed by using Nei-Li similarity coefficients permitted separating the two *C. gloeosporioides* species groups, with a 44% similarity (Fig. 3). Bearing in mind the relatively cosmopolitan habit of the fungus, and its ability to infect flowers of citrus species (data not shown), these low similarity levels in the *C. gloeosporioides* population could be explained by a long association with the source crop or the geographic isolation of the hosts.

The Nei-Li similarity coefficient detected a low homogeneity index (0.62) for the group of mango isolates. In Colombia there is no knowledge of the attributes of the components of the pathogen population; nevertheless, various studies have indicated that the *C. gloeosporioides* population causing anthracnose in several fruit groups is highly variable (Denoyes-Rothan *et al*., 2005), an aspect that contributes to the high degree of difficulty in implementing effective and lasting control strategies (Ospina and Osorio, 2005). High levels of complexity and genetic heterogeneity in *C. gloeosporioides* have been described previously (Freeman *et al*., 1998; Freeman *et al.,* 2001; Afanador *et al*., 2003; Abang *et al.,* 2005), and some studies suggest that these could also be due to the presence of a perfect state. Alternatively, the *C. gloeosporioides* population studied may contain migrant genotypes from other fruit species not included in this study, which have adapted to their new host; this possibility has been discussed in prior studies (Peres *et al.,* 2005) and recently demonstrated by MacKenzie *et al.* (2009) in strawberry, and warrants further exploration in future studies.

Conclusions

It was found that *C. acutatum* is the species causing anthracnose en Tahiti lime and tamarillo; the population of the pathogen includes two genetically distinct groups according to the host. The *C. acutatum* group corresponding to tamarillo was characterized for being homogeneous, apparently clonal and with a narrow genetic base, while the population from the Tahiti lime showed greater heterogeneity and diversity.

We found that *C. gloeosporioides* is the pathogen causing anthracnose in mango, which was also isolated from Tahiti lime flowers. The population of this species was highly differentiated according to the source host and exhibited considerable heterogeneity, indicating its high potential for genetic change.

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Literature cited

Abang, M.M., O. Fagbola, K. Smalla, and S. Winter. 2005. Two genetically distinct populations of *Colletotrichum gloeosporioides* Penz. from yam (*Dioscorea* spp.). J. Phytopathol. 153, 137-142.

Afanador, L., D. Minz, M. Maymon, and S. Freeman. 2003. Characterization of Colletotrichum isolates from tamarillo, pasiflora and mango in Colombia and identification of a unique species from genus. Phytopathol. 93(5), 579-587.

Arauz, L.F. 2000. Mango anthracnose: economic impact and current options for integrated management. Plant Dis. 84(6), 600-611.

Burdon, J. and J. Silk. 1997. Sources and patterns of diversity in plant pathogenic fungi. Phytopathol. 87(7), 664-699.

Denoyes-Rothan B., G. Guérin, E. Lerceteau-Köhler, and G. Risser. 2005. Inheritance of a race-specific resistance to *Colletotrichum acutatum* in *Fragaria* x *ananassa*. Phytopathol. 95, 405-412.

Freeman, S. and T. Katan. 1997. Identification of *Colletotrichum* species responsible for anthracnose and root necrosis of strawberry in Israel. Phytopathol. 87, 516-521.

Freeman, S., T. Katan, and E. Shabi. 1998. Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. Plant Dis. 82, 596-605.

Freeman, S., D. Minz, E. Jurkevitch, M. Maymon, and E. Shabi. 2000. Molecular analysis of *Colletotrichum* species from almond and other fruits. Phytopathol. 90, 608-614.

Freeman, S., D. Minz, M. Maymon, and A. Zveibil. 2001. Genetic diversity within *Colletotrichum acutatum sensu* Simmonds. Phytopathol. 91, 586-592.

Ganley, R.J. and E. Bradshaw. 2001. Rapid identification of polymorphic microsatellite loci in a forest pathogen, *Dothistroma pini*, using anchored PCR. Mycol. Res. 105, 1075-1078.

Hantula, J., M. Dusabenyagasani, and R. Hamelin. 1996. Random amplified microsatellites (RAMS) – a novel method for characterizing genetic variation within fungi. Eur. J. Plant Pathol. 26, 159-166.

Johnston, P. and D. Jones. 1997. Relationship among *Colletotrichum* isolates from fruit rots assessed using rDNA sequences. Mycologia 89, 420-430.

Kelemu, S., J. Badel, C. Moreno, J. Miles, S. Chakraborty, C. Fernandes, and M. Charchar. 1997. Biodiversity, epidemiology and virulence of *Colletotrichum gloeosporioides*. I. Genetic and pathogenic diversity in *Colletotrichum gloeosporioides* isolates from *Stylosanthes guianensis*. Trop. Grasslands 31, 387-392.

Kelemu, S., D. Skinner, J. Badel, C. Moreno, C. Rodríguez, C. Fernandes, M. Charchar, and S. Chakraborty. 1999. Genetic diversity in South American *Colletotrichum gloeosporioides* isolates from *Stylosanthes guianensis*, a tropical forage legume. Eur. J. Plant Pathol. 105, 261-272.

Liyanage, H., M. Millan, and H. Kistler. 1992. Two genetically distinct populations of *Colletotrichum gloeosporioides* from citrus. Phytopathol. 82(11), 1371-1376.

MacKenzie, S.J., T. Seijo, D. Legard, L. Timmer, and N. Peres. 2007. Selection for pathogenicity to strawberry in populations of *Colletotrichum gloeosporioides* from native plants. Phytopathol. 97, 1130-1140.

MacKenzie, S.J., N. Peres, M. Barquero, L. Arauz, and L. Timmer. 2009. Host range and genetic relatedness of *Colletotrichum acutatum* isolates from fruit crops and leatherleaf fern in Florida. Phytopathol. 99, 620-631.

Mahuku, G. 2004. A Simple extraction method suitable for PCR based analysis of plant, fungal, and bacterial DNA. Plant Mol. Biol. Rep. 22, 71-81.

Mahuku, G.S., M. Henríquez, J. Muñoz, and R. Buruchara. 2002. Molecular markers dispute the existence of the Afro-Andean group of the bean angular leaf spot pathogen, *Phaeoisariopsis griseola*. Phytopathol. 92, 580-589.

McKay, S.F., S. Freeman, D. Minz, M. Maymon, M. Sedgley, G. Collins, and E. Scott. 2009. Morphological, genetic, and pathogenic characterization of *Colletotrichum acutatum*, the cause of anthracnose of almond in Australia. Phytopathol. 99, 985-995.

Osorio, J.A. 2000. Aspectos epidemiológicos y de manejo de la antracnosis de los cítricos. pp. 41-48. In: Memorias del Seminario Nacional sobre Enfermedades de los Cítricos. Pereira, Colombia.

Osorio, J.A., M.F. Torres, J. Hio, P. Lizarazu, P. Castro, J. Romero, and J. Argüelles. 2005. Detección y análisis de la distribución de infecciones latentes de *Colletotrichum acutatum* J. H. *Simmonds* en arbolés de frutales tropicales. Fitopatol. Colomb. 29(2), 45-52.

Ospina, C. and J. Osorio. 2005. Caracterización de poblaciones del género *Colletotrichum* causante de la antracnosis de los cítricos. Fitopatol. Colomb. 29(1), 15-22.

Páez, A.R. 1995. Uso de variedades tolerantes: alternativa para el manejo de antracnosis (*Colletotrichum gloeosporioides*) en mango (*Mangifera indica*). Ascolfi-Informa 21, 36-39.

Peres, N., L. Timmer, J. Adaskaveg, and J. Correll. 2005. Lifestyles of *Colletotrichum acutatum*. Plant Dis. 89, 784-796.

Reyes, A., J. Osorio, E. Martínez, and J. Hio. 2007. Estudio de la relación fenológica reproductiva y el comportamiento de las infecciones latentes de *Colletotrichum acutatum* en tomate de árbol. Fitopatol. Colomb. 31(2), 37-42.

Rohlf, F.J. 2000. NTSYSpc: Numerical taxonomy and multivariante analysis system. Versión 2.1. Department of Ecology and Evolution, State University of New York; Exeter Software, Setauket, NY.

Talhinhas, P., S. Sreenivasaprasad, J. Neves, and H. Olivera. 2002. Molecular and phenotypic analyses reveal association of diverse *Colletotrichum acutatum* groups and a low level of *C. gloeosporioides* with olive antracnose. Appl. Environ. Microbiol. 71(6), 2987-2998.