Molecular and Morphological Characterization of *Colletotrichum gloeosporioides* Isolates Causing Anthracnose of Cashew (*Anacardium occidentale* L.) in Côte d'Ivoire

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Abstract

Anthracnose is one of the main cashew diseases in all cashew-growing countries. In Côte d'Ivoire, all diagnostic studies of this disease have been limited to morphological identification of pathogens. Although rapid and less costly, this identification technique remains highly inaccurate. The aim of this study was to use both morphological and molecular techniques to confirm the identity of the pathogen and to evaluate the virulence of different isolates. Isolations were therefore made from symptomatic samples of anthracnose collected from four cashew genotypes throughout the country's growing zone. The isolates obtained were characterised based on morphology and then a rapid PCR diagnosis was performed using a pair of primers specific to the *Colletotrichum gloeosporioides* complex. Finally, tests to establish the pathogenicity of the isolates were carried out in a greenhouse. A total of 146 isolates of *Colletotrichum* were isolated from leaves, flower panicles, apples, and cashew nuts. These isolates had cylindrical conidia with a length ranging between 11.56 ± 0.23 and 12.81 ± 0.17 and a width varying between 4.07 ± 0.01 and 4.77 ± 0.07. These characteristics were statistically identical between agroecological zones. In addition, isolates had cottony dense or flat mycelium with a grey or white coloration. PCR amplification reactions produced an amplicon size of 380 bp confirming that isolates are *C. gloeosporioides* species. Pathogenicity tests established the pathogenic nature of *C. gloeosporioides* isolates and DBOUL-302 was the most virulent among the isolates tested. This highly virulent isolate can serve as a model for resistance screening in cashew breeding programmes or for finding effective fungicides against anthracnose.

Keywords: Anthracnose; cashew; rapid diagnosis; PCR; pathogenicity; virulence

INTRODUCTION

Colletotrichum is a genus of microscopic fungi in the Glomerellaceae family and the Ascomycota division. It comprises 16 species complexes, including the *Colletotrichum gloeosporioides* complex, and 15 singleton species (Liu *et al*., 2022). This genus contains a large number of species that can be phytopathogenic, endophytic, saprobic, or pathogenic to humans (Sharma and Kulshrestha, 2015; Díaz-González *et al*., 2020; Liu *et al*., 2022). *Colletotrichum* comprises important plant pathogenic species and is one of the ten most studied plant pathogenic fungi (Dean *et al*., 2012). Phytopathogenic species of the *Colletotrichum* genus are ubiquitous hydrophilic species with a wide host range (Damm *et al*., 2013; Talhinhas, 2023; Thao *et al*., 2024). They attack plant production, either directly by contaminating a large number of fruits or indirectly by causing necrosis on leaves, thus reducing the plant's ability to photosynthesise (Kouamé *et al*., 2011; N'Guettia *et al*., 2013; Cruz-Lagunas *et al*., 2020).

Cashew trees are grown mainly for their cashew nuts, which are one of the leading export crops in West Africa (Kolliesuah *et al*., 2020). This crop is the main cash crop for the population of the savannah areas of Côte d'Ivoire. Since 2015, Côte d'Ivoire has been the world's leading cashew nut producer. National production of raw nuts, which was 700,000 tonnes in 2015, has risen to more than one million tonnes in 2022. This represents around a quarter of the world's raw nut production (FIRCA, 2022). The majority of cashew plantations in Côte d'Ivoire are established by direct seeding of cashew nuts. The allogamous nature of the cashew tree means that plantations contain a wide variety of trees with unknown characteristics. These cashew trees grown by direct seeding, also known as the 'unknown' genotypes, are characterised by their low yield of cashew nuts per hectare and their susceptibility to insect pests and diseases (N'Depo *et al*., 2017; Silué *et al*., 2017a; Ouali-N'goran *et al*., 2020; Soro *et al*., 2022). Anthracnose is one of the main diseases affecting cashew trees and is widespread in all cashew orchards in Côte d'Ivoire (Soro *et al*., 2015, 2022). It is a cryptogamic disease. As the germination and growth of fungi are strongly linked to environmental factors such as temperature and relative humidity, the weather conditions observed throughout the cashew production zone by Amani *et al*. (2023) have been defined by these authors as potentially conducive to disease. Anthracnose affects cashew trees in several countries and occurs on leaves, flower panicles, nuts, and cashew apples (Silué *et al*., 2017a; Wonni *et al*., 2017; Muntala *et al*., 2020; Veloso *et al*., 2022). In Benin, it can lead to cashew nut yield losses between 54 and 73% (Houndahouan *et al*., 2018). In Côte d'Ivoire, to provide producers with high-performance planting material, varietal selection has led to the dissemination of three high-yielding genotypes codified LA X3264, LA X4297, and LA Z330 (Kouakou *et al*., 2020). These genotypes are recognised by growers under codes A24, A27, and A30, respectively. However, the selection process for these genotypes focused on cashew nut yield and quality parameters. This selection did not consider the requirement of resistance of these genotypes to identified pests and diseases. Consequently, these genotypes could therefore turn out to be just as susceptible as the 'unknown' genotypes. Diagnostic studies of cashew anthracnose in Brazil have revealed that *C. gloeosporioides* species complex are the pathogens responsible for this disease (Dos Santos *et al*., 2019; Veloso *et al*., 2021). In Côte d'Ivoire, all diagnostic studies of the disease have been limited to the morphological characteristics of pathogens. The morphological characteristics are still not very accurate (Thao *et al*., 2024). Hence, it is important to use molecular tools. Developing resistant cashew genotypes would also be an effective way to control this disease. This will necessarily involve rigorous screening programmes using the most virulent *Colletotrichum* pathotypes. The initial step in this process is to carry out pathogenicity tests to select virulent pathotypes. This study employs both morphological and molecular techniques to confirm the identity of the pathogen and evaluates the virulence of different isolates, providing critical insights for future disease management strategies.

MATERIALS AND METHODS

Plant material

Fungal pathogens were isolated from leaves, flower panicles, apples, and cashew nuts samples that showed anthracnose symptoms. These samples were collected in plantations on 'unknown' genotypes, in nurseries, and in wood parks on the three high-yielding genotypes (A24, A27, and A30), which are the genotypes disseminated and ground in Côte d'Ivoire. These genotypes (unknown and high-yielding) were used for pathogenicity testing of isolates in the greenhouse. Plants of the 'unknown' genotype were obtained by seeding cashew nuts harvested from plantations. Plants of high-yielding genotype were supplied by an approved cashew nut nursery located in Toumodi (Central of Côte d'Ivoire). They were obtained by grafting with buds from these three genotypes.

Collection of anthracnose symptomatic samples

Samples of cashew tree organs showing symptoms of anthracnose as described by Silué *et al*. (2017a), and Soro *et al*. (2022) were collected throughout the cashew nut production zone in Côte d'Ivoire. This production area covered six agroecological zones (AEZs) of the country. Samples were collected from 97 sites, including plantations, nurseries, and wood parks (Figure 1).

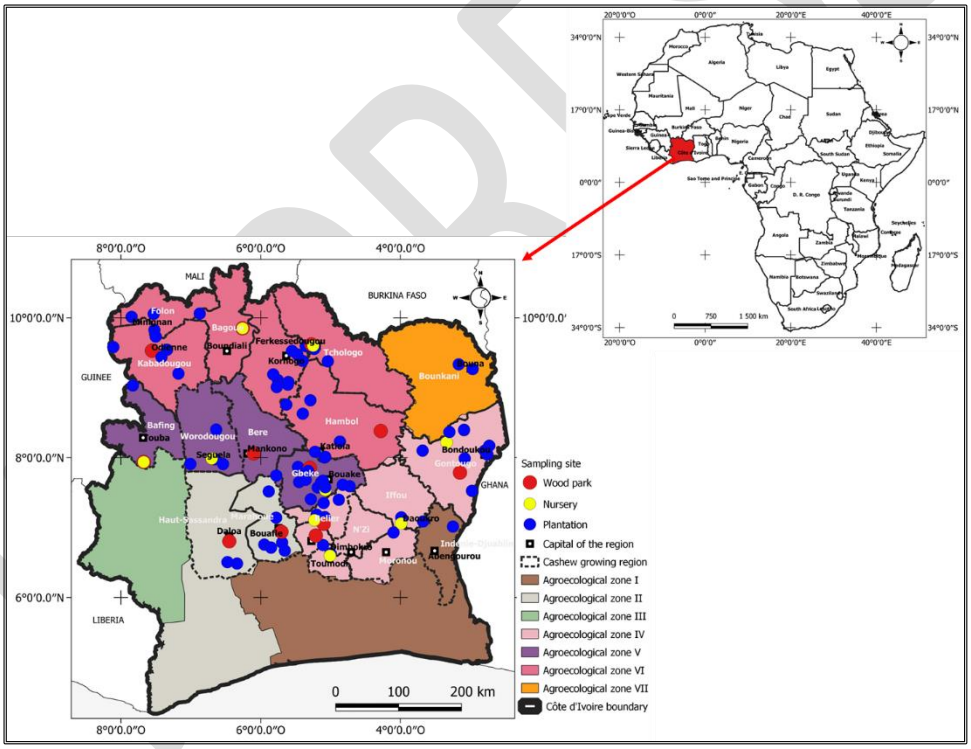

Figure 1. Map of sampling sites for cashew organs showing symptoms of anthracnose

Samples were collected in plantations in two phases. The first sampling was carried out between October and December during the pre-flowering phase of cashew trees. The second sampling was carried out in March during cashew fruiting. Samples were collected in nurseries and wood parks in July 2022 and 2023. The cashew plants were observed along the two diagonals of each site to look for any symptoms of anthracnose on cashew organs. Then, 3 to 5 samples of each organ showing symptoms of anthracnose were collected per site, stored in envelopes and transported to the laboratory in a refrigerated cooler for pathogen isolation and identification.

Isolation of pathogenic fungi

Pathogen isolation was carried out under a laminar flow hood and began with harvesting symptomatic tissue from the leading edge using sterile scalpels. The sampled materials were then sterilised successively in 70% alcohol for 1 min and in 3% sodium hypochlorite for 3 min. After being rinsed three times with sterile distilled water, the sampled materials were then blotted on sterile blotting paper in a laminar flow hood and placed onto PDA (Potato Dextrose Agar) medium in Petri dishes (90 mm diameter). The resulting cultures were incubated at 25-28°C for 48-72 h (Silué *et al*., 2017a; Dembélé *et al*., 2020). The growing fungi were purified by successive subculturing from the leading edge mycelium to a new PDA medium. After one month of incubation, *Colletotrichum* isolates were identified by microscopic observations using the identification key of Barnett and Hunter (1972).

Purification of *Colletotrichum* isolates by single spore culture

Isolates developed from conidia were obtained using the vortex spore suspension method described by Senanayake *et al*. (2020) with some modifications. To achieve this, *Colletotrichum* isolates were subcultured onto Agar medium at 20% (w/v) to encourage sporulation at the expense of an abundance of mycelium. After 10 days of incubation, conidial suspensions were obtained by pouring 5 ml of sterile distilled water onto the surface of the new cultures. Each surface was then scraped with a sterile Pasteur Pipette. The resulting suspension was transferred to a centrifuge tube, vortexed, and diluted to 10^{-3}. 10 µl of this diluted conidial suspension was spread in Petri dishes containing 15 ml Agar medium. The cultures were incubated for 24 hours. A portion of Agar medium bearing a germinated conidium was removed and inoculated onto a new PDA medium. All this experiment was carried out under a laminar flow hood. The single conidium cultures were incubated under laboratory conditions between 25-28°C and observed as they grew.

Morphological characterization of *Colletotrichum* isolates

The characterization of *Colletotrichum* isolates began with sampling a mycelial disc (5 mm in diameter) from the margin of the colony resulting from a conidium. Each mycelial disc was subcultured in the middle of a PDA medium in Petri dishes. Three dishes were subcultured for each isolate. The dishes were sealed and incubated for 7 days in the laboratory between 25-28°C. The morphological characteristics studied were the appearance of the mycelium, the coloration of the mycelium on the front and reverse of Petri dishes, and the average linear growth rate (ALGR). The average linear growth rate was determined from the equation used by Jahan *et al*. (2013) :

$$ALGR = \frac{C_7 - C_0}{7}$$

Where, C_0: is the initial diameter of the colony (C_0 = 5 mm) and C_7: is the diameter of the colony in the seventh day of incubation.

Microscopic characterization was carried out after 21 days of incubation. A fragment of mycelium was removed from each isolate and then mounted between the slide and coverslip. A light microscope model AmScope T660C with a camera connected to a computer was used for observation. The AmScope application (version X64 of 2019) was used to measure the length and width of 30 conidia taken randomly from each isolate.

Molecular identification of *Colletotrichum gloeosporioides* isolates

The molecular identification of isolates within the *Colletotrichum gloeosporioides* complex was performed using the primers SDColl_F1 (TTGCTTCGGCGGGTAGGGTC) and SDColl_R1 (ACGCAAAGGAGGCTCCGGGA) specific to this complex and developed by Kamle *et al*. (2013). 10 isolates were selected randomly for this molecular identification. The

genomic DNA was extracted using the Wizard Genomic Kit (Kit ® Promega). DNA extraction was performed from mycelia of 7 to 10 days old harvested from a PDA medium. The protocol developed by Freitas (2016) was followed for this DNA extraction. DNA yield and purity were assessed using a Nanodrop. PCR reactions were performed in a 25 µL mixture containing 5 µL of Fire Hot Mix, 2.5 µL of each of the two primers SDColl_F1 (10 µM) and SDColl_R1 (10 µM), 5 µL of genomic DNA diluted 1:20 and 10 µL of sterile distilled water. Two controls were considered for this PCR: a positive control (+ve) from the study done by Bougoum *et al*. (2023) and a negative control (-ve) consisting of sterile distilled water. The positive control was isolated from mango fruit and identified as *Colletotrichum gloeosporioides* by Bougoum *et al*. (2023). The PCR was performed as follows, an initial step of 5 min at 95°C, 30 cycles of 30 s at 95°C, 45 s at 58°C, and 1 min 30 s at 72°C, followed by 5 min at 72°C. PCR products were visualised by electrophoresis in a 1% (w/v) agarose gel (Ultra Pure TM agarose, Invitrogen, Spain) and 0.5 X of 1 x Tris-Acetate-EDTA (TAE) buffer. Finally, a 100 bp ladder (Solis Biodyne Data Sheet) was used to determine the molecular size of PCR amplicons.

Pathogenicity and virulence tests of *C. gloeosporioides* isolates

Preparation of plant material

The pathogenicity and virulence of isolates were determined on young leaves (2 weeks old) of the three high-yielding genotypes and on young leaves (2 weeks old) of the 'unknown' genotype. The first step of this experiment was to put the plants with young leaves in specially prepared micro-greenhouses covered with transparent plastic. The whole set-up was maintained in a greenhouse (5º21'25.25" N, 3º54'12.87" W). The inside and outside of the micro-greenhouses were regularly watered with distilled water for forty-eight hours. The temperature and the relative humidity inside the micro-greenhouse were recorded with a Data Logger (Type EL-USB-2 from LASCAR electronics). The temperature was between 25 and 35°C while the relative humidity was between 70 and 95%.

Preparation of inoculum

All the isolates identified by the molecular study were used for pathogenicity and virulence tests except the positive control from the study of Bougoum *et al*. (2023). The fungal inoculum was obtained for each isolate by adding 10 ml of sterile distilled water to a 21-day-old fungal culture on a PDA medium. The surface of the mycelium was scraped with a sterile Pasteur Pipette. The suspension solution was transferred to a sterile tube, vortexed, and filtered. The concentration of conidia was estimated using a Malassez hematimeter and adjusted to 10^6 conidia/ml. Then, 1 ml of a prepared 1% Dextrose + Agar solution was added to the conidial suspension to facilitate their adhesion to cashew leaves during inoculation (Silué *et al*., 2017a, 2018).

Inoculation of cashew plants in the greenhouse

The cashew plants were inoculated gently (without wounding) following the method used by Silué *et al*. (2018). It consisted of spraying the leaves of 2 weeks old with inocula concentrated at 10^6 conidia/ml using hand sprayers. Three plants with an average of six leaves were inoculated for each isolate. Each leaf was inoculated on both sides with 1 ml of inoculum. Control plants received an inoculum containing sterilised distilled water + 1% Dextrose and Agar solution. The experiment was repeated three times. The parent isolates were used the first two times, and the third time, the isolates were reisolated after inoculation. Finally, the inoculated plants were incubated in the same micro-greenhouse and sprayed with water 3 times per day during the first week while avoiding water run-off on the leaves.

Determination of the pathogenicity and virulence of isolates

The pathogenicity of the isolates was verified by the presence of typical anthracnose symptoms. The date of appearance of the first symptoms was also noted. The virulence of isolates was

determined 30 days after inoculation by the incidence and severity index of anthracnose symptoms obtained on leaves in the greenhouse. Incidence was determined per plant from the average ratio of the number of leaves showing symptoms to the total number of leaves inoculated. While severity was assessed using a scoring scale defined by Groth *et al*. (1999) and Cardoso *et al*. (2004): 0: no symptoms; 1: 1-5%; 3: 6-10%; 5: 11-25%; 7: 26-50%; 9 > 50% of leaf area infected. Finally, the severity index was determined per plant for each isolate using the equation of Kranz (1988), taking into account these scores:

$$Is = \frac{\Sigma(xi.ni)}{N.Z} x\ 100$$

Where, Is: is disease severity index, xi: is disease severity i on the leaf, ni: is number of leaves of severity i, N: is total number of leaves inoculated and Z: is highest score on the scale, which is 9.

Data analysis

The data obtained on the morphological characteristics and virulence of the isolates were analysed using R software version 4.3.2. The length and width of conidia, the average linear growth rate of mycelia, the incidence and severity index of anthracnose symptoms were subjected to an ANOVA test. Before that, both Shapiro–Wilk test and Bartlett test were used to check normality and homogeneity of variances, respectively. These tests were performed using 'shapiro.test' and 'bartlett.test' functions, respectively. In case of a significant difference after ANOVA test, the Newman-Keuls post hoc test was used to separate the means into homogeneous subgroups at a significance level of 5% by using 'SNK.test' function of the package 'agricolae'.

RESULTS

Morphology of isolates of *Colletotrichum*

From symptomatic anthracnose samples, 146 isolates of *Colletotrichum* were identified after isolation. These isolates showed cylindrical conidia with rounded and/or pointed ends and an occasional presence of septa (Figure 2).

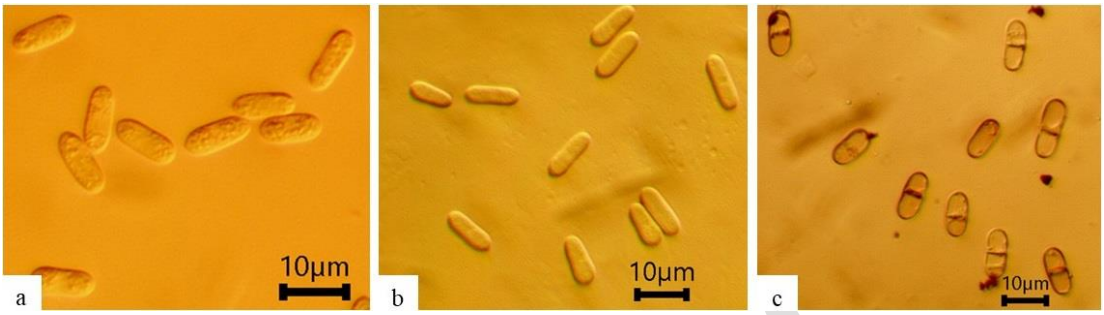

Figure 2**.** Conidial shape of *Colletotrichum* isolates (GX400); a: cylindrical conidia rounded at both ends and not partitioned, b: cylindrical conidia with one end rounded and the other pointed and not partitioned, c: partitioned conidia.

The average length of conidia was 12.62 µm and an average width of 4.71 µm. The average linear growth of the mycelium onto PDA medium was 0.91 cm/day (Table 1). Analysis of variance of the length and width of conidia and the average linear growth rate of the mycelium revealed that the origin zone of isolates did not influence these morphological characteristics of *Colletotrichum* infecting cashews in Côte d'Ivoire.

Table 1: Morphological characteristics of *Colletotrichum* isolates according to agroecological zone

Agroecological zone (AEZ)	Number of isolates	Percentage of isolates	Conidia length (µm)	Conidia width (µm)	ALGR (cm/day)
AEZ II	11	7.53	12.44 ± 0.36	4.68 ± 0.15	0.90 ± 0.02
AEZ IV	43	29.45	12.81 ± 0.17	4.68 ± 0.07	0.91 ± 0.01
AEZ V	33	22.60	12.72 ± 0.21	4.70 ± 0.10	0.90 ± 0.01
AEZ VI	57	39.04	12.48 ± 0.13	4.77 ± 0.07	0.90 ± 0.01
AEZ VII	2	1.37	11.56 ± 0.23	4.07 ± 0.01	0.94 ± 0.13
Average			12.62 ± 0.09	4.71 ± 0.04	0.91 ± 0.01
F value			1.224	0.998	0.224
p-value			0.304	0.411	0.913

The average values of the morphological traits are not significantly different between the agroecological zone according to ANOVA test.

Macroscopic observation of isolates grown from one conidium revealed that 94.70% of isolates had cottony dense mycelia and 5.30% had flat mycelia. The mycelium of isolates was greenish grey or dark grey on the front of Petri dishes with a black reverse (21.19%). A second group had a whitish-grey mycelium on the front of Petri dishes with a white reverse with a black centre (31.13%). Finally, the last group had a white mycelium on the front of Petri dishes and a white reverse with a black inoculation point (47.68%) (Figure 3).

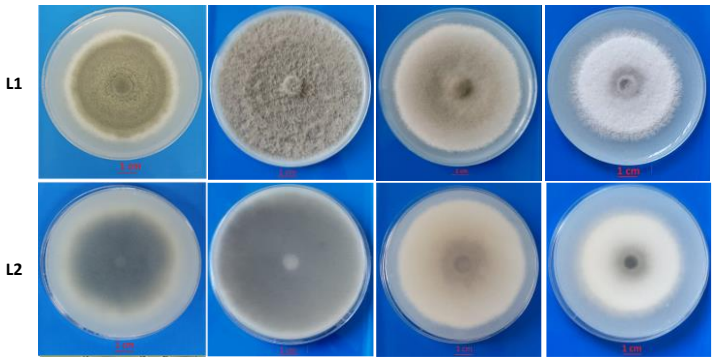

Figure 3. Macroscopic appearance of *Colletotrichum* isolates on PDA medium; L1: front pictures of isolates, L2: reverse pictures of isolates.

Molecular identification of fungal isolates

The PCR assay carried out using primers specific to the *Colletotrichum gloeosporioides* species complex produced an amplicon size of 380 bp in 10 isolates, including the positive control, meaning nine isolates from this study. No amplicons were observed with isolate TBONL-296 and with the negative control. Figure 4 shows the results of electrophoresis on 1% agarose gel.

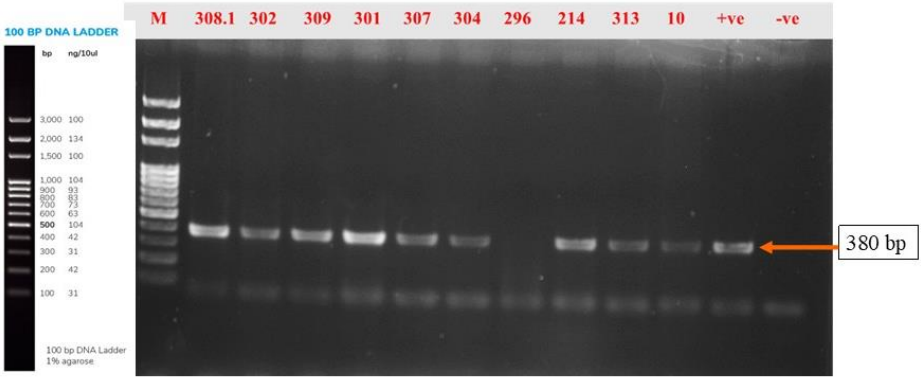

Figure 4. Agarose gel showing amplification in isolates with the pair of primers specific to *Colletotrichum gloeosporioides*; M: 100 bp ladder (Solis Biodyne), 308.1 to 304 and 214 to 10: isolates that presented amplicons, 296: isolate that did not present an amplicon, +ve: positive control, -ve: negative control.

Pathogenicity and virulence of *Colletotrichum gloeosporioides* isolates

The nine isolates of *C. gloeosporioides* inoculated were pathogenic by inducing necrosis on cashew leaves in the greenhouse. These necroses appeared in two forms: dotted and small brown patches (Figure 5). For all isolates, necrosis appeared on cashew leaves at 2 days post-inoculation.

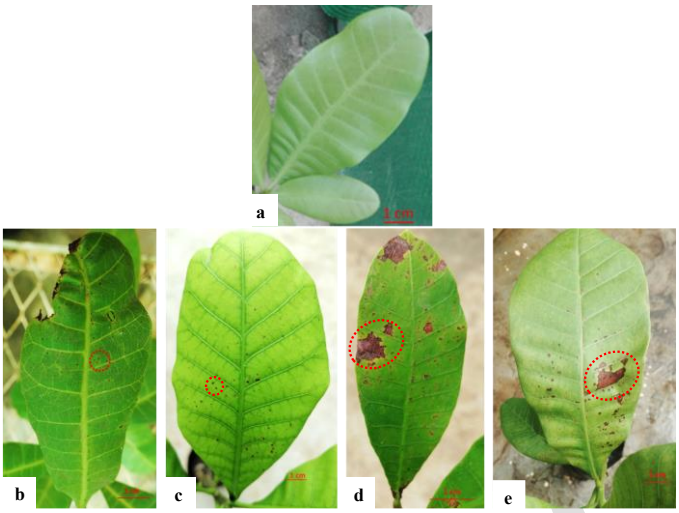

Figure 5. Symptoms caused by *C. gloeosporioides* isolates on cashew leaves in the greenhouse; a: control and asymptomatic cashew leaves; b and c: necroses as dotted lines obtained on leaf blades of 'unknown' and high-yielding genotypes respectively; d: necroses as small brown patches located mainly on the leaf margin, obtained on high-yielding genotypes; e: necroses as small brown patches located on leaves veins of high-yielding genotypes

For the virulence of isolates, analysis of variance of the incidence and severity index of anthracnose revealed a significant difference between isolates, with p-values of 0.002854 and 0.006364, respectively. Incidence ranged from 15.07 to 82.05%. The Newman-Keuls post hoc test revealed three homogeneous groups (Figure 6A). The mean severity index was 11.06%. Isolate TBODL-10 had the lowest severity index with 1.67%. In contrast, isolate DBOUL-302 was the most severe with 22.34% of the severity index (Figure 6B). Thus, isolate DBOUL-302 emerged as the most virulent of the nine *C. gloeosporioides* isolates tested.

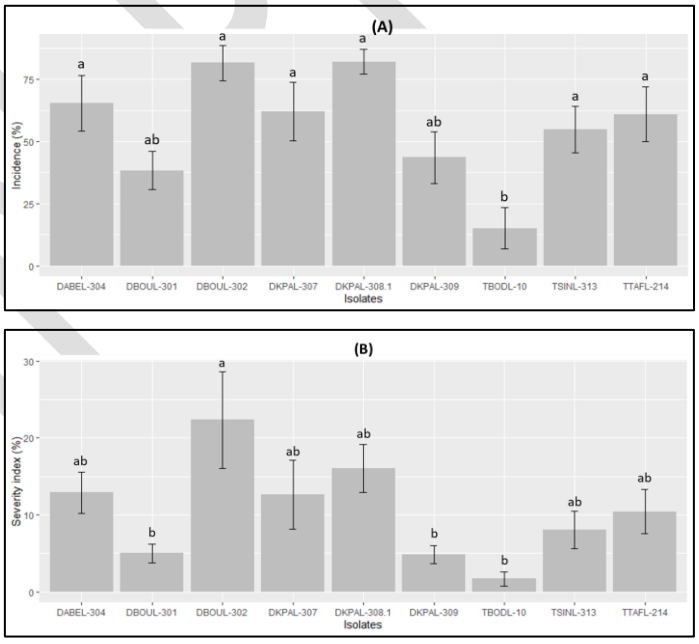

Figure 6. Incidence (A) and severity index (B) of anthracnose on cashew leaves inoculated in the greenhouse.
The different letters above the histograms designate the grouping classes according to the Newman-Keuls test at the 5% significance level. Classes surmounted by the same letters are not significantly different.

DISCUSSION

Anthracnose remains one of the main biotic constraints on cashew tree cultivation in several countries worldwide, particularly in Côte d'Ivoire. The country could look for effective methods to control this disease and to maintain its position as the world leader in cashew nut production. These control methods could involve effective and sustainable fungicides or the development of resistant genotypes to anthracnose. Either of these methods requires preliminary studies to identify the causal agent and screen the most virulent pathotypes. This study was carried out to identify and characterise the causal agent of anthracnose in cashew and then screen isolates based on virulence. 146 isolates of *Colletotrichum* were obtained from leaves, flower panicles, apples, and cashew nuts showing anthracnose symptoms. Microscopic observation of purified isolates growing on PDA medium revealed only conidia with a cylindrical shape. This cylindrical shape of the conidia is characteristic of some species of *Colletotrichum* genus (Dos Santos *et al*., 2019; Dembélé *et al*., 2020; Liu *et al*., 2022). In this study, the average length of conidia was 12.62 µm and an average width of 4.71 µm. The conidia size found in our study is similar to that obtained by Silué *et al*. (2017a) but different to that obtained by Dembélé *et al*. (2020). In their study focused on the morphological characterization of *Colletotrichum* isolates infecting mango, Dembélé *et al*. (2020) observed conidia longer than 15 µm and wider than 4.8 µm. This difference could be linked to the crop or the *Colletotrichum* species complexes infecting the crop. Indeed, Dembélé *et al*. (2020) identified two complexes, *C. gloeosporioides* and *C. acutatum*. *C. acutatum* contains species with spindle-shaped conidia. The morphological study also revealed that isolates of *Colletotrichum* infecting cashews in Côte d'Ivoire have cottony dense or flat mycelia with a greenish grey, dark grey, whitish grey, or white colour. These morphological parameters would be specific to the infected cultures and would depend on parameters in the incubation zones in Petri dishes. Nevertheless, the cottony appearance of the mycelium is dominant in *Colletotrichum* species (Ehui *et al*., 2019; Dembélé *et al*., 2020; Muntala *et al*., 2020). Amplification with primers specific to the *C. gloeosporioides* species complex revealed amplicons of approximately 380 bp in nine isolates from our study. These nine isolates are therefore *C. gloeosporioides* according to Kamle *et al*. (2013) and Bougoum *et al*. (2023). The absence of an amplicon with isolate TBONL-296 could mean that this isolate is not a *C. gloeosporioides* and could belong to another complex. On the other hand, this isolate could be a cryptic species of *C. gloeosporioides* that cannot be identified using these specific primers, which would highlight the limitations of these primers in identifying *C. gloeosporioides*. Furthermore, *C. gloeosporioides* is a species complex with around 22 species (Weir *et al*., 2012). Consequently, a precise identification study of the *C. gloeosporioides* species infecting cashews in Côte d'Ivoire should be carried out. In Brazil, the study done by Veloso *et al*. (2018) identified seven species of *C. gloeosporioides* infecting this crop. These are *C. chrysophilum*, *C. fragariae*, *C. fructicola*, *C. gloeosporioides* sensu stricto, *C. queenslandicum*, *C. siamense* and *C. tropicale*. The nine isolates inoculated in the greenhouse induced necrosis on cashew leaves. These *C. gloeosporioides* isolates are therefore pathogenic. The re-isolated isolates were identical to the parent isolates. Koch's postulate was verified, confirming that *C. gloeosporioides* is responsible for cashew anthracnose in Côte d'Ivoire, as reported in Ghana (Muntala *et al*., 2020), Burkina faso (Wonni *et al*., 2017), Brazil (Veloso *et al*., 2018) and Cameroun (Dooh *et al*., 2021). Isolate DBOUL-302 proved to be the most virulent. Further studies should be carried out to determine whether this high virulence is linked to higher sporulation or host specificity. Also, this isolate may be used in the development of cashew genotypes resistant to anthracnose and/or in other screening programmes such as the search for effective fungicides. To date, the practices adopted by farmers to control cashew disease are based on agronomic and chemical methods (Silué *et al*., 2017b; Soro *et al*., 2020). However, potential biocontrol strategies against anthracnose on cashews have been identified (Silué *et al*., 2018; Soro *et al*., 2019).

CONCLUSION

This study identified *Colletotrichum gloeosporioides* as the species complex responsible for cashew anthracnose in Côte d'Ivoire. These *Colletotrichum* isolates had cylindrical conidia with rounded and/or pointed ends and the occasional septa. These conidia had an average length of 12.62 µm and an average width of 4.71 µm. The mycelium had two aspects (cottony dense and flat) with a front coloration that varied from grey to white. The morphological characteristics of isolates were statistically identical from one agroecological zone to another. Amplification with primers specific to *C. gloeosporioides* produced an amplicon size of 380 bp, confirming the molecular identity of isolates. Inoculation tests established the pathogenic nature of *C. gloeosporioides* isolates on cashew leaves. Isolate DBOUL-302 was the most virulent with 82.05% and 22.34%, respectively for incidence and severity index. However, given that *C. gloeosporioides* represents a species complex containing several species, future studies should employ multilocus sequence analysis to precisely identify species within this complex and assess the efficacy of resistant cashew varieties against virulent isolates.

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CONFLICT OF INTEREST
The authors declare that they have no known a conflict of interest that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS
S.N.A.: writing – original draft, methodology, investigation, data analysis, conceptualization. S.S.: conceptualization, writing – review and editing, supervision, funding acquisition. D.Z.O.: investigation, methodology, conceptualization. T.A.: investigation, methodology. W.I.: provision of working materials for molecular study, supervision. A.K.: supervision. K.D.: supervision, funding acquisition.

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