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Current status of *Colletotrichum capsici* strains, causal agents of Brown blotch disease of cowpea in Burkina Faso

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Brown blotch disease, caused by *Colletotrichum capsici*, is an important disease of cowpea with a significant yield losses ranging from 42 to 100% in West Africa. In this study, a specific polymerase chain reaction (PCR) primer set CC1F1/CcapR was used to characterize and to study the phylogenetic relationship of thirty eight strains of *Colletotrichum* species. This primer set is capable of amplifying only *C. capsici* from different fungal structures and provide a powerful tool for *C. capsici* detection in brown blotch disease in cowpea. Phylogenetic analysis from neighbor-joining (NJ) showed a high genetic variability in the rDNA-ITS region of the isolates. The isolates formed four groups or clusters on the basis of specific fragment analysis. Groups I, II, and III consist of strains containing specific region length of twenty one nucleotides and were considered as variant 1 of *C. capsici*. Group IV was a heterogeneous and consists of variants 1, 2, 3, and 4 of *C. capsici*.

Key words: Cultivars, internal transcribed spacer (ITS) sequence, *Colletotrichum* species.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of main grain legume crops grown in sub-Saharan Africa in terms of cultivated areas, production, and consumption (Singh et al., 2002; Ajeigbe, 2006). In Burkina Faso, cowpea is a staple food crop, which is grown essentially for human food and nutrition. Cowpea production is subjected to abiotic stresses, such as drought, heat, and poor soil fertility and to various biotic agents including soil borne and seed borne, fungal pathogens, which cause diseases leading to a significant yield losses and reduction in grain density (Katile et al., 2010). Of the diseases, Brown blotch caused by *Colletotrichum capsici/truncatum* has been cited to be of great importance not only in Burkina Faso, but also in other parts of the world. In a worldwide perspective, *C. capsici* pathogen causes a disease commonly known as anthracnose on a wide range of

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plants, including legumes, vegetables, and small fruits (Sérémé, 1999; Banerjee et al., 2007; Torres-Calzada et al., 2011). Recently, strains of *C. capsici* and *Colletotrichum dematium f. truncatum* have been described as the same species (Hyde et al., 2009) and are now considered to be synonym of *Colletotrichum truncatum* (Damm et al., 2009). The genus *Colletotrichum* includes several species, such as *Colletotrichum graminicola*, *C. capsici*, *C. truncatum*, *Colletotrichum gloeosporioides*, *C. dematium*, *Colletotrichum destructivum,* and *Colletotrichum coccodes*, which may occur as endophytes, saprobes or pathogens (Hyde et al., 2009). *Colletotrichum* is a cosmopolitan fungus and includes a number of economically important plant pathogenic fungi, occurring predominantly in tropical and subtropical regions on a wide range of crops (Sharma et al., 2014a)

Working on brown blotch disease of cowpea in Nigeria, Emechebe (1986) identified 8 races of *C. capsici.* Four of these races were reported to be specific to habitats in Guinea and Sudan Savannah ecologies. The four other races were reported to be common in rain forest zones. The spread of the pathogen can be facilitated by high humidity and heavy rains during the growing season. The identification of *Colletotrichum* species has been reported to be difficult due to morphological similarities of the pathogen races (Hyde et al., 2009). In Burkina Faso, Sérémé (1999) reported 12 pathogenic groups of *Colletotrichum* spp., including all *C. capsici* which are associated with brown blotch disease. The existence of these pathogen races in Burkina Faso create confusion especially when quick, accurate and proper pathogen identification is needed.

Traditionally, identification and classification of *Colletotrichum* spp. Have been based on the morphological characters, pathogenicity tests, and biochemical approaches (N'Guettia et al., 2013; Saxena et al., 2014; Chai et al., 2014; Enyiukwu et al., 2014). In most cases, there were no clear differences in conidial or appressorial size and shape among these species.

Thus molecular characterization is essential for completing the morphological description of *Colletotrihum* spp. (Sharma et al., 2014a, b; Enyiukwu et al., 2014). The rDNA region of *Colletotrichum* spp. has been investigated for species identification and phylogenetic relationships (Cannon et al., 2012). The internal transcribed spacer (ITS) markers have also been successfully used for detection of many species including plants, fungi and bacteria (Cros et al., 1993; Shivaprakash et al., 2011). Some ITS markers notably C.capR/C.capF and CC1F1/CC2R2 have been used for *C. capsici* species detection and phylogenetic analysis (Banerjee et al., 2007; Chandra et al., 2009; Torres-Calzada et al., 2011).

In this paper, species specific polymerase chain reaction (PCR) primer pair CC1F1/CcapR were used to characterize and study the phylogenetic relationship of different strains of *Colletotrichum* spp. responsible of brown blotch disease in cowpea in Burkina Faso.

MATERIALS AND METHODS

Collection of *Colletotrichum* isolates

Colletotrichum isolates were obtained from infected cowpea tissues (leaf, stem and pod) from farmer's fields in seven different sites, situated in three agro-ecological zones of Burkina Faso, namely Saria, Kamboinse, and Kouare (North Soudanian zone), Farako Ba and Gaoua (Soudanian zone), and Pobe Mengao and Bani (Sahelian zone) during the period from October to November, 2013. The collected tissues materials were surface sterilized in 70% (v/v) ethanol for 1 min followed by immersion in sodium hypochlorite (NaOCl) 1% (v/v) for 5 min and three successive rinses in sterilized distilled water. The samples were left to dry under the laminar air cabinet for 1 h. Then small pieces (approximately 5 × 5 mm) from the margins of infected tissues were transferred to Petri dishes containing three layers of wet blotter papers. Petri dishes containing samples were incubated at 28°C under 12 h/12 h light: dark for 7 to 9 days.

Identification of *Colletotrichum* spp.

Colletotrichum spp. (complex *C. capsici/truncatum*) were identified based on morphological characters of the acervuli and conidia produced on the infected tissues on the blotter papers under the stereomicroscope and compound microscopes based on the identification key established by Marthur and Kongsdal (2003). Then, using a sterile loop, pure isolates were further placed onto potato dextrose agar (PDA) containing streptomycin (0.3 µg/L of PDA). The plates were incubated at 24°C for 7 days under ultraviolet (UV) light of alternating 12 h light and darkness to obtain pure culture.

Purification of *Colletotrichum* single spore isolates

To obtain fresh single spore pure culture of *Colletotrichum* isolates, distilled water (100 ml) was added to PDA and 200 µl of the suspension obtained were spread on new PDA plates and incubated for 24 to 48 h as previously described. Then three to five single cells from each isolate were again transferred onto new PDA. After seven days of growth, single spore pure cultures were stored at 20°C until used.

Molecular characterization

DNA extraction

The Flinders Technology Associates (FTA) plant cards were used for fungal DNA extraction. The fungi suspension of 100 µl containing approximately 10^3 to 10^6 spores/ml was applied as a single spot on the paper, and the paper was air-dried for 1 to 2 h at room temperature. The spotted FTA plant cards were stored at room temperature until use. Using a Harris Micro Punch instrument, discs of 2-mm in diameter were taken from spots made on the FTA plant card. The sample discs were each placed in separate Eppendorf tubes. The discs from each FTA plant card were washed and air-dried for 1 h at room temperature following the manufacturer's instruction (www.whatman.com).

ITS amplification and sequencing

PCR detection assay was conducted using the genus *Colletotrichum*

specific primer CC1F1 5'ACCTAACTGTTGCTTCGGCG-3' (Chandra et al., 2009) and the species *C. capsici* specific primer CcapR 5'-CCCAATGCGAGACGAAATG3' (Torres-Calzada et al., 2011), which gave a specific band size of 425 bp. The PCR mixture of 25 µl contained 1 FTA disk, 5 µl of Buffer (Flexer) 5X, 0.5 µl of dNTP (10 nM), 18.42 µl of distilled H_2O, 0.5 µl of each primer (5 µm), and 0.08 µl of DNA Go Taq (Promega). The PCR was performed using a thermocycler Master Cycler Gradient at 95°C for 5 min for initial denaturation, followed by 25 cycles of denaturation at 94°C for 30s, primer annealing at 62°C for 30s and extension at 72°C for 2 min. The final extension was set at 72°C for 5 min. Eight microlitters of the PCR products was analyzed on 1.5% agarose gel stained with 5 µl of ethidium bromide. After approximately 45 to 60 min of running at 70 to 80 mV, the gel was visualized. The PCR positive products were then sent to Beckman Coulter Genomics service (USA) for sequencing.

Molecular identification and phylogenetic analysis

BioEdit software was used for the Clustal W Multiple sequence alignment, and BLAST for species identification in the NCBI GenBank. Due to complications in distinguishing C*. capsici*, *C. truncatum* and *C. dematium* (Hyde et al., 2009), specific fragment of *C. capsici* was targeted on the BioEdit multiple sequence alignment software and was compared with that in the NCBI database. The phylogenetic tree was produced using DARwin 6.0.4 software.

RESULTS

The isolates of *Colletotrichum* spp. which were identified from the lesions of cowpea tissues were identified as *C. capsici* based on size and shape of conidia. The isolates of *C. capsici* showed differences in morphological characteristics such as colony color (brown, orange, black or grey or white), conidial shape and size (Figure 1). Conidia of fungal isolates were typically ellipsoidal and hyaline, and produced acervuli. Our results indicated that mycelium of *C. capsici.* from cowpea varied and could be aerial, compact or cottony (Figure 1). The size of conidia also varied within the isolates (Table 1).

The present study characterized thirty eight isolates of *Colletotrichum* spp. using molecular approaches; specific region in the International Transcribed Spacer regions of *C. capsici* was targeted and was used to determine phylogenetic relationships between these fungal strains.

The ITS sequence analysis of the 38 isolates of *Colletotrichum* spp. showed that all the strains were *C. capsici* (Table 2). Among the strains, 92% contained the specific sequence of *C. capsici* (Torres-Calzada et al., 2011) and were designated as variant 1. In total, four variants of *C. capsici* were identified on the basis of the sequence analysis of species (Table 3).

The four variants of *C. capsici* identified (Figure 2) in this study are in alignment with four physiological races identified by Emechebe (1986) which are specific to Guinea and Sudan Savanna habitats. The species *capsici* variant 1 represented the most, with 92% of the strains, and was distributed over all agro-ecological zones of the country. But variants 2, 3 and 4 seemed to be specific to the Sudanian zone (Gaoua).

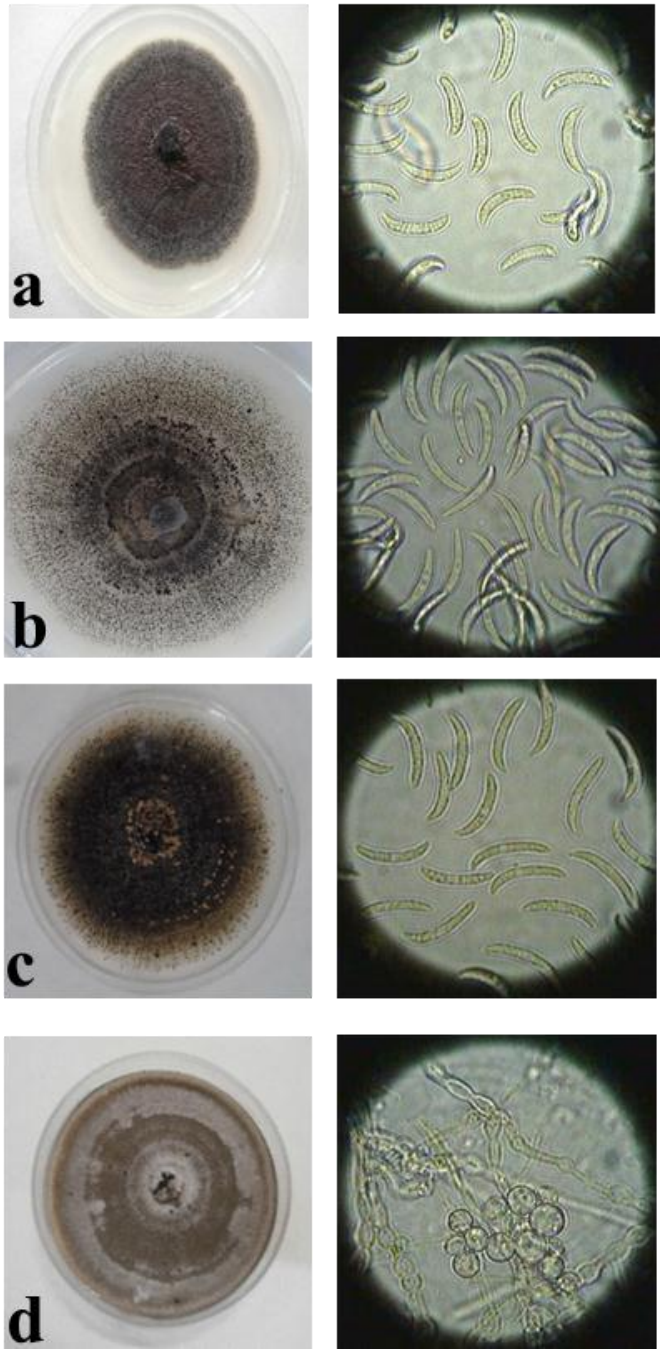

Figure 1. Different morphological aspects and close view of conidia of four *C. capsici* isolates in PDA culture; a: 03-KO-1 (*var.* 1); b: 35-GA-5 (*var*. 2); c: 42-GA-7 (*var.* 3), d: 20-KB-5 (*var.* 4).

Based on the phylogenetic relationship of the 38 clones, 4 clusters at a distance coefficient of 0.1 (Figure 3) were formed. The major cluster I had sixteen isolates with 65% similarity. Three of the identified variants of *C. capsici* belonged to the heterogeneous cluster IV.

Table 1. Comparatives morphological characters of eight *C. capsici* isolates, 10 days on PDA.

Isolate	Location	Organ	Conidia length (µm)	Morphological characters		
				Colony colour	Acervulus	Mycelia form
03-KO-1	Kouare	Pod	20	Brown	Abundant	Compact
20-KB-5	Kamboinse	Stem	-	Brown-white	Absent	Cottony
35-GA-5	Gaoua	Pod	28	Orange	Abundant	Cottony
42-GA-7	Gaoua	Pod	23	Orange	Abundant	Aerial
71-FA-6	Farako-Ba	Pod	22	Brown	Abundant	Compact
74-BA-1	Bani	Pod	21	Brown	Abundant	Aerial
77-PM-1	Pobe	Pod	21	Black	Abundant	Compact
96-SA-2	Saria	Stem	20	Orange	Abundant	Cottony

Table 2. Molecular identification of *Colletotrichum* spp. single spores isolates from cowpea in Burkina Faso.

Colletotrichum spp. isolate	Organ	Location	NCBI identification	GenBank accession number	Specific sequence analysis identification	Variants
001-KO-1	Pod	Kouare	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
003-KO-1	Pod	Kouare	*C. capsici*	KM213014 (99% id.)	*C. capsici*	*Var. 1*
005-KB-1	Pod	Kamboinse	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
006-KB-1	Pod	Kamboinse	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
013-KB-3	Stem	Kamboinse	*C. capsici*	HQ271457 (99% id.)	*C. capsici*	*Var. 1*
016-KB-3	Stem	Kamboinse	*C. capsici*	KM213014 (99% id.)	*C. capsici*	*Var. 1*
020-KB-5	Stem	Kamboinse	*C. capsici*	HM197759 (85% id.)	*C. capsici*	*Var. 2*
024-GA-2	Stem	Gaoua	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
025-GA-3	Pod	Gaoua	*C. capsici*	KM213014 (99% id.)	*C. capsici*	*Var. 1*
026-GA-3	Pod	Gaoua	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
027-GA-3	Pod	Gaoua	*C. capsici*	J185787 (99% id.)	*C. capsici*	*Var. 1*
028-GA-3	Pod	Gaoua	*Colletotrichum sp.*	HQ130691 (99% id.)	*C. capsici*	*Var. 1*
033-GA-4	Pod	Gaoua	*C. capsici*	KM213014 (99% id.)	*C. capsici*	*Var. 1*
035-GA-5	Pod	Gaoua	*C. capsici*	HM197759 (92 % id.)	*C. capsici*	*Var. 3*
036-GA-5	Pod	Gaoua	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
042-GA-7	Pod	Gaoua	*C. capsici*	HM191710 (99 % id.)	*C. capsici*	*Var. 4*
043-GA-7	Pod	Gaoua	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
045-GA-8	Pod	Gaoua	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
047-GA-9	Stem	Gaoua	*C. truncatum*	JQ936245 (99% id.)	*C. capsici*	*Var. 1*
051-GA-10	Stem	Gaoua	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
053-GA-10	Stem	Gaoua	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
058-FA-2	Leaf	Farako-Ba	*C. capsici*	KM213014 (99% id.)	*C. capsici*	*Var. 1*

Table 2. Contd.

059-FA-3	Leaf	Farako-Ba	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
060-FA-3	Leaf	Farako-Ba	*C. capsici*	HQ271451 (99% id.)	*C. capsici*	*Var. 1*
062-FA-4	Stem	Farako-Ba	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
064-FA-4	Stem	Farako-Ba	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
071-FA-6	Pod	Farako-Ba	*C. capsici*	KM213014 (98% id.)	*C. capsici*	*Var. 1*
072-FA-6	Pod	Farako-Ba	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
074-BA-1	Pod	Bani	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
077-PM-1	Pod	Pobe-Mengao	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
082-PM-3	Stem	Pobe-Mengao	*C. capsici*	HQ271457 (99% id.)	*C. capsici*	*Var. 1*
083-PM-3	Stem	Pobe-Mengao	*C. capsici*	HQ271457 (99% id.)	*C. capsici*	*Var. 1*
085-PM-3	Stem	Pobe-Mengao	*C. capsici*	HQ271457 (99% id.)	*C. capsici*	*Var. 1*
089-PM-5	Stem	Pobe-Mengao	*C. capsici*	HQ271457 (99% id.)	*C. capsici*	*Var. 1*
090-PM-5	Stem	Pobe-Mengao	*C. capsici*	HQ271457 (99% id.)	*C. capsici*	*Var. 1*
091-PM-5	Stem	Pobe-Mengao	*C. capsici*	HQ271457 (99% id.)	*C. capsici*	*Var. 1*
092-SA-1	Stem	Saria	*C. capsici*	HM197759 (99 % id.)	*C. capsici*	*Var. 1*
096-SA-2	Stem	Saria	*C. capsici*	HQ271457 (99% id.)	*C. capsici*	*Var. 1*

Table 3. rDNA sequence comparison of the specific fragment from the internal transcribed spacer (ITS) region of the 38 *Colletotrichum capsici* species isolated from cowpea plants.

Variant of *C. capsici*	Sequence variability in the specific region	Seq. identity GenBank	Representative clone
Variant 1 (C. capR specific fragment)	-AACATTTCGTCTCGCATTGG(T)G	92 to 99%	045-GA-8
Variant 2	-ACCATTTCGCCTCGGATTGGG	92%	035-GA-5
Variant 3	-AACATTCCGCTT-GGATTGGG	88%	042-GA-7
Variant 4	-TACACTTCGCCTCGGATTGGG	85%	020-KB-5

Variants 2, 3, and 4 had 71% similarity with variant 1 in cluster IV. The sequences similarities of the 38 strains of *C. capsici* were consigned in Table 4.

DISCUSSION

In this study, thirty eight *Colletotrichum* spp. isolates were characterized using molecular approaches. The present findings have revealed that there exist high level of variation between the rDNA-ITS region of *Colletotrichum* spp. in Burkina Faso.

High number of clades obtained on the dendrogram shows a strong intraspecific differentiation within the *C. capsici* species. All the four clusters were representative of the three agroecological zones and the distribution of the isolates was not depending on the geographic area indicating a high genetic diversity at all locations. Kumar et al. (2010) observed the same results; working with *C. falcatum* from sugarcane in India. Such variations could be due to similar climatic conditions, which might favor the coexistence of the isolates.

The analysis of sequence identity between *C.*

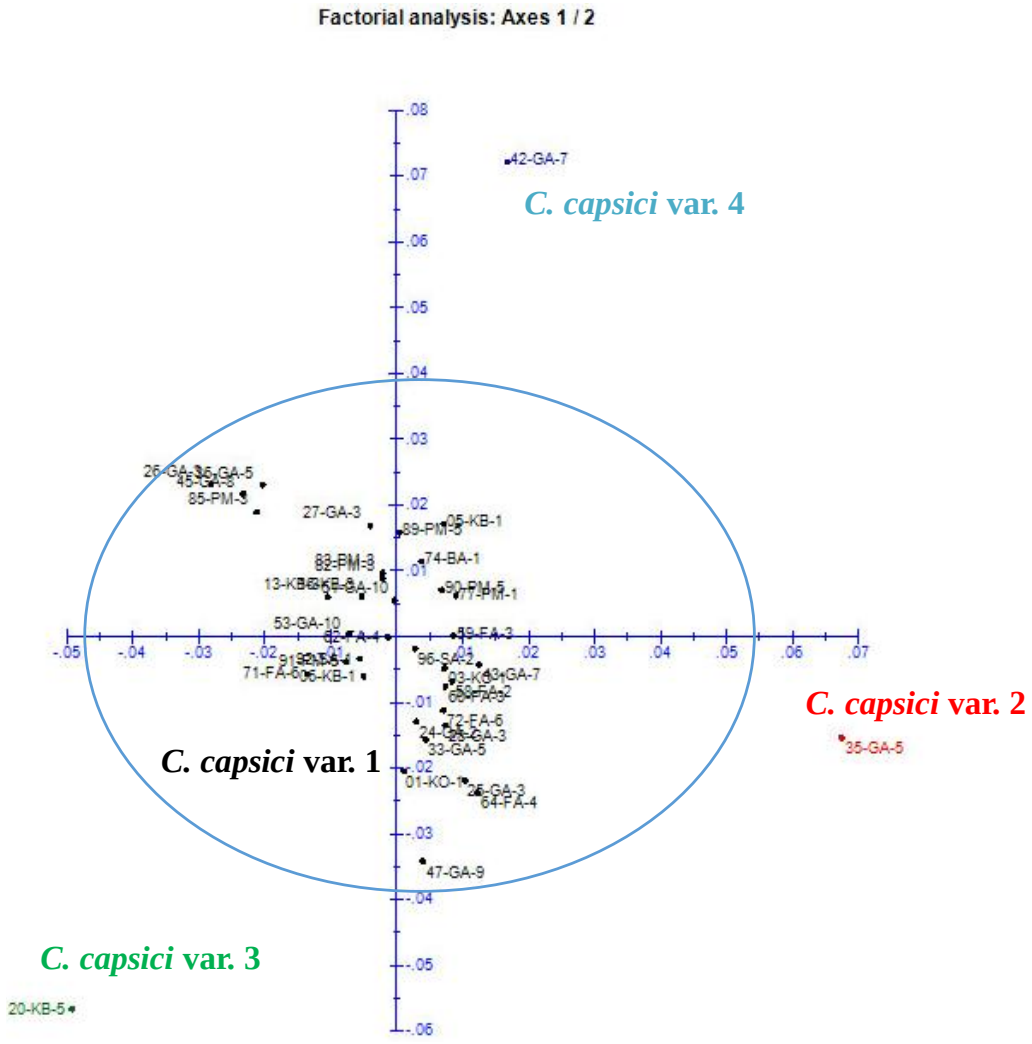

Figure 2. Distribution of *Colletotrichum capsici variant*.

Capsici strains ranged from 0.773 to 0.974 (Table 3). This similarity indicates a high penalty for closely related sequences. The highest degree of sequence identity was observed between strains 96-SA-2 and 91-PM-5 (0.974), 92-SA-1 and 77-PM-6 (0.961), and the lowest degree (0.773) was observed between strains 20-KB-5 (variant 3) and 28-GA-3 (variant 1). *C. capsici* isolates from Saria (North soudanian) were very close to those from Pobe Mengao (Sahelian).

Working with Anthracnose disease on chilli fruits, using Randomly Amplified Polymorphic DNA (RAPD), Kumar et al. (2015) demonstrated genetic variability among isolates of *C. capsici* from different locations in India. Contrary to our study, they found a low value of similarity coefficient. The difference in similarity coefficients between this study and Kumar et al. (2015) could be due to the fact that ITS sequences cannot be used to distinguish closely related species.

The concept of a species in the genus *Colletotrichum* is not well defined due to insufficient variation in classical descriptive criteria and difficulties in dealing with pathogens of similar morphology, but different host specificity (Sutton, 1992). The molecular study of *C. capsici* strains provides criteria that permit differentiation of strains or species with similar conidial morphology. The examination of highly conserved rDNA, along with other criteria represents a useful approach of addressing taxonomic uncertainties (Sherriff et al., 1994; Bailey et al., 1996; Sexena et al., 2014). The phylogenetic relationship of the strains of *C. capsici* showed that the variants 2, 3 and 4 may be evolved from variant 1 from a mutation of two or three nucleotides in the specific fragment region.

In a previous report, *C. capsici* strains from cowpea in Burkina Faso were different based on both biomorphological and biochemical characters (Sereme, 1999).

In the current study, the use-fulness of rDNA-ITS markers was demonstrated to distinguish and establish the phylogenetic relationship of different strains of *C. capsici* isolated from cowpea plants in Burkina Faso. This

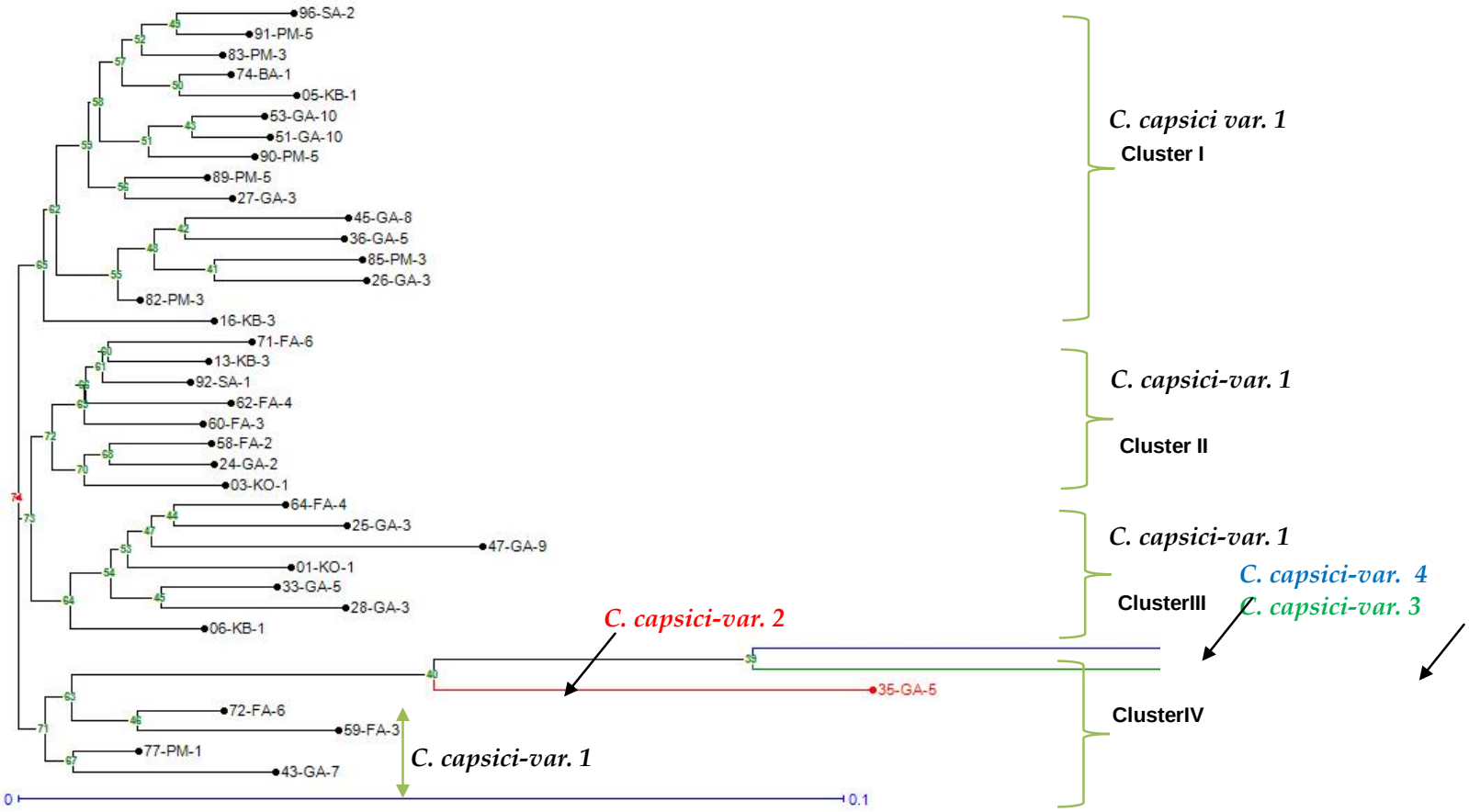

Figure 3. Phylogenetic relationship of 38 strains of *C. capsici* based on NJ/UnWeighted method. The distance coefficient = 0.1.

research provides a quick and accurate tool for specific PCR detection of *C. capsici*, causative agents of brown blotch disease in cowpea. In this study, the ITS markers were recognized to be phylomarkers for intra and extra-specific relationship within species population as per Lei et al. (2012), and for validation of species status as per the study by Dabert (2006). Thus, findings were presented, which will help future researchers to avoid confusion when distinguishing the *C. capsici* from other *Colletotrichum* spp. infecting cowpea.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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Table 4. Sequence identity/similarity Matrix of *Colletotrichum capsici* strains from cowpea in Burkina Faso.

Seq->	01-KO	03-KO	05-KB	06-KB	13-KB	16-KB	20-KB	24-GA	25-GA	26-GA	27-GA	28-GA	33-GA	35-GA	36-GA	42-GA	43-GA	45-GA…
01-KO-1	ID																	
03-KO-1	0.946	ID																
05-KB-1	0.914	0.917	ID															
06-KB-1	0.943	0.948	0.926	ID														
13-KB-3	0.932	0.948	0.914	0.964	ID													
16-KB-3	0.957	0.929	0.919	0.957	0.941	ID												
20-KB-5	0.780	0.778	0.758	0.803	0.785	0.797	ID											
24-GA-2	0.933	0.928	0.906	0.944	0.933	0.939	0.778	ID										
25-GA-3	0.944	0.934	0.924	0.951	0.941	0.953	0.774	0.919	ID									
26-GA-3	0.923	0.907	0.913	0.930	0.920	0.935	0.779	0.915	0.910	ID								
27-GA-3	0.925	0.916	0.933	0.946	0.936	0.941	0.780	0.927	0.930	0.950	ID							
28-GA-3	0.920	0.913	0.906	0.946	0.925	0.932	0.773	0.914	0.928	0.918	0.929	ID						
33-GA-5	0.951	0.937	0.910	0.960	0.943	0.957	0.786	0.931	0.949	0.937	0.941	0.955	ID					
35-GA-5	0.867	0.846	0.842	0.871	0.855	0.887	0.816	0.854	0.868	0.861	0.872	0.855	0.874	ID				
36-GA-5	0.930	0.909	0.910	0.939	0.939	0.948	0.787	0.919	0.933	0.960	0.941	0.916	0.932	0.872	ID			
42-GA-7	0.792	0.792	0.779	0.810	0.792	0.800	0.833	0.791	0.782	0.795	0.793	0.783	0.795	0.821	0.786	ID		
43-GA-7	0.906	0.906	0.909	0.924	0.924	0.921	0.770	0.944	0.907	0.906	0.916	0.907	0.913	0.850	0.906	0.786	ID	
45-GA-8	0.928	0.916	0.915	0.941	0.932	0.944	0.790	0.917	0.926	0.948	0.948	0.916	0.932	0.865	0.960	0.783	0.906	ID

Table 4. Contd.

Seq->	47-GA	51-GA	53-GA	58-FA	59-FA	60-FA	62-FA	64-FA	71-FA	72-FA	74-BA	77-PM	82-PM	83-PM	85-PM	89-PM	90-PM	91-PM	92-SA	96-SA
47-GA-9	**ID**																			
51-GA-10	0.907	**ID**																		
53-GA-10	0.902	0.978	**ID**																	
58-FA-2	0.903	0.921	0.915	**ID**																
59-FA-3	0.873	0.895	0.893	0.917	**ID**															
60-FA-3	0.905	0.928	0.933	0.931	0.898	**ID**														
62-FA-4	0.907	0.926	0.935	0.938	0.902	0.962	**ID**													
64-FA-4	0.939	0.935	0.939	0.939	0.911	0.941	0.941	**ID**												
71-FA-6	0.903	0.932	0.930	0.944	0.900	0.949	0.949	0.939	**ID**											
72-FA-6	0.885	0.905	0.896	0.927	0.927	0.890	0.897	0.919	0.897	**ID**										
74-BA-1	0.888	0.942	0.937	0.932	0.936	0.911	0.913	0.926	0.915	0.928	**ID**									
77-PM-1	0.906	0.943	0.939	0.953	0.915	0.951	0.958	0.948	0.953	0.923	0.937	**ID**								
82-PM-3	0.886	0.920	0.918	0.914	0.895	0.909	0.912	0.921	0.918	0.922	0.914	0.934	**ID**							

Table 4. Contd.

83-PM-3	0.898	0.955	0.948	0.916	0.906	0.912	0.914	0.928	0.918	0.920	0.960	0.938	0.936	**ID**						
85-PM-3	0.905	0.923	0.923	0.905	0.902	0.903	0.905	0.916	0.923	0.892	0.910	0.927	0.927	0.925	**ID**					
89-PM-5	0.905	0.960	0.957	0.923	0.913	0.914	0.921	0.944	0.930	0.916	0.953	0.950	0.934	0.966	0.934	**ID**				
90-PM-5	0.903	0.974	0.967	0.915	0.896	0.931	0.928	0.933	0.941	0.899	0.944	0.946	0.920	0.951	0.934	0.962	**ID**			
91-PM-5	0.900	0.957	0.957	0.918	0.911	0.921	0.918	0.937	0.925	0.909	0.955	0.938	0.931	0.969	0.927	0.966	0.955	**ID**		
92-SA-1	0.909	0.939	0.939	0.946	0.907	0.967	0.967	0.946	0.967	0.905	0.924	0.969	0.920	0.923	0.920	0.930	0.941	0.932	**ID**	
96-SA-2	0.902	0.962	0.957	0.930	0.906	0.926	0.926	0.939	0.932	0.907	0.942	0.948	0.925	0.962	0.927	0.960	0.960	**0.974**	0.934	**ID**

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