

Full Length Research Paper

Differential reaction of cowpea genotypes to brown blotch disease (*Colletotrichum capsici*) in Burkina Faso

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Thirty six *Colletotrichum capsici* (L.) single spore isolates associated with brown blotch disease in cowpea were collected from three agro-ecological zones of Burkina Faso from October to November 2014. To identify the most virulent strains, cowpea genotypes KVx61-1, KVx396-4-5-2-D and Moussa Local was inoculated with each isolate. The results showed that isolates 096-SA-2, 071-FA-6 and 079-PM-2 were the most virulent, respectively, in North Soudanian, Soudanian and Sahelian zones. To identify brown blotch disease resistant cowpea, each of the isolates was used to inoculate 41 different cowpea genotypes. Inoculated cowpea plants were evaluated for brown blotch disease severity at 7, 14 and 21 days after inoculation. Highly significant differences ($P < 0.001$) were found among genotypes, isolates and their interactions. Seven cowpea genotypes including KN-1, Moussa Local, Donsin Local and Melakh were identified as resistant and present specific resistance to the isolates. These genotypes can be used to improve cowpea resistance to brown blotch disease in Burkina Faso.

Key words: Cultivars, pathotypes aggressiveness, disease resistance.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important grain legume crop in Sub-Saharan Africa (Adegbite and Amusa, 2008). The crop is grown worldwide with an estimated cultivation area of about 12 million hectares

annually with the production of over 10 million metric tons a year (FAOSTAT, 2011). About 65 to 70% of the world's cowpea grain production occurs in the west and the central part of Sub-Saharan Africa. Cowpea provides

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food and nutrition for humans and feed for livestock. However, in Burkina Faso and other African countries, cowpea production is subject to many biotic constraints such as soil-borne and seed-borne fungal pathogens including *Colletotrichum capsici*, the causal agent of brown blotch disease, which can result in yield losses of between 42 and 100% in tropical agriculture (Sereme, 1999; Banerjee et al., 2007; Adegbite and Amusa, 2008; Torres-Calzada et al., 2011). *C. capsici* is a major constraint to many crop production causing severe losses both in pre- and postharvest decay (N'Guettia et al., 2013; Saxena et al., 2014; Chandra et al., 2009).

Infection by *C. capsici* in cowpea is particularly devastating due to its hemibiotrophic nature (Hyde et al., 2009). Additionally, the pathogen occurs in different races. For instance, Emechebe (1986) identified eight races of *C. capsici* associated with brown blotch disease in Nigeria; four out of the eight races were specific to Guinea and Sudan Savanna while the remaining four were to the rainforest area. Sereme (1999) reported 12 pathogenic groups of *Colletotrichum* spp. including *C. capsici* associated with brown blotch disease in Burkina Faso. Based on molecular characterization with a specific primer pair, Thio et al. (2016) identified four variants of *C. capsici* in Burkina Faso.

Several *C. capsici* management techniques including cultural control, use of plant extracts (Sereme, 1999; Mark et al., 2015; Mark and Channya, 2016), use of chemicals, biological control (Chacko and Gokulapalan, 2015) and use of tolerant and resistant cultivars (Adebitan et al., 1992; Amusa et al., 1994) have been recommended. However, no single specific management program can eliminate brown blotch disease in cowpea (Obi and Barrusa-Vargas, 2014). Breeding for resistance is also the most economical for growers as well.

Therefore, host plant resistance remains the most viable option in managing the brown blotch disease in cowpea (Adebitan et al., 1992; Fery and Singh, 1997; Enyiukwu et al., 2014; Obi and Barrusa-Vargas, 2014).

In this study, spraying method was used to study the pathogenicity and resistance of cowpea genotypes to *C. capsici* strains originating from different locations in Burkina Faso.

MATERIALS AND METHODS

Collection of isolates of *Colletotrichum* spp.

Cowpea plant parts (leaves, stems, pods) naturally infected by *Colletotrichum* spp. were collected from farmers' fields in three agro-ecological zones of Burkina Faso during the period from October to November 2013. The locations included Saria, Kamboinse and Kouaré (North Soudanian zone), Farako Bâ and Gaoua (Soudanian zone), and Pobe Mengao (Sahelian zone). The cowpea plant 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. After disinfection, the materials were allowed to dry under laminar flow hoods for 15 min. Tissues of approximately 4 mm² in size were placed in Petri dishes containing moistened blotting paper. Plates were incubated in alternating cycles of light/darkness (12 h/12 h) for 7 to 9 days at 28°C. The *Colletotrichum* species were identified based on the growth habit characteristic and morphology of acervuli and conidia and on available identification keys (Marthur and Kongsdal, 2003).

Sporulating cultures of *C. capsici* were aseptically transferred in Petri dishes containing potato dextrose agar (PDA), which was previously autoclaved for 15 min at 121°C and then supplemented with streptomycin (0.3 µg/l of PDA) to prevent bacterial contamination. The Petri dishes were incubated at 24°C under UV light in alternating cycles of light/darkness (12 h/12 h) for 7 days.

Single spore production

A pure single spore culture was obtained from each *C. capsici* isolate after 7 days of growth on PDA. 100 ml of distilled water were added to the fresh fungal culture, and 200 µL of the suspension was spread on PDA media culture and incubated for 12 to 24 h depending on cell germination and growth. Three to five single cells or mycelium from each isolate were transferred to new Petri dishes containing PDA. After 7 days of growth, pure cultures of single spore were stored at 20°C before use.

Pathogenicity tests

A total of 36 single spores *C. capsici* (Table 1) were used to carry out pathogenicity tests in greenhouse conditions. These isolates were used for artificial inoculation of three differential cowpea genotypes namely KVx61-1, KVx396-4-5-2-D and "Moussa Local". The response of these three cowpea lines have been identified by Sereme (1999). Cowpea seeds were sterilized in 1% sodium hypochlorite (NaOCl) for 5 min, rinsed three times successively with distilled water, and dried on laminar flow hoods for 24 h. Fifteen to twenty seeds were sown in duplicates in 2.5 L pots containing sterilized soil mixture of sand and forest soil in a 1:2 ratios (v/v). Fourteen days after sowing (DAS), cowpea plants were inoculated (the whole surface of plants) with a concentration of approximately 10⁶ spores/ml of each *Colletotrichum* isolate. To favor spores penetration inside the plant, the inoculated plants were maintained at 22 ± 1°C in humid (RH > 78%) conditions for 5 days. Then, the cowpea plants were examined for brown blotch disease symptoms after 7, 14 and 21 days after inoculation (DAI).

The disease severity index was based on a 1 to 5 scale, where 1 = no symptoms, 2 = small spots of brown blotch in the stem, 3 = coalescent spots on the stem, 4 = coalescent spot with presence of acervuli but surviving plant, 5 = withered stem and plant death (Figure 1) (Sereme, 1999), and on the formula proposed by Allen et al. (1981) as follows:

$$I = \frac{\sum (X_i - 1) n_i}{[E (X_i) - 1] N} \times 100$$

Where: X_i = the note of disease for each plant; n_i = individual number of category X_i ; N = total number of observed plant; $E (X_i)$ = scale range.

Analysis of variance (ANOVA) was performed using Gen Stat 12th edition on disease incidence and severity data. All the data for disease severity were subjected to Arc sine transformation before

Table 1. List of *C. capsici* single spores isolates used for the pathogenicity test in this study.

Name of <i>Colletotrichum</i> isolates	Organ	Cowpea genotype	Sites of collection	Agro ecological zone
003-KO-1	Pod	KVx61-1	Kouare	North Soudanian
006-KB-1	Pod	KVx61-1	Kamboinse	North Soudanian
009-KB-2	Pod	KVx396-4-5-2D	Kamboinse	North Soudanian
013-KB-3	Stem	KVx780-8	Kamboinse	North Soudanian
017-KB-4	Stem	KVx780-8	Kamboinse	North Soudanian
020-KB-5	Stem	Komcalle	Kamboinse	North Soudanian
021-KB-5	Stem	Komcalle	Kamboinse	North Soudanian
023-GA-1	Stem	KVx61-1	Gaoua	Soudanian
024-GA-2	Stem	KVx61-1	Gaoua	Soudanian
026-GA-3	Pod	KVx61-1	Gaoua	Soudanian
030-GA-4	Pod	KVx61-1	Gaoua	Soudanian
035-GA-5	Pod	KVx61-1	Gaoua	Soudanian
036-GA-5	Pod	KVx61-1	Gaoua	Soudanian
039-GA-6	Pod	KVx396-4-5-2D	Gaoua	Soudanian
043-GA-7	Pod	KVx396-4-5-2D	Gaoua	Soudanian
044-GA-8	Pod	KVx396-4-5-2D	Gaoua	Soudanian
045-GA-8	Pod	KVx396-4-5-2D	Gaoua	Soudanian
047-GA-9	Stem	IT98K-205-8	Gaoua	Soudanian
053-GA-10	Stem	Komcalle	Gaoua	Soudanian
054-FA-1	Leaf	Variety hybride 1	Farako-Ba	Soudanian
057-FA-2	Leaf	Variety hybride 1	Farako-Ba	Soudanian
059-FA-3	Leaf	Variety hybride 1	Farako-Ba	Soudanian
060-FA-3	Leaf	Variety hybride 1	Farako-Ba	Soudanian
064-FA-4	Stem	KVx61-1	Farako-Ba	Soudanian
069-FA-5	Stem	KVx61-1	Farako-Ba	Soudanian
071-FA-6	Pod	KVx61-1	Farako-Ba	Soudanian
074-BA-1	Pod	Local variety	Bani	Sahelian
077-PM-1	Pod	KVx61-1	Pobe-Mengao	Sahelian
079-PM-2	Pod	KVx61-1	Pobe-Mengao	Sahelian
084-PM-3	Stem	KVx61-1	Pobe-Mengao	Sahelian
086-PM-4	Stem	KVx61-1	Pobe-Mengao	Sahelian
090-PM-5	Stem	KVx61-1	Pobe-Mengao	Sahelian
092-SA-1	Stem	Variety hybride 2	Saria	North soudanian
093-SA-1	Stem	Variety hybride 2	Saria	North soudanian
096-SA-2	Stem	Variety hybride 2	Saria	North soudanian
097-SA-2	Stem	Variety hybride 2	Saria	North soudanian

analysis. The Fisher's least significant difference test was used to compare mean values of disease severity. XLSTAT 2013 3.4 software was used for ascendance classification.

Seedling resistance evaluation of brown blotch disease

Forty one cowpea lines were screened for their resistance or susceptibility to brown blotch disease under greenhouse conditions. The seeds were previously surface sterilized in sodium hypochlorite (NaOCl) 1% (v/v) for 5 min and air dried for 24 h before sowing. A mixture of sand and field soil (v/v=1:2) was autoclaved at 121°C for 1 h and used to fill up 75% of the 10 L pot . Two replicates were set

up for each cowpea accession and for each *C. capsici* isolates in a split plot design. The most virulent strain of *C. capsici* from each Agro-ecological zone of the country, designated Ccap-PO, Ccap-FA and Ccap-SA were used for plant inoculation (Figure 2).

About 15 to 20 seeds were sowed in each pot for plant growth. The spore suspension from fresh fungi culture of 7 days was prepared and adjusted to a concentration of 10^6 spores/ml. The *C. capsici* suspension was applied by spraying over the entire surface of the individual plant and plant incubated for symptoms notation. Disease incidence and severity were determined using the same methods described for the pathogenicity test. Seedlings were also scored for disease severity using a modified scale (Sereme, 1999) as shown in Table 2.



Figure 1. Brown blotch disease annotation symptoms on 1 to 5 scale.

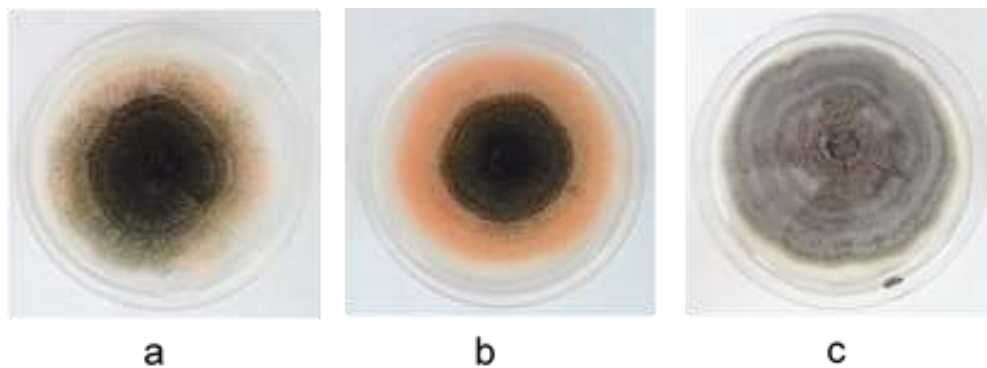


Figure 2. Colony surface of strains: a: Ccap-PO; b: Ccap-FA and c: Ccap-SA, in PDA media, after 7 days of growth.

Table 2. Designation of resistance level based on disease severity score.

Severity scale	Severity (%)	Resistance level
0	S = 0	Symptomless (SL)
1	$1 \leq S \leq 6$	Highly resistant (HR)
2	$6 < S \leq 10$	Resistant (R)
3	$10 < S \leq 20$	Moderately resistant (MR) or moderately susceptible (MS)
4	$20 < S \leq 50$	Susceptible (S)
5	$S > 50$	Highly susceptible (HS)

RESULTS AND DISCUSSION

Pathogenicity testing

Thirty six single spore isolates of *C. capsici* from natural

infected cowpea plants have been used for pathogenicity test in greenhouse conditions. 14 days after inoculation, all the plants presented symptoms of brown blotch disease. The aggressiveness of brown blotch disease depended on the isolate and cowpea line. Seven

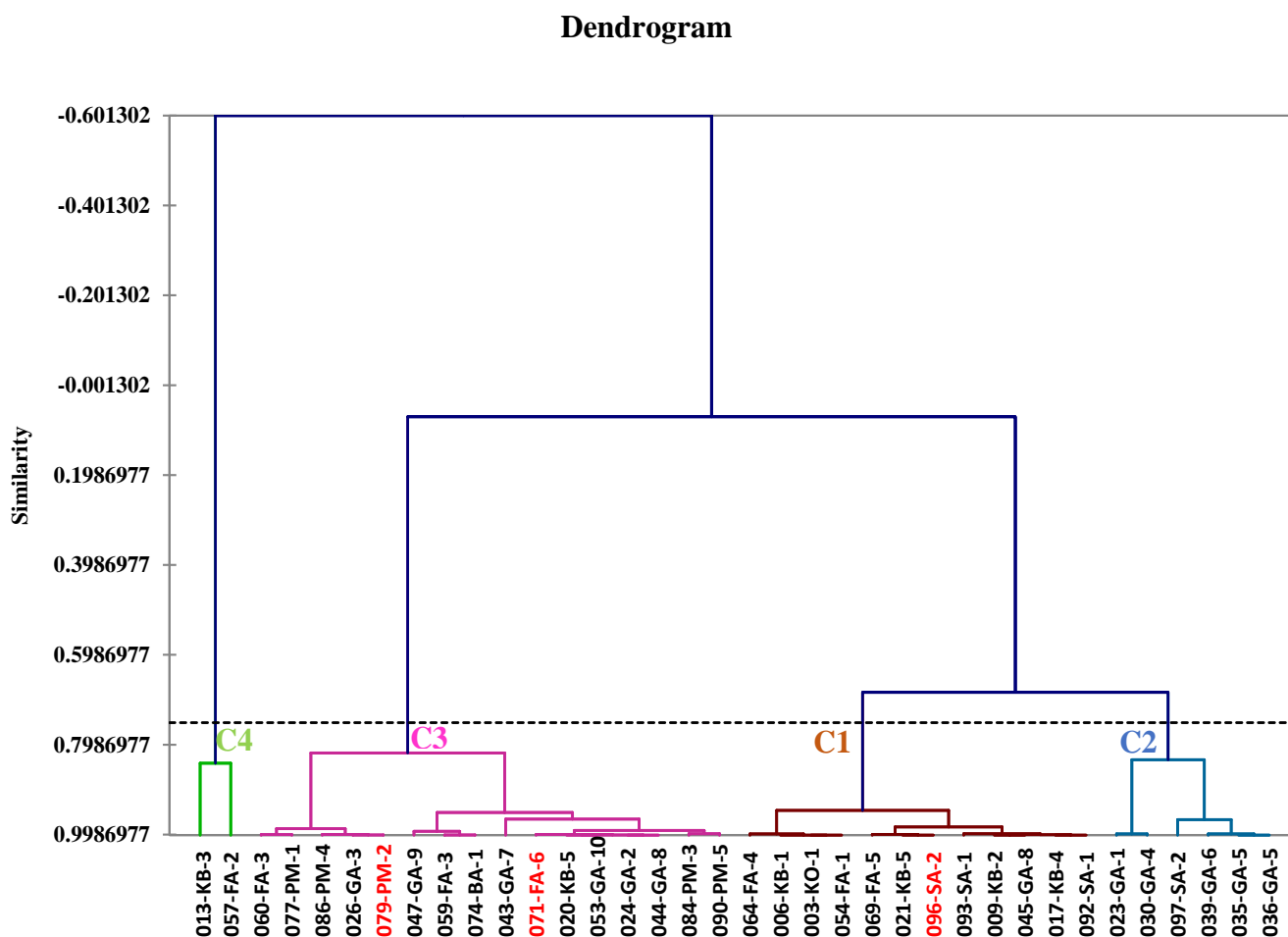


Figure 3. Ascendance hierarchical classification of pathotypes (four classes were grouped at 75% of Pearson correlation coefficient).

pathogenic groups or races of *C. capsici* were identified based on their virulence or aggressiveness. Therefore, Agglomerative Hierarchical Clustering (AHC) analysis was used to predict 4 classes of pathogens in terms of disease severity at 75% of Pearson correlation coefficient (Figure 3). This analysis also indicated a high similarity coefficient within class at 84 (49%) against 15 (51%) for between classes. Thus, the Classes 1 and 2 contain the most pathogenic strains of *C. capsici*. All of the isolates of Saria were identified as the most virulent and are associated with the cowpea genotype K VX396-4-5-2D (Tables 3 and 4). Among these isolates, the strain 096-SA-2 was the most devastating with 78, 82 and 10% of severity, respectively, for K VX61-1, K VX396-4-5-2D and Moussa Local (Table 3). Isolates 096-SA-2, 079-PM-2 and 071-FA-6 were the most aggressive for the North Soudanian, Sahelian and Soudanian agro-ecological zone, respectively. Disease severity and incidence varied across the three agro-ecological zones. These three isolates are newly designated as Ccap-SA (096-SA-2),

Ccap-PO (079-PM-2) and Ccap-FA (071-FA-6).

The analysis of disease incidence and severity due to the virulent pathogens showed a highly significant difference ($P < 0.001$) among the isolates, genotypes and their interactions (Table 5). Among the three cowpea lines used for the test, K VX61-1 (28% severity) was the most susceptible followed by K VX396-4-5-2D (18%) while Moussa Local (7%) seemed to be resistant.

Sereme (1999) reported that the most pathogenic groups of *Colletotrichum* spp. associated with brown blotch disease of cowpea were from Soudanian zone and were collected on the site of Farako Ba. The results of this study indicate a progression of *C. capsici* virulence around the country. The most pathogenic strains belonged to the *C. capsici* variant 1 which were identified by using internal transcribed spacer (ITS) phylo markers in a previous study (Thio et al., 2016). The strains 035-GA-5 and 020-KB-5 presented the lowest values of aggressiveness and correspond, respectively to variants 2 and 4 of *C. capsici*.

Table 3. *C. capsici* strains virulence associated to brown blotch disease on three cowpea genotypes at 14 days after inoculation.

Singlaspore isolate	Cowpea cultivar			Severity	Pathogenic group
	KVx61-1	KVx396-4-5-2D	Moussa Local		
096-SA-2***	64	71	2	46 ^a	1 (S S R)
097-SA-2	38	89	0	42 ^a	1
092-SA-1	51	61	0	37 ^a	1
093-SA-1	52	69	0	40 ^a	1
090-PM-5	54	2	10	22 ^b	2 (S R R)
003-KO-1	30	39	0	23 ^b	1
079-PM-2**	25	0	12	13 ^{cde}	2
009-KB-2	26	25	1	17 ^{bc}	1
071-FA-6*	28	0	2	10 ^{cdefg}	2
077-PM-1	24	0	5	10 ^{cdefg}	2
084-PM-3	34	0	15	16 ^{bcd}	2
086-PM-4	3	1	15	16 ^{bcd}	3 (R R R)
060-FA-3	26	0	4	10 ^{cdefg}	2
074-BA-1	26	5	0	10 ^{cdefg}	2
006-KB-1	25	22	2	17 ^{bcd}	1
059-FA-3	35	4	0	13 ^{cde}	2
024-GA-2	32	0	0	11 ^{cdef}	2
021-KB-5	20	5	0	8 ^{defghi}	2
057-FA-2	17	1	30	16 ^{bcd}	4 (S R S)
030-GA-4	0	21	5	9 ^{cdefghi}	5 (R S R)
043-GA-7	2	0	3	2 ^{ghi}	3
039-GA-6	7	0	5	4 ^{efghi}	3
045-GA-8	1	0	0	0 ⁱ	3
036-GA-5	5	0	0	2 ^{ghi}	3
044-GA-8	4	2	0	2 ^{fghi}	3
054-FA-1	0	5	3	3 ^{fghi}	3
026-GA-3	7	1	5	5 ^{efghi}	3
047-GA-9	2	0	0	1 ^{hi}	3
064-FA-4	0	0	0	0 ⁱ	3
069-FA-5	2	2	0	2 ^{ghi}	3
013-KB-3	1	0	6	2 ^{fghi}	3
017-KB-4	0	4	0	1 ^{ghi}	3
020-KB-5	0	0	0	0 ⁱ	3
023-GA-1	0	6	0	2 ^{fghi}	3
053-GA-10	5	0	0	2 ^{ghi}	3
035-GA-5	0	0	0	0 ⁱ	3
No treatment	0	0	0	0 ⁱ	3
Pathotype virulence mean	18 ^a	12 ^b	3 ^c		

Means followed by the same letter in a column are not significantly different at $P \leq 0.05$. ***Most pathogenic strain from agro-ecological zone.

A specific reaction of cowpea genotypes to *C. capsici* isolates

The screening of 41 cowpea cultivars in greenhouse condition involves identifying potential source of cowpea resistance. The results indicated highly significant difference ($P < 0.05$) in disease severity among cultivars,

isolate effects and their interactions (Table 6). These results indicate high genetic variability in cowpea cultivars to brown blotch disease. All cowpea cultivars present approximately the same incidence at 14 DAI which is not significant. The observations also showed that the cowpea cultivars were more severely affected 21 days after inoculation than 14 DAI. The current study indicated

Table 4. *C. capsici* strains virulence associated to brown blotch disease on three cowpea genotypes at 21 days after inoculation.

Single spore isolate	Cowpea cultivar			Severity	Pathogenic group
	KVx61-1	KVx396-4-5-2D	Moussa Local		
096-SA-2***	78	82	10	57 ^a	1 (S S R)
097-SA-2	58	98	0	52 ^a	1
092-SA-1	68	88	0	52 ^a	1
093-SA-1	62	86	5	51 ^a	1
090-PM-5	81	5	11	33 ^b	2 (S R R)
003-KO-1	51	44	0	32 ^b	1
079-PM-2**	48	5	31	28 ^{bc}	4 (S R S)
009-KB-2	34	41	3	26 ^{bc}	1
071-FA-6*	39	19	18	25 ^{bc}	6 (S S S)
077-PM-1	50	2	22	25 ^{bc}	4
084-PM-3	54	7	15	25 ^{bcd}	2
086-PM-4	46	1	26	25 ^{bcd}	4
060-FA-3	40	10	24	24 ^{bcd}	4
074-BA-1	46	17	8	24 ^{bcd}	1
006-KB-1	34	29	6	23 ^{bcd}	1
059-FA-3	45	9	0	1 ^{8cdef}	2
024-GA-2	44	1	0	15 ^{defg}	2
021-KB-5	20	21	1	14 ^{efgh}	1
057-FA-2	10	1	31	14 ^{efgh}	7 (R R S)
030-GA-4	2	22	5	10 ^{fghi}	5 (R S R)
043-GA-7	18	4	1	10 ^{fghi}	2
039-GA-6	8	11	5	8 ^{fghi}	3 (R R R)
045-GA-8	10	12	0	8 ^{ghi}	3
036-GA-5	5	15	0	7 ^{ghi}	3
044-GA-8	14	2	1	6 ^{ghi}	3
054-FA-1	8	7	3	6 ^{ghi}	3
026-GA-3	8	1	5	5 ^{ghi}	3
047-GA-9	11	4	0	5 ^{hi}	3
064-FA-4	6	5	0	4 ^{hi}	3
069-FA-5	6	6	0	4 ⁱ	3
013-KB-3	1	3	6	3 ⁱ	3
017-KB-4	4	4	2	3 ⁱ	3
020-KB-5	9	0	0	3 ⁱ	3
023-GA-1	0	6	1	3 ⁱ	3
053-GA-10	8	0	0	3 ⁱ	3
035-GA-5	1	4	0	2 ⁱ	3
Pathotype virulence mean	28^a	18^b	7^c		

Means followed by the same letter in a column are not significantly different at $P \leq 0.05$ *** or ** or * = most pathogenic strain from agro-ecological zone; R= Resistant, S= Susceptible.

that 76% of cultivars tested were susceptible to brown blotch disease. The results revealed that among the 41 cowpea lines screened, the cultivar KN-1 showed a specific resistance to the three pathotypes. It was consistently brown blotch disease resistant and should be a good source of resistance genes for susceptible cowpea lines. However, KN-1 cultivar did show

symptoms of brown blotch on the stem. No other genotype was completely resistant to the disease. MELAKH, Moussa Local, Donsin Local, Djouroum Local, Pobe Local and 58-57 appeared to have some level of tolerance (MR/MS) to one or two of the three isolates (Table 7). Cultivars KN-1 and Moussa Local showed a stable level of resistance to brown blotch disease during

Table 5. ANOVA of mean disease incidence in cowpea at 14 days after inoculation due to *C. capsici* virulence.

Source of variation	df	Mean square	F value	Pr > F (LSD 5%)
Cultivars	2	34907.8	260.12	< 0.001***
Isolates	36	3142.7	23.42	< 0.001***
Cultivars X Isolates	72	1575.5	11.74	< 0.001***
Grand mean	24			
CV%	47.3			

***Highly significant difference; CV = Coefficient of variation; df = Degree of freedom; Pr = Probability of F value.

Table 6. ANOVA of genetic variability of cowpea cultivars in brown blotch disease severity at 21 days after inoculation.

Source of variation	df	Mean square	F value	Pr > F (LSD 5%)
Genotypes	40	729.3	3.01	< 0.001**
Isolates	3	6877.1	28.35	< 0.001**
Genotypes X Isolates	120	371.4	1.53	0.006*
Grand mean	20			
CV%	77.3			

**Highly significant difference; CV = Coefficient of variation; df = Degree of freedom; Pr = Probability of F value.

Table 7. Differential reaction of 41 cowpea cultivars at 14 and 21 days after inoculation in *C. capsici* pathotypes.

Cowpea line	Resistance level at 14 DAI			Resistance mean	Resistance level at 21 DAI			Resistance mean
	<i>Ccap-PO</i>	<i>Ccap-FA</i>	<i>Ccap-SA</i>		<i>Ccap-PO</i>	<i>Ccap-FA</i>	<i>Ccap-SA</i>	
KN-1	HR	HR	HR	2 ^a	R	HR	R	6 ^a
Moussa local	MR/MS	HR	HR	5 ^a	MR/MS	R	R	8 ^b
MELAKH	HR	HR	HR	6 ^a	HR	MR/MS	R	7 ^b
Donsin local	HR	SL	HR	4 ^a	MR/MS	HR	R	8 ^b
Djouroum local	R	HR	HR	5 ^a	R	R	MR/MS	10 ^b
Pobé local	R	R	HR	5 ^a	MR/MS	MR/MS	HR	10 ^b
58-57	HR	R	HR	3 ^a	HR	S	R	9 ^b
IT99K-573-2-1	HR	MR/MS	HR	6 ^a	HR	S	HR	11 ^c
503/46-13	SL	MR/MS	MR/MS	8 ^b	SL	S	MR/MS	15 ^c
IT95K-499-35	HR	S	MR/MS	9 ^b	R	S	MR/MS	12 ^c
Komcallé	HR	S	MR/MS	11 ^c	R	S	MR/MS	14 ^c
KVx61-1	HR	S	R	11 ^c	MR/MS	S	R	15 ^c
KVx65-114	R	MR/MS	MR/MS	9 ^b	MR/MS	MR/MS	MR/MS	14 ^c
KVx780-1	HR	S	HR	9 ^b	MR/MS	S	R	14 ^c
IT95M-190	HR	MR/MS	R	7 ^b	MR/MS	MR/MS	MR/MS	13 ^c
KVx780-9	MR/MS	S	S	19 ^c	MR/MS	S	S	21 ^d
Gorom local	HR	HR	MR/MS	5 ^a	MR/MS	MR/MS	S	14 ^c
Gourgou	HR	S	MR/MS	17 ^c	MR/MS	S	MR/MS	19 ^c
HTR	R	MR/MS	HR	8 ^b	MR/MS	S	MR/MS	17 ^c
NS-1	HR	MR/MS	MR/MS	8 ^b	HR	MR/MS	S	13 ^c
TN88-63	R	S	HR	9 ^b	MR/MS	S	MR/MS	14 ^c
TVU14676	HR	MR/MS	R	7 ^b	R	MR/MS	MR/MS	11 ^c
503/46-48	MR/MS	S	MR/MS	20 ^c	S	HS	S	36 ^d
503/46-72	S	S	MR/MS	25 ^d	HS	S	S	29 ^d
524B	R	S	MR/MS	13 ^c	MR/MS	S	HS	27 ^d

Table 7. Contd.

B301	HR	HR	HS	15 ^c	R	MR/MS	HS	27 ^d
CB46	MR/MS	S	MR/MS	15 ^c	MR/MS	S	S	23 ^d
IT81D-994	HR	MR/MS	S	15 ^c	R	MR/MS	HS	26 ^d
IT82D-849	HR	S	MR/MS	16 ^c	R	S	S	28 ^d
IT93K-503-1	MR/MS	R	S	18 ^c	MR/MS	S	HS	26 ^d
IT95K-627-4	R	MR/MS	S	13 ^c	R	S	S	23 ^d
IT98K-205-8	MR/MS	S	S	16 ^c	MR/MS	S	HS	30 ^d
KVx30-309-6G	HS	R	HR	18 ^c	HS	MR/MS	HR	22 ^d
KVx396-4-5-2D	R	MR/MS	HS	24 ^d	R	S	HS	32 ^d
KVx404-8-1	MR/MS	S	S	24 ^d	S	S	HS	36 ^d
KVx421-2J	S	S	S	22 ^d	S	S	S	27 ^d
KVx525	S	S	S	23 ^d	S	S	S	29 ^d
KVx745-11P	S	S	S	29 ^d	HS	HS	S	43 ^d
KVx771-106	S	S	S	22 ^d	S	S	S	35 ^d
KVx775-33-2G	S	S	S	20 ^d	S	S	S	27 ^d
Bambey-21	S	S	MR/MS	22 ^d	S	S	S	26 ^d
Pathotype virulence mean	12 ^b	20 ^a	18 ^a	13	17 ^b	26 ^a	29 ^a	24

SL= Symptomless; HR=Highly Resistant; R= Resistant; MR/MS= Tolerant =Moderately Resistant or moderately susceptible; S= Susceptible; HS= Highly Susceptible; DAI= Day after inoculation; Means followed by the same letter (a, b, c and d) in a resistance mean column belong to the same susceptibility class.

the last decade (Sereme, 1999).

They might have revealed a polygenic resistance to the disease. All of the new improved varieties; Gourgou, KVx98K-205-8, Komcalle, Tiligre (KVx775-33-2G), Nafi (KVx771-11P) and IT93K-503-1 from IITA were susceptible to brown blotch. The cultivars IT99K-573-2-1 and 503/46-13 showed highly specific resistance, respectively, to two (Ccap-PO and Ccap-SA) and one (Ccap-PO) of the three pathotypes. They could be also used for resistance gene incorporation to susceptible genotypes depending on agro-ecological zone. The cultivar IT81D-994, IT82D-849 and KVx61-1 were previously reported susceptible to brown blotch disease (Adebitan et al., 1992; Sereme, 1999) corresponding with the results.

Disease severity appeared highly dependent on plant age or period of disease evaluation. According to Health (1996), age-related resistance of certain cultivars of cowpea to disease appears to be due to a delay in parasite-specific resistant genes activation. Early evaluation of disease incidence and severity could influence susceptibility class designation. 21 days after inoculation is allowed presented the best reaction of the interaction, genotype-pathotypes in term of disease severity. In conclusion, the study clearly demonstrated that resistance or susceptibility depends on cowpea cultivars, the pathotypes specific resistance and the stage of cowpea plant development and may be consistent with the presence of different genes; recessive, dominant or both can control resistance

(Pakdeevaporn et al., 2005; Mahasuk et al., 2009).

The identification of new sources of resistance from cowpea cultivars is a decisive step in managing the brown blotch disease in Burkina Faso. These results will significantly contribute in designing good programs for marker assisted selection in cowpea resistance to *C. capsici*.

In this study, potential cowpea cultivars presenting a specific resistance to the pathotypes of *C. capsici* and associated with the three agro-ecological zones of the country were identified.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Adebitan SA, Ikotun T, Dashiell KE, Singh SR (1992). Use of three inoculation methods in screening cowpea genotypes for resistance to two *Colletotrichum* species. *Plant Dis.* 76(10):1025-1028.
- Adegbite AA, Amusa NA (2008). The major economic field disease of cowpea in the humid agro-ecologies of South-western Nigeria. *Afr. J. Biotechnol.* 7(25):4706-4712.
- Allen DJ, Emechebe AM, Ndimande B (1981). Identification of resistance in cowpea to diseases of the African savannas. *Trop. Agric.* 58(3):267-274.
- Amusa NA, Ikotun T, Osikanlu YOK (1994). Screening cowpea and soybean cultivars for resistance to anthracnose and brown blotch disease using phytotoxic metabolite. *Afr. Crop Sci. J. (Kenya)* 2(2):221-224.

- Banerjee AK, Arora M, Murty USN (2007). How far is ITS2 reliable as a phylogenetic marker for the mosquito genera? *Elect. J. Biol.* 3(3):61-68.
- Chacko ST, Gokulapalan C (2015). *In vitro* study of fungicides and biocontrol agents against *Colletotrichum capsici* causing anthracnose of chilli (*Capsicum annuum* L.). *Int. J. Appl. Pure Sci. Agric. (IJAPSA)* 1(5):93-98.
- Chandra NC, Udaya SAC, Niranjana SR, Prakash HS, Mortensen CN (2009). Anthracnose disease of chilli pepper. *Techn. Bull.* P 15.
- Emechebe AM (1986). Cowpea pathology. In grain legume improvement. Program Annual report 1985. IITA, Ibadan, Nigeria pp. 69-100.
- Enyiukwu DN, Awurum AN, Ononuju CC, Nwaneri JA (2014). Biology and management strategies of cowpea anthracnose disease caused by *Colletotrichum* species. *Greener J. Biochem. Biotechnol.* 1(2):052-065
- Fery RL, Singh BB (1997). Cowpea genetics: A review of recent literature. in *Advances in cowpea research*, edited by Singh BB, Mohan Raj DR, Dashiell KE, Jackai LEN. Copublication of IITA and JIRCAS. IITA, Ibadan, Nigeria pp. 13-29.
- FAOSTAT (2011). <http://faostat.fao.org/faostat/>
- Hyde KD, Cai L, McLenzie EHC, Yang YL, Zhang JZ, Prihastuti H (2009). *Colletotrichum*: A catalogue of confusion. *Fungal Diversity* 39:1-17.
- N'Guettia MY, Hortense Atta Diallo HA, Kouassi N, Coulibaly F (2013). Diversité morphologique et pathogénique des souches de *Colletotrichum* sp. responsables de l'anthracnose de la mangue en Côte d'Ivoire. *J. Anim. Plant Sci.* 18(3):2775-2784.
- Mahasuk P, Taylor P WJ, Mongkolporn O (2009). Identification of two new genes conferring resistance to *Colletotrichum acutatum* in *Capsicum baccatum*. *Phytopathology* 99:1100-1104.
- Mark WA, Channya KF, Chimbekujwo IB, Bristone B (2015). Control *Colletotrichum capsici* of cowpea in the Savanna using ASH. *GJBAHS* 4(1):136-141.
- Mark WA, Channya KF (2016). Control of *Colletotrichum capsici* (Pathogen of Brown Blotch of Cowpea in the Savanna) Using Garlic Oil. *Int. J. Res. Agric. For.* 3(1):22-29.
- Marthur SB, Kongstal O (2003). *Common Laboratory Seed Health Testing Methods for Detecting Fungi*. Published by the International Seed Testing Association (ISTA) P 425.
- Obi VI, Barrusa-Vargas JJ (2014). Situation of biofungicides reconnaissance, a case of anthracnose disease of cowpea. *Am. J. Plant Sci.* 5(9):10.
- Pakdeevaporn P, Wasee S, Taylor PWJ, Mongkolporn O (2005). Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in *Capsicum*. *Plant Breed.* 124(2):206-208.
- Sereme P (1999). La maladie des taches brunes du niébé (*Vigna unguiculata*) au Burkina Faso : connaissance des agents pathogènes impliqués et développement de méthodes de lutte. Thèse de Doctorat d'Etat ès-Sciences Naturelles, Université de Cocody, UFR Biosciences, Côte d'Ivoire P. 15.
- Thio IG, Zida EP, Sawadogo M, Sereme P (2016). Current status of *Colletotrichum capsici* strains, causal agents of Brown blotch disease of cowpea in Burkina Faso. *Afr. J. Biotechnol.* 15(5):96-104.
- Torres-Calzada C, Tapia-Tussell R, Quijano-Ramayo A, Martin-Mex R, Rojas-Brito D (2011). A species-specific polymerase chain reaction assay for rapid and sensitive detection of *Colletotrichum capsici*. *Mol. Biotechnol.* 49:48-50.