Scientific Research Publishing

Open Journal of Applied Sciences, 2022, 12, 1837-1855
https://www.scirp.org/journal/ojapps
ISSN Online: 2165-3925
ISSN Print: 2165-3917

Morphological and Biometric Diversity of *Colletotrichum capsici* Isolates, Causal Agent of Cowpea Brown Blotch Disease (*Vigna unguiculata* (L.) Walp) in the Sudano-Sahelian Zone of Cameroon

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How to cite this paper: Yoyo, F.D.M., Gonne, S., Kosma, P., Pierre, T.F., Liliane, I., Arlette, Z.Z., Pierre, M., Noel, M.F.M., David, A., Yvonne, D., Dieudonné, G., Ohlson, E.W. and Timko, M.P. (2022) Morphological and Biometric Diversity of *Colletotrichum capsici* Isolates, Causal Agent of Cowpea Brown Blotch Disease (*Vigna unguiculata* (L.) Walp) in the Sudano-Sahelian Zone of Cameroon. *Open Journal of Applied Sciences*, **12**, 1837-1855.
https://doi.org/10.4236/ojapps.2022.1211127

Received: October 12, 2022
Accepted: November 14, 2022
Published: November 17, 2022

Abstract

Cowpea [*Vigna unguiculata* (L.) Walp] is an important legume in the midst of about 170 species of its genus because it is an important source of protein and other essential nutrients for humans and animals. Its production faces many constraints such as the cowpea brown blotch disease caused by *Colletotrichum capsici* which contributes in wet conditions of the field to losses ranging from 42% to 100%. This study was conducted to identify *Colletotrichum capsici* isolates responsible for cowpea brown blotch disease and to determine their diversity in the Sudano-Sahelian zone of Cameroon. Identification and isolation were made from cowpea organ samples on the Potato Dextrose Agar (PDA) medium and, morphological and biometric characteristics such as: the colony color, the mycelium shape, the abundance of acervules, the presence or absence of saltations, the mycelial growth rate, the conidia length and width were used to assess the diversity. The results obtained indicate that 55 *Colletotrichum capsici* isolates have been identified in the Sudano-Sahelian zone of Cameroon. Statistical analysis showed that there is a sig-

nificant difference between isolates. Isolates showed multiple colony colours and were brown coloured as presented by 36.36% of isolates, compact mycelium is found in 56.36% of isolates, 56.36% of isolates have abundant acervulis, and saltations were absent in 45.45% of *C. capsici* isolates. The mycelial growth rate is between 6.69 mm/d and 12.33 mm/d. The principal component analysis (PCA) made indicated that there are differences between the observed and measured characteristics. The Hierarchical Ascending Classification (HAC) was done and 10 morphotypes of *C. capsici* in the Sudano-Sahelian zone were identified.

Keywords

Cowpea, Isolates, *Colletotrichum capsici*, Diversity, Sudano-Sahelian Zone

1. Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is an important legume in the midst of about 170 species of its genus because it's an important source of protein and other essential nutrients for humans and animals [1]. Second African legume after groundnuts, its production faces many constraints such as insect attacks, drought, diseases... There are many diseases as cowpea brown blotch disease caused by *Colletotrichum capsici* which is a very important disease of cowpea cultivation [*Vigna unguiculata* (L.) Walp] [2]. The typical symptoms characterizing it are observed at all stages of plant development, causing seed yield losses of the order of 75% [3]. Described as a disease subservient to the Sudanese and forested areas of cowpea cultivation in Africa [4], brown blotch disease is thought to be caused by a complex of [*Colletotrichum capsici* (Syd.), Butler & Bisby] and [*Colletotrichum truncatum* (Schein) Andrus & Moore] [5]. But authors such as Emechebe, [6]; Emechebe *et al.* [7] et Emechebe & Florini D. A. [8] established that *Colletotrichum capsici* is the main pathogen of cowpea brown blotch disease because it contributes more than 90% in infections caused by it. Thus described as a disease of the Sudanese zones, the cowpea brown blotch disease is also encountered in the Sudano-Sahelian zone of Cameroon. This disease can cause wet losses in the field ranging from 42% to 100% [9].

However, the interest of this study is to highlight the diversity that would exist between *Colletotrichum capsici* isolates, causal agent of the cowpea brown blotch disease in sudano-sahelian zone of Cameroon. Thus, following this introductive part, the structural organization of this work focuses on: Material and Methods: it was a question of presenting by describing the plant material used, the process of identification and isolation of isolates, the procedure of morphological and biometric characterization, and the data analysis, results and discussions presenting the proportions of isolates obtained by division, the morphological and biometric characteristics of the isolates, the diversity of isolates through their spatial arrangement in relation to the study parameters with a

hierarchical ascending classification, a conclusion summarizing the results, a recommendation indicating the resulting research.

2. Materials and Methods

2.1. Plant Material

The plant material used in this study was constituted by organs (stems, leaves and pods) of cowpea plants with symptoms of cowpea brown blotch disease. They were collected in forty-four villages in seven divisions in the northern and far northern regions of the Sudano-Sahelian zone of Cameroon. The collection was done in the cowpea production basins according to the arrangement of the cardinal points [10]. These samples of diseased organs were transported to the plant pathology laboratory of the regional agricultural research center of Maroua.

2.2. Identification and Isolation of *Colletotrichum capsici* Isolates

The infected organs collected were cut with scapel and then superficially desinfected with sodium hypochlorite (NaOCl) 1% for 5 minutes and immersed in 70% alcohol (v/v) for one minute followed by three successive rinses with sterile distilled water [11] [12]. After rinsing, the disinfected fragments were dried for at least two hours on sterile absorbent paper and then placed in the petri dishes previously dressed with three layers of blotting paper moistened with sterile distilled water. The boxes were incubated for 5 to 7 days at room temperature in the laboratory under alternating light and dark (12h/12h) [13]. After the incubation period, the cultured samples were taken under a stereo microscope for identification of *Colletotrichum capsici*. Identification was made on the basis of the morphological characteristics of *Colletotrichum capsici* using the identification key defined by Mathur and Kongsdal (2003) [14]. After identification, isolates were transplanted onto the Potato Dextrose Agar (PDA) culture medium supplanted by streptomycin and ampicilin and incubated for seven days for purification [15].

The single spores production was made from previously isolated and purified population isolates. A diameter of 5 mm conidian mass of each population isolate was transferred to 10 ml of sterile water. The mixture was stirred at the vortex and 1ml of the suspension was removed and added to 9 ml of sterile distilled water contained in a tube. The action was redone twice to obtain a less concentrated suspension (10^{-3} of the initial concentration) [16]. One to two drops of the suspension was sprayed on PDA medium. After sixteen to 24 hours of incubation, 3 to 4 sprouted conidia were individually transplanted onto PDA.

2.3. Morphological and Biometric Characterization of Single Spores Isolates of *C. capsici*

Morphological characterization was done based on qualitative and quantitative characteristics from the work of other scientists on different species of *Colletotrichum* spp. [17] [18] [19].

After purification of the single spore isolates, a 5 mm diameter fragment of each of the isolates was transferred and deposited in the centre of the petri dishes containing the PDA medium. The incubation of the cultures was done at room temperature of the laboratory (23˚C - 25˚C). The morphological characteristics selected for the study focused on the colony colour, the mycelial shape, the abundance of acervulis and the presence or absence of saltations [20]. Observations and notations were made on the seventh day after incubation of single spores isolates. The biometrics used for the study included mycelial diameter, daily radial growth rate, conidia length and width [21]. The reverses of petri dishes containing the single spores isolates were previously crossed by two diagonals (x, y) perpendicular to each other. The measurement of the evolution of the colony was taken every other day until the sixth day.

The mycelial diameter was calculated by the formula: $D = \frac{d_1 + d_2}{2} - d_0$ [18].

The radial growth rate was calculated by the formula:

$$Rgr = \left(DMJ_1 - DMJ_0\right)/n + \left(DMJ_2 - DMJ_1\right)/n + \ldots + \left(DMJ_n - DMJ_{n-1}\right)/n \quad [22]$$

where DMJ_n is the average growth diameter on the nth day of measurement.

The daily radial growth rate (DMJ_n) is the average daily increase in crop diameter; it's calculated by the formula:

$DMJ_n = (d_1 + d_2)/2$ where d_1 is the first diameter and d_2 is the second diameter measured on the n^{th} day.

DMJ_0 = 5 mm = diameter of the transplanted explant.

On the tenth day after transplanting, 10 ml of sterile water was poured into the culture and after stirring, the suspension was returned to the test tubes. A drop was mounted on a micrometer and the reading was made using the Motic microscope at magnification 400. Thus, measurements (Length and width) of conidia were taken on 30 conidia per strain [23].

2.4. Data Analysis

The data was entered into the Excel 2010 spreadsheet. SPSS software was used for qualitative data analysis and STATGRAPHICS Centurion XVI.II software for quantitative data. These data were subjected to analysis of variance (ANOVA) and separate means using Fisher's smallest significant difference test (PPDS) at the 5% probability threshold [24] for quantitative data and the khi two test for qualitative data. Using the averages of the measured and scored parameters, the principal component analysis was performed using XLSTAT 2007 software.

3. Results and Discussions

3.1. Results

The results obtained from this study are presented under labels such as identification of *Colletotrichum capsici* isolates in Cameroon, their morphological characters, biometric characters, spatial arrangement and similarity.

3.1.1. Identification of *Colletotrichum capsici* isolates in Cameroon

After collection, 151 (one hundred and fifty-one) samples of plant organs were collected. Of the samples collected, fifty-five (55) isolates of *Colletotrichum capsici* were identified and isolated (Table 1). At least one isolate of *C. capsici* was identified in each division: 5.45% in Benoue, 7.27% in Mayo-Tsanaga, 10.90% in Mayo-Sava, 16.36% in Mayo-Louti, 14.55% in Mayo-Danay, 21.81% in Mayo-Kani and 23.64% in Diamare.

Table 1. Single spores isolates of *C. capsici* identified in the Sudano-Sahelian zone of Cameroon.

Isolates	Setting Organ	Obtening Site	Geographical Coordinates			Division
			North Latitude	East Longitude	Altitude	
135-MABE-101	Stem	Mayo-dadi	09˚10'66.2"	013˚26'52.3"	754	Benoue
139-NGBE-141	Stem	Ngong	09˚07'69.1"	013˚30'52.8"	1045	
138-NGBE-133	Pod	Ngong	09˚08'66.4"	013˚30'52.8"	1005	
026-MAMT-011	Stem	Mandaka	10˚42'71.1"	013˚50'63.4"	2545	Mayo-Tsanaga
028-MAMT-031	Stem	Mandaka	10˚43'99.8"	013˚50'95.7"	2474	
040-ZAMT-151	Stem	Zamai	10˚38'39.9"	013˚53'77.5"	2044	
042-MOMT-171	Stem	Mokola	10˚45'28.5"	013˚48'87.6"	2614	
017-TOMS-022	Leaf	Tokombere	10˚50'30.6"	014˚06'85.9"	1575	Mayo-Sava
017-TOMS-023	Pod	Tokombere	10˚50'30.6"	014˚06'85.9"	1575	
017-TOMS-021	Stem	Tokombere	10˚50’30.6"	014˚06'85.9"	1575	
019-TOMS-041	Stem	Tokombere	10˚50'30.6"	014˚06'85.9"	1575	
021-TOMS-051	Stem	Tokombere	10˚50'26.4"	014˚06'90.5"	1558	
021-MEMS-061	Stem	Meme	10˚58'50.4"	014˚11'69.5"	1420	
002-SOML-021	Stem	Soukoundou	09˚50'76.2"	013˚52'36.4"	1170	Mayo-Louti
005-GUML-041	Stem	Guider	09˚50'62.3"	013˚51'97.5"	1174	
012-GUML-111	Stem	Guider	09˚50'32.5"	013˚51'49.8"	1208	
011-GUML-101	Stem	Guider	09˚50'76.2"	013˚52'36.4"	1170	
010-SOML-091	Stem	Soukoundou	09˚51'62.3"	013˚51'97.5"	1181	
010-SOML-092	Leaf	Guider	09˚51’62.3"	013˚51'97.5"	1181	
009-SOML-083	Pod	Soukoundou	09˚50'62.1"	013˚51'97.7"	1187	
013-GUML-123	Pod	Guider	09˚50'31.5"	013˚51'51.5"	1220	
015-SOML-143	Pod	Soukoundou	09˚50'62.3"	013˚51'97.5"	1234	
054-DIMD-071	Stem	Djaolone	10˚40'12.2"	014˚53'09.2"	1131	Mayo-Danay
049-SAMD-031	Stem	Sadjakbe	10˚03'67.1"	014˚54'88.3"	1140	
048-SAMD-021	Stem	Sadjakbe	10˚03'55.0"	014˚50'56.6"	1147	
047-SAMD-012	Leaf	Sadjakbe	10˚50'54.2"	014˚54'96.2"	1139	
049-SAMD-032	Leaf	Sadjakbe	10˚03'67.1"	014˚54'88.3"	1142	

Continued

052-DIMD-062	Leaf	Djaolone	10˚40'03.6"	014˚53'09.2"	1142	
057-TIMD-103	Pod	Tiliga	10˚40'12.2"	014˚53'09.2"	1143	
051-SAMD-053	Pod	Sadjakbe	10˚40'03.6"	014˚53'09.2"	1141	
116-LAMK-191	Stem	Lara	10˚70'86.2"	014˚28'54.8"	2645	Mayo-Kani
109-GUMK-121	Stem	Guidiguis	010˚07'60.00"	014˚41'59.99"	1240	
118-KAMK-212	Leaf	Kaele	10˚21'23.3"	014˚13'92.2"	1518	
107-TOMK-103	Pod	Touloum	10˚65'44.6"	014˚50'18.2"	1142	
111-LAMK-193	Pod	Lara	10˚70'86.2"	014˚28'54.8"	1540	
117-KAMK-203a	Pod	Kaele	10˚90'36.3"	014˚29'29.0"	1515	
122-LAMK-253	Pod	Lara	10˚14'98.6"	014˚12'75.2"	1532	
118-KAMK-213	Pod	Kaele	10˚21'23.3"	014˚13'92.2"	1518	
123-LAMK-263	Pod	Lara	10˚15'04.7"	014˚12'74.9"	1540	
117-KAMK-203b	Pod	Kaele	10˚90'36.3"	014˚29'29.0"	1515	
114-KOMK-173	Pod	Kourbi	10˚80'36.1"	014˚36'71.6"	2554	
121-MOMK-243	Pod	Mouda	10˚21'21.2"	014˚13'90.3"	1513	
097-GADI-231	Stem	Gayak	10˚35'27.5"	014˚18'57.5"	1319	Diamare
085-DODI-111	Stem	Dougouf	10˚56'54.7"	014˚28'16.1"	1175	
083-GRDI-091	Stem	Groubelen	10˚31'84.8"	014˚10'82.4"	1472	
080-SADI-061	Stem	Salak	10˚27'15.0"	014˚14'88.1"	1383	
086-DODI-121	Stem	Dougouf	10˚65'54.5"	014˚82'16.1"	1172	
089-MADI-151	Stem	Maroua	10˚35'27.1"	014˚18'57.3"	1148	
090-MADI-161	Stem	Maroua	10˚27'15.0"	014˚14'88.1"	1383	
094-GADI-201	Stem	Godola	10˚42'09.6"	014˚18'04.1"	1588	
085-DODI-112b	Leaf	Dougouf	10˚56'54.7"	014˚28'16.1"	1175	
085-DODI-112a	Leaf	Dougouf	10˚56'54.7"	014˚28'16.1"	1175	
076-GUDI-023	Pod	Guiring	10˚37'27.1"	014˚22'13.7"	1268	
079-GUDI-053	Pod	Guiring	10˚37'27.6"	014˚22'19.4"	1263	
089-MADI-153	Pod	Maroua	10˚35'27.4"	014˚18'57.1"	1148	

Isolates were isolated from each type of organ collected in the field. Of the isolates identified, 50.92% are from the stem organ, 14.3% are isolated from the leaf organ and 34.55% are isolated from the pod organ.

3.1.2. Characteristics of Single Spores Isolates of *C. capsici*

1) Morphological characters

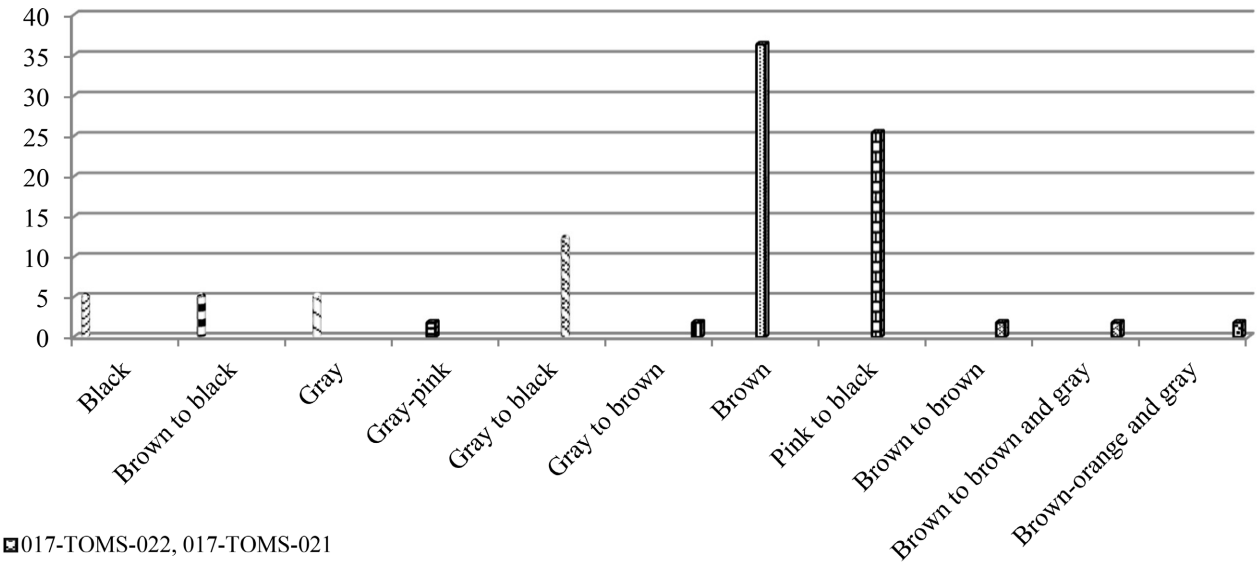
The results of the analysis of the colour data showed that there is a highly significant difference ($P < 0.05$) between isolates for colony colour. The colors

found are: black, brown-black, gray, gray-pink, gray to black, gray to brown, brown to black, brown to brown and gray, brown-orange and gray, and brown to black (Figure 1). 1.81% of isolates are brown-orange and grey, and 36.36% of isolates are brown.

The results of the data analysis reveal that there is a highly significant difference ($P < 0.05$) between isolates for mycelial shape (Figure 2). Indeed, seven forms of mycelial are observed in *C. capsici* isolates: compact, compact to air, compact to cottony, aerial to compact, cottony to compact, cottony to air and aerial to cotton. However, the compact form is observed in 56.36% of isolates while the cotton to compact and aerial to cotton forms are observed in only 1.81% of *C. capsici* isolates.

The results of the data analysis showed that there is a highly significant difference ($P < 0.05$) between *C. capsici* isolates for acervulean abundance (Figure 3). Acervulis are absent in 1.81% of isolates, rare in 9.09% of isolates, scarce in 3.64%, very abundant in 12.7% of isolates, very abundant and well differentiated in 16.36% and abundant in 56.36% of *C. capsici* isolates.

The results of the analysis of the saltation data revealed that there is a highly



017-TOMS-022, 017-TOMS-021

121-MOMK-243,085-DODI-111, 085-DODI-112a

086-DODI-121,076-GUDI-023,107-TOMK-103

083-GRDI-091

116-LAMK-191, 028-MAMT-031, 002-SOML-021, 005-GUML-041,009-SOML-083, 013-GUML-123, 010-SOML-092, 010-SOML-091, 047-SAMD-012, 049-SAMD-032,057-TIMD-103, 118-KAMK-212,123-LAMK-263,097-GADI-231, 089-MADI-153, 080-SADI-061, 085-DODI-112b, 090-MADI-161,138-NGB

026-MAMT-011, 040-ZAMT-151, 042-MOMT-171, 017-TOMS-023,021-TOMS-051, 015-SOML-143, 054-DIMD-071, 052-DIMD-062,048-SAMD-021, 051-SAMD-053, 117-KAMK-203b, 079-GUDI-053,012-GUML-111

139-NGBE-141, 011-GUML-101, 049-SAMD-031, 116-LAMK-191, 122-LAMK-253, 118-KAMK-213,111-LAMK-193

109-GUMK-121

117-KAMK-203a

019-TOMS-041

114-KOMK-173

Figure 1. Colony color.

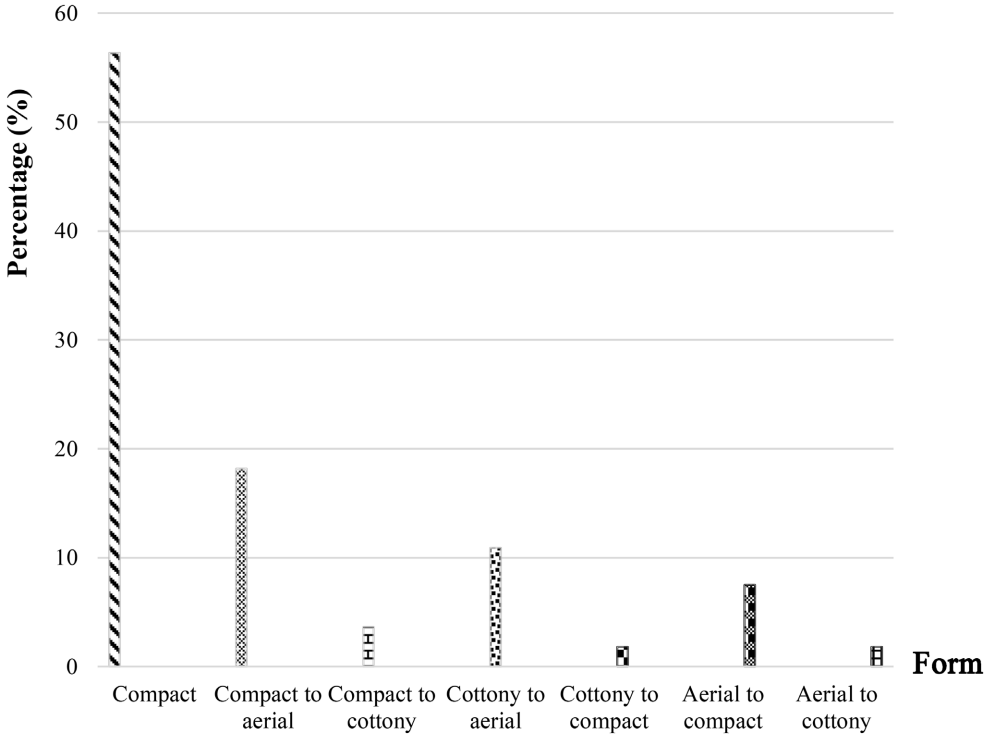


Figure 2. Mycelial form.

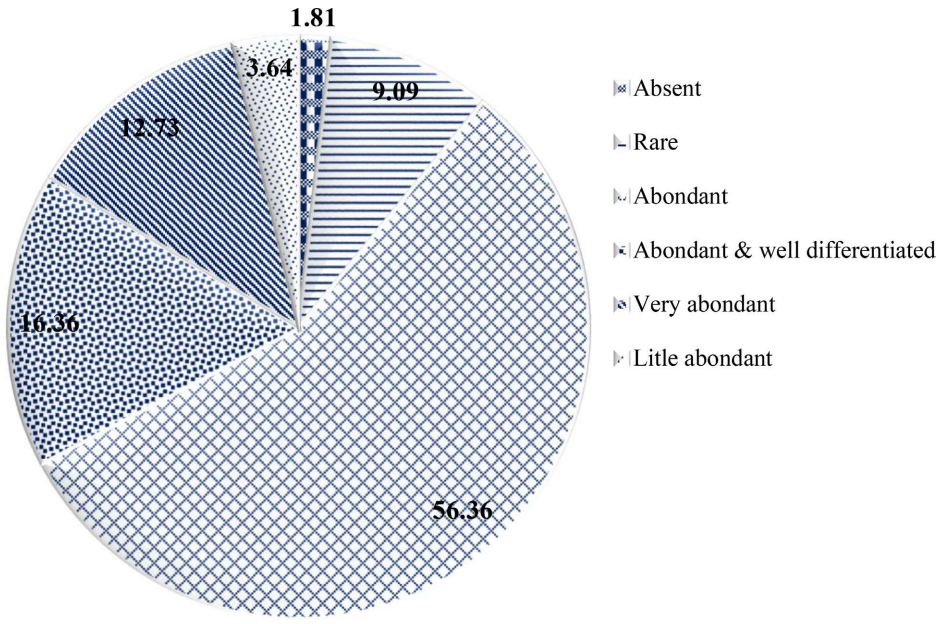


Figure 3. Abundance of acervulis.

significant difference ($P < 0.05$) between *C. capsici* isolates in the presence or absence of saltations (Figure 4). Saltations are absent in 45.45% of isolates, present and poorly differentiated in 36.36% of isolates, present and well differentiated in 14.55% of isolates and, present and undifferentiated in 3.63% of *C. capsici* isolates.

2) Biometrics

The results of the analysis of mycelial diameter data showed that there is a statistically significant difference between isolates at 2 DAR ($P < 0.05$), 4 DAR ($P < 0.05$) and 6 DAR ($P < 0.05$). At 2 DAR, the mean mycelial diameter is 20.90 mm and isolate 089-MADI-153 has the smallest value (15.50 mm) in diameter mycelial while isolate 090-MADI-161 has the largest value (26.33 mm) in diameter mycelial. At 4 DAR, the average mycelial diameter is 44.23 mm. Isolate 089-MADI-153 has a smaller value (36.16 mm) and isolate 005-GUML-041 has the highest value (52.00 mm) in mycelial diameter. At 6 DAR, the average mycelial diameter is 66.23 mm and isolates 054-DIMD-071, 021-MEMS-061 and 048-SAMD-021 have the smallest (58.16 mm) and highest value (73.16 mm) in mycelial diameter, respectively.

In addition, the results of the analysis of the growth rate data showed that there is a statistically significant difference ($P < 0.05$) and the average growth rate is 11.03 mm/day. Isolate 005-GUML-041 has 6.69 mm/d (lowest value) and isolate 118-KAMK-212 has 12.33 mm/d. Multiple tests revealed 30 homogeneous groups. Table 2 presents the mycelial growth rate of *C. capsisi* isolates.

The results of the analysis of conidia length data showed that there is a statistically significant difference ($P < 0.05$) between *C. capsici* isolates for conidia length at 95% confidence. The conidia length of *C. capsici* varies between 48.10 ± 7.28 µm and 72.79 ± 1.05 µm with an average length of 64.31 ± 5.20 µm (Figure 5). Multiple scope tests matched 28 homogeneous groups.

The results of the analysis of conidia width data showed that there is a statistically significant difference ($P < 0.05$) between *C. capsici* isolates at 95% confidence. The width varies between 4.41 µm and 7.71 µm. However, the average width of the conidia is 5.30 ± 0.51 µm. Isolate 114-KOMK-173 has the smallest width value (4.41 ± 0.17 µm) and isolate 002-SOML-021 is the isolate with the

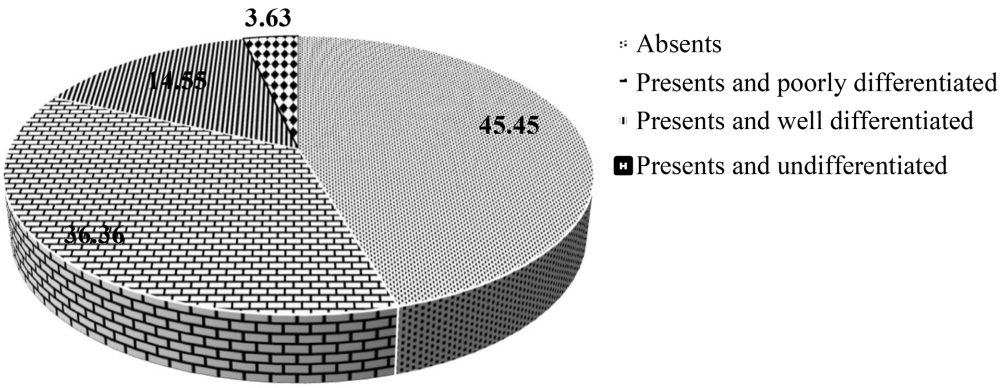


Figure 4. Saltations of *C. capsici* isolates (%).

Table 2. Mycelial growth rate (Mgr) of *C. capsisi* isolates.

Isolats	Mgr (mm/d)	Isolats	Mgr (mm/d)	Isolats	Mgr (mm/d)	Isolats	Mgr (mm/d)	Isolats	Mgr (mm/d)
005-GUML-041	6.69^{a}	107-TOMK-103	10.56^{c-j}	019-TOMS-041	10.95^{e-o}	085-DODI-112b	11.35^{g-r}	021-TOMS-051	11.83^{l-r}
094-GADI-201	9.14^{b}	118-KAMK-213	10.58^{c-j}	086-DODI-121	10.97^{e-o}	122-LAMK-253	11.36^{g-r}	040-ZAMT-151	11.91^{l-r}
089-MADI-153	9.69^{bc}	117-KAMK-203a	10.58^{c-j}	049-SAMD-031	11.03^{e-p}	123-LAMK-263	11.39^{h-r}	042-MOMT-171	11.92^{l-r}
054-DIMD-071	9.69^{bc}	028-MAMT-031	10.64^{c-j}	117-KAMK-203b	11.06^{e-p}	047-SAMD-012	11.39^{h-r}	026-MAMT-011	11.94^{m-r}
021-MEMS-061	9.70^{b-d}	083-GRDI-091	10.75^{c-k}	010-SOML-092	11.08^{e-p}	017-TOMS-023	11.42^{i-r}	097-GADI-231	12.00^{n-r}
085-DODI-111	10.08^{b-e}	089-MADI-151	10.81^{c-l}	051-SAMD-053	11.14^{e-p}	138-NGBE-133	11.45^{i-r}	010-SOML-091	12.05^{o-r}
085-DODI-112a	10.21^{b-f}	076-GUDI-023	10.86^{c-m}	116-LAMK-191	11.22^{f-q}	015-SOML-143	11.56^{j-r}	109-GUMK-121	12.06^{o-r}
079-GUDI-053	10.22^{b-g}	111-LAMK-193	10.86^{c-m}	135-MABE-101	11.22^{f-q}	048-SAMD-021	11.58^{j-r}	011-GUML-101	12.08^{p-r}
002-SOML-021	10.33^{c-h}	121-MOMK-243	10.86^{d-m}	057-TIMD-103	11.25^{f-q}	090-MADI-161	11.67^{k-r}	080-SADI-061	12.08^{p-r}
012-GUML-111	10.44^{c-i}	052-DIMD-062	10.89^{e-n}	017-TOMS-021	11.28^{g-r}	013-GUML-123	11.69^{k-r}	017-TOMS-022	12.19^{qr}
049-SAMD-032	10.47^{c-i}	009-SOML-083	10.92^{e-o}	114-KOMK-173	11.29^{g-r}	139-NGBE-141	11.83^{l-r}	118-KAMK-212	12.33^{r}

highest width value (7.71 ± 1.86 µm) (Figure 6). Multiple scope tests matched 16 homogeneous groups.

Spatial arrangement of measured parameters

The principal component analysis (PCA) made it possible to visualize that there are differences between the observed and measured characteristics: colony color (Cco), mycelial shape (Fmy), acervulean abundance (Aac), saltations (Sal), growth rate (Vcr), conidia length, conidia width (Figure 7). However, the representation of isolates studied following the evaluated traits shows that the distribution of these isolates is diversified in the Sudano-Sahelian zone. Thus, the isolates analyzed in the F1 × F2 biplot are visible at 47.15% and, yet 23.95% and 23.95% in the F1 and F2 axes respectively (Figure 8). Thus, there are ten (10) clouds grouping the isolates of said characters. Such a result shows that these traits are very important for discriminating against the *C. capsici isolates* studied. The traits used for the study were used by several scientists to characterize *Colletotrichum* spp. isolates, such as Smith and Black [25], Appiah-kubi *et al.* [26].

The first cloud shows that isolates 135-MABE-101, 138-NGBE-133, 139-NGBE-141, 042-MOMT-171, 009-SOML-083, 010-SOML-092, 015-SOML-143, 049-SAMD-032, 052-DIMD-062, 122-LAMK-253, 123-LAMK-263, 111-LAMK-193 are related by mycelial shape, saltations, colony colour and conidia width.

The 2nd cloud reveals that isolates 026-MAMT-011, 028-MAMT-031, 04-ZAMT-151, 017-TOMS-023, 021-TOMS-051, 013-GUML-123, 012-GUML-111, 011-GUML-101, 010-SOML-091, 047-SAMD-012, 057-TIMD-103, 049-SAMD-031, 048-SAMD021, 116-LAMK-191, 109-GUMK-121, 118-KAMK-212, 117-KAMK-203b, 097-GADI-231, 083-GRDI-091, 085-DODI-112b, 080-SADI-061, 090-MADI-151 and 094-GADI-201 are grouped around the abundance of acervulis, the rate of radial growth and conidia length.

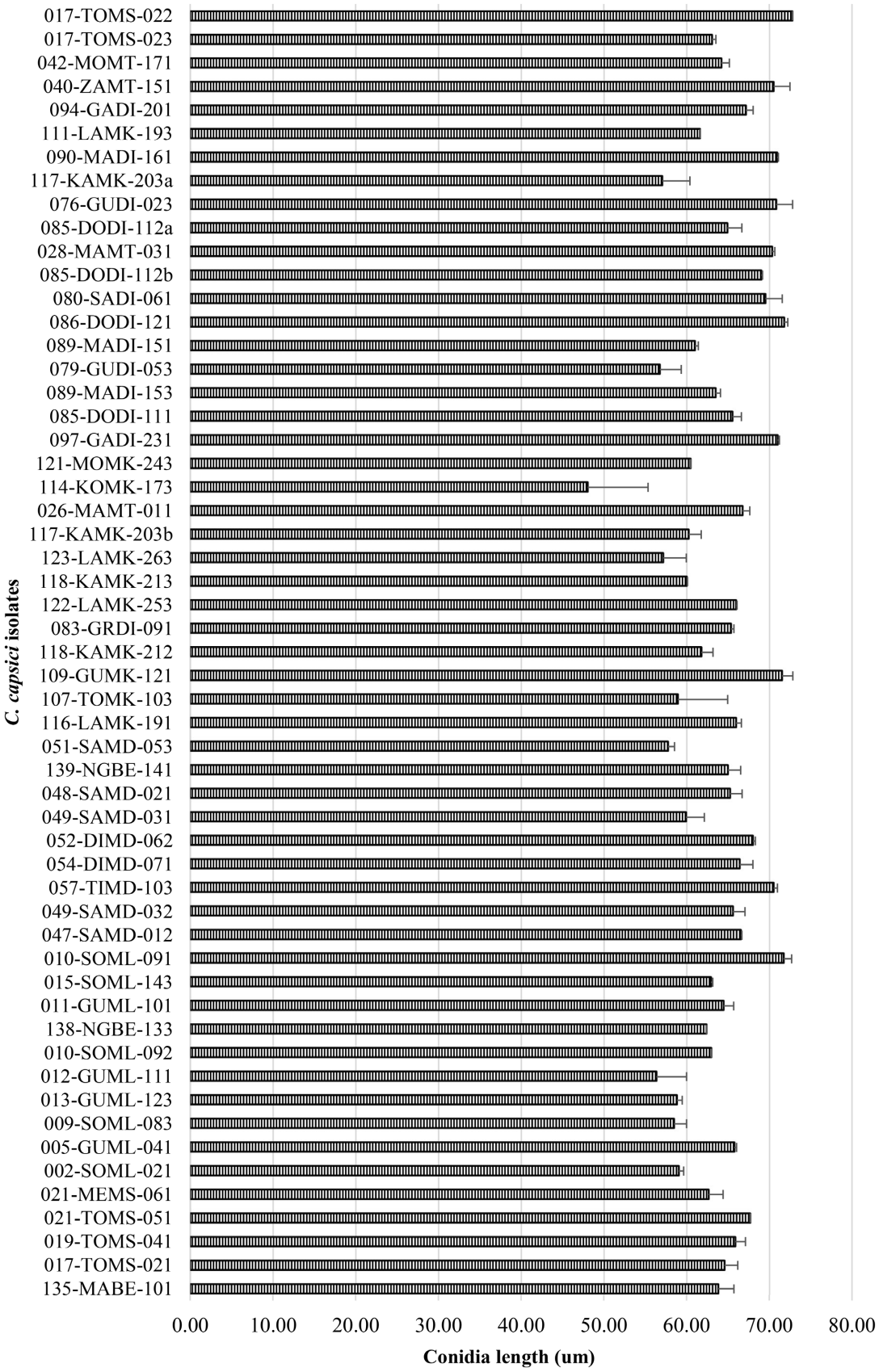


Figure 5. Conidia length.

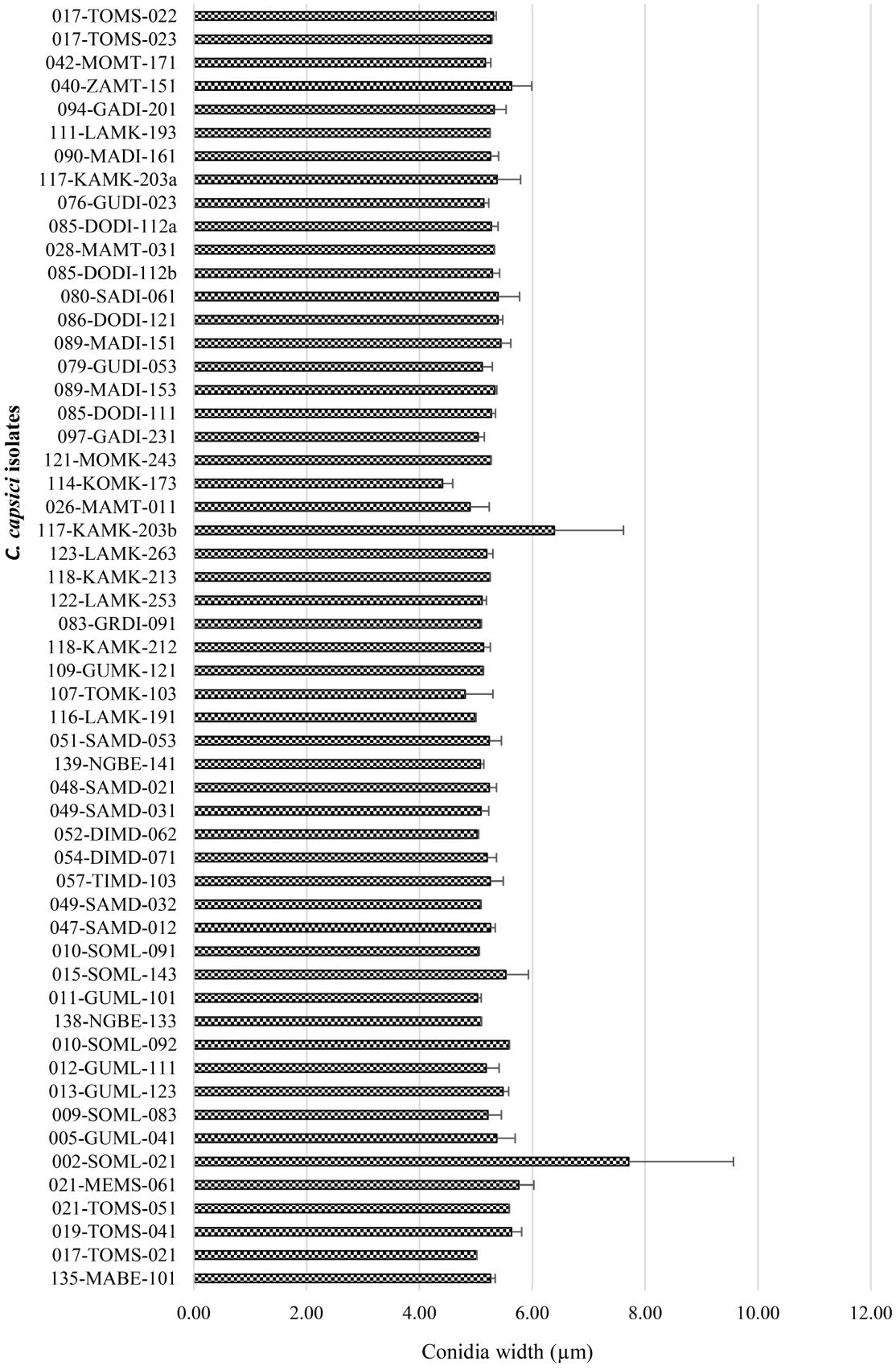
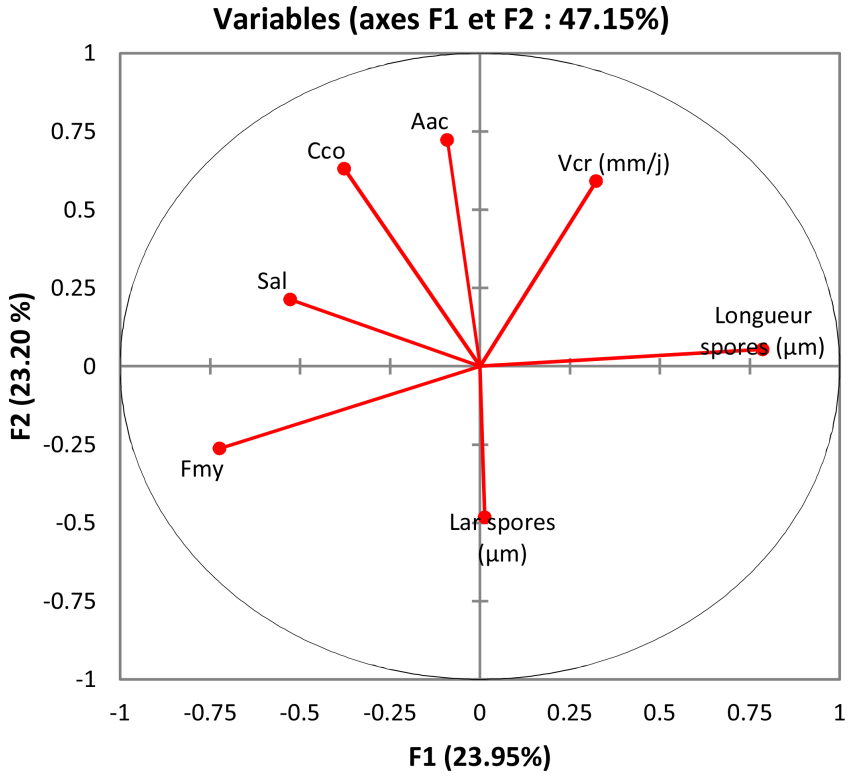


Figure 6. Conidia width.



Cco: Color of the colony; Aac: Abundance of acervules; Sal: Saltations, Fmy: Mycelium form; Vcr: Radial growth rate, Lar spores: Spore width; Long spores: Spore length.

Figure 7. Spatial arrangement of the measured parameters.

The 3rd cloud presents isolates 017-TOMS-22, 017-TOMS-021, 021-MEMS-061, 107-TOMK-103, 085-DODI-111, 076-GUDI-023, 086-DODI-121 and 085-DODI-112a clustering around both conidia length and width.

The fourth, fifth and sixth clouds reveal only one isolate each; including isolates 019-TOMS-041, 002-SOML-021 and 005-GUML-041. Isolates 002-SOML-021 and 005-GUML-041 are closer to conidia width while isolate 019-TOMS-041 is closer to acervulis abundance.

The seventh cloud shows that isolates 054-DIMD-071, 051-SAMD-053, 089-MADI-151, 079-GUDI-053 and 089-MADI-153 cluster around the mycelial shape.

The eighth, ninth and tenth clouds each reveal a single isolate including 117-KAMK-203a, 114-KOMK-173 and 121-MOMK-243 respectively. Isolate 114-KOMK-173 is closer to the colony color and presence of saltations; 117-KAMK-203a isolate is close to the presence of saltations while 121-MOMK-243 isolate is close to mycelial form.

The hierarchical bottom-up classification (**Figure 9**) revealed the existence of ten (10) classes of isolates including several morphotypes of *C. capsici*, thus confirming the previous principal component analysis. Morphotypes 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 comprise 23.64%, 41.82%, 14.55%, 1.82%, 1.82%, 1.82%, 9.09%, 1.82%, 1.82% and 1.82% of isolates, respectively.

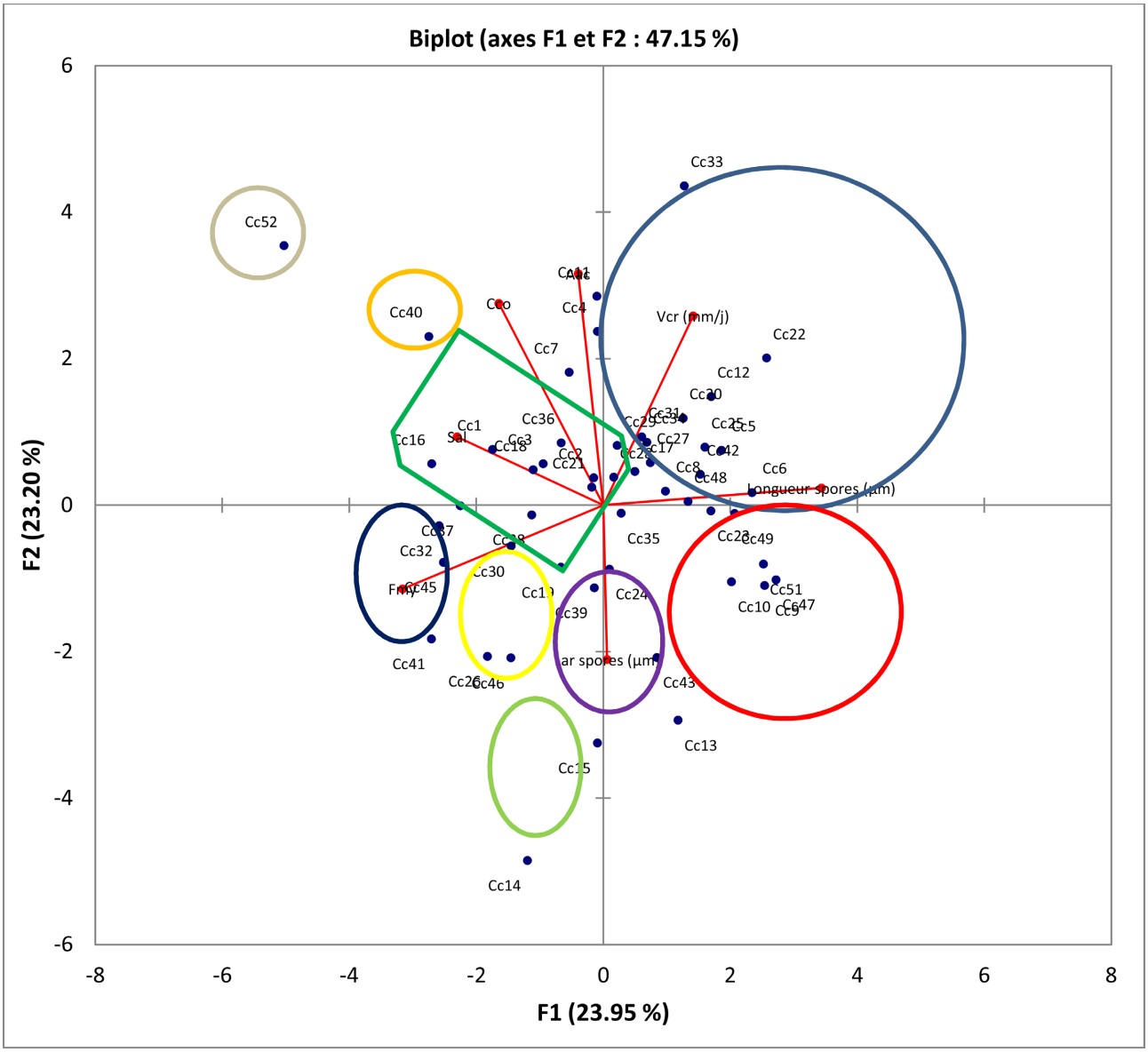


Figure 8. Spatial arrangement of isolates relative to parameters.

3.2. Discussions

After collection, 151 samples of plant organs were obtained. Of the 151 samples, 51 single spores isolates were isolated. These results show that isolates could not be identified and isolated at each collection site. However, Thio's work (2016) [1] shows that at least one isolate has been identified in each of the collection sites.

From the same work, 44.44% of isolates were identified from the stem organ, 8.33% from the leaf organ and 47.22% from the pod organ compared to 50.92%, 14.3% and 34.55% respectively from the stem, leaf and pod organs in the present study. However, it should be noted that both in this study and in the other the high percentage of isolate comes from the stem organ, followed by the pod organ.

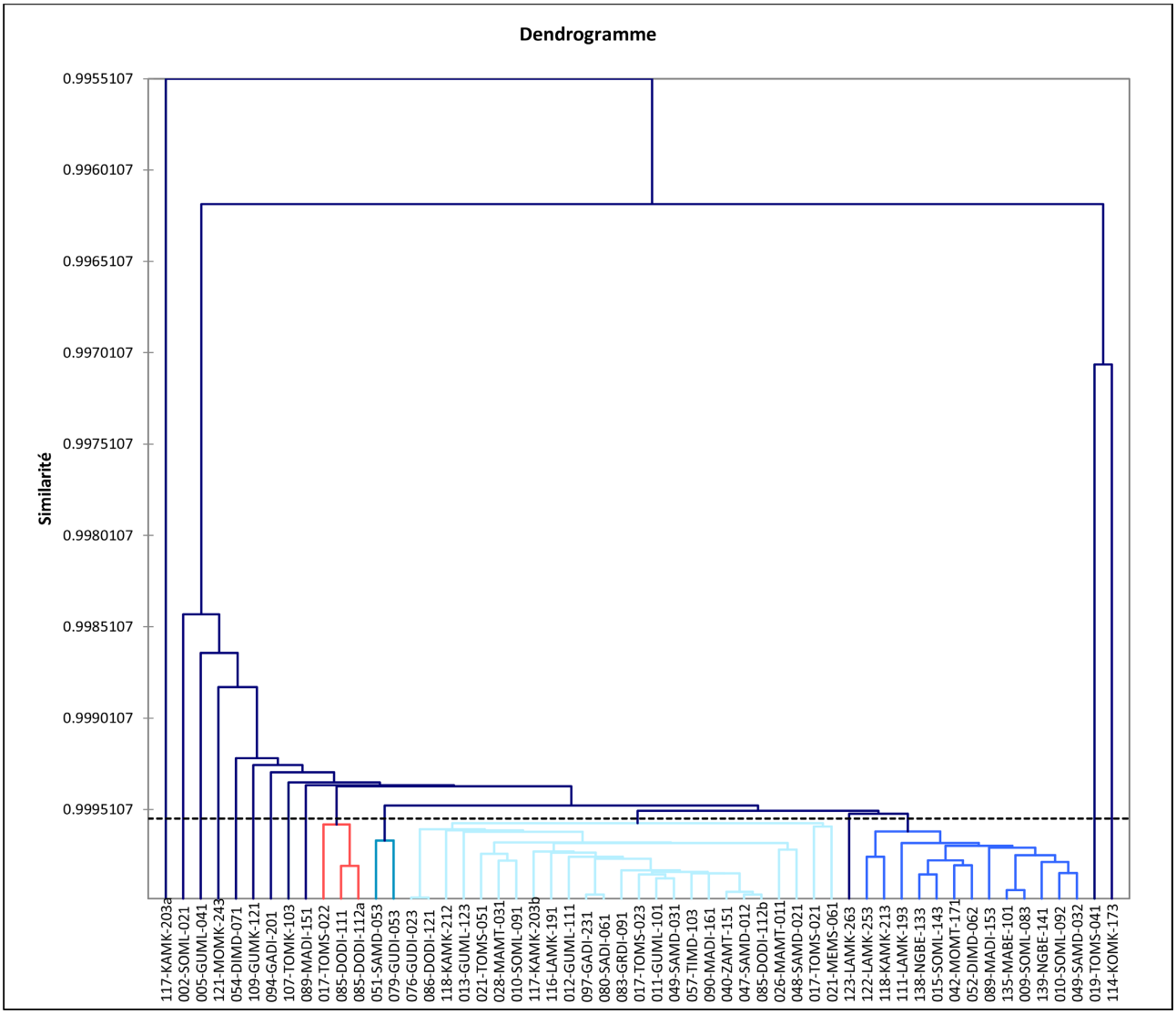


Figure 9. Hierarchical bottom-up classification of Colletotrichum isolates.

This could be explained by the fact that *Colletotrichum capsici* isolates come more from stems [25]. Also, the infectious process is highly developed during flowering and fruit formation [27].

The results of this study indicate that there are various colony colors of *Colletotrichum capsici*: black, brown-black, brown-orange, brown, black, gray colors. These colors are characteristic of *C. capsici* and C. gloeosporiodes [28] [12].

This colour variation could be related either to the host plant or to the nature of the isolate or the environmental conditions of the isolate. The identification of Colletotrichum species is based on the morphocultural characteristics coupled with the origins of the isolates. However, several isolates of Colletotrichum show great variations in culture. [29].

Of the identified *C. capsici* isolates, 56.36% have abundant acervulis, which is much lower than the proportion (90%) observed by Thio (2018) [30]. In addition, Sereme [2] indicated that the abundance of acervulis is a discriminating

character for the characterization of *C. capsici*.

Various forms of the mycelial have been noted such as compact, cottony, airy... These results corroborate those obtained by Ehui *et al.* [12].

The differences established between isolates by the different parameters confirm their use by several scientists including Ehui *et al.* [12]; Appiah *et al.* [26]; Sereme [2] characterized *Colletotrichum spp* isolates using these parameters. The revelation of several homogeneous groups of isolates indicates that there is a morpho-metric diversity of *Colletotrichum capsici* in the Sudano-Sahelian zone of Cameroon.

The average growth diameter on the sixth day is 66.23 mm and smaller than that (68 mm) obtained by Sereme [2] and the average daily growth rate is 11.03 mm/day. These values are higher than those obtained by Ehui *et al.* [12] during the characterization of *C. gloeosporiodes* Penz manihotis causative agent of cassava anthracnose.

During his study, the average diameter was 65.40 mm and the radial growth rate was 9.20 mm/day. The average value of the growth diameter is higher than that obtained (57.87 mm) by Kuete *et al.* [17] when the radial growth rate is higher than that obtained by the same author (12.39 mm/day).

The conidia dimensions (length and width) observed in *C. capsici* isolates are not similar to those reported in other Colletrotrichum [18] [19], Appiah *et al.* [26] and similar to those reported in other Colletotrichum and fungal species Sereme [2] [12] [22].

Indeed, conidia lengths and widths ranged from 48.10 to 72.79 µm and 4.41 µm and 7.71 µm respectively. These values do not fall within the range described by Yun *et al.* [31]; who demonstrated that *C. capsici* conidia had dimensions ranging from 13.21 µm to 16.21 µm for length and from 1.79 to 3.28 µm for width.

4. Conclusion

Morphological and biometric variability could be preliminary for characterizing *Colletotrichum capsici*. With diversity in morphological characters, conidial measurements, radial growth, mycelial colour in culture, presence or absence of saltations, it's obvious that there are several morphotypes of isolates of *Colletotrichum capsici*. Also, the similarities shown among some of the isolates from different divisions of sudano-sahelian zone of Cameroon suggest a common origin for some of them.

5. Recommendation

Following the results obtained from this study, it will be wise to evaluate the pathogenicity of this diversity of isolates and to conduct their Biomolecular characterization.

Acknowledgements

The authors are thankful the Kirkhouse Trust Project for the material support

provided for this work.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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