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Research article

Cultural and morphological characteristics of *Colletotrichum sublineolum* isolates infecting sorghum in eastern Ethiopia

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ABSTRACT

Colletotrichum sublineolum is the most destructive pathogen causing sorghum anthracnose worldwide. The fungus is known to have highly variable pathotypes. A characteristic study of pathogen is important to document the change occurring in population as variability in morphology indicates the existence of different pathotypes. Controlled condition experiment was conducted to examine cultural and morphological characteristics of *C. sublineolum* isolates infecting sorghum in eastern Ethiopia. Sorghum leaves showing symptoms of anthracnose were collected from five districts through survey. To study the characteristics of *C. sublineolum*, single-spore isolates representing isolate collection districts were selected from the stock cultures and cultivated on potato dextrose agar. Culture growth, colony color, elevations, texture and margin, conidial diameter and shape were used to characterize isolates. The isolates were varied significantly in many aspects. Colony colors were differed from light-gray to gray, purple-gray to cottony-gray, white to salmon-whit, plum-pink to beige and rosy brown on upper side of the petri dishes. The mean culture growth of *C. sublineolum* isolates showed highly significant ($P < 0.01$) variations among each other and ranged from 15 to 44 mm eight-days after incubation. Most of the isolates were produced hyaline, smooth walled, falcate conidia but without septa. Conidial diameter of *C. sublineolum* isolates showed variations with width and length ranged from 2.97 to 6.01 μm and 10.01–27.75 μm, respectively. Most isolates had smooth colony margin and few had undulated margin. This finding revealed that substantial variations were observed among *C. sublineolum* isolates and the existence of variable characteristic showed the presence of several sub-species of the pathogen infecting sorghum in different agro-ecologies of eastern Ethiopia.

1. Introduction

Anthracnose, caused by *C. sublineolum*, is one of the most destructive diseases of sorghum (*Sorghum bicolor* L. Moench), especially in warm and humid areas of the world [1]. Sorghum anthracnose is among serious diseases in all sorghum growing areas of Ethiopia [2]. It is also highly prevalent and very severe disease of sorghum in eastern Ethiopia [3]. *C. sublineolum* is the only *colletotrichum* species that has been confirmed to cause foliar, stem, panicle and grain anthracnose on sorghum [4]. The foliar infection occurs at any stage of plant growth and may cause yield losses from 20% up to 80% [5,6]. The primary mode of reproduction is by means of lunate to falcate

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hyaline conidia, which are produced on sickle-shaped conidiophores [7] called falcate conidia [8].

C. sublineolum is considered to be a very heterogeneous species, primarily based on the large number of pathotypes that have been described based on deferential virulence to host [9]. Isolates of *C. sublineolum* can vary considerably in morphology based on different conditions [10,11]. Many resistant cultivars have been developed but lose their resistance due to changes in the virulence patterns and pathogenicity of the *Colletotrichum* pathotypes [12]. Most studies have indicated the presence of many physiological races of *Colletotrichum* strains infecting sorghum [13]. More than 40 pathotypes have been reported from different geographical areas of the world [5,10,14]. Based on morphological and cultural characteristics [10], identified 9 morphological groups from 50 isolates collected from major sorghum producing areas in Nigeria [15]. also found five morphological groups within five isolates gathered from various infected plant parts. Moreover [16], were reported 22 pathotypes from 37 isolates examined. In addition [17], established 13 new pathotypes from 87 isolates collected from Arkansas.

Significant intra-population variation in colony growth, pigmentation and conidial size can occur within isolates sub-cultured from a colony initiated from a single conidium. Some cultures from a single conidium form distinct mycelial sectors that differ genetically from the original isolate [18]. However, morphological variation is independent of pathogenic or genetic variation [19]. Colonies are usually shades of gray interspersed with moist patches when sporulation occurs [20]. The conidiophores, produced in great quantity inside the acervuli are erect and hyaline, but without septa. The falcate conidia are produced in dark-colored acervuli, which occur in the center of the lesions on host tissue and *in vitro* cultures [21]. The falcate conidia of *C. sublineolum* isolates (18.5–27.5 x 3–4.5 μm) are tapering at both ends [20].

Variability studies are important to document the changes occurring in populations and individuals as variability in morphological and physiological traits indicate the existence of different pathotypes [22,23]. Studies of *C. sublineolum* isolates collected at different time and location generate important knowledge and help to trace the origins of disease outbreaks locally and globally [24]. Such knowledge could also aid understanding of the epidemiology of anthracnose disease. In addition, to devise effective management strategies of sorghum anthracnose, understanding the cultural and morphological characteristics of the pathogenic organism is very essential; and it is also an important input in resistance breeding development programs. Therefore, this study was aimed to determine morphological and cultural variability of *C. sublineolum* isolates infecting sorghum in eastern Ethiopia.

2. Materials and methods

2.1. Description of sample collection areas

Sample collection was conducted in five major sorghum growing districts (Haramaya, Babile, Girawa, Tullo and Odabultum) of eastern Ethiopia during the 2017 main cropping season. The districts differed in their ecological features. Haramaya, Babile, and Girawa districts were found in eastern Hararghe, while Tullo and Odabultum districts were located in western Hararghe, eastern Ethiopia. They are located between 40°77' to 42°32' E and 8°90' to 9°90' N, with elevations ranging from 1405 to 2481 m above sea level.

2.2. Sample collection

Sorghum leaves showing typical symptoms of anthracnose were collected from five major sorghum growing districts of eastern Ethiopia. During sampling survey, a total of 125 sorghum fields (25 fields per district) were considered. Based on the importance of the crop, five representative peasant associations were purposively selected from each district. The collection sites represented different geographic locations at varying elevation and climatic condition. Five farmers field located within 5–10 km interval were sampled per peasant associations and five symptomatic sorghum leaves were collected from each field. Specimens were pressed and packed in paper bags, labeled with all required information and transported to Haramaya University Plant Pathology Laboratory for isolation, identification and characterization of the causal organism of sorghum anthracnose.

2.3. Isolation and identification of C. sublineolum

Samples of sorghum leaves with typical anthracnose symptoms were cut into pieces, surface disinfected in 5% sodium hypochlorite solution for 3 min and rinsed in sterilized distilled water. The surface-sterilized leaf pieces were allowed to dry in laminar flow hood before plating. Then the specimens placed on oatmeal agar (OMA) amended with antibiotics (streptomycin) and incubated at 25 °C for 7 days. Developing fungal colonies were sub-cultured on potato dextrose agar (PDA) plates. Pure cultures of more than 120 single-spore isolates representing different geographic locations were found and these cultures were maintained in PDA slants at 4 °C for further studies as described by Ref. [25]. The macroscopic and microscopic characteristics of *C. sublineolum* isolates were studied based on cultural and morphological features.

2.4. Cultural and morphological characteristics

To study the cultural characteristics of *C. sublineolum* isolates, a total of 25 single-spore isolates representing isolate collection districts were randomly selected from the stock cultures and cultivated on PDA at 25 °C. After 5 days of incubation, 6 mm mycelia plugs were taken from the actively growing edge of each isolate and transferred to the center of a PDA plate, and again incubated at 25 °C. The Petri plates were arranged in completely randomized design (CRD) with three replications. Radial growths of each isolate were

estimated from colony diameter measurements (mm) taken at two perpendicular planes on the reverse side of the plate. Radial growth (mm day^{-1}) of isolates were recorded at 24 h intervals consecutively for seven days [25,26]. In addition, Colony texture, colony elevation, upper and reverse side colony color and colony margins were recorded. The colony color was described using RGB (red, green and blue) color chart [27].

The conidial size (length and width) and shape were assessed under light microscope at eyepiece lens magnification of ×10 and objective lens magnification of ×40 (mg = ×400) [28]. The conidial diameter [length and width (μm)] of isolates were measured using ocular micrometer. The average size of 30 conidia was calculated per isolate to determine conidial size of *C. sublineolum* isolates.

2.5. Pathogenicity test

In order to prove Koch's postulate, pathogenicity test was carried out on detached and intact sorghum leaves.

2.6. Pathogenicity test using detached leaf

Five *C. sublineolum* isolates (GiJg1, HaDk3, TuOn1, BbAb2 and ObMk5) that represented the districts where isolates were collected was tested using detached sorghum leaves. The codes of these isolates were given from district, peasant association and field number, respectively. Five isolates (one isolate per district) were randomly selected from the stock cultures and grown on PDA at 25 °C. As described by Ref. [29], the suspension of conidia of each isolate was prepared by suspending mycelia scraped from 7 days old culture in sterile distilled water and stirred vigorously for 90 s and then filtered through two layer cheese cloth. The concentration of spore suspension was adjusted to 1×10^5 ml^{-1} by using haemacytometer before inoculation.

Healthy sorghum leaves were collected from sorghum field at Haramaya University crop research field. The leaves were washed and surface-sterilized using 5% sodium hypochlorite solution for 30 s and rinsed three times in sterile distilled water. The leaves were cut and placed in Petri dishes lined with four layers of sterilized and moisten tissue papers. The leaves were sprayed with spore suspensions of each isolate and **incubated** at 25 °C until typical symptoms of anthracnose were observed. Non-inoculated controls were treated with sterile-distilled water. The experiment was arranged in a completely randomized design (CRD) with three replications. Incubation period was recorded as days between inoculation and the appearance of the first symptoms of sorghum anthracnose. The causative agent in the diseased leaf parts was re-isolated and the characteristics were compared with original culture [29].

Table 1
Cultural characteristics of *C. sublineolum* isolates growing on potato dextrose agar and incubated at 25 °C for 10 days.

Isolate[a]	Colony color		Colony texture	Colony margin	Colony elevation
	Upper side	Reverse side			
GiJg4	Light-gray	Goldenrod	Fluffy	Smooth	Raised
GiJg1	White	Sandy-brown	Fluffy	Undulated	Raised
GiMg2	Beige	Dim-gray	Velvet	Smooth	Flat
GiMg5	Ivory-white	Dim-gray	Velvet	Smooth	Flat
GiEt5	Gray	Tan-brown	Velvet	Smooth	Flat
GiEt3	Light-gray	Goldenrod	Velvet	Smooth	Flat
GiHj2	Light-gray	Dim-gray	Velvet	Smooth	Flat
GiRj1	Gray	Tan-brown	Velvet	Undulated	Flat
GiHj4	Purple-gray	Tan-brown	Fluffy	Smooth	Raised
HaBg5	Light-gray	Light-goldenrod	Fluffy	Smooth	Flat
HaIo4	Gray	Saddle-brown	Velvet	Smooth	Flat
HaHa1	Purple-gray	Wheat-brown	Velvet	Smooth	Flat
HaDk3	Salmon-white	Burly-wood	Velvet	Undulated	Flat
HaHa4	Plum-pink	Wheat-brown	Velvet	Undulated	Flat
HaIo1	Gray	Dim-gray	Velvet	Smooth	Flat
TuKk4	Light-gray	Dim-gray	Fluffy	Undulated	Flat
TuHl2	Light-gray	Saddle-brown	Velvet	Undulated	Flat
TuOn1	Gray	Light-yellow	Velvet	Undulated	Flat
TuHm1	Light-gray	Tan-brown	Fluffy	Smooth	Raised
ObMk5	Gray	Saddle-brown	Velvet	Smooth	Flat
ObOs3	Beige	Light-goldenrod	Velvet	Smooth	Raised
ObOr4	White	Saddle-brown	Fluffy	Smooth	Raised
BbAb2	Light-gray	Light-yellow	Fluffy	Smooth	Raised
BbTl2	Rosy-brown	Sandy-brown	Velvet	Smooth	Flat
BbIf4	Cottony-gray	Goldenrod	Fluffy	Smooth	Raised

[a] **Gi, Ha, Tu, Ob and Ba** = Isolates collected from Girawa, Haramaya, Tullo, Odabultum and Babile districts of different peasant associations, respectively. Names of peasant associations in listed representative isolates include: Jg = Jirugemechu, Mg = Meyragudina, Hj = Hulajeneta, ET = Ejersatobota, Rj = Rassajeneta, Bg = Biftugeda, Io = Ifaoromia, Ha = Harroadi, Dk = Direkebso, Kk = Kufakas, Hl = Hundelafto, On = Odanegaya, Hm = Hakamulis, Mk = Mekanisa, Os = Odabaso, Or = Odaroba, Ab = Abdiboch, Tl = Tulla, If = Ifaa.

2.7. Pathogenicity test under greenhouse conditions

Pathogenicity of five representative isolates of *C. sublineolum* was tested in the greenhouse at Rare, Haramaya University. The experiment was conducted using universally susceptible sorghum cultivar BTX-623 which was maintained by Bako Agricultural Research Center. Seeds of cultivar BTX-623 were surface disinfected with 70% ethanol and rinsed with sterile distilled water. Seeds were planted in plastic pots filled with autoclave-sterilized media mixture (black soil: sand: farmyard manure in a ratio of 3:2:2 volume) [30] and kept in the greenhouse. The treatment was arranged in a completely randomized design (CRD) with four replications. The experiment had 6 treatments (five isolates X BTX-623) and one control (non-inoculated seeds).

The suspension of conidia of each isolate was prepared by suspending mycelia scraped from 7 days old culture (section 2.5.1). The three week old plants were spray-inoculated with a conidial suspension of each isolate using a hand sprayer. Observations were made regularly for the appearance of sorghum anthracnose symptoms. Data, starting from two days after inoculation, was recorded for incubation period (time in days from inoculation to appearance of first necrotic lesion).

2.8. Data analysis

Colony diameter (mm) and conidial size (μm) data of *C. sublineolum* isolates were subjected to analysis of variance using SAS software version 9.1. For treatments having significant differences, LSD test at $P<5\%$ probability level was used for mean comparisons among treatments.

3. Results

3.1. Cultural characteristics

The cultural characteristics of *C. sublineolum* isolates showed variation in colony color, margin, radial growth, elevations and textures (Table 1, Fig. 1). Ten different types of upper side colony color were obtained from the 25 representative *C. sublineolum* isolates evaluated. Most (68%) of the isolates had upper side gray colony color. These gray colony colors also varied from light-gray to gray, and purple-gray to cottony-gray. White, ivory-white, salmon-whit, plum-pink, beige and rosy-brown upper side colony colors were also observed on the other isolates. On the other hand, isolates had dim-gray, saddle-brawn to sandy-brown, tan-brown to wheat-brown, goldenrod to light-goldenrod and light-yellow colors when viewed on the reverse side of the plates (Table 1).

The isolates showed marked variation in colony margin, texture and elevation when grown on PDA. From the 25 *C. sublineolum*

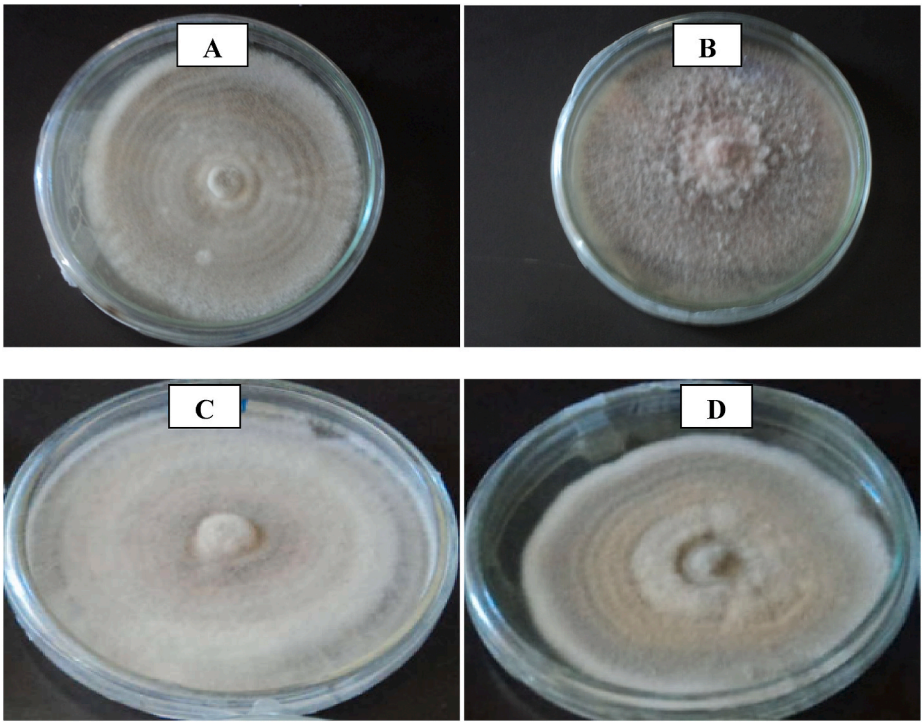

Fig. 1. Cultural characteristic variations of *C. sublineolum* isolates collected from Girawa district of ejersatobota **(A)**, Girawa district of hulajeneta **(B)**, Tullo district of hakamulis **(C)** and Haramaya district of ifaoromia **(D)** farmers association, Eastern Ethiopia, grown on potato dextrose agar at 25 °C for 10 days.

isolates, 72% of the isolates had smooth colony margins and only 28% of the isolates had undulated colony margins. The isolates also showed variations in colony elevations among the collection sites. Seventeen isolates had flat colony elevation, while the remaining eight isolates were characterized by raised colony elevation. Colony textures observed among isolates were velvet and fluffy types. Velvety texture was noted in 16 isolates, while the other nine isolates showed fluffy texture (Table 1, Fig. 1). These variations in colony margin, elevations and textures clearly showed that how much this pathogen was variable in different sorghum growing areas of eastern Ethiopia.

The mean radial growth of *C. sublineolum* isolates on PDA during eight-days of incubation at 25 °C showed highly significant ($P < 0.01$) variation among each other (Table 2). The colony diameter ranged from 15 to 44 mm at eight-day incubation period. Isolates TuHm1, GiHj4 and BbIf4 showed the highest radial colony growth of 43.5, 41.33 and 40 mm, respectively. Conversely, isolates GiHj2, HaHa4, HaIo4 and HaHa1 showed the lowest radial growth of 15, 15.17, 15.83 and 16.75 mm, respectively (Table 2). The variations in culture growth of the isolates could be due to its heterogeneous nature of the pathogen, among other factors.

3.2. Morphological characteristics

Majority (60%) of *C. sublineolum* isolates infecting sorghum had falcate shaped conidia, while some isolates had variable conidial shapes ranging from cylindrical (24%) to oval (16%) shapes (Table 3, Fig. 2). Isolates produced hyaline and smooth-walled conidia without septa when it was grown on PDA. *C. sublineolum* isolates showed pointed and rounded apices. The variations could implied that *C. sublineolum* have different pathotypes that can cause anthracnose on sorghum in eastern Ethiopia.

Differences in conidial dimensions were also exhibited by *C. sublineolum* isolates when grown on PDA at 25 °C. The width and length of conidia were ranged from 2.97 to 6.01 μm and 10.01–27.75 μm, respectively. The highest (27.75 μm) mean conidial length was recorded from isolate GiMg2, followed by ObMk5 (27.70 μm) and GiJg1 (27.05 μm). The lowest (10.01 μm) conidial length was recorded from isolate GiEt3. On the other hand, the widest conidial width (6.01 μm) was measured on GiJg1 isolate, followed by ObMk5 (5.82 μm) and TuHl (5.52 μm). On the contrary, the thinnest conidial width (2.97 and 2.98 μm) was measured on BbAb2 and HaDk3 isolates, respectively (Table 3).

Generally, there were many variations in sizes and shapes of conidia among *C. sublineolum* isolates tested. The morphological variations among isolates could depend on variations with sorghum genotypes and weather conditions where the crop was grown. The results clearly showed that there was variation among *C. sublineolum* isolates under different geographic areas of sorghum production

Table 2
Mean colony diameter (mm) of *C. sublineolum* isolates grown on potato dextrose agar incubated at 25 °C for eight days.

Isolate[a]	Colony diameter (mm) at different days after inoculation and incubation						
	2 days	3 days	4 days	5 days	6 days	7 days	8 days
GiJg4	3.67^{k}	5.67^{g}	9.17^{l}	12.42^{j}	16.33^{jk}	18.75^{l}	20.50^{k}
GiJg1	9.50^{abc}	13.17^{bc}	16.67^{bcde}	19.00^{fgh}	23.33^{ef}	26.92^{fgh}	30.12^{efgh}
GiMg2	4.33^{k}	11.33^{de}	13.50^{ij}	18.50^{gh}	21.67^{fgh}	28.17^{ef}	29.67^{fghi}
GiMg5	9.17^{bcd}	12.00^{cd}	15.50^{efgh}	18.50^{gh}	22.17^{efgh}	25.00^{hij}	29.12^{fghi}
GiEt5	7.67^{fghi}	10.67^{ef}	14.83^{h}	18.00^{h}	22.00^{efgh}	25.83^{ghi}	30.00^{efgh}
GiEt3	9.17^{bcd}	13.50^{b}	16.08^{defg}	18.00^{h}	20.50^{hi}	24.83^{hijk}	28.33^{ghi}
GiHj2	2.25^{l}	4.33^{h}	5.67^{m}	8.83^{k}	11.50^{l}	12.33^{n}	15.00^{l}
GiRj1	7.83^{efgh}	12.00^{cd}	15.33^{gh}	18.83^{gh}	21.83^{fgh}	26.83^{fgh}	30.83^{defg}
GiHj4	6.00^{j}	12.00^{cd}	17.00^{bcd}	23.50^{c}	29.50^{b}	34.17^{c}	41.33^{ab}
HaBg5	8.83^{cde}	13.50^{b}	17.50^{bc}	23.00^{cd}	27.00^{c}	30.67^{d}	35.00^{c}
HaIo4	1.75^{l}	4.75^{gh}	5.50^{m}	8.83^{k}	12.50^{l}	13.00^{mn}	15.83^{l}
HaHa1	8.33^{def}	10.83^{def}	11.83^{k}	12.33^{j}	13.00^{l}	15.17^{m}	16.75^{l}
HaDk3	10.50^{a}	13.83^{b}	17.83^{b}	20.00^{efg}	24.17^{de}	28.00^{efg}	31.83^{def}
HaHa4	7.33^{fghi}	10.83^{def}	11.08^{k}	12.00^{j}	13.00^{l}	14.08^{mn}	15.17^{l}
HaIo1	7.33^{fghi}	10.83^{def}	13.17^{j}	15.33^{i}	18.42^{ij}	22.67^{k}	26.58^{ij}
TuKk4	2.17^{l}	5.67^{g}	6.33^{m}	11.67^{j}	15.67^{k}	19.83^{l}	25.00^{j}
TuHl2	7.00^{hji}	11.17^{de}	15.17^{gh}	18.00^{h}	20.50^{hi}	23.17^{jk}	26.50^{ij}
TuOn1	8.17^{def}	12.00^{cd}	14.67^{hi}	18.00^{h}	21.00^{gh}	23.83^{ijk}	30.67^{defgh}
TuHm1	10.17^{ab}	15.17^{a}	22.00^{a}	27.83^{a}	34.00^{a}	41.17^{a}	43.50^{a}
ObMk5	8.13^{defg}	11.00^{def}	15.42^{fgh}	19.75^{fg}	23.08^{efg}	25.25^{hij}	27.58^{hij}
ObOs3	7.42^{fghi}	10.67^{ef}	16.08^{defg}	20.58^{ef}	26.08^{cd}	29.92^{de}	32.50^{cde}
ObOr4	6.83^{hij}	10.58^{ef}	14.58^{hi}	19.08^{fgh}	23.50^{ef}	26.83^{fgh}	30.33^{efgh}
BbAb2	$7.08g^{hi}$	10.83^{def}	16.33^{cdefg}	19.67^{fgh}	23.83^{def}	26.75^{fgh}	30.00^{efgh}
BbTl2	6.67^{ij}	9.92^{f}	16.58^{cdef}	21.50^{de}	26.08^{cd}	30.08^{de}	33.50^{cd}
BbIf4	10.33^{a}	13.50^{b}	21.00^{a}	26.00^{b}	33.00^{a}	37.67^{b}	40.00^{b}
Mean	7.11	10.79	14.35	17.97	21.75	25.24	28.59
CV (%)	9.26	6.86	5.06	5.72	6.36	5.57	6.59

[a] **Gi, Ha, Tu, Ob and Ba** = Isolates collected from Girawa, Haramaya, Tullo, Odabultum and Babile districts of different peasant associations, respectively. Names of peasant associations in listed representative isolates include: Jg = Jirugemechu, Mg = Meyragudina, Hj = Hulajeneta, ET = Ejersatobota, Rj = Rassajeneta, Bg = Biftugeda, Io = Ifaoromia, Ha = Harroadi, Dk = Direkebso, Kk = Kufakas, Hl = Hundelafto, On = Odanegaya, Hm = Hakamulis, Mk = Mekanisa, Os = Odabaso, Or = Odaroba, Ab = Abdiboch, Tl = Tulla, If = Ifaa. Mean values with the same letter(s) within a column are not significantly different from each other at 5% probability level of significance.

Table 3
Conidial shape and dimension of *C. sublineolum* isolates grown on **potato dextrose agar** incubated at 25 °C for 10 days.

Isolate[a]	Conidial shape	Dimension of conidia (mean ± SD)[b]			
		Conidial length (μm)		Conidial width (μm)	
		Mean	Range	Mean	Range
GiJg4	Falcate	23.28 ± 3.91	16.20–32.40	3.96 ± 1.19	2.70–5.45
GiJg1	Falcate	27.05 ± 3.20	21.60–35.10	6.01 ± 1.33	4.05–8.10
GiMg2	Falcate	27.75 ± 1.77	27.00–32.40	5.40 ± 0.88	4.05–8.10
GiMg5	Falcate	23.09 ± 3.35	16.20–29.70	3.86 ± 1.24	2.70–5.40
GiEt5	Falcate	26.44 ± 2.89	21.60–32.40	4.14 ± 0.95	2.70–5.40
GiEt3	Oval	10.01 ± 2.41	5.40–13.50	3.86 ± 1.34	2.70–8.10
GiHj2	Falcate	26.54 ± 1.76	25.65–32.40	5.40 ± 0.88	2.70–8.10
GiRj1	Falcate	18.67 ± 6.06	10.80–35.10	5.12 ± 1.37	2.70–8.10
GiHj4	Cylindrical	11.64 ± 2.51	8.10–18.90	4.28 ± 1.20	2.70–5.40
HaBg5	Falcate	26.21 ± 3.98	18.90–35.10	3.03 ± 1.26	1.89–5.40
HaIo4	Falcate	20.20 ± 5.08	2.70–29.70	3.49 ± 1.12	2.70–5.40
HaHa1	Falcate	26.00 ± 3.82	21.60–35.10	3.49 ± 1.11	2.70–5.40
HaDk3	Falcate	26.13 ± 3.73	3.73–35.10	2.98 ± 0.98	2.16–5.40
HaHa4	Falcate	13.22 ± 3.33	5.40–21.60	5.12 ± 1.46	2.70–8.10
HaIo1	Cylindrical	10.71 ± 2.45	8.10–16.20	3.45 ± 0.99	2.70–5.40
TuKk4	Cylindrical	10.89 ± 2.75	5.40–16.20	3.77 ± 1.46	2.70–8.10
TuHl2	Falcate	13.04 ± 2.40	8.10–18.90	5.52 ± 1.66	2.70–8.10
TuOn1	Oval	11.36 ± 2.33	8.10–16.20	3.72 ± 1.12	2.70–5.40
TuHm1	Cylindrical	10.52 ± 1.96	8.10–13.50	3.77 ± 1.42	2.70–8.10
ObMk5	Falcate	27.70 ± 4.08	18.90–35.10	5.82 ± 1.36	2.70–8.10
ObOs3	Cylindrical	11.36 ± 1.96	8.10–13.50	3.59 ± 1.16	2.70–5.40
ObOr4	Oval	11.73 ± 2.91	8.10–18.90	3.62 ± 1.08	2.70–5.40
BbAb2	Falcate	26.12 ± 3.19	21.60–35.10	2.97 ± 0.63	2.16–4.05
BbTl2	Cylindrical	10.80 ± 1.91	8.10–13.50	5.31 ± 1.39	2.70–8.10
BbIf4	Oval	10.89 ± 1.97	8.10–13.50	3.63 ± 1.03	2.70–5.40
Mean		18.45 ± 8.48		4.24 ± 1.55	

[a] **Gi, Ha, Tu, Ob and Ba** = Isolates collected from Girawa, Haramaya, Tullo, Odabultum and Babile districts of different peasant associations, respectively. Names of peasant associations in listed representative isolates include: Jg = Jirugemechu, Mg = Meyragudina, Hj = Hulajeneta, ET = Ejersatobota, Rj = Rassajeneta, Bg = Biftugeda, Io = Ifaoromia, Ha = Harroadi, Dk = Direkebso, Kk = Kufakas, Hl = Hundelafto, On = Odanegaya, Hm = Hakamulis, Mk = Mekanisa, Os = Odabaso, Or = Odaroba, Ab = Abdiboch, Tl = Tulla, If = Ifaa. [b]SD = Standard deviation.

in eastern Ethiopia.

3.3. Pathogenicity test using detached leaf

The inoculated detached leaves of sorghum showed typical anthracnose disease symptom similar to those symptoms observed under natural infection. Purple, red, circular to elliptical necrotic spots and tan or black spots with acervuli were the common symptoms observed on sorghum leaves. However, incubation periods varied among isolates on detached leaf test. The parts of the leaves that served as controls did not showed any symptoms.

3.4. Pathogenicity test under greenhouse

The results of the test revealed that all evaluated isolates caused typical sorghum anthracnose symptoms on the leaves of artificially inoculated BTX-623 sorghum variety; and the incubation period of the five *C. sublineolum* isolates were found variable. The shortest (5 days) incubation period was recorded on GiJg1 and HaDk3 isolates, while the longest (7 days) incubation period was recorded on BbAb2 isolate. Re-isolation of artificially inoculated sorghum leaves were results in the same pathogen and confirmed the pathogenic role of this organism.

4. Discussion

Isolates of *C. sublineolum* collected from different districts of sorghum growing areas of eastern Ethiopia showed considerable variation in cultural and morphological characteristics. The existences of different *C. sublineolum* feature could indicate the presence of several pathotypes (strains) of the pathogen infecting sorghum in different agro-ecologies. This might imply that *C. sublineolum* is a highly variable pathogen in sorghum growing areas of the country; which undermines crop breeders' efforts to develop resistant variety [10,11]. indicated that isolates of *C. sublineolum* from different locations, lesions, or even the single-conidial derivatives from single-lesion cultures can vary considerably in morphology [5]. also reported that significant variation in colony growth, pigmentation, and conidial size occurred within isolates sub-cultured from a colony initiated from a single conidium. The diversity within the species of *C. sublineolum* is large, as revealed by morphological characters and molecular markers, as well as by physiology, competitive ability and pathogenicity [21,31,32].

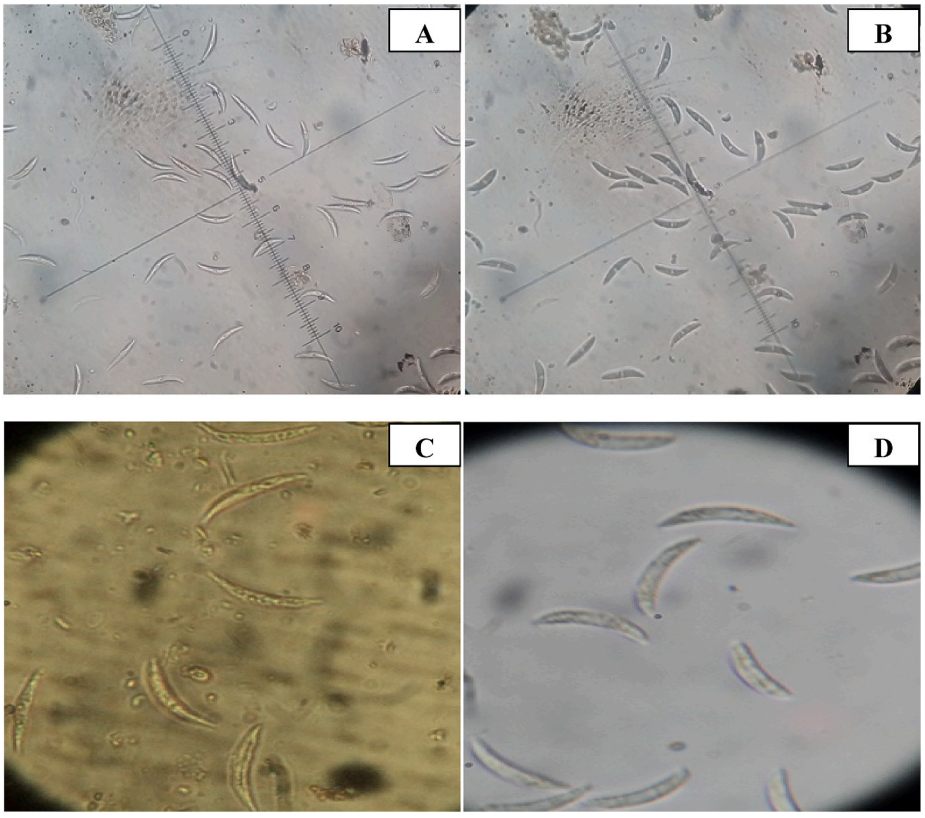

Fig. 2. Conidia of *C. sublineolum* isolates infecting sorghum at Girawa district of hulajeneta **(A)**, Babile district of abdiboch **(B)**, Girawa district of meyragudina **(C)** and Haramaya district of direkebso **(D)** farmers association, eastern Ethiopia observed under light microscope.

Cultural variations in colony color and margin among *C. sublineolum* isolates might be due to variable effects of environment and an attempt by the newly formed pathogen strains to overcome resistant varieties of sorghum. In line with this finding [33], reported that the presence of different *C. sublineolum* mycelial color on upper and reverse side may confirm the presence of newly evolving strains [25]. also reported that there were variations among *C. sublineolum* isolates in colony margin and color in Ethiopian *C. sublineolum* isolates [1]. Indicated that based on cultural variation, colony color of *C. sublineolum* isolates ranged **from** white to pink and dark-gray to clear-gray [26]. also reported that mycelia color of all the *C. sublineolum* isolates were gray but with varying color intensities and at the centers of colony, different colors ranging from black, gray, dirty white, cream and brown were observed.

In the current investigation, marked differences in radial growth were observed among the *C. sublineolum* isolates. Variations in radial growth of the isolates could be due to its heterogeneous nature of the pathogen. Similarly [1], reported different radial growth rates among *C. sublineolum* isolates [25]. also noted that radial mycelial growth varied considerably among isolates from different sampling sites and even between isolates of the same sampling site. Moreover [34], showed variations in colony growth of isolates within the *Colletotrichum* species.

Variable morphology characterizes anthracnose pathogen infecting sorghum [1,25,35]. The present study also confirmed the presence of different conidial morphology among *C. sublineolum* isolates studied. Morphological variations of conidia among isolates could depend on variations with sorghum genotypes and weather conditions, among other possible factors [25]. Indicated that morphological differences in conidial shape of *C. sublineolum* isolates obtained from two districts could be due to certain environmental conditions and sorghum genotypes, which play significant roles in genetic and physiological changes within the pathogenic strains. In addition [1], asserted that variation in conidial shape may be due to attempt by the pathogen to overcome panicle resistance to infection.

The *C. sublineolum* isolates obtained from different districts of eastern Ethiopia revealed variations in conidial sizes and produces falcate, oval and cylindrical shape [26]. also reported that from the same colony, there were many variations in sizes of conidia among the leaf isolates, and *C. sublineolum* isolates produced falcate and oval conidia [21]. Suggested that in solid culture media, falcate type of conidia was observed. In addition [20,21], reported that this pathogen can produce oval conidia. Moreover, the presence of many strains of *C. sublineolum* coincides with the pathogen-host evolution and differentiation theory which states that as the number of host species increases through breeding for resistant varieties of the same host, the pathogens of the same species feeding on these different hosts also change in their genetic characteristic after a given period of time [26].

Pathogenicity test was carried out for sorghum anthracnose (*C. sublineolum*) isolated from infected sorghum leaves collected at five districts of eastern Ethiopia. The symptoms observed under artificial conditions were similar to natural symptom of sorghum

anthracnose in the field. However, the tested *C. sublineolum* isolates exhibited variations in incubation period. **These differences showed** that the isolates varied in their genetic and physiological attributes. Related to the present study [30], found different incubation periods for *C. sublineolum* isolates evaluated against sorghum genotypes [13]. also observed symptom response variations from one species/strain of the *Colletotrichum* isolate to the other. These variations clearly indicated that *C. sublineolum* is variable in its nature throughout sorghum growing areas of the world.

5. Conclusions

C. sublineolum isolates collected from sorghum fields of eastern Ethiopia had great variations in cultural and morphological characteristics. Colony growths of sorghum anthracnose isolates showed significant variation among each other. The pathogen also exhibited many differences in sizes and shapes of conidia among tested isolates. In general, the isolates predominantly showed falcate conidia, light-gray colony color and smooth colony margin when grown on potato dextrose agar media. Findings of the study indicated that *C. sublineolum*, a causative agent of sorghum anthracnose, is a very heterogenouse pathogen. Such research data on the variability of *C. sublineolum* isolates could serve as baseline information on the future prospects of sorghum breeding program for anthracnose resistance in eastern Ethiopia. Hence, the breeding program of sorghum in Ethiopia should aim at developing anthracnose resistant genotypes against *C. sublineolum* isolates, along with a mixture of isolates to evaluate the disease reactions and to identify sorghum resistant genotypes. However, **characterization** of the pathogen population based on morphological and cultural technique is not adequate enough to properly distinguish different isolates of *C. sublineolum*. Since this technique influenced by incubation conditions, ages of culture, and also it is time taking and labor intensive activity, molecular techniques could overcome these limitations and characterize the genetic variability of fungal population much more precisely.

Author contribution statement

Girmay Aragaw; Alemayehu Chala; Habtamu Terefe: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

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