



Research Article

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Physiological Races of *Colletotrichum lindemuthianum* from South and Central Ethiopia

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Abstract

Bean anthracnose (*Colletotrichum lindemuthianum*) is one of the major diseases of common bean (*Phaseolus vulgaris*) in Ethiopia causing up to 63% yield loss. The current study was initiated with the objective to identify races of *C. lindemuthianum* prevailing in major bean producing areas in south and central Ethiopia. Thirty isolates of *C. lindemuthianum*, collected from Damot Gale, Halaba Special, Adama, Boset, Hawassa Zuria and Boricha woredas in 2017 were inoculated on 12 differential cultivars in greenhouse using Completely Randomized Design (CRD). Results revealed the presence of 17 physiological races (pathotypes), of which only three were previously reported from Ethiopia. Race 9 was the most dominant race across the surveyed areas. Four of the 17 races (3047, 2260, 2225 and 2073) were able to infect the highly resistant differential cultivar G2333 indicating that the Ethiopian *C. lindemuthianum* populations might be composed of highly virulent races. The differential cultivars Michelite, Mexico 222 and PI 207262 showed the most susceptible reaction to the tested races, while no differential cultivar was immune to the pathogen races. In conclusion, the current work demonstrated the existence of highly variable isolates of *C. lindemuthianum* that incite bean anthracnose in Ethiopia. The generated results of race identification would serve as important inputs for future development of host resistance against bean anthracnose in Ethiopia and elsewhere having similar agro-ecologies. Therefore, for the future race identification study need to be continued with more advanced techniques and by including more isolates across agro-ecology gradients.

Keywords

Bean anthracnose, *Colletotrichum lindemuthianum*, Differential cultivars, *Phaseolus vulgaris*, Physiological races, Ethiopia

Introduction

Bean anthracnose caused by *Colletotrichum lindemuthianum* (Sacc & Magnus) Briosi & Cavara, is one of the most important seed borne diseases of common bean in the world [1-3]. Depending on the cultivar and environmental conditions, anthracnose infections can drastically reduce crop yield [4]. Fernandez, et al. [5] reported that infection of susceptible cultivars, like Mexican-142 and Awash-1, under favorable environmental conditions leads to 100% grain yield loss. In Africa, the disease is common in Burundi, Democratic Republic of Congo, Ethiopia, Kenya, Rwanda, Tanzania and Uganda [6]. In Ethiopia it is a major production constraint in bean-growing regions [3], causing a yield loss of up to 63% [7].

Colletotrichum lindemuthianum exists in many physiological forms in all bean-growing regions of the world and infects bean genotypes from both bean gene pools, i.e. Andean and Mesoamerican (Mahuku and Riascos, 2004) [8]. According to CIAT [9], the fungus is known to have races that vary across countries, regions, locations and varieties. The highest diversity and variation are reported from Latin America, which is the center of origin of common beans [10].

Since east African regions are considered as the secondary center of diversity for common bean, due to co-evolution of the *C. lindemuthianum* pathogen and its host, the East African highland regions are expected to have a high variability of *C. lindemuthianum* [11]. The existence of high pathogenic variability and emergence of new pathogen races results in continuous breakdown of host resistance [12].

Knowledge on race variability of the pathogen population is a prerequisite for developing durable resistance in bean varieties [6]. Co-evolution of the pathogen and its host in the Andean and Mesoamerican gene pools provides a useful

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means of identifying appropriate sources of resistance, since common bean genotypes originating from one gene pool are more likely to express resistance to pathogenic races than another gene pool [13,14]. In Ethiopia, some efforts have been done to identify the type of races that occur in Ethiopia where Tesfaye (2003) gave an account of eight races of *Colletotrichum lindemuthianum* from Ethiopia and compared them to six races brought from Southern Africa. But as previous studies in this topic are very limited in Ethiopia, it is important to first characterize the *Colletotrichum lindemuthianum* isolates collected from major bean growing areas of the country to develop resistant common bean genotypes. Therefore, the current study aimed at filling the gap by assessing race variability among the *Colletotrichum lindemuthianum* populations of south and central Ethiopia.

Materials and Methods

Disease survey

A field survey for *Colletotrichum lindemuthianum* isolate collection was conducted in 2017 main season in two regions (Oromia and SNNPR) at six districts, i.e., Damot Gale, Halaba Special (Choroko 2 kebele), Adama (Melkassa), Boset (Kechachula), Hawassa Zuria (Hawassa Agricultural Research Center) and Boricha (Figure 1). The survey districts were systematically selected based on production potential and previous reports on bean anthracnose. Two to three farmers' fields were randomly identified per district for the field disease survey and isolate collection. Five sampling quadrats (1 m²) were used on each farmer's field to collect data on anthracnose incidence and severity as follows:

$$\text{Disease Incidence (DI)} = \frac{\text{Number of infected plant/s in the quadrat}}{\text{Total number of the plants in the quadrat}} \times 100\%$$

Severity of bean anthracnose was recorded on leaves based on CIAT (1987), a standard system for the evaluation of bean germplasm (Table 1).

Sample collection

In each location, samples depicting typical bean anthracnose symptoms were randomly considered, and from each identified representative plants, three leaves were collected. Collected leaves were immediately wrapped on paper towels to absorb their moisture, placed in paper bags and labeled with pencil. Finally, three representative samples from a single common bean leaf with typical anthracnose symptoms from each field were picked for race analysis.

Isolation of the pathogen

Isolation of the pathogen was conducted at Melkassa Agricultural Research Center (MARC) Plant Pathology Laboratory. Leaf samples were washed in running tap water and leaf pieces or patches with 2-5 mm size were cut with sterilized pair of scissors and surface-sterilized with 2.5% sodium hypochlorite (chlorox, NaOCl) solution for 2-3 minutes and rinsed three times with sterile distilled water (SDW). Since collected samples could possibly carry more than one strain of a pathogen or even more pathogens, effort was made to spatially resolute a spot with typical anthracnose symptom from a single leaf, and then pieces were cut out to maintain the genetic purity of *Colletotrichum lindemuthianum* isolates. Then, the pieces were placed on sterilized paper towel for 5-10 minutes; and five such pieces were aseptically transferred into a 9 cm Petri dish with potato dextrose agar (PDA). The cultures were incubated at 20 °C under continuous fluorescent light for 10 days. Sub-culturing was done by taking a 3 mm mycelial plug from the PDA, which was visually

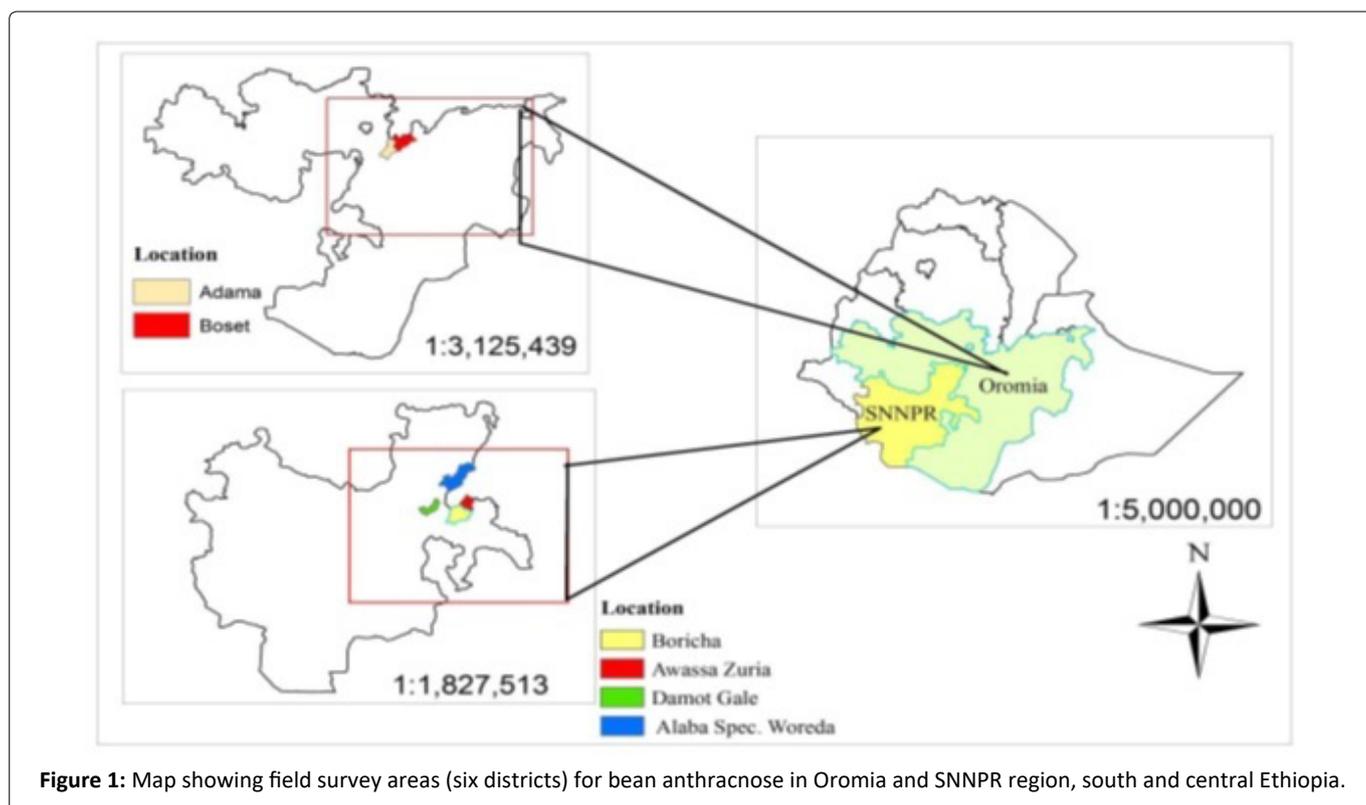


Table 1: Symptom evaluation of common bean anthracnose severity scale according to CIAT (1987).

Disease Scale	Common bean plant part affected
1	No visible disease symptoms
3	Presence of very few and small lesions, mostly on the primary vein of the leaf's lower side or on the pod, that covers approximately 1% of the surface area
5	Presence of several small lesions on the petiole or on the primary and secondary veins of the leaf's lower side. On the pods, small (less than 2 mm diameter) round lesions, with or without reduced sporulation, cover approximately 5% of the pod surface areas
7	Presence of numerous enlarged lesions on the lower side of the leaf. Necrotic lesions can also be observed on the upper leaf surface and on petioles. On the pods the presence of medium sized (larger than 2 mm in diameter) lesions are evident but also some small and larger lesions generally with sporulation and that cover approximately 10% of pod surface area may be found
9	Severe necrosis on 25% or more of the plant tissue is evident as a result of lesions on the leaves, petioles, stem, branches, and even on the growing point which often results in death of the plant tissues. The presence of numerous, large, sporulating, sunken cankers can result in pod malformation, low seed number, and death of the pod

free from any contamination, and transferring into new Petri dishes containing fresh PDA. Then continuous sub-culturing was made until pure cultures with no contamination were obtained. While culturing, streptomycin sulfate (5 mg L⁻¹ of PDA) was used to suppress bacterial colonial growth. In this way, a total of 30 isolates were purified and obtained from the survey areas for race identification.

Inoculum preparation for race identification

Two-week-old pure cultures were flooded with 50 mL of sterile distilled water (SDW) and the spores were scraped off using a fine brush. Flooding was repeated three times, each time using fresh sterile distilled water to get most of the conidia from the culture. Then the suspension was poured into a beaker and mixed thoroughly. Spore suspensions from all cultures of the same isolate were mixed and passed through double layer cheesecloth (44*36 thread/sq. inches) to retain fragments of mycelia and culture medium. The concentration of the spore suspension was adjusted to 1.2 × 10⁶ spores per mL of sterile distilled water using hemocytometer. One drop of Tween 20 was added per 100 mL spore suspension and mixed thoroughly before inoculation.

Planting common bean differentials

Common bean seeds of 12 differential cultivars were surface sterilized with 0.1% chlorox solution for three minutes, washed or rinsed thoroughly with sterile distilled water and dried at room temperature (25 °C ± 2). Then the seeds were sown in plastic pot filled with 3 kg mix of sun-dried sterile topsoil, manure and sand in 2:1:1 ratio, respectively, and pots placed in greenhouse. Five seeds per pot were sown in completely randomized design, and each plant was considered as a replicate for each common bean differential cultivars and for each isolate of the pathogen.

Inoculation

Fourteen-day-old common bean seedlings were used for inoculation. All the 12 differential cultivars were inoculated separately with the prepared separate isolate suspension (60 × 10⁶ spores per pot) using a hand sprayer on both the abaxial and adaxial surfaces of the leaves until suspension runoff at a time. A control (plants inoculated with sterile distilled water alone) was included for each set of the differentials

inoculated with an isolate. Inoculated plants were covered with transparent polythene sheets to maintain high relative humidity. The polythene sheet was removed five days after inoculation. After removal of polythene sheet, pots were kept in the greenhouse and maintained at temperature of 18-33 °C and relative humidity ranging from 72 to 95%.

Race determination

The reaction [susceptible reaction (+) and resistant reaction (-)] of each common bean differential cultivar to each isolate was assessed 15 days after inoculation, i.e., 30 days after planting. For the purpose of consistency, only the primary leaves of each plant were evaluated. After evaluation of all differential cultivars, each isolate was assigned a name (race number) based on the binary nomenclature system by adding the numerical binary values of the susceptible varieties together. A binary number is equal to 2ⁿ, where n is equivalent to the place of the cultivar within the differential series order as tabulated below (Table 2). The sum of all binary numbers of cultivars with susceptible reactions (i.e., $y = 2^n + 2^{(n+1)} + 2^{(n+2)} + \dots + 2^{(n+11)}$; where n = 0) gives a specific race number or name (y) [15].

Results and Discussion

Disease Survey

Surveyed areas had elevation ranging between 1436 and 1958 m.a.s.l. Bean anthracnose incidence in the study areas ranged from 33 to 67% (Figure 2). The highest anthracnose incidence (66.7%) was recorded in Hawassa and Boricha, followed by Damot Gale (58.5%) and the lowest (33.3%) was in Melkassa. These results are in contrast to the finding of Habtu, et al. [16] who reported high level of bean anthracnose in the Rift Valley (Melkassa) and low level of bean anthracnose in Sidama (Hawassa).

The mean anthracnose severity score across the surveyed areas ranged from 25 to 60%. The highest (60%) severity score was recorded in Damot Gale and Boricha areas and the lowest (25%) at Melkassa. These results were not in agreement with the observation of Tesfaye [17] who reported higher anthracnose severity in the western zone and Rift Valley areas of the country, suggesting a possible shift in bean anthracnose intensity across the country. Seed born nature of

Table 2: Standard differential cultivars of common bean used to characterize *Colletotrichum lindemuthianum*, their binary codes, resistance genes and gene pool.

Differential Cultivar	Seed Type	Notation	Binary Code	Resistance Gene	Gene Pool
Michelite	S	0	1	Co-11	MA
MDRK	L	1	2	Co-1	A
Perry Marrow	L	2	4	Co-1 ³	A
Cornell 49-242	S	3	8	Co-2	MA
Widusa	L	4	16	Co-1 ⁵	A
Kaboon	L	5	32	Co-1 ²	A
Mexico 222	S	6	64	Co-3	MA
PI 207262	S	7	128	Co-4 ³ Co-9	MA
TO	S	8	256	Co-4	MA
TU	S	9	512	Co-5	MA
AB 136	S	10	1024	Co-6	MA
G2333	S	11	2048	Co-4 ² Co-5 ² Co-7	MA

S: Small seeded; L: Large seeded; MA: Middle American; A: Andean; Source: Awale, et al. (2007)

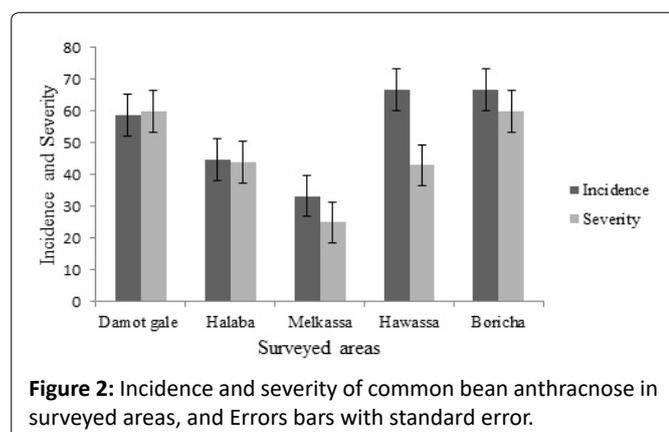


Figure 2: Incidence and severity of common bean anthracnose in surveyed areas, and Errors bars with standard error.

the disease [18] and uncontrolled exchange of common bean seeds for planting across the country lead to such deviation on the incidence and severity of bean anthracnose.

Race determination

The reaction of bean differential cultivars to selected isolates was variable. Out of the 30 isolates of *Colletotrichum lindemuthianum* collected, a total of 17 physiological races (pathotypes) were identified (Table 3). Race designation gives an indication of the variability of the pathogen [6]. Out of 17 races identified by the current study, only three races, i.e., race 1011 at Areka, race 898 at Bako, and race 128 at Ziway, were previously reported in Ethiopia [17].

Race 9, which was identified from Boricha, Hawassa and Halaba districts, was the most dominant physiological race, followed by the typical races 2260, 272 and 1011. Similar result was reported by Ansari, et al. [19] who reported race 9 as the most widespread race in Argentina. The same authors indicated that race 9 included isolates originating from four different countries. On the other hand, Gonçalves, et al. [20] stated that race 73 was the most common and widespread race in Santa Catarina State in Brazil.

When *C. lindemuthianum* races identified in the current work are assigned to geographic region, four races (385, 3047, 9 and 587) were from Boricha; two races (2260 and 272) from Melkassa; 12 races (272, 321, 9, 1172, 898, 128,

465, 73, 1250, 34, 1011 and 2260) from Hawassa; two races (2225 and 1011) from Wolayta; and two races (2073 and 9) were from Halaba. This suggests the richness of the Rift Valley region with reference to *C. lindemuthianum* race variability. The differences among the pathogen populations in different areas might reflect the differences in the agricultural practices employed and common bean germplasm used; that would affect the selection and adaptation processes, in the different agro-ecologies [6].

Of the 17 *C. lindemuthianum* races identified in the current work, four races, i.e. 3047, 2260, 2225 and 2073, were able to infect the highly resistant differential cultivar G2333. Susceptibility of this cultivar to isolates of *C. lindemuthianum* has rarely been reported [21]. Thus, the result strongly suggested that *C. lindemuthianum* populations in Ethiopia could possibly be composed of highly virulent races that may cause much damage even to resistant/tolerant germplasms. Besides causing bean anthracnose on G2333, race 3047 also successfully infected the differential cultivars TU, TO, PI 207262, Mexico 222, Kaboon, Perry Marrow, MDRK and Michelite. Furthermore, race 2260, 2225 and 2073 managed to break the resistance of the differential cultivars PI 207262, Mexico 222, Widusa, Perry Marrow, Kaboon, Cornell 49-242 and Michelite (Table 3).

No single differential cultivar was resistant to all of the isolates collected and tested. Nevertheless, the differential cultivar AB 136 was a relatively more resistant cultivar than the rest, considering the number of races (only two, i.e. 1172 and 1250) that infected it. This observation is similar with findings of Sicard, et al. [22] and Gonzalez, et al. [23] who reported the differential cultivar AB 136, containing resistance two genes Co-6 and Co-8 as resistant to many isolates of *C. lindemuthianum* originating from different parts of the world. The differential cultivars Perry Marrow, G 2333 and TU were susceptible to three, four and four races, respectively, and ranked second and third most resistant cultivars to the pathogen races in the current experiment (Figure 3). A similar result was reported previously by Cláudia, et al. [24], where the cultivars AB 136 and G 2333 were found resistant to races collected from Paraná State of Brazil. Likewise, the

Table 3: Reaction of 12 common bean differential cultivars to *Colletotrichum lindemuthianum* isolates in the greenhouse.

Isolates	Differential cultivars												Race Name
	Michelite	MDRK	Perry Marrow	Cornell 49242	Widusa	Kaboon	Mexico 222	PI 207262	TO	TU	AB 136	G 2333	
Boricha207(1)	+	-	-	-	-	-	-	+	+	-	-	-	385
Boricha207(2)	+	+	+	-	-	+	+	+	+	+	-	+	3047
Boricha207(3)	+	-	-	-	-	-	-	+	+	-	-	-	385
Boricha205	+	-	-	+	-	-	-	-	-	-	-	-	9
Boricha204	+	+	-	+	-	-	+	-	-	+	-	-	587
Kechachule 2	-	-	+	-	+	-	+	+	-	-	-	+	2260
Seed Multi	-	-	-	-	+	-	-	-	+	-	-	-	272
Block 1(2)	-	-	-	-	+	-	+	-	+	-	-	-	272
Block 1(3)	+	-	-	-	-	-	+	-	+	-	-	-	321
Block 2(2)	+	-	-	+	-	-	-	-	-	-	-	-	9
Block 1(1)	+	-	-	-	-	-	+	-	+	-	-	-	321
Block 2(3)	-	-	+	-	+	-	-	+	-	-	+	-	1172
Wolaita p-6	+	-	-	-	+	+	-	+	-	-	-	+	2225
Wolaita p-7	+	+	-	-	+	+	+	+	+	+	-	-	1011
Halaba p-3	+	-	-	+	+	-	-	-	-	-	-	+	2073
Halaba p-2	+	-	-	+	-	-	-	-	-	-	-	-	9
HRC 305	-	+	-	-	-	-	-	+	+	+	-	-	898
HRC 315	-	-	-	-	-	-	-	+	-	-	-	-	128
HRC 314	+	-	-	-	+	-	+	+	+	-	-	-	465
HRC 9	+	-	-	+	-	-	+	-	-	-	-	-	73
HRC 319	+	-	-	+	+	-	+	+	+	-	-	-	465
HRC 7	-	+	-	-	-	+	+	+	-	-	+	-	1250
HRC 318	-	+	-	-	-	+	-	-	-	-	-	-	34
HRC 8	-	+	-	-	-	+	-	-	-	-	-	-	34
HRC 6	+	+	-	-	+	+	+	+	+	+	-	-	1011
HRC 12	+	-	-	+	-	-	+	-	-	-	-	-	73
HRC 2	-	-	+	-	+	-	+	+	-	-	-	+	2260
HRC 1	-	+	-	-	-	+	+	+	-	-	+	-	1250
HRC 310	-	+	-	-	-	+	+	+	-	-	+	-	1250
HRC 308	-	+	-	-	-	+	-	-	-	-	-	-	34

*(+) susceptible reaction, (-) resistant reaction

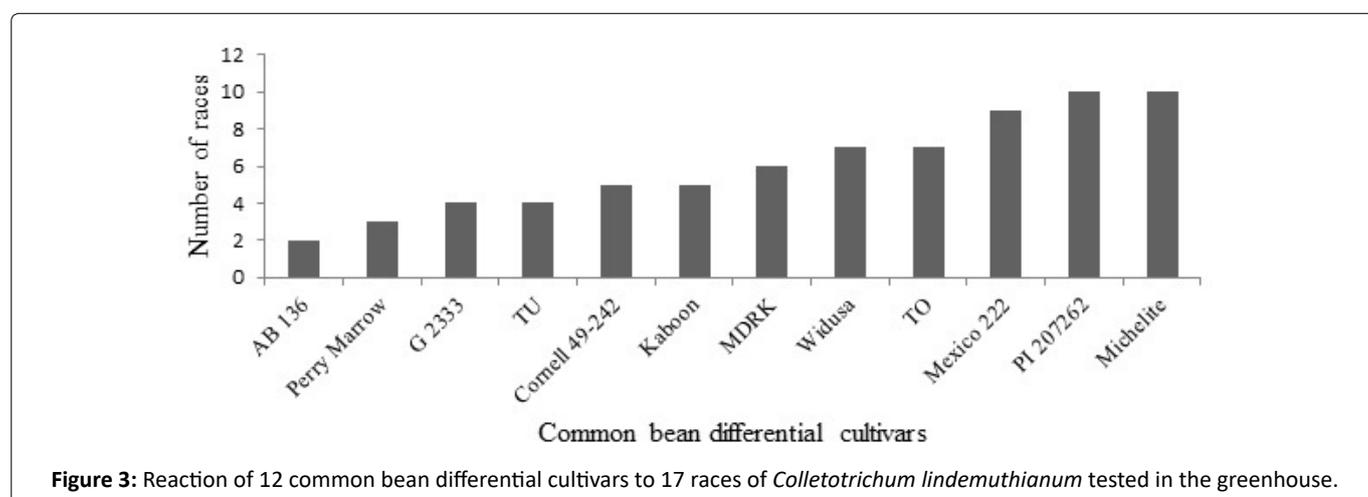


Figure 3: Reaction of 12 common bean differential cultivars to 17 races of *Colletotrichum lindemuthianum* tested in the greenhouse.

differential cultivar TU was also reported to be susceptible to four isolates of *C. lindemuthianum* in another study [19].

On the other hand, the differential cultivars Michelite and PI 207262 were found to be the most susceptible differential cultivars showing susceptible reaction to 10 races, followed

by Mexico 222, TO and Widusa, which were susceptible to 9, 7 and 7 races in that order. More or less similar results were also reported by Cláudia, et al. [24] and Ansari, et al. [19]. The differential cultivars Michelite and Mexico 222 showed susceptible reaction to all of the 10 isolates collected from

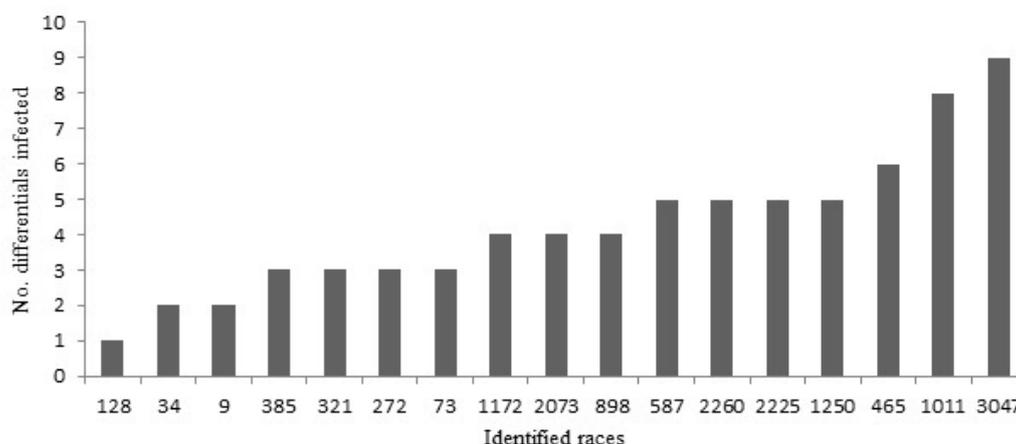


Figure 4: Virulence of identified races of *Colletotrichum lindemuthianum* collected from south and central Ethiopia to common bean differential cultivars tested in the greenhouse.

Grosso State, Brazil [25]. Also, according to Gonzaz (2016) [26], the bean differential cultivar Michelite was reported susceptible to all *C. lindemuthianum* isolates, which were collected from Arumeru, Karatu, Mbulu and Babati rural areas.

Of the 17 *C. lindemuthianum* races identified in the current work, race 3047 and race 1011 were the most 'cosmopolitan' races, infecting nine and eight differential cultivars, respectively, whereas races 128, 34 and 9 were 'narrow ranged', infecting a maximum of two cultivars, out of the total 12 cultivars (Figure 4).

With reference to bean gene pool, among the 17 identified races, 11 races (3047, 587, 2225, 1011, 2073, 465, 898, 1250, 2260, 1172 and 272) were pathogenic to the common bean differential cultivars having gene pool from both Mesoamerican and Andean origin. It is common to see races infecting differential cultivars having both gene pools as reported in Gonzaz [26]. Race 34 was pathogenic only to the differential cultivars from Andean gene pool, and races 385, 9, 321, 73 and 128 were pathogenic only to Mesoamerican gene pool. In line with this result, race 73, which infected only the Mesoamerican origin in this study, was also shown to be pathogenic to the differential cultivars from Mesoamerican origin [27,24]. Most of the isolates collected from Argentina and Central America were predominantly pathogenic to the Mesoamerican cultivars, while isolates collected from Africa, Europe, the Dominican Republic and South America showed a higher frequency of pathogenicity to the Andean cultivars than to the Mesoamerican differential cultivars [19].

Conclusion

The current study indicated that Ethiopian *Colletotrichum lindemuthianum* populations were highly variable as evidenced by the occurrence of 17 races of the pathogen from 30 isolates collected from five districts of south and central Ethiopia. Both highly virulent as well as less virulent races were observed. Current results also suggested that the *Colletotrichum lindemuthianum* population in Ethiopia could possibly be composed of highly virulent races that can

cause much damage even to resistant/tolerant germplasm. The differential cultivar AB 136 was relatively the most resistant cultivar as it was susceptible only to two races. The differential cultivars Michelite and PI 207262 were the most susceptible differential cultivars as both showed susceptible reaction to 10 races. With reference to gene pool, race 34 was the only race that was pathogenic only on the differential cultivar from Andean gene pool, whereas races 385, 9, 321, 73 and 128 were pathogenic only on the differential cultivar from Mesoamerican gene pool. In conclusion, the current study suggested that the Ethiopian population of *Colletotrichum lindemuthianum* shall be regularly monitored for emergence of new races and race identification study need to be continued with more advanced techniques, i.e. molecular characterization method and by including more isolates across agro-ecological gradients of Ethiopia.

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