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Physiological characterization and evaluation of common bean genotypes against anthracnose (*Colletotrichum lindemuthianum* (Sacc. and Magnus) Lams-Scriber) races in West Shewa, Ethiopia

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ABSTRACT

Common bean (Phaseolus vulgaris L.) is an important legume crop in the daily diet and good source of cash and food nutrients. Despite its economic significance, anthracnose disease caused by Colletotrichum lindemuthianum (Sacc. and Magnus) Lams-Scriber is one of the most destructive diseases and causes significant yield losses in common bean growing areas. The pathogen possesses a very high degree of pathogenic variability, but there are lack of knowledge of population race structure and variability of the pathogen in West Shewa Zone, Ethiopia. Therefore, the present study was conducted to identify C. lindemuthianum races and evaluation of common bean genotypes against the virulent isolates. Race analysis was carried out by inoculating 51 isolates on to the 12 differential hosts. Among 51 isolates, 15 physiological races were identified and designated as races 19, 67, 68, 73, 82, 99, 128, 154, 296, 389, 589, 649, 1033, 1403 and 1703 of which 10 of them (19, 67, 82, 99, 154, 389, 649, 1033, 1403 and 1703) were new races identified for the first time in Ethiopia. The most virulent race was 1703 made seven differential hosts' resistant genes ineffective and the least virulent race was 128, which conquer only one resistant gene of the differential cultivar. There were no races found to be pathogenic to differential cultivar G2333 and the differential cultivar, Michelite was found to be the most susceptible with virulence gene frequency of 64.7%. Besides, differential cultivar Widusa, TO and AB136 were highly resistant while G2333 was the highest resistant to all isolates. Among 36 common bean genotypes screened against seven virulent races, 19.4% were resistant and 16.2% highly susceptible to the virulent races. Thus, the effective differential cultivars and common bean genotypes could serve as sources of resistant genes under West Shewa Zone, Ethiopia. In general, the results of this study are very promising for enhancing common bean production and improving food security in study area.

Key words: Common bean, Anthracnose, Colletotrichum lindemuthianum, Disease intensity, Physiological races.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important legume crop in the daily diet of more than 300 million people of the world's population (Hadi *et al.,* 2006; Meziadi *et al.*, 2016). It is one of the most important crops that provide protein. In particular, in developing countries, the importance of common bean is beyond limit as a source of cash and full food nutrients (Popelka *et al*., 2004; Hadi *et al*., 2006; Akhavan *et al.,* 2013; Meziadi *et al.*, 2016). Globally, common bean is cultivated on nearly 28 million hectares, producing about 20 million tons annually. It is major staple food in Eastern and Southern Africa and is recognized as the second source of human dietary protein after maize and third most important source of calories after maize and cassava (Kelly *et al.,* 2004; Ndee, 2013).

In Ethiopia, over 3.3 million household producers grow common beans as an important food crop and source of cash. The major common bean producing areas of Ethiopia are central, eastern and southern parts of the country (CSA, 2014). In 2013/14, total common bean production in the country was about 4,574,116 quintals on approximately 326,465 hectares of land (2.6% of the grain crop area) (CSA, 2014). Between the years, 2004-2012, income from the exported common bean almost tripled from USD 18 to 50 million (EEPA, 2012). Despite its economic significance and wide area of production, the national annual average yield of common been obtained in the year 2013/2014 is 1.5 tons/ha which is lower than the potential yield 4.5 tons/ha (CSA, 2014).

The low national yield could be attributed to various constraints like moisture stress, low adoption of improved agricultural technologies, low soil fertility, pests, lack of improved seed varieties, poor cultural practices, shortage of land and environmental degradation (Legesse *et al.,* 2006; Kutangi *et al.,* 2010; Meziadi *et al.*, 2016). Among constraints, bean anthracnose disease caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Lams-Scriberis is one of the most devastating seed-borne diseases of common bean (*Phaseolus vulgaris* L.) in Ethiopia and cause a yield loss of 90-100% in many beans growing regions of the world (Ansari *et al*., 2004). Yield loss incurred due to bean anthracnose in Ethiopia is estimated to be about 63% (Tesfaye, 1997).

The spread of pathogen, the ubiquity of the inoculum and high costs have made management of disease impracticable for growers but use of genetically resistant cultivar is the most effective, efficient, economical and ecologically safe management strategy for anthracnose management (Esteban *et al*., 2003; Meziadi *et al.*, 2016). However, the wide natural variability of *C. lindemuthianum* and the potential for the appearance of new virulent races presents a major challenge for bean breeders (Mahuku *et al*., 2002). Hence, to develop durable resistant variety, adequate knowledge of the race structure and distribution of *C. lindemuthianum* is still insufficient in Ethiopia. Therefore, the current study was conducted to identify *C. lindemuthianum* races and evaluation of common bean genotypes against the virulent isolates.

METHODOLOGY

Physiological race characterization of *C. lindemuthianum*

Sample collection

The samples were collected from the six major common bean growing districts viz. Ambo, Toke Kutaye, Bako Tibe, Elu Gelan (Ejeji), Dendi and Nono districts in West Shewa Zone of Oromia Regional State in Ethiopia; in the main cropping season of 2015. Common bean pods and leaves with pronounced anthracnose symptoms were collected from naturally infected bean plants at 5-10 km intervals on the main and feeder roads in each randomly selected fields. Out of 71 fields inspected, the samples were collected only from 55 fields that exhibited the disease symptom, while no disease symptom was observed in the remaining 16 fields Anthracnose infected pods and leaves were cut with sterilized scissors and placed in labelled envelope the transported to Ambo Plant Protection Research Canter's (APPRC) Laboratory for race identification. Samples were preserved in a refrigerator at 4^{o}C until further use.

Isolation of *C. lindemuthianum* from diseased samples

The samples collected from different fields and cultured on standard PDA medium in three replications. These pieces of infected plant tissue were surface sterilized and placed on Potato Dextrose Agar (PDA) medium. To restrict bacterial growth, PDA was amended with 100 mg/l antibiotics Chloramphenical (CAM), and then poured into Petri plates. Colonies of *C. lindemuthianum* start growing on standard PDA after three days of culturing. The colonies showed morphological

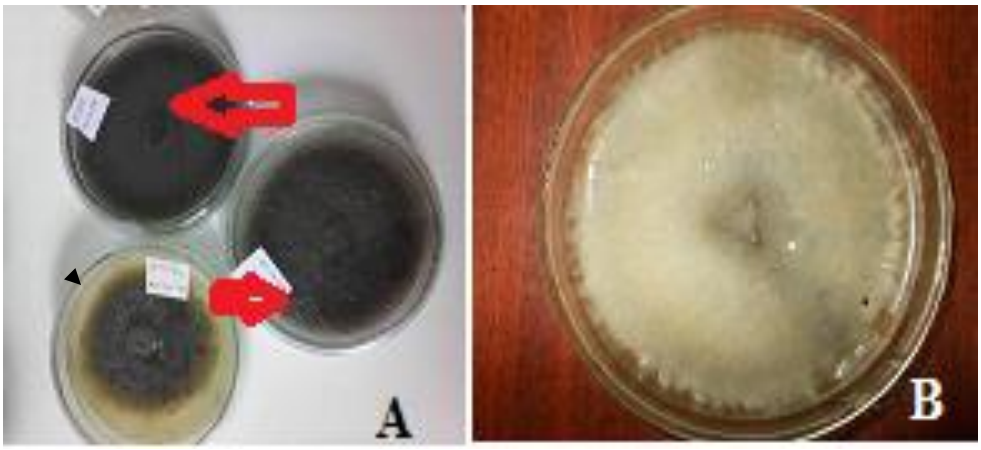

Figure 1 Cultures of *C. lindemuthianum* growing on PDA media

characteristics of gray at a young stage and became dark black with very compact mycelia growth at their late stage (Fig.1A) where as some of them are white dirty to dark (Fig.1B). Thus, developed colonies on culture medium were identified under a microscope for the development of a pure culture of *C. lindemuthianum*. The spores of isolates were hyaline, cylindrical with both obtuse and base narrow and without septa and uni-nucleated. Out of 55 samples cultures on PDA, 31 cultures (one culture/sample/field) developed into a pure culture whereas the diseases sample from 24 fields failed to grow on the culture media.

Development and multiplication of single-pustule

Each of the pure culture isolates of *C. lindemuthianum* flooded with a drop of distilled water (10 ml) and the spores scraped off using fine brush smoothly and then the suspension poured into a beaker by refining within cheese cloth, allowed soaking for few minutes. A 0.1ml of spore suspension was pipette from a beaker with a fine pipette tip for further serial dilution. The spore concentration was determined under a microscope by using haemacytometer after serial dilution. The serial dilution continued until the required concentration of spores reached. After the concentration adjusted through serial dilution, 50µl of the diluted suspension of conidia were pipette out and transferred to fresh acidified PDA medium, where they were maintained until sporulation. A sterile glass rod was used to spread conidia evenly on the surface of the media. Petri-dishes were incubated at approximately 22±1^{o}C for 2-3 days.

After 24 to 48 h, mono-conidial spore isolates germinated were transferred to fresh acidified medium with a sterile needle (Wang-Ching and Wen–Hsiung, 1996). The germinated single spores were maintained on PDA under continuous fluorescent light to promote better sporulation (Nicholsen and Warren, 1981). Colonies produced from a single spore were maintained as a pure isolate. A total of 87 single pustule isolates were developed for further investigation on differential cultivars and genotype infection analysis.

Randomly selected 51 pure culture of single pustule isolates were multiplied for the inoculation of differential cultivars and genotypes. Each pure culture of isolates was sub-cultured by using a sterile needle. Four to five pieces (1-2cm diameter) of agar plugs with actively growing fungus were cut and placed in the plates containing the PDA. The plates were then kept in inoccupation chamber for 4 days in dark and then exposed to light to continuous fluorescent light to promote better sporulation. Three to six cultures of each isolate were raised in order to obtain enough inoculum.

Preparation of spore suspension for inoculation

Out of 87, a single pustule isolates was developed, 51 were selected for physiological race characterization. These were selected to represent isolate collected from all surveyed districts in the zone. To get enough spore concentration for further test, each of pure cultures was flooded with 100 ml of distilled water then the spores scraped off using a fine brush and the suspension poured into a beaker by purifying with cheese cloth.

This was repeated about 3 times, each time using fresh distilled water. Conidia suspensions from all cultures of the same isolate were mixed. Using a haemocytometer, the concentration of the spore suspension was adjusted to1.2 x10^{6} spores/ml. This procedure was applied to all isolates. One drop of Tween 20 was added per 100 ml of inoculum and mixed thoroughly before inoculation.

Table 1 Differential lines used to characterize races of anthracnose in common bean

Differential lines	Host genes	Place of cultivar	Gene pool	Binary number	Growth habit
Michelite	---	0	MA	1	II
Michigan Dark Red Kidney	Co – 1	1	A	2	I
Perry Marrow	Co – 1^3	2	A	4	II
Cornell 49242	Co – 2	3	MA	8	II
Widusa	Co – 1^5	4	A	16	I
Kaboon	Co – 1^2	5	A	32	II
Mexico 222	Co – 3	6	MA	64	I
PI 207262	Co – 4^3, Co – 9	7	MA	128	III
TO	Co – 4	8	MA	256	I
TU	Co – 5	9	MA	512	III
AB136	Co – 6, Co – 8	10	MA	1024	IV
G2333	Co – 4^2, Co-5, Co – 7	11	MA	2048	IV

Differential cultivars

To distinguish the races of different *C. lindemuthianum* isolates, the set of 12 internationally recognized anthracnose race differential proposed by CIAT (Pastor-Corrales, 1991) were used. The set consists of 12 cultivars, each with a designated binary number and their characteristics (Kelly and Vallejo, 2004) are outlined in Table 1. The number or race designation is given to an isolate was determined by the cultivars of the differential set that were infected by that isolate. The sum of the numbers assigned to each infected cultivar was the race number.

Seed multiplication

To get enough amounts of seed for race identification, race differential seeds obtained from CIAT (Centro Inter-nacianal de Agricultura Tropical) were multiplied in a controlled atmosphere in the greenhouse of Plant Protection Research Centre of Ambo and Holleta Agricultural Research Centre in 2015.

Inoculation of differential cultivars

Five plants of each differential lines and one susceptible variety Awash-1 were planted in clay pots separately in the greenhouse. The clay pot contains a mixture of soil, humus and sand at a ratio of 2:1:1. The susceptible variety Awash-1 was used to determine the viability of spores inoculated on the differential cultivars. Spores from each isolate were suspended at a concentration of 1.2×10^6spores/ml and sprayed on the 10 day old bean plants. Each experiment was inoculated and the whole experiments were repeated at least twice. Whole plant inoculations were done using a hand sprayer. Leaves were sprayed on both the abacial and adacial surfaces with inoculum until run off. All the differential cultivars in each set were inoculated with one isolate at a time. A control (plants inoculated with clean distilled water as well as water with two drops of tween 20) was included in each set of the plants inoculated with an isolate. Inoculated beans were covered with transparent polyethylene sheets/bags in order to maintain a relative humidity of approximately 92-95% and 13-26 ^{o} C for four days in a growth chamber.

Data Collection

Symptoms evaluation on inoculated plants was commenced on 10 days after inoculation, based on a 1-9 severity scale, depicting the percentage of the leaf covered with anthracnose lesions as described by Balardin *et al.* (1990). Score description of 1-3 represents the degree of resistance while 4-9 susceptible. A variety that was considered susceptible had 60% (or 3/5) of its inoculated plants exhibiting same disease, scores within the susceptibility range (Ansari *et al*., 2004). After evaluation of all varieties, 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 (CIAT, 1988).

Analysis of virulence frequency.

The virulence frequency expresses the proportion of virulent isolates per gene. Based on the calculated value of virulence gene frequency, the level of effectiveness of the resistance genes was classified using the grouping system developed by Felsenstein and Jaser (2000) (Table 2).

Table 2: Classification of the effectiveness of genes as a function of virulence frequency in the pathogen population

Virulence gene frequency (%)	Resistance level
0-10	Very high
>10-20	High
>20-50	Moderate
>50	Low/no

Source: Felsenstein and Jaser, (2000)

Table 3 Common bean cultivar (genotypes) used for resistivity test against selected races in 2015-16

S/N	Variety	S/N	Variety	S/N	Variety	S/N	Variety
1	Argane	11	Gofta	20	Ibado	29	Morka
2	Dimtu	12	Nasir	21	Melke	30	KAT B9(Dandesu)
3	Cranscope	13	Brazil-1	22	Chore	31	Red Walayita
4	SER-125	14	SAB-736	23	A/Melka	32	Tinike
5	Dinknesh	15	Awash-2	24	Deme	33	Roba-1
6	DRK	16	Bole	25	Kufanzik	34	GobeRasha
7	Batu	17	Dursitu	26	GLP-2	35	Awash-1
8	Beshbesh	18	SAB-737	27	Nazirat-2	36	Ser-119
9	Ayenew	19	Chercher	28	Ecab -0081	37	MelkaDima Glp-2
10	SAB-632						

Analysis of pathogenicity index

Pathogenicity is the ability of the pathogen to interfere with one or more of the essential functions of the plant, thereby causing disease. The pathogenicity and resistivity index was evaluated with previously developed by Balardin *et al.* (1990). For each *C. lindemuthianum* race inoculated on common bean seedling was computed by dividing the number of bean landrace genotypes with a susceptible reaction to the total number of landrace genotypes of bean under study (Balardin *et al.,* 1990).

Evaluation of common bean cultivars (genotypes) using identified races

A total of 36 common bean genotypes and Awash-1(check) were tested for their resistance against identified virulent races (Table 3). The seeds were obtained from national low land pulse improvement program of Melkassa Agriculture Research Centre, Melkassa, Ethiopia. A CRD with three replications were used. Ten-day-old seedlings (the first leaf is fully expanded and the second leaf is just emerged to grow) were inoculated with spores of virulent races. The same inoculation and evaluation methods as mentioned above for the differential test were applied. Cultivars were considered as resistance when they exhibited 1 to 3 = resistant in 1-9 scale otherwise they are susceptible according to the scale developed by Balardin *et al*. (1990).

RESULTS AND DISCUSSION

Race identification of *Colletotrichum lindemuthianum*

Cultural and microscopic characterization of *C. lindemuthianum*

Out of 71 common bean fields assessed, the disease was detected only in 55 fields. Of the samples collected from the 55 fields, only sample taken from 31 fields were developed as a pure culture in artificial media. A total of 87 single pustule isolates (three isolates per sample) were developed from these viable cultures. A randomly selected 51 isolates (two or one per site sample) were used for further physiological race characterization. The culture media that were incubated at 22±1^{o}C showed good mycelium and sporulation under the continuous fluorescent light. Pastor-Corrales and Tu (1989) reported that the temperature requirement for optimum conidial production on culture media ranges 18-25^{0}C; while above 30^{0}C limit both the growth and conidial production. Unicellular conidia are formed on unbranched unicellular conidiophores grouped in acervuli. The isolates consistently produced very sparse

to dense colonies which varied in mycelia growth pigmentation from white to gray to dark black. Colonies at their young stage were gray and became dark black with very compact mycelia growth at their late stage while on some media showed central dark black surrounded by whitish colour of mycelia (Fig. 2A-F). Batureine (2009) demonstrated that cultures of *C. lindemuthianum* growing on pure PDA media showing differences in sporulation colour within a stage of culture. The present results confirmed with the Fitsum *et al*. (2014) demonstration of anthracnose pathogen isolated from infected common bean on PDA culture media showed morphological characteristic of central blackish with hallo surrounding of whitish mycelia during sporulation. Spores of isolates were hyaline, cylindrical with both obtuse or with base narrow and abbreviate. Spores were without septa and uni-nucleated. Hall (1994) stated that *C. lindemuthianum* spores were characterized by hyaline to gray hyphae, which become dark with very compact aerial mycelium upon maturity.

Physiological races identification of *C. lindemuthianum*

Race investigation provided essential information in determining the range of pathogenic variation in a specific area, for screening resistant varieties, before the pathogen became a threat to crop production in a specific area. Race characterization of *C. lindemuthianum* was determined using 12 internationally recognized anthracnose race differential proposed by CIAT (Pastor-Corrales, 1991).

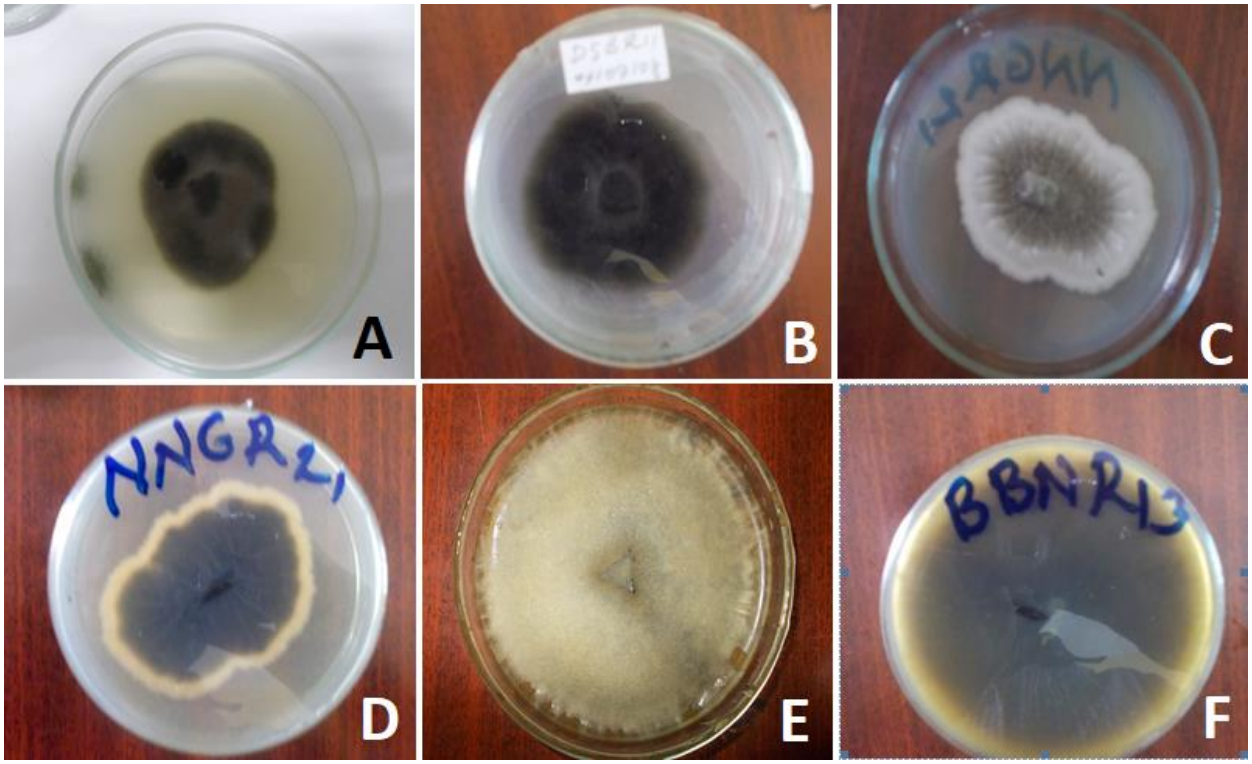

Figure 2 Cultures of *C. lindemuthianum* grown in PDA: Arial view of culture plates - A, B, C and E, while D and F - Reverse view of culture plates.

Table 4: Physiological races of *C. lindemuthianum* identified from the surveyed district of West Shewa zone in 2015.

District	Altitude range m. a. s. l.	Sample collected	Number of isolate	Race identified
EluGelan	1642-1771	7	11	589, 1703, 82, 128, 19
BakoTibe	1619-1725	8	14	589, 389, 296, 154, 128, 99, 82, 73, 19
Dendi	2080-2311	6	9	1703, 649, 389, 296, 154, 99
Ambo	2180-2461	3	6	649, 68, 99
TokeKutaye	2052-2208	2	4	73,68
Nono	1531-1636	5	7	67, 589, 1033, 1403
Total	1531-2461	31	51	15

In this study, 51 tested isolates were categorized into 15 physiological races based on reaction recorded on the differential cultivars, of which 10 new races were identified for the first time and 5 races reported earlier by Tesfaye (1997) and Yesuf (2005) in Ethiopia. The physiological races identified were 19, 67, 68, 73, 82, 99, 128,154, 296, 389,589, 649, 1033, 1403 and 1703, of which the new races were 19, 67, 82, 99, 154, 389, 649, 1033, 1403 and 1703 (Table 4).While the other five races (68, 73, 128, 296 and 589) were already reported. There was a variation of physiological races across the location of the inspected six districts. The highest numbers of races were identified from Bako Tibe followed by Dendi and EluGelan. The corresponding total numbers of races detected are 9, 6 and 5, respectively. The lowest number of races, two (2) were identified from Toke kutaye followed by Ambo and Nono 3 races each. There is no any common race identified in all the districts. The common/shared races were three, which were identified in three districts.

Among the six races identified in Dendi, 5 (83.3%) races were obtained from widely grown local cultivar Boloda. Likewise, most of the identified races were obtained from Naser (10 races) and Tibe (9 races) which indicates one race could attack many common bean cultivars and vice versal. Compatible with the result, Ansari *et al*, (2004) approved similar isolate of the pathogen was confirmed to attack many other *Phaseolus* beans. This was probably due to the pathogenic variability of *C. lindemuthianum* increased through time and the pathogen becomes virulent in common bean production area. Yesuf (2005) reported the introduction of several types of bean germplasm from various sources for the bean improvement program and the availability of local landraces of *Phaseolus* beans as well as weather conditions and cropping practices may be the possible causes of variations within the pathogen population in different bean growing agro-ecological areas.

To summarize, the reality beyond the variation might be due to *C. lindemuthianum* exhibit a high degree of variability and new races pathogenic to previously resistant cultivars were frequently generated through the recombination of nuclear genes during sexual reproduction, exchange of genetic materials in somatic cells, mutation, or by extra chromosomal variations. Similarly, pathogens, which reproduce sexually (like *Colletotrichum* species), are expected to producevariants more readily than the asexual ones (Ogallo, 1991).

Virulence spectrum of physiological race of *C. lindemuthianum*

Pathogenicity of 15 physiologically distinct races of *C. lindemuthianum* identified from six districts had pathogenic index range of 8.3% to 58.3% (Tables 5 & 6).

Table 5 : Physiological races of *C. lindemuthianum* found in 2015 and their reactions on 12 common bean differential cultivars.

Number of isolates	Differential cultivars												Race designation
	A	B	C	D	E	F	G	H	I	J	K	L	
2	+	+	-	-	+	-	-	-	-	-	-	-	19
3	+	+	-	-	-	-	+	-	-	-	-	-	67
5	-	-	+	-	-	-	+	-	-	-	-	-	68
2	+	-	-	+	-	-	+	-	-	-	-	-	73
4	-	+	-	-	+	-	+	-	-	-	-	-	82
5	+	+	-	-	-	+	+	-	-	-	-	-	99
2	-	-	-	-	-	-	-	+	-	-	-	-	128
3	-	+	-	+	+	-	-	+	-	-	-	-	154
3	-	-	-	+	-	+	-	-	+	-	-	-	296
3	+	-	+	-	-	-	-	+	+	-	-	-	389
8	+	-	+	+	-	-	+	-	-	+	-	-	589
3	+	-	-	+	-	-	-	+	-	+	-	-	649
1	+	-	-	+	-	-	-	-	-	-	+	-	1033
1	-	-	-	-	-	-	-	+	+	-	+	-	1403
6	+	+	+	-	-	+	-	+	-	+	+	-	1703

+=Susceptible; - =Resistant; A-L= Differential cultivars and their respective binary values (in parenthesis): A-Michelite (1); B-Michigan dark red kidney (2); C- Perry marrow (4); D- Cornell 49-242 (8); E- Widusa (16); F- Kaboon (32); G-Mexico 222 (64); H-PI 207-262 (128); I- TO (256); J- TU (512); K- AB 136 (1024); L- G 2333 (2048).

Table 6 Virulence spectrum and occurrence of races of *C. lindemuthianum*

Race type	Differential line infected (Virulence spectrum effective resistance genes)	No of isolates	Frequency (%)	Pathogenicity index
19	Michelite, M DRK, Widusa	2	3.9	25
67	Michelite ,M DRK, Mexico 222	3	5.9	25
68	Perry marrow, Mexico 222	5	9.8	16.7
73	Michelite ,Cornell 49-242, Mexico 222	2	3.9	25
82	M DRK, Widusa, Mexico 222	4	7.8	25
99	Michelite, M DRK, Kaboon, Mexico 222	5	9.8	33.3
128	PI 207-262	2	3.9	8.3
154	M DRK ,Cornell 49-242,Widusa, PI 207-262	3	5.9	33.3
296	Cornell 49-242, Kaboon, TO	3	5.9	25
389	Michelite ,Perry marrow, PI 207-262 ,TO	3	5.9	33.3
589	Michelite , Perry marrow ,Cornell 49-242, Mexico 222 , TU	8	15.7	41.7
649	Michelite ,Cornell 49-242, PI 207-262, TU	3	5.9	33.3
1033	Michelite ,Cornell 49-242, AB 136	1	1.96	25
1403	PI 207-262,TO, AB 136	1	1.96	25
1703	Michelite, M DRK ,Perry marrow, Kaboon, PI 207-262, TU , AB 136	6	11.8	58.3

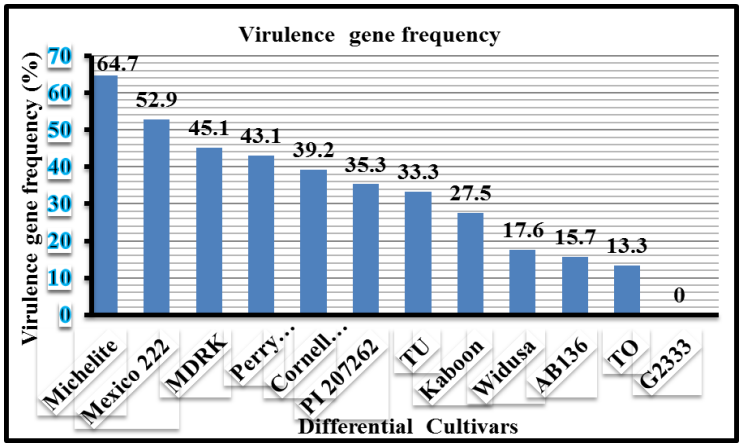

Figure 3: Reaction of common bean differential cultivars to the tested isolates

NB. Cultivar MDRK, Perry Marrow, Widusa and Kaboon are of Andean origin whereas Michelite, Cornell 49242, Mexico 222, PI 207262, To, TU, AB 136 and G 2333 are of Mesoamerican origin.

The most pathogenic race was 1703 with a pathogenic index of 58.3% making seven (7) resistant genes of differential cultivars ineffective. Likewise, the second most virulent race was 589 with pathogenicity index of 41.7%, which was virulent on five specific resistant gene possessed by the differential lines of common beans. While races 99, 154, 389 and 649 were the third most virulent which defeat 4 resistant gene and the least virulent physiological race was 128 which conquered only one resistant gene of deferential cultivar (Table 6). In contrary, no races were found to be virulent to cultivars G2333. This is likely due to the presence of three carrier resistance genes namely Co-4^2, Co-5 and Co-7 in G2333 that inhibited the pathogen. Kelly *et al.* (1994) and Pastor-Corrales *et al. (*1994) reported G2333 was resistant to almost all the European, American and Africa isolates/races. Similarly, Yesuf (2005) also verified that rare susceptibility of G2333 cultivar to *C. lindemuthianum.* The frequency of *C. lindemuthianum* races obtained in this study ranged from 1.96% to 15.7%. The most predominant and widely distributed races identified were 589, 1703, 99 and 82 with a frequency rate of 15.7%, 11.8%, 9.8% and 7.8%, respectively. The next most frequent races were 67, 154, 296, 389 and 649 with 5.9% frequency each, while races 19, 73 and 128 with a frequency rate of 3.9%. The lowest frequency of 1.96% was recorded on races 1403 and 1033 (Tables 5 and 6).

Virulence frequency of *C. lindemuthianum* isolates to resistant genes

The differential cultivars with the resistant gene were infective to most of the isolates tested. The differential cultivar response to *C. lindemuthianum* isolate interaction a resistance or susceptibility range with virulence gene frequency in the range of 0% to 64.7%.

The differential cultivar, Michelite was found to be the most susceptible followed by Mexico 222 with the virulence frequency of 64.7% and 52.9% respectively. These cultivars were susceptible to 33 and 27 different isolates of *C. lindemuthianum* correspondingly. Likewise, the differential cultivars Michigan Dark Red Kidney, Perry Marrow, Cornell 49242, PI207262 and TU were found to be moderately resistance to *C. lindemuthianum* isolates with virulence gene frequency of 45.1%, 43.1%, 39.2%, 35.1% and 33.3%, respectively.

On the other hand, the differential cultivar Widusa, TO and AB136, which carries resistance gene co-1[5], co-4 and "co-6 co-8" were only infected by 9, 7 and 8 different *C. lindemuthianum* isolates with virulence frequency of 17.6%, 13.3% and 15.7 %, respectively. According to this evaluation, these differential cultivars were highly resistant to different *C. lindemuthianum* isolates. All of the isolates were found to be non-pathogenic to G2333 differential cultivar which consists of resistant genes "Co-42, Co-5, Co-7" (Fig. 3).

This difference in resistance among the differentials or avirulence and virulence in *C. lindemuthianum* are genetically controlled and determined by conjugation of the gene-for-gene model. Common bean (*Phaseolus vulgaris L.*) has different pre-existing structural or chemical defence mechanisms that are present in plants before the pathogen attack and induced structural or chemical defences activated by the presence of the pathogen in the host tissue to *C. lindemuthianum (*Agrios, 2005). This made the cultivar response to pathogen different from each other.

To summarize, according to the resistance level developed by Felsenstein and Jaser (2000), the lowest resistance of gene observed by cultivar Michelite (64.7%) and Mexico 222 (52.9%). While the moderate resistance of gene to the *C. lindemuthianum* seen by differential cultivar Michigan Dark Red Kidney (45.1%), Perry Marrow (43.1%), Cornell 49242 (39.2%), Kaboon (27.5%), PI 207262 (35.3%) and TU (33.3%) whereas high resistance of gene response observed by differential cultivar Widusa (17.6%), TO (13.3%) and AB136 (15.7%). The highest gene resistance response detected on differential cultivar G2333, which was not infected by any of *C. lindemuthianum* isolates evaluated.

Reaction of common bean genotypes to virulent races of *C. lindemuthianum*

The use of resistant varieties is pivotal to effective, economical and environmentally friendly strategy of managing *C. lindemuthianum* especially for small-scale farmers (Esteban *et al*., 2003; Meziadi *et al.,* 2016). The advantage of host plant resistance is that once the technology has been developed, it is packaged in the seed that is easier to disseminate or deploy, which does not require any additional or specialized handling on the part of the farmers (Mahuku *et al.,* 2009). However, this has been complicated by the presence of several forms or races of the fungus due to pathogenic variability and the fact that plants resistant to one race may be susceptible to another (Bost, 2013).

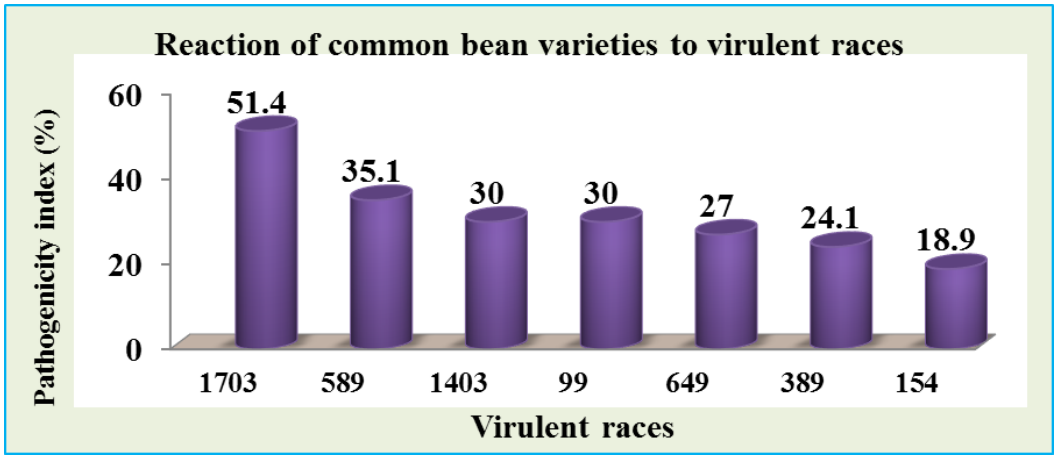

Figure 4 Reaction of common bean genotypes to virulent races

The present evaluation to identify effective genotypes of common bean varieties against virulent races of *C. lindemuthianum* were done under controlled environment in Ambo Plant Protection Research Centre. The evaluation involves thirty six (36) different common bean genotypes and susceptible checks, Awash-1 were screened against seven identified virulent races of *C. lindemuthianum*. The reaction of common bean varieties to the identified races in green house revealed that most of the varieties were resistant to the virulent races evaluated. Moreover, seven of the varieties such as Deme, Bole, Nazirat-2, Ecab-0081, Glp-2, Ayenew and Brazil-1 were resistant to all races under evaluation with disease score of 1-3 (Balardin *et al.,* 1990). In the same way, 20 (54.05%) of the tested genotypes were only susceptible to one or two virulent races. In contrary, six varieties such as Awash-1 (susceptible check) (100%), Roba-1 (71.4%), SEN-125 (71.4%), Nasir (71.4%), Ibbado (71.4%) and Awash-2 (71.4%) responded highly susceptible to the virulent races.

On the other hand, race designated as 1703 was the most virulent over the evaluated common bean cultivars with pathogenicity index of 51.4% and made nineteen (19) improved variety genes ineffective (Fig. 4). The second most virulent isolate was race designated as 589, which break the resistant genes of 13 genotypes. Likewise, isolate designated as races 1403, 99, 649, 389 and 154 were made 11, 11, 10, 9 and 7 common bean resistant genes ineffective, respectively.

To encircle, effective and efficient differential cultivars such as G2333, Widusa, TO and AB136, and common bean improved varieties like Deme, Bole, Nazirat-2, Ecab-0081, Glp-2, Ayenew and Brazil-1, could serve as sources of resistant genes and varieties under West Shewa. Therefore, based on the results, incorporation of these aforementioned effective genotypes into cultivars provides desirable agronomic characters in the breeding program to enhance common bean yield through reducing cost of production and improving food security in Ethiopia.

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