

*Full Length Research Paper*

## **Races of *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara in major bean growing regions in Tanzania**

**Masunga Mpeguzi\*, Nchimbi-Msolla Susan, Robert Mabagala and Luseko Amos Chilagane**

Department of Crop Science and Horticulture, Sokoine University of Agriculture, P. O. Box 3005,  
Chuo Kikuu Morogoro, Tanzania.

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Breeding for resistant varieties has been shown to be the most suitable method to control bean anthracnose caused by *Colletotrichum lindemuthianum* though the method is challenged by the existence of many races of the pathogen. This work focused on characterizing races of *C. lindemuthianum* from potential bean agro ecological zones of Tanzania using a set of differential bean cultivars. From 144 anthracnose infected bean samples collected, 50 pure isolates were obtained and characterized whereby 42 races were identified. The most virulent race identified was race 3610 from the Southern Highland zone of Tanzania while the least virulent was characterized as race 0. Race 2 was the most widely spread (4.2 %) found in Northern, Southern highland and in Eastern zones of Tanzania. The work confirms that G2333 can still be used as a potential donor of resistant genes to varieties that are to be grown in Northern, Eastern and Lake zones but not for the varieties from Southern Highland and Western zones of Tanzania since isolates from these zones overcame resistant genes *Co-4<sup>2</sup>*, *Co-5*, *Co-7* in G2333. 95% of all races which were identified are new and were not specific to either Mesoamerican or Andean origin common bean.

**Key words:** *Colletotrichum lindemuthianum*, races, resistant genes, differential cultivars, Anthracnose.

### **INTRODUCTION**

Bean anthracnose is one of the most serious diseases of common bean caused by the fungal pathogen *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara in areas where common bean is grown. The disease has been reported to cause losses of up to 100% if not well controlled (Opio et al., 2001; Markell et al., 2012). In Tanzania, the work of Allen et al. (1998) in Northern Tanzania reported that, for each 1% increase in

anthracnose incidence, seed yield decreases by 9 kg/ha while Shao and Teri (1985) and Mwalyego (1991) reported loss due to anthracnose to be up to 100% if the disease is not well controlled. According to Opio et al. (2001), the effective control measures to reduce losses caused by bean anthracnose is the use of resistant varieties. This has been proven to be cost effective and requires less skill for farmers to apply it (Mahuku et al.,

\*Corresponding author. E-mail: [mmpeguzi@gmail.com](mailto:mmpeguzi@gmail.com).

2002; Abraham and Mashilla, 2018).

Several genes from both Meso-Americans and Andean bean origin have been shown to offer resistance to anthracnose disease of common bean to anthracnose disease (Méndez-Vigo et al., 2005). The Mesoamerican genes include *Co-2*, *Co-3* (and its alleles *Co-3<sup>2</sup>*, *Co-3<sup>3</sup>*, *Co-3<sup>4</sup>*, and *Co-3<sup>5</sup>*), *Co-4* (and its alleles *Co-4<sup>2</sup>*, *Co-4<sup>3</sup>*), *Co-5* (and its allele *Co-5<sup>2</sup>*), *Co-6*, *Co-11*, *Co-16*, *Co-17*, *Co-u*, and *Co-v* while the Andean genes include: *Co-1* (and its alleles *Co-1<sup>2</sup>*, *Co-1<sup>3</sup>*, *Co-1<sup>4</sup>*, and *Co-1<sup>5</sup>*), *Co-12*, *Co-13*, *Co-14*, *Co-15*, *Co-x*, *Co-w*, *Co-y*, and *Co-z* (Kelly and Vallejo, 2004; de Lima Castro et al., 2017). But the effective choice and introgression of resistant genes to the host (common bean) in order to develop a resistant variety depends on the knowledge of the variability of *C. lindemuthianum*.

Mahuku and Riascos (2004), Kelly and Vallejo (2004), Silva et al. (2007), and Vidigal et al. (2007) reported that *C. lindemuthianum* has a high rate of evolution resulting in emergence of the new race of the pathogen from time to time. This causes resistance breakdown for the already developed resistant varieties because the resistance of common bean to *C. lindemuthianum* is very specific, so that a resistance gene from a bean genotype confers protection against specific isolates or races of *C. lindemuthianum*. Therefore, before breeding for anthracnose resistant varieties, it is important to study and identify the available races of *C. lindemuthianum* which is helpful in planning suitable breeding strategies for varieties with durable resistance and to design effective gene pyramids and deployment of resistant cultivars over time and space.

In Tanzania, although some of the works to develop resistant varieties to bean anthracnose have been done, information on *C. lindemuthianum* variability and the race identification is still limited for effective breeding for the anthracnose disease. Therefore, the present work focused on studying the variability of *C. lindemuthianum* in major bean growing regions of Tanzania and identifying races of *C. lindemuthianum* available in such areas using differential bean cultivars as proposed by Pastor-Corrales (1991).

## MATERIALS AND METHODS

### Collection of bean sample infected with anthracnose

Collection of common bean samples infected with anthracnose disease was done in five bean growing zones in Tanzania (Figure 1) during 2016/2017 bean growing season. Selection of zone was based on potential common bean production. In each zone, the distance between sampling fields was separated by a distance of 2 to 5 km. A minimum of five fields were sampled per zone. In the field, quadrants of 3 m x 3 m were used to randomly collect anthracnose symptomatic bean plants. A total number of four subplots identified by quadrants per field were established and, in each subplot four to nine plants were sampled. The collected infected plant parts were pods, leaves and stems. The pod samples collected were pressed well and stored in the herbarium, while

other bean pods of the same plant were preserved in silica gel. Leaves and stems were pressed well in the herbarium before they were taken to the plant pathology laboratory at Sokoine University of Agriculture, Morogoro for isolation and characterization of *C. lindemuthianum*.

### Pathogen isolation and inoculum preparation

Pathogen isolation was performed from the well-developed anthracnose diseased bean pods and leaves. Selected samples were sterilized for two minutes using 2% sodium hypochlorite (NaOCl) solution and rinsed well using sterile distilled water. Small cuts of 5cm long were made on the symptomatic areas and the cut placed on the prepared V8 growth media (17 g micro agar, 3 g of calcium carbonate, 200 ml of V8 juice and 800 ml of distilled water mixed together and autoclaved at 120°C and 2 bars of pressure for 15 min). The culture media were then incubated in the dark room at 22 to 24°C for seven days. From well grown isolates, mono-conidial isolates were obtained by picking pin-point colonies from the media using a sterile needle, and then sub-cultured on fresh V8 medium for purification of cultures using the single spore technique; and then single spore isolates were incubated aseptically. The standard spore suspensions for each obtained fungal isolate were prepared following the procedure of (Pastor-Corrales et al., 1995) and were stored at -4°C before inoculation to the differential bean cultivar.

### Raising differential cultivars and inoculation

A set of differential cultivars as described by Pastor-Corrales (1991) (Table 1) obtained from the Bean Improvement Program at Sokoine University of Agriculture (SUA) were planted in pots in the screen house at the Department of Crop Science and Horticulture, SUA. At fourteenth days after sowing, each differential cultivar seedling was inoculated separately by standard *C. lindemuthianum* inoculum prepared from the obtained isolate. The spore concentration used were quantified and adjusted to spore concentrations of  $1.2 \times 10^6$  spores/ml (Pastor-Corrales et al., 1995) using the hemocytometer (Neubauer-Precis Chamber) and the light microscope. The inoculation was done using the hand sprayer and the experiment was replicated three times.

In order to maintain the relative humidity of approximately 95% for the infection to take place, each plant was covered by the transparent polythene sheets for four days after inoculation followed by regular management such as watering and fertilizer application.

### Data collection and data analysis

Symptom evaluation and scoring of anthracnose disease severity on inoculated bean plants was done eight days after inoculation using a scale of 1-9 previously used by Balardin et al. (1990). The average disease scores were obtained and the disease reaction type categories were determined. Cultivars with scores of 1-3 were regarded as resistant while cultivars with scores of 4-9 were regarded as susceptible (Barladin et al., 1990). Race identification was done as the sum of binary numbers of all differential cultivars showing susceptible reaction to the particular isolate.

## RESULTS

Phenotypic characterization of *C. lindemuthianum* done by using set of differential cultivars as proposed by Pastor-Corrales (1991) was able to identify 50 isolates

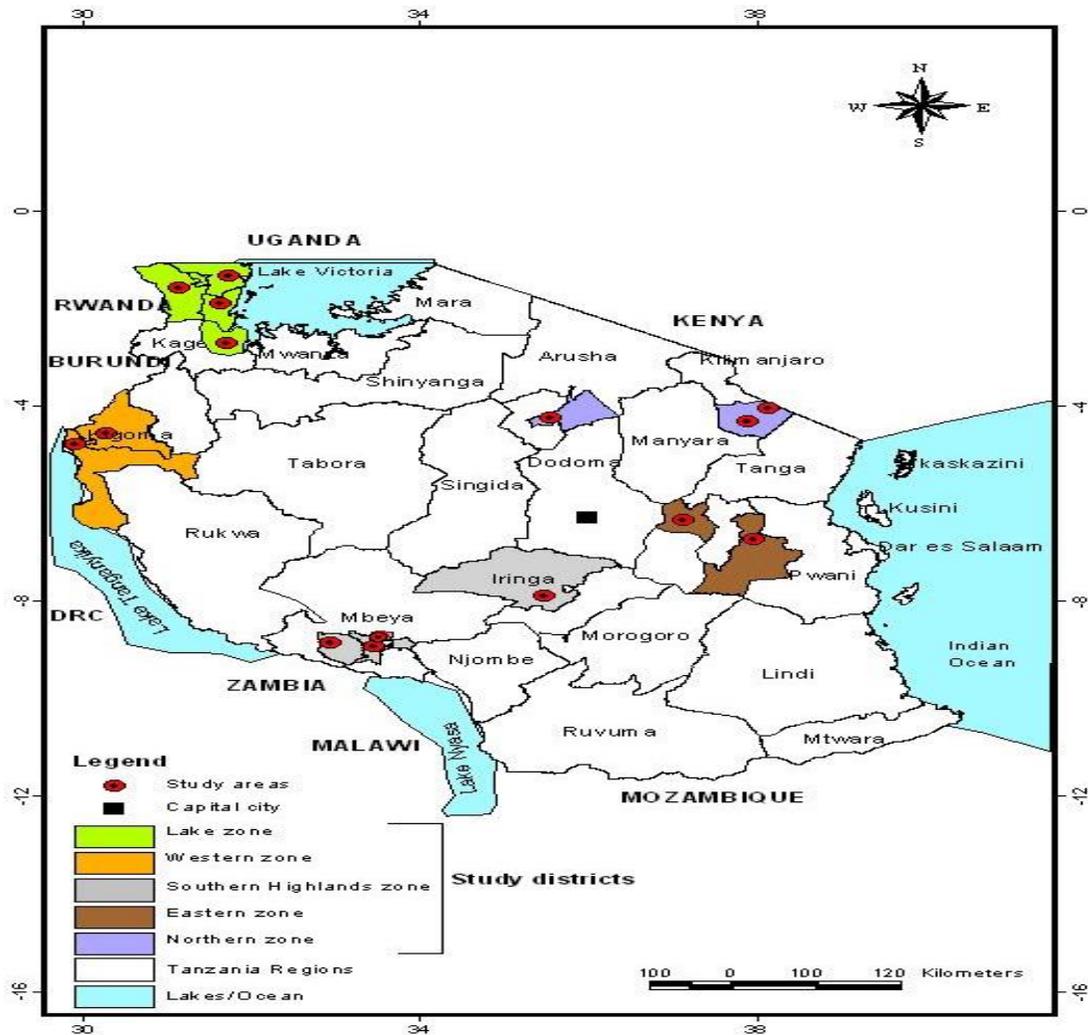


Figure 1. The map of Tanzania showing regions where isolates were collected.

Table 1. Anthracnose differential series, resistance genes, host gene pool, and the binary number of each cultivar used to characterize races of anthracnose in common bean.

Differential cultivar	Host genes	Place of cultivar	Gene pool	Binary number	Growth habit
Mitchelite	-	0	MA	1	II
Michigan Dark Red Kidney	<i>Co1</i>	1	A	2	II
Perry marrow	<i>Co-1<sup>3</sup></i>	2	A	4	II
Cornel 49242	<i>Co-2</i>	3	MA	8	II
Widusa	<i>Co-9</i>	4	A	16	II
Kaboon	<i>Co-1<sup>2</sup></i>	5	A	32	II
Mexico 222	<i>Co-3</i>	6	MA	64	II
PI	<i>Co-4<sup>3</sup>, Co-9</i>	7	MA	128	III
TO	<i>Co-4</i>	8	MA	256	I
TU	<i>Co-5</i>	9	MA	512	III
AB136	<i>Co-6, Co-8</i>	10	MA	1024	IV
G2333	<i>Co-4<sup>2</sup>, Co-5, Co-7</i>	11	MA	2048	IV

MA= Middle American gene pool; A= Andean gene pool of *Phaseolus vulgaris*. Binary numbers:  $2^n$ , where n is equivalent to the place of the cultivar within the series (0-11). Growth habit: I = Determinate; II = Indeterminate bush; III = Indeterminate bush with weak main stem and prostrate branches; IV = Indeterminate climbing habit.

Source: Pastor-Corrales (1991).

**Table 2.** The number of isolates and races of *Colletotrichum lindemuthianum* identified in each zone by phenotypic characterization.

Zone	Number of isolates	Races identified
Eastern zone	6	2, 31, 39, 129, 716, 770
Northern Zone	10	0, 2, 62, 91, 167, 277, 344, 524
Western Zone	15	0, 105, 166, 191, 274, 398, 661, 832, 849, 1510, 1805, 1891, 2061, 2614, 3068
Lake zone	8	0, 9, 101, 112, 128, 133, 1176, 1271
Southern Highland Zone	11	0, 2, 128, 316, 776, 944, 1696, 2434, 2566, 3264, 3610

from 144 bean samples collected. Of these 50 isolates obtained, 15 were from Western zone, 8 from Lake zone, 10 from Northern zone, 11 from Southern highland zone and 6 isolates were from Eastern zone (Table 2). Further it was possible to identify 42 races out of 50 isolates obtained (Tables 2 and 3). The most virulent race was race 3610 which was collected from the Southern Highland zone while the least virulent isolate was characterized as race 0, found in the Northern, Lake, Western and Southern Highland zones (Tables 2 and 3). From the Eastern zone, six isolates of *C. lindemuthianum* were identified out of all samples which were collected (Table 2). No isolate from the Eastern zone was able to infect the differential cultivar G2333 while differential cultivar Michigan Dark Red Kidney was the most affected differential cultivar with the four isolates. From the Eastern zone, the least virulent isolate was race 2 while the most virulent was characterized as race 770 which affected the differential cultivars Michigan Dark Red, TO, TU and AB136 (Table 3). From the Northern bean growing zone of Tanzania, among a total of 10 isolates collected, 8 races of *C. lindemuthianum* isolates were characterized (Table 2). These isolates affected almost all the differential cultivars except AB136 and G2333. Differential cultivars Michigan dark red kidney, Perry Marrow, Cornell and Widusa were highly infected with four isolates from the northern zone. The most virulent isolate was characterized as race 524 while the least was characterized as race 0 (Table 3). From the western zone, 15 isolates were obtained (Table 2). All differential cultivars were affected by these isolates including the most resistant differential cultivar G2333, which was affected by two isolates while Perry Marrow was the most affected differential cultivar. From this zone, the most virulent race was race 3068 while the least virulent race was race 0 (Table 3).

A total of eight isolates of *C. lindemuthianum* were obtained from the Lake zone (Table 2). These isolates could not affect differential cultivars G2333, TO and TU while Michelite was the most affected differential cultivar by four isolates (Table 3). The most virulent isolate from the Lake zone was characterized as race 1271 while the least virulent isolate from this zone was characterized as race 05 (Tables 2 and 3). From the Southern highland zone, 11 isolates were obtained in which (Table 2). Michelite was the only differential cultivar not affected by

any isolate from this zone; while TU was the most affected differential cultivar affected by four isolates (Table 3). Isolates from this zone were able to infect the differential bean cultivar G2333 (Table 3). The most virulent isolate from the Southern Highland zone was characterized as race 3610 which infected differential bean cultivars Widusa, TU, AB136 and G2333; while the least virulent isolate was characterized as race 0 (Table 3).

Among the differential bean cultivars used, Perry Marrow was the most susceptible differential cultivar and it was infected by 42% of all *C. lindemuthianum* isolates collected while differential bean cultivar G2333 was the most resistant cultivar to most of the collected isolates; it was affected by only 14 % of isolates collected, and these were from the Western and Southern Highland zones (Tables 2 and 3).

Of all the races identified in bean growing zones of Tanzania, race 2 was the most common with high frequency of 4.8 % and widely distributed within bean growing zones; and it was distributed in Northern, Southern Highland and in Eastern zones, while race 128 was only distributed in Lake and Southern Highland zones. Race 3610 collected from the Southern Highland zone was the most virulent isolate compared to all isolates collected from bean growing zones while the least virulent race identified was characterized as race 0.

## DISCUSSION

The phenotypic characterization of *C. lindemuthianum* in major bean growing areas in Tanzania revealed the existence of races and the variability of *C. lindemuthianum*. This study reveals new races of *C. lindemuthianum* which were not reported before. Earlier studies by Ansari et al. (2004) identified five races (0, 2, 12, 38 and 192) of *C. lindemuthianum* in Tanzania whereby only races 0 and 2 were also reported by this work distributed in all five zones.

This study confirms the evolution of *C. lindemuthianum* with time in Tanzania. The work of Mwalyego (1991) identified races 6, 28, 60, 63, 98, 155, 182, 287, 618, 958, 1478, 1515 and 1678 whereby out of them no races were reported in this study.

In this study 96% of the races identified from the

**Table 3.** Reaction of differential cultivars to the isolates of *Colletotrichum lindemuthianum* and identification of races.

Isolate number	Differential cultivars												Race name
	MT	MDR	PM	CL	WD	KB	Mex	PI	TO	TU	AB 136	G2333	
LZ02	R	R	R	R	R	R	R	R	R	R	R	R	0
WZ06	R	R	R	R	R	R	R	R	R	R	R	R	0
NZ06	R	R	R	R	R	R	R	R	R	R	R	R	0
NZ07	R	R	R	R	R	R	R	R	R	R	R	R	0
SHZ01	R	R	R	R	R	R	R	R	R	R	R	R	0
NZ09	R	R	R	R	R	R	R	R	R	R	R	R	0
NZ10	R	S	R	R	R	R	R	R	R	R	R	R	2
SHZ09	R	S	R	R	R	R	R	R	R	R	R	R	2
EZ02	R	S	R	R	R	R	R	R	R	R	R	R	2
LZ07	S	R	R	S	R	R	R	R	R	R	R	R	9
EZ03	S	S	S	S	S	R	R	R	R	R	R	R	31
EZ06	S	S	S	R	R	S	R	R	R	R	R	R	39
NZ05	R	S	S	S	S	S	R	R	R	R	R	R	62
NZ08	S	S	R	S	S	R	R	R	R	R	R	R	91
LZ03	S	R	S	R	R	S	S	R	R	R	R	R	101
WZ01	S	R	R	S	R	S	S	R	R	R	R	R	105
LZ05	R	R	R	R	S	S	S	R	R	R	R	R	112
LZ08	R	R	R	R	R	R	R	S	R	R	R	R	128
SHZ08	R	R	R	R	R	R	R	S	R	R	R	R	128
EZ01	S	R	R	R	R	R	R	S	R	R	R	R	129
LZ01	S	R	S	R	R	R	R	S	R	R	R	R	133
WZ10	R	S	S	R	R	S	R	S	R	R	R	R	166
NZ01	S	S	S	R	R	S	R	S	R	R	R	R	167
WZ09	S	S	S	S	S	S	R	S	R	R	R	R	191
WZ05	R	S	R	R	S	R	R	R	S	R	R	R	274
NZ03	S	R	S	R	S	R	R	R	S	R	R	R	277
SHZ10	R	R	S	S	S	S	S	R	S	R	R	R	316
NZ04	R	R	R	S	S	R	S	R	S	R	R	R	344
WZ03	R	S	S	S	R	R	R	S	S	R	R	R	398
NZ11	R	R	S	S	R	R	R	R	R	S	R	R	524
WZ11	S	R	S	R	S	R	R	S	R	S	R	R	661
EZ04	R	R	S	S	R	R	R	S	R	R	R	R	716
EZ05	R	S	R	R	R	R	R	R	S	S	R	R	770
SHZ03	R	R	R	S	R	R	R	R	S	S	R	R	776
WZ07	R	R	R	R	R	R	S	R	S	S	R	R	832
WZ13	S	R	R	R	S	R	S	R	S	S	R	R	849
SHZ07	R	R	R	R	S	S	R	S	S	S	R	R	944
LZ04	R	R	R	S	S	R	R	S	R	R	S	R	1176
LZ06	S	S	S	R	S	S	S	S	R	R	S	R	1271
WZ12	R	S	S	R	R	S	S	S	S	R	S	R	1510
SHZ06	R	R	R	R	R	S	R	S	R	S	S	R	1696
WZ15	S	R	S	S	R	R	R	R	S	S	S	R	1805
WZ14	S	S	R	R	R	S	S	R	S	S	S	R	1891
WZ04	S	R	S	S	R	R	R	R	R	R	R	S	2061
SHZ11	R	S	R	R	R	R	R	S	S	R	R	S	2434
SHZ02	R	S	S	R	R	R	R	R	S	S	R	S	2566
WZ08	R	S	S	R	S	S	R	R	R	S	R	S	2614
WZ02	R	R	S	S	S	S	S	S	S	S	R	S	3068
SHZ04	R	R	R	R	R	R	S	S	R	R	S	S	3264

Table 3. Contd.

SHZ05	S	R	R	R	S	R	R	R	R	S	S	S	3610
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NZ = Northern Zone, SHZ = Southern Highland Zone; LZ = Lake Zone, WZ = Western zone and EZ = Eastern Zone; R = resistance reaction, S = Susceptible reaction; MT = Michelite, MDR = Michigan Dark Red Kidney, PM = Perry Marrow, CL = Cornell 49242, WD = Widusa, KB = Kaboon, Mex = Mexico 222, PI = PI 207262.

collected isolates have not been reported before in Tanzania. The previous work done by Ansari et al. (2004) to study the variability of *C. lindemuthianum* of isolates from Tanzania who also identified five races (0, 2, 12, 38 and 192) out of 8 isolates which were characterized and race 0 and 2 were common races which were also identified from this work. This could be due to the extensive collection of isolates which was done during this study; it covers large area growing common bean. Some new races could have evolved from when the last study was conducted in the country till now when this study was conducted. Most of the virulent isolates were found in areas where the weather condition favors the growth and development of the pathogen (Mohamed, 2013). For example, the most virulent isolate characterized as race 3610 was found in the Southern Highland zone. This zone was characterized by very cool and humid conditions to most of its areas during the bean growing season (Tanzania Meteorological Agency, 2017). Such conditions favor growth and developments of *C. lindemuthianum* which may influence its variability as it have been reported by Tu (1983) and Mohammed (2013).

This study shows that *C. lindemuthianum* in Tanzania is not specialized to either Meso-American or Andean common bean gene pool only. The collected isolates were able to affect differential bean cultivars of both Meso-American and Andean gene pool. For example isolate SHZ05 from the Southern Highland zone was able to overcome both Andean resistance gene *Co-9* in Widusa and Meso-American resistance genes (*Co-4<sup>2</sup>*, *Co-5*, *Co-7*) in G2333, *Co-6* and *Co-8* in AB136 and *Co-5* in TU. Similar work of Balardin et al. (1990) and Balardin et al. (1997) indicated that some races contain virulence factors to both Meso-American and Andean gene pools and they infect both Andean and Meso-American bean gene pools. In the areas with large host diversity of both Mesoamerican and Andean at the same time it highly influences the pathogen diversity such as isolates collected from Western zone. Therefore, in order to develop the durable resistance variety, it is important to incorporate more than one resistant gene by gene pyramiding technique as it was also proposed by Balardin and Kelly (1998), Kelly and Vallejo (2004), Pastor-Corrales et al. (1995) from both Meso-American and Andean sources so as to provide the broader range and durable resistance variety.

Out of 12 differential cultivars used, G2333 was the

most resistant differential bean cultivar affected by only 7 isolates from the Western and Southern Highland zones only. Previous work reported that the presence of three resistant genes (*Co-4<sup>2</sup>*, *Co-5* and *Co-7*) in G2333 offer broader resistance to a wide range of *C. lindemuthianum* races (Young et al., 1998; Mahuku et al., 2002). Therefore based on races identified in Northern, Eastern and the Lake zones, G2333 can be used as a potential donor for resistance genes in bean breeding program for the varieties to be grown in those areas, according to Mwalyego (1992a) who reported 15 separate isolates and none of them was pathogenic to G2333 bean cultivar. But varieties that are to be grown in the Southern Highland and Western zones should be incorporated with genes for resistance from other sources. de Lima Castro et al. (2017) reported Paloma cultivar as a new source of resistance gene to common bean anthracnose. This cultivar has a new dominant gene conferring resistance to anthracnose, which is independent of *Co-1*, *Co-2*, *Co-3<sup>4</sup>*, *Co-4*, *Co-4<sup>2</sup>*, *Co-4<sup>3</sup>*, *Co-5*, *Co-6*, *Co-12*, *Co-13*, *Co-14*, *Co-15* and *Co-16* resistance genes identified previously; this gene is named *Co-Pa*. Therefore, in the future Paloma cultivar can be included in the list of differential cultivars before being recommended as the donor source of resistant genes to the cultivars grown in the Western and Southern Highland zone. Also more characterization work should be done on (*Co-Pa*) resistant genes in Paloma.

## Conclusion

This study indicates that the pathogen, *C. lindemuthianum* which causes anthracnose disease, *C. lindemuthianum* shows variability in major bean growing areas of Tanzania. The pathogen seems to be widely distributed in all bean growing areas with the most virulent isolate from the Southern Highland zone. The best control method for the disease in these potential bean growing areas is using the host resistance. It is recommended to use *Co-4<sup>2</sup>*, *Co-5* and *Co-7* from G2333 to develop resistant varieties to be grown in the all zones except in Southern and Western zone. The research should also focus on characterizing other genes that can offer resistance to races from Western and Southern Highland zones that were able to overcome resistance genes in G2333 such as *Co-Pa* in cultivar Paloma.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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