Analysis of Western Kenya Isolates of *Colletotrichum lindemuthianum* Casual Agent of Bean Anthracnose using Pathogenicty and BOX-AIR Analysis

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Abstract

Anthracnose disease of common bean (Phaseolus vulgaris), caused by Colletotrichum lindemuthianum, is one of the major causes of yield losses. This pathogen is known to vary greatly in its pathogenicity challenging its control strategies which include, mainly development of resistant cultivars. The objective of this study was to investigate the pathogenic and genetic diversity among the C. lindemuthianum isolates collected from Bungoma, Kitale, Kakamega, Nandi, Busia, Kisii, Siaya of Western Kenya. One hundred and twenty two C. lindemuthianum isolates were obtained from bean pods with anthracnose symptoms collected from farmers' fields during the two surveys. Virulence on a standard set of 12 common bean differential varieties and BOX-AIR analysis was used to asses the variability of the isolates. High levels of pathotypic (74pathotypes) were identified among 122 isolates, revealing that C. lindemuthianum is a highly diverse pathogen. Races 0 was the most frequent race and occurred in most regions. The most virulent races were less frequent and occurred in a single region only. 14 races broke the resistance of the most resistant cultivar G2333 indicating their high virulence. BOX-AIR analyzes performed on 60 of the 122 isolates revealed great genetic diversity, clustering the isolates into 8 groups. The races that clustered together were not the same nor from same region of collection. Due to the high virulence and genetic variation exhibited by C. lindemuthianum, breeding for more resistance genes appears to be the best option for developing cultivars with durable anthracnose resistance in Western Kenya.

Keywords: *Anthracnose, Common Bean, Virulence, Genetic Variability, BOX- analysis*

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important legume food crop widely cultivated and consumed in Kenya. Total area under common bean cultivation is estimated to be 910,478 hacters with average production of 0.5 tonnes ha^{-1} (FAO, 2008).However this production is much less compared to the yield potential of up to 1–2 tonnes ha^{-1} (Muasya, 2001). Further, yield has been declining at an average rate of 6.8 percent (MOARD, 2004). Bean anthracnose a seedborne disease caused by a cosmopolitan fungi *Colletotrichum lindemuthianum* (Sacc & Magnus) Lams. – Scrib. (Barrus, 1911) is one of the major causes of yield loss. The fungus causes dark brown sunken lesions on all above ground parts including the seeds (Jerba *et al.*, 2005). Currently, more than 75% small scale farmers in Western Kenya sow uncertified farm-saved seeds from previous crop season because they are economical and readily available (Opile *et al.*, 2003; Otsyula *et al*., 2004). The farm-saved seeds are uncertified and their quality is compromised by bean anthracnose among other factors. Anthracnose infected seeds if planted significantly cause blemishes on pods, distortion and discoloration of the seed (del Río & Bradley, 2002) leading to reduction of the crop stands, seed quality, marketability and yield (Fernandez *et al.*, 2000). Seed infections as low as 0.01- 0.1% can cause very severe yield losses exceeding 90% depending on the agro-ecological and weather conditions (Yesuf & Sangchote, 2007). Previous results from Tanzania estimated that for each 1 percent increase in anthracnose incidence, seed yield decreased by 9kg/ha (Allen *et al*., 1998).

Use of host resistance and chemical methods are among the most effective anthracnose control strategies. However, the use of these methods is challenged by the highly variable nature of the pathogen which brings about continuous breakdown of resistance in the bean cultivars (Bigirimana & Hofte, 2001). This challenge has made it difficult to develop effective anthracnose resistant cultivars. Additionally indiscriminate uses of chemical control lead to degradation of the environment and development of fungicidal resistance. *C. lindemuthianum* exists in many physiological forms in all bean growing regions and new ones reportedly keep emerging time after time (Mahuku & Riascos, 2004; Nkalubo, 2006; Sharma *et al.*, 2008). Ombiro *et al*. (2002) acknowledge the existence a new race, race 485 in Rongai, Kenya, on addition to the previously existing six races; Beta, Alpha, Gamma, Delta, Epsilon and Lambda.

This means that race composition in any given area is continuously changing and thus continuous monitoring of their composition is necessary. Also, development of resistant cultivars depends to a large extend on levels of pathogenic variability exhibited by the pathogen therefore study on the variability of *C. lindemuthianum* in Western Kenya was necessary. However use of pathogenicity test to characterize the pathogen is long, tedious and subject to environmental conditions. Use of molecular markers has become very popular in pathogen characterization because they are environment-neutral, fast and more informative in assessing variability within organisms. BOX-AIR technique a Rep-PCR analysis is one of the molecular markers that have been used to study genetic diversity (Mahuku & Riascos, 2004; Bardas *et al.*, 2009; Mwesigwa, 2009). In Kenya studies on diversity of the pathogen using molecular markers has not been done. The objective of this investigation was to characterize *C. lindemuthianum* isolates in Western Kenya using differential cultivars and BOX analysis. This could direct breeding efforts towards long-term resistance to anthracnose.

Materials and Methods

Fungal Collection and Isolation

The study was carried out in seven major bean growing regions of Western Kenya: Bungoma, Kakamega, Kitale, Nandi, Busia, Kisii and Siaya between November 2008 and July 2010. Systematic random sampling was used to select bean fields at regular intervals of 4-10 km along accessible routes. Five mature bean pods with pronounced anthracnose symptoms were collected at 10-20 m intervals in every field. From each diseased pod small pieces of infected (0.5 cm^2) parts were cut, held in running tap water for 30 seconds, surface sterilized by immersion in an aqueous solution of commercial bleach (10 % v/v) for 60 seconds and rinsed twice in sterile, distilled water. The pieces were plated aseptically on a labeled Potato dextrose agar (PDA) petridishes and incubated in complete darkness at 20-21^oC for 7 days. Streptomycin sulphate (0.1 gl^{-1}) was added to the inoculated media to suppress bacterial growth. The pathogen was identified based on its morphological characteristics (Mathur & Kongsdal, 2003).

Single spore isolates were prepared as described by Ombiri *et al.* (2002). Small pieces of hyphae from the distinct colonies that showed growth and morphology of typical *Colletotrichum lindemunthianum* fungi were transferred onto PDA media and incubated for 7 days in darkness at 20-21^oC. The culture plates were flooded with sterile distilled water then spores were dislodged by scrapping the culture surface with a spatula. Two to three milliliter conidial suspension were aseptically spread on Water Agar (WA) in Petri dish and incubated in darkness at 20-21^oC for 24 hours. The cultures were examined under a microscope at 25 X magnification for germination of conidial on WA. Three germinated conidia per culture-plates were removed using a thin isolation needle and aseptically plated separately on labeled PDA plates. After 4 to 5 days the cultures were sub cultured on PDA plates and incubated for 14 days. 122 single spore cultures replicated three times were obtained and stored at 4 ^oC for further study.

Virulence of 122 isolates was determined using pathogenicity tests on 12 defined common bean differential cultivars (Table 1) obtained from Centro Internacional De Agricultura Tropical (CIAT), Cali Colombia and multiplied in an anthracnose-free green house under strict supervision to avoid cross-pollination and seed mixture. The inoculums were prepared from 14 days old single spore cultures. The cultures were flooded with 5ml sterile distilled water, the surface scrapped with a sterile glass rod and the spores were filtrated through a double layer of cheese cloth to remove the mycelia mass. Spore concentration was calibrated to 1.2 $x10^6$ spores per milliliter using a haemacytometer and suspended in Tween 80 (0.05%, V/v). Primary leaf stage differential seedlings were sprayed with inoculum suspension on both sides of the leaves, petiole and stem until run off. The inoculated plants were kept in moistened chambers at 21 ± 2^oC temperatures with more than 95% relative humidity for 48 hours and then transferred to a green house with a temperature ranging from 21-24^oC and relative humidity of about 70%. Disease reactions were scored on the 7th day after inoculation based on a 1-5 disease severity descriptive scale (Ombiri *et al.*, 2002) where : 1- no symptoms; 1.5 - Light infection (pin point lesions); 3 - Moderate infection (up to 5 small to large sunken lesions-about 3mm); 5 - severe infection (large deep lesions- larger than 3mm- and /or death). Plants showing the reaction type between 1.0 and 1.5 were considered resistant while those showing reaction types above 1.5 were considered susceptible. To obtain a virulence data, disease severity index (DSI) of each isolate was computed using the following formula (Ombiri *et al.*, 2002):

$$DSI= (1 X n_1 + 1.5 X n_2 +3 X n_3 +5 X n_4) /N$$

Where 1, 1.5, 3 and 5 are the severity scales; n_1 to n_4 refers to the number of seedlings with the respective severity scale; N refers to the total number of seedlings examined per variety per isolate.

Based on the isolates virulence to the 12 standard bean differential cultivars, different physiological races of *C. lindemuthianum* were identified in Western Kenya using binary nomenclature system (Pastor-Corrales, 1991). Briefly, each differential cultivar had an assigned number (2^n), where n corresponds to the order number of the cultivar within the 12 standard bean differential series. The race designation was obtained by summing the 2^n values of all cultivars compatible with the isolate used for inoculation.

BOX Analysis

The 122 *C. lindemuthianum* mycelia was obtained for DNA extraction (Mesquita *et al*., 1998). Briefly, a 2mm culture plug from an actively growing margins of 14 days old single spore cultures were used to inoculate 50 ml of Potato Dextrose Broth media (PDB) flask. The flasks were incubated under constant agitation at 108 rpm at 22-25 ^{O}C in the dark for 7 days. Mycelium were harvested by filtration through cheese cloth, surface dried, frozen in liquid nitrogen and ground to a fine powder using a sterilized mortar and pestle. DNA was extracted from 100mg of freeze-dried ground mycelia (Balardin *et al*., 1997). Extracted DNA was dissolved in 100 ul of TE and stored at – 20^{O}c. DNA Concentration was estimated in 1 % agarose gel and stained with ethidium bromide. The extracted DNA was amplified **(**Bardas *et al*. 2009) using BOX-AIR primer 5'CTACGGCAAGGCGACGCTGAC G3'. Amplification reaction were performed in a final volume of 20 ul with Bioneer AccuPower ^{TM} PCR premix, 150 ng DNA, 0.2mM BOX primer and 18 µl molecular grade water. To ensure that amplification products were not primer artifacts genomic DNA was omitted from the control reaction. DNA amplification was performed in a thermal cycler programmed according to the following thermal profile: 1 initial denaturation cycle at 95 ^{O}C for 7 min, 30 step cycles - denaturation 94 ^{O}C for 1 min, annealing at 53 ^{O}C for 1 min and extension at 65 ^{O}C for 8 min and by a final extension step at 65 ^{O}C for 15 min. The amplification was conducted twice for each isolate. The amplified PCR products were separated by electrophoresis at 70 V for 2.5 hours on 2 % agarose gel 0.5x TBE running buffer. The products were detected by staining with 0.5 % ethidium bromide. A 100 bp DNA ladder was used as a molecular weight marker. The gels were visualized under UV light (Fotodyne Ultraviolet Trans- illuminator; Fotodyne Inc., New Berlin, WI, USA) before being photographed. The reaction was run in duplicate and only 60 isolates of 122 isolates which had well defined and reproducible bands were analyzed.

To generate a BOX binary data, each polymorphic band in every sample was considered a locus with two alleles: presence or absence of amplified fragments. Each band was then scored as a binary character (1 for presence and 0 for absence). The binary data was used to calculate pair wise genetic distances with the Similarity for Qualitative Data Program (SIMQUAL) in the Numerical Taxonomy and Multivariate Analysis System for personal computer (NTSYS-pc (version 2.1 (NTSYS, New York, NY, USA). Simple Matching co-efficient (SM) were used to compute the distances and thus SM similarity coefficient data was generated. The SM was used to construct a dendogram by the Unweighted Pair Group Method with Arithmetic Average (UPGMA) clustering method and tree program in NTSYS.

Results

Virulence Analysis

Disease scores were not significantly (P>0.05) different between replicates of the inoculated seedlings. All the 12 differential cultivars were susceptible to the isolates (Figure 1). The binary system of race classification based on pathogenicity testing assigned the 122 isolates to 74 physiological races (Table 1). Based on the race distribution (Table 2) in the study regions the races were categorized into two: Category one of 57 races which occurred only in the region where collected such as race 4045 found in Kisii only. Category two of 17 races each found in two or more regions such as race 0 found in five different regions.

Based on pathogenicity of the pathogen on the differentials the races were categorized into four. Category one (9 races) of Andean origin as they predominantly attacked beans of Andean gene pool such as race 6. Category two (16 races) of Meso American origin as they predominantly attacked beans of Meso American gene pool such as race 64. Category three (97 races) of a wider range of virulence as they predominantly infected beans of both Andean and Meso American origin such as race 21. Category four (1 race) which had no compatible reaction with any of the differential cultivars and was assigned race zero (0).

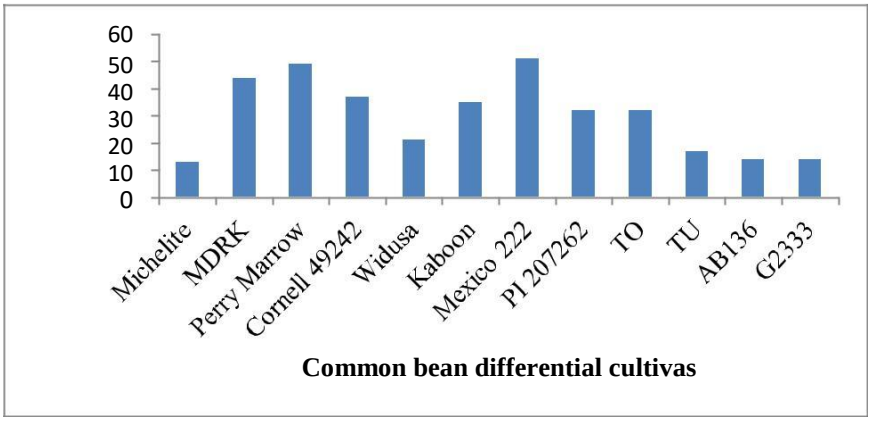

Figure 1. Susceptibility of different common bean differential cultivars to the 122 solates Table 1. The reaction of the differential bean cultivars to the *C. lindemuthianum* isolates collected from W. Kenya

Race	*Differential cultivars*												*Race*	**Differential cultivars**											
	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**H**	**i**	**j**	**k**	**l**
0	r	r	r	r	r	r	r	r	r	r	r	r	320	r	r	r	r	r	r	s	R	s	r	r	r
3	s	s	r	r	r	r	r	r	r	r	r	r	324	r	r	s	r	r	r	s	R	s	r	r	r
4	r	r	s	r	r	r	r	r	r	r	r	r	358	r	s	s	r	r	s	s	R	s	r	r	r
6	r	s	s	r	r	r	r	r	r	r	r	r	379	s	s	r	s	s	s	s	R	s	r	r	r
8	r	r	r	s	r	r	r	r	r	r	r	r	448	r	r	r	r	r	r	s	S	s	r	r	r
14	r	s	s	s	r	r	r	r	r	r	r	r	449	s	r	r	r	r	r	s	S	s	r	r	r
16	r	r	r	r	s	r	r	r	r	r	r	r	456	r	r	r	s	r	r	s	S	s	r	r	r
18	r	s	r	r	s	r	r	r	r	r	r	r	485	s	r	s	r	r	s	s	S	s	r	r	r
21	s	r	s	r	s	r	r	r	r	r	r	r	531	s	s	r	r	s	r	r	R	r	s	r	r
22	r	r	s	r	s	r	r	r	r	r	r	r	544	r	r	r	r	r	s	r	R	r	s	r	r
23	s	s	s	r	s	r	r	r	r	r	r	r	548	r	r	s	r	r	s	r	R	r	s	r	r
36	r	r	r	r	r	s	r	r	r	r	r	r	550	r	s	s	r	r	s	r	R	r	s	r	r
38	r	s	s	r	r	s	r	r	r	r	r	r	552	r	r	r	s	r	s	r	R	r	s	r	r
46	r	s	s	s	r	s	r	r	r	r	r	r	558	r	s	s	s	r	s	r	R	r	s	r	r
48	r	r	r	r	s	s	r	r	r	r	r	r	608	r	r	r	r	r	s	s	R	r	s	r	r
55	s	s	s	r	s	s	r	r	r	r	r	r	622	r	s	s	s	r	s	s	R	r	s	r	r
64	r	r	r	r	r	r	s	r	r	r	r	r	648	r	r	r	s	r	r	r	S	r	s	r	r
68	r	r	s	r	r	r	s	r	r	r	r	r	776	r	r	r	s	r	r	r	R	s	s	r	r
70	r	s	s	r	r	r	s	r	r	r	r	r	968	s	r	r	s	r	r	s	S	s	s	r	r
72	r	r	r	s	r	r	s	r	r	r	r	r	1028	r	r	s	r	r	r	r	R	r	r	s	r
80	r	r	r	r	s	r	s	r	r	r	r	r	1110	r	s	s	r	s	r	s	R	r	r	s	r
86	r	s	s	r	s	r	s	r	r	r	r	r	1189	s	r	s	r	r	s	r	S	r	r	s	r
92	r	r	s	s	s	r	s	r	r	r	r	r	1280	r	r	r	r	r	r	r	R	s	r	s	r
94	r	s	s	s	s	r	s	r	r	r	r	r	1463	s	s	s	r	s	s	r	S	s	r	s	r
96	r	r	r	r	r	s	s	r	r	r	r	r	2054	r	s	s	r	r	r	r	R	r	r	r	s
98	r	s	r	r	r	s	s	r	r	r	r	r	2124	r	s	r	s	r	r	s	R	r	r	r	s
102	r	s	s	r	r	s	s	r	r	r	r	r	2504	r	r	r	s	r	r	s	S	s	r	r	s
104	r	r	r	s	r	s	r	s	r	r	r	r	2540	r	r	s	s	r	s	s	S	s	r	r	s
110	r	s	s	s	r	s	s	r	r	r	r	r	2816	r	r	r	r	r	r	r	R	s	s	r	s
130	r	s	r	r	r	r	r	s	r	r	r	r	3073	s	r	r	r	r	r	r	R	r	r	s	s
134	r	s	s	r	r	r	r	s	r	r	r	r	3080	r	r	r	s	r	r	r	R	r	r	s	s
162	r	s	r	r	r	s	r	s	r	r	r	r	3136	r	r	r	r	r	r	s	R	r	r	s	s
192	r	r	r	r	r	r	s	s	r	r	r	r	3216	r	r	r	r	s	r	r	s	r	r	s	s
200	r	r	r	s	r	r	s	s	r	r	r	r	3447	s	s	s	r	s	s	s	r	s	r	s	s
256	r	r	r	r	r	r	r	r	s	r	r	r	3529	s	r	r	s	r	r	s	s	s	r	s	s
258	r	s	r	r	r	r	r	r	s	r	r	r	3546	r	s	r	s	s	r	s	s	s	r	s	s
267	s	s	r	s	r	r	r	r	s	r	r	r	4045	r	r	s	s	r	r	s	s	s	s	s	s
284	r	r	s	s	s	r	r	r	s	r	r	r	4049	s	r	r	r	s	r	s	s	s	s	s	s

a-Michelite (1); b-MDRK(2); c-Perry Marrow(4); d-Cornell 49-242(8); e-Widusa(16); f-Kaboon(32); g-Mexico 222(64); h-PI 207262(128); i-TO(256); j-TU(512); k-AB136(1024); l-G2333(2047). s- Compatible reaction; r- incompatible reaction (CIAT, 1988)

Table 2. Distribution of C. lindemuthianum races among 122 isolates collected from 7 regions of Western Kenya

Region	No. of isolates	Races detected
Bungoma	24	0; 0; 0; 4; 4; 8; 16; 64; 64; 64; 70; 70; 94; 94; 110; 130; 162; 192; 320; 449; 544; 544; 548; 548; 1463; 3546.
Kakamega	20	0; 0; 0; 48; 64; 70; 96; 98; ; 200; 200; 304; 558; 648; 968; 1028; 1110; 1189; 2054; 2504; 2540.
Kitale	14	0; 8; 8; 18; 21; 68; 324; 379; 608; 622; 776; 1280; 2816; 3216.
Nandi	14	3; 4; 14; 14; 16; 16; 80; 96; 104; 267; 284; 358; 552; 3447.
Busia	19	0; 22; 36; 38; 38; 46; 70; 92; 200; 448; 448; 448; 456; 456; 456; 550; 550; 3073; 3529.
Kisii	11	6; 36; 38; 72; 102; 134; ; 256; 258; 550; 4045; 4049.
Siaya	19	0; 4; 8; 38; 38; 38; 46; 70; 86; 134; 256; 256; 448; 456; 456; 531; 2124; 3080; 3136.

Molecular Analysis

From the 122 isolates, only 60 were successfully analyzed using BOX-AIR molecular markers. BOX primer replicates yielded the same banding profiles (Plate1). The polymorphic bands were scored and a dendrogram with 8 separate clusters (I-VIII) was obtained (Figure 2). The number of races within a cluster ranged from 41 races in cluster II to 1 race in clusters III, VI, VII and VIII. The multisolate clusters such as cluster I and II consisted of different races from different regions.

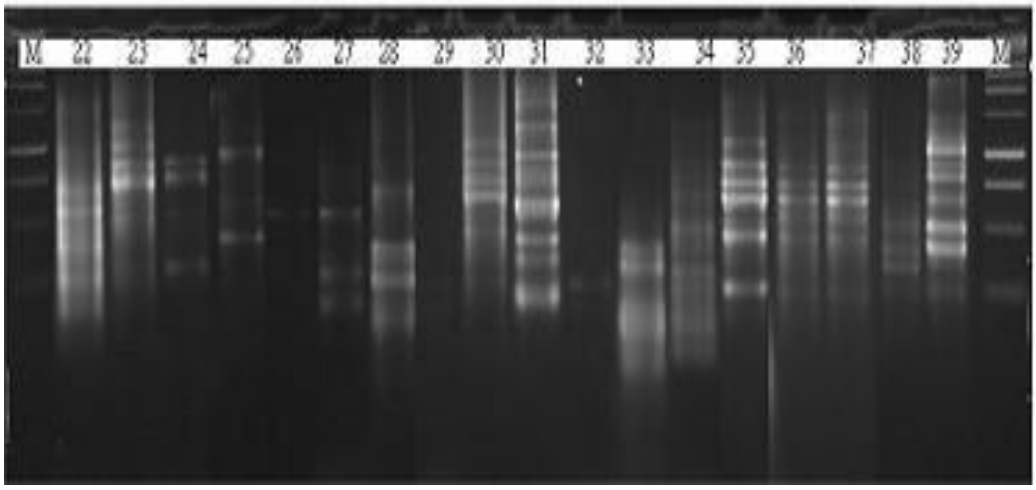

Plate 1. Part of PCR amplification products of genomic DNA of 60 selected races characterized from W. Kenya C. lindemuthianum isolates. M- Molecular weight Markers of 100kb DNA ladder

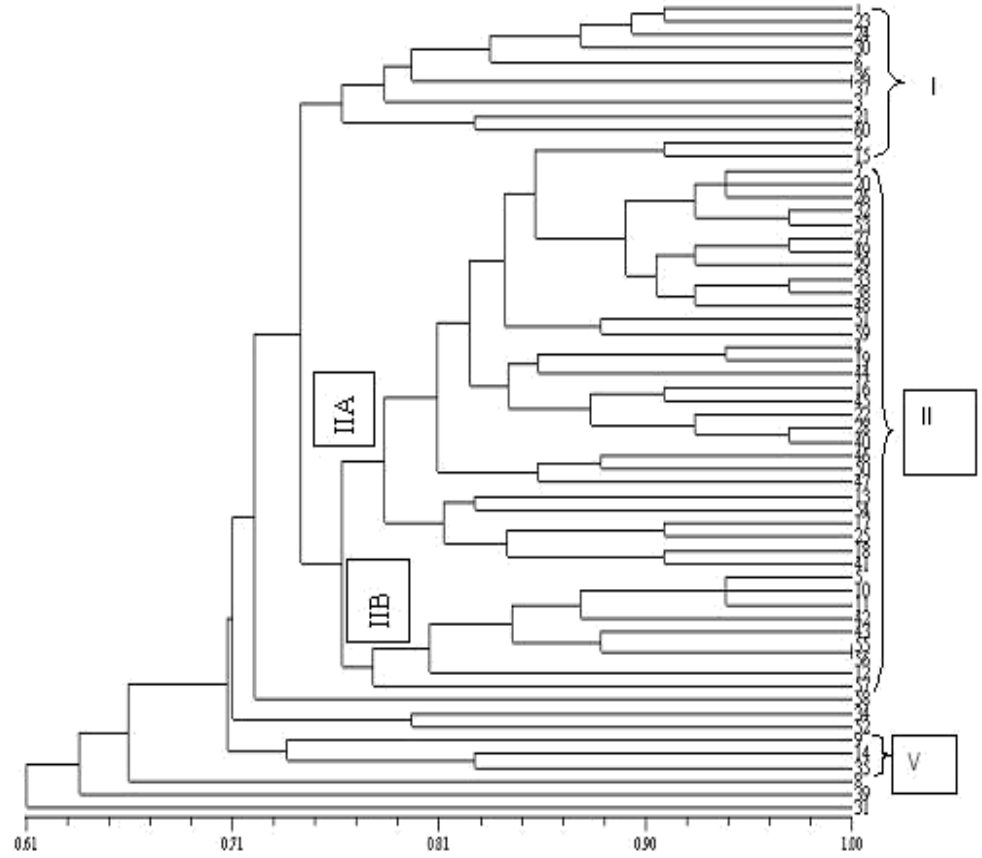

Figure 2. Dendogram showing similarities of 60 C. lindemuthianum isolates based on molecular polymorphism generated by BOX-AIR DNA analysis

Discussion

Virulance Characterization

The 122 isolates studied were designated into 74 races based on different patterns of virulence on the 12 differential cultivars. This was a high number of races which indicates high race diversity of the pathogen in Western Kenya. This is in agreement with Damasceno *et al.*, (2007), Mahuku *et al*. (2004) and Ansari *et al*. (2004) who have identified high levels of pathogenicity diversity of 10 races among 48 isolates, 90 pathotypes among 200 isolates and 30 races among 74 isolates respectively. In Kenya, Ombiri *et al*. (2002) has found one race in 4 isolates collected from Rongai Nakuru. The number of races detected in this study was high than in the previous seven races designated as race 17, 2, 38, 23, 1, 55 and 485 in Kenya (Kinyua, 1976; Mwangi, 1986; Gathuru & Mwangi, 1991). Only race 38 identified in the present study resembled the earlier ones. This implies that the pathogen in Western Kenya increasingly become diverse in virulence. Mahuku *et al.* (2004), Kelly *et al*. (2004) and Vidigal *et al*. (2007) have also indicated that *C. lindemunthianum* has high rates of evolution and new races keep emerging from time to time. Races of lower numbers were the most frequent and widely distributed such as races 0and 70. This agrees with the previous studies by Damasceno *et al.* (2007) who reported that widely spread races 5, 12, 73 and 81 of *C. lindemunthianum* infected a smaller number of differential cultivars.

Most mesoamerican races such as 44049 and 3080 were highly virulent. This indicates that high pathogen virulance was obseved in Mesoamerican races as they were of large number among the isolates and restricted to regions of collection. Therefore there is need for adequate planning and observation in the regions of occurance of the meso American races and host cultivars, to delay the dissemination or arrival of new races, and consequently, prolong the useful life of the cultivars indicated for cropping.

The differential cultivars used comprised four Andean and eight Meso American cultivars and all of them showed susceptibility responses to the 122 isolates hence presence of 12 virulence factors. This shows that races obtained in this study had more virulent genes against the differential cultivars since even the most resistant bean cultivars of Meso American gene pool were attacked. Susceptibility of cultivars TU, AB 136 and G2333 to *C. lindemuthianum* had rarely been reported around the world but in this study these cultivars were found to be susceptible to 14 isolates each. Mahuku *et al.*,2004 also

observed 33 races break the resistance of AB136 while races 3481, 3545, 3977 and 3993 break the resistance of G2333. This study show that stable resistance to *C.lindemunthianum* in Western Kenya might not be found in the primary gene pool. Mutation within the pathogen population can lead to a loss of avirulence factors producing a compatible reaction with previously resistant genes such as those in cultivars AB136 and G2333. The pathotype diversity displayed is a reflection of the dynamic nature of preexisting pathotypes and continued evolution of pathoypes in response to the introduction of new resistantance genes (Fabre *et al*., 1995; McDonald *et al.,* 2002). Therefore alternative sources of resistance must be sought.

Nine isolates had no compatible interaction with any of the differential cultivars and were assigned race zero (0). They did not fit into any of the two host gene groups. This reveals that there are more susceptible genes in the bean cultivars of Western Kenya that have not been incorporated in the differential cultivars. The breeders need to note this and consider incorporating more genes to the differential cultivars used in the race characterization.

BOX Analysis

The BOX-AIR primer generated products of 800-2072 bp and DNA polymorphism among 60 isolates. This BOX DNA amplicons pattern shows high polymorphism indicating the highly variable nature of the pathogen. The high molecular diversity was also observed by Mahuku and Riascos (2004) and Bardas *et al*. (2009).

In the dendogram the races that clustered together were not the same nor from same region of collection. This indicates that the genetic structure of *C. lindemunthianum* reveals no geographical differentiation in Western Kenya. These results are supported by the conclusion of Balardins *et al*. (1997), and Fabre *et al*. (1995) who reported that genetic structure of *C. lindemunthianum* reveal no geographical differentiation. This has important implications in deployment of resistace genes and directing programs tasked with developing anthracnose resistant cultivars. The clustering of isolates by region of collection is difficult because of free interchange of seeds (Opole *et al.,* 2003) infected with *C. lindemunthianum* spores among regions of Western Kenya. This agrees with previous studies that gene flow, sexual, asexual and parasexual reproduction (Damasceno *et al*., 2007), coupled with selection over time and driven by introduction of new varieties with new sources of resistance play major roles in generating and mantaining the high genetic variation observed in this pathogen.

Conclusion

Pathogenic diversity was expressed by occurrence of 74 races of the pathogen from122 different isolates. Both highly virulent as well as less virulent races were realized. 14 races were highly virulent as they were able to infect the highly resistant cultivar, G2333. Further, nine isolates were unable to cause infection or their severity scores were 1.5 or less; hence considered under resistant reactions and subsequently classified as race 0 (zero). 12 virulance factors obtained since all the cultivars succumbed to the isolates. Western Kenya population of *Colletotrichum lindemuthianum* should be regularly monitored for emergence of variable pathotypes since the current study revealed new races that were never found by previous researchers (Ombiri *et al.,* 2002).

Molecular markers are used extensively to differentiate fungal pathogens and when combined with virulence data, these markers often elucidate pathogen genetic diversity and evolutionary relationships of the plant pathogens (Bardas *et al*., 2009). This informaton provide a better view of the specific pathogen's variability, leading to more efficient control strategy (Mahuku and Riascos, 2004). The current study used Box and virulence markers to show that there exists high virulence and molecular diversity of *Colletotrichum lindemunthianum* in Western Kenya.

Recommendations

There is need to expand the scope of this study to cover the whole country so as to document the diversity of the *C. lindemuthianum* in Kenya.

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References

Allen, D. J., Buruchara, R. A., & Smithson, J. B. (1998). Diseases of Common Bean. In. D. J. Allen, & J. M. Lenne, (CIAT Eds.), *The pathology of Food and Pasture Legumes. CAB International*, (pp 179-265).

Ansari, K. I., Palacios, N., Araya, C., Langin, T., Egan, D., & Doohan, F. M. (2004): Pathogenic and genetic variability among *Colletotrichum lindemuthianum* isolates of different geographic origins. *Plant Pathology, 53,* 635-642. Blackwell Publishing, Ltd.

Bardas, G. A., Koutita O., & Kate T. (2009). Molecular diversity and assessment of biological characteristics of Greek *Colletotrichum lindemunthianum* populations. *Journal of Phytopathology, 157*, 311-318.

Barrus, M. F. (1911). Variation of varieties of beans in their susceptibility to anthracnose. *Phytopathology, 1*, 190-195.

Bigirmana, J., Fontaine, R., & Hofte, M. (2001). Bean anthracnose virulence of *Colletotrichum lindemuthianum* isolated from Burundi, Central Africa. *Plant diseases, 84*, 491.

Balardin, R. S., Jarosz A. M., & Kelly, J. D. (1997). Virulence and Molecular Diversity in from South, Central and North America. *Phytopathology, 87*, 1184-1191.

Centro International de Agricultura Tropical (CIAT) (1987). *Standard System for the evaluation of bean germplasm. Schoonhoven, A. Van and M.A. Pastor-Corrales (complilers).* Cali, Colombia.

Damasceno, K. J., Silva E., De Souza E. A., & Ishikawa F. H. (2007). Characterization of isolates from the state of Minas Gerais, Brazil. *Journal of Phytopathology, 155*, 241-247.

del Rí,o L. & Bradley, C. (2002). *Anthracnose of dry beans. North Dakota State University Extension Service.* Fargo, North Dakota 58105. p. 1233.

Fabre, J. V., Julien, J., Parisot, D., & Dron, M. (1995). Analysis of diverse isolates of *Colletotrichum lindemuthianum* infecting common bean using molecular markers. *Mycology Research, 99*, 429-435.

Food and Agricultural Organization (FAO) (2008). *Food Security Statistics*. www.fao.org 1995-2003.

Gathuru, E. M., Mwangi, S. F. M., & Mwangi, (1991). Importance of the disease and the Genus *Colletotrichum*. In R. A. Buruchara, R.A. (Ed.). *Proceedings of the first Pan African working group meeting on anthracnose of anthracnose of beans*. Ambo, Ethiopia. Feburuary 17-23. CIAT African Workshop series No. 15, pp 43-50.

Jerba, V. F., Rodella, R. A., & Furtado, E. l. (2005). Relationship between leaf structure and *Glomerella cingulata* f.sp. *phaseolus* preinfection Pesqui. *Agropecu. Bras*., pp 217-223.

Kinyua, G. K. (1976). The occurrence of race alpha of *Colletotrichum lindemuthianum* in Kisii district of Kenya. *Ministry of Agriculture (NHRS-Thika) report*. pp. 3.

Mathur, S. B., & Kongsdal, O. (2003). Common Laboratory Seed Health Testing Methods for Detecting Fungi (1st ed.). Bassersdorf, CH, Switzerland: International Seed Testing Association.

Mahuku, G. S., & Riascos, J. (2004). Virulence and Molecular diversity within *Colletotrichum lindemunthianum* isolates from Andean and Mesoamerican bean varieties and regions. *European Journal of Plant Pathology, 110*, 253-263.

McDonald, B. A., & Linde, C. (2002). Pathogen Population genetics, evolutionary potential and Durable resistance . *Annual Review of Phytopathology, 40*, 349-379.

Ministry of Agriculture and Rural Development (MOARD) (2004). *Annual Report*. Nairobi, Kenya.

Muasya, R. M. (2001). *Crop Physiological Analysis of Seed Quality Variation in Common Beans (Phaseolus vulgaris L.)* (Ph.D. Thesis). Wageningen University, The Netherlands.

Mwangi, S. F. M. (1986). *Pathogenic variation in Colletotrichum lindemuthianum (ssacc. And Magn.) scrib. the cause of bean anthracnose and its implication to Kenya* (Msc. Thesis). University of Nairobi, p 96.

Nkalubo, S., Melis, R., Laing, M. D., & Opi, F. (2007). Yield losses associated with anthracnose disease on Ugandan – market class dry bean cultivars. *African crop science society, 8*, 869-874.

Pastor-Corrales, M. A. (1991). Estandarizacion de variedades diferenciales y de designacion de razes de *Colletotrichum lindemuthianum*. *Phytopathology, 81*, 694 .

Ombiri, J., Zinkernagel, V., Gathura, E. M., Achwanya, O. (2002). First report of race 485 of *Colletotrichum lindemuthianum* in Kenya and its implication in bean resistance breeding. *Gettenbauwissenschft, 67*(3), 81-85.

Opole, R. A., Mathenge, P. W., Auma, E. O., Van Rheenen, H. A., Almekinders, C. J. M. (2003). On-farm seed production practices of common bean (*Phaseolus vulgaris* L.) *African Crop Science Conference Proceedings, 6*, 722-725.

Otsyula, R., Rachier, G., Ambitsi, N., Juma, R., Ndiya, C., Buruchara, R., & Sperling, L. (2004). The use of informal seed producer groups for moving root-rot resistant varieties during periods of acute stress. In L. T. Sterling, J. M. Remington, J.M Haugen, & S. Nagoda, (Eds.), *Addressing seed Security in Disaster Response: Linking Relief with Development.* Cali, Colombia: International Center for Tropical Agriculture. ISBN: 958: 694-071-3.

Sharma, P. N., Kumar, A., Sharma, O. P., Sud, D., & Tyagi, P. D. (2008). Pathogenic Variability in *Colletotrichum lindemuthianum* and Evaluation of Resistance in *Phaseolus vulgaris* in the North Western Himalayan Region of India. *Journal of Phytopathology, 36*, 30-34.

Vidigal, F. P. S., Gonc¸alves-Vidigal M. C., Kelly J. D. and Kirk. W. W., (2007). Sources of Resistance to Anthracnose in Traditional Common Bean Cultivars from Parana´ , *Brazil. Journal of Phytopathology, 155*, 108-113.

Yesuf, M., & Sangchote, S. (2007). Survival and tansmission of Colletotrichum lindemuthianum fom naturally infected common bean seeds to the seedlings. *Tropical Science, 47*(2), 96-103.