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Response of French bean genotypes to *Colletotrichum lindemuthianum* and evaluation of their resistance using SCAR markers

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Common bean anthracnose caused by *Colletotrichum lindemuthianum* is one of the major biotic constraints to production of French beans (*Phaseolus vulgaris* L.) in Kenya. This study aimed at screening French bean genotypes in relation to their response to common bean anthracnose in order to identify potential sources of germplasm for breeding. The genotypes were tested in three sites (Kakamega, Mwea and Kutus) where results revealed a significant genotype by environment interaction effect ($p \leq 0.05$) which emphasizes the strong influence of the growing conditions on the expression of host resistance. Physiological characterization identified a total of 14 distinct races out of 16 successfully plated isolates, revealing a very high diversity of *C. lindemuthianum* in Kenya. Six races have not been reported in previous studies in the country and are considered as new races, that is, races 84, 141, 246, 515, 576 and 768. Andean race 401 was the most virulent race with a virulence index of 67% among the genotypes. Molecular analysis using six sequence characterized amplified region (SCAR) markers revealed polymorphism among the genotypes. The SCAR markers SBB-14, SH-18 and SAB-03 have shown to be useful for marker assisted selection (MAS) of the target resistance genes. This study has also identified locally improved breeding lines as potential donors for resistance breeding to *C. lindemuthianum* in Kenya.

Key words: Anthracnose, sequence characterized amplified region (SCAR), marker assisted selection (MAS).

INTRODUCTION

French bean (also referred to as the snap or green bean) is valued for its production of tender, thin pods containing small seeds (Singh et al., 2015). It is a type of common bean (*Phaseolus vulgaris* L.), a crop grown widely as a source of protein (Broughton et al., 2003; Blair et al., 2007). The immature legumes have pods that are very tender and thin, with small seeds, features that make them to be considered as vegetables (Allaire and Brady,

2010). French bean is valued for its protein (23%); the pods are also rich in calcium (Ca), phosphorous (P) and iron (Fe). They are packed with dietary fiber which contains high levels of vitamin A, a strong antioxidant that fights against high cholesterol, heart disease and cancer. They also contain vitamin B6 (pyridoxine), thiamin (vitamin B-1), and vitamin C (Anderson et al., 2009). Locally in Kenya the green leaves are also used as vegetables (Mwaninki, 2017).

Anthracnose caused by the Ascomycete *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib is among the main diseases of the common bean (*Phaseolus vulgaris* L.) in Kenya (Balardin et al., 1997). The seed-borne pathogen is a major production constraint affecting common beans worldwide, especially in areas that experience high relative humidity and moderate temperatures (Singh and Schwartz, 2010; Conner et al., 2019). Severe infections of anthracnose in common bean can lead to almost complete crop loss because the pathogen produces masses of conidia that are capable of being easily disseminated to healthy tissues (Fernández et al., 2000; Sharma et al., 2008; Padder et al., 2017). Successful dissemination of the spores to the pods produces lesions that reduce the marketable yield of French beans (Singh et al., 2015). From the pods, the inoculum that is deposited on the seed acts as a main source of inoculum in the subsequent crop (Melotto and Kelly, 2000).

Management of the pathogen can be achieved through clean seed programs although this is not a viable option for many farmers in Africa because they lack well organized dry bean seed production systems (Ferreira et al., 2013). The French bean seed systems are well organized resulting in low incidences of anthracnose transmission through seed, although inoculum transmission from neighboring dry bean farms is likely to occur in susceptible cultivars. In this regard, majority of farmers in Kenya mainly rely on fungicides to reduce production and post-harvest losses to this disease (Wahome et al., 2011). The continued use of chemicals leads to emergence of disease resistant pathogen races, increased production costs and negative effect on the environment and human health (Burkett-Cadena et al., 2008). Several other control measures have been applied including cultural methods such as crop rotation, intercropping, elimination of plant debris, adjustment of planting dates, use of compost, and blending heterogeneous cultivars which have shown to reduce disease severity (Deeksha et al., 2009). However, these management practices come with several challenges and therefore, the use of host plant resistance is the most economical and environmentally sustainable method for controlling anthracnose of beans.

The successful development of anthracnose resistant cultivars depends on the understanding of the levels of variability in the pathogen and in the host. Populations of *C. lindemuthianum* comprise a collection of races, as recognized by their interaction with known major genes for host resistance (Batureine, 2009). A set of twelve differential varieties have been established as a means of characterizing the race structure of any given population of *C. lindemuthianum*; some of these races have arisen in the host's Andean gene pool, and others in the Mesoamerican gene pool (Melotto and Kelly, 2000; Gonçalves-Vidigal et al., 2009). Currently, nearly 250 races have been identified, 74 of which have been recorded in Kenya (Mogita et al., 2011a; Musyimi, 2014; De Lima et al., 2017). The dynamic race structure of *C. lindemuthianum* populations has implications for the deployment of resistance genes, particularly in Africa where the majority of farmers are unable to purchase pathogen-free seeds or fungicides (Otsyula et al., 2004; Mogita et al., 2011b).

Anthracnose resistance in common bean is governed by monogenic independent genes that are identified by the *Co* symbol (Kelly and Vallejo, 2004), although quantitative resistance *loci* (QRL) has also been reported (Oblessuc et al., 2014; González et al., 2015). Seventeen numbered genes (*Co-1* to *Co-17*) and other unnumbered genes (*Co-u*, *Co-w*, *Co-x*, *Co-y*, *Co-z*, *CoPv02c*$^{3\text{-}X}$, *CoPv02c*$^{7\text{-}X}$, *CoPv02c*$^{19\text{-}X}$, *CoPv0c*$^{2449\text{-}X}$ and *CoPv09c*$^{453\text{-}C}$) have been mapped in common bean (Geffroy et al., 2008; Lacanallo et al., 2010; Ferreira et al., 2013; Campa et al., 2014; Coimbra-Gonçalves et al., 2016; Zuiderveen et al., 2016). The resistance in all the *loci* is dominant except for the recessive *co-8* gene. Furthermore, co-localization of the anthracnose resistance gene and genes for angular leaf spot and rust resistance have been reported offering additional resistance to other pathogens (Gonçalves-Vidigal et al., 2011; Sousa et al., 2015; Valentini et al., 2017).

The mapping of the *Co* genes has majorly been achieved using molecular markers which offer an opportunity for marker-assisted selection (MAS) to enhance anthracnose resistance in common bean. A number of sequence characterized amplified polymorphism (SCAR) markers, random amplified polymorphic DNA (RAPD) markers and single nucleotide polymorphism (SNP) markers that are tightly linked to anthracnose resistance genes have been reported (Young and Kelly, 1997; Alzate-Marin et al., 2000; Melotto and Kelly, 2000; Garzón et al., 2008; Vallejo and Kelly, 2009). These markers offer new opportunities

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for the identification of genes for resistance and have been used successfully in common bean breeding programs (Miklas et al., 2003; Ragagnin et al., 2003; Njuguna, 2014). This study was therefore undertaken to determine the response of Kenyan French bean germplasm to *C. lindemuthianum* under natural environment, to determine their resistance to local races and analyze these entries for their banding patterns to molecular markers that are linked to specific anthracnose resistance genes. This information is important to unlock opportunities for the development of French bean cultivars with complex resistance through MAS.

MATERIALS AND METHODS

Plant material

The germplasm panel was made up of 33 entries comprising 16 commercial French bean genotypes commonly grown in Kenya, 3 local breeding lines, 2 landraces and the 12 standard differential cultivars for *C. lindemuthianum*. The French bean seed was sourced from various seed companies, research organizations and the National Gene bank of Kenya; while the seeds for the differential cultivars were sourced from the International Center for Tropical Agriculture (CIAT-Uganda). The differential cultivars were used as checks in the study because their gene pools and resistance genes have been documented (Kelly and Vallejo, 2004; Ferreira et al., 2013).

Field screening experimental sites

The plant materials were planted at three sites (Mwea, Kutus and Kakamega) in Kenya, chosen to provide a contrasting set of environments. Mwea and Kutus represent French bean growing zones in Kenya, while Kakamega represented a bean disease hot spot site based on previous studies in Kenya (Mogita et al., 2011b; Arunga et al., 2012; Kimno et al., 2016). The three sites experience a bimodal pattern of rainfall: the long rains season from March to June and the short rains season from September to December. The Kakamega site, located at the Non-Ruminant Research Institute (34°46´E, 0°16´N, elevation of 1555 meters above sea level), receives an annual rainfall of 1950 mm per year while the local mean temperature is 21°C. The experiments in Mwea were set up at the Kenya Agricultural and Livestock Research Organization (KALRO) Center in Mwea (37°20´E, 0°37´S; elevation of 1159 meters above sea level), with a total annual mean rainfall of about 850 mm and a mean air temperature of 22°C. The experiments in Mwea and Kakamega were set up during the long and short rain seasons in 2017. During the short season, the disease incidence in Mwea was extremely low and therefore, another site (Kutus), representing another French bean growing zone was included during the long rains of 2018. Kutus and Mwea sites are found in Kirinyaga County, a major French bean production region in Kenya. The Kutus site (35°37'E, 0°01´S; elevation of 1287 meters above sea level) climate is classified as tropical. The average annual temperature is 21°C and receives 1095 mm rainfall annually.

Experimental layout and data analysis

The experiments were set out using a randomized complete block design, with three replicates included in each environment. Each entry was represented by a 4.8 m long single row at a spacing of 45 cm between rows and 15 cm between plants. The dry bean cultivar GLP 585 was used as a guard row. Disease severity was assessed on a whole plot basis, and was quantified using the 1-9 CIAT scale as described by Pastor-Corrales et al*.,* (1998), in which a score of 1-3 is taken to indicate a high level of resistance, 4-6 a moderate level of resistance and 7-9 susceptibility (Table 1). A disease severity index (DSI) was calculated as follows: $(n_1 + 2*n_2 + 3*n_3 + 4*n_4 + 5*n_5 + 6*n_6 + 7*n_7 + 8*n_8 + 9*n_9)/N$, where 1 through 9 represent the disease score of individual plants and n_1 through n_9, the number of plants exhibiting a specific severity score (Ombiri et al., 2002). N represents the total number of plants examined per entry. The disease severity index (DSI) data collected from the field was subjected to analysis of variance (ANOVA) using routines implemented in SAS 9.4 software for Windows (Version 9.4. Cary, NC: *SAS* Institute Inc; 2014), considering four environments (Kakamega season 1, Kakamega season 2, Mwea and Kutus) and 34 entries as fixed factors. Means were compared using the Tukey's honestly significant difference metric at a confidence level of 95%.

Physiological characterization of *C. lindemuthianum*

A total of 16 diseased leaf samples with signs of attack, that is, lower leaf surface with brick red to purplish red discoloration on the leaves and dark brown eyespots on the pods (Figure 1a) were collected from the four contrasting enviroments. Furthermore, purposeful sampling was employed to collect eight more samples from farmers' fields around the study sites making a total collection of 16 samples. These samples were transferred to University of Embu Research Laboratory for isolation as described by Pastor-Corrales et al. (1998).

Isolates of *C. lindemuthianum* collected from the field were cultured on Potato Dextrose Agar (PDA) media (Figure 1b). For single spore isolation, successful colonies were plated on tap water agar (TWA) for 3 days and single hyphae picked under a stereo microscope and cultured on new PDA media. Colonies that failed to sporulate on PDA were cultured on V8 media to enhance sporulation. Incubation was done for 21 days at 22°C in alternating 12 h of light and darkness. Inoculum was prepared from 21 days old monospore cultures by adding 5 ml of distilled water on the surface of the culture and scrapped gently with a brush. Thereafter, the spore suspension was sieved using a cheese cloth and the inoculum was adjusted to a concentration of 1.2×10^6 spores/ml using a hemocytometer (Mahuku et al., 2002).

For race identification, screening of differential cultivars was carried out in a screen house. For each of the isolate, 12 differential cultivars were sown in seedling trays of five seedlings per row in three replicates. The growing media was made up of loam soil, manure and sand in the ratio of 2:1:1, respectively. Fourteen days after sowing, plants were inoculated with monosporic cultures of *C. lindemuthianum*. Using a small sprayer, the spores were sprayed on the stem and leaves of the plants until runoff. After inoculation, the plants were incubated and maintained in a moist chamber (20–22˚C, 95% relative humidity) for three days, and thereafter, maintained at 20-28˚C for 7 days. After this period, the disease symptoms were scored visually for anthracnose severity index on the leaves using a CIAT scale of 1-9 (Pastor-Corrales et al., 1998). Identification of the races was based on the susceptibility of each differential cultivar to the isolates of the pathogen using a binary nomenclature as described by Pastor-Corrales (1991). In summary, each differential cultivar has an assigned number (2^n), where n corresponds to the order number of the cultivar within the 12 standard bean differential series (Table 2). The race designation

Table 1. The CIAT disease evaluation scale (1-9) for assessing severity of *C. lindemuthianum.*

Reaction rating	Category	Description
1	Resistant	No visible symptom
2	Resistant	1% of the leaf veins affected, visible only on the lower leaf surface
3	Resistant	3% of the leaf veins affected, visible only on the lower leaf surface
4	Moderately resistant	1% of the leaf veins affected, visible on both surfaces of the leaves
5	Moderately resistant	3% of the leaf veins affected, visible on both surfaces of the leaves
6	Moderately resistant	Leaf veins affected, visible on both leaf surfaces, and the presence of some lesions on stems, branches, and petioles
7	Susceptible	Necrotic spots on most of the leaf veins and in a large part of the adjacent mesophyll tissue, which ruptures, as well as the presence of abundant lesions on the stem, branches, and petioles
8	Susceptible	Necrotic spots on almost all the leaf veins and very abundant on stems, branches, and petioles, leading to ruptures, leaf shedding, and reduction of plant growth
9	Susceptible	Most of the plant is dead

Figure 1. Field infected pods (A) and Isolates plated on PDA media (B).

was obtained as the sum of all binary numbers of cultivars with susceptible reactions.

Screening French bean accessions for resistance to different races of *C. lindemuthianum*

Identified races in the differential cultivars were re-isolated to obtain monosporic cultures by plating the samples on TWA and single hyphae obtained under microscope. Each single hyphae was plated on PDA and V8 media for sporulation for 21 days at 22°C in alternating 12 h of light and darkness. In order to screen the French bean genotypes, a modified detached leaf technique was used as described by Tu (1986). The detached leaf technique was earlier used to confirm the races of the collected samples and results obtained were similar to the analysis based on whole plants and therefore the method was suitable for screening the French bean accessions. The modified detached leaf technique involved inoculating detached leaves in clean Petri dishes in the laboratory. The procedure started with preparing clean plastic Petri dishes by lining them with a thin layer of cotton that was moistened with sterile water. In the evening hours (cool weather), young trifoliate leaves were carefully excised from three weeks old seedlings by pinching

Table 2. List of twelve anthracnose differential cultivars and their characteristics.

S/N	Differential cultivar	Resistant gene (s)	Binary no.	Gene pool	Growth habit[a]
1	Michelite	*Co-11*	1	Middle American	II
2	MDRK	*Co-1*	2	Andean	I
3	Perry Marrow	*Co-1*^{3}	4	Andean	II
4	Cornell 49-242	*Co-2*	8	Middle American	II
5	Widusa	*Co-1*^{5}, *Co-3*^{3}	16	Andean	I
6	Kaboon	*Co-1*^{2}	32	Andean	II
7	Mexico 222	*Co-3*	64	Middle American	I
8	PI 207262	*Co-4*^{3}, *Co-3*^{3}	128	Middle American	III
9	TO	*Co-4*	256	Middle American	I
10	TU	*Co-5*	512	Middle American	III
11	AB 136	*Co-6, co-8*	1024	Middle American	IV
12	G2333	*Co-3*^{5,} *Co-4*^{2}, *Co-5*^{2}	2048	Middle American	IV

Growth habit[a]: I = Determinate; II = Indeterminate bush; III = Indeterminate bush with weak main stem and prostrate branches; IV = Indeterminate climbing habit.

with a finger at the petiole. They were then placed in triplicates in Petri dishes with the lower sides of the leaves facing up. The monosporic spore suspension was sieved using a cheese cloth, and the inoculum was adjusted to a concentration of 1.2 x 10^6 spores/ml using a hemocytometer for each race. The inoculum was sprayed gently onto the leaves using a hand sprayer. The Petri dishes were then placed on the research laboratory tables (University of Embu) at room temperature (25 ± 2°C) for 10 days. The leaves were moistened daily using sterile water to prevent the leaves from wilting. The leaves were assessed on the 10th day post-inoculation for anthracnose severity by scoring the pathogen reaction from three trifoliate leaves (Figure 2) on a scale of 1-9 (Pastor-Corrales et al*.*, 1998).

Molecular analysis of French bean genotypes using SCAR markers

Seeds of the French bean accessions and the differential cultivars were sown in plastic pots in a greenhouse maintained at 25 ± 5°C at the University of Embu, Kenya. The growth media comprised of loam soil, manure and sand in the ratio of 2:1:1, respectively. Each pot was supplied with Diammonium Phosphate (DAP) fertilizer. The pots were watered daily to field capacity. For DNA extraction, three-week old young leaves for each French bean genotype and differential cultivars were collected. DNA was extracted according to Mahuku and Riascos (2004). The DNA samples were then amplified using six SCAR markers that are linked to anthracnose genes that have some levels of resistance in Kenya (Table 3). A total PCR reaction volume of 10 µl was used composing of 5 ηg/ul DNA, 20uM of each specific reverse and forward primer, 5x Bioline MyTaq Reaction buffer (5 mM dNTPs, 15 mM, $MgCl_2$, stabilizers and enhancers), 1.2 units of *Taq* DNA Polymerase (Bioline) made up to the volume using molecular grade water. PCR reactions were performed using the following regime: an initial hybridization step at 95°C for 1 min; followed by 35 cycles of denaturation step at 95°C for 30 s, annealing step ranging from 60-65°C for 1 min (Table 5), a third extension step at 72°C for 2 min, followed by a final extension at 72°C for 5 min. After amplification, a volume of 2 µl of 6x DNA loading dye (New England Biolabs-NEB) was added to each amplicons and resolved on 1.2% agarose gel containing ethidium bromide, run in 1x Sodium borate buffer at 100 volts for 2 h. The DNA bands were viewed under ultraviolet light and analyzed as binary data by recording 1 and 0 for presence and absence of the marker, respectively.

RESULTS AND DISCUSSION

Reaction of French beans and differential cultivars to anthracnose under field conditions

According to the ANOVA, there were significant differences ($p \leq 0.05$) in the severity of anthracnose infection both among the genotypes and environments. The significant genotype by environment interaction effect emphasizes the strong influence of the growing conditions on the expression of host resistance which is agreement with work conducted by Kiptoo et al. (2020). The highest mean DSIs were experienced at Kakamega (long rains season: 4.0, short rains seasons: 2.64), with a lower disease pressure experienced at Mwea (1.71) and Kutus (1.64). In the Kakamega long rains season experiment, 10 of the 17 French bean genotypes revealed moderate resistance (mean DSI 4-6) while 4 were completely susceptible (7-9). Only 3 genotypes showed resistance (1-3) (Table 4). The entries which showed consistency with respect to resistance were the landrace GBK032921, three breeding lines (T19, MU#2 and MU#13) and two commercial French bean cultivars (Julia and Monel). Among the differential cultivars, Perry Marrow (*Co-1*^{3}), Kaboon (*Co-1*^{2}), Widusa (*Co-1*^{5}, *Co-3*^{3}), TU (*Co-5*), AB136 (*Co-6*, *co-8*) and G2333 (*Co-4*^{2}, *Co-5*^{2}, *Co-3*^{5}) showed the most consistent level of resistance across the four environments. Differentials of the

Figure 2. Detached leaf technique A=infected leaves of a susceptible sample B=un-infected leaf sample from a resistant sample.

Table 3. List of SCAR markers linked to anthracnose resistance genes in common bean that were used in the study.

SCAR marker[a]	*Locus*	Size (bp)	Parental control genotype	Chromosome	Distance (cM)	Tm (°C)[b]	Sequence
SY20	*Co-4*	830	TO	Pv08	0.0	65	F:AGCCGTGGAAGGTTGTCAT R:CCGTGGAAACAACACACAAT
SH-18	*Co-4²*	1150	G2333	Pv08	4.2	65	F:CCAGAAGGAGCTGATAGTACTCCACAAC R:GGTAGGCACACTGATGAATCTCATGTTGGG
SAS-13	*Co-4²*	900	G2333	Pv08	0.4	65	F:CACGCACCGAATAAGCCACCAACA R:CACGGACCGAGGATACAGTGAAAG
SBB-14	*Co-4²*	1050/ 1150	G2333	Pv08	5.9	65	F:GTGGGACCTGTTCAAGAATAA TAC R:TGGCGCACACCATCAAAA AAGGTT
SAB-03	*Co-5*	450	TU	Pv07	5.9	65	F:TGGCGCACACATAAGTTCTCA CGG R:TGGCGCACACCATCAAAA AAGGTT
SZ-20	*Co-6*	845	AB136	Pv08	7.1	60	F:ACCCCTCATGCAGGTTTTTA R:CATAATCCATTCATGCTC ACC

[a]Source: Bean Improvement Cooperative; [b] Annealing temperature based on Laboratory troubleshooting at University of Embu

Mesoamerican gene pool exhibited high anthracnose resistance compared to those of the Andean gene pool. Similar results have been observed for other common bean pathogens in Kenya and East Africa (Kimno et al., 2016; Chilagane, 2017). The resistance exhibited across the different environments by differential cultivars Widusa, AB136 and G2333 is likely to reflect their harbouring of several *Co* genes, since their resistance is effective against multiple races of the pathogen (Alzate-Marin et al., 1997; Gonçalves-Vidigal et al., 1997; Young et al., 1998; Alzate-Marin et al., 2000; Vallejo and Kelly, 2009; De Lima et al., 2017). It is therefore important to consider gene pyramiding in resistance breeding against anthracnose.

The French bean varieties currently grown in Kenya expressed little resistance to anthracnose. Nevertheless,

Table 4. Response of French bean genotypes to *C. lindemuthianum* grown under field conditions in four environments.

S/N	Genotypes	Kakamega	Kakamega	Mwea	Kutus
		Long rains	Short rains	Long rains	Short rains
1	Amy	5.0^{a-c}	2.0^{b-d}	2.0^{a}	2.0^{ab}
2	Belcampo	5.3^{a-c}	2.0^{b-d}	1.0^{a}	2.0^{ab}
3	Blazer	5.7^{a-c}	2.0^{b-d}	1.0^{a}	4.0^{a}
4	Boston	4.3^{a-c}	2.7^{b-d}	3.0^{a}	3.0^{ab}
5	Edge	7.0^{ab}	3.7^{a-d}	1.7^{a}	3.0^{ab}
6	Enclave	7.0^{ab}	3.7^{a-d}	1.7^{a}	3.3^{ab}
7	Fanaka	5.0^{a-c}	2.7^{b-d}	1.0^{a}	2.3^{ab}
8	Goal	3.0^{a-c}	2.3^{b-d}	1.7^{a}	2.3^{ab}
9	Hawaii	4.0^{a-c}	2.0^{b-d}	1.7^{a}	2.0^{ab}
10	Julia	2.7^{a-c}	1.5^{d}	1.0^{a}	2.0^{ab}
11	Konza	4.5^{a-c}	2.0^{b-d}	1.0^{a}	2.0^{ab}
12	Lomami	6.7^{ab}	3.0^{b-d}	1.5^{a}	1.5^{ab}
13	Monel	1.7^{bc}	2.0^{b-d}	1.3^{a}	2.3^{ab}
14	Source	3.7^{a-c}	2.0^{b-d}	1.0^{a}	2.7^{ab}
15	Tahoe	7.0^{a}	2.4^{a}	1.0^{a}	2.3^{ab}
16	Venda	4.3^{a-c}	3.5^{a}	2.0^{a}	-
17	GBK 032921	2.3^{bc}	1.3^{d}	1.0^{a}	2.0^{a}
18	GBK 032952	2.7^{a-c}	5.0^{a-c}	1.5^{a}	2.0^{a}
19	T19	3.3^{a-c}	2.0^{b-d}	1.0^{a}	3.0^{ab}
20	MU#03	2.0^{bc}	3.0^{b-d}	1.5^{a}	2.0^{a}
21	MU#13	2.7^{a-c}	1.7^{cd}	1.0^{a}	1.7^{a}
22	Michelite	2.0^{b-d}	2.0^{a}	1.7^{a}	2.0^{b-d}
23	MDRK	3.3b^{cd}	2.5^{a}	2.0^{a}	3.3b^{cd}
24	Perry Marrow	1.3^{d}	-	1.7^{a}	1.3^{d}
25	Cornell 49-242	7.0^{a}	2.7^{a}	2.3^{a}	7.0^{a}
26	Widusa	1.3^{d}	2.0^{a}	2.0^{a}	1.3^{d}
27	Kaboon	2.0^{b-d}	1.0^{a}	1.7^{a}	2.0^{b-d}
28	Mex 222	3.3^{b-d}	3.5^{a}	3.3^{a}	3.3^{b-d}
29	PI 207262	5.3^{ab}	1.5^{a}	1.7^{a}	5.3^{ab}
30	TO	5.3^{ab}	2.3^{a}	2.0^{a}	5.3^{ab}
31	TU	1.0^{d}	-	2.0^{a}	1.0^{d}
32	AB136	1.3^{d}	1.0^{a}	1.5^{a}	1.3^{d}
33	G2333	1.0^{d}	1.0^{a}	1.0^{a}	1.0^{d}
	Means	4.0^{a}	2.7^{b}	1.8^{c}	1.6^{c}
	P value	*<.0001*	*<.0001*	*0.0425*	*0.7798*

Means sharing the same letter are not different at P ≤ 0.05 according to Tukey's test. S/N 1-16 = commercial French beans, 17-18 = landraces, 19-21 =breeding lines & 22-33 = differential cultivars. (-) = missing data due to confounding effects of other diseases.

the resistance expressed by some commercial cultivars, landraces and local breeding materials could be exploited as a basis for breeding French beans because, in French beans, yield is a complex trait brought about by additional pod quality traits (Singh et al., 2015). Furthermore, the differential cultivar Widusa, which is also a French bean from Europe (Drijfhout and Davis, 1989), can be exploited as a resistant donor in Kenya. Widusa is classified as an Andean differential cultivar but pedigree evidence suggests that it could be of Mesoamerican origin (Gonçalves-Vidigal and Kelly, 2006), supporting the importance of Mesoamerican genes in anthracnose resistance in Kenya. Further, ANOVA depicted significant environment main effect and a significant genotype by

Table 5. Characterization of *C. lindemuthianum* isolates using the differential cultivars used to *identify races.*

Isolate	Differential Cultivars[a]												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Race**
K1[b]	S[c]	R[d]	R	R	R	R	S	R	R	R	R	R	65
K2	R	S	R	R	R	R	R	R	S	S	R	R	768
K3	S	R	S	S	S	R	R	S	S	R	R	R	141
K4	S	R	R	R	R	R	S	R	R	R	R	R	65
K5	R	R	R	R	R	R	R	R	R	R	R	R	0
K7	R	R	R	R	R	R	R	R	S	R	R	R	256
E1	R	R	S	R	S	R	S	R	R	R	R	R	84
E2	R	S	R	R	R	R	R	R	R	R	R	R	2
E3	R	R	R	R	R	R	S	R	R	S	R	R	576
Kt1	S	S	R	R	R	R	R	R	R	S	R	R	515
Kt2	S	R	R	R	S	R	R	S	S	R	R	R	401
Kt3	S	R	R	S	R	R	S	R	R	R	R	R	73
Kt4	R	S	S	R	R	S	R	R	R	R	R	R	38
M2	S	R	R	R	S	R	S	R	R	R	R	R	81
M3	S	R	R	R	R	R	S	R	R	R	R	R	65
M4	S	S	S	R	S	S	R	R	R	R	R	R	55

[a]Differential cultivars: 1-Michelite, 2-MDRK, 3-Perrymarrow, 4-Cornel 49-242, 5-Widusa, 6-Kaboon, 7-Mex 222, 8-PI 207262, 9-TO, 10-TU, 11-AB136, 12-G2333. [b]Collected from: K-Kakamega, E-Embu, Kt-Kutus, M-Mwea. [c]S- Susceptible scale 4-9. [d]R-resistant scale 1-3.

environment interaction effect which emphasizes the strong influence of the growing conditions on the expression of host resistance. This is likely driven by the effect of the environment on the growth and dispersal of the pathogen. The high DSIs observed at the Kakamega site during the long rains season probably reflect the prevalence of warm, humid conditions coupled with high rainfall during the growing season. The ideal conditions for the growth of the *C. lindemuthianum* pathogen are an air temperature range of 13-26°C, the availability of abundant moisture and frequent rainfall which encourages the dispersion of conidia (Schwartz et al*.*, 2005; Sharma et al*.*, 2008). In contrast, Mwea and Kutus sites experience a much lower rainfall and are generally less humid, conditions which are less favourable for the development of anthracnose. These results therefore support the studies by Kimno et al. (2016) who recommended Kakamega as a hotspot for screening common bean diseases.

Physiological characterization of *C. lindemuthianum*

Evaluation using specific races was considered important in this study given the confounding effects brought about by other pathogens as observed in the field. Race characterization will also help one to understand the composition of races in the country to assist in breeding for resistance since anthracnose exhibits a vertical/qualitative form of resistance (Miklas et al*.*, 2006). The current study identified 14 distinct races from 16 successfully plated isolates revealing a high level of diversity of *C. lindemuthianum* out of which 43% were considered new races in Kenya (Table 5). Similar results have been identified in the neighboring country, Tanzania where 96% of the 42 races were considered new obtained from 50 isolates (Mpeguzi et al*.*, 2020). Previous studies conducted in Kenya by Musyimi (2014) identified 12 races most of them being different from the races that were obtained in the other studies too. Race 65 may be considered as widespread as it was identified in Kakamega and Mwea. Races 65 and 73 have also been documented to be prevalent in Brazil (Vieira et al*.*, 2018). Furthermore, race zero (0) was also identified in the present study as it could not infect any of the twelve anthracnose differential cultivars as previously reported in Kenya (Mogita et al*.*, 2011b) and Uganda (Nkalubo, 2006).

There is a need therefore, to review the current set of differential cultivars to include a susceptible cultivar in the differential set that can distinguish this race especially in Eastern Africa which is considered to be a secondary center of diversity for common beans (Allen and Edje, 1990; Wortmann, 1998; Sperling, 2001).

The race composition in the current study was predominantly Andean (70%). This could be probably due

to a large number of French bean genotype used in the study that have been reported to be largely of the Andean origin (Arunga et al*.,* 2015, Arunga and Odikara, 2020). In addition, the common bean composition in the country has been reported to be of Andean origin as well (Asfaw et al*.,* 2009). This supports the work carried out by Baradin and Kelly (1998) where they discovered that races isolated from Andean cultivars have a broader virulence on germplasm of the Andean gene pool and a reduced virulence on Mesoamerican varieties, which suggests a co-evolution between the pathogen and the host. Among the differential cultivars, the most affected cultivar was Michelite having been overcome by seven (7) of the 14 isolates with cultivars AB136 and G2333 being the most resistant as they were not affected by any of the isolates. The susceptibility observed in cultivar Michelite has also been reported to be situated on the lower tire of the differential cultivars as a susceptible check that also succumbed to seven isolates. Similar to field results, high resistance was observed in the Mesoamerican genotypes as compared to the Andean genotypes with the exemption of the cultivar Kaboon *(Co-1).* Race characterization revealed that cultivar MDRK succumbed to five races including race 768, first to be reported in the country. Resistance exhibited by the cultivars AB136 and G2333 is likely to reflect their harbouring of several Co genes.

Virulence of 14 races of *C. lindemuthianum* on French bean genotypes

The evaluated French bean genotypes showed different virulence reactions to the 14 *C. lindemuthianum* races used in this study (Table 6). Races 401 and 84 were the most virulent across all the genotypes while race 768 was the least virulent race. Among the French bean cultivars, Lomami was the most susceptible cultivar as it was infected by 86% of the races; whereas cultivar Boston, breeding lines MU#3, MU#13 and T19 were the most resistant. The results for the breeding lines demonstrated that the greenhouse/laboratory results were not very different from the field experiment. The breeding lines were resistant to most Mesoamerican races except for Andean races 2, 38 and 84. These breeding lines were obtained from one breeding program which utilized resistance genes from both gene pools including dry beans (Arunga et al*.*, 2015). This probably resulted in the introgression of the Mesoamerican genes into the French bean background.

Among the differential cultivars, the high susceptibility observed in cultivar Michelite was as expected given that the cultivar is situated on the lower tire of the differential cultivars as a susceptible check. Similar to field results, race characterization revealed high resistance among the differential cultivars of the Mesoamerican origin compared to those of the Andean origin with the exemption of the cultivar Kaboon. Cultivar Kaboon was only susceptible to two Andean races in the current study, that is, races 33 and 55. Susceptibility to race 55 has been reported in previous studies (Alzate-Marin et al., 1997). According to Melotto and Kelly (2000), the major dominant resistance gene present in the cultivar Kaboon is an allele of the *Co-1* gene, called *Co-1*^{2}. The authors revealed the importance of this gene in common bean breeding programs, as it gives resistance to both Andean and Central American races recently identified in Michigan. Similarly, cultivar Kaboon was resistant under field conditions across the four environments. Given the great composition of the French bean genotypes in Kenya are of the Andean origin, this cultivar can be used for gene introgression to improve resistance to *C. lindemuthianum*. Andean differential genotypes possess locus (*Co-1*), the only most utilized locus in the cultivars of this origin conferring resistance to *C. lindemuthianum* (Melotto and Kelly, 2000; Zuiderveen et al*.*, 2016). This could be the bottleneck facing genotypes within this gene pool. The resistance exhibited by the cultivars AB136 and G2333 is likely to reflect their harbouring of several *Co* genes.

Further, the study employed a better, simple and rapid screening method/technique to ensure efficient selection of resistant materials. Detached leaf technique has been deployed in the past as an alternative to conventional screening techniques in screen houses and has been reported to be successful with similar results (Rezene et al*.*, 2018). This is particularly important where seeds are limited and there is a need to preserve the whole plant for further evaluation. The method can also be carried out in the lab reducing the need for installation of screen houses. In common beans, the method has been used in screening for ALS and anthracnose (Tu, 1986; Rezene et al*.*, 2018). The technique has also been employed in other crops such as maize against southern corn leaf blight (Aregbesola et al*.*, 2020), soybean against white mold (Twizeyimana et al*.*, 2007) and *Fusarium* in wheat (Browne and Cooke, 2004).

Molecular markers analysis

All the six (6) SCAR markers used in this study generated polymorphisms between each parent and the resistant and susceptible genotypes which were specific for the *loci* to which they are linked. However, only SAB-03, SZ-20 and SH-18 markers were allele-specific (Table 7). The marker SY-20$_{830bps}$ a dominant marker reported to be very specific and tightly linked to the *Co-4 locus* at 0.0 cM (Costa et al*.*, 2010), could not discriminate the other alleles at this *locus* among the 12 differential set. This is

Table 6. Response of French bean genotypes, Landraces and Breeding lines to *C. lindemuthianum* races identified in Kenya.

S/N	Genotype	*Colletotrichum lindemuthianum races*														
		0	**2**	**38**	**55**	**65**	**73**	**81**	**84**	**141**	**256**	**401**	**515**	**576**	**768**	**RI%**
1	Amy	S	S	R	R	R	R	S	R	S	R	S	R	R	R	64
2	Belcampo	R	R	S	R	R	S	R	S	R	R	R	R	S	R	71
3	Blazer	R	S	S	R	R	R	R	R	R	S	S	S	R	R	57
4	Boston	R	R	R	S	R	R	R	R	R	R	S	R	R	R	86
5	Edge	R	R	R	S	R	R	R	S	R	R	S	S	R	R	71
6	Enclave	S	R	R	S	R	R	R	R	R	R	S	S	S	R	64
7	Fanaka	R	S	S	R	R	S	R	R	S	R	S	R	R	R	64
8	Goal	R	R	R	R	S	R	R	S	S	S	S	R	R	R	64
9	Hawaii	S	S	S	R	R	S	R	S	S	S	S	R	R	R	43
10	Konza	S	S	S	R	R	R	R	S	R	S	S	R	S	R	50
11	Lomami	S	S	R	S	S	S	S	S	S	S	S	S	R	S	14
12	Monel	S	R	R	S	S	R	R	R	R	R	S	S	R	S	57
13	Source	S	S	R	S	R	R	R	S	R	R	R	R	S	R	64
14	T19	R	R	R	S	S	R	R	R	R	R	R	R	S	R	86
15	MU#3	S	R	R	R	R	S	R	R	R	R	R	R	R	R	79
16	MU#13	R	S	S	R	R	R	R	S	R	R	R	R	R	R	79
17	GBK 032921	S	R	R	R	R	S	R	S	R	S	R	R	R	R	71
18	GBK 032952	S	R	S	R	S	S	R	S	S	R	S	R	R	R	57
Virulence Index (%)		42	42	37	32	21	37	16	53	32	32	63	26	26	11	

[a]Physiological races classified according standard system of classification. [b]RI = Resistance index. [c]Disease reactions: R = Resistant, S = susceptible. [d]VI = Virulence Index.

in agreement with similar work done by Beraldo et al., (2009). Figure 3C shows the clear band of cultivar TO (*Co-4*) (No 9), the tagged *locus* for the marker and the thicker bands of the alternative alleles possessed by cultivars PI 207262 (*Co-4*^{3}) and G2333 (*Co-4*^{2}) No 8 and12 respectively. This is in agreement with Vieira et al. (2018) who did similar work in Brazil. The marker however, did not detect the possible gene for resistance among the French bean genotypes used in this study.

Linkage to the *locus Co-6* in chromosome Pv 07 was evaluated using the marker SZ-20$_{845\ bps}$ linked at a distance of 7.1 cM derived from the differential cultivar AB 136 (Queiroz et al., 2004). The marker was only identified with the differential cultivar AB 136 revealing its specificity (Figure 3D). Cultivars Mitchelite (*Co-11*), MDRK (*Co-1*), Kaboon (*Co-1*^{2}), TO (*Co-4*), TU (*Co-5*) and G2333 (*Co-3*^{5}, *Co-4*^{2}and *Co-5*) amplified an unspecific band close to the *locus*. Among the French bean genotypes, the marker was detected in three cultivars Edge, Blazer and T19 (Tables 6 and 7). The locus *Co-6* has been known to offer broad-based resistance to most of the Andean races in the Latin America (23, 31, 64, 69, 73, 81, 89 and 453) but less effective against races of Mesoamerican origin (Gonçalves-Vidigal et al., 1997;Gonçalves-Vidigal et al., 2001). Thus, the *Co-6* locus can be utilized in breeding programs for French beans in Kenya after successful

Table 7. Evaluation of genotypes using SCAR markers.

S/N	Genotype	SCAR marker					
		SY-20	SZ-20	SAB-03	SBB-14**	SH-18	SAS-13
1	Amy	0	0	0	0	0	1
2	Belcampo	0	0	0	0	0	1
3	Blazer	0	0	1	0	0	0
4	Boston	0	0	1	0	0	1
5	Edge	0	1	0	0	0	1
6	Enclave	0	0	1	0	0	1
7	Fanaka	0	0	1	0	0	0
8	Goal	0	0	0	0	0	0
9	Hawaii	0	0	0	0	0	0
10	Julia	0	0	0	0	0	0
11	Konza	0	0	0	0	0	0
12	Lomami	0	0	0	0	0	0
13	Monel	0	0	0	0	0	0
14	Source	0	0	0	0	0	1
15	Tahoe	0	0	0	0	0	1
16	Venda	0	0	0	0	0	0
17	GBK 032 921	0	0	0	0	0	0
18	GBK 032 952	0	0	1	0	0	0
19	MU#03	0	0	1	0	0	1
20	MU#13	0	0	1	0	0	1
21	T19	0	0	0	0	0	1
22	Michelite	0	0	0	0	0	0
23	MDRK	0	0	0	0	0	0
24	Perry Marrow	0	0	0	0	0	1
25	Cornell 49-242	0	0	0	1	0	1
26	Widusa	0	0	0	0	0	0
27	Kaboon	0	0	1	0	0	0
28	Mex 222	0	0	0	0	0	0
29	PI 207262	1	0	0	0	1	0
30	TO	**1***	0	0	1	0	1
31	TU	0	0	**1***	0	0	1
32	AB136	0	**1***	0	1	0	1
33	G2333	1	0	1	**1***	**1***	**1***

S/N 1-16 = commercial French beans,17-18= landraces, 19-21 = breeding lines and 22-33 = differential cultivars. SBB-14** Co-dominant marker where 1-1150_{bps}, 0-1050_{bps}. 1*parent genotype; 1- presence of gene 0-absence of gene.

marker validation. The SCAR marker SAS-13_{900bps} tightly linked to the *Co-4*^{2} locus at 0.4 cM (Young et al*.,* 1998; Kelly et al*.,* 2003), was detected in two differential cultivars PI 207262 (*Co-4*^{3}) and G2333 (*Co-4*^{2}) specific to the tagged locus and one unspecific amplification in differential cultivar Widusa (*Co-1*^{5}, *Co-3*^{3}) (Figure 3A). Of the nine French bean genotypes under evaluation identified by the *only* local French bean breeding lines were consistent under field evaluation and on the reaction with specific races. Recent studies have shown that SAS-13_{900c} is not specific for *Co-4* allele (Awale and Kelly, 2001; Alzate-Marin et al., 2007) but, has been known to detect *Co-4* locus consistently regardless of the type of allele as was confirmed in this study.

The SCAR marker SBB-$14_{1050/1150bps}$ a codominant marker also linked to the gene *Co-4*^{2} revealed consistency in detecting the *Co-4 locus* among the three differential cultivars PI 207262 (*Co-4*^{3}) to (*Co-4*) and

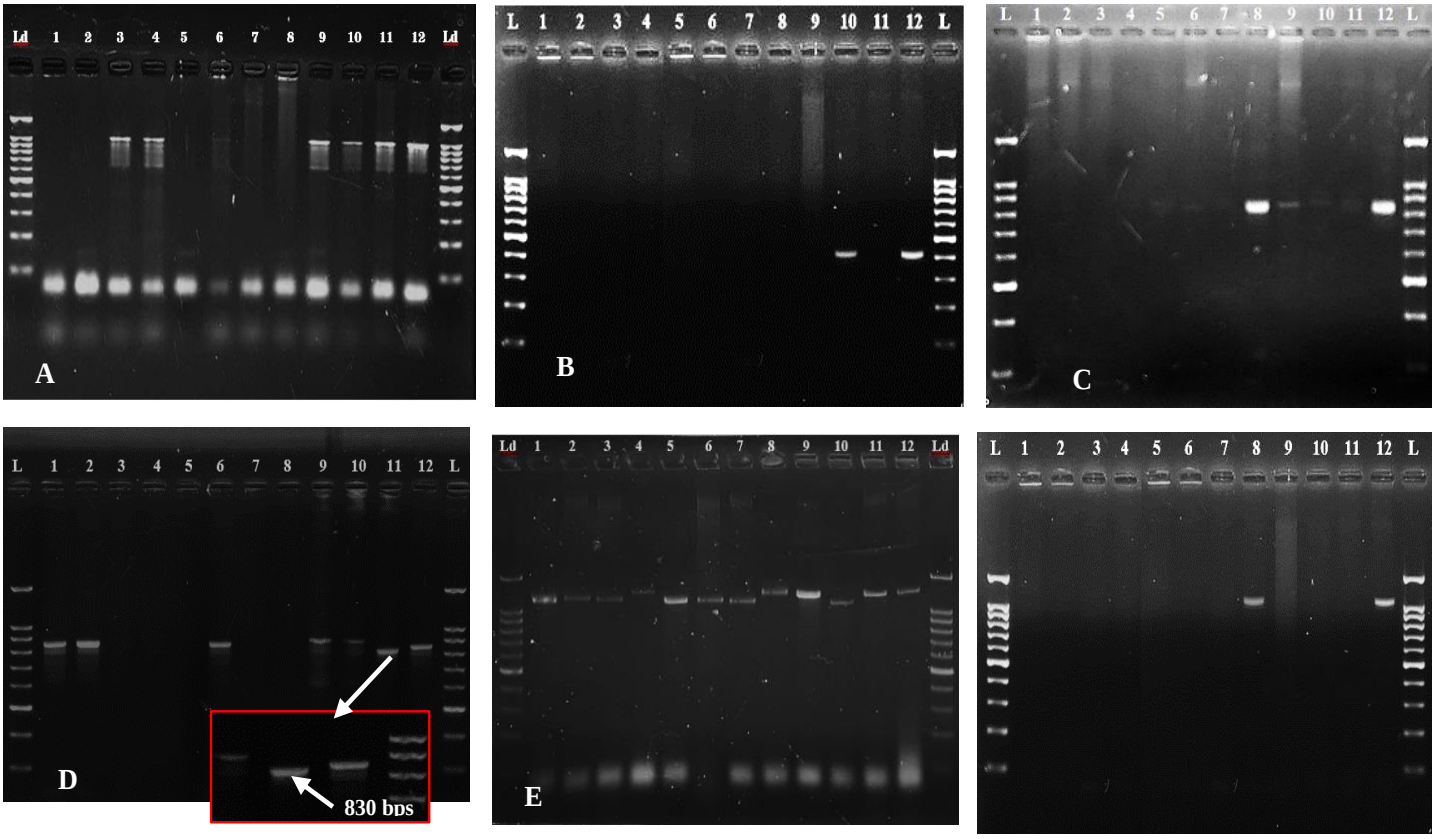

Figure 3. Amplification of molecular markers associated with the anthracnose resistance genes *Co-4*, *Co-5* and *Co-6* in differential cultivars; (A) SCAR marker SAS-13_{900bps} for *Co-4*^{2}, (B) SCAR marker SAB-03_{400bps} for *Co-5*, (C) SCAR marker SY-20_{830bps} for *Co-4*, (D) SCAR marker SZ-20_{850bps} for *Co-6*, (E) SCAR marker SBB-$14_{1050/1150\ bps}$ for *Co-4*^{2} and (F) SCAR marker SH-$18_{1100bps}$ for *Co-4*^{2}. L/Ld- 100bps DNA Ladder. Cultivars (1) Michelite, (2) MDRK, (3) Perry marrow, (4) Cornel 49-242, (5) Widusa, (6) Kaboon, (7) Mex222, (8) PI 207262, (9) TO, (10) TU, (11) AB136, (12) G2333.

G2333 (*Co-4*^{2}) (Figure 3E). However, amplification of cultivars Cornell 49-242 (*Co-2*) and AB136 (*Co-6* and *Co-8*) was unusual and none of the other genotypes were detected. The marker SH-$18_{1100bps}$ a dominant marker revealed consistency in detecting the *Co-4*^{2} *locus* among the differential cultivars PI 207262 (*Co-4*^{3}) and G2333 (*Co-4*^{2}); however, none of the other genotypes was identified with the marker. The *Co-4 locus* is the most utilized *locus* in common bean for anthracnose resistance. Its allele (*Co-4*^{2}) has been shown to provide greater resistance as compared to the original *Co-4* and *Co-4*^{3} allele and is among the broadest-based resistance genes described in common bean (Balardin and Kelly, 1998; Silvério et al., 2002; Vaz Bisneta and Gonçalves-Vidigal, 2020).

SAB-03_{400bps} maker that tags the gene *Co-5* at a distance of 5.9 cM (Vallejo and Kelly, 2001) was detected in the anthracnose differential cultivars TU (*Co-5*) and G2333 (*Co-5*) as expected (Figure 3B). The marker was detected in eight other genotypes, that is, five French beans cultivars, two breeding lines and one local cultivar (Table 7). Only the local breeding lines in the MU series revealed consistency with the marker both in the field and response to specific races.

The *Co-5* gene has not been widely deployed in the past in resistance breeding programs but, has been identified as one of the most effective genes in a survey of races of *C. lindemuthianum* from Central America and Mexico that is predominantly Mesoamerican (Balardin et al., 1997). However, the combination of *Co-5* and *Co-4* has been seen as the most effective in providing broadest resistance to *C. lindemuthianum* races. Thus, the comparative advantage of the limited use of *Co-5* in breeding makes the *locus* even more valuable to breeders for gene pyramiding bean programs.

SCAR markers tightly linked to anthracnose resistance genes offer another means to detect specific genes and for marker assisted selection as has been revealed in this study. Combination of *Co-4*^{2} and *Co-5* has been shown to offer the highest and broad resistance spectrum to

anthracnose as revealed in cultivar G2333 which agrees with the worked done by Sousa et al., (2015). The consistency of the local breeding lines with the markers could be traced to the fact that these breeding lines were obtained from one breeding program which utilized resistance genes from both gene pools including dry beans (Arunga et al., 2015). This may have led to the introgression of the Mesoamerican resistant genes into the French bean background that has been known to offer broader resistance as compared to the Andean genotypes. Four of the six SCAR markers tested in the present work (SH18 and SBB-14 for *Co-4*^{2}, SAB-03 for *Co-5*, and SY-20 for *Co-4*), have shown to be useful for assisted selection of the target resistant genes. They were specific for the *loci* to which they are linked, even though some did not discriminate alleles from the same *locus*, except for SH18, which was shown to be specifically linked to *Co-4*^{2}. However, more robust molecular markers such as SSRs, CAPS and SNPs should be developed for future selection of French bean genotypes for resistance to *C. lindemuthianum* as the current set of SCAR markers were only useful to very few French bean genotypes in Kenya.

Conclusion

The current study has revealed that, the race composition of *C. lindemuthianum* in Kenya is continuously changing and highly variable, hence the need for continuous evaluation. The highest composition of the races was of the Andean origin that could probably be due to the large number of French beans used in this study that are predominantly Andean, evidence for co- evolution between *C. lindemuthianum* and the host within the two centers of origin. Local breeding lines in the MU series could offer a good basis of gene introgression in Kenya, a great advantage in reducing linkage drag common with introgression from dry beans. Identification of race zero (0) in this study is another evidence that there is a need to review the current set of differential cultivars to include a susceptible cultivar in the differential set that can distinguish this race. Use of molecular markers proved effective and can assist future breeding efforts focusing in identification of other possible resistance *loci* to buffer against resistance breakdown through gene pyramiding especially in combination with both field evaluation and race characterization.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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