CELL BIOLOGY & DEVELOPMENT
Volume 6, Number 1, June 2022 E-ISSN: 2580-4499
Pages: 20-31 DOI: 10.13057/cellbioldev/v060104

Introgression of anthracnose resistance gene(s) into common bean (*Phaseolus vulgaris*)

GONZAZ KYARUZI KAZIMOTO, SUSAN F. NCHIMBI-MSOLLA, ROBERT B. MABAGALA♥
Sokoine University of Agriculture. P.O. Box 3005, Morogoro, Tanzania. Tel./fax.: + 255-23-2603511-4, ♥email: mabagala@sua.ac.tz

Manuscript received: 21 March 2022. Revision accepted: 6 June 2022.

Abstract. *Kazimoto GK, Nchimbi-Msolla SF, Mabagala RB. 2022. Introgression of anthracnose resistance gene(s) into common bean (*Phaseolus vulgaris*). Cell Biol Dev 6: 20-31.* Common bean anthracnose disease caused by the fungus *Colletotrichum lindemuthianum* causes significant yield losses. It is most destructive in areas with cool temperatures and high humidity (90-100%). The study aimed to introduce resistance genes into adapted but susceptible local cultivars of Masai Red and Soya Njano using conventional breeding methods. Five races of *C. lindemuthianum* were isolated and named from thirty-two common bean-diseased plant samples collected from Northern Tanzania. The sources of resistant genes were bean cultivars G2333 and AB136. Early developed populations were evaluated under field conditions in high altitude and humid environments at Bashnet in the Manyara region, in the Northern highlands of Tanzania. Both F2 and F3 populations of Soya Njano x G2333 were segregated for *C. lindemuthianum* resistance at a 9:7 ratio. Such segregation implied that two dominant epistatic genes conferred from G2333, the resistance being in the mode of epistatic gene interaction. The crosses between Masai Red x G2333 and F2 and F3 populations segregation ratio was 10:6, implying two dominant resistant genes were transferred to developed populations. The F2 and F3 progenies obtained from crossing Soya Njano and AB136 showed a ratio of 3:1. The F2 progenies from a cross between Masai Red and AB136 were segregated at a ratio of 3:1 and F3 progenies were 3:1. The 3:1 ratio confirmed single dominant gene inheritance conferred to developed progenies. The heritability (h^2) from populations of Soya Njano x G2333 and Masai Red x G2333 was between 0.41 and 0.45. While Soya Njano, Masai Red, and A136 were between 0.2 and 0.53, which implied moderate heritability. F2 and F3 populations developed need further testing using MAS to confirm the presence of resistant genes. Multi-location testing should be done to verify the resistance levels of the developed bean population in later generations.

Keywords: *Colletotrichum*, Morogoro, *Phaseolus*, resistance gene

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is an annual crop that belongs to the family Fabaceae. The genus *Phaseolus* comprises 55 species and is an important grain legume grown within the boundary between two climatic zones, the tropics, and subtropics, with its primary center of diversity in Mexico, Southern Peru, Bolivia, and Argentina (Debouck 1994). The Portuguese later introduced it in East Africa and Brazil (Jones and Mejia 1999).

The domesticated bean species individually constitute a primary gene pool with its wild ancestral form. The wild bean's distribution northwards and southwards led to the formation of two geographically distinct gene pools in Meso America and the Andean (Broughton et al. 2003). Domestication of the common bean gave rise to several domesticated races of beans, and each of the two gene pools became the origin of races. Meso-American gene pool races were in Durango, Guatemala, and Jalisco, while the Andean gene pool was in New Granada, Peru, and Chile (Hillocks et al. 2006). A wide diversity of common bean cultivars is available in developing countries for production and crop improvement for adaptation to biotic and abiotic stress. The crop expresses wide variability in maturity ranging from 60-150 days (Blair et al. 2007). According to CIAT (2013), the common bean is the most vital grain legume in human diets. In sub-Saharan Africa, over 200 million people grow beans as a primary staple food and the most crucial source of calories after maize (Beebe et al. 2012). It enhances health-promoting aspects of the diet, thus vital in mitigating health risks for diseases such as obesity, cancer, diabetes, and heart disease (Raatz 2013).

The world's largest common bean producers are India, Brazil, Myanmar, and Mexico (FAOSTAT 2014). In Africa, Tanzania is the leading producer contributing 4.9% of the production (FAOSTAT 2014). However, the production of common beans in various parts of the world faces a few major biotic and abiotic constraints. Biotic stresses are caused by fungi, bacteria, viruses, and insect pests. The abiotic bean production constraints include macronutrients such as nitrogen [N] and phosphorus [P], micronutrients deficiency, such as excessive rain/flooding, drought, heat, and cold stress factors, each of which causes yield loss significantly (Beebe et al. 2012).

All agricultural zones in Tanzania are constrained by incidences of diseases and insect pests, both in the field and in storage. Major diseases are angular leaf spot (*Pseudocercospora griseola*) and anthracnose (*Colletotrichum lindemuthianum*), and Common bacterial blight (*Xanthomonas phaseoli).* Insect pests include bean stem maggot (*Ophiomyia* spp.), bean aphids (*Aphis fabae*),

bean leaf beetle (*Ootheca benningseni* (*Acanthosceli*), bean bruchids (*Acanthoscelides obtectus*) (Nyambo 2009).

Under favorable climatic conditions, anthracnose is a devastating common bean seed-borne disease. It causes significant yield loss in susceptible bean cultivars worldwide, resulting in 80-100% yield losses (Sharma et al. 2007). Infections can be quite destructive when climatic conditions are favorable to the pathogen. Economic yield losses can be as high as 100% (Davide and de Souza 2009). The yields are about three times as high in developed countries, such as U.S.A and Canada, compared to the developing countries (Porch et al. 2013). According to FAOSTAT (2015) estimates for 2013, the world bean production was 1235 kg/ha, while the yield for Africa was 799 kg/ha and 885 kg/ha for Tanzania. The yield potential is 1500 to 3000 kg/ha under reliable rainfall (Hillocks et al. 2006).

Several anthracnose management strategies, including planting mixtures of bean cultivars (Mwesigwa 2009), have been advocated to alleviate the deleterious effects of anthracnose disease on bean productivity. However, the success remains low due to smallholder producers' unaffordable cost of practices and labor constraints. Genetic resistance is the most cost-effective means of controlling the disease (Miklas et al. 2006; Tryphone et al. 2013). Developing well-adapted resistant bean cultivars is an effective alternative management option to control anthracnose (Kelly and Vallejo 2009).

This study aimed at introducing genes conferring resistance against anthracnose into popular local bean cultivars' Masai Red' and 'Soya Njano' to improve their productivity.

MATERIALS AND METHODS

Study location

This work was conducted in Long village, about 62 km west of Babati Town, Tanzania. The field experiment was conducted in a farmer's field located at S 04°13.815; E35° 27.090, at an elevation of 2,187 meters above sea level. The village experiences a bimodal rainfall pattern. Mid-November to mid-January is the predominant season for growing common beans in Babati rural areas.

Bean plant materials

Two locally adapted cultivars,' Masai Red' and 'Soya Njano,' were used as female parents. They were collected from farmers at Upper Kitete Village, Arusha Region, Tanzania. Unfortunately, both varieties succumb to bean anthracnose infection. The G2333 and AB136 are resistant to bean anthracnose disease and were used as donor parents. G2333 and AB 136 cultivars were used as male parents. The seed types of these parental genotypes are shown in Figure 1. Both donor-parent bean cultivars were obtained from the Sokoine University of Agriculture (SUA), Tanzania, bean breeding program. Some of their characteristics are given in Table 1. The segregating bean seeds in F1, F2, and F3 generations obtained by crossing Masai Red x G2333 are shown in Figure 2, representing other closes such as Soya Njano x G2333, Soya Njano x AB136, Masai Red x AB136, which also had to segregate bean seeds.

Generation of breeding lines

Hybridization

Purifying "Masai Red" and "Soya Njano" was done by planting 5 seeds of each variety in plastic pots. Two seeds harvested from a single plant were used in making crosses. Two seeds were sown per pot filled with sterilized forest soil, and thinning was done two weeks after planting. Six pots were used per variety for crossing establishment. Recipients, as well as donor parents, were planted in a staggered mode at an interval of seven to fourteen days. First, donor lines were planted seven to fourteen days before planting recipient parents. Diammonium Phosphate (DAP) at a rate of 60 kg/ha was used during sowing. Urea was top dressed at a rate of 20 kg N/ha. Watering was done throughout the time of the experiment.

Crosses were made between adapted local cultivars (Masai Red and Soya Njano) susceptible female parents with donor lines G2333 containing complementary genes Co-4², Co-5, and Co-7 and AB 136, carrying complementary genes Co-6, co-8. Crosses were as follows: Masai Red x G2333, Masai Red x AB136, Soya Njano x G2333, and Soya Njano x AB136 to get F1 populations (Figure 3).

Pollination was performed by rubbing the pollinated stigma of the male flower on the female flower. The hooking technique removed the donor parent's pollinated stigma using forceps and hooked it against the recipient parent flower (CIAT 1989). Before new emasculation, forceps were sterilized by dipping in alcohol to avoid contamination. The F1 progenies obtained by crossing were harvested from each cross separately and then grown to advance them to F2 and F3 populations.

Table 1. Bean cultivars used for the current study and some of their key phenotypic characteristics (Pastor-Corrales et al. 1994)

Genotype	Growth habit	Source of seed	Seed size	Seed color	Flower color	Reaction to anthracnose
Soya-Njano 1	Determinate Erect, bush	Upper Kitete	Medium	Yellow	Pale pink	Susceptible
Soya-Njano 2	Indeterminate	Upper Kitete and Slahamo	Small- round	Pale- Yellow	Pale pink	susceptible
Masai Red	Indeterminate	Upper Kitete	Small	Deep red	White	Susceptible
G2333	Indeterminate	SUA-Morogoro	Small	Maroon	White	Resistant
AB136	Indeterminate	SUA-Morogoro	Small	Red	White	Resistant

The recipient parent cultivars Masai Red and Soya Njano

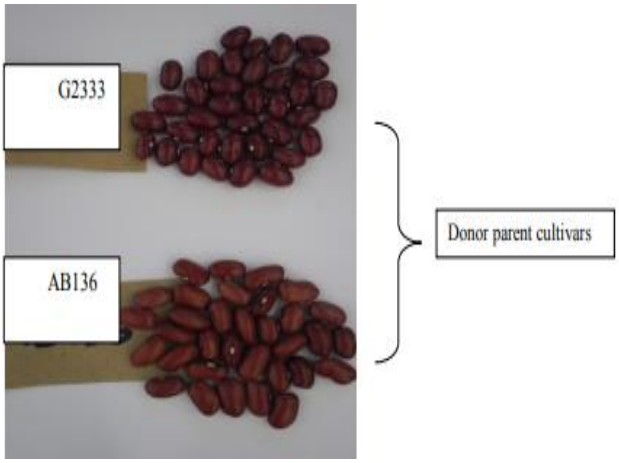

Figure 1. Bean cultivars used as donor parents G2333 and AB36 in hybridization

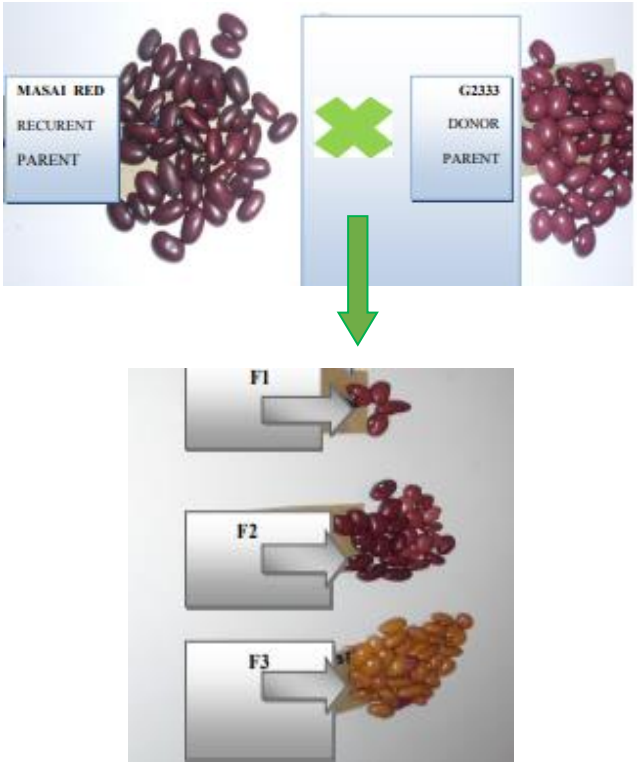

Figure 2. Crosses between Masai Red and G2333, F_1, F_2, and F_3 populations developed from them

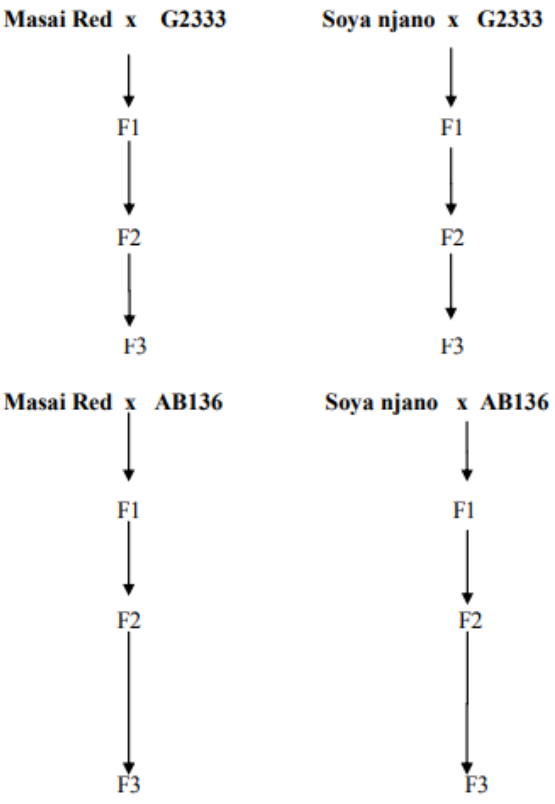

Figure 3. Crossing design for transferring anthracnose resistance genes to *Colletotrichum lindemuthianum* to susceptible bean parent cultivars

Screening for disease resistance

Collection of Colletotrichum lindemuthianum *isolates*

Stem, leaf, and pod samples bearing fresh symptoms of anthracnose infection were collected from farmers' fields in areas with natural infections across different agro-ecologies in Arumeru, Karatu, and Babati rural districts in northern Tanzania and Mvomero District in the Morogoro region.

The collected samples were placed between folds of paper bags and stored under normal room temperature at 24°C. Paper bags with diseased samples were kept open, unpiled, and separated to control decay. Each sample was labeled carefully to depict the names of varieties from which samples were picked, sites, villages, districts, and the region's name. GPS was used to mark each site's altitude, latitude, and longitude coordinates.

Medium and inoculum preparation

The fungal isolates were grown in the Petri plates at 24°C on a V8 medium composed of V8 juice (200 mL), $CaCO_3$ (3.0 g), Bato Agar (15 g), streptomycin (10 mg), and distilled autoclaved H_2O (1000 mL). Single spore isolates were established employing a standard procedure with modification according to Munda et al. (2009). First, the pathogens were isolated from well-developed and fresh lesions of bean pods and stems. Then, small pieces of infected tissue were cut between diseased and healthy tissue, at least five pieces, for a single culture. Next, the pieces were surface sterilized in alcohol 7% for less than 1 min. Next, the pieces were dipped with sterile forceps into 2% NaOCl (Sodium hypochlorite) for 3 minutes. Next, the

pieces were transferred aseptically into sterile distilled water and washed 3 times before being blotted dry on a sterile paper towel. Lastly, the pieces were transferred into prepared media within a petri dish and arranged, leaving isolation space between each other. The Petri dishes containing V8 medium containing the pieces were sealed and incubated at 24°C (Pastor-Corrales et al. 1994).

Inoculation

Seed inoculation procedure

Six single spore isolates of the coded isolates 2CRHOT, 3ASLAHMO, 6ASAR14, 1C-BASH-L, and 2D-1 collected from Long Village in Bashnet, were used. Each isolate was stored in V8 media at 4°C. Eight Petri plates with V8 medium without antibiotics were planted with a single spore of each isolate for multiplication. Inoculum-containing spore suspensions of 1.2 x 10^6 spores/mL for inoculating test plants were prepared from ten-day-old spore cultures according to the procedure described by Mahuku et al. (2002).

Seed inoculation

Four seeds of each differential cultivar were germinated by being placed in humid plates with more than 92% relative humidity at 25°C for 5 to 7 days. Germinated seeds were dip inoculated in a calibrated spore suspension of 1.2 x 10^6 spores/ml for 5 min in 200 mL (Bigirimana and Höfte 2001). The inoculated seeds were placed on humid plates and incubated in a dark room. After 2 days, the seedlings were at the emergence stage. They were transferred to trays, covered by a thin layer of sterilized soil, and incubated at 19-22°C with relative humidity above 92%.

Seedling inoculation

Planting parent cultivars and new populations

The experiment consisted of parent cultivars, derived F1, F2, and F3 populations, and bean differential cultivars planted in non-replicated and unrandomized plots. The plot size was 2 rows, each 2.75 m in length. The inter-row spacing was 50 cm, and intra hill spacing was 20 cm. Plots were planted with a donor, recipient cultivars, F1, F2, and F3 populations. Each row was a plot with 12 plants for a parent, spreader cultivars, and F1 populations. The F2 and F3 populations were planted in two rows per plot. The tested plant populations were irrigated every evening on rain-free days to provide high relative humidity conditions for 7 days after inoculation.

Seedling inoculation

The inoculum was prepared from spore suspension derived from 2D-1 Long Ayt. isolate cultures, raised on V8 medium, and kept in the darkness for ten days at 24°C. The ten days old sporulated cultures were flooded with 0 mL of sterile distilled water and scraped from the plates using a toothbrush. The spore suspensions were filtered through a four-layered gauze cloth. A hemocytometer was used to calibrate spore concentration to 1.2 x 106 spores/mL (Mahuku et al. 2002). Seedling inoculation was made by spraying with the aqueous conidial suspension on 14-day-old seedlings in the field. During the inoculation and incubation period, temperatures and relative humidity ranged between 20 to 21°C and 96 to 100%, respectively.

Disease score

The reaction of plants to *C. lindemuthianum* was evaluated 7 to 10 days post-inoculation. The disease score was done using a scale of 1-9 where (Van Schoonhoven and Pastor-Corrales 1987) seedlings with no visible symptoms (severity value 1) or showing limited necrotic lesions (severity values 2 to 3) were considered resistant. Seedlings with large sporulating lesions (severity values 4 to 8) or dead (severity value 9) were considered susceptible. A set of 12 common bean anthracnose standard differential cultivars was used to confirm the pathogenic identity of the *C. lindemuthianum* isolates.

Data analysis

GenStat statistical package was used to compute means, variance, standard deviation**,** standard error, and regression coefficient of variation between variables. The disease means scores of parents, F1, F2, and F3 populations were generated and used in estimating the narrow sense heritability of parents using regression analysis. Chi-square was used to compare the segregation of F2, and F3, populations to Mendelian ratios. Genetic gain for disease resistance was computed using a procedure proposed by Zobel and Talbert (1991), cited by Abengmeneng et al. (2015).

The selection differential (S) was estimated as below.

(1) $S = Xs - X\mu$

(2) Mid- parent = $\frac{P1 + P2}{2}$

(3) Deviation = grand mean score – mid-parent disease score

(4) % age deviation = $\frac{\text{Deviation x 100 \% Percentage deviation}}{\text{Mean}}$

(5) % age gained = % deviation x h^2

(6) Genetic gain G = % age gain x h^2 (percentage gain x heritability)

Where,

h^2: Narrow sense heritability.

S: Selection differential (difference between the mean of selected individual and the population mean

Xμ: Mean of population,

Xs: Mean phenotypic value after selection (sample mean),

Genetic gain (ΔG) was estimated as:

G = Percentage gain;

Where,

h^2: Narrow sense heritability;

Deviation x 100% percentage gain = percentage deviation x heritability

Naming of *Colletotrichum lindemuthianum* races

Four seeds of each differential cultivar were germinated and dipped into inoculate suspension of six different isolates. Six sets of differential cultivars were used. The germinated inoculated seeds were placed in a plastic tray and then covered with a thin layer of sterilized soil. Trays were placed in a growth chamber and watered daily for 14 days. Disease scoring was performed 10 days after sowing

(Bigirimana and Höfte 2001). The disease score data was used to determine susceptible and resistant cultivars. The races derived from different *C. lindemuthianum* isolates were distinguished by using a set of differential cultivars. This set consisted of 12 cultivars, each with a designated binary number as follows: Michelite, 1; Michigan Dark Red Kidney, 2; Perry Marrow, 4; Cornell 49-242, 8; Widusa, 16; Kaboon, 32; Mexico 222, 64; PI 207262, 128; To, 256; Tu, 512; AB136, 1024; and G2333, 2048. The sum of the numbers assigned to each infected cultivar of the differential set determined race designation (Pastor-Corales et al. 1994).

RESULTS AND DISCUSSION

Pathogen in Isolates

Thirty-two isolates of *C. lindemuthianum* were isolated from different common bean samples of infected bean cultivars from 26 sites within an elevation ranging between 1390-2197 meters above sea level (Table 2).

In Babati Rural, Karatu, and Arumeru districts, the samples were drawn from bush types (Lyamungo 90, JESCA), semi-climber (Canadian wonder), and climbing bean types (Masai Red). The common bean cultivars collected, such as Lyamungo 90, Soya Njano, JESCA, Canadian wonder, and farmer, were all infected with *C. lindemuthianum.* The common bean-diseased plant samples collected were as indicated in Figure 4.

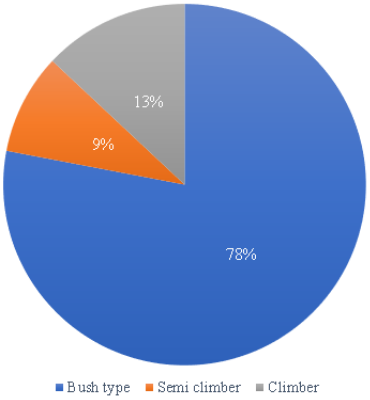

Figure 4. Common bean cultivars diseased samples percentage infected by *Colletotrichum lindemuthianum*, their growth habit and distribution as collected from Babati, Karatu, Mbulu, Arumeru, and Mvomero in Morogoro

Table 2. Collected isolates of common bean anthracnose disease from Babati, Karatu, Mbulu, Arumeru, and Mvomero Districts, Tanzania

Name of site	Sample code name	Variety	Growth habit	Gene pool	Altitude in m.absl
Bashnet-Manyara	1ABASH	JESCA (Punda)	Bush type	Andean	2189
Long /Endaw	1BASH	Farmer's variety	Bush type	Andean	2193
Long	1CBASH	Experimental variety	Climber	Mesoamerican	2195
Long	2A	Masai Red	Climber	Mesoamerican	2197
Endaw	2B	Masai Red	Climber	Mesoamerican	2186
Endaw	2C	Lyamungo 90	Bush type	Andean	2187
Endaw	2D	Farmer's var.	Bush	Andean	2183
Endaw	3A	Farmer's var.	Bush type	Andean	2146
Bony	3B	Farmer's var.	Bush type	Andean	2156
Bony	3D	Farmer's var.	Bush type	Andean	2166
Bony	3E	Farmer's var. (black grains)	Semi climb**er**	Mesoamerica	2170
Masquaroda-Mbulu	1AMASQ	Lyamungo 90	Bush	Andean	1895
Mbulu	1BMBLU	Lyamungo 90	Bush	Andean	1942
Simba	1A1KMS	Farmer's variety	Semiclimber	Mesoamerica	1498
Kambiya Simba	1BKMS	Farmer's variety	Climber	Mesoamerica	1523
Kainam	1A-2KAI	Farmer's variety	Bush	Andean	1544
Kambi ya samba	1CKMS	Soya Njano	Bush type	Andean	1533
Kambi ya Simba	1DKMBS14	Farmer'svariety	Semiclimber	Mesoamerica	1534
Kambi ya Simba	1EKMBS	Farmer's variety	Bush	Mesoamerican	1537
Rhotia	1ARhotia	Soya Njano	Bush	Andean	1613
Rhotia	2BRHOT	Soya Njano	Bush	Andean	1624
Rhotia	2CRHOT-KR	Soya Njano	Bush	Andean	1621
Slahhamo	3ASLAMO	Soya Njano	Bush	Andean	1532
Slahhamo	3BSLAMO	"Bwana shamba" Canadian wonder	Bush	Andean	1548
Upper Kitete	4AUPKIT	Soya Njano	Bush	Andean	1534
Upper Kitete	4BUPKIT	Soya Njano	Bush	Andean	1717
Upper Kitete	4CUPKIT	Soya Njano	Bush	Andean	1720
Upper Kitete	4DUPKIT	Soya Njano	Bush	Andean	1720
KITETE	5AKIT	Soya Njano	Bush	Andean	1749
SARI	6ASAR	Lyamungo 90	Bush	Andean	1411
SARI	6BSAR	Lyamungo 90	Bush	Andean	1399
Mgeta	ANyd-	Lyamungo 90	Bush	Andean	1645
Mgeta	ANyd-	Farmer's variety	Bush	Andean	1645

Key: m. a.s.l: meters above sea level

Pathogenicity test

Pathogenicity test and race naming of Colletotrichum lindemuthianum *under growth chamber condition*

A total of nine pure single spores were obtained from 32 diseased samples collected. Six out of nine isolated single spores of *C. lindemuthianum* were named using twelve standard differential cultivars.

Pathogenicity and race classification of Colletotrichum lindemuthianum *isolates*

Six *C. lindemuthianum* isolates showed pathogenicity undergrowth chamber conditions on 12 differential bean cultivars, as indicated in Table 3. Six of the 12 bean differential cultivars showed susceptibility to at least one of the collected *C. lindemuthianum* isolates.

The reactions of a set of common bean differential cultivars to 6 isolates of *C. lindemuthianum* allowed the identification of the races 21, 37, 55, 161, and 533 from Karatu, SARI (Arumeru) and Babati districts. The bean cultivar Michelite was susceptible to all *C. lindemuthianum* isolates collected from Arumeru, Karatu, Mbulu, and Babati rural.

In general, isolates collected around the study infected both Andean and Mesoamerica cultivars MDRK, Perry marrow, Widusa, and Kaboon; on the Mesoamerican cultivar Michelite, Mexico222, PI 207262, TO, and TU. None of the isolates infected cultivars Cornel 49242, Mexique 222, AB136, and G2333. According to Mahuku et al. (2002), susceptibility to *C. lindemuthianum* of G2333 and AB136 was not frequently reported. In addition, they were resistant under screen house and field conditions when the pathogenicity test was conducted. The isolate found in Northern Tanzania differed from those reported by (Ansari et al. 2004) and those reported by Drone and Bailey (1999) regarding bean differential cultivars it infected.

Pathogenicity testing of Colletotrichum lindemuthianum *under field conditions at Bashnet Manyara*

The results in Table 4 showed the compatibility of 2D-1 Long Ayt isolate of bean *C. lindemuthianum* to a set of 12 bean differential cultivars which allowed naming race 161. Isolate 2D-1 Long Ayt (161) was compatible with bean cultivars Michelite, Kaboon, and PI 207262; the other nine differential cultivars were not infected. However, this race exhibited compatibility with both indeterminate and determinate bean cultivars. The differential cultivars not infected by race 161 were MDRK, Perry Marrow, Widusa, G2333, AB136, Cornell 49242, Mexico 222, TO, and TU. However, an isolate designated 161 was not among the listed isolates collected from Africa and other parts of the world, as reported by (Ansari et al. 2004). It was also not in the list of the previous isolates collected in the southern highlands of Tanzania (Dron and Bailey 1999). These results implied that isolates collected at Bashnet consisted of *C. lindemuthianum* named race 161, which was not previously reported.

Introgression of anthracnose resistance in preferred varieties

Four genotypes were grown and crossed under screen house conditions during the 2014-2015 growing seasons at SUA. Two genotypes were resistant donors, and two were recipient cultivars. The general observation in Table 5 showed the cross performances between Soya Njano x G2333, which gave 37 seeds, and Masai Red x G2333 had the lowest number of seeds (15). The inadaptability of G2333 could have contributed to the environment during crossing work. The number of seeds obtained for F1 ranged from 15-37, and for F2, were from 45 to 138. Masai Red x AB136 gave the highest number of seeds (138). Both recurrent and donor parents were climbers; compared to Soya Njano x G2333 had the lowest (45). The F3 ranged from 34 to 61 seeds, as indicated (Table 5). These results comply with the reported results on common bean plants by (Porch and Jahn 2001). Therefore, the Soya Njano, Masai x AB136, crosses in F2 and F3 produced many seeds due to AB136 good environmental adaptability and successful crosses.

Table 3. Pathogenicity test of *Colletotrichum lindemuthianum* under growth chamber conditions

Gene pool	Isolate reaction on common bean differentials												Race
	A	B	C	D	E	F	G	H	I	J	K	L	
	M	A	A	M	M	A	M	M	M	M	M	M	
Isolate Codes													Designation
2CRHOT	S	R	S	R	S	R	R	R	R	R	R	R	21
3ASLAHMO	S	R	S	R	S	R	R	R	R	S	R	R	533
6ASAR14	S	R	S	R	R	S	R	R	R	R	R	R	37
1C-BASH-L	S	R	R	R	R	S	R	R	R	R	R	R	161
2D-1 Long Ayt	S	R	R	R	R	S	R	S	R	R	R	R	161
1b-Bashnet-Bony	S	S	S	R	S	S	R	R	R	R	R	R	55

Note: Differential cultivars of common bean and their binary values (in parentheses): A, Michelite (1); B, Michigan dark red, kidney (2); C, Perry marrow (4); D, Cornell 49242, (8); E, Widusa (16); F, Kaboon,(32); G, Mexique 222 (64); H, PI 207262 (128); I, TO (256); J, TU (512); K, AB136 (1024); and L, G2333 (2048). M=Mesoamerica, A=Andean gene pools, R=resistant, S=Susceptible. 2CRHOT: RHOT- Karatu Isolate; 3ASLAHMO: Slahmo-Karatu Isolate; 6ASAR14 SARI-Arusha Isolate; 1C-BASH-L;2D-1 Long Ayt. and 1b Bashnet-Bony were all from Bashnet

Table 4. Pathogenicity of *Colletotrichum lindemuthianum* under field conditions at Bashnet Manyara, Tanzania

Differential cultivars	Genes conferring resistance	Place of cultivar	Binary number	Gene pool	disease score *	Isolate reaction*
Michelite (A)	-	0	1	MA	5	S
MDRK (B)	Co – 1	1	2	A	1	R
Perry Marrow (C)	Co – 1^3	2	4	A	1	R
Cornel 49242 (D)	Co – 2	3	8	MA	1	R
Widusa (E)	Co – 9	4	16	MA	3	R
Kaboon (F)	Co – 1^2	5	32	A	5.5	S
Mexico 222 (G)	Co – 3	6	64	MA	3	R
PI 207262 (H)	Co – 4^3, Co – 9,	7	128	MA	4	S
TO (I)	Co – 4	8	256	MA	3	R
TU (J)	Co – 5	9	512	MA	1	R
AB 136 (K)	Co – 6, Co-8	10	1024	MA	1	R
G 2333 (L)	Co – 4^2, Co-5, Co-7	11	2048	MA	1	R
Race designation			161			

Note: Binary number of a specific race was computed by summating susceptible cultivars' binary numbers. MA; Middle American gene pool; A: Andean gene pool of *Phaseolus vulgaris*. Binary number; 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 stems and prostrate branches; IV = Indeterminate climbing habit.* Disease score and *bean differential cultivars reaction on isolates

Table 5. Number of seeds of F_1, F_2, F_3obtained from crossing the donor for anthracnose disease resistance and the adapted parents of common bean local cultivars

Parent material	No. of crosses	F_1 seeds	F_2 seeds	F_3 seeds
Soya Njano x G2333	58	37	45	40
Soya Njano x AB136	42	25	89	61
Masai Red x G2333	74	15	57	42
Masai Red x AB 136	42	22	138	34

Note: Inheritance pattern of anthracnose resistance in early populations of crosses of Soya Njano, Masai Red, and G2333, AB136 segregation ratios

Crosses made between Soya Njano, Masai Red and G2333

The parental cultivars Soya Njano, Masai Red, G2333, and AB136, developed populations (F1, F2, and F3) were tested for bean anthracnose disease resistance under field conditions. Results showed that Soya Njano plants were all susceptible, as indicated in plate 1d, while all plants in the donor parent cultivar G2333 population were resistant. Plants in the F1 generation were all resistant to bean anthracnose disease. The F2 generation segregation ratio of Soya Njano x G2333 was 9R: 7S Resistant: Susceptible (χ^2 = 0.01, P= 0.872) and F3 generation plants of had segregation ratio of 9R : 7S (χ^2 = 0.001, P = 0.979) (Table 6). The segregation data from F2 and F3 populations indicated that G2333 carried two dominant resistance genes. The results of bean cultivar G2333 conferring two were reported by Young and Kelly (1996). Similar results were reported by (Campa et al. 2011). However, the segregation ratio of 9: 7 implied that two pairs of genes with duplicate recessive epistasis were expressed by the heterozygous dominant individuals phenotypically distinguishable from other possible genotypes obtained from the studied populations (Burns 1980). González et al. (2015) reported that either additive main, epistatic, or both effects function concurrently; those are responsible for controlling anthracnose disease resistance in beans.

The F1 plant population was all resistant to *C. lindemuthianum*, indicating that a dominant gene was responsible for resistance. The F2 plant population segregation was fitted to 10R: 6S ratio (5R) Resistant and (3S) susceptible (χ^2 = 0.714; P > 0.05) and F3 population segregation ratio was 10R : 6S ratio (χ^2 = 0.002; P > 0.05) in (Table 6). These results revealed that two dominant genes conferred resistance to the developed populations of Masai Red and G2333. Similar inheritance was reported by Pastor-Corrales et al. (1994) on the presence of two dominant independent genes in G2333 controlling resistance to *C. lindemuthianum*. However, the segregation of 9: 7, 10:6 (5:3) ratios expressed two epistatic genes relationships that could correspond to the ratios found in this study, as reported by (Diering and Tomas 2001). Other findings showed that G2333 was a three-gene pyramided cultivar, with genes at different loci conferring resistance independently (Vallejo and Kelly 2009). According to Mahuku et al. (2002), genetic resistance to some pathotypes of *C. lindemuthianum* is conferred by various single, duplicate, or complementary dominant genes. However, bean cultivar G2333 was reported to be capable of controlling more than 380 races in different areas (Pastor-Corrales et al. 1994).

Crosses made between Soya Njano, Masai Red and AB136

The donor parent AB136, Soya Njano, Masai Red recipient bean cultivars, and F1, F2, and F3 populations derived from crosses made between Soya Njano, Masai

Red, and AB136 were inoculated with race 161. Soya Njano and Masai Red plants were all susceptible to *C. lindemuthianum*, as indicated in Figures 5A and 5B. The spreader row of Lyamungo 90 was susceptible, as shown in Figures 5C and 5D. The donor parent AB136 plants were all resistant. The F1 plants were all resistant to common bean anthracnose disease; this indicated that the dominant gene controlled resistance in AB136. The F2 plants segregation fitted to 3: 1 ratio ($X^2 = 3.56$, $P = 0.04$) (Table 7). The F3 population showed a significant difference from the F2 population at 0.05 probability. It exhibited that AB136 conferred a single dominant gene, similar to other studies (Gonçalves-Vidigal et al. 2001). The F3 population segregation ratio for resistance of *C. lindemuthianum* was also 3: 1 ($\chi^2 = 0.44$; $P < 0.50$). In the crosses between Masai Red and AB136, the F2 population segregation was 3R:1S ratio ($\chi^2 = 4.19$; $P < 0.05$) and F3 population segregated at a ratio of 3R: 1S ($\chi^2 = 0.55$; $P < 0.50$) (Table 8). The results conformed with other studies (Alzate-Marin et al. 1997) on the resistance of *C. lindemuthianum* races 89 and 64. All F1 populations developed from the crosses exhibited resistance, implying that resistance was due to dominant genes transferred into the derived generation. This study reported the presence of two independent resistant genes of AB136 to pathogen race 73 of *C. lindemuthianum*, where the (Co-6) gene was the dominant gene and a recessive gene assigned with the genetic symbol co-8 (Alzate- Marin et al. 1997).

Heritability estimation

The estimated narrow sense heritability ranged between 0.42 and 0.46 in populations derived from crosses between Soya Njano, Masai Red with G2333, and Masai Red with AB136 was 0.22 (Table 8 and Figure 3). These results implied the presence of moderate heritability in developed populations. Similar results were reported by (Poletine et al. 2006), that medium magnitude narrow sense heritability value, even at that moderate magnitude, indicated the possibility of success in obtaining resistant genotypes in derived populations. For example, the population derived from Soya Njano with AB136 had a heritability of 0.53 (53%) that showed moderate heritability, $R^2 = 0.29$ coefficient of determination (Figure 6).

Populations of Masai Red x AB136, Soya Njano x G2333, and Masai Red x G2333 were 5%-15%. The moderate narrow sense heritability in developed F2 from Soya Njano x AB136 implied that the mean performance of the developed populations has regressed at 53% towards the mean of the previous resistant generation (Stanfield 1991). When heritability for a trait is high, selection using phenotypic traits is effective (Campa et al. 2014).

Table 6. Segregation ratios for resistance and susceptible progenies in parental cultivars and their developed populations to *Colletotrichum lindemuthianum* under field conditions

Pedigree	Number of plants			Segregation		
	Generation	Resistant	Susceptible	Ratio (R:S)	χ^2	Probability
Soya Njano	P1	0	12	…….	…….	…….
G2333	P3	12	0	…….	…….	…….
Soya Njano x G2333	F1	12	0	…….	…….	…….
Soya Njano x G2333	F2	12	10	9:7	0.016	0.872
Soya Njano x G2333	F3	13	10	9:7	0.001	0.979
Masai Red	P2	0	12	…….	…….	…….
G2333	P3	12	0	…….	…….	…….
Masai Red x G2333	F1	12	0	…….	…….	…….
Masai Red x G2333	F2	15	6	10 : 6	0.714	0.296
Masai Red x G2333	F3	13	8	10 : 6	0.002	0.955

Note: χ^2: Chi test, P1= Soya Njano, P2: Masai Red, P3: G2333, P3: G2333; F1- F3: Soya Njano x G2333 and Masai Red x ABG2333

Table 7. Segregation ratios for resistance and susceptibility in parental cultivars and their derived populations to *Colletotrichum lindemuthianum* under field conditions

Pedigree	Number of plants			Segregation		
	Generation	Resistant	Susceptible	Ratio	χ^2	Probability
Soya Njano	P1	0	12	…….	……	…….
AB136	P4	12	0	…….	…….	…….
Soya Njano x AB136	F1	12	0	…….	…….	…….
Soya Njano x AB136	F2	14	10	3:1	3.56	0.04
Soya Njano x AB136	F3	13	6	3:1	0.44	0.50
Masai Red	P2	0	11	…….	…….	…….
AB136	P4	12	0	…..	…..	…….
Masai Red x AB136	F1	12	0	……..	……	…..
Masai Red x AB136	F2	13	10	3:1	4.19	0.05
Masai Red x AB136	F3	15	7	3:1	0.55	0.50

Note: χ^2: Chi test, P1: Soya Njano, P2: Masai Red, P3: G2333, P4: AB136, F1-F3: Soya Njano x AB136 and Masai Red x AB136

Table 8. Heritability in narrow sense estimation for *Colletotrichum lindemuthianum* in derived F2 and F3 populations

Generations (F2& F3)	Mean disease Score							
	F2	F3	SDEVF2	SDEF3	P-value	b (h^2)	A	R^2
P1 X P3	3.55	4.05	1.37	1.73	0.13	0.42	2.57	0.12
P2 X P3	2.67	3.62	1.65	1.96	0.07	0.46	2.38	0.15
P1 X P4	3.53	2.84	1.6	1.63	0.02	0.53	0.92	0.29
P2 x P4	4.17	3.22	1.93	1.61	0.73	0.22	2.18	0.05

Note: P1= Soya Njano, P2: Masai Red, P3: G2333, P4: AB136, R^2 Regression determination, F2, F3: Filial generation 2 and 3, SDEV: Standard deviation of F2 and F3, b: Coefficient of X slope; stands for (h^2) heritability in the narrow sense. P: P value; A: y-intercept

Figure 5. Pictures (A-D) show diseased bean plants with common bean anthracnose symptoms on different parts of the plants. A. Masai Red- pod symptoms; B. Soya Njano – pod symptom; C. Lyamungo 90 (diseases spreader variety); D. Lyamungo at vegetative stage

Comparisons of parents, F1, F2, and F3 for common bean anthracnose disease resistance

The mean disease scores of crosses of F1, F2, and F3 generated populations from Soya Njano, Masai Red with G2333 and mid-parent are presented in Figures 7A and 7B. The results show a reduction of disease reaction in the F_1 populations based on recipients and mid-parent resistance performance. The F2 populations of Masai Red x G2333 mean score were 2.7 more resistant than the mid-parent mean score of 3.8. The susceptible bean cultivars mean disease scores were between 6.0-6.5, which implied high susceptibility to common bean anthracnose. Abengmeneng et al. (2015) reported that genotypes with mean disease resistance above the population's mean performance are recommended for selection and use as seed. According to the results in Figures 7C and 7D, Soya Njano x, AB136 (F3) and Masai Red x AB136 (F3) exhibited improvement of resistance in developed populations to *C. lindemuthianum* and Soya Njano xG2333, the Masai Red x G2333 (F2 and F3) showed equal performance as mid-parent. Abengmeneng et al. (2015) reported that only genotypes whose phenotypes were approximate to the population mean were good for selection as resistant plants and fit in the Northern zone environment.

Estimation of genetic gain

The results in Table 9 showed that the mean anthracnose disease score was 6.0 for Masai Red and Soya Njano was between 6.0 to 6.5. The donor bean cultivar G2333's mean disease score was 1.5, and AB136 was 1.5. The maximum genetic gain through selection depends on the phenotypic variations present in the base population and maintained in the following cycles through selection (Janick 2010). However, the genetic gain in the F2 and F3

populations of Soya Njano and G2333 genetic gain is between 0.2 and 2.0. The F2 populations derived from Soya Njano, Masai Red, and AB136 genetic gain were absolute and ranged between (-0.1 to 0.8). Negative succession per season represented the stabilization of the genetic gain in disease resistance, as displayed by the figures close to zero (Chiorato et al. 2010). These results showed different levels of resistance to common bean anthracnose disease, hence, high potential genotypes resistant to *C. lindemuthianum* for selection.

Genetic gain results in Table 9 showed increased genetic gain through moderate narrow sense heritability. The results concur with the findings reported by Souza et al. (2014) that genetic gain depends on the availability of moderate to high heritability and a useful amount of genetic variation. Population breeding methods such as line development by standard backcrossing, pedigree, or bulk and recurrent selection were suggested as most perfectly suited to long-term genetic gains. However, these methods require sufficient time (Cowling 1996).

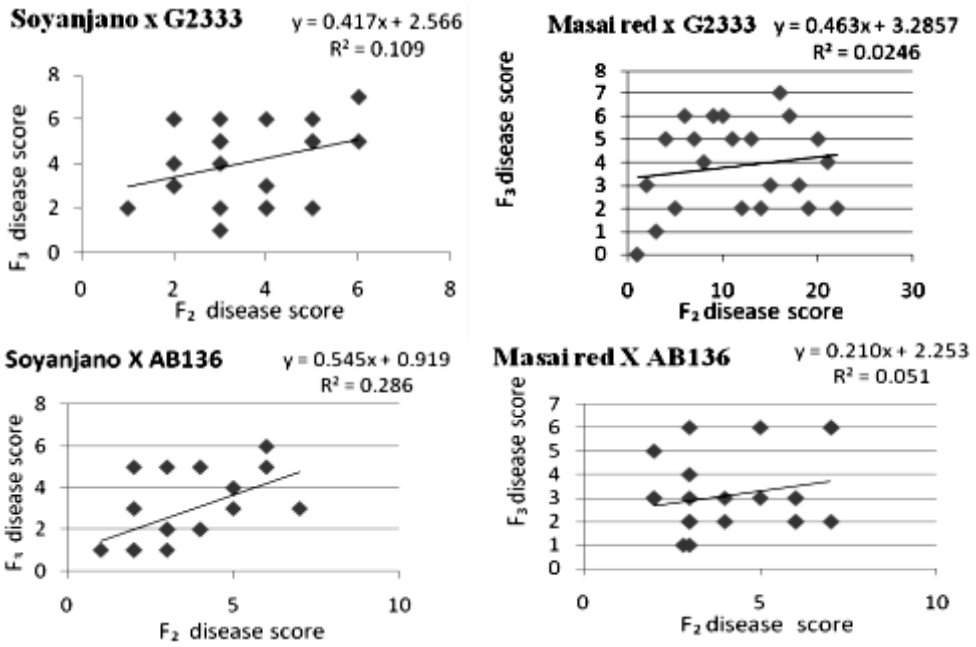

Figure 6. Heritability determination of the *resistance of C. lindemuthianum* using F3 populations to F2. P1: Soya Njano, P2: Masai Red, P3: G2333, P4 AB136, Regression graphs on the inheritance of F2 and F3 populations

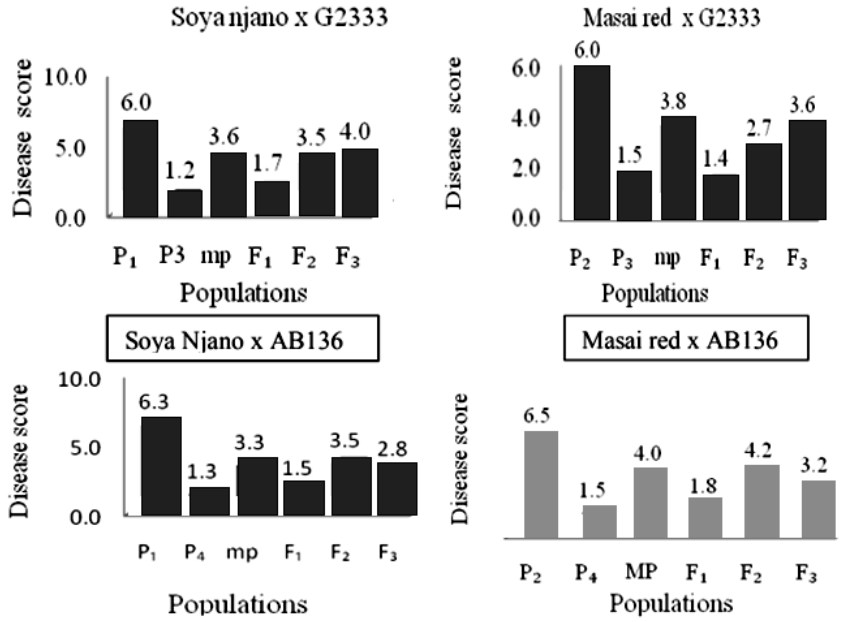

Figure 7. Comparison of mid-parents F1, F2, and F2 generations for resistance of Common-bean Anthracnose disease. Key: P1: Soya Njano, P2: Masai Red, P3: G2333, P4: AB136

Table 9. Genetic gain for *Colletotrichum lindemuthianum* resistance estimation in F2 and F3 derived populations

Parent material		Mpt	Mean disease score (1-9)	deviation/F-mid parent	Grand mean disease scores	Percentage deviation	Heritability in h^2	% age gained	Genetic gain (scores)
Soya Njano			6.0						
Masai Red			6.0						
G2333			1.5		1.5				
Soya Njano x G2333	F2	3.6	3.5	-0.1	3.5	-2.9	0.42	1.2	0.5
Masai Red x G2333	F2	3.8	2.7	-0.3	3.5	-8.6	0.46	3.9	1.8
Soya Njano x G2333	F3	3.6	4.0	-0.1	3.5	-2.9	0.26	0.7	0.2
Masai Red x G2333	F3	3.8	3.6	-0.3	3.5	-8.6	0.48	4.1	2.0
Grand mean			3.5						
Soya Njano			6.3						
Masai Red			6.5						
AB136			1.5		1.5				
Soya Njano xAB136	F2	3.3	3.5	0.1	3.4	2.9	0.53	1.6	0.8
Masai Red x AB136	F2	4	4.2	-0.6	3.4	-17.6	0.22	-3.9	-0.9
Soya Njano xAB136	F3	3.3	2.8	0.1	3.4	2.9	0.52	1.5	0.8
Masai Red x AB136	F3	4	3.2	-0.6	3.4	-17.6	0.24	-4.2	-1.0
Grand mean			3.4						

Note: Mpt: mid-parent, F: Filial generation

In conclusion, Race 161 was determined from diseased bean samples collected at Bashnet, with pathogenicity testing conducted under field conditions. Donor parents of bean cultivars G2333 and AB136 exhibited resistance to *C. lindemuthianum* pathogens when tested under field conditions, hence are potential donor parents. Other potential donor bean cultivars were MDRK, Perry Marrow, Widusa, Cornell 49 242, Mexico 222, TO, and TU, which exhibited resistance under field testing at Bashnet. The genes for resistance against common bean anthracnose disease were successfully introgressed into adapted bean cultivars Soya Njano and Masai Red using conventional breeding. The results showed two genes introgressed from resistant parents G2333 and one dominant gene from AB136. Heritability in the narrow sense of common bean anthracnose disease resistance was moderate in the developed populations in this study. However, the results showed that selecting resistant genotypes from the derived populations was possible.

REFERENCES

Abengmeneng CS, Ofori DA, Jamnadass R, Akromah R, Kumapley P. 2015. Estimation of heritability and genetic gain in height growth in *Ceiba pentandra*. Afr J Biotechnol 14 (22): 1880-1885.

Alzate-Marin AL, Baía GS, de Paula Júnior TJ, de Carvalho GA, de Barros EG, Moreira MA. 1997. Inheritance of anthracnose resistance in common bean differential cultivar, AB 136. Plant Dis 81: 996-998. DOI: 10.1094/PDIS.1997.81.9.996.

Ansari KI, Palacios N, Araya C, Langin ETD, Doohan FM. 2004. Pathogenic and genetic variability among *Colletotrichum lindemuthianum* isolates of different geographic origins. Plant Pathol 53 (5): 635-642. DOI: 10.1111/j.0032-0862.2004.01057.x.

Beebe S, Rao I, Mukankusi C, Buruchara R. 2012. Improving Resources Use Efficiency and Reducing Risk of Common Bean Production in Africa, Latin America, and Caribbean. International Center for Tropical Agriculture (CIAT), Cali.

Bigirimana J, Höfte M. 2001. Bean anthracnose, inoculation methods, and influence of plant stage on resistance of *phaseolus vulgaris* cultivars. Phytopathology 149: 403-408. DOI: 10.1111/j.1439-0434.2001.tb03870.x.

Blair MW, Fregene MA, Beebe SE, Ceballos H. 2007. Marker-assisted selection in common bean and cassava. In: Guimaraes EP, Ruane J, Scherf BD, Sonnino A, Dargie JD (eds.). Marker Assisted Selection: Current Status and Future Perspective in Crops, livestock, Forestry and Fish. Food and Agriculture Organization of the United Nations, Rome, Italy.

Broughton WJ, Hern'andez G, Blair M, Beebe S, Gepts P, Vanderleyden J. 2003. Beans (*Phaseolus* spp.) model food legumes. Plant and Soil 252: 55-128. DOI: 10.1023/A:1024146710611.

Burns WG. 1980. The Science of Genetics. Macmillan Publishing Company, NewYork.

Campa A, Giraldez R, Ferreira JJ. 2011. Genetic analysis of the resistance to eight anthracnose races in the common bean differential cultivar Kaboon. Phytopathology 101: 757-764. DOI: 10.1094/PHYTO-11-10-0296.

Campa A, Rodríguez-Suárez C, Giraldez R, José JF. 2014. Genetic analysis of the response to eleven *Colletotrichum lindemuthianum* races in a RIL, population of common bean (*Phaseolus vulgaris* L.). Plant Biol 14: 115-119. DOI: 10.1186/1471-2229-14-115.

Chiorato AF, Carbonell SAM, Vencovsky R, Silva Fonseca Júnior H, Pinheiro JB. 2010. Genetic gain in the breeding program of common beansat IAC from 1989 to 2007. Crop Breed Appl Biotechnol 10: 4-7. DOI: 10.1590/S1984-70332010000400007.

CIAT. 1989. Bean production problems in the tropics. 2nd ed. In: Schwartz HF, Pastor-Corrales MA (eds). Cali, Colombia. CIAT.

CIAT. 2013. The Impacts of CIAT's Collaborative Research. International Center for Tropical Agriculture, CIAT, Cali, Colombia.

Cowling WA. 1996. Plant breeding for stable agriculture. J R Soc West Aust 79: 00-00.

Davide LMC, de Souza EA. 2009. Pathogenic variability within race 65 of *Colletotrichum lindemuthianum* and its implications for common bean breeding. Crop Breed Appl Biotechnol 9: 23-30. DOI: 10.12702/1984-7033.v09n01a04.

Debouck DG. 1994. Beans (*Phaseolus* spp.). International Board for Plant Genetic Resource, Rome, Italy.

Diering DA, Tomas PM. 2001. Inheritance of Male sterility in *Lesquerella fendleri*. J Am Soc Hortic Sci 126 (6): 738 743. DOI: 10.21273/JASHS.126.6.738.

Dron M, Bailey JA. 1999. Improved Control of Bean Anthracnose Disease in Latin America and Africa Through Increased Understanding of Pathogen Diversity. CTA.

Food and Agricultural Organization of the United Nations (FAOSTAT). 2014. Dry Bean Statistical Database. [http://faostat3.fao.org] site visited on 17/6/2016.

Food and Agricultural Organization of the United Nations (FAOSTAT). 2015. State of Diversity of Major and Minor Crops. The Second Report on the State of the World's.

Gonçalves-Vidigal MC, Sakiyama NS, Vidigal Filho PS, Amaral Júnior AT, Poletine JP, Oliveira VR. 2001. Resistance of common bean cultivar AB 136 to Races 31 and 69 of *Colletotrichum lindemuthianum*: The Co-6 locus. Crop Breed Appl Biotechnol 1 (2): 99-104. DOI: 10.13082/1984-7033.v01n02a01.

González AM, Yuste-Lisbona FJ, Rodiño AP, De Ron AM, Capel C, García-Alcázar M, Lozano R, Santalla M. 2015. Uncovering the genetic Arc hitecture of *Colletotrichum lindemuthianum* resistance through QTL mapping and epistatic interaction analysis in common bean. Plant Sci 6: 141. DOI: 10.3389/fpls.2015.00141

Hillocks RJ, Madata CS, Chirwa R, Minja EM, Msolla SN. 2006. *Phaseolus* bean improvement in Tanzania 1959-2005. Euphytica 150: 225-231. DOI: 10.1007/s10681-006-9112-9.

Janick J. 2010. Plant Breeding Reviews; A John Willey and Sons, Inc, Hoboken, New York. DOI: 10.1002/9780470880579.

Jones AL, and Mejia, D. 1999. *Phaseolus vulgaris*: Beans; Production; Processing; Harvesting; Postharvest Losses; Pest Control. Food and Agriculture Organization of the United Nations, Rome, Italy.

Kelly JD, Vallejo VA. 2009. New insights into the anthracnose resistance of common bean landrace G2333. Hortic J 2: 29-33. DOI: 10.2174/1874840600902010029.

Mahuku GS, Jara CE, Cajiao C, Beebe S. 2002. Sources of resistance to *Colletotrichum lindemuthianum* in the secondary gene pool of *Phaseolus vulgaris* and in crosses of primary and secondary gene pools. Plant Dis 86: 1383-1387. DOI: 10.1094/PDIS.2002.86.12.1383.

Miklas PN, Kelly JD, Beebe SE, Blair MW. 2006. Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. Euphytica 147 (2): 105-131. DOI: 10.1007/s10681-006-4600-5.

Munda A, Radisek S, Šuštar-Vozli J, Javornik B. 2009. Genetic variability of *Colletotrichum lindemuthianum* isolates from Slovenia and resistance of local *Phaseolus vulgaris* germplasm. J Plant Dis Prot 116 (1): 23-29. DOI: 10.1007/BF03356282.

Mwesigwa JB. 2009. Diversity of *Colletotrichum lindemuthianum* and Reaction of Common Bean Germplasm to Anthracnose Disease. [Thesis]. Faculty University of Makerere, Uganda.

Nyambo B. 2009. Tanzania Agricultural Sector Development Program (ASDP) Integrat ed Pest Management Plan, (IPMP) Revised Version. MAFS publications, Dar es Salaam, Tanzania.

Pastor-Corrales MA, Erazo OA, Estrada EI, Singh SP. 1994. Inheritance of anthracnose resistance in common bean accession G 2333. Plant Dis 78: 959-962. DOI: 10.1094/PD-78-0959.

Poletine JP, Gonçalves-Vidigal MC, Vidigal Filho PS, Gonela A, Schuelter AR. 2006. Genetic inferences in common bean differential cultivarsto *C. lindemuthianum* Race 69. Ciencias Agrarias Londirna 3: 393-398. DOI: 10.5433/1679-0359.2006v27n3p393.

Porch TG, Beaver JS, Debouck DG, Jackson SA, Kelly JD, Dempewolf H. 2013. Use of wild relatives and closely related species to adapt common bean to climate change. J Agron 3: 433-461. DOI: 10.3390/agronomy3020433.

Porch TG, Jahn M. 2001. Effects of high-temperature stress on micro sporogenesis in heat-sensitive and heat-tolerant genotypes of *Phaseolus vulgaris*. Plant Cell Environ 24 (7): 723-731. DOI: 10.1046/j.1365-3040.2001.00716.x.

Raatz S. 2013. Nutritional value of Dry Beans, Retrieve from [http://beaninstitute. com/healthbenefits/nutritionalvalueofdrybeans]. Site visited on 25/5/2013.

Sharma PN, Padder BA, Sharma OP, Pathania A, Sharma P. 2007. Pathological and molecular diversity in *Colletotrichum lindemuthianum* (bean anthracnose) across Himachal Pradesh, a North-Western Himalayan State of India. Australas Plant Pathol 36: 191-197. DOI: 10.1071/AP07013.

Souza DA, Pereira FAC, Dias JA, Leite ME, Bosco dos Santos J. 2014. Reaction of common bean progenies to white mold derived from recurrent selection. Ciência Rural 44: 4-7. DOI: 10.1590/S0103-84782014000400001.

Stanfield WD. 1991. Theory and Problems of Genetics. McGraw – Hill Companies, New York.

Tryphone MG, Chilagane LA, Protas D, Kusolwa PM, Nchimbi-Msolla S. 2013. Marker assisted selection for common bean diseases improvements in Tanzania prospects and future need. In: Andersen SB (eds.). Plant Breeding from Laboratories to Fields. InTech, Rijeka.

Vallejo V, Kelly JD. 2009. New insights into the anthracnose resistance of common bean landrace G 2333. Hortic J 2: 29-33. DOI: 10.2174/1874840600902010029.

Van Schoonhoven A, Pastor-Corrales MA. 1987. Standard System for the Evaluation of Bean Germplasm. (CIAT), Cali, Colombia.

Young RA, Kelly JD. 1996. Characterization of the genetic resistance to *Colletotrichum lindemuthianum* in common bean differential cultivars. Plant Dis 80: 650-654. DOI: 10.1094/PD-80-0650.