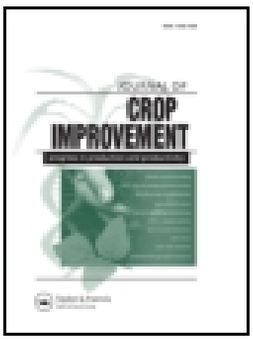

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Resistance to *Pythium* root rot and anthracnose among Kenyan common bean genotypes and marker-assisted introgression of resistance genes

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is an important food legume crop and major protein source in Kenya and many parts of the world. However, the yields are low due to the diseases like anthracnose caused by *Colletotrichum lindemunthianum* and *Pythium* root rots. The study, therefore, sought to screen Kenyan common bean genotypes for anthracnose and *Pythium* resistance and to improve the resistance of local landraces to the two diseases. Local landraces and commercial cultivars were screened for resistance under greenhouse conditions for *Pythium* and *in vitro* for anthracnose. The results showed that majority of the landraces were moderately resistant to anthracnose and *Pythium* root rot. The study further utilized molecular markers linked to anthracnose and *Pythium* resistance, respectively, to determine their potential to detect specific resistance genes among the test genotypes. None of the markers amplified the test genotypes except the control genotypes pointing toward different resistance genes. Further, cultivars G2333 and KK8 were used as donor parents for anthracnose and *Pythium* resistance, respectively, in a marker-assisted backcross breeding program involving three high-yielding farmer-preferred landraces. A number of lines were developed that carry both resistance genes. These lines can be advanced for release as new cultivars to enhance common bean production in Kenya.

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KEYWORDS
Common bean landraces; resistance; *Pythium*; anthracnose; backcross; molecular markers

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an important legume crop in East Africa for direct consumption and as a source of income to many smallholder farmers (Odera 2016). Despite being a major source of protein to many households, biotic and abiotic factors limit its production (Sharma 2012). Diseases are the principal constraint of common bean production followed by pests and low soil fertility (Rodríguez De Luque and Creamer 2015), although other production constraints exist including poor market

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access, high cost of inputs, low farm-gate prices, large number of cartels and fluctuations in market prices (Katungi et al. 2009; Odera 2016; Wanjala et al. 2019). Furthermore, low adoption rates for improved varieties by the farmers predispose the crop to some diseases like *Pythium* root rot and common bean anthracnose, which can cause severe yield losses (Nzungize et al. 2011a; Beebe et al. 2013). Few studies have focused on the identification of sources of resistance among common bean genotypes in Kenya especially the western part of the country, a major common bean production area in Kenya.

Bean anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams. Scrib, can affect all aerial plant parts at all stages of development (Agrios 2005; Halvorson 2015). Symptoms appear on aerial parts of the plant causing black shrunken lesions with spores on pods (Kelly and Vallejo 2004). The disease is more prevalent in temperate and subtropical climates with its development favored by moderate temperature with excessive humidity (Sharma et al. 2019; Anunda 2021). Breakdown of resistance has been observed due to several pathogen races as well as diversity within the same race (Sharma et al. 2019). The pyramiding of different race-specific resistance alleles could be used as a strategy for developing broad and durable resistance to a large number of races (Souza et al. 2014). A number of genes that confer resistance to anthracnose in common bean have been characterized (*Co-1* to *Co-17, Co-u, Co-w, Co-x, Co-y, Co-z, CoPv02c3-X, CoPv02c7-X, CoPv02c19-X, CoPv0c2449-X*, and *CoPv09c453-C*) and molecular markers linked to the genes developed to aid crop improvement (Campa et al. 2014; Campa, Trabanco, and Ferreira 2017; Coimbra-Gonçalves et al. 2016; Zuiderveen et al. 2016). The use of resistant cultivars is the most successful, efficient, and safe approach of managing anthracnose in common beans (Meziadi et al. 2016). Classical breeding methods have been used previously in Kenya resulting in long periods of cultivar development (Blair et al. 2007). Marker technology offers new ways to fasten cultivar development with more precision and time reduction in release of improved bean varieties (Miklas et al. 2006).

Bean root rot caused by *Pythium* spp. is another most damaging disease affecting common beans in East and Central Africa (Wortmann et al. 1998). The pathogen causes a wet rot of the seedling, either before or after emergence where the pith of the stem is attacked leading to yellowing of leaves, wilting, and eventually death. Some *Pythium* species are favored by cool temperatures, whereas others are favored by warm temperatures but are all favored by wet conditions (Owen-Going et al. 2008). Like anthracnose, the use of resistant bean varieties is the major effective, economical, and environmentally sustainable strategy to control *Pythium* root rot disease (Papias et al. 2016). Resistance to *Pythium* root rot is conditioned by a single dominant gene (Otsyula et al. 2003; Nzungize et al. 2011b). The cultivars RWR 719, AND1062, and SCAM-80-CM/15 have been used as donors for

resistance against the virulent and predominant *Pythium* spp. in breeding programs in East and Central Africa region (Otsyula et al. 2003). Variety KK8 was developed from a breeding program involving the aforementioned genotypes and a SCAR marker linked to the resistance was developed and being utilized for marker-assisted selection (MAS) (Mahuku et al. 2007; Nzungize et al. 2011b). Furthermore, a study by Okii et al. (2017) showed that multiple pathogen co-infections on common beans are responsible for complete crop losses in susceptible bean varieties. This necessitates breeding for disease resistance by targeting multiple pathogens simultaneously through pyramiding resistance genes in a single genotype for a broader and durable resistance (Mondo, Kimani, and Narla 2019).

Marker-assisted selection enables selection at seedling stage by reducing the number of resistance tests, identification of resistant genotypes in absence of the pathogen (Miklas et al. 2006; Collard and Mackill 2008; Ferreira et al. 2012; Ndee 2013; Uwera et al. 2021). The selection of populations using molecular markers tightly linked to specific genes increases the efficiency of breeding programs (Gupta, Langridge, and Mir 2010). However, gene flow within and between common bean genepools and races as a result of out-crossing in farmers' fields or crossing programs in formal breeding could result in intermediate phenotypes that do not correspond well to any of the single race or genepool divisions (Beebe et al. 2001; Islam et al. 2004; Blair et al. 2007).

Introgression of multiple disease resistance into commercial genotypes has been achieved in Kenya through marker-assisted gamete selection (Musyimi 2014). However, there is no documented breeding efforts that focuses on characterization and improvement of common bean local landraces in Kenya. Therefore, there is a need to characterize common bean germplasm for resistances in order to identify/develop potential markers that can be used for selection of the resistant varieties (Anunda 2021). This study aimed at characterizing anthracnose and *Pythium* root rot resistance in landraces and commercial cultivars grown in western Kenya and developing breeding lines that combine resistance to the two pathogens.

2. Materials and methods

2.1. *Plant materials*

The germplasm was made up of 98 common bean genotypes including 89 landraces collected across Kenya and 9 commercial varieties (Table 1 and Figure 1). The landraces and locally released cultivars (GLP 2, GLP 585, CAL 194, KK2, KK8, Chelelang, and Tasha) were collected from different agro-ecologies in the following administrative Counties in Kenya: Kakamega, Bungoma, Busia, Trans-Nzoia, Vihiga, Kisii, Siaya, Migori Bomet, Nandi,

Table 1. Seed characteristics of common bean genotypes evaluated for *Pythium* root rot and anthracnose resistances.

Seed type	Genotypes	Seed type	Genotypes
Black medium oval	Landrace 76	Pink large oval	Landrace 57
Black Calima large cuboid	Landrace 5, 6	Purple medium oval	Landrace 20
Black Calima large kidney	Landrace 72	Red & white large round	Landrace 75
Black Calima medium oval	Landrace 2	Red Calima large cuboid	Landrace 4, 79
Brown medium oval	Landrace 52, 53	Red Calima large kidney	CAL 96, Chelelang, GLP2, Tasha
Brown small oval	Landrace 12, 13, 15	Red Calima large oval	Landrace 83, CAL 194
Brown-speckled large cuboid	Landrace 67	Red Calima medium cuboid	KK8
Brown-speckled large Kidney	Landrace 77	Red Calima small oval	Landrace 3, 81
Brown-speckled large round	Landrace 69	Red large cuboid	Landrace 32
Brown-speckled medium kidney	Landrace 64	Red large kidney	Landrace 49, 74
Brown-speckled medium oval	Landrace 61, 66	Red large oval	Landrace 56
Brown-speckled medium round	Landrace 68	Red large round	Landrace 54
Brown-speckled small round	Landrace 60	Red medium cuboid	Landrace 70
Cream large oval	Landrace 59	Red medium kidney	G2333, Landrace 58
Cream medium oval	Landrace 14, 26, 51, 86	Red medium oval	Landrace 33, 35, 36, 82
Dark green large round	Landrace 16	Red small kidney	GLP585, KK22
Dark green medium round	Landrace 1	Red small oval	Landrace 29, 31, 34, 37, 38, 39, 41, 42, 44, 46
Dark Red large cuboid	Landrace 28, 43, 48	Red small round	Landrace 71
Dark Red large round	Landrace 47	White medium cuboid	Landrace 10
Dark Red medium kidney	Landrace 80	White small oval	Landrace 9, 11
Dark Red medium oval	Landrace 50	White small round	Landrace 8
Dark Red small oval	Landrace 27, 30, 40, 45, 78, 84, 85	White speckled large kidney	Landrace 65, 87, 89
Dark Red small round	Landrace 73	White speckled medium kidney	Landrace 62, 63, 88
Grey large oval	Landrace 19	White speckled medium oval	Landrace 90
Grey small oval	Landrace 17, 18, 21	Yellow large cuboid	Landrace 23
Pink medium kidney	Landrace 55	Yellow medium round	Landrace 22, 24, 25

West Pokot, Nakuru, Kiambu, Embu, and Meru. Simple random sampling design was used in germplasm collection. Cultivar CAL 96 (Mukalazi 2004) was used as a susceptible check for *Pythium* root rot, G2333 a resistant check for anthracnose (Kelly et al. 1998; Mahuku et al. 2002), and two resistant checks for *Pythium* root rot (KK8 and KK22) (Otsyula 2010; Mukalazi 2004). CAL 96 and G2333 were obtained from the International Center for Tropical Agriculture (CIAT) in Uganda.

Figure 1. Seed type of the landraces and cultivars used in the study. LR = Landrace.

2.2. Phenotypic screening for resistance to *Pythium* root rot

Isolate MS61 obtained from the CIAT Laboratory in Uganda was used for phenotypic screening for resistance to *Pythium* root rot. The study used a characterized isolate because root rots are caused by a complex of soil-borne pathogens including *Fusarium solani, Pythium* spp., *Rhizoctonia solani,* and *Macrophomina phaseoli*. The MS61 isolate is the most virulent and has been used in studies by Otsyula (2010) and Nzungize et al. (2011b). The inoculum, which had been previously stored on filter paper, was reactivated on Corn Meal Agar (CMA) media. These were later sub-cultured on Potato Dextrose Agar (PDA) for high mycelia growth. For subsequent tests, the isolate was plated on a filter paper and stored at −20°C. A growth media made of autoclaved millet grains (300 g) mixed with 200 ml of water in autoclaveable bags was prepared following the Abawi, Ludwig, and Gugino (2006) protocol. The finger millet was left to cool in a sterilized laminar flow before mixing with one culture plate of the fully active growing mycelia. These were left to ferment and colonize the millet for 12 days at room temperature under sterile conditions. The infested finger millet was then transferred to sterilized soil media at a ratio of 1:8 v/v that was placed in wooden trays measuring 42 cm by 72 cm in a greenhouse at the Kenya Agricultural and Livestock Research Organization (KALRO) in Kakamega, Kenya (Otsyula 2010; Mondo, Kimani, and Narla 2019). The trays were then covered by a polyethylene sheet and incubated for 7 days for the pathogen to colonize the soil. Seeds of the test genotypes were thereafter sown in the inoculated soil in trays which were randomly placed in the greenhouse. Disease scoring was conducted 21 days after sowing by uprooting the plants, washing the roots and checking for symptoms. Disease rating was based on incidence, calculated as the percentage number of plants showing symptoms

of the disease, and severity was measured using a scale of 1–9 according to Schoonhoven and Pastor-Corrales (1987). A score of 1–3 was considered resistant, 4–6 moderately resistant, and 7–9 as susceptible.

2.3. *Screening for resistance to anthracnose in common bean*

Ten samples of anthracnose infected leaves and pods were collected from farmers' fields in Western Province in Kenya, through a simple random sampling method. The samples were transferred to the KALRO, Kakamega lab for isolation as described by Pastor-Corrales, Jara, and Singh (1998) and cultured on PDA media. The inoculum was further plated on Tap Water Agar (TWA) for 72 hrs to allow for sporulation. Single hyphae were later picked under a stereo microscope and cultured on new PDA media for sporulation. These plates were incubated at 22°C in alternating 12-hour light and darkness for 21 days. Races identification was performed by screening anthracnose differential cultivars using the detached leaf method (Rezene et al. 2018). The races were identified based on the susceptibility of each differential cultivar to each isolate using a binary nomenclature as described by Pastor-Corrales (1991). Thereafter, the inoculum was prepared by suspending a mixture of the sporulating cultures in distilled water to a final concentration of 1.2×10^6 spores per ml, mixed with Tween-20 (10 µl/100 ml). The middle leaflet of the first trifoliate leaf of each 21-day old plant raised in a screen house was detached from each test genotype. The detached leaves were placed in petri dishes containing moistened paper towels to create a humid environment inside the petri dish and the inoculum sprayed with the aid of a hand sprayer according to Rezene et al. (2018). The petri dishes were randomized on a lab bench with three replications and incubated on a laboratory bench at 27°C. Humidity in the petri dishes was monitored and maintained using applications of watering every three days throughout the incubation period.

2.4. *Screening common bean genotypes using molecular markers linked to resistance genes*

DNA pooled from five plants per genotype was extracted from first trifoliate leaf tissues of 14-day-old plants using the CTAB method as described by Afanador, Haley, and Kelly (1993). DNA from all tested genotypes were amplified using a set of four Sequence Characterized Amplified Regions (SCAR) markers linked to anthracnose and *Pythium* resistance genes. The SCAR marker $PYAA19_{800}$ was used to screen for *Pythium* resistance gene *Pyult1* (Mahuku et al. 2007), while SH18, SBB14, and SAB3 were used to screen for the presence of anthracnose resistance genes *Co-4*^{2} (Beebe et al.

Table 2. Molecular markers linked to *Pythium* root rot and anthracnose resistance genes.

Marker	Size (Bp)	Primer sequences	Tagged locus	Annealing temperature
SH18	1100	CCA GAA GGA GCT GAT AGT ACT CCA CAA C GGT AGG CAC ACT GAT GAA TCT CAT GTT GGG	*Co-4*^{2}	65°C
SBB14	1150/1050 codominant	GTG GGA CCT GTT CAA GAA TAA TAC GTG GGA CCT GGG TAG TGT AGA AAT	*Co-4*^{2}	67°C
SAB3	400	TGG CGC ACA CAT AAG TTC TCA CGG TGG CGC ACA CCA TCA AAA AAG GTT	*Co-5*	65°C
PYAA19	800	TTA GGC ATG TTA ATT CAC GTT GG TGA GGC GTG TAA GGT CAG AG	*Pyult1*	63°C

2001; Kelly et al. 2003) and *Co-5* (Table 2) (Beebe et al. 2001; Kimani et al. 2005), respectively.

The PCR amplifications were performed in a 10 µL final volume containing 5 ng DNA, 20 µM of each forward and reverse primer, puReTaq Ready-To-Go PCR beads (GE Healthcare) dissolved in 25 µL of molecular water. These beads contain stabilizers, BSA, dNTPs, 2.5 units of puReTaq DNA polymerase, and a reaction buffer. When the bead is reconstituted to a 25 µL final volume, the concentration of each dNTP is 200 µm in 10 mM Tris-HCl, 10 mM KCl, and 1.5 mM $MgCl_2$. Polymerase Chain Reaction (PCR) was conducted in a thermocycler using the following regime: initial denaturation (94°C/5 min), followed by 30 cycles of denaturation (94°C/10 s), primer pair-specific annealing step and an extension step (72°C/2 min), and was completed by a final extension step (72°C/5 min). The amplicons were separated by electrophoresis through a 1.2% agarose gel containing 0.5 µg/mL ethidium bromide and viewed in a trans-illuminator. The gel picture obtained was scored as (1) for the presence or (0) for the absence of the respective band.

2.5. Marker-assisted introgression of anthracnose and *Pythium* resistances into local landraces

Three common bean landraces, Sugar 1 (Landrace 90), Sugar 2 (landrace 89), and Sugar 3 (landrace 88), that are maintained at KALRO, Kakamega, were utilized in a marker-assisted backcross breeding program as recurrent parents, while the varieties G2333 and KK8 were used as donor parents for anthracnose (*Co-4*^{2}) and *Pythium* root rot (*Pyult1*) resistances, respectively. The three landraces were selected based on earliness in maturity and farmer preferences in the region. A backcross breeding program was used to develop a breeding population combining resistance to anthracnose and *Pythium* root rots. The landraces were hybridized through emasculation and pollen deposited on the stigma (Genchev 2007). Initially, a three-way crossing scheme was utilized and later progressed in a backcross breeding scheme as shown in Figure 2. The SCAR

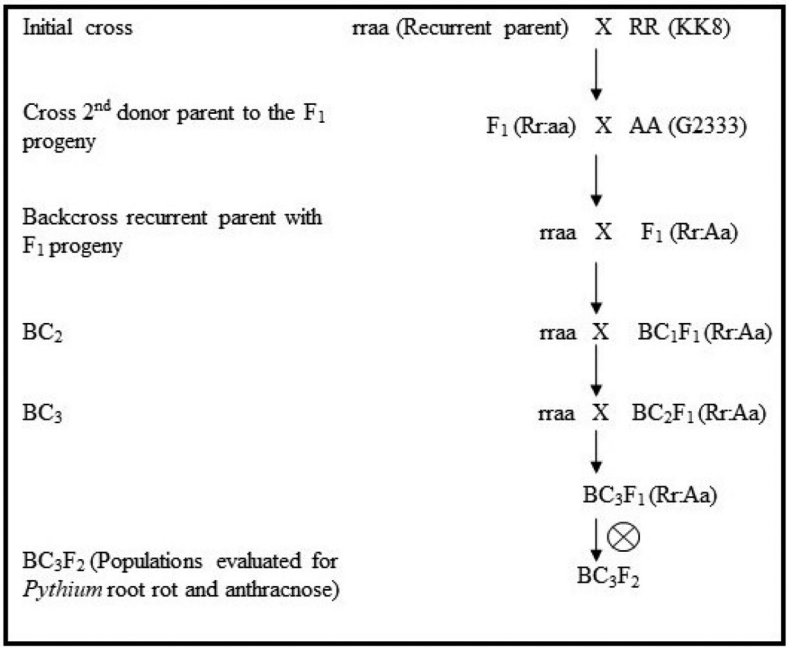

Figure 2. Backcrossing scheme for introgression of resistance to *Pythium* root rot and anthracnose into common bean landraces.

markers $PYAA19_{800}$ was used to confirm successful introgression of *Pythium* root rot resistance while SH18 was used to confirm successful introgression of anthracnose resistance in every generation. The BC_3F_2 populations were phenotyped using MS61 isolate of *Pythium* and three physiological races of anthracnose together with a mixture of the races (170, 815, 1286, and mixture). The screening procedures are as described in sections 2.2 and 2.3, respectively.

2.6. *Data Analysis*

The molecular data were subjected to the chi-square test to assess the gene segregation pattern of the populations in the three different crosses. Comparison between the molecular marker data and the phenotypic expression of the diseases was performed through a correlation study.

3. Results

3.1. Response of common bean genotypes to *Pythium* root rot under greenhouse conditions

Virulence tests showed different disease severity levels depending on the variety screened but the same mode of reaction per variety was observed across the replications. All test materials were susceptible to *Pythium* root rot

under greenhouse conditions except landrace 58, Landrace 75, and the check cultivars (CAL 194, KK8, KK22). However, 42.5% of the genotypes showed moderate resistance (Table 3). In terms of seed classes, a majority of the small-seeded landraces were moderately resistant as compared to the large-seeded varieties.

3.2. *Response of common bean genotypes to anthracnose using the detached leaf method*

Generally, anthracnose disease symptoms started appearing on the second week after inoculation. The 10 anthracnose isolates led to identification of three races of *C. lindemuthianum* (170, 815, and 1286). The results showed that most genotypes were moderately resistant to the mixed inoculum except for four landraces and the resistant check (G2333). A total of 33.7% of the accessions were resistant to anthracnose, 41.3% were moderately resistant while 25% were susceptible (Table 4).

3.3. *Molecular marker analysis*

The *Co-4*^{2} gene targeted by the SCAR markers $SH18_{1100bps}$ and $SBB\text{-}14_{1050/1150bps}$ was only detected in G2333 which was similar to *Co-5* gene targeted by $SAB3_{400bps}$ marker as shown in Figure 3 (Gel 1, Gel 2, and Gel 3). For the case of *Pythium* root rot, the $PYAA19_{800bps}$ that is linked to *Pyult1* gene was not detectable in all the germplasm except KK8 and KK22

Table 3. Response of common bean genotypes to *Pythium* root rot isolate MS61 under greenhouse conditions.

Disease Reaction	Genotypes
Susceptible	CAL 96, Landraces 1, 2, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 20, 23, 24, 26, 28, 30, 33, 34, 36, 36, 37, 39, 40, 42, 43, 47, 51, 54, 55, 60, 61, 64, 65, 67, 69, 70, 71, 73, 74, 76, 77, 80, 82, 83 & 84
Moderately resistant	GLP2, Landraces 3, 5, 6, 8, 18, 21, 22, 25, 27, 29, 31, 32, 38, 41, 44, 45, 46, 48, 49, 50, 52, 53, 56, 57, 59, 62, 63, 66, 68, 72, 78, 79, 81, 85, 86, 87, 88, 89 & 90
Resistant	CAL 194, KK8, KK22, Landraces 58 & 75

Table 4. Response of common bean genotypes to a mixed isolate of anthracnose.

Disease Reaction	Genotypes
Susceptible	Chelelang, Tasha, Landraces 4, 5, 13, 15, 19, 20, 32, 33, 37, 53, 54, 60, 61, 66, 69, 75, 83, 86, 88, 89 & 90
Moderately resistant	Landraces 2, 3, 6, 9, 10, 14, 17, 23, 24, 26, 27, 28, 29, 30, 36, 40, 41, 42, 44, 45, 47, 49, 55, 56, 57, 59, 62, 63, 64, 65, 74, 76, 79, 80, 81, 84, 85 & 87
Resistant	G2333, Landraces 1, 8, 11, 12, 16, 18, 21, 22, 25, 31, 34, 35, 38, 39, 43, 46, 48, 50, 51, 52, 58, 67, 68, 70, 71, 72, 73, 77, 78 & 82

(Figure 3, Gel 4). The marker was not detected even in the resistant landraces (58 and 75).

3.4. Marker-assisted introgression of *Pythium* root rot and anthracnose resistance genes

The first cross involving the resistance donors and the recurrent genotypes (Sugar 1, Sugar 2 and Sugar 3) yielded 20–30 F_1 seeds. Subsequently, BC_1 to BC_3 seeds were obtained as detailed in Table 5. The lines with varying single genes were not advanced beyond each tested generation and only plants with the two genes were advanced. A total of 20 F_1 plants from "SUGAR 1/KK8," 20 plants from "SUGAR 2/KK8," and 30 plants from "SUGAR 3/KK8" were used as pollen recipients for a three-way cross with the anthracnose resistance donor parent to obtain SUGAR/KK8//G2333. The three-way cross yielded 78 seeds that were sown in the next season to develop BC_1 population in a marker-assisted backcross breeding program. The molecular markers $PYAA19_{800}$ and SH18 that were utilized for foreground selection, amplified the expected fragment of 800 bp and 1100 bp, respectively (Figure 4). For the second backcross (BC_2), 33 plants were selected using both markers to develop BC_3 population (Figure 5) which was later selfed to obtain BC_3F_2 population. A total of 100 BC_3F_2 plants possessed the two resistance loci were obtained for phenotyping. Segregation ratios at BC_3F_2 were 9:16 (resistant: susceptible). The results show that the observed ratios and the calculated ratios are not significantly different at the

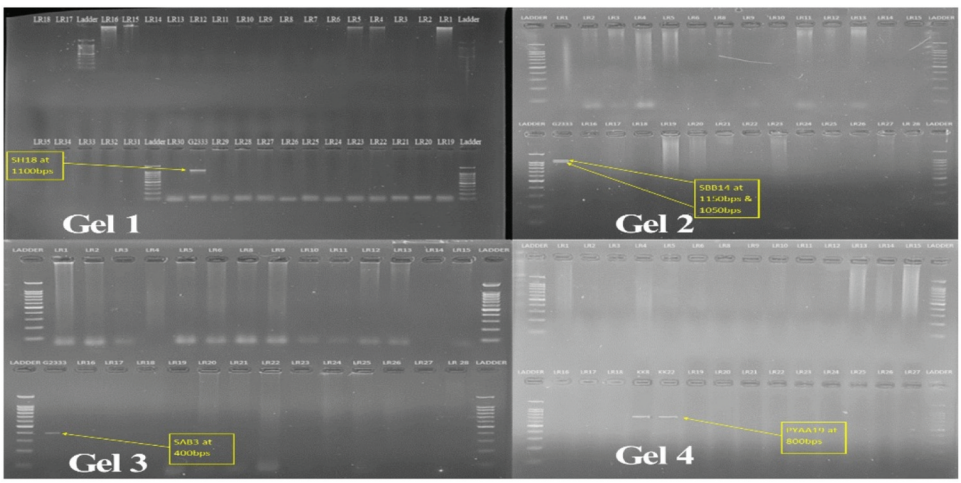

Figure 3. Gel 1: Amplification of SH18 linked to anthracnose resistance gene *Co-4*^{2}. Gel 2: Amplification of SBB14 associated with the *Co-4* anthracnose resistance gene. Gel 3: Amplification of SAB3 associated with the *Co-5* anthracnose resistance gene. Gel 4: Amplification of PYAA19 associated with the *Pyult1 Pythium* root rot resistance gene.

Table 5. Frequencies of phenotypic and genotypic classes for *Pythium* root rot and anthracnose resistances.

Population	No. of selected BC_3 plants	No. of screened BC_3 F_2 plants	No. of resistant BC_3 F_2 plants	No. of susceptible BC_3 F_2 plants	Expected ratio	P Value	X^2
SUGAR 1/ KK8// G2333	12	32	19	13	9:16	0.229	1.444
SUGAR 2/ KK8// G2333	10	37	21	16	9:16	0.275	1.190
SUGAR 3/ KK8// G2333	14	105	59	46	9:16	0.091	2.864

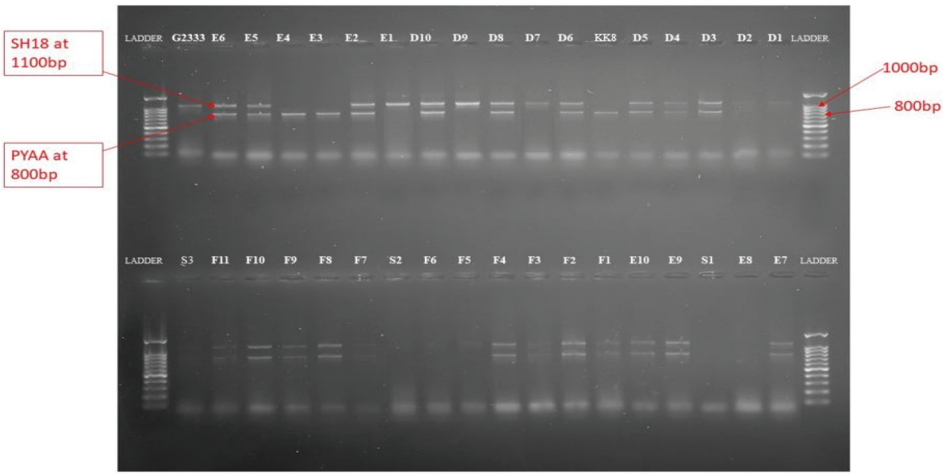

Figure 4. Amplification of SH18 and PAA1900 associated with the anthracnose resistance gene (*Co-4*^{2}) and *Pythium* root rot resistance gene (*Pyut1*) in BC_2 populations; KEY: D1-10: Sugar 1 progenies, E1-8: Sugar 2 progenies, F1-11: Sugar 3 progenies, S1: Sugar 1, S2: Sugar 2, S3: Sugar 3.

0.05 level of significance in BC_3F_2 populations, where $P < 0.229$ for SUGAR 1/KK8//G2333 crosses, $P < 0.275$ for SUGAR 2/KK8//G2333 crosses, and $P < 0.091$ for SUGAR 3/KK8//G2333 crosses molecular marker screening. The chi-square values from these findings show that there are no significant differences between the observed and expected ratios at the given probability levels as shown in Table 5. Phenotypic screening data were compared with the molecular marker screening data to assess the reliability of MAS as a tool in breeding for disease resistance. The correlation coefficient between phenotypic and molecular screening for anthracnose was 0.846 while that for Pythium root rot was 0.923. The high and positive correlation coefficient indicates that one has high chance of success in using molecular markers for screening for disease resistance; hence, one can rely on molecular marker data to equally determine the phenotype.

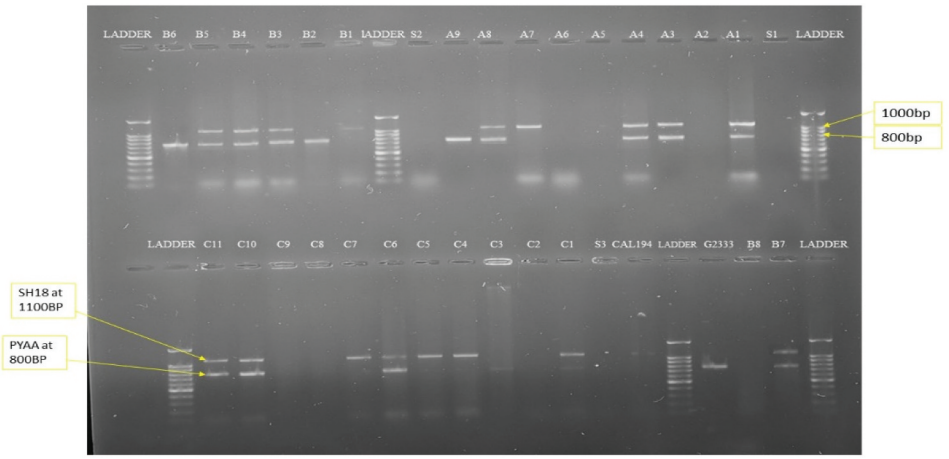

Figure 5. Amplification of SH18 and PAA1900 associated with the anthracnose resistance gene (*Co-4*^{2}) and *Pythium* root rot resistance gene (*Pyut1*) in BC_3F_2 populations; KEY: A1-9: Sugar 1 progenies, B1-8: Sugar 2 progenies, C1-11: Sugar 3 progenies, S1: Sugar 1, S2: Sugar 2, S3: Sugar 3.

The 100 BC_3F_2 populations, recurrent parents, and the two resistance donors were jointly evaluated against three races of anthracnose (Races 170, 815, 1286), a mixture of the three races and MS61 *Pythium* root rot isolate. The results showed that the resistant anthracnose donor (G2333) was resistant to all the three races while *Pythium* resistant check variety (KK8) was resistant to MS61 isolate. In addition, the 100 BC_3F_2 lines were resistant to all anthracnose races under study, although 11 lines were susceptible to the mixed inoculum and 38 lines were susceptible to MS61 isolate. Molecular data in Figure 5 showed that all the 99 selected lines carried both markers (Table 6).

4. Discussion

4.1. Resistance of common bean landraces to anthracnose and *Pythium* root rot in *Kenya*

A number of Kenya landraces showed moderate resistance to *Pythium* root rot similar to Otsyula (2010) who reported moderate resistance among small

Table 6. Phenotypic evaluation of the selected BC_3F_2 populations containing resistance to *Pythium* root rot and anthracnose.

		Anthracnose races									
		170		815		1286		Mixed races		*Pythium* root rot	
Population	SH18 + PYAA19	R	S	R	S	R	S	R	S	R	S
SUGAR 1/KK8//G2333	19	19	0	19	0	19	0	18	1	13	6
SUGAR 2/KK8//G2333	21	21	0	21	0	21	0	19	2	14	7
SUGAR 3/KK8//G2333	59	59	0	59	0	59	0	51	8	34	25

S: susceptible, R: Resistant

seeded landraces in Kenya. However, the study contradicted Mukankusi et al. (2010) who found no moderate resistance among landraces in Uganda. This moderate resistance explain why farmers are still cultivating these landraces. The findings further suggest that small seeded size may be linked to moderate resistance to *Pythium* root rot which may is because of an insertion of a single locus for resistance in moderately resistant small-seeded background which masks the expression of polygenic resistance (Otsyula 2010). Small-seeded genotypes of Middle American origin were reported by Beebe (1981) as tolerant to *Pythium* root rot compared to large-seeded Andean types. Findings by Abawi and Pastor-Corrales (1990) show that *Pythium* root rot resistance is qualitatively controlled with most known resistance sources being small-seeded bean varieties.

Two landraces showed resistance during the phenotypic screening which contradicts with Anunda, Nyaboga, and Amugune (2019) study where none of the landraces screened were resistant. These landraces could act as potential sources of resistance once mapping for the resistance genes has been conducted to find the source of resistance. Furthermore, the study confirmed that CAL 96 is highly susceptible to *Pythium* as reported earlier by Nzungize et al. (2011b) and KK8 that was developed for *Pythium* root rot resistance still possess the resistance. In this study, each variety maintained its relative expression of resistance or susceptibility on both roots and hypocotyls across the replications which is supported by Li, You, and Barbetti (2014). In addition, Dramadri et al. (2020) found that the resistance or susceptibility of a given bean variety was similar at every screening season. Previous studies show that MS61 isolate has been used to identify general resistance to a wider spectrum of *Pythium spp*. (Otsyula 2010; Nzungize et al. 2011b; Dramadri et al. 2020; Amongi et al. 2020), hence its use in this study. However, it is important to conduct further screening of the genotypes under field conditions for resistance to other root rot pathogens because of the possibility of various root rot pathogens occurring in the same field Paparu et al. (2018).

Anthracnose disease symptoms were first observed in the second week after inoculation, similar to previous observations (Mahuku, Jara, and Castellanos 2003; Pereira et al. 2014). In the preliminary experiments, this study revealed new races (170, 815 and 1286) which were not reported before by Kamiri et al. (2021). The differential cultivars carry different resistant genes; Michelite (*Co-11*), MDRK (*Co-1*), Perry Marrow ($Co\text{-}1^3$), Cornell (*Co-2*), Widusa ($Co\text{-}1^5$), Kaboon ($Co\text{-}1^2$), Mexico 222 (*Co-3*), PI 207262 ($Co\text{-}3^3$ & $Co\text{-}4^3$), TO (*Co-4*), TU (*Co-5*), AB136 (*Co-6* & *Co-8*), and G2333 ($Co\text{-}4^2$, *Co-5* & *Co-7*). New races are used to identify new sources of resistance to anthracnose. Most of the small-seeded genotypes were moderately resistant to anthracnose as compared with the large-seeded genotypes in Kenya. However, in order to target durable resistance to anthracnose in a breeding program, this study considered the use of mixtures of different races as it has been reported to have synergistic effects

resulting in increased disease symptoms (Aliyu, Balogun, and Gbadebo 2013; Tembo 2016; Falleiros et al. 2018; Ogunsola et al. 2021). Furthermore, this study used the detached leaf method to facilitate screening the same plant using different races. The method has previously proved to reduce possible interaction among races of the same pathogen or different pathogens during inoculation, reduction in costs, and working time (Rezene et al. 2018). Data from the evaluation of landraces informed the study about resistance sources that were later used to initiate a breeding program. Crosses between accessions with complementary resistance spectra could be used to develop lines with wider resistance spectra (Ferreira et al. 2008).

4.2. *Molecular marker analysis*

The SCAR markers linked to anthracnose resistance at *Co-4* and *Co-5* loci did not amplify DNA from any of the Kenyan landraces despite some of them showing resistance phenotypically pointing toward the possibility of other genes that confer resistance. These findings therefore call for further screening of the germplasm to explore other loci that confer resistance to anthracnose. The possibility of exploiting molecular markers that are associated with the *Co-5* and the *Co-4*^{2} in improving anthracnose resistance in beans was investigated and reported by Garzón, Ligarreto, and Blair (2008). The *Co-4*^{2} gene was only detectable in G2333 by the SCAR markers SH18 (1150 bp) which is linked in coupling phase with *Co-4* locus at 4.27 ± 2.37 cM and SBB14 (150/1050 bp) linked at 5.89 ± 1.93 cM from the *Co-4* gene (Beebe et al. 2001; Kelly et al. 2003). The SAB3 (400 bp) maker linked to the *Co-5* gene at a distance of 5.9 cM (Beebe et al. 2001) was amplified in the anthracnose differential cultivar G2333.

The DNA amplification using the SCAR Marker PYAA19 at a band size of 800 bp was only observed in KK8 and KK22 (RWR 719). The resistances observed in Landraces 58 and 75 suggest the presence of other loci conferring resistance to *Pythium ultimum* since the marker could only detect the presence of the *Pyult1* gene linked to Pythium root rot resistance in KK22 (RWR 719) as reported by Namayanja et al. (2014). *Pythium* root rot, being controlled by a single dominant gene (Mahuku et al. 2007; Nzungize and Lyumugabe 2012), could be the reason why there are low numbers of varieties showing resistance. This is also supported by PYAA19$_{800}$ being tightly linked at 1.5 cM to the resistance gene (Mahuku et al. 2007). A study by Anunda, Nyaboga, and Amugune (2019) found that there were no resistant landraces among the germplasm screened; however, there was a high genetic variability among the susceptible and moderately resistant varieties. This confirms findings by Namayanja et al. (2014) that there could be more loci that contribute to the moderate resistances observed. The use of one marker in this study limits the identification of other loci that may be

conferring resistance to *Pythium* root rot hence there is a need to develop other markers from previous studies that can tag these specific loci (Nyakio et al. 2015).

4.3. Introgression of *Pythium* root rot and anthracnose resistance through MAS

The major common bean diseases in Kenya are root rots and anthracnose that have seriously limited the number of varieties grown by farmers as most landraces are completely susceptible to these diseases affecting many smallerholder farmers in East Africa (Mohammed 2013; Anunda 2021). Marker-assisted backcrossing has been proven to be a fast and efficient way to improve one or two traits in preferred common bean cultivars (Muhamba et al. 2013; Okii et al. 2017; Chukwu et al. 2020).

The F_1 generation resulting from the crosses suggested that resistance to *Pythium* and anthracnose was inherited as a dominant trait in the genotypes with simple inheritance patterns. This corresponds with Lema, Demissie, and Rezene (2021) and Namayanja et al. (2014) findings. The results further show that there is a probability of choosing plants with both genes, a single gene for each disease as reported by Lema, Demissie, and Rezene (2021). In the development of inter-gene pool multiple-parent genotypes, Okii et al. (2017) showed the effectiveness of marker-assisted selection in pyramiding of resistance genes together with improved agronomic qualities.

The *Co-4*^{2} and *Co-5* genes were introgressed in a susceptible cultivar by Alzate-Marin et al. (2004) in a backcross breeding program using RAPD markers. The two dominant markers used in this study were polymorphic between the resistant and susceptible parents and successfully used to distinguish the populations that had the targeted genes from those that did not. The only challenge that was encountered was the SCAR markers provided limited information at the loci they tag because they are dominant in nature; hence, it was only possible to tell whether a given allele is present or not at a given locus but could not distinguish the heterozygote from the homozygotes within the population. Homozygotes and heterozygotes are able to be differentiated by the use of codominant markers at early stages hence eliminating the necessity of further genotyping for the fixed allele allowing the breeder to focus on fewer segregating alleles in subsequent generations (Kiryowa 2015).

The cultivar G2333 was resistant to all the races of anthracnose used in this study. This translated to most of the lines developed using this cultivar being resistant to these races, hence illustrating the value of gene pyramiding as G2333 is known to possess *Co-4*^{2}, *Co-5,* and *Co-7* genes (Vallejo and Kelly 2009). The consistent phenotypic and genotypic results in this study confirmed the reliability of molecular markers in selection for disease resistance.

5. Conclusion

The study identified some resistant landraces that can serve as resistance donor parents in a common bean breeding program after successful gene mapping. The study further utilized linked molecular markers for developing breeding lines for further selection under field conditions, yield tests, and subsequent release as new cultivars.

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