Marker Assisted Gamete Selection for Multiple Disease Resistance in Andean Bean Genotypes and Characterization of *Colletotrichum lindemuthianum* in Kenya

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DECLARATION

The work contained in this thesis is my original work. It has not been submitted for the award of any degree or its equivalent in this university or in any other institution of higher learning.

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To all in pursuit of Knowledge

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LIST OF ABBREVIATIONS

ALS	Angular leaf spot
ANTH	Anthracnose
BCMNV	Bean common mosaic necrotic virus
BCMV	Bean common mosaic virus
BGMV	Bean golden mosaic virus
BGYMV	Bean golden yellow mosaic virus
BSM	Bean stem maggot
CBB	Common bacterial blight
CIAT	Centro Internacional de Agricultura Tropical
DAI	Days after inoculation
ECABREN	Eastern and Central Africa Bean Research Network
FAO	Food and Agriculture Organisation
MAS	Marker assisted selection
NARS	National Agricultural Research Systems
PABRA	Pan-African Bean Research Alliance
PBS	Phosphate buffered saline
PDA	Potato dextrose agar
PNW	Pacific northwestern
RAPD	Randomly amplified polymorphic DNA
RR	*Pythium* root rot
SABRN	Southern Africa Bean Research Network

SCAR	Sequence characterised amplified region
SHT	Southern highlands of Tanzania
SSD	Single seed descent
USA	United States of America
YDCA	Yeast dextrose calcium carbon agar

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ABSTRACT

Resistant bean varieties can reduce yield losses especially in widespread low input production systems in eastern Africa and enhance production stability. Pathogenic variation for major diseases has received limited attention in eastern Africa. Marker technology has presented new opportunities to accelerate cultivar development with more precision. Gamete selection method is a recent, efficient and effective breeding method that facilitates simultaneous improvement of multiple traits in common bean. The objectives of this study were to (i) determine pathogenic variation of *Colletotrichum lindemuthianum* in Kenya, (ii) introgress genes for resistance to angular leaf spot, anthracnose, common bacterial blight and *Pythium* root rot into four susceptible large seeded bean varieties, and (iii) evaluate and select resistant plants and families from F_1 and $F_{1.2}$ populations under field conditions. Anthracnose diseased materials were collected from bean growing regions in Kenya. Donor and the recipient parents were phenotyped and genotyped to confirm presence or absence of resistance genes. Sixteen F_1 populations were generated from crosses between four multiparent male gametes and four susceptible female varieties following the gamete selection breeding method. The F_1 and F_1-derived F_2 ($F_{1.2}$) families were evaluated for angular leaf spot, anthracnose, common bacterial blight and Pythium root rot resistance in the field at Kabete and Tigoni. Agronomic traits were also

recorded. Twelve physiological races of *Colletotrichum lindemuthianum* were identified. Three markers (SAB-3, SH-13 and SU-91) were polymorphic. PYAA-19 the marker for root rot was not polymorphic. Of the 89 multi-parent plants screened with markers for angular leaf spot (SH-13), anthracnose (SAB-3) and common bacterial blight (SU-91), three plants were positive for three markers, 8 for two markers, while all others had one or no markers present. Among the four male gametes constituted, G10909/G2333//AND 1062 /VAX6 F_{1s} showed intermediate resistance to angular leaf spot (4.8). MEX54/G2333//RWR719/VAX6 had intermediate resistance to anthracnose (5.2) and common bacterial blight (4.7). G10909/G2333//RWR719/VAX6 was resistant to root rot (2.1). Among the four commercial varieties introgressed, New Rosecoco F_{1s} were intermediate resistant to the four diseases. All the F_{1s} showed a resistance of 2.2 against *Pythium* root rot. All populations showed intermediate resistance to anthracnose (5.5), angular leaf spot (5.0) and common bacterial blight (4.9). Among the male gametes, progenies of G10909/G2333//RWR719/VAX6 were the best yielding with mean of 4095 kg ha^{-1}. Among the female commercial varieties used in the final cross, F_1 progenies of New Rosecoco were the best yielding with a mean of 4194.5 kg ha^{-1}. $F_{1.2}$ families of MEX54/G2333//RWR719/VAX6 gave the highest yields of 3202 kg ha^{-1}. $F_{1.2}$ families with Kenya Umoja as the female parent were the

best yielding with a mean yield of 3320 kg ha^{-1}. The population that produced lines with combined resistance to 3 diseases and high yield potential was MEX54/G2333//RWR719/VAX6///Kenya Umoja. The anthracnose pathogen *Colletotrichum lindemuthianum* population in Kenya is quite varied and there is need for continuous and extensive characterisation. Use of markers further improved precision and efficiency of gamete selection method. Families derived from crosses with New Rosecoco and Kenya Umoja seem to offer the best potential of developing new lines with multiple disease resistance, high grain yield and commercial grain type.

CHAPTER 1 : INTRODUCTION

1.1 Importance of common bean

Common bean (*Phaseolus vulgaris* L.) is one of the most important grain legumes in the world because of its commercial value, extensive production, consumer use, and nutrient value (being a source of carbohydrate, protein, minerals and vitamins) (Liebenberg and Pretorius, 1997; Pastor- Corrales *et al*., 1998; Singh, 1999). Millions of small-scale farmers in Africa rely on the production and sale of beans in local markets and urban areas to provide an important source of household income (Mwale *et al*., 2008). Beans offer good prospects in export markets providing opportunities to earn foreign currency (CIAT, 1981; Mauyo *et al*., 2007). Globally, common bean is the leading grain legume crop with about 12 million metric tons produced annually on more than 14 million hectares. This is 30% of the total pulse production and 85% of the production area sown to all *Phaseolus* species in the world (Singh, 2001). Latin America is the largest producer, with some 5.5 million metric tons, with Brazil and Mexico being by far the major producers. Africa is the second most important region, producing about 2.5 million metric tons, with Uganda, Kenya, Rwanda, Burundi, Tanzania, and D.R. Congo playing major roles (FAO 2005, 2006 and 2007; Wortmann *et al*., 1998). As a source of food, over 200 million people in the sub-Saharan Africa depend on common bean as a primary staple. Common bean is a source of

complex carbohydrate, protein (20-25%), minerals (Ca, Cu, Fe, Mg, Mn, Zn), vitamins (folate) and amino acids (lysine and methionine) for more than 300 million people in the tropics (Liebenberg and Pretorius, 1997). Pulses contribute 20% of per capita total protein intake in Kenya (Kimani *et al*., 2001). In Eastern, Central and Southern Africa, common bean (*Phaseolus vulgaris* L.) is the most important grain legume providing food and income to at least 100 million people (CGIAR at www.cgiar.org, CIAT at www.ciat.org).

1.2 Common bean production trends in eastern Africa

World annual production, including both dry and snap bean, exceeds 21 million metric tons, which represents more than half of the world's total food legume production (Miklas *et al*., 2006). Common bean is primarily produced in tropical low-income countries which account for over three quarters of the annual world production (Singh, 2001). Among Kenya's agricultural commodities, common bean comes second only to maize as a food crop (Gethi *et al.*, 1997). Although there has been an increase in bean production due to expansion into marginal agricultural lands, productivity per unit area of land has continued to decline (Nderitu *et al*., 1997). Typical bean yields obtained on farmers' fields are only 20% to 30% of the actual yield potential for most commercial dry bean varieties (Wortmann *et al.*, 1998). These low yields are attributed to a number of constraints, most important of which are diseases, insect pests, low soil fertility

and periodic water stress (Allen *et al*., 1989; Otsyula *et al*., 1998). Land allocated to dry bean cultivation in Kenya has been increasing with the exception of 2010 (Table 1.1). Yield has also increased over the years with the exception of 2011. Consequently production in 2010 reduced as area under common bean reduced in the same year. However an upward trend is evident in bean production over the years.

Table 1.1 Common bean production trends in Kenya 2008-2012

Year	Parameter		
	Area (Ha)	Yield (Kg ha^{-1})	Production (t)
2008	641,936	412.8	265,006
2009	960,705	484.4	465,363
2010	689,377	566.6	390,598
2011	1,036,738	557.2	577,674
2012	1,058,920	579.7	613,902

Source: FAOstat at www.fao.org 2008-2012

Uganda's area under beans has increase over the years as yields reduced. However production has increased over the years save for 2011 (Table 1.2). Tanzania's area under common bean, yield and production has fluctuated over the years. Rwanda has steadily increased area allocated to common beans save for 2010. However Rwanda had the highest yields in 2010 between 2008 and 2012. Bean production in Rwanda has increased over the years. Burundi's area under common bean, yield and production has fluctuated over the years.

Table 1.2 Common bean production trends in eastern Africa community 2008-2012

Year	Uganda			Tanzania		
	Area (Ha)	Yield (Kg ha^{-1})	Prod. (tonnes)	Area (Ha)	Yield (Kg ha^{-1})	Prod. (tonnes)
2008	896,000	491.1	440,000	749,540	761.5	570,750
2009	925,000	488.6	452,000	868,310	891.1	773,720
2010	952,000	486.3	463,000	1,208,690	717.7	867,530
2011	983,460	455.0	447,430	737,661	916.3	675,948
2012	1,060,000	401.3	425,400	800,000	975.0	780,000

Source: Computed from data from FAO data base 2008-2012.
Units: Area: Ha; Yield: Kg ha^{-1}; Production: Tonnes

Table 1.2 Continued…

Year	Rwanda			Burundi		
	Area (Ha)	Yield (Kg ha^{-1})	Prod. (tonnes)	Area (Ha)	Yield (Kg ha^{-1})	Prod. (tonnes)
2008	336,577	915.1	308,000	215,000	882.1	189,661
2009	345,851	944.1	326,532	220,000	922.4	202,934
2010	319,252	1025.8	327,497	215,000	937.4	201,551
2011	341,819	968.8	331,166	264,163	759.7	200,673
2012	479,899	902.0	432,857	340,752	604.4	205,944

Source: Computed from data from FAO data base 2008-2012.
Units: Area: Ha; Yield: Kg ha^{-1}; Production: Tonnes

In terms of average production area, Uganda is the leading producer of common bean in eastern Africa community followed by Tanzania and then Kenya (Table 1.3). However, in terms of production ha^{-1}, Tanzania comes second after Rwanda, with Burundi taking third position.

Table 1.3 Average bean production in eastern Africa community 2008-2012

Country	Average production Area (Ha)	Average yield (kg ha^{-1}).
Kenya	877,535	520.1
Uganda	963,292	464.5
Tanzania	872,840	852.3
Rwanda	364,680	951.2
Burundi	250,983	821.2

Source: Table 1.2 above

1.3 Problem statement

In several African countries, bean consumption can be as high as 50 kg per person per year and is the only constant source of protein for some resource limited smallholder farmers (Voysest, 2000). The high nutritive value and high consumption rates makes common bean an important crop among people in many of the developing countries of Africa (Voysest, 2000). Despite its importance, bean yields in developing countries are among the lowest in the world, with average yields of 0.5 t ha^{-1} (FAO, 2007) compared to 1 to 2 t ha^{-1} commonly reported in experimental sites and up to 4 t ha^{-1} reported in the USA (Voysest, 2000). Productivity is severely constrained by biotic and abiotic stresses, which are compounded by the low genetic yield potential of available cultivars (Beebe and Pastor-Corrales, 1991). Major biotic constraints to productivity include angular leaf spot, anthracnose, root rots, bean common mosaic virus (BCMV/NV) and common bacterial blight. Major abiotic stress factors include low soil fertility and drought. Resistant bean varieties can reduce

yield losses especially in widespread low input production systems in eastern Africa and enhance production stability in diverse and adverse environments and poor soil conditions. However development of improved varieties in Kenya has traditionally followed classical breeding methods. This approach resulted in long periods of cultivar development (up to 12 years) and heavy reliance on unpredictable environmental conditions. Common bean breeders often face the challenge to improve an array of agronomically important traits, including yield, maturity, plant type, quality characteristics, and resistance to biotic and abiotic factors causing crop losses and affecting product quality (Singh, 2004). Pathogenic variation for major diseases has received limited attention in eastern Africa. Race diversity for major bean pathogens is poorly understood. This has further reduced efficiency of breeding programs because it is difficult to develop varieties with resistance targeted to the most virulent and most widely distributed disease races.

1.4 Justification

Dry bean production mainly occurs under low input agriculture on small-scale farms in developing countries. Beans produced by these resource-poor farmers are more vulnerable to attack by diseases because they can hardly afford alternative disease management strategies. The development and use of resistance cultivars to address the risk posed by diseases is the most effective,

economical, and environmentally sound strategy for disease control. Resistant bean varieties can reduce yield losses especially in wide spread low input production systems common with smallholder, resource limited farmers in eastern Africa (CIAT, 2003; Mahuku *et al.,* 2004; Otsyula *et al.,* 2005; Miklas *et al.*, 2006; Kimani and Mwang'ombe, 2007). The advantage of host plant resistance is that once the technology has been developed, it is packaged in seed which is easier to disseminate and deploy, and does not require any additional or specialized handling on the part of the farmers, other than what they normally do to grow their crops (Mahuku *et al*., 2009). There are few market preferred-bean cultivars with combined resistance to angular leaf spot, anthracnose, common bacterial blight and root rot in Kenya which prompted efforts to breed for resistance in the susceptible but highly adaptable and preferred cultivars. Marker technology has presented new opportunities to accelerate cultivar development with more precision and reduce duration to release of improved bean varieties (Milkas *et al.*, 2006). Integration of marker-aided breeding with conventional approaches can speed up, increase precision and effectiveness of been breeding and facilitate pyramiding of desirable genes. Several markers linked to resistance genes for major diseases in eastern Africa have been identified. Gamete selection method is a recent, efficient and effective breeding method that facilitates simultaneous improvement of multiple traits in common

bean. It has been proposed that use of markers can further improve precision and efficiency of this approach. Marker assisted gamete selection ensures precision in tracking pyramided genes by markers. Sources of resistance as well as markers linked to resistant genes are now available making this endeavor a possibility. Gene pyramiding has been suggested as a strategy for stabilizing resistance against variable plant pathogens (Nelson, 1978).

1.5 Overall objective

The overall objective of this study was to apply marker-assisted gamete selection in pyramiding genes for resistance to angular leaf spot, anthracnose, common bacterial blight and *Pythium* root rot and introduce these genes into susceptible, but popular, large seeded bean varieties.

1.5.1 Specific objectives:

The specific objectives were to

Determine pathogenic variability of *Colletotrichum lindemuthianum* in bean growing areas of Kenya.

Evaluate parental lines for disease polymorphism using markers and phenotyping.

Conduct early generation selection for combined resistance to angular leaf spot, anthracnose, root rots and common bacterial blight and other agronomic traits.

1.6 Hypothesis

1. There are no differences in virulence of Kenyan races of *Colletotrichum lindemuthianum* on local bean germplasm.
2. There are no differences in efficiency, effectiveness and/or precision of phenotypic and marker assisted gamete selection methods.
3. Combined genetic resistance to angular leaf spot, root rot, anthracnose and common bacterial blight and other agronomic traits is not expressed in genotypes of Andean gene pool.

CHAPTER 2 : REVIEW OF LITERATURE

2.1 Origin and domestication of common bean

Of the 50-60 wild *Phaseolus* species of American origin (Neotropics) only five, namely, common (*P. vulgaris*), year-long (*P. polyanthus*), scarlet runner (*P. coccineus*), tepary (*P. acutifolius*), and Lima bean (*P. lunatus*) have been domesticated. Each domesticated species constitutes a primary gene pool with its wild ancestral form. Secondary and tertiary gene pools may exist for all the domesticated species, depending on the phylogenetic events that lead to the formation of the biological species (Delgado Salinas, 1985; Debouck, 1991; Gepts and Debouck, 1991; Debouck, 1999, 2000). Among these species, common bean is the most widely grown, occupying more than 85% of production area sown to all *Phaseolus* species in the world (Singh, 2001). Diversity among *Phaseolus* species in relation to common bean is organised into primary, secondary, tertiary, and quaternary gene pools (Debouck and Smartt, 1995; Debouck, 1999, 2000). The primary gene pool of common bean comprises both cultivars and wild populations. The latter are the immediate ancestors of common bean cultivars (Weiseth, 1954; Miranda C., 1967; Gentry, 1969; Berglund-Brücher and Brücher, 1976; Kaplan, 1981; Brücher, 1988; Kami *et al*., 1995). The secondary gene pool of common bean comprises *P. coccineus*, *P. costaricensis* Freytag & Debouck, *P. polyanthus.* The tertiary gene pool of common bean comprises *P. acutifolius* and

P. parvifolius Freytag (Singh, 2001). Domestication of common bean occurred within at least two major geographic locations: Mesoamerica or Middle American (e.g., Mexico and Central America) and Andean South America (e.g., Peru, Bolivia, and northern Argentina) (Gepts, 1988). The major centres of domestication correspond to the two major gene pools of common bean, the Mesoamerican and Andean gene pools. Cultivated varieties of this crop are derived from either of these two locations, and are referred to as being Middle American, Andean, or hybrid bean genotypes. Mesoamerican beans typically have small (<25 g 100 seed-weight^{-1}) to medium (25–40 g 100seed-weight^{-1}) size seeds while Andean beans usually have large seeds (>40 g 100 seed-weight^{-1}) (Evans, 1973; 1980; Gepts and Bliss, 1985; Khairallah *et al.*, 1990; Singh *et al.,* 1991b). On the basis of agronomic and morphological characteristics, and geographical distribution within their domestication range in Latin America, three races have been identified in each gene pool, namely Chile, Nueva Granada, and Peruin Andean; and Durango, Jalisco and Mesoamerica in Middle American cultivars (Singh *et al.*, 1991a; 1991b).

2.2 Botanical characteristics

Common bean (*Phaseolus vulgaris L)*, includes dry or field beans grown for the dry edible seeds, and snap beans, also known as French or string beans, grown for the immature fruits. Common beans are seed propagated, true diploids

(2n=2x=22) and have the estimated size of genome of 637 Mbp or 0.66 pg/IC (Arumuganathan and Earle, 1991). It is an annual leguminous plant that belongs to the genus, *Phaseolus,* with pinnately compound trifoliolate large leaves. It is largely a self-pollinated plant though cross-pollination is possible if the stigma contacts with pollen coated bee when extended. Seeds are non-endospermic and vary greatly in size and colour from the small black wild type to the large white, brown, red, black or mottled seeds of cultivars, which are 7-16 mm long (Cobley and Steele, 1976). Common bean shows variation in growth habits from determinate bush types to indeterminate upright or viny bush types, vigorous climbing types. The bush type bean grows 20-60 cm tall with most of its pods held above the ground. It is the most predominant type grown in Africa, and is relatively short season crop, maturing in 60 days from seeding in a tropical climate and yielding between 700-2000 kg ha^{-1} on average. If supported, climbing beans may grow 2-3m tall. Common beans take 100-120 days to mature at mid-elevations and the yield can be as high as 5000 kg ha^{-1}. (van Schoonhoven and Pastor-Corrales, 1987; Buruchara, 2007).

2.3. Ecological requirements

Common bean is a warm-season crop that does not tolerate frost or long periods of exposure to near-freezing temperatures at any stage of growth. Usually high temperatures do not affect it if adequate soil water is present, although high

nocturnal temperatures will inhibit pollination. At very high temperatures (>30°C), the crop can set little seeds or shed many flowers and buds, which reduces yield (Fageria, Baligar and Jones 1997). The crop requires moderate amounts of rainfall (300 – 600 mm) but adequate amounts are essential during and immediately after the flowering stage. Dry weather is desirable for maturation of the crop and for harvesting but late rains may discolour the beans and lower their grade and market value (Gomez, 2004). Generally, common bean is considered a short-season crop with most varieties maturing in a range of 65 to 110 days from emergence to physiological maturing (Buruchara, 2007). Maturity period occurs up to 200 days after planting amongst climbers that are used in cooler upland elevations (Graham and Ranalli, 1997).

2.4 Common bean production in eastern Africa

Common bean was introduced in East Africa, by the Portuguese in the 16th century. It has since spread into many parts of Africa. In East Africa, beans are rated as the second most important source of human food dietary protein, and the third most important source of calories of all agricultural commodities produced (Pachico, 1993). Common bean is an important component of production systems and a major source of protein for the poor in Eastern Africa. Common bean is largely grown for subsistence primarily by small-scale farmers mainly women (Wortmann *et al*., 1998). Common beans are produced in a range

of cropping systems, mainly in association with maize, banana, roots and tubers, sorghum or millet (Allen and Edje, 1990) mostly under rainfed-low input systems. In recent years, the crop production trend has not kept pace with the annual growth rate (estimated above 2 percent) in population in some countries due to a number of biotic, abiotic and socio-economic constraints (Kambewa, 1997; and Xavery *et al*., 2006). Drought is the main abiotic constraint and is common across eastern Africa. In terms of area, Kenya is the leading producer of common bean in the region and indeed in Africa followed by Uganda and then Tanzania (FAO, 2008). However, in terms of production, Kenya comes second after Uganda, with Tanzania maintaining its third position. Common bean yields are higher in Uganda, than in Kenya because of a relatively favourable biophysical environment (such as weather condition) in Uganda compared to Kenya (Katungi *et al.,* 2009). A high degree of diversity (in terms of growth habits, seed shape, size and colour) exists but the most common bean varieties grown in Africa are of bush type with small to medium sized seeds. Bush type common bean is preferred to the climbing type because of it low cost production requirements and convenience for market production. The climbers dominate the highland areas, where population density is high and land is limiting. The traditional growing areas include Burundi, Rwanda, and DR of Congo and to a lesser extent in south-western highlands of Uganda, western highlands of Ethiopia, Kenya and

Malawi (Wortmann *et al*., 1998; Allen and Edje, 1990). In the last six years, climbing bean varieties (Vunikingi, Umubano, Gisenyi, Flora, Gisenyi, and Ngwinurare), originally introduced in Rwanda in the late 1980s, have extended to other countries like Tanzania, Kenya, Angola, and Madagascar and expanded within the traditional growing countries (Katungi *et al*., 2009; Kimani, 2005). Nevertheless, climbing beans still account for a small share of land under beans compared to bush type. Bush types are popular in areas where commercial bean production has gained importance because of their early maturing characteristics.

2.5 Constraints to bean production

2.5.1 Abiotic constraints

Common bean suffers from both abiotic and biotic production constraints (Graham, 1978; Schwartz and Pastor-Corrales, 1989; Singh, 1992; Wortmann *et al*., 1998; Zaumeyer and Thomas, 1957). The most widely distributed abiotic constraints are low soil fertility, particularly deficiency of nitrogen, phosphorus, and zinc, as well as toxicities or aluminium and manganese (Singh, 2001). Similarly, drought is among the most widely distributed and endemic abiotic problems affecting bean production in many regions of the world, especially in northern Brazil, the central and northern highlands of Mexico, the Rift Valley of East Africa, and the intermountain regions of the USA. Complete crop failures

under dry land conditions in these regions are not uncommon (Singh, 2001). High temperatures (>30°C day and/or>20°C night) in tropical lowlands (below 650m elevation) and at higher latitudes (e.g., California, Colorado, Idaho, Nebraska, Washington, and Wyoming in the USA) can severely limit bean production. Low temperatures (below 15°C), as well a frost at the beginning and end of the growing season in the highlands (above 200m elevation) of Latin America and at higher latitudes (e.g., >40°C in the USA and Canada) also can reduce bean yields (Singh, 2001).

2.5.2 Biotic constraints

Pathogens that cause bacterial blights, [i.e. common bacterial blight (CBB), halo blight (HB) and bacterial brown spot (BBS)], fungal diseases such as anthracnose, rust, angular leaf spot, ascochyta, scab, mildew, white mold, and root rots as well as viral diseases such as bean common mosaic virus, bean golden mosaic virus, and bean common mosaic necrotic virus, contribute to reducing dry bean production (Liebenberg *et al*., 2002). Among biotic stresses, angular leaf spot [caused by *Phaeoisariopsis griseola* (Sacc.) Ferr.], anthracnose [caused by *Colletotrichum lindemuthianum* (Sacc.& Magnus) Lams.-Scrib.], and rust are considered among the most widely distributed foliar fungal diseases that cause severe yield losses of common bean in the Americas, Africa, and other parts of the world (Singh, 2001).

Pests such as Mexican bean beetle, aphids, bean pod weevil, leafhoppers, bruchids, root-knot nematodes, white spider mites, red spider mites and bean fly cause considerable damage on bean plants (Brick and Grafton, 1999; Singh, 1999c).

2.5.2.1 Angular leaf spot

Angular leaf spot, caused by the fungus *Phaeoisariopsis griseola* an imperfect fungus that belongs to the *Moniliales* order and *Stilbaceae* family is currently the most economically important and widely distributed disease in tropical and sub tropical areas causing losses in dry bean yields by as much as 50 to 80%, when susceptible varieties are planted. *P. griseola* infects all above ground parts of the bean plant, with the most notable symptoms being on leaves, where leaf lesions start as small, brown or grey spots that become angular and necrotic, being confined by leaf veins. Leaf spots eventually coalesce, causing premature defoliation. Pod symptoms consist of circular to elliptical red-brown lesions. This results in shriveled seeds of reduced size and quality. In the Great Lakes Region of Africa, losses attributed to angular leaf spot have been estimated to be around 374 800 t (Schwartz *et al*., 1981; Saettler, 1991; Correa *et al*., 1994; Liebenberg and Pretorius, 1997; Wortmann *et al.*, 1998; de Jesus Junior *et al*., 2001, Stenglein *et al.*, 2003;).

2.5.2.2 Anthracnose

2.5.2.2.1 The anthracnose pathogen (*Colletotrichum lindemuthianum*)

The ascomycete *Colletotrichum lindemuthianum* (Sacc. and Magn.), the causal agent of Bean anthracnose, is considered one of the most economically important fungal pathogens of common beans in Kenya. It infects all aerial parts like leaves, stems and pods and is seed transmitted. The disease can result in total crop loss (Pastor-Corrales and Tu, 1989; Opio *et al*., 2001) especially when infected seed is planted and favourable conditions for the establishment and growth of the pathogen exist. The disease is transmitted through seed hence it is widely spread. Common bean anthracnose was first described and recorded in 1875 on plant specimens which had been obtained from Germany. However, *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scrib. had been collected by mycologists as early as 1843. The fungus is known to have races that vary from, country, region, location, and variety (CIAT, 1997). Today, the disease is reportedly one of the most important and widely distributed throughout the world. It is found in Latin America, Asia, Europe, USA and Africa (Ansari, 2004). In Africa, it is particularly important in Uganda, Kenya, Tanzania, Rwanda, Burundi, Ethiopia and DR of Congo.

2.5.2.2.2 Classification and biology of *Colletotrichum lindemuthianum*

Colletotrichum lindemuthianum is an ascomycete and produces its conidia in acervuli. This fungus belongs to the genus *Colletotrichum*, order *Melanconiales*, family *Melanconiaceae* and section *Hyalosporae* (Alexopoulos, 1962). The fungus is found in nature in a conidial (imperfect) stage, but can overwinter as mycelia or conidia. The pathogens' perfect stage, *Glomerella cingulata* is rarely found in nature. Its conidia are oval shaped and dark brown in colour (Agrios, 1997). On the host, they form pink masses of conidia packed into the acervuli. *Colletotrichum lindemuthianum* differs from other species in this genus by its growth characteristics and a dark pigmentation on cultures (Tesfaye, 2003).

2.5.2.2.3 Epidemiology of *Colletotrichum lindemuthianum*

Colletotrichum lindemuthianum survives in bean crop residue and seed (Barrus, 1921; Tu, 1983; Pastor-Corrales and Tu, 1989). In areas where beans are continuously cropped, previous seasons inoculum can initiate epidemics of anthracnose (Dillard *et al*., 1993). Although plant residues contribute greatly to pathogen survival and distribution, infected seed serves an important role in the long distance distribution of the anthracnose pathogen. The pathogen can remain viable in seed for 3-5 years and the farmers who retain seed from previously grown crop (like those in most parts of Kenya), most probably contribute to the carry over and spread of the disease (Tesfaye, 2003).

2.5.2.2.4 Management strategies

Several strategies can be used to manage anthracnose, but planting genetically resistant cultivars is most effective, least expensive, and easiest for farmers to adopt. The main drawback to resistant cultivars is the possible breakdown of resistance caused by the adaptation of the pathogen to host resistance.

2.5.2.3 Common bacterial blight

Common bacterial blight, the most important bacterial disease of common bean, is widespread from tropical to temperate bean growing environments (Yoshii, 1980; Singh 2001). Common bacterial blight is ranked the fourth most important bean disease in Africa (Wortmann *et al*., 1998). It is caused by the soil pathogen *Xanthomonas campestris* pv. *phaseoli* (Xap). Common bacterial blight is systemic (Burkholder, 1921) and seed transmitted (Aggour *et al*., 1989b). Seed transmission plays an important role in the development of the endemic (Weller & Saettler, 1980). Relative humid and warm growing conditions register very high losses on susceptible cultivars. Susceptible cultivars accumulate large bacterial populations, these moving faster through vascular tissue than do common bacterial blight-resistant genotypes (Singh and Muñoz, 1999). Common bacterial blight causes 20 to 60% yield losses depending on disease pressure, environmental condition, and cultivar. In Africa losses of 220,000 tons year^{-1} are reported; of these 146,000 tons are lost in Eastern Africa (Wortmann *et al*,

1998). Moreover, severe common bacterial blight adversely affects seed quality including size, shape, colour, and germination. Pod quality is also reduced. Thus, the marketability of infected seed and its distribution out of the production region can be limited (Marquez *et al.*, 2007). Common bacterial blight-infected seeds with visible symptoms can lose their colour and their value is thus lowered (Singh and Muñoz, 1999). However, planting infected seeds does not necessarily result in systemic transmission of the bacteria from the vascular tissue of the grown plants to the new seeds (Aggour *et al*., 1989b). Bacteria can survive for months on plant debris left on the soil and in seeds (Gilbertson *et al*., 1990). Severity of yield losses varies according to the cultivar, levels of infection, environment, and stage of crop growth (Singh and Muñoz, 1999). Heavy and early infection, high humidity, temperatures fluctuating between 20 and > 25°C, and alternatively dry and wet weather can cause more than 40% yield loss in susceptible cultivars (Serracin *et al*., 1991). Other factors influencing disease severity are photoperiod (Arnaud Santana *et al*., 1993a), inoculation method and bacterial concentration (Aggour *et al*., 1989a), and stage of crop maturity at infection (Coyne and Schuster, 1974). A *Phaseolus* genotype may also show resistance in leaves, but susceptible in pods, or vice versa; it may also be resistant to some strains of the bacterium but susceptible to others (Aggour *et al*., 1989a). Disease incidence can be reduced by intercropping, for example with

maize (*Zea mays L.*) (Fininsa, 1996) or by chemicals such as copper hydroxide and potassium methyldithiocarbamate, particularly when applied early (Singh and Muñoz, 1999). However, chemical control does not significantly reduce pod infestation nor is seed yield increased (Weller and Saettler, 1976). Because no satisfactory chemical control of common bacterial blight is available, cultivar resistance is thus the most effective long-term control strategy (Sanders & Schwartz, 1980), and is pivotal to all other common bacterial blight control measures, including integrated disease-and-crop management practices.

2.5.2.4 Bean root rot

Bean root rots are widely distributed and economically important on common bean in central and South America, Africa and other areas (Abawi and Pastor-Corrales, 1990; Singh, 2001; Kelly *et al*., 2002). It is caused by a complex of soil-inhabiting fungi. These soil-borne fungi include Fusarium root rot (*Fusarium solani* f. sp. *phaseoli), Fusarium* wilt or yellows *(Fusarium oxysporum* f. sp. *phaseoli),* Rhizotonia root rot (*Rhizoctonia solani), Pythium* wilt and seed rot (*Pythium* spp.), charcoal rot or ashy stem blight (*Macrophomina phaseolina)*, black root rot (*Thielaviopsis basicola)*, and Aphanomyces root rot (*Aphanomyces eufeches* f. sp. *phaseoli)* (Abawi & Pastor-Corrales, 1990). The most important ones being *Fusarium solani* f. sp*. phaseoli* and *Pythium ultimum* var*. ultimum* (Rusuku *et al.,* 1997; Spence, 2003; Tusiime, 2003). Resistance to *Fusarium solani*

is complex and is conditioned by two or more genes (Schneider *et al.,* 2001; Romans-Aviles, 2005; Mukankusi *et al*., 2011), whereas, *Pythium ultimum* resistance is controlled by a single dominant gene, marked by a dominant SCAR marker-PYAA19$_{800}$ (Otsyula *et al*., 2003; Mahuku *et al*., 2005; Otsyula, 2010). Root rot symptoms due to *Fusarium* spp. and *Pythium spp.* (the two pathogens show similar symptoms) include poor seedling establishment, damping-off at seedling stage, stunted growth, chlorosis, premature defoliation, death of severely infected plants, foliar blight or pod rot, and lower yield (Abawi and Pastor-Corrales, 1990; Román-Avilés *et al.,* 2003; Abawi *et al.,* 2006). The two pathogens occur concurrently in farmer's fields (Tusiime, 2003). *Pythium* species are spread worldwide (Paul, 2004). Over the last 20 years, there has been an increase in the importance of *Pythium* bean root rots in several countries of Eastern and Central Africa, such as Burundi, the DR of Congo, Kenya and Uganda (Otsyula *et al*., 2003). For example, in Western Kenya and in Rwanda, many farmers stopped growing beans between 1991 and 1993 due to a severe outbreak of root rots, which caused serious food shortages and price increases beyond the reach of many resource-poor households (Nekesa *et al*., 1998). In Rwanda, western Kenya and south western Uganda, *Pythium* spp. are the fungal pathogens most frequently associated with severe root rot epidemics (Rusuku *et*

al., 1997). Yield losses of up to 70% in commercial bean cultivars have been reported in Rwanda and Kenya (Otsyula *et al*., 2003).

2.5.2.5 Bean common mosaic

Bean common mosaic virus (BCMV, a potyvirus) in most bean production regions of the world, and bean golden mosaic virus (BGMV, a geminivirus) which occurs in tropical and subtropical Central America, coastal Mexico, the Caribbean, Brazil and Argentina cause severe yield losses in common bean. Curly top (caused by beet curly top virus, a geminivirus) in the western and Pacific Northwestern (PNW) USA, and bean yellow mosaic virus (a potyvirus) in the PNW, Europe, Middle East, North Africa, and Asia also can cause severe yield losses in susceptible cultivars (Singh, 2001).

2.5.2.6 Other constraints

Among the insect pests, Bean fly (*Ophiomyia phaseoli* Tryon) is by far the most damaging insect pest of common bean in Africa (Abate and Ampofo, 1996; Wortmann *et al*., 1998). Bean stem maggot is the insect pest of greatest concern. It is widespread and especially serious during late planting and when conditions for seedling growth are not favourable. Aphids are found throughout Africa. Aphids are a pest of importance because of their role in transmitting the bean common mosaic virus. Bruchids cause heavy post-harvest losses and a

consequent heavy loss of profit (Wortmann *et al*., 1998). The bean weevil *Zabrotes subfasciatus* Boheman (in warm tropical and subtropical environments) and *Acanthoscelides obtectus* (in cool and temperate environments) cause severe losses when dry beans are not properly stored (Singh, 2001). Leafhoppers *Empoasca kraemeri* Ross & Moore (in the tropics and subtropics) and *E. fabae* (Harris) (in the temperate and cooler environments) are the most widely distributed problems, especially in relatively drier areas (Singh, 2001). Bean pod weevil (*Apion godmani* Wagner and *A. aurichalceum* Wagner) causes severe damage in the highlands of Mexico, and in Central America. In the highlands of Mexico and the USA, Mexican bean beetle (*Epilachna varivestis* Mulsant) also causes severe leaf damage, especially in late maturing cultivars (Singh, 2001).

2.6. Sources of resistance to target bean diseases

Several sources of resistance to bean anthracnose have been found. The well documented sources of anthracnose resistance include AB136, G2641, PI207262, G2333, Cornell 49-242, Mex222, G811, Mex227, and Ecuador 299 (Graham and Ranalli, 1997). Pastor-Corrales *et al*., (1994) showed that only the G 2333 line was resistant to 380 isolates of *C. lindemuthianum*. This line was resistant to all the Brazilian isolates and all the European and North American races (Pastor-Corrales and Tu, 1989; Balardin and Pastor-Corrales, 1990; Balardin *et al*., 1990). Resistance in G 2333 is controlled by two independent dominant genes with

equivalent effects (Pastor-Corrales *et al*., 1994). In addition, Young *et al*., (1998) detected in G 2333, three different dominant resistance genes, *Co-4*^{2} allele, at the *Co-4* locus, *Co-5* and *Co-7*. G2333 has the broadest known resistance and carries three complementary genes that confer resistance against more than 90% of known *C. lindemuthianum* races (Young *et al*., 1997, Mahuku *et al.,* 2002).

With the objective of identifying good sources of angular leaf spot resistance, the Centro Internacional de Agricultura Tropical (CIAT) evaluated materials from the primary and secondary gene pools of common bean under field conditions (Mahuku *et al*., 2003; Buruchara & Bua, 1999; Pastor-Corrales *et al*., 1998). Potential sources of resistance were indentified including MAR 1, MAR 2, MAR 3, Mexico 54, BAT 332, G 5686, G 10474, G 10909, AND 277, ESAL 550, Cornell 49-242. Previous studies conducted by CIAT (International Centre for Tropical Agriculture) demonstrated that cultivar Mexico 54 is resistant to most African ALS isolates so far characterized (CIAT, 1996; Anonymous, 1996a; 1997). Out of the 163 isolates in Africa, it was resistant to 158. This cultivar is therefore a potential source of resistance in breeding work, particularly in Africa (Pastor-Corrales *et al*., 1998; Mahuku *et al*., 2002; Mahuku *et al.,* 2003).

Moderate resistance to common bacterial blight has been found in common bean; comparatively higher levels in some scarlet runner bean (*P. coccineus*) and

the highest levels in tepary bean (*P. acutifolius*). Hybridization between *P. vulgaris and P. acutifolius* by embryo rescue was initiated at CIAT, Palmira, Colombia, in 1989. XAN 159, XAN 160 and XAN 161 were developed from a *P. vulgaris X P. acutifolius* (PI 319443) population received from the University of California-Riverside (Singh and Muñoz, 1999). Mejia-Jimenez *et al*., (1994) successfully hybridized common and tepary (*P. acutifolius* A. Gray) bean and produced CBB-resistant interspecific breeding lines (IBLs). Singh and Muñoz, (1999) selected IBLs with pyramided common bacterial blight resistance, from the populations developed by Mejia-Jimenez *et al*., (1994) namely VAX 1, VAX 2, VAX 3, VAX 4, VAX 5, and VAX 6. The VAX lines with combined resistance from *P. vulgaris* and *P. acutifolius* possess the highest level of CBB resistance developed to date (Singh *et al*., 2001).

Otsyula *et al*., (1998) evaluated 400 genotypes in the field for resistance to root rots for over six seasons. 374 entries were from Kenyan germplasm collection, and 26 introductions previously identified as resistant in Rwanda. Of the 374 local accessions, only GLP X92 was found to be tolerant. Many of those which were resistant in Rwanda were found to be resistant or tolerant in western Kenya. Sixteen genotypes found to be resistant (R) or tolerant (T) to root rots in western Kenya were: RWR 719 (R), MLB-49-89A (R), Ihumure (R), SCAM 80-CM/15 (R), RWR 1092 (R), MLB-40-89A (R), RWR 1059 (R), RWR 432 (R), MLB-48-

89A (R), GLP X92 (T), MLB-17-89A (T), SCAM 80-CM/5 (T), MLB-39-89A (T), MCD 221 (T), RWR 868 (T). Nzungize *et al* (2012) adds AND 1055 and AND 1062 to the above list of *Pythium* root rot resistant varieties. Previous screen house and field evaluations carried out in Kenya, Rwanda and Uganda by Buruchara and Kimani (1999) identified a few bean lines with resistance properties to *Pythium* root rot disease. Among those lines, were the genotypes RWR 719 and AND1062.

2.7. Breeding beans for resistance to diseases in eastern Africa

Presently, breeding for angular leaf spot resistance in Uganda and many other developing countries is based on conventional methods, which are limited by the lengthy screening procedures and also reliance on favourable environmental factors (Namayanja *et al*., 2006). The market-class dry bean which are large-seeded and red or mottled seeds are the most preferred by the market in Uganda, and are sold on local markets for cash and exported to earn foreign exchange (Nkalubo *et al.,* 2009). RAPD OPE04 marker linked to the *Phg-2* resistance gene has been used in Uganda in selecting resistant and susceptible segregating populations (Namayanja *et al*., 2006). Mongi *et al* (2009) crossed five of the most preferred landraces (Kablanketi, Masusu, Kigoma, Salundo and two improved varieties DRK and Kabanima released in 1980's from Southern Highlands of Tanzania (SHT) having susceptibility to anthracnose and angular leaf spot with cultivar AB 136 and Mexico 54, carrying resistance genes *Co-6,* and

Phg-2 respectively. Progenies were evaluated under high disease pressure in a screen house and field. The pedigree selection method was used to identify superior genotypes, for generation advancement. Thirteen advanced lines derived from crosses made were evaluated against three local checks in a randomized complete block design with three replications for yield and other agronomic traits. Resistant levels of the advanced lines were not statistically significant different ($P>0.05$) but yielding ability differed significantly ($P<0.05$). The advanced lines showed relative high levels of resistance which, in most cases was similar to that of resistant parents. Two genotypes, PB507009 and PB507078 showed the highest yields with general adaptability and were identified for promotion under farmer field conditions. Mkandawire *et al* (2004), used a Kenyan strain of *X. campestris* pv. *phaseoli,* X21, in pathogen-host-coevolution studies. The rep-PCR fingerprints of *X. campestris* pv. *phaseoli* strains revealed that *X. campestris* pv. *phaseoli* is a heterogeneous pathovar composed of three genetically distinct genotypes: two found in East Africa. Their results also showed the two East African *X. campestris* pv. *phaseoli* genotypes to be more pathogenic on Andean than Middle American beans.

National Agricultural Research Systems (NARS) in the region have released varieties with resistance to one or more diseases (Kimani *et al*., 2005). For example, Awash 1 with resistance to rust was released in Ethiopia in 1989;

Raozin' Alaotora, released in Madagascar in 1995, is resistant to rust, ascocyta, and angular leaf spot; CAL 143, released in Malawi, is tolerant to angular leaf spot; Vunikingi, a climber resistant to *Fusarium* root rot, was released in Rwanda in 1985; Uyole 98, released in Tanzania, is resistant to anthracnose; while K131, released in Uganda and Zambia, is resistant to bean common mosaic virus and bean common mosaic necrosis virus. Between 1992 and 1996, 69 bush or climbing bean cultivars were released and disseminated by bean researchers in eight eastern Africa countries (David, 1997; David *et al*., 2000). Several others were in advanced evaluations. The released varieties showed improved performance in on-station and on-farm trials and were superior to local landraces in grain yield or resistance to diseases, pests, or had another important trait (Kimani *et al*., 2005; Buruchara *et al*., 2011). Yield advantage varied with countries and test environments. For example, yield improvement of new bush bean cultivars over the check (often a previously released cultivar) was 5% to 69% in Ethiopia; 14% to 33% in Kenya; 5% to 33% in Rwanda; 8% to 18% in Tanzania, 51% in DR of Congo, and 22% in Uganda. The maximum increases for climbers ranged from 15% to 30% in Rwanda and 2% to 35% in Tanzania. In western Kenya, the introduction of root-rot-tolerant climbing beans and bush beans increased bean yields in farmers' fields from less than 0.2 t ha^{-1} to over 0.8 t ha^{-1} per season (Nekesa *et al*., 1998). K132 out yielded the local improved

cultivar, K20, by 38%, and a popular landrace, 'Kanyebwa', by 35% in farmers' fields in Mbale under intercropped conditions (David *et al*., 2000). Another new release, K131, out-yielded K20 by 79% and 'Kanyebwa' by 69% in the same trials (Kimani *et al*., 2005). In collaboration with the Harvest Plus Challenge Program, several crosses for large-seeded bush and climbing beans have been performed, to combine higher mineral content with other useful traits such as resistant to diseases (such as angular leaf spot and bean common mosaic necrotic virus) (Kimani *et al*., 2005). One hundred new crosses involving three thousand families which combine drought tolerance and high mineral content in small-seeded beans were created and evaluated. Crosses to combine drought tolerance, iron content, and angular leaf spot resistance in small-seeded bush beans were made at CIAT HQ. Similar crosses are being made in DR Congo and in Kenya (PABRA, 2007). Twenty five varieties in total were released in seven countries. Twelve new varieties were released in four SABRN countries: DR Congo (three varieties resistant to angular leaf spot, bean stem maggot, and bean common mosaic necrotic virus and tolerant to low soil fertility); Mozambique (two varieties, tolerant to low soil fertility, resistant to rust and angular leaf spot); Swaziland (five varieties, tolerant to rust and low soil fertility); and South Africa (two varieties, resistant to rust). Thirteen varieties have been released from the ECABREN region and include: four in Kenya (MAC13, MAC 34 and MAC 64 and

AFR 708 for low soil fertility), two in Uganda, (RWR 2075-NABE 14, RWR 1946-NABE 13) which are resistant to root rots and tolerant to low soil fertility; and seven in Ethiopia (STTT-165-92-Chore, NZBR-2-5, RAB 484-Dinkesh, XAN 310-Melka Dima, G843-Haramaya, STTT 165-95-Hirna and STTT (PABRA, 2007).

2.8. Pathogenic variation

2.8.1 *Collectotrichum lindemuthianum*

The bean anthracnose fungus (*Collectotrichum lindemuthianum*) is an ascomycete that is highly variable both genetically and physiologically, with over 100 races characterized worldwide. (Schwartz *et al.,* 1982; Pastor-Corrales and Tu, 1989; Kelly *et al.,* 1994; Restrepo, 1994; Sicard *et al.,* 1997; Balardin and Kelly, 1998; and Mahuku *et al.,* 2002). The existence of physiological specialization or pathogenic races was first observed by Barrus in 1911 when he described the differential ability of *C. lindemuthianum* isolates to infect a group of bean varieties. Since then, several races of this fungus have been reported in literature (Menezes and Dianese, 1988; Gathuru and Mwangi 1991; Kelly *et al*., 1994; TU, 1994; Vilarinhos *et al*., 1995; Barladin *et al*., 1997; CIAT, 1997; Mesquita *et al*., 1998; Gonzalez *et al*., 1998; Tesfaye, 2003; Ansari *et al*., 2004; Sartorato *et al*., 2004 and Mahuku and Riascos, 2004). These researchers have shown a high variability of the pathogen using the international set of bean differentials for the classification of *C. lindemuthianum* races (section 3.3.4 Table

7). Alzate-Marin *et al.,* (2004) for example identified a total of 50 *C. lindemuthianum* pathotypes in Brazil between 1994 and 2002, whereas Mahuku and Riascos (2004) identified 90 races from 200 *C. lindemuthianum* isolates collected from Andean and Mesoamerican bean varieties and regions. Virulence diversity of this pathogen has also been reported in some areas of Africa (Tesfaye, 2003) and Europe where common bean has not traditionally been grown, and where climatic conditions differ from those of the two centres of origin of its host. Butare (unpublished data) identified 42 races of this pathogen out of 53 isolates from Rwanda. Sartorato *et al.*, (2004) used 24 races of *C. lindemuthianum* on 23 bean genotypes and only five bean genotypes were found resistant to all the races, the rest of the beans showed diverse reaction to the races. Generally, one bean cultivar may be resistant to some races, but not others (CIAT, 1997). The high variability may also render resistant germplasm susceptible when exposed to other races or populations of the pathogen. This high pathogenic variability observed in this fungus may be mostly the result of i) the pathogen-host co-evolution (Alzate-Marin et al., 1999), ii) association of *C. lindemuthianum* with various crops and iii) the different environmental conditions found from region to region. This variability of the fungus has been cited as the primary reason for the economic importance of anthracnose in many

areas of Central and Eastern Africa, as well as Latin America (Garrido and Cova 1989).

In Kenya six races namely alpha, beta, delta, epsilon, gamma and lambda have been reported by (Kinyua, 1976; Mwangi, 1986; Gathuru and Mwangi, 1991). Ombiri *et al* (2002) using the 12 international anthracnose differential cultivars and the binary system (CIAT, 1987; Buruchara, 1991) equated alpha, beta, delta, epsilon, gamma and lambda to race 17, 2, 23, 1, 38 and 55 respectively. Gathuru and Mwangi (1991) conducted studies on pathogenic variation in *C. lindemuthianum*. Thirty six isolates were collected from nine districts of Kenya. Isolates were cultured and inoculated on bean differentials: 'Michelite', 'Perry Marrow', 'Michigan Dark Red Kidney', 'Emerson 847', 'Kaboon', 'Cornell 49-242', 'Processor' and 'Canadian Wonder'. Eleven isolates were grouped as beta, eight as gamma, and five as epsilon, two as delta and one as alpha according to the system proposed by Hubbeling (1957). Ombiri *et al* (2002) using the 12 international anthracnose differential cultivars and the binary system (CIAT, 1987; Buruchara, 1991) equated beta, gamma, epsilon, delta and alpha to race 2, 38, 1, 23, and 17 respectively. Nine isolates did not fit in any of the known races. The system of characterizing the isolates proposed by Hubbeling (1957) used by the researchers was limited in that some isolates could not be identified. Researchers at CIAT (1987) came up with a system where they defined a group

of 12 common bean differentials to be used internationally in order to facilitate the exchange of information and of resistant germplasm. At the same time, a binary system of classification was proposed. With the utilization of the 12 differential cultivars coupled with binary system for race designation, it is possible to characterize all to the existing races by designating them a number. Ombiri *et al.* (2002) collected anthracnose-infected bean seeds of variety `Rosecoco' from Rongai in formerly Nakuru District and obtained four isolates of *Collectotrichum lindemuthianum*. All the four isolates exhibited more or less similar response to the differential cultivars, and identified as race 485. This was the first report of race 485 in Kenya. However the fungus has also been reported in Burundi without consideration of physiologic races (Bigirimana *et al*., 2000). According to the Binary system of classification (Buruchara, 1991; TU, 1992 and 1994), the six races (alpha, beta, delta, epsilon, gamma and lambda) and some uncharacterized races have been designated as race 17, 2, 38, 23, 1 and 55 respectively. Race 2 has also been reported in Uganda by Mwesigwa (2009).

2.8.2 Pathogenic variation in other diseases

Previous studies have revealed high levels of pathogenic and genetic variation in *Phaeoisariopsis griseola* (Buruchara, 1983; Correa-Victoria, 1988; Guzman *et al.*, 1995; Maya *et al.*, 1995; Pastor-Corrales & Jara, 1995; Boshoff *et al.*, 1996; Chacon *et al.*, 1997; Pastor-Corrales *et al.*, 1998; Busogoro *et al.*, 1999; *Mahuku*

et al., 2002b). For example, Marin-Villegas (1959) identified 13 pathotypes among 33 isolates from Colombia, while with Brazilian isolates, Paula & Pastor-Corrales (1996) identified 21 pathotypes among 27 isolates and Aparicio (1998) identified 30 pathotypes among 66 isolates (Mahuku *et al.*, 2002). A high level of pathogenic variability has also been reported in the Kenyan isolates of *P. griseola.* Monda (1995) identified 11 pathogenic groups from 15 isolates. Wagara (2005) characterised 100 isolates into 44 physiological races. Wagara (1996) grouped 18 isolates into 15 races.

Pathogenic variation has been reported among strains of *X. campestris* pv. *phaseoli* (Schuster *et al*., 1973; Valladares-Sanchez *et al*., 1979; Opio *et al*., 1996). However, unequivocal evidence for existence of races of *X. campestris* pv. *phaseoli*, identifiable on common bean differential genotypes, has yet to be found (Gilbertson and Maxwell, 1992). This is consistent with the fact that common bacterial blight resistance is a quantitative trait (Saettler, 1989; Gilbertson and Maxwell, 1992; Singh and Muñoz, 1999.). *X. campestris* pv. *phaseoli* strains have been differentiated into pathogenic races based on reactions on tepary bean (*P. acutifolius*) lines (Zapata, 1990; Opio *et al*., 1996), but the significance of this variability in terms of common bean remains to be established.

A study was conducted in Kenya, Rwanda and Uganda to identify the causal agents of root rots (Mukalazi, 2004). The species isolated from bean samples affected by root rot symptoms included: *Pythium nodosum* Bhatn, *Pythium echinulatum* Matthews, *Pythium pachycaule* Shtayeh, *Pythium oligandrum* Drechsler, *Pythium acanthicum* Drechsler, *Pythium chamaehyphon* Sideris, *Pythium folliculosum* Paul, *Pythium indigoferae* Butler, *Pythium irregulare* Buisman, *Pythium lutarium* Shtayeh, *Pythium macrosporum* Vaartaja, *Pythium myriotylum* Drechsler, *Pythium paroecandrum* Drechsler, *Pythium torulosum* Coker, *Pythium vexans* de Bary, *Pythium zingiberis* Takah, *Pythium graminicola* Subraman, *Pythium spinosum* Sawada, *Pythium ultimum* Trow, *Pythium arrhenomanes* Drechsler, *Pythium catenulatum* Matthews, *Pythium diclinum* Tokun, *Pythium dissotocum* Drechsler, *Pythium rostratum* Butler, *Pythium salpingophorum* Drechsler and *Pythium deliense* Meurs. One hundred and thirty-four *Pythium* isolates obtained from root rot affected areas in Kenya and Rwanda were characterized by sequencing. Out of 134 isolates characterized, 22 species were identified. Fifteen of the 22 species were recovered from Kenya. They included *Pythium acanthicum, Pythium chamaehyphon*, *Pythium folliculosum*, *Pythium indigoferae*, *Pythium irregular, Pythium lutarium*, *Pythium macrosporum*, *Pythium myriotylum*, *Pythium paroecandrum*, *Pythium torulosum*, *Pythium vexans*, *Pythium zingiberis*, *Pythium graminicola*, *Pythium spinosum*,

Pythium ultimum with *Pythium vexans*being the most frequent species, followed by *Pythium torulosum, Pythium irregular* and *Pythium ultimum* however overall *Pythium ultimum* is the most frequent species in the region (Buruchara *et al.,* 2004).

2.8.3 Breeding methods

Beans are self-pollinated and thus breeding methods for autogamous crops are employed. Pedigree selection and its modifications is the most commonmethod used by bean breeders to develop improved cultivars. An important limitation of pedigree selection is the amount of time needed to develop new cultivars (Fehr, 1987). In order to preserve horticultural and seed traits of snap and dry edible bean cultivars, plant breeders have often utilized backcross breeding and its modifications to incorporate simply inherited traits. This selection method is also well suited for marker-assisted selection (Miklas *et al*., 2003; Miklas, 2007). This breeding method is not useful, however, for the improvement of quantitatively inherited traits such as seed yield or tolerance to abiotic stress. If multiple generations can be grown each year, bulk breeding can be used to rapidly advance bean populations. This approach would be most appropriate for crosses between elite lines within a market class where little segregation for seed type or adaptation would be expected. If the bulked populations are grown in the target environment, some natural selection may occur for traits of

economicvalue. Kelly *et al*., (1998a) recommended the use of single seed descent (SSD) when working with crosses between elite lines within a market class. The procedure provides a way to maintain genetic variability while advanced-generation lines are produced.Recurrent selection permits the accumulation of favourable alleles as the result of recombination ineach cycle of selection.

Singh (1994) proposed the use of gamete selection to simultaneously select common beans for multiple traits.Gamete selection allows screening and selection of dominant and co-dominant alleles during hybridisation and immediately after production of final multiple-parent F_1 hybrids. Simultaneous improvement of multiple traits such as seed yield, disease resistance, and growth habit among others can be realised using multi-parent crosses. This is achieved by combining multiple genes simultaneously in a procedure called gene pyramiding. This method presents the breeder with many privileges not available when using the conventional methods among them identification of promising lines in early generations, thus avoiding the loss of valuable time and resources. Molecular markers may facilitate gamete selection in the identification of early-generation populations that continue to possess the desired alleles (Singh *et al*., 1998). Among the assumptions made in gamete selection are that the male parents of the final crosses are heterozygous, and therefore heterogametic. In contrast, the females (commercial susceptible varieties) are homozygous and

homogeneous for most traits and therefore homogametic. Thus they produce only one type of gamete. This implies that any differences observed in the F_{1s} are due to the difference in the male parents which is a result of the different resistance genes present in the male parents.Gamete selection proved to be successful in the development of high-yielding, erect bean lines with resistance to leafhoppers and five diseases (Singh *et al*., 1998). Asensio-S.-Manzanera *et al*. (2006) also used gamete selection to develop breeding lines with resistance to common bacterial blight and halo blight.

2.8.4 Marker assisted breeding

Marker-assisted Selection (MAS) involves using the presence/absence of a marker as a substitute for or to assist on phenotypic selection, in a way which may make it more efficient, effective, reliable and cost-effective compared to the more conventional plant breeding methodology (Miklas *et al.,* 2006). Marker-assisted selection provides an effective and efficient breeding tool for detecting, tracking, retaining, combining, and pyramiding disease resistance genes. Several markers linked to resistance genes for major diseases in eastern Africa have been identified (Table 2.1). Marker-assisted selection (MAS) has been employed in CIAT headquarters for several years to obtain resistance to viruses, first BGYMV and then BCMV (CIAT, 2007). This activity was transferred to Africa, and was expanded significantly to include selection for genes for resistance to

other diseases. More than 1000 plants were assayed in Uganda for resistance genes (both recessive and dominant) against BCMV and BCMNV. Markers developed at CIAT headquarters to detect the gene for resistance to *Pythium* found in RWR 719 (a Rwandan bred line) were also tested in Uganda on 111 backcrossed progeny and 54 families derived from double crosses. Meanwhile, at headquarters, 282 F_2-derived families were tested for the presence of anthracnose-resistance genes derived from G 2333. During the 2007–08 period, two SCAR genetic markers for resistance to bean rust were identified by CIAT. This helps plant breeders to screen plants in local germplasm that carry the resistant gene. Thirty-eight lines selected for rust resistance were propagated in the greenhouse and in the field in Kenya, and sent to Ethiopia, Madagascar and Rwanda (PABRA, 2009). In SABRN and ECABREN countries during the 2006 to 2007 period, there was identification and validation of new SCAR markers which were associated with resistance to *Pythium* root rots and angular leaf spot in key resistance sources. Systematic use of molecular markers in selection for specific resistance genes against bean common mosaic necrotic virus, anthracnose, angular leaf spot and *Pythium* root rots was initiated at the biotechnology laboratory at Kawanda (PABRA, 2007).

Table 2.1 Resistant genes and their markers

Resistance source	Disease	Linked marker	Tagged locus	Linkage distance	Size (bp)/ Orientation	Reference
G2333	Anthracnose	SAB-3	*Co5*	12.98cM	400 cis	Vallejo and Kelly, 2001
Mex 54 G10909	Angular leaf spot	SH-13	*phg-1*	5.6cM	520 cis	Queiroz et al., 2004a
AND 1062 RWR 719	*Pythium* root rot	PYAA19	Dominant gene in AND 1062	1.5cM	800 cis	Muhuku 2005
VAX 6	Common bacterial blight	SU 91	Major QTL (XAN 159)	0.5cM	700 cis	Pedraza et al., 1997

CHAPTER 3 : PATHOGENIC VARIATION OF *COLLETOTRICHUM LINDEMUTHIANUM* IN MAJOR BEAN GROWING AREAS OF KENYA

3.1 Abstract

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cav., is one of the major constraints to bean production in Kenya. This pathogen is particularly important in relatively cool, wet production areas of tropical and temperate regions. Crop losses can be severe or total, especially when susceptible cultivars are grown in an environment that is favourable to the pathogen. The most effective and economic way to manage anthracnose of common bean is the use of host plant resistance. However, effective problem oriented breeding for resistance to anthracnose requires understanding pathotypes present in the common bean growing areas of interest. Updated information on pathogenic variation and geographical distribution of *C. lindemuthianum* in Kenya is lacking. The objective of this study was to characterize Kenyan population of *Colletotrichum lindemuthianum* and determine its geographical distribution. A set of 12 international common bean anthracnose differentials were used to assess the pathogenic diversity of 31 *C. lindemuthianum* strains isolated from western, rift valley, central, eastern and

coastal regions of Kenya. Six sources of resistance and four Kenyan commercial varieties were screened for resistance to infection by isolates of *C. lindemuthianum*. The first trifoliate leaf was inoculated with a spore concentration of 1.2 x 10^6 ml^{-1}. Plants were maintained at 20°C ±2°C and 90-100% relative humidity for 48 hours. Symptoms were evaluated 12, 17 and 21 days after inoculation. The isolates were characterized into 12 pathogenic races of *Colletotrichum lindemuthianum*. Of the 12 races identified, seven (1, 2, 17, 23, 38, 55 and 485) had been previously identified, while five (65, 73, 81, 87 and 89) were new. Races 65 (8 of 31 isolates) and race 73 (4 of 31 isolates) were the most frequent in surveyed regions. G2333 and AB 136 were highly resistant to all the isolates. The other sources of resistance namely G10909, MEX54, AND 1062, RWR719 and VAX6 and four commercial cultivars namely New Rosecoco, Kenya Umoja, GLP1004 and Canadian Wonder showed high compatibility with most of the races. Differential varieties AB 136 and G 2333 can be used in breeding programs in Kenya as they were resistant to all the races identified. Future bean breeding efforts should consider the new racial diversity of *C. lindemuthianum* because the four commercial varieties used in this study were highly susceptible.

3.2. Introduction

Anthracnose, caused by *C. lindemuthianum* is one of the most destructive bean diseases in the cool highlands of east and central Africa. It is particularly important in Uganda, Kenya, Tanzania, Rwanda, Burundi, Ethiopia and DR Congo. Genetic and physiological variability of *C. lindemuthianum* has been observed in different parts of the world where beans are grown (Pastor-Corrales and Tu, 1989; Beebe and Pastor-Corrales1991). More than 100 races of the pathogen have been characterized worldwide (Garrido-Ramirez and Romero-Cova, 1989; Pastor-Corrales and Tu, 1989; Rava *et al*., 1993; Balardin *et al*., 1997). The race structure of *C. lindemuthiaum* is highly variable and new ones reportedly keep emerging (Leaky and Simbwa-Bunya, 1972; Pastor-Corrales and Tu, 1987, Nkalubo, 2006). Association of the pathogen with various crops and the different environmental conditions found from region to region may lead to the high pathogenic variability which has been observed in this fungus. This diversity may mostly be the result of the pathogen-host co-evolution (Alzate-Marin *et al.*, 1999). Due to the highly variable nature of the pathogen and the continual emergence of new pathogen races, genetic resistance in the host is not durable (Bigirimana and Hofte, 2001). This factor is a big threat against creating bean cultivars with durable resistance to anthracnose (Alzate-Marin *et al*., 2001).

A number of races may be found in each producing region, which may hinder the development of cultivar resistance to *C. lindemuthianum* (Rava *et al.*, 1994). No known host resistance genes are effective against all known races of the pathogen. One way of making the resistance more stable is to pyramid or incorporate several resistance genes into a single line. Currently, races of *C. lindemuthianum* are classified by inoculating isolates onto a universal set of 12 differential cultivars. The aim of this study was to characterize Kenyan population of *Colletotrichum lindemuthianum* and determine its geographical distribution.

3.3 Materials and Methods

3.3.1 Disease survey and collection of diseased materials

Anthracnose Diseased materials were collected in three field surveys (Table 3.1, Fig 3.1) conducted during the short rain season (December 2010) and long rains (June 2011) in major bean growing regions in Kenya. Fifty-five samples of diseased materials were collected from 35 districts in western, Eastern, Nyanza, Rift Valley, Central Highlands and the upper coastal bean growing regions of Kenya (Table 3.2, Fig. 3.2). Samples were collected from naturally infected bean plants in farmers' fields during the early to middle stages of disease development because they were easier to examine and isolate (Fig 3.3`a'). The materials were placed in brown bags and envelopes, labeled and placed in cool

boxes and delivered to the Plant Pathology Laboratory, Dept. of Plant Science and Crop Protection, at Upper Kabete University of Nairobi.

Table 3.1 Collection of *Colletotrichum lindemuthianum* infected bean material

Surveys	Season	Dates	No. of Samples
Western	Short rains	1st – 7th Dec 2010	21
Central	Short rains	19th – 24th Dec 2010	19
Mt. Kenya + Coast	Long rains	7th – 12th June 2011	15
Total			55

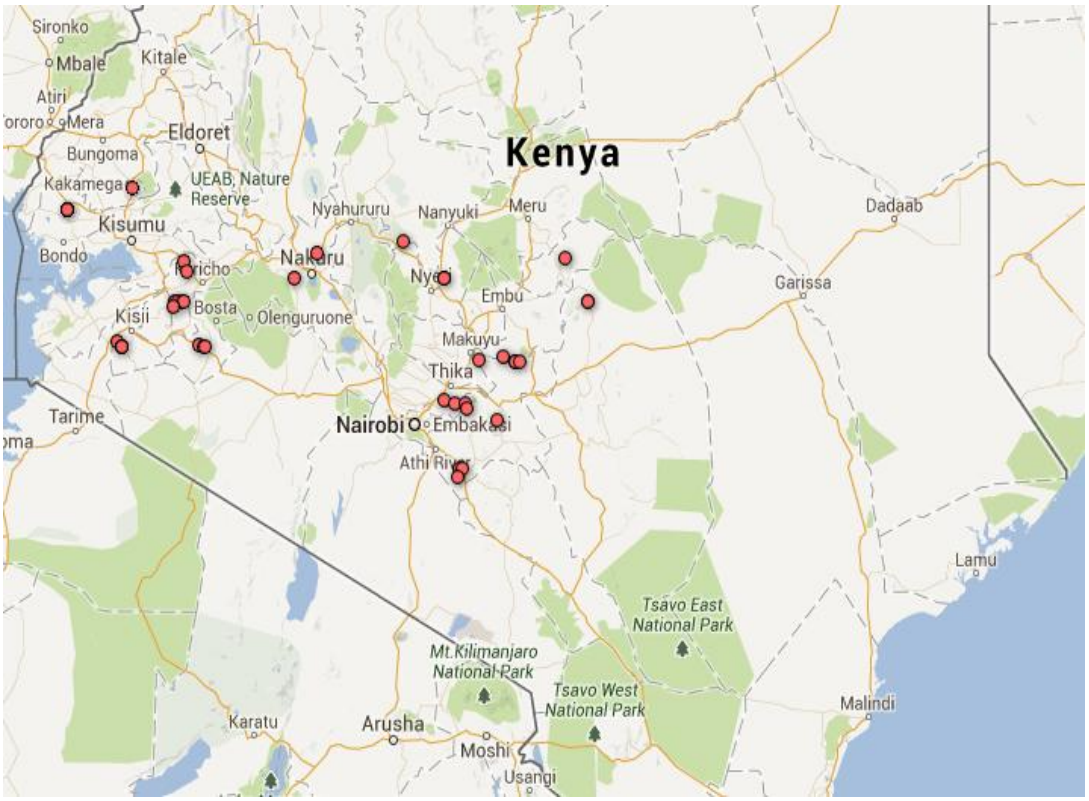


Figure 3.1 Map of Kenya showing areas surveyed for prevalence of bean disease and collection of diseased material. The red spots on the map indicate these sites.

Table 3.2 Altitude, GPS coordinates and agro-ecological zones of disease collection sites

County	District	AEZ	SAMPLE CODE	GPS COORDINATES		
				Elev.(m)	Longitude	Latitude
Kericho	Bureti	UM1	2pa	1613	35° 04' E	0° 31' S
		UM4	2pb	1736	35° 05' E	0° 29' S
		UM1	2pc	1727	35° 06' E	0° 29' S
		UM1	2pd	1768	35° 08' E	0° 29' S
		UM2	2pe	1783	35° 09' E	0° 29' S
Kakamega	Kakamega	UM1	3pa	1517	34° 46' E	0° 16' N
Kericho	Kericho	LH1	4pa	1949	35° 09' E	0° 13' S
		LH1	4pb	1985	35° 10' E	0° 17' S
Bomet	Bomet	LH2	5pa	1928	35° 15' E	0° 46' S
		LH3	5pb	1964	35° 18' E	0° 47' S
		LH4	5pc	1937	35° 16' E	0° 47' S
Siaya	Siaya	LM1	10pa	1271	34° 18' E	0° 07' N
		LM2	10pb	1272	34° 18' E	0° 07' N
		LM3	10pc	1288	34° 18' E	0° 07' N
		LM4	10pd	1263	34° 18' E	0° 07' N
Nakuru	Nakuru	UM3	11pa	1944	36° 07' E	0° 10' S
Kisii	Mugirango	UM1	14pa	1710	34° 40' E	0° 45' S
Gucha		UM1	16pa	1664	34° 42' E	0° 47’ S
		UM1	16pb	1687	34° 42' E	0° 47’ S
		UM1	16pc	1688	34° 42' E	0° 47’ S
		UM3	16pd	1722	34° 42' E	0° 47’ S
Nakuru	Njoro	LH3	22pa	2139	35° 57’ E	0° 20’ S
Kiambu	Thika	UM4	25pa	1525	37° 08' E	0° 98' S
Laikipia	Narumoru	LH4	26pa	1994	37° 02' E	0° 20' S
		LH4	26pb	2006	37° 02' E	0° 19' S
Maragua	Murang’a	UM3	27pa	1509	37° 09' E	0° 95' S
		UM2	27pb	1491	37° 10' E	0° 95' S
Meru	Meru	UM1	28pa	1354	37° 65' E	0° 29' S
		UM2	28pb	2017	37° 55' E	0° 12' S
		UM3	28pc	1354	37° 65' E	0° 29' S
Kiambu	Kiambu	UM1	29pa	1628	36° 85' E	1° 16' S
Nyeri	Othaya	LH3	30pa	1870	36° 93' E	0° 53' S
		LH3	30pb	1911	36° 87' E	0° 55' S
		LH3	30pc	1894	36° 90' E	0° 54' S
Murang'a	Murang'a	UM4	31pa	1454	37° 07' E	0° 69' S

County	District	AEZ	SAMPLE CODE	GPS COORDINATES		
				Elev.(m)	Longitude	Latitude
		UM2	31pb	1402	37° 12' E	0° 71' S
		LH1	31pc	1624	37° 02' E	0° 68' S
		UM2	31pd	1401	37° 11' E	0° 69' S
Kibirigwi	Kirinyaga	UM2	34pa	1440	37° 17' E	0° 52' S
Kirinyaga	Kerugoya	UM2	35pa	1461	37° 28' E	0° 51' S
Kabete	Kiambu	LH3	44pa	1844	36° 44' E	0° 05' S
Taita Taveta	Wundanyi	LH2	45pa	1468	38° 21’ E	3° 24’ S
		UM1	45pb	1462	38° 21’ E	3° 24’ S
		UM3	45pc	1444	38° 21’ E	3° 24’ S

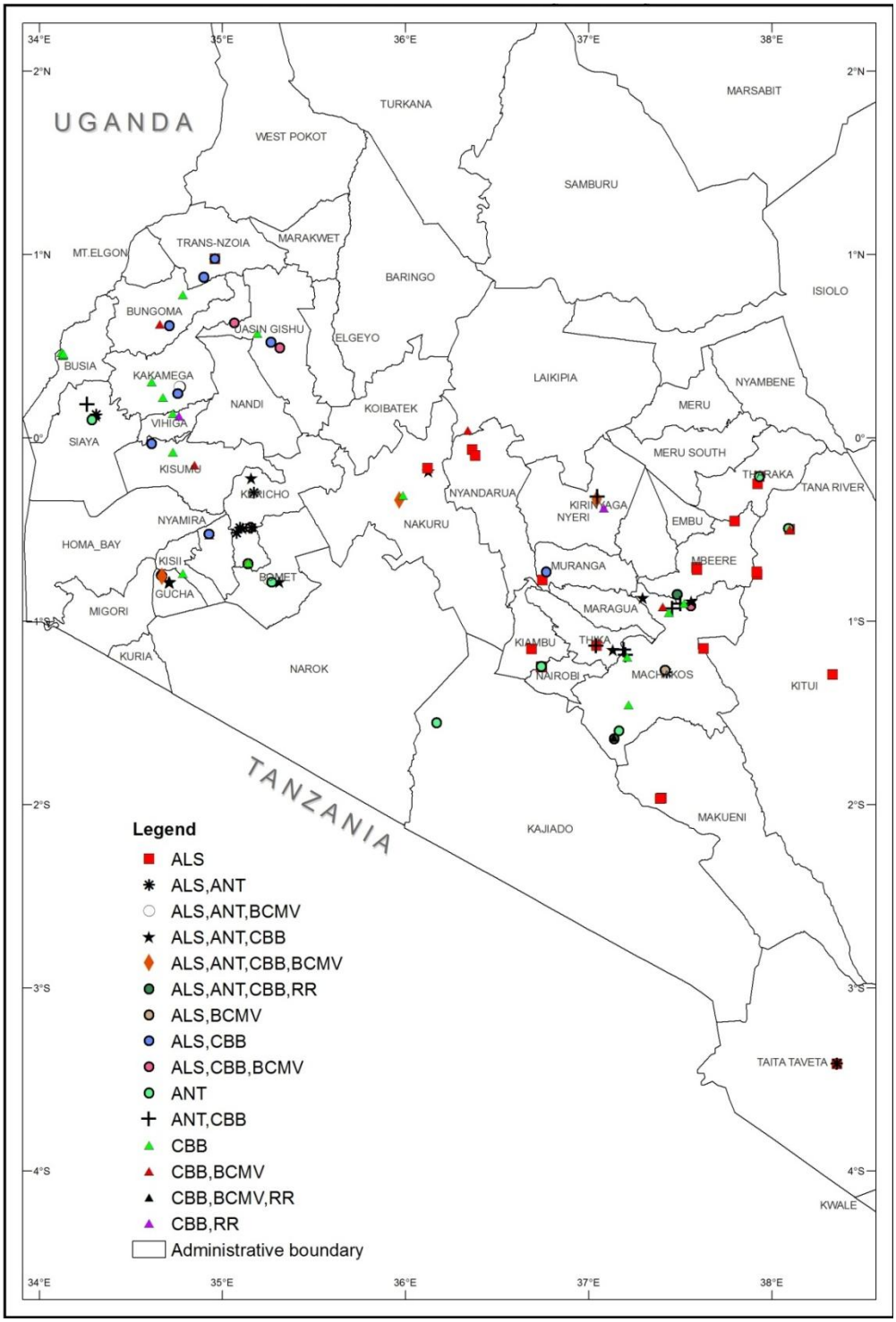


Figure 3.2 Map of Kenya showing common bean growing areas. The areas are further marked with the diseases that were found there.

3.3.2 Media preparation.

Colletotrichum lindemuthianum was isolated on potato dextrose agar (PDA). Standard PDA medium was prepared by mixing 39g of potato dextrose agar (PDA) in one litre of distilled water. To sterilize the media it was autoclaved at 120°C and 1 bar of pressure for 20 minutes. To prevent bacterial contamination, when the media had cooled, 100µl of each of the antibiotics chloromphenical (CAM) and ampicillin (AMP) at concentrations of 40µl/ml and 13µl/ml respectively were added and then mixed thoroughly and there after poured into 9cm Petri-dishes and allowed to set.

3.3.3 Pathogen isolation.

The pathogen *Colletotrichum lindemuthianum* was isolated from infected bean pods collected during the field surveys. Isolations were done in the Plant Pathology Laboratory, Department of Plant Science and Crop Protection, Upper Kabete Campus University of Nairobi. Pathogen mycelia was preserved in filter paper and desiccated in silica gel before storage at -20°C. Well developed pod lesions (Fig 3.3 `a') were carefully cut and surface sterilized with 1% sodium hypochlorite for 2 minutes and allowed to completely dry before plating on PDA medium. Using laminar flow hood to ensure a sterile environment, the fungus was placed on medium in sterile petri dishes. The plates were then placed upside down in an incubator (22-25°C). The upside down position avoids contamination

of the cultures by the water that forms on the leads. After five days, the fungus was sub-cultured from the base plate (initial culturing plate) by cutting small pieces of agar with the fungus and placing them onto a new plate. The fungus was ready for inoculation after 10 days (Fig 3.3 `b'). The fungal plates should be less than one month old from the date of plating when it's time to inoculate the plants.

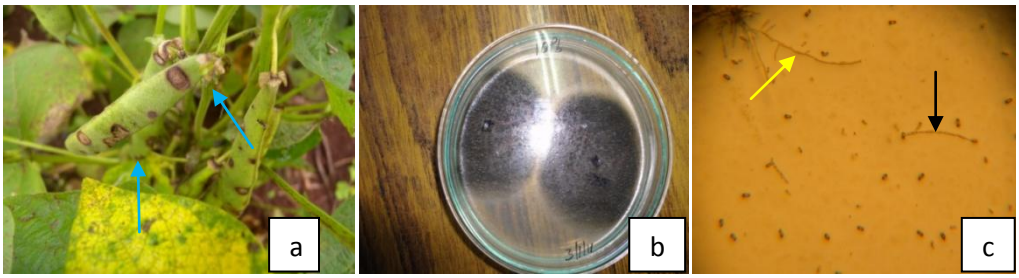


Figure 3.3 Anthracnose lesions on bean pods (a), *Colletotrichum lindemuthianum* growing on PDA media (b), single and germinating spores of *Colletotrichum lindemuthianum*(c)

3.3.4 Anthracnose differentials seed increase

Seeds of 12 international bean anthracnose differentials were obtained from Dr. Merion Liebenberg, ARC Potchestroom (South Africa), Annet Namayanja, NARO (Uganda) and from CIAT, Kawanda (Uganda) (Table 3.3, Fig 3.4). Three seeds of each differential variety were grown in plastic pots of diameter 19.5-22 cm in the green house at Kabete Field Station (Fig 3.5). The pots were filled with sterile soil mixed with sterile sand and decomposed chicken manure in 3:1:1. Diammonium phosphate (18:46:0), at a rate of 1g kg^{-1} of soil was used at planting. The plants

were top dressed with calcium ammonium nitrate (26% N) at the rate of 1g kg^{-1} of soil and NPK (17:17:17) at the rate of 1g kg^{-1} of soil. Pots were watered to field capacity at planting and subsequently as required.

Table 3.3 Anthracnose differential series, resistance genes, host gene pool, and the binary number of each cultivar used to characterize races of anthracnose in common bean

Differential Cultivar	Resistance Genes	Place of Cultivar	Gene Pool*	Binary Number*	Growth Habit*
Mitchelite	-	0	MA	1	II
MDRK	*Co – 1*	1	A	2	I
Perry Marrow	*Co – 1^3*	2	A	4	II
Cornel 49242	*Co – 2*	3	MA	8	II
Widusa	*Co – 1^5, Co – 3^3*	4	A	16	I
Kaboon	*Co – 1^2*	5	A	32	II
Mexico 222	*Co – 3*	6	MA	64	I
PI 207262	*Co – 4^3, Co – 3^3*	7	MA	128	III
TO	*Co – 4*	8	MA	256	I
TU	*Co – 5*	9	MA	512	III
AB 136	*Co – 6, Co – 8*	10	MA	1024	IV
G 2333	*Co – 4^2, Co – 5, Co-5^2, Co – 7*	11	MA	2048	IV

MA: Middle American gene pool; **A**: Andean gene pool of *Phaseolus vulgaris*. **Binary numbers**: 2^n, where n is equivalent to the place of the cultivar within the series (0-11). The sum of cultivars with susceptible reaction will give the binary number of a specific race. For example race 48 is virulent on Widusa (2^n = 24=16) and Kaboon (2^n =25=32). Growth habit: **I** = Determinate; **II** = Indeterminate bush; **III** = Indeterminate bush with weak main stem and prostrate branches; **IV** = Indeterminate climbing habit.

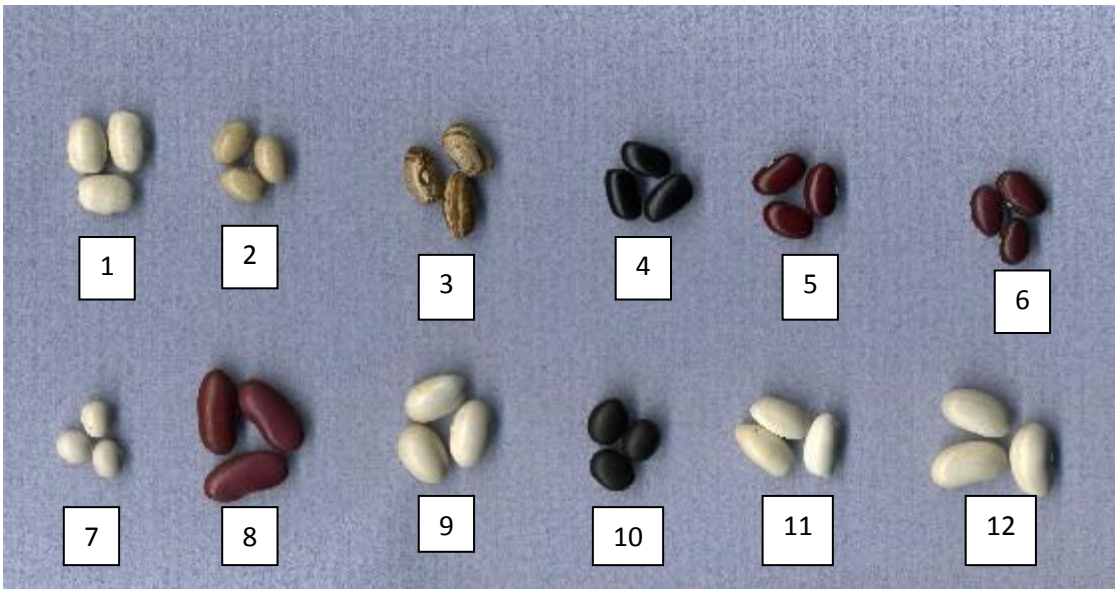


Figure 3.4 Seed size and colour of the International bean anthracnose differentials.
1=Mexico 222, 2=PI 207262, 3=TO, 4=TU, 5=AB136, 6=G2333, 7=Michelite, 8=MDRK, 9=Perry Marrow, 10=Cornell 49242, 11=Widusa, 12=Kaboon.

Figure 3.5 Anthracnose differentials seed increase in the green house

3.3.5 Multiplication of isolates and Inoculum preparation

Inoculum for isolates was increased on sterile snap bean pods to enhance sporulation. Both ends of snap bean pods were cut off, washed and autoclaved at 121°C for 30 minutes. At the same time, potato dextrose agar was separately prepared as explained in section 5.3.2. About 20ml of PDA was poured into 10cm sterile disposable plastic plates when it had cooled. Five of the autoclaved pods were then placed horizontally in each plate. Using sterile needle or scalpel, 5-6 pieces (1-2cm diameter) of agar plugs with actively growing fungus (Fig 3.3 `c') were cut and placed in the plates containing the sterile pods. The plates were then kept in darkness for 4 days, and then exposed to light for 10 more days. Five to six cultures of each isolate were raised in order to obtain enough inoculum.

3.3.6 Preparation of spore suspension for inoculation

The plate cultures were flooded with known amount of distilled water, the spores scraped off using a fine brush and the suspension poured into a beaker and mixed thoroughly. This was repeated about 3 times, each time using fresh distilled water to get most of the conidia from the culture. Conidia suspensions from all cultures of the same isolate were mixed. Using a haemocytometer, the concentration of the spore suspension was standardized by adjusting it to $1.2x10^{6}$ spores ml^{-1} (Pastor-Corrales *et al.*, 1995).

3.3.7 Inoculation

The virulence phenotype of each monosporic isolate was confirmed by inoculating isolates onto a universal set of 12 differential cultivars. Twelve, 17 and 21 days-old bean seedlings were sprayed with a concentration of 1.2×10^6 spores ml^{-1}. Whole plant inoculation was done using a hand sprayer. Leaves were sprayed on both the abaxial and adaxial surfaces with inoculum until runoff. All the differential cultivars in each set were inoculated with one isolate at a time. The plants were incubated and maintained in a mist chamber for 48 hours, at $20^{\circ}C \pm 2^{\circ}C$ and 90-100% relative humidity. Plants were scored for reaction to infection 12, 17 and 21 days after inoculation, using a CIAT 1-9 scale (Schoonhoven and Pastor-Corrales, 1987) presented in Table3.4. Races were assigned a cumulative numerical value for each susceptible differential cultivar based on an established binary system (CIAT, 1988; Pastor-Corrales, 1991).

Table 3.4 Disease scoring scale

Score	Description
1	Plants with no symptoms
3	Plants with 5-10% of the leaf area with lesions
5	Plants with 20% of with lesions and sporulation
7	Plants with up to 60% of with lesions and sporulation, associated with chlorosis and necrosis
9	90% of leaf area with lesions, frequently associated with early loss of the leaves and plant death

Genotypes with a score of 3 or less were considered resistant, 4-6, intermediate and a score greater than 6 were considered susceptible.

3.4 RESULTS

3.4.1 Prevalence of common bean diseases in Kenya

According to the disease survey of 2010 and 2011 (Fig. 2), angular leaf spot caused by *Phaeoisariopsis griseola* was the most prevalent disease in all the farms visited. Bean anthracnose caused by *Colletotrichum lindemuthianum* was second in prevalence across the regions. Common bacterial blight was third most frequent disease in the farms visited. Loitokitok and Njukini regions of the Rift valley were particularly highly infested by *Xanthomonas axonopodis* pv. *Phaseoli* (Xap). *Pythium* root rot was the least occurring with high incidences recorded in Western Kenya and parts of Taita Taveta County.

3.4.2 Anthracnose differential seed increase

Among the set of 12, G 2333 and Cornell yielded highly (Table 3.5). Other Mesoamerican varieties also yielded highly compared to their Andean varieties which did not yield as highly. When grown in the greenhouse the performance of the entire differentials set was way better than when grown in the screen house.

Table 3.5 Anthracnose differential seeds increase

Genotype	Number of seeds harvested
Michelite	1021
Cornell	1057
G2333	1523
T.U	1221
P.I	1108
AB 136	945
T.O	669
Widusa	742
Mexico 222	635
Michigan Dark Red Kidney	847
Perry Marrow	532
Kaboon	462

3.4.3 Identification of races of *Colletotrichum lindemuthianum*

Twelve race groups of *Colletotrichum lindemuthianum* were characterized from a total of 31 isolates indicating high variability of the pathogen in the bean growing areas of Kenya. These races were capable of infecting several differential common bean genotypes for anthracnose of both Andean and Mesoamerican gene pools (Table 3.6). T.U, AB 136 and G2333 were resistant to all the races identified (Table 3.6 and 3.7). Differentials PI 207262 and T.O were only susceptible to race 485. Cornell 49-242 reacted positively to 2 races. Widusa, MDRK and Perry Marrow showed positive reaction to 5 races each. MEX 222 reacted positively to 6 races. Mitchellite, which is considered the most susceptible differential cultivar with no known anthracnose resistance gene, was

susceptible to ten races. Race 1 and 2 infected one differential each while race 485 was able to infect half of the differential cultivars (this was the highest frequency observed) indicating that it was the most virulent.

Table 3.6 Reaction of differential cultivars to *C. lindemuthianum* isolates from bean growing districts of Kenya

	Differential Cultivars*												
Isolate	A	B	C	D	E	F	G	H	I	J	K	L	Race
1	S	R	R	R	R	R	S	R	R	R	R	R	65
2	S	R	R	R	S	R	S	R	R	R	R	R	81
3	S	R	R	R	S	R	R	R	R	R	R	R	17
4	R	S	S	R	R	S	R	R	R	R	R	R	38
5	S	R	S	R	R	S	S	S	S	R	R	R	485
6	S	R	R	S	S	R	S	R	R	R	R	R	89
7	S	R	R	R	R	R	S	R	R	R	R	R	65
8	S	R	R	S	R	R	S	R	R	R	R	R	73
9	S	R	R	S	R	R	S	R	R	R	R	R	73
10	S	R	R	R	R	R	S	R	R	R	R	R	65
11	S	S	S	R	S	S	R	R	R	R	R	R	55
12	R	S	S	R	R	S	R	R	R	R	R	R	38
13	S	R	R	R	R	R	S	R	R	R	R	R	65
14	S	S	S	R	S	R	S	R	R	R	R	R	87
15	S	R	R	R	R	R	S	R	R	R	R	R	65
16	S	R	R	S	S	R	S	R	R	R	R	R	89
17	S	R	R	R	S	R	S	R	R	R	R	R	81
18	S	S	S	R	S	R	R	R	R	R	R	R	23
19	S	R	R	S	R	R	S	R	R	R	R	R	73
20	S	R	R	S	R	R	S	R	R	R	R	R	73
21	S	R	R	R	R	R	S	R	R	R	R	R	65

	Differential Cultivars*												
Isolate	A	B	C	D	E	F	G	H	I	J	K	L	Race
22	S	R	R	R	R	R	R	R	R	R	R	R	1
23	S	S	S	R	S	R	S	R	R	R	R	R	87
24	S	R	R	S	S	R	S	R	R	R	R	R	89
25	S	S	S	R	S	R	R	R	R	R	R	R	23
26	R	S	S	R	R	S	R	R	R	R	R	R	38
27	S	R	R	R	R	R	S	R	R	R	R	R	65
28	S	R	R	R	R	R	S	R	R	R	R	R	65
29	S	R	R	R	S	R	R	R	R	R	R	R	17
30	R	S	R	R	R	R	R	R	R	R	R	R	2
31	S	S	S	R	S	R	S	R	R	R	R	R	87

*Binary numbers (in parenthesis)of bean differential cultivars used to characterize races of *C. lindemuthianum*: A- Michelite (1); B- Michigan Dark Red Kidney (2); C-Perry Marrow (4); D- Cornell 49-242 (8); E- Widusa (16); F- Kaboon (32); G- Mexico 222 (64); H- PI207262 (128); I- TO (256); J- TU (512); K- AB 136 (1024); L- G 2333 (2048). S = Susceptible, R = Resistant (Pastor-Corrales, 1991).

Table 3.7 Number of isolates characterized and number of differentials resistant or susceptible to the races

Race	# of isolates	Differential cultivar	# of differentials	
			Resistant	Susceptible
65	8	Michelite	2	10
73	4	MDRK	7	5
38	3	Perry-Marrow	7	5
87	3	Cornell49-242	10	2
89	3	Widusa	7	5
17	2	Kaboon	9	3
23	2	Mexico 222	6	6
81	2	PI	11	1
1	1	TO	11	1
2	1	TU	12	0
55	1	AB136	12	0
485	1	G2333	12	0
12	31			

3.4.4 Distribution of races of *Colletotrichum lindemuthianum* in Kenya

The most widely spread race that could be considered of importance in Kenyan breeding programs in future are races 65 and race 73 (each identified in 8 counties) (Table 3.8 and Fig. 3.6). Races 485, 55, 1 and 2 were the least frequent with each of them being identified in one county. However race 485 was the most virulent and could be a serious problem in future (Table 3.6).

Table 3.8 Geographic distribution of the races of *Colletotricum lindemuthianum* in Kenya

Isolate	Race	County	District	Isolate	Race	County	District
2Pa	65	Kericho	Bureti	26Pa	87	Laikipia	Narumoru
5Pb	65	Bomet	Bomet	2Pe	87	Kericho	Bureti
4Pa	65	Kericho	Kericho	10Pd	87	Siaya	Siaya
10Pa	65	Siaya	Siaya	28Pb	89	Meru	Meru
16Pb	65	Kisii	Gucha	28Pa	89	Meru	Meru
30Pc	65	Nyeri	Othaya	27Pb	89	Maragua	Murang'a
26Pb	65	Laikipia	Narumoru	30Pa	17	Nyeri	Othaya
						Taita	
31Pd	65	Murang'a	Murang'a	45Pa	17	Taveta	Wundanyi
22Pa	73	Nakuru	Njoro	25Pa	23	Kiambu	Thika
35Pa	73	Kirinyaga	Kerugoya	29Pa	23	Kiambu	Kiambu
31Pc	73	Murang'a	Murang'a	2Pb	81	Kericho	Bureti
27Pa	73	Maragua	Murang'a	5Pc	81	Bomet	Bomet
3Pa	38	Kakamega	Kakamega	10Pb	1	Siaya	Siaya
						Taita	
4Pb	38	Kericho	Kericho	45Pc	2	Taveta	Wundanyi
16Pc	38	Kisii	Gucha	44Pa	55	Kabete	Kiambu
				11Pa	485	Nakuru	Nakuru

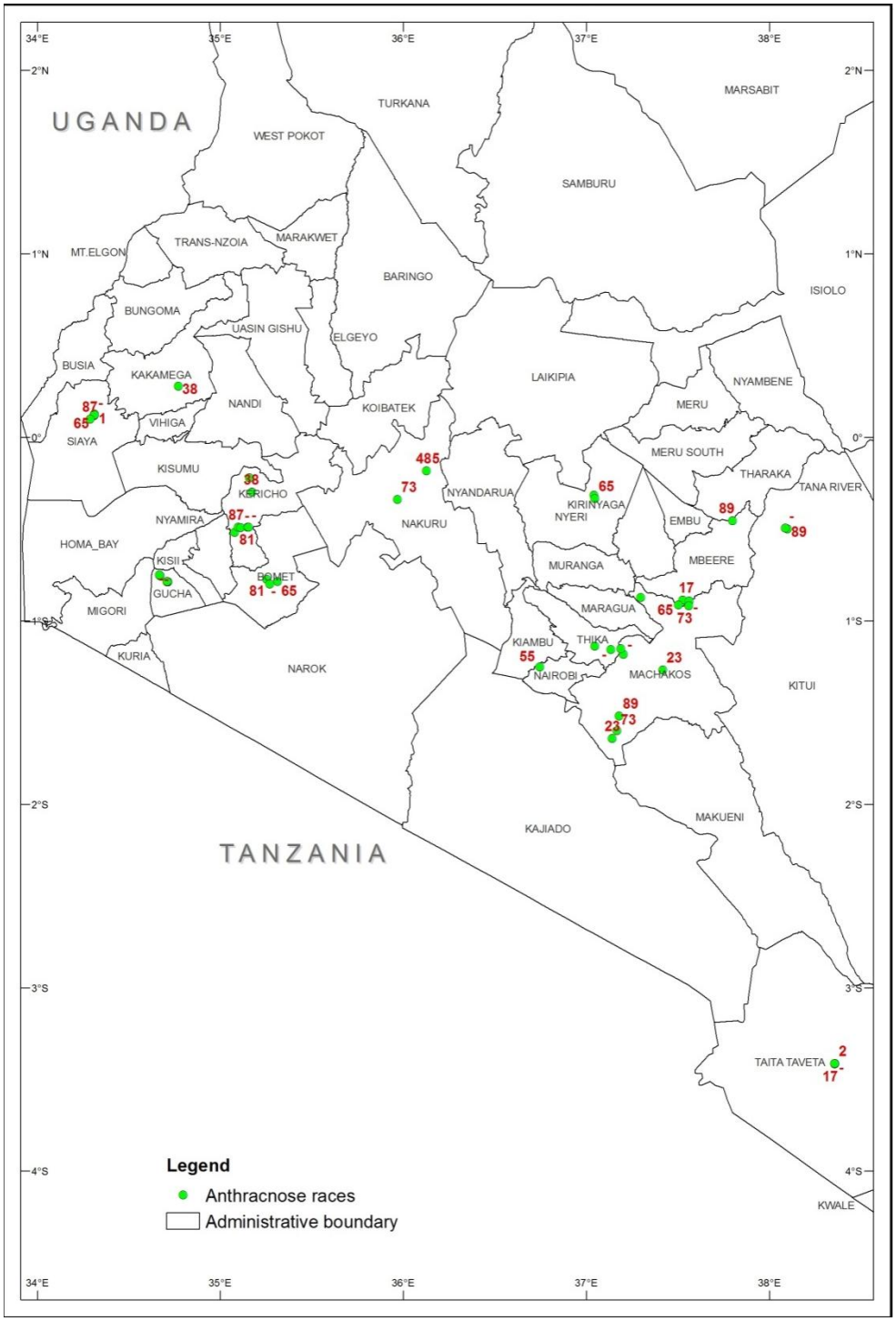


Figure 3.6 Map of Kenya showing distribution of *Colletotrichum lindemuthianum* races

Kenya has six major agro-ecological zones: Upper highland (UH) 10-15°, Lower highland (LH) 15-18°, Upper midland (UM) 18-21°, Lower midland (LM) 21-24°, Lowland (L)>24° and Coast Lowlands (CL)>24°. These zones are associated with corresponding temperature variations ranging from freezing to 40°C. Lower Highland 3 (LH3), Upper Midland 1 (UM1) and Upper Midland 2 (UM2) showed the highest pathogen diversity with four races each (Fig. 3.7). Lower Highland 1 (LH1), Lower Highland 4 (LH4) and Upper Midland 3 (UM3) were next with three races each. Upper Midland 4 (UM4) was the only agro-ecological zone with two races. Lower Highland 2 (LH2), Lower Midland 1(LM1), Lower Midland 2 (LM2), and Lower midland 4 (LM4) had one race each. Of interest to note were all the Lower Midlands which had only one race each. This shows not many races have colonized these agro-ecological zones. This uniformity is not seen in any other agro-ecological zone. The Upper Midlands together had 13 races while the lower highlands had 11 races.

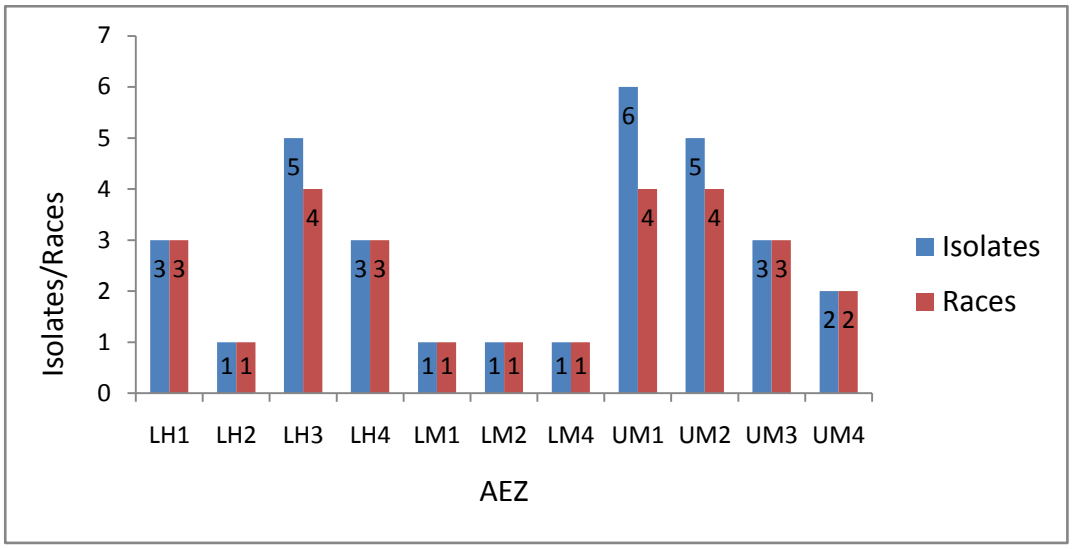


Figure 3.7 *Colletotrichum lindemuthianum* isolates and races distributed across agro-ecological zones

3.4.5 Reaction of parental lines to *Colletotrichum lindemuthianum* races

G 2333 was highly resistant to all the races with scores of 1.0 to 1.7 (Table 3.9). MEX 54 had predominantly intermediate reaction with most of the races. However it was resistant to races 17, 38, 23, 1, 38, 17 and 2. G 10909, AND 1062, RWR 719 and VAX 6 had intermediate reaction to all the races. The commercial varieties had intermediate to susceptible reaction to all the races.

Table 3.9 Reaction of parental genotypes to infection by races of *C. lindemuthianum*

		Donor Parents						Recipient Parents			
Race	Origin	MEX 54	G10909	G2333	AND 1062	RWR 719	VAX 6	Kenya Umoja	New Rosecoco	Mwezi Moja	Canadian Wonder
65	Bureti	5.9	5.2	1.4	5.8	3.9	5.7	5.3	6.2	5.3	5.7
81	Bureti	4.2	6.4	1.0	6.0	6.1	6.2	5.7	7.6	6.0	6.1
17	Othaya	2.2	3.9	1.0	5.2	3.0	3.3	4.2	4.2	4.8	4.9
38	Kakamega	5.2	4.2	1.0	5.9	3.4	4.2	5.2	6.4	5.3	5.5
485	Nakuru	7.7	7.9	1.4	7.7	6.9	7.0	8.6	8.1	8.6	7.7
89	Meru	5.9	6.1	1.1	6.4	6.5	6.4	7.4	6.7	7.6	6.4
65	Bomet	4.9	5.9	1.7	5.8	6.3	6.2	6.9	7.6	6.2	5.8
73	Njoro	6.4	6.4	1.0	6.5	6.8	5.9	6.4	6.0	6.3	6.2
73	Kerugoya	6.4	6.0	1.0	6.3	5.4	6.4	6.1	7.7	6.0	6.1
65	Kericho	5.2	5.7	1.0	6.1	7.0	5.7	5.9	6.8	5.7	6.2
55	Kabete	5.7	6.9	1.0	5.6	5.7	5.2	5.7	5.7	5.4	7.6
38	Kericho	2.8	5.9	1.0	5.3	3.3	4.8	4.8	5.2	5.3	5.4
65	Siaya	4.9	6.6	1.0	5.7	6.1	5.8	5.8	5.9	5.4	6.3
87	Narumoru	6.8	6.0	1.0	7.2	6.9	6.3	7.1	7.8	7.4	7.0
65	Gucha	4.9	5.8	1.1	5.9	5.5	6.1	7.0	6.3	5.7	7.6
89	Meru	7.7	6.7	1.0	7.5	6.8	6.0	8.1	7.7	6.8	7.4
81	Bomet	7.2	6.8	1.0	6.1	6.4	6.7	6.4	6.2	7.2	7.0
23	Thika	2.7	3.4	1.2	5.2	3.2	4.9	4.2	5.5	6.0	6.3
73	Murang'a	6.4	7.4	1.0	6.4	6.6	6.1	6.6	5.9	6.9	6.0
73	Maragua	6.4	7.0	1.0	6.0	6.8	5.7	6.5	5.7	6.1	6.5
65	Othaya	5.6	6.5	1.0	6.1	5.7	6.0	6.0	6.1	6.0	6.8
1	Siaya	2.2	3.3	1.0	5.4	3.1	3.0	4.1	4.2	4.9	5.2
87	Bureti	6.7	7.6	1.0	7.4	6.7	6.7	7.6	6.7	6.4	6.7
89	Maragua	6.1	6.8	1.0	6.8	5.9	5.9	7.6	6.0	7.6	6.7
23	Kiambu	2.4	3.4	1.1	5.3	4.8	3.9	4.8	4.8	5.3	5.4
38	Gucha	2.3	3.6	1.0	5.2	4.9	5.3	5.4	4.9	5.5	5.7
65	Narumoru	5.4	6.7	1.0	5.5	5.4	5.7	5.3	6.8	6.6	5.9
65	Murang'a	4.9	6.9	1.3	5.4	5.5	6.5	5.2	5.4	6.5	5.8
17	Wundanyi	1.9	3.2	1.0	6.0	3.2	3.4	4.9	5.3	4.1	4.8
2	Wundanyi	1.3	3.0	1.0	5.2	3.1	3.1	4.2	4.1	4.8	4.2
87	Siaya	6.6	6.1	1.0	7.5	6.7	7.0	7.7	6.5	7.6	7.1

3.5 DISCUSSION

The first set of seeds was received from CIAT Kawanda (Uganda). Conspicuously missing among the differential seeds was Kaboon. Nevertheless these seeds were planted immediately for seed multiplication. A second supply of the seeds (full set of 12 differentials) were received from Dr. Merion Liebenberg and planted immediately. But no Kaboon survived. Ms. Annet Namayanja provided a third full set of the 12 differentials. At the third attempt Kaboon survived but only after being pre-germinated. This account partly explains why Kaboon trailed behind the other differentials in the number of seeds attained. When grown in the greenhouse the performance of the entire differentials set was way better than when grown in the screen house. The high temperatures in the greenhouse facilitate accelerated and vigorous growth. The green house is a controlled environment. Plants do not have a lot of constraints. Green house plants were planted in pots therefore plant to plant competition for water and nutrients was minimized. Water run-off and underground seepage of nutrients was also minimal.

Accessions AB 136 and G 2333 have also been reported resistant to almost all the European and American isolates (Schwartz *et al*., 1982; Balardin *et al*., 1990; Kelly *et al*., 1994; Pastor-Corrales *et al*., 1994). The differentials AB 136 and G2333 that showed the highest resistance have been used in many breeding

programs around the world as sources of resistance. G2333 has three pyramided genes*Co – 4*^{2}*, Co – 5, Co-5*^{2}*,* AB 136 has two resistance genes *Co – 6, Co – 8* and Tu has the resistance gene *Co – 5* (Table 3.3). Pastor-Corrales *et al.* (1994, 1995) also reported breakdown of resistance in MEX 222 to Middle Latin and American races of *C. lindemuthianum*. Breakdown of resistance of cultivar Cornell 49-242 (*Are* gene) has also been reported by Fouilloux, (1976), Kelly *et al*. (1994) and TU (1994). The Andean differentials were found to be more susceptible compared to their Mesoamerican differentials. This is because Andean differential cultivars possess only one locus (*Co-1*) that confers resistance to bean anthracnose (Melotto and Kelly, 2000). Each differential cultivar carries different allele of *Co-1* locus. Eleven independent anthracnose resistance genes (*Co*-genes) have been described in common bean; 10 genes from Mesoamerican germplasm and one from Andean germplasm (Young and Kelly 1996; Alzate-Marin *et al.* 1997, 2003, 2007; Melotto and Kelly 2000; Kelly and Vallejo 2004; Gonçalves-Vidigal and Kelly 2006; Gonçalves-Vidigal *et al.* 2007).

Agro-ecological zones Lower Highland, Upper Midland and Lower Midland are in zone III which occurs at elevations between 900-1800m with annual rainfall of 950 and 1500mm. These can be termed as the common bean zone as beans grow at altitudes between 600-1950m with a rainfall of 300-400mm per crop cycle. Common beans grow within a range of temperatures of 17.5-27°C. Lower

Highland and Upper Midland agro-ecological zones have cooler temperatures of 15-21°. This explains the high pathogen diversity observed in these areas as the cooler temperatures and high humidity are conducive for the C*olletotrichum lindemuthianum* pathogen. The Lower Midlands had fewer races of *Colletotrichum lindemuthianum* because its temperature of 21-24° is high for common beans which require 17.5-27°. The anthracnose pathogen also cannot thrive under such drier and hot conditions. Beans (*Phaseolus vulgaris*) growing regions in Kenya are Eastern, Western, Nyanza, Rift valley and Central.

G2333, was highly resistant to all the isolates. This could be attributed to the presence of three pyramided genes *Co-4*^{2}, *Co-5* and *Co-7*. The other five donor parents, not specific for anthracnose resistance and the recipient parents showed high disease incidences. This was according to expectation as they do not have any of the anthracnose resistance genes (*Co-1 to Co-10*). Kenyan commercial varieties showed intermediate reaction to races identified in this study (Table 3.9).

3.6 CONCLUSION

This study revealed high variability among the Kenyan population of *Colletotrichum lindemuthianum*. Continuous characterisation is vital as importance of different races change with time. Currently races 65 and 73 are wide spread and of importance in breeding programs. Race 485 was the most virulent and should be researched more to avoid epidemics in future (Table 3.7).

CHAPTER 4 : EVALUATION OF COMMON BEAN GENOTYPES FOR MOLECULAR MARKER AND DISEASE POLYMORPHISM

4.1 Abstract

Hybridization in a breeding program is premised on accurate identification of parental lines with contrasting but complimentary characters desired in subsequent populations and varieties. Choice of parents requires long duration of reliable and thorough evaluation of parental sources nurseries against stress factors for adaptation and other desired traits. DNA markers can not only enhance the reliability and precision of evaluations but also can shorten the duration of field and greenhouse evaluations. Useful markers discriminate one donor parent from another and the donor parents from the commercial varieties. The objective of this study was to i) determine the potential of available markers for the identification of parental genotypes with different genes for resistance to bean diseases and for subsequent use in a marker assisted gamete selection breeding program and ii) to evaluate parental lines for disease polymorphism using markers and phenotyping. Ten parental lines (six donor and four recipient genotypes) were screened for polymorphism with seven sequence characterized amplified region (SCAR) markers linked to genes for resistance to angular leaf spot, anthracnose, root rot, and common bacterial

blight. The donor parental lines Mexico 54 and G10909 for angular leaf spot, G2333 for anthracnose, AND 1062 and RWR 719 for *Pythium* root rots and VAX 6 for resistance to common bacterial blight were tested for markers linked to resistance genes. Mexico 54 and G10909 was tested for $OPE04_{709}$ (*Phg*-1 gene), SH-13 (*Phg*-1 gene) and $SNO2_{890}$; G 2333 was used to validate SAB-3 (*Co-5* gene), SAS-13 ($Co\text{-}4^2$ gene) and SBB-14 ($Co\text{-}4^2$ gene) linked to resistance genes. SAP6 and SU91 were used to validate VAX 6 plants, while AND1062 and RWR 719 were tested for PYAA. Four commercial varieties with no known resistance genes were tested for polymorphism with the seven markers. Three markers (SAB-3, SH-13 and SU-91) showed polymorphism among the plants and were effective selection tools. However, $PYAA_{19}$ the marker for root rot had no amplification during PCR reaction and was ineffective in selecting for gene(s) conferring resistance to *Pythium* root rot. SH-13 was selected for the angular leaf spot parents, SAB-3 was selected for G2333, SU-91 for VAX6 and $PYAA_{19}$ was the marker used for *Pythium* root rot parents. All the parental genotypes were inoculated with isolates of angular leaf spot, anthracnose, *Pythium* root rot and common bacterial blight for comparison. G10909 and MEX54 showed a resistant reaction to infection by *Phaeoisariopsis griseola.* G2333 was resistant to all the isolates tested. AND1062 and RWR719 showed superior resistance to *Pythium* root rot. Disease phenotyping of VAX 6 confirmed marker data that indeed it is

resistant to common bacterial blight. The outstanding commercial varieties were Kenya Umoja which was resistant to angular leaf spot, common bacterial blight, and GLP 24 which was resistant to angular leaf spot and anthracnose. GLP 1004 was found to be resistant to common bacterial blight. The disease reactions confirmed the resistance of the six donor parents and the susceptibility of the four commercial varieties.

4.2 Introduction

Parental selection for each trait to be combined is based on thorough evaluations and reliable data obtained from contrasting environments (Singh, 1994). Parental lines for breeding programs are selected from across races (Singh *et al.*, 1991) and gene pools (Singh, 1988, 1989) with variation in maturity, plant type, and seed characteristics. In addition, parents should show some affinity for these traits with cultivars being improved. It is therefore useful to know a priori the combining ability of donor parents with commercial cultivars to be improved for seed yield and other traits during the designing of crosses (Singh, 1994). The 7 markers used in this study are sequence characterized amplified region (SCAR) markers. SCAR markers were used for selection purposes because they have proven to be reliable, reproducible, robust, and easily scored when a single polymorphic band is generated (Gu *et al*., 1995, Melotto *et al*., 1996). Generally, markers should be validated by testing their effectiveness in determining the

target phenotype in independent populations and different genetic backgrounds, which is referred to as 'marker validation' (Cakir *et al.*, 2003; Collins *et al.*, 2003; Jung *et al.*,1999; Langridge *et al.*, 2001; Li *et al.*, 2001; Sharp *et al.*, 2001). In other words, marker validation involves testing the reliability of markers to predict phenotype. This indicates whether or not a marker could be used in routine screening for marker assisted selection (Ogbonnaya *et al.*, 2001; Sharp *et al.*, 2001). Markers should also be validated by testing for the presence of the marker on a range of cultivars and other important genotypes (Sharp *et al.*, 2001; Spielmeyer *et al.*, 2003). For markers to be most useful in breeding programs, they should reveal polymorphism in different populations derived from a wide range of different parental genotypes (Langridge *et al.*, 2001). Pastor-Corrales *et al.* (1994) showed that only the G 2333 line was resistant to 380 isolates of *C. lindemuthianum*. This line was resistant to all the Brazilian isolates and all the European and North American races (Pastor-Corrales and Tu, 1989; Balardin and Pastor-Corrales, 1990; Balardin *et al.*, 1990). G2333 has the broadest known resistance and carries three complementary genes that confer resistance against more than 90% of known *Colletotrichum lindemuthianum* races (Young *et al*., 1997, Mahuku *et al.,* 2002). Previous studies conducted by CIAT (International Centre for Tropical Agriculture) demonstrated that cultivar Mexico 54 is resistant to most African ALS isolates so far characterized (CIAT,

1996; Anonymous, 1996a; 1997). Out of the 163 isolates in Africa, it was resistant to 158. The co-evolution of the ALS pathogen with bean gene pools has been sufficiently documented (Guzman *et al*. 1995; Mahuku *et al*. 2002; Pastor-Corrales and Jara, 1995). G10909 was identified as having high levels of resistance to *Phaeoisariopsis griseola* under field conditions (Pastor-Corrales *et al*., 1998) and under greenhouse conditions using *P. griseola* pathotypes of diverse origin (Mahuku *et al*., 2003). Due to the co-evolution of *Phaeoisariopsis griseola* with the large and small seeded bean gene pools, combining Andean and Mesoamerican resistance genes into the same background (gene stacking) is a strategy most likely to provide lasting resistance to ALS disease. This is because it makes it statistically very difficult for a pathogen to evolve that would overcome all the resistance genes simultaneously, thus the use of MEX 54 (Mesoamerican) and G 10909 (Andean). Otsyula *et al.* (1998) found RWR 719, a late maturing introduction from Rwanda to be preferred by farmers in western Kenya. AND 1062 and RWR 719 are known to possess resistance to *Pythium* which is controlled by a single dominant gene (Otsyula *et al*., 2003; Nzungize *et al*., 2011).

FTA® is an acronym for fast technology for analysis of nucleic acids. It was originally developed by Burgoyne and Fowler at Flinders University in Australia in the 1980s as a means of protecting nucleic acid samples from degradation by

nucleases and other processes. Whatman® licenses the FTA® technology from Flinders University. They offer a line of products using this technology, most notably filter paper cards. The filter paper cards also known as FTA® cards contain chemicals that lyse cells, denature proteins and protect nucleic acids from nucleases, oxidation, and UV damage. Because nucleases are inactivated, the DNA is essentially stable when the sample is properly dried and stored. FTA® Cards are stored at room temperature before and after sample application.The aim of this study was to i) determine the potential of available markers for the identification of parental genotypes with different genes for resistance to bean diseases and for subsequent use in a marker assisted gamete selection breeding program and ii) to evaluate parental lines for disease polymorphism using markers and phenotyping.

4.3 Materials and Methods

4.3.1 Plant Materials

Some characteristics of 10 parental lines used in this study are shown in Table 4.1. The genotypes include resistance sources and recipient commercial varieties. All the local susceptible cultivars are of Andean origin while the resistant sources are of Meso-American origin (save for AND 1062) with different growth habits and seed colour.

Table 4.1 Characteristics of bean parental lines used in this study

Parents	Days to germination	Days to flowering	Growth habit	Flower colour	Seed colour	Gene pool	Days to maturity	Susceptibility	Resistance
MEX 54	7	43	IV	Purple	Cream beige	MA	82	CBB, BCMV	ALS
G10909	7	43	IV	White	Small red	MA	78	CBB, BCMV	ALS
G2333	6	47	IV	White	Small red	MA	85	CBB,BCMV	ANTH
RWR 719	8	52	II	White	Small red	MA	92	ALS,ANTH, BCMV	Root rot
AND 1062	7	34	I	White	Red kidney	A	69	CBB,BCMV	Root rot
VAX 6	6	40	I	White	Small red	MA	80	ANTH,ALS	CBB
Kenya Umoja	7	38	IIa	White	Red mottled	A	80-85	ANTH	ALS, CBB
New Rosecoco	7	40	II	White	Red mottled	A	80-85	ANTH	CBB
GLP 1004	7	41	I	White	Purple speckled	A	85	Halo blight	Tolerant to drought and bean fly
GLP 24	7	38	II	White	Red kidney	A	95	BCMV, Rust	ALS, ANTH

Ma= Mesoamerican A=Andean ALS=angular leaf spot ANTH=Anthracnose BCMV=bean common mosaic CBB=common bacterial blight

Source: Singh *et al*.,1992, Otsyula *et al*., 1998, Mukalazi *et al*.,2001 and Singh *et al*., 2001 and from this study.

4.3.2 Validation for marker polymorphism

4.3.2.1 DNA Collection

Two seeds per genotype were planted in plastic pots of diameter 19.5-22 cm in the green house at Kabete Field Station. The pots were filled with sterile soil mixed with sterile sand and decomposed chicken manure in 3:1:1. At planting diammonium phosphate (18:46:0), at a rate of 1g kg^{-1} of soil was used. Pots were watered to field capacity and subsequently as required. At the first trifoliate stage, for every genotype or plant that was to be sampled, a leaf was collected and crushed (using a pestle) on to the FTA plant saver card (while placing cutting mat underneath the card and covering the leaf with the card's flap) until the plant's chlorophyll sips onto the opposite side of the card (Figure 4.1 `a').

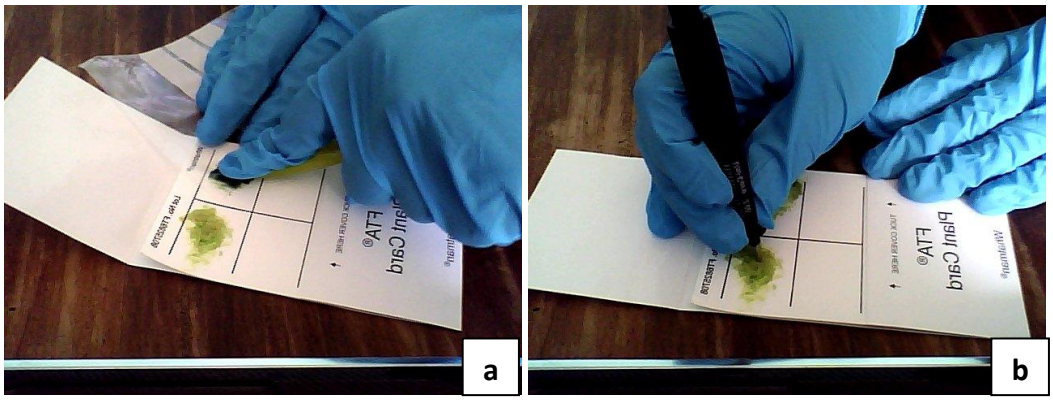


Figure 4.1 Use of FTA Card for DNA extraction

Cell membranes and organelles were lysed and the released nucleic acids were entrapped in the fibers of the matrix. The nucleic acids remain immobilized and were preserved for transport, immediate processing or long-term room

temperature storage. Two-mm diameter punches were then removed from FTA card (Figure 4.1 `b') for subsequent DNA extraction and molecular analysis. Captured nucleic acids were ready for purification and amplification. Each spot was labeled with the name or the initials of the plant it was collected from. This was repeated for all the materials that are to be collected and allowed to dry. Care was taken to avoid contamination during sampling.

4.3.2.2 DNA Extraction

Using a 1.2mm or 2.0mm diameter Harris unicore punch or other paper punch a sample disc was taken from the dried spot (Figure 4.1 `b') and put in a PCR amplification tube. 100µl of FTA purification reagent was then added to the PCR tube. The punch was rinsed in ethanol between transfers to prevent cross contamination. To disrupt the debris and aid in washing off the cards the tubes were shaken for 5 minutes at room temperature. Using a pipette all used FTA purification reagent was removed and discarded. A total of 2 washes with FTA purification reagent were done. 100µl of 1X TE Buffer (10mM Tris-HCl**,** 0.1mM EDTA, pH 8.0) (Appendices 1-3) was added to each tube inverted and left for 5 minutes at room temperature. All used TE^{-1}buffer was removed with a pipette and discarded. A total of 2 washes with TE^{-1}buffer were done. All the liquid was removed before performing analysis. The disk was allowed to air dry.

4.3.2.3 PCR

Bioneer Accupower PCR premix kit (Bioneer, Corp, Korea, www.Bioneer.com) was used. Only primer and water were added to the leaf disc prepared above. All primers were diluted using de-ionised water to a working concentration of 10µM. To the PCR premix tube, 1µl of the forward primer and 1 µl of the reverse primer was added and 18 µl of water. The blue solution was then transferred to PCR amplification tube containing the disc. The disc is considered to be 0µl. This makes a volume of 20µl per reaction. The disc is now ready for amplification. Amplifications were performed in Applied Biosystems 2720 Thermal Cycler. The amplifications conditions used are shown below;

Table 4.2 Molecular markers for detection of resistance genes and their amplification conditions

Marker	Fragment size (bp)	Primer sequence	Thermocycler conditions
SU-91	820	F 5'-CCA CAT CGG TTA ACA TGA GT-3' R 5'-CCA CAT CGG TGT CAA CGT GA-3'	34 cycles of 10s at 94 ^{o}C, 40s at 58 ^{o}C, and 120s at 72 ^{o}C; followed by one cycle of 5 minutes at 72 ^{o}C
SAB-3 *co-5*	400	F 5'-TGG CGC ACA CAT AAG TTC TCA CGG-3' R 5'-TGG CGC ACA CCA TCA AAA AAG GTT-3'	1 cycle of 3 mins at 94 ^{o}C; 30 cycles of 10s at 94 ^{o}C, 30s at 65 ^{o}C, 2 minutes at 72 ^{o}C; followed by 1 cycle of 5 mins at 72 ^{o}C
SH-13 *Phg-1*	520	F 5'-GAC GCC ACA CCC ATT ATG TT-3' R 5'-GCC ACA CAG ATG GAG CTT TA-3'	35 cycles of 30s at 94 ^{o}C, 60s at 59 ^{o}C, and 90s at 72 ^{o}C

4.3.2.4 Gel electrophoresis

An0.8% (w/v) Agarose gel was prepared by adding 1.2g of agarose to 100ml of 1X TBE (Appendix 4). The mixture was then heated in the microwave for 2 min. to boiling to allow the agarose to dissolve. As the agarose cooled, the casting assembly (2325 Galileo Unit, Galileo Bioscience) was set up by tapping the exposed edges. 0.5µl/µl of Ethidium bromide was added to the cooled agarose. Gently the agarose was poured and the combs inserted. This was left for 30 minutes at room temperature to allow the agarose to solidify. Once ready the combs were removed and the horizontal electrophoresis unit filled with 500ml of 1X TBE (Appendix 4). The masking tape was then removed from the edges of the casting unit and gently put into the electrophoresis unit. Caution was taken to place the tray in the correct orientation as DNA moves toward the positive electrode (red). The first well was filled with 5µl of the ladder and the rest filled with the samples (PCR products). The lid was then replaced onto the electrophoresis unit and connected to the power pack. The gel was allowed to run for 90 minutes at a voltage of 100V. The gel was then placed in the UV illuminator for viewing and capturing the images (Coolpix P6000, Nikon Corp., Japan).

4.3.3 Phenotypic validation of parental genotypes for disease polymorphism

Six donor parents (MEX 54, G 10909, G 2333, AND 1062, RWR 719 and VAX 6) and four recipient parents (Kenya Umoja, New Rosecoco, GLP 1004 and GLP 24) were planted in plastic pots of diameter 19.5-22 cm in a greenhouse at Kabete Field Station. Potting media was soil mixed with sand and chicken manure in the ratio of 3:1:1 respectively. Diammonium phosphate (18:46:0), at a rate of 1g kg^{-1} of soil was used at planting. Calcium ammonium nitrate (26% N) at the rate of 1g kg^{-1} of soil was used for top dressing at flowering stage. Irrigation and weeding was done as necessary. Naturally infected diseased materials of common bean were collected in bean fields at Kabete Field Station. Disease causal agents were isolated from these materials.

4.3.3.1 Angular Leaf Spot

Angular leaf spot was isolated from diseased leaves where symptoms were most recognizable. Lesions on leaves appeared as brown spots with a tan or silvery centre that were initially confined to tissue between major veins, giving it an angular appearance. A close look at lesions on the underside of a leaflet with a magnifying lens revealed tiny dark tufts (synnemata) protruding from lesions. In the laboratory using a dissecting microscope spores were transferred from the lesions to V-8 agar. Using a sterile needle a small piece of agar was used to touch a spore and transfer onto V-8 agar. These plates were incubated for 24h at 24 °C.

Germinating spores were observed with a stereo microscope and transferred onto V-8 agar after 24 h. To make inoculum, sporulating cultures were scraped using a sterile blade and the number of spores determined using a haemocytometer. Spore concentration was adjusted to 10^4/ml using distilled water. Using a hand sprayer, inoculum was sprayed onto both surfaces of leaves at 14 days. The plants were maintained in a moist chamber 100% RH for four days. The chamber was then removed and disease reactions are rated after 7 days as recommended (CIAT, 1987).

4.3.3.2 Anthracnose

Pods with very sunken, circular, chocolate brown to black lesions with a raised dark margin surrounded by a thin zone of reddish tissue were collected for disease isolation. The procedure followed for media preparation, isolation, inoculums preparation and inoculation was outlined in Chapter 3 (sections 3.3.2 to 3.3.3 and sections 3.3.5 to 3.3.7).

4.3.3.3 Common bacterial blight

Common bacterial blight infested seeds were incubated in sterile phosphate buffered saline (PBS), pH 7.4 for 6 h at 28°C. Serially diluted homogenate was streaked onto both the semi selective medium (MXP) for *Xanthomonas campestris* pv *phaseoli* and yeast dextrose calcium carbonate agar (YDCA) media. MXP was prepared following the procedure described by Claflin *et al*. (1987).

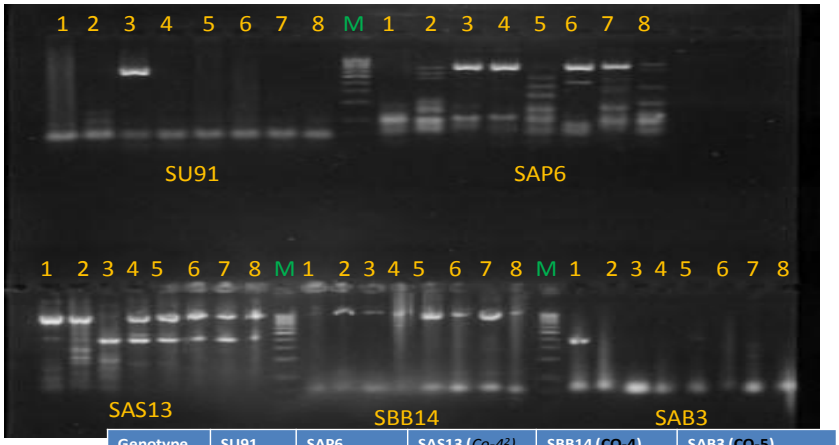
YDCA was prepared as described by Vidaver (1967). The plates were incubated for 5 days at 28°C. Colonies that hydrolyse starch on MXP were assumed to be *Xanthomonas campestris* pv *phaseoli* and were transferred to YDCA. Yellow mucoid colonies which had a translucent area surrounding them were transferred onto YDCA and incubated at 28°C. Leaves of 18-day old seedlings were injured and sprayed with *Xanthomonas campestris* pv *phaseoli* inoculum at a concentration of about 5x107 cfu/ml. Dilution was done using phosphate buffer (0.01M; pH 7.2). CIAT standard scale (CIAT, 1987) was used for evaluation.

4.4 RESULTS

4.4.1 Marker polymorphism in parental lines

For angular leaf spot, $OPE0_4$ was dropped as it could not discriminate between the parents (Table 4.3). SNO_2 was also a poor marker as it was only absent in G 2333 but present in the rest. For the purpose of this work, SH-13 was selected as the marker for *phg-1* because it was present in both angular leaf spot donor parents MEX 54 and G 10909 and absent in all the recipients except GLP1004. SAB-3 was selected as the marker for *co-5* gene for Anthracnose resistance because it was only present in G 2333 and G 10909 but absent in all the other genotypes. SAS-13 and SBB-14 were dropped as they did not discriminate between and among the donor and recipient parents. SU-91 was the best marker among all the markers tested. It was present in VAX 6, the common bacterial

blight source, and absent in all the other parents. SAP-6 was dropped because it was present in most of the parents.PYAA19 the marker for root rot does not appear in Table 4.3 as the band obtained was not consistent with expected size (800 bp).

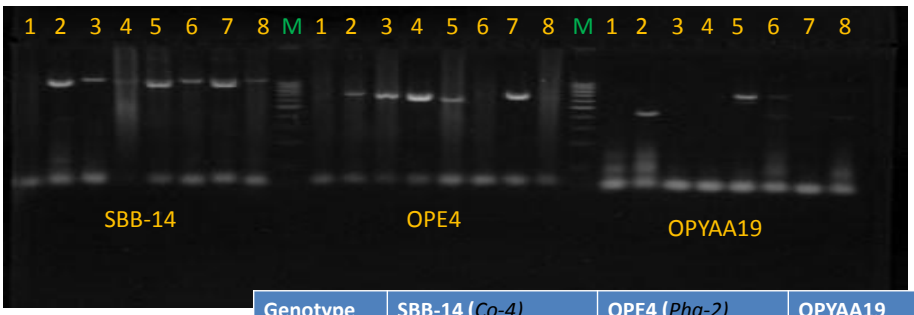


1. G2333
2. BRB191
3. VAX6
4. MEX54
5. G10909
6. AND1062
7. RWR719
8. KATB1

M. 100BP ladder

Genotype	SU91 700bp	SAP6 820bp	SAS13 (*Co-4²*) 950bp	SBB14 (CO-4) 1050/1150bp	SAB3 (CO-5) 400bp
G2333	-	-	+	+	+
BRB191	-	-	+	+	-
VAX6	+	+	-	+	-
MEX54	-	+	+	+	-
G10909	-	-	+	+	-
AND1062	-	+	+	+	-
RWR719	-	+	+	+	-
KATB1	-		+	+	-

Figure 4.2 PCR amplifications and gel scoring for resistance markers in donor parents



KEY

1. G2333
2. BRB191
3. VAX6
4. MEX54
5. G10909
6. AND1062
7. RWR719
8. KATB1

M. 100BP ladder

Genotype	SBB-14 (*Co-4*) 1050/1150bp	OPE4 (*Phg-2*) 700bp	OPYAA19 800bp
G2333	+	-	-
BRB191	+	+	-
VAX6	+	+	-
MEX54	+	+	-
G10909	+	+	+
AND1062	+	-	+
RWR719	+	+	-
KATB1	+	+	-

Figure 4.3 PCR amplifications and gel scoring for resistance markers in donor parents

4.4.2 Disease phenotyping to validate resistance

All 10 parental lines were inoculated with isolates of *Phaeoisariopsis griseola*, *Colletotrichum lindemuthianum*, *Xanthomonas campestris* pv. *phaseoli* and *Pythium* spp. from Kabete. Reactions of the parental lines to infection are presented in Table 4.4. G 10909 demonstrated high level of resistance to angular leaf spot and anthracnose (score of 1) and intermediate resistance to *Pythium* root rot. However, it was susceptible to common bacterial blight. MEX 54 and G 2333 both showed high level of resistance to angular leaf spot and anthracnose, and intermediate reaction to both common bacterial blight and *Pythium* root rot.

RWR 719 was resistant to anthracnose and *Pythium* root rot, but had intermediate reaction to angular leaf spot and common bacterial blight. AND 1062 was resistant to *Pythium* root rot, showed intermediate reaction to angular leaf spot and common bacterial blight but susceptible to anthracnose. VAX 6 was resistant to common bacterial blight and intermediate for the other diseases. Kenya Umoja was resistant to common bacterial blight and angular leaf spot, but susceptible to anthracnose and *Pythium* root rot. New Rosecoco was susceptible to anthracnose but intermediate for the other diseases. GLP 24 was resistant to angular leaf spot and anthracnose but showed intermediate disease reaction to common bacterial blight and *Pythium* root rot. GLP 1004 was resistant to common bacterial blight and showed intermediate disease reaction to the other three diseases.

Table 4.3 Reaction of 10 parental lines to inoculation with disease pathogens

Genotype	Angular leaf spot	Anthracnose	Common bacterial blight	*Pythium* root rot
G10909	1.0	1.0	7.4	5.2
MEX54	1.0	1.0	5.7	4.7
G2333	1.0	1.0	5.0	6.6
RWR719	5.5	1.0	4.0	1.0
AND1062	6.6	8.1	4.0	1.0
VAX6	6.6	3.6	1.4	3.4
Kenya Umoja	1.0	7.1	2.3	7.4
New Rosecoco	5.8	9.0	3.4	6.6
GLP24	1.0	2.5	5.9	5.7
GLP1004	5.2	4.7	2.8	5.0

4.5 DISCUSSION

The disease reactions corresponded with the marker-selection data. The genotypes that were found to have the markers for the resistance genes also showed low disease reactions with the pathogens. G10909 and MEX54 showed a resistant reaction to infection by *Phaeoisariopsis griseola.* This indicated high level of resistance to angular leaf spot (Table 4.4). G2333 which was the proposed donor parent for anthracnose based on its pyramided three genes and resistance that have not been so far broken by many African races proved ideal for our breeding program as it was highly resistant. AND1062 and RWR719 showed superior resistance to *Pythium* root rot. Disease phenotyping of VAX 6 confirmed marker data that indeed it is resistant to common bacterial blight. The outstanding commercial varieties were Kenya Umoja which was resistant to angular leaf spot common bacterial blight, and GLP 24 which had resistant reaction to angular leaf spot and anthracnose. GLP 1004 was found to be resistant to common bacterial blight.

4.6 CONCLUSION

Both approaches use of markers and disease phenotyping are important and strengthen each other in a breeding program. The choice of which approach to follow depends on costs, availability of markers and/or disease differential, the skills for marker use or for handling the pathogen and number of traits being introgressed and the ease of tracking them.

CHAPTER 5 : EARLY GENERATION MARKER ASSISTED GAMETE SELECTION FOR MULTIPLE DISEASE RESISTANCE IN ANDEAN BEAN GENOTYPES

5.1 Abstract

Although gamete selection has been used in breeding common bean for multiple constraint resistance, identification of multi-parent male gamete with desired alleles during population development and subsequent selection has relied on conventional field, greenhouse and laboratory phenotyping for biotic stress factors. Use of DNA markers to improve precision and enhance effectiveness and efficiency of this new procedure in breeding bean for multiple constraint resistance has not received much attention. The objectives of this study were to determine the potential of using DNA markers to pyramid genes for resistance to angular leaf spot, anthracnose, common bacterial blight and *Pythium* root rot into susceptible commercial varieties, and ii) to evaluate and select for multiple disease resistance and agronomic traits in the segregating F_1 populations and F_2 families. Four multi-parent male gametes with genes for resistance to angular leaf spot, anthracnose, common bacterial blight and *Pythium* root rot were crossed to four susceptible commercial varieties creating 16 F_1 segregating populations at Kabete Field Station. Four markers, SAB-3 for anthracnose, SH-13

for angular leaf spot, SU-91 for common bacterial blight and PYAA-19 for root rots, were used to screen 89 plants of one male gamete (MEX54/G2333//RWR719/VAX6) for desired alleles before crossing with the final female parent. The 16 F_1 populations were subsequently evaluated at Kabete Field Station and KARI-Tigoni during the 2012 long rain season. Selected $F_{1.2}$ plants were further evaluated at Kabete Field Station during the 2012/2013 short rain season. Results showed that 12 of 89 plants of one double-cross male gamete had two or more genes for resistance to the four diseases. No positive amplification was observed with the PYAA-19 marker for *Pythium* root rot. All the F_1s showed a resistance of 2.2 against *Pythium* root rot. All the populations showed intermediate resistance to anthracnose at 5.5. Resistance to angular leaf spot was scored at 5.0 while resistance to common bacterial blight was scored at 4.9. Among the four commercial varieties used for introgression, New Rosecoco F_1s showed intermediate resistance to the four diseases of interest in this study**.** F_1s of G10909/G2333//RWR719/VAX6 with yields of 4095 kg ha^{-1} were the highest. In the case of commercial varieties, F_1s of New Rosecoco yielded highest with 4194.5 kg ha^{-1}. In $F_{1.2}$ plants of MEX54/G2333//RWR719/VAX6 gave the highest yields of 3202 kg ha^{-1}. Kenya Umoja $F_{1.2}$s among the commercial varieties yielded highly with yields of 3320 kg ha^{-1}. The population that produced lines with combined resistance to diseases and high yield potential was

MEX54/G2333//RWR719/VAX6///Kenya Umoja. Markers were effective in identifying resistant segregants in early generation. Selection under disease pressure is a vital as a proof of concept even if the presence of resistance genes ha**s** been confirmed by markers.

5.2 Introduction

Bean improvement in eastern Africa has been achieved mainly by conventional breeding methods. However, these methods are time-consuming, labor-intensive and inefficient for pyramiding resistance genes due to the need for multiple inoculations. Marker-assisted breeding offers different strategies to overcome these difficulties. Marker-assisted selection (MAS) can provide an effective and efficient breeding tool for detecting, tracking, retaining, combining, and pyramiding several disease resistance genes into a single host genotype. MAS offers significant advantages in cases where phenotypic screening is particularly expensive or difficult, including breeding projects involving multiple genes, recessive genes, or late expression of the trait of interest. When the main objective of plant breeding, as for the case in this study, is the introgression of one or more favourable alleles/genes from a donor parent into an elite variety, MAS can be very useful. For common bean, PCR-based RAPD and SCAR markers linked with more than 20 disease resistance genes have been obtained to date. Gamete selection based on F_1-derived families that come from crosses that are

multiple-parent, heterogeneous, and heterogametic, has been used effectively in breeding common bean for multiple constraint resistance (Singh, 1994). Molecular markers may facilitate gamete selection in the identification of early-generation populations that continue to possess the desired alleles (Singh *et al*., 1998). However, the use of molecular markers to improve efficiency of gamete selection has not received much attention. The objectives of this study were to: i) pyramid genes for resistance to angular leaf spot, anthracnose, common bacterial blight and *Pythium* root rot into male gametes and introgress them into susceptible commercial varieties, ii) evaluate and select for multiple disease resistance and agronomic traits in the segregating F_1 population and $F_{1.2}$ families.

5.3 Materials and Methods

5.3.1 Germplasm

Sixteen F_1 segregating populations were developed from crosses among four multi-parent male gametes combining genes for resistance to angular leaf spot, anthracnose, root rot and common bacterial blight and four susceptible commercial varieties in the greenhouse at Kabete Field Station. The four male gametes were MEX54/G2333//RWR719/VAX6, MEX 54/G2333//AND1062/VAX6, G10909/G2333//RWR719/VAX6, and G10909/G2333//AND1062/VAX 6. In these crosses, MEX 54 and G 10909 were used as donor parents for angular leaf spot. G 2333 was the source of anthracnose resistance genes. AND 1062 and RWR 719

were the sources of resistance to root rot. VAX 6 was the donor parent for bacterial blight resistance. MEX 54, G10909, G2333, VAX and RWR 719 are of Mesoamerican origin. AND 1062 is of Andean origin.

G2333 is an indeterminate strong climber with small red seeds. It belongs to the Mesoamerican gene pool and is highly resistant to anthracnose races in various regions of the world (Singh *et al*., 1992).

Mexico 54 has medium sized purple seed, with an indeterminate growth habit.

G10909 is a medium red-seeded climbing bean genotype from the highlands of Guatemala.

The genotype RWR 719 is a small seeded variety of Mesoamerican gene pool that is resistant to all species of *Pythium* while AND1062 is medium maturing and the only large seeded variety resistant to *Pythium* (Mukalazi et al., 2001). RWR 719 is resistant to both *Fusarium solani* and *Pythium ultimum* root rots which occur concurrently in farmer's fields (Otsyula *et al*., 1998).

VAX 6 is one of the six lines resistant to common bacterial blight developed by Shree Singh at CIAT in the late 1990s. The VAX lines with combined resistance from *P. vulgaris* and *P. acutifolius* possess the highest level of CBB resistance developed to date (Singh *et al*., 2001).

Canadian Wonder (GLP 24) developed in 1984 at KARI-Thika grows well between 1200-1800 m asl. It matures in 3 months and yields between 1.3-1.8 t ha^{-1}. It has

shiny dark reddish purple seeds, recommended for medium rainfall areas, resistant to angular leaf spot (ALS) and anthracnose but susceptible to bean common mosaic virus (BCMV) and rust (www.infonet-biovision.org).

Mwezi Moja (GLP 1004) developed at University of Nairobi grows best at an elevation of 1200-1600m and takes 2-3 months to mature. It yields between 1.2-1.5t ha^{-1}. It is well suited for the drier semi-arid low rainfall areas and also performs well in medium rainfall areas during short rains. It has large beige or light brown speckled purple seeds and is tolerant to drought and bean fly but susceptible to halo blight (www.infonet-biovision.org).

AFR 708, commonly known as Kenya Umoja is a large red mottled developed at University of Nairobi best suited for altitude of 1000-1900m above sea level. It is a bush type IIa that matures in 80-85 days (www.infonet-biovision.org).

E8 commonly known as New Rosecoco is a large red mottled developed at University of Nairobi. It is best suited for altitude of 1100-2000m above sea level. It is a bush type II that matures in 80-85 days (www.infonet-biovision.org).

5.3.2 Population development

Study populations were developed following the crossing scheme presented in Figure 5.1. To develop a multi-parent male gamete, six donor parents having genes for resistance to foliar diseases and root rots were crossed. This involved single and double crosses aimed at concentrating resistance genes into a male

gamete i.e. (A×B) x (C×D). Anthracnose and angular leaf spot donors were crossed together while common bacterial blight and *Pythium* root rot donors were crossed in all possible combinations. The following lines which posses associated markers for resistance to angular leaf spot (Mexico 54, G10909), anthracnose (G2333), *Pythium* root rots (RWR 719, AND 1062) and common bacterial blight (VAX 6) were used to generate single and double cross male gamete. These gametes were used in the final cross with the susceptible commercial varieties.

The structure of the crosses was as follows:

Commercial variety///angular leaf spot/anthracnose//common bacterial blight / root rots

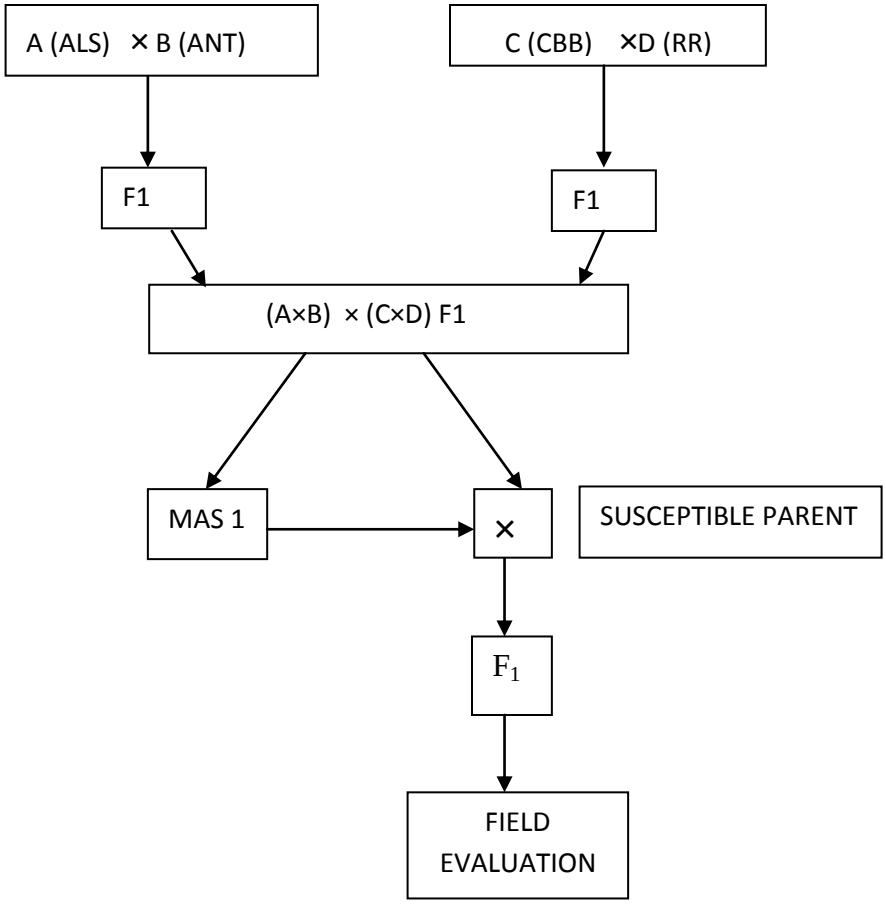


Figure 5.1 Crossing scheme for marker assisted pyramiding of disease resistance genes insusceptible commercial varieties.

The scheme above is an illustration of the single and double crosses that was done to generate the male gametes and the final F_1 between the male gamete and the commercial. Artificial cross pollination was done by emasculation and

hook method (Buishand, 1956). The stigma of the female is receptive at least 2 days before and 1 day after anthesis. Bumpy buds showing colour and that would open the next day were chosen on the female parent. The flower bud to be pollinated was held in one hand between the thumb and forefinger. Holding a pair of fine tipped forceps in the other hand; the standard was detached from below with the forceps and bend backward. The keel was pulled off piece by piece and the stamens were removed. For the supply of pollen an open flower was picked from intended sources. To expose the thickly pollinated stigma the wings were pressed downwards. The two stigmas were then rubbed against each other and hooked. A tag was tied to the pollinated female flower bearing the name of the female parent followed by the name of the male parent. Because homozygous individuals produce only one type of gamete no special precaution was required in making single crosses between homozygous parents (donor parents) (Singh, 1994). However plant-to- plant or pair-wise hybridizations for multi-parental crosses were effected following a systematic numbering of each female (susceptible commercial variety) and male gametes.

The target was 700 seeds for each of the four male gametes:

i. MEX 54/G2333// RWR 719/VAX 6
ii. MEX 54/G2333//AND 1062/VAX 6
iii. G10909/G2333//RWR 719/VAX 6
iv. G10909/G2333//AND 1062/VAX 6

A minimum of 200 F_1 seeds was produced for each single cross (Singh, 1994). After gene pyramiding, the double cross (heterogametic male parent) was screened for desirable dominant or co-dominant markers in the Molecular Breeding Laboratory at Kabete before or during flowering.

5.3.3 Marker-assisted selection of resistant plants of MEX54/G2333//RWR719/VAX6

To confirm the presence of resistance genes in the male gamete plants, the molecular markers SH-13, SAB-3, PYAA-19 and SU-91 respectively were used. Female parents were planted alongside 89 seeds of the double cross MEX54/G2333//RWR1062/VAX6 gamete in the screen house at the rate of one seed per pot. For potting media, soil was mixed with sand and chicken manure in the ratio of 3:1:1 respectively. Diammonium phosphate (18:46:0), at a rate of 1g kg^{-1} of soil was used at planting. At flowering calcium ammonium nitrate (26% N) at the rate of 1g kg^{-1} of soil was used for top dressing. Irrigation and weeding was done as necessary. At the first trifoliate stage, leaves were collected separately, one leaf per plant, and labelled accordingly. In the screen house the collected leaf was crushed onto FTA card as described in section 4.3.2.1 and again labelled according to the plant number where it was collected. After drying, the cards were taken to the laboratory where a punched sample was taken from each spot where a leaf was crushed and processed through the procedure outline in

section 4.3.2.2. The PCR cycling conditions were similar to those in section 4.3.2.3 as well as gel preparation and running.

5.3.4 Plant-to-plant pairwise introgression of resistance genes to commercial varieties

For the final cross, the commercial varieties were used as female parents for hybridization. Because such female parents often are homozygous and homogeneous for most traits, they are homogametic (i.e., produce a single type of gamete). Thus, because of their uniform genetic background, they serve as a filter or screen against which differences among male gametes produced by the donor crosses (i.e., the male parent) are revealed (Singh, 1994). The commercial varieties were selected based on the absence of markers for the resistance genes implying that they were susceptible to the target disease. Plant-to-plant or pair-wise hybridizations was done following a systematic numbering of each female and male plant. This maximised gene recombination among all parents involved in each population and helped develop a separate F_1-derived family from each recombination event (i.e., zygotic seed) (Singh, 1994). All the four male gametes, namely: i) MEX54/G2333//RWR719/VAX6 ii) MEX54/G2333//AND1062/VAX6 iii) G10909/G2333//RWR719/VAX6 and iv) G10909/G2333//AND1062/VAX6 were crossed with all the four commercial cultivars Kenya Umoja, New Rosecoco, GLP

1004 and GLP 24 in all possible combinations generating the following 16 populations;

1. GLP1004///G10909/G2333//AND1062/VAX6
2. GLP1004///G10909/G2333//RWR719/VAX6
3. GLP1004///MEX54/G2333//AND1062/VAX6
4. GLP1004///MEX54/G2333//RWR719/VAX6
5. New Rosecoco///G10909/G2333//AND1062/VAX6
6. New Rosecoco///G10909/G2333//RWR719/VAX6
7. New Rosecoco///MEX54/G2333//AND1062/VAX6
8. New Rosecoco///MEX54/G2333//RWR719/VAX6
9. GLP24///G10909/G2333//AND1062/VAX6
10. GLP24///G10909/G2333//RWR719/VAX6
11. GLP24///MEX54/G2333//AND1062/VAX6
12. GLP24///MEX54/G2333//RWR719/VAX6
13. Kenya Umoja///G10909/G2333//AND1062/VAX6
14. Kenya Umoja///G10909/G2333//RWR719/VAX6
15. Kenya Umoja///MEX54/G2333//AND1062/VAX6
16. Kenya Umoja///MEX54/G2333//RWR719/VAX6

5.3.5 Field evaluation of 16 segregating populations

The 16 F_1 populations were evaluated at Kabete Field Station and KARI-Tigoni during the 2012 long rain season. $F_{1.2}$ families selected at the two sites were subsequently evaluated in progeny row trial at Kabete Field Station during the 2012/2013 long rain season.

Table 5.1 Description of the trial sites

Trial site	Locality	Average temperature	Rainfall	Soils	AEZ	Elevation (m. asl)
Kabete Field Station	1° 15' S 36°44' E	13.7°C –min 24.3°C -max	1100mm to 1200mm	Clay loam	LH3	1940
KARI-Tigoni	10° 55' S 33° 57' E	11.3°C –min 22.8°C -max	1100mm to 1200mm	Sandy clay loam	LH1	2095

Source: UoN Met. Data; KARI-Tigoni Met. Data

The experiment was laid out in a split plot design with three replicates (Table 5.2). Male gametes were the main plots and the commercial varieties (female parent) the subplot. Each subplot had 4 rows measuring 3m long. Spacing was 10cm within rows and 50cm between rows. Diammonium phosphate (18:46:0), at a rate of 130 kg ha^{-1} was used at planting. Supplemental sprinkler irrigation was supplied at Kabete Field Station three times during the season. The crop at KARI-Tigoni was rainfed. Manual weeding was done on demand. Data on disease reaction, plant vigour, days to flowering and days to maturity were based on evaluation of all the plants in a row.

Table 5.2 Description of traits measured on F_1 plants grown in Kabete and Tigoni during 2012 long rain season

Trait (units)	Description
Disease severity	Area of plant tissues affected by the disease-causing-organism and expressed as the percentage of the total amount of tissues.
Vegetative adaptation (vigor)	Evaluation done when plants reach their maximum development. Effect of growth habit on plant vigor is considered.
Days to flowering (days)	Number of days from planting to the day when 50% of the plants within a plot had at least one flower
Days to maturity (days)	Number of days from planting to harvest maturity
Pods per plant (count)	Number of pods per plant
Seeds per pod (count)	Number of seeds per pod
Seed size (g)	Weight in grams of 100 randomly chosen seeds
Seed yield (g/m^2)	Total weight of seeds per plot

Source: Barladin 1998

5.4 Results

5.4.1 Population development

Approximately 100 F_1 seeds were produced for each single cross and more than 500 seeds were produced for each double cross (Table 5.3). However, more than 1000 seeds were realised for MEX54/G2333//RWR719/VAX 6. For the final crosses, more than 500 seeds were also attained for each cross with three cross combinations attaining more than 1000 seeds.

Table 5.3 Cross combinations and the seeds produced

Crosses	Target seeds	Seeds harvested
Single cross		
MEX 54 /G2333	50	204
G 10909/G2333	50	130
AND1062/VAX 6	50	156
RWR 719 /VAX 6	50	241
Double cross (Male gamete)		
MEX54/G2333//AND1062/VAX 6	100	854
MEX54/G2333//RWR719/VAX 6	100	1103
G 10909/G2333//RWR719/VAX 6	100	832
G 10909/G2333//AND1062/VAX 6	100	563
Final F_1		
Kenya Umoja///Mex 54 / G2333 // AND 1062 / VAX 6		752
New Rosecoco///Mex 54 / G2333 // AND 1062 / VAX 6		935
Mwezi Moja///Mex 54 / G2333 // AND 1062 / VAX 6		1132
Canadian Wonder///Mex 54 / G2333 // AND 1062 / VAX 6		830
Kenya Umoja///Mex 54 / G2333 // RWR 719 / VAX 6		1050
New Rosecoco///Mex 54 / G2333 // RWR 719 / VAX 6		588
Mwezi Moja///Mex 54 / G2333 // RWR 719 / VAX 6		696
Canadian Wonder///Mex 54 / G2333 // RWR 719 / VAX 6		657
Kenya Umoja///G10909 / G2333 // AND 1062 / VAX 6		592
New Rosecoco///G10909 / G2333 // AND 1062 / VAX 6		857
Mwezi Moja///G10909 / G2333 // AND 1062 / VAX 6		954
Canadian Wonder///G10909 / G2333 // AND 1062 / VAX 6		976
Kenya Umoja///G10909 / G2333 // RWR 719 / VAX 6		743
New Rosecoco///G10909 / G2333 // RWR 719 / VAX 6		852
Mwezi Moja///G10909 / G2333 // RWR 719 / VAX 6		1065
Canadian Wonder///G10909 / G2333 // RWR 719 / VAX 6		984

5.4.2 Amplification of DNA markers for selection of resistant plants from MEX/G2333//RWR719/VAX6

Of the 89 male gamete plants screened for angular leaf spot, anthracnose, common bacterial blight and *Pythium* root rot, 3 plants had 3 resistance genes, 8 had 2, 36 had 1 and 42 had none (Table 5.4). SAB-3 the marker for anthracnose resistance gene *co-5* was detected in 30 plants out of the 89 sampled; this was the highest frequency (Figure 5.2 and 5.3). SU-91 the marker for common bacterial blight resistance genes was next with 21 detections (Figure 5.4 and 5.5) while only 10 plants out of 89 tested positive for angular leaf spot resistance gene *phg 2*. (Figure 5.6 and 5.7). PYAA the marker for root rot had no positive amplification (Figure 5.8 and 5.9). The band obtained was not consistent with expected size (800 bp). A total of 11 plants with either three or two desired resistance genes were selected for introgression into Kenya Umoja (Table 5.4).

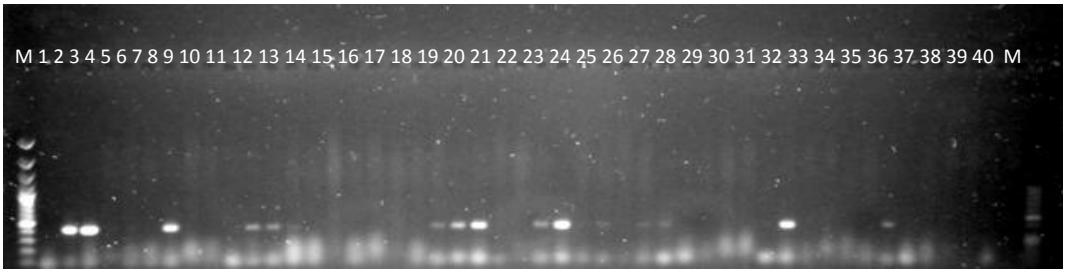


Figure 5.2 Amplification of SAB-3 marker in the first 48 plants (Strip I) (1.0-5.8).

On figures 5.2 to 5.9 `M' indicates the ladder (molecular weight).

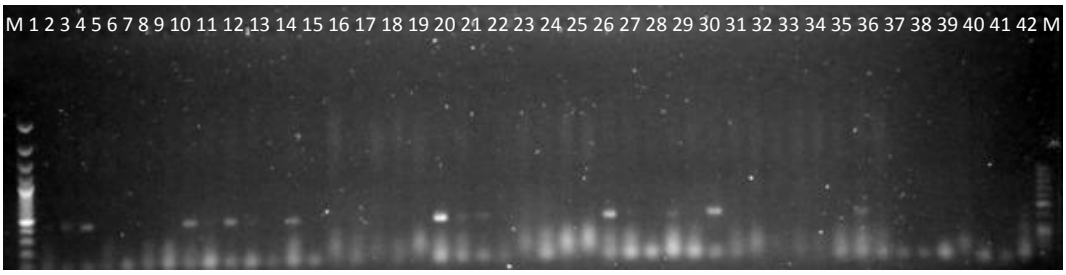


Figure 5.3 Amplification of SAB 3 marker in the second set of 48 plants (Strip II) (5.9-9.9)

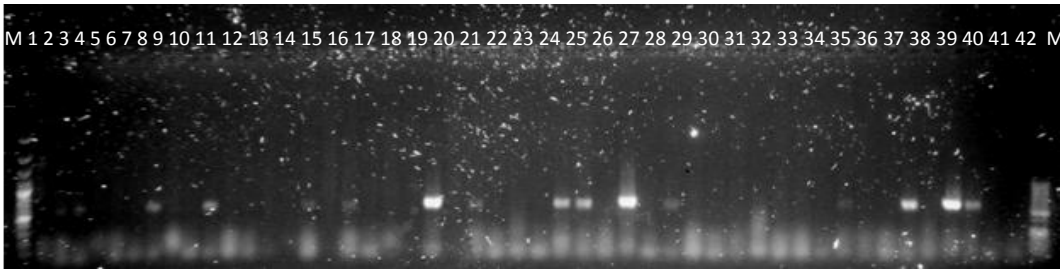


Figure 5.4 Amplification of SU 91 marker in the first 48 plants (Strip I) (1.0-5.8)

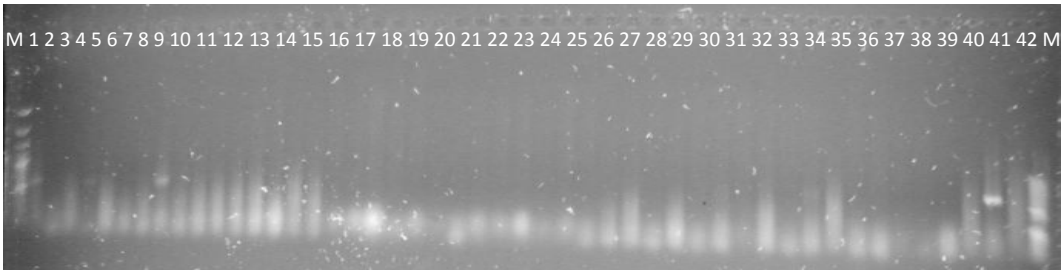


Figure 5.5 Amplification of SU 91 marker in the second set of 48 plants (Strip II) (5.9-9.9)

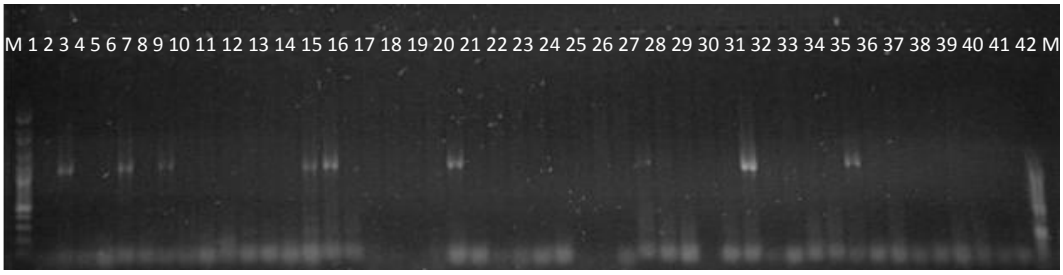


Figure 5.6 Amplification of SH 13 marker in the first 48 plants (Strip I) (1.0-5.8)

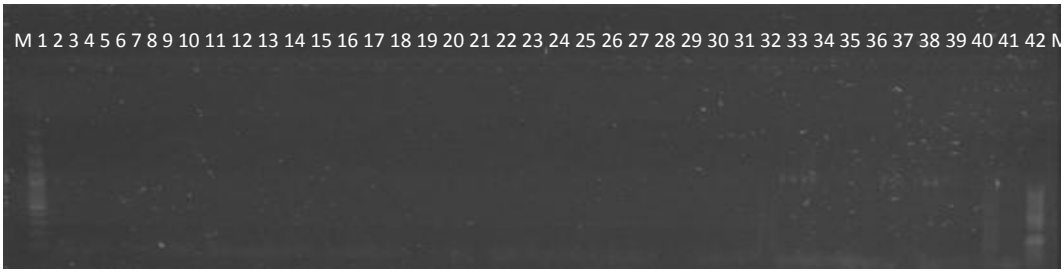


Figure 5.7 Amplification of SH 13 marker in the second set of 48 plants (Strip II) (5.9-9.9)

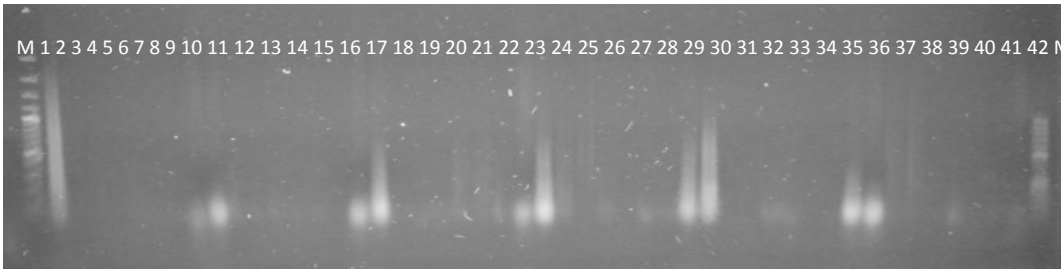


Figure 5.8 Amplification of PYAA19 marker in the first 48 plants (Strip I) (1.0-5.8)

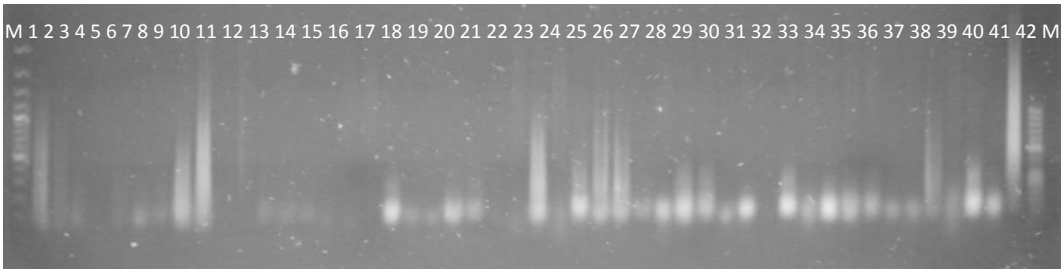


Figure 5.9 Amplification of PYAA-19 marker in the second set of 48 plants (StripII) (5.9-9.9)

Table 5.4 Identification of disease resistance genes in

MEX54/G2333//RWR719/VAX6 gamete

			DISEASE AND MARKERS				
	Plant	Strip/Gel	ANTH	CBB	ALS	RR	No. of
Serial No.	No.	lane	SAB 3	SU 91	SH 13	PYAA19	Markers
1	1.0	2	-	-	-	-	0
2	1.1	3	+	+	+	-	3
3	1.2	4	+	+	-	-	2
4	1.3	5	-	-	-	-	0
5	1.4	6	-	-	+	-	1
6	1.5	7	-	-	-	-	0
7	1.6	8	+	+	+	-	3
8	1.7	9	-	-	-	-	0
9	1.8	10	-	-	-	-	0
10	1.9	11	-	+	-	-	1
11	2.0	12	+	-	-	-	1
12	2.1	13	+	-	-	-	1
13	2.2	14	+	-	-	-	1
14	2.3	15	-	-	+	-	1
15	2.4	16	-	+	+	-	2
16	2.5	17	-	-	-	-	0
17	2.6	18	-	+	-	-	1
18	2.7	19	-	-	-	-	0
19	2.8	20	-	-	-	-	0
20	2.9	21	+	-	-	-	1
21	3.0	22	+	+	+	-	3
22	3.1	23	+	-	-	-	1
23	3.2	24	-	+	-	-	1
24	3.3	25	-	-	-	-	0
25	3.4	26	+	-	-	-	1
26	3.5	27	+	-	-	-	1
27	3.6	28	-	+	-	-	1
28	3.7	29	+	+	-	-	2
29	3.8	30	-	-	-	-	0
30	3.9	31	+	+	-	-	2
31	4.0	32	+	-	-	-	1
32	4.1	33	-	+	-	-	1

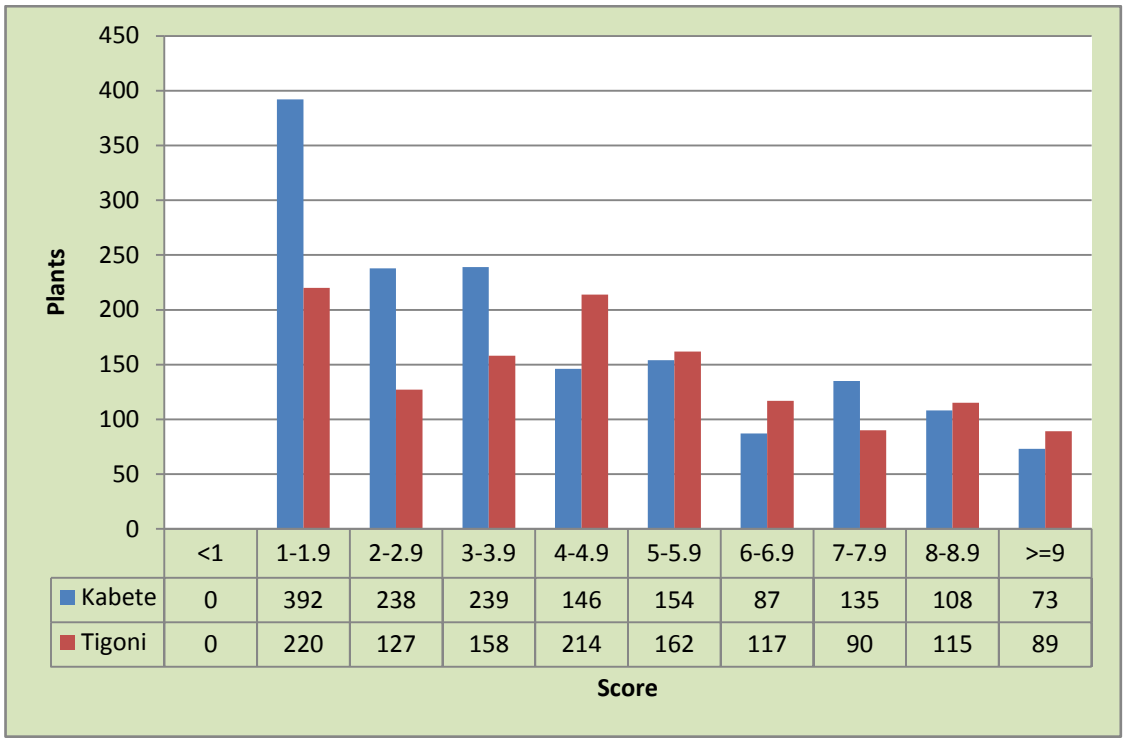
Serial No.	Plant No.	Strip/Gel lane	DISEASE AND MARKERS				No. of Markers
			ANTH	CBB	ALS	RR	
			SAB 3	SU 91	SH 13	PYAA19	
33	4.2	34	-	-	-	-	0
34	4.3	35	-	-	-	-	0
35	4.4	36	-	-	+	-	1
36	4.5	37	-	-	-	-	0
37	4.6	38	+	-	-	-	1
38	4.7	39	-	-	-	-	0
39	4.8	40	-	-	-	-	0
40	4.9	41	-	+	+	-	2
41	5.0	42	-	-	-	-	0
42	5.1	43	+	-	-	-	1
43	5.2	44	-	+	-	-	1
44	5.3	45	-	-	-	-	0
45	5.4	46	-	+	-	-	1
46	5.5	47	-	+	-	-	1
47	5.6	48	-	-	-	-	0
48	5.7	49	-	-	-	-	0
49	5.8	2	+	+	-	-	2
50	5.9	3	+	-	-	-	1
51	6.0	4	+	-	-	-	1
52	6.1	5	-	+	-	-	1
53	6.2	6	-	-	-	-	0
54	6.3	7	-	-	-	-	0
55	6.4	8	-	-	-	-	0
56	6.5	9	+	+	-	-	2
57	6.6	10	-	-	-	-	0
58	6.7	11	+	-	-	-	1
59	6.8	12	+	-	-	-	1
60	6.9	13	-	-	-	-	0
61	7.0	14	+	-	-	-	1
62	7.1	15	-	-	-	-	0
63	7.2	16	-	-	-	-	0
64	7.3	17	-	-	-	-	0
65	7.4	18	-	-	-	-	0
66	7.5	19	-	-	-	-	0
67	7.6	20	-	-	-	-	0
68	7.7	21	+	-	-	-	1

			DISEASE AND MARKERS				
	Plant	Strip/Gel	ANTH	CBB	ALS	RR	No. of
Serial No.	No.	lane	SAB 3	SU 91	SH 13	PYAA19	Markers
69	7.8	22	+	-	-	-	1
70	7.9	23	+	-	-	-	1
71	8.0	24	-	-	-	-	0
72	8.1	25	-	-	-	-	0
73	8.2	26	-	-	-	-	0
74	8.3	27	-	-	-	-	0
75	8.4	28	-	-	-	-	0
76	8.5	29	+	-	-	-	1
77	8.6	30	-	-	-	-	0
78	8.7	31	-	-	-	-	0
79	8.8	32	+	-	-	-	1
80	8.9	33	-	-	-	-	0
81	9.0	34	+	-	-	-	1
82	9.1	35	-	-	-	-	0
83	9.2	36	-	+	-	-	1
84	9.3	37	-	-	-	-	0
85	9.4	38	-	-	-	-	0
86	9.5	39	-	-	+	-	1
87	9.6	40	-	+	+	-	2
88	9.7	41	+	-	-	-	1
89	9.8	42	-	-	-	-	0
MEX 54	9.9	43	-	-	+	-	1
G 2333	10.0	44	+	-	-	-	1
RWR 719	10.1	45	-	-	-	-	0
VAX 6	10.2	46	-	+	-	-	1
Kenya	10.3	47	-	-	-	-	0
Umoja							
+ve Control		48					
-ve Control		49					
Total			+= 30	+= 21	+= 10	+= 0	

5.4.3 Field evaluation of segregating population

5.4.3.1 Vegetative vigour

Male gamete and commercial variety had significant effect on vigour at $P<0.01$. The two way interactions between male gamete and location and commercial variety and location had significant effect on vigour both at $P<0.01$. The three way interaction between male gamete, commercial variety and location had significant effect on vigour at $P<0.01$ (Appendix 6). The range for vigour for the 16 F_1 populations was 1 to 9 in both locations (Figure 5.10). Most of the plants were very vigorous (1 to 3). The mean vigour among the populations ranged from 3.3 to 4.7 in Kabete. In Tigoni the mean vigour among the populations ranged from 3.6 to 4.5. The range for vigour of the parental lines was varied across both locations.



	<1	1-1.9	2-2.9	3-3.9	4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	>=9
Kabete	0	392	238	239	146	154	87	135	108	73
Tigoni	0	220	127	158	214	162	117	90	115	89

Figure 5.10 Frequency distribution for F_1s vigour

In Kabete the most vigorous genotypes were AND 1062 and RWR 719 with their vigour ranging from 2 to 4. The least vigorous parental lines in Kabete were New Rosecoco (5-6) and GLP 1004 (5-7). In Tigoni the most vigorous genotypes were G 10909 (2-5) and GLP 1004 (2-6). AND 1062 (4-5) and MEX 54 (4-5) were the poor performers in Tigoni (Table 5.5). The mean vigour was 4.1 and a variance of 5 to 7. Plants at Kabete were more vigourous than plants in Tigoni. Mean score vigour for Kabete was 3.7 while that for Tigoni was 4.4. The F_1s constituted using MEX54/G2333//RWR719/VAX6 as the male gamete had the most vigorous vegetative growth with a mean of 3.8. In Kabete the populations with

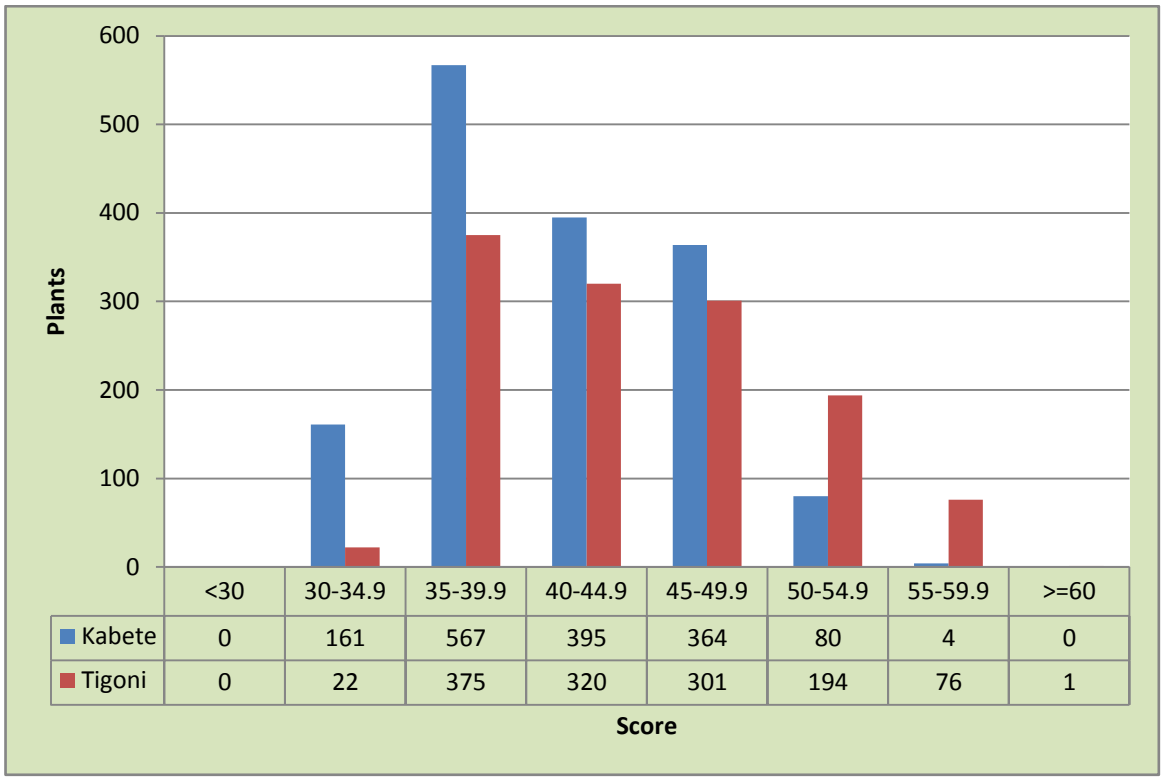
G10909/G2333//RWR719/VAX6 double cross had the highest vegetative growth with a mean score of 3.5 while in Tigoni, MEX54/G2333//RWR719/VAX6 population had the most vigorous plants with a mean score of 4.2. Among the commercial varieties, Kenya Umoja populations were the most vigourous with a mean of 3.6. Across both locations Kenya Umoja had the most vigorous populations with a mean of 3.0 in Kabete and a mean of 4.2 in Tigoni. Among the populations Kenya Umoja///MEX54/G2333//RWR719/VAX6 was the most vigourous with 3.5.

Table 5.5 Vegetative vigour of F_1 populations grown at Kabete and Tigoni during the 2012 long rain season

Male gamete	KABETE	TIGONI	MEAN
G10909/G2333//AND1062/VAX6	3.6	4.3	3.9
G10909/G2333//RWR719/VAX6	3.5	4.8	4.2
MEX54/G2333//AND1062/VAX6	4.4	4.3	4.3
MEX54/G2333//RWR719/VAX6	3.4	4.2	3.8
Commercial variety			
GLP1004	4.0	4.6	4.3
New Rosecoco	3.9	4.5	4.2
GLP24	4.0	4.3	4.2
Kenya Umoja	3.0	4.2	3.6
F_1			
GLP1004///G10909/G2333//AND1062/VAX6	3.4	4.7	4.0
GLP1004///G10909/G2333//RWR719/VAX6	3.1	5.3	4.2
GLP1004///MEX54/G2333//AND1062/VAX6	6.1	4.5	5.3
GLP1004///MEX54/G2333//RWR719/VAX6	3.4	3.7	3.6
New Rosecoco///G10909/G2333//AND1062/VAX6	3.2	4.1	3.6
New Rosecoco///G10909/G2333//RWR719/VAX6	3.5	4.9	4.2
New Rosecoco///MEX54/G2333//AND1062/VAX6	4.9	4.4	4.7
New Rosecoco///MEX54/G2333//RWR719/VAX6	4.0	4.7	4.3
GLP24///G10909/G2333//AND1062/VAX6	4.0	4.6	4.3
GLP24///G10909/G2333//RWR719/VAX6	4.6	4.8	4.7
GLP24///MEX54/G2333//AND1062/VAX6	3.7	3.8	3.8
GLP24///MEX54/G2333//RWR719/VAX6	3.7	4.0	3.9
Kenya Umoja///G10909/G2333//AND1062/VAX6	3.6	4.0	3.8
Kenya Umoja///G10909/G2333//RWR719/VAX6	2.8	4.3	3.6
Kenya Umoja///MEX54/G2333//AND1062/VAX6	3.1	4.2	3.7
Kenya Umoja///MEX54/G2333//RWR719/VAX6	2.6	4.3	3.5
Mean	3.7	4.4	4.1
LSD (0.05)	1.4		
CV%	21.0		

5.4.3.2 Days to flowering

Male gamete did not have a significant effect on days to flowering (Appendix 6). However, commercial variety had a significant effect on flowering at $P<0.01$. Interaction between male gamete and location had no significant effect on flowering. Interaction between commercial variety and location had significant effect on flowering at $P<0.01$. The three way interaction between male gamete, commercial variety and location had significant effect at $P<0.01$ on flowering (Appendix 6). Duration to 50% flowering among the populations varied from 32 days to 51 days in Kabete. In both locations a majority of plants took 35 to 50 days to flower (Figure 5.11).



	<30	30-34.9	35-39.9	40-44.9	45-49.9	50-54.9	55-59.9	>=60
Kabete	0	161	567	395	364	80	4	0
Tigoni	0	22	375	320	301	194	76	1

Figure 5.11 Frequency distribution for F_1s days to flowering

In Tigoni the populations took from 33 days to 55 days to flower (Table 5.6). In Kabete the earliest population to flower was GLP1004///MEX54/G2333//RWR 719/VAX6 taking 30-51 days. In Tigoni the population that flowered late was Kenya Umoja///G10909/G2333//AND1062/VAX6 taking 35-59 days. All the parental lines took 48 days to flower except Kenya Umoja which took 50 days. The mean flowering time was 42.3 days with a variance of 19 to 32 in Kabete and 29 to 41 in Tigoni. The mean flowering time in Kabete was 40.9 days while in Tigoni it was 43.8 days. The populations took between 32 days and 52 days to flower. Male gamete used did not influence the populations flowering as they all had an

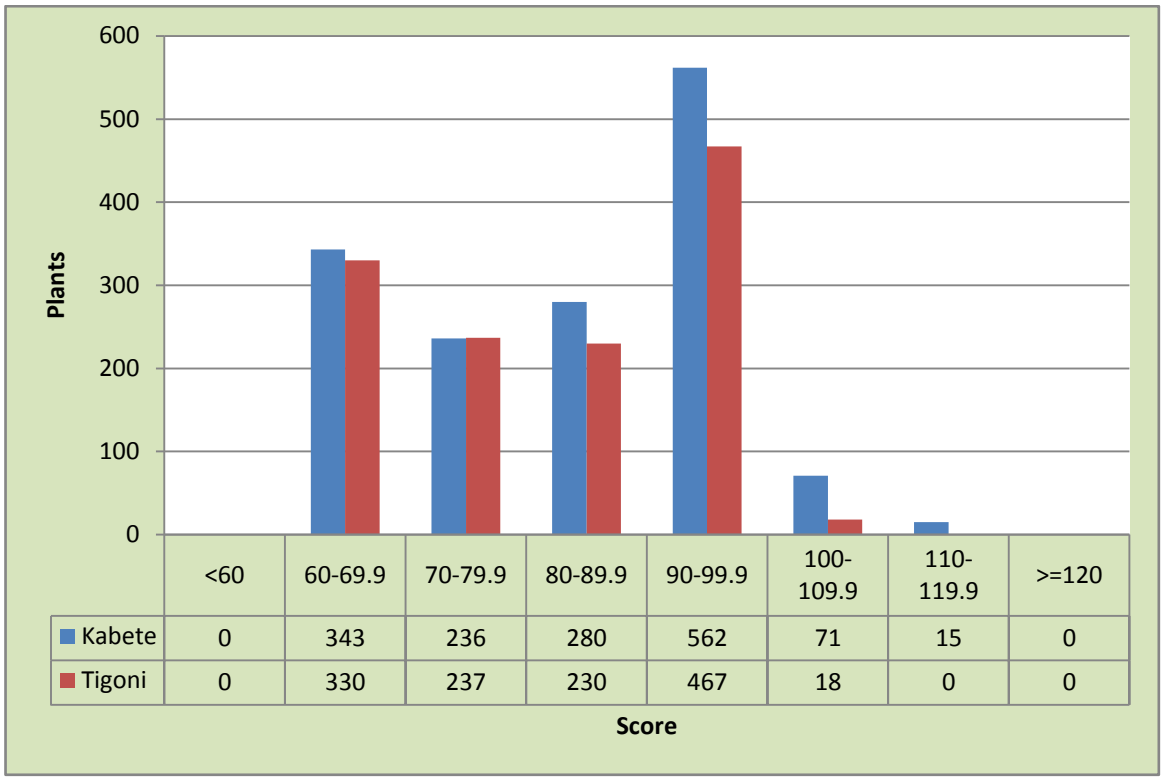
average of 42 days to flowering. The same uniformity was evident in both locations as populations in Kabete took 40 days to flower save for G10909/G2333//AND1062/VAX6 that took 41 days to flower. In Tigoni all the populations took 43 days to flower save for MEX54/G2333//AND1062/VAX6 that took 44 days to flower. Commercial variety did not have a significant effect on days to flowering as all the F_1 from different commercial varieties all flowered in 42 days. In Kabete populations flowered in 40 days save for populations with Kenya Umoja that flowered in 41 days. In Tigoni GLP1004 and Kenya Umoja F_1s flowered in 44 days while New Rosecoco and GLP24 F_1s flowered in 43 days. Among the populations GLP1004///G10909/G2333//RWR719/VAX6, which flowered in 41.1 days was the earliest and GLP1004///G10909/G2333//AND 1062/VAX6 the latest (43.4).

Table 5.6 Flowering of F_1 populations grown at Kabete and Tigoni during the 2012 long rain season

Male gamete	KABETE	TIGONI	MEAN
G10909/G2333//AND1062/VAX6	41.3	43.8	42.6
G10909/G2333//RWR719/VAX6	40.6	43.7	42.1
MEX54/G2333//AND1062/VAX6	40.7	44.3	42.5
MEX54/G2333//RWR719/VAX6	40.8	43.5	42.2
Commercial variety			
GLP1004	40.3	44.0	42.1
New Rosecoco	40.7	43.5	42.1
GLP24	40.9	43.7	42.3
Kenya Umoja	41.4	44.0	42.7
F_1			
GLP1004///G10909/G2333//AND1062/VAX6	40.9	45.8	43.4
GLP1004///G10909/G2333//RWR719/VAX6	39.6	42.7	41.1
GLP1004///MEX54/G2333//AND1062/VAX6	40.1	43.3	41.7
GLP1004///MEX54/G2333//RWR719/VAX6	40.8	43.9	42.4
New Rosecoco///G10909/G2333//AND1062/VAX6	41.6	42.7	42.2
New Rosecoco///G10909/G2333//RWR719/VAX6	40.7	44.3	42.5
New Rosecoco///MEX54/G2333//AND1062/VAX6	40.3	44.0	42.1
New Rosecoco///MEX54/G2333//RWR719/VAX6	40.3	43.3	41.8
GLP24///G10909/G2333//AND1062/VAX6	41.3	42.0	41.7
GLP24///G10909/G2333//RWR719/VAX6	40.5	43.5	42.0
GLP24///MEX54/G2333//AND1062/VAX6	41.0	45.4	43.2
GLP24///MEX54/G2333//RWR719/VAX6	40.9	44.0	42.5
Kenya Umoja///G10909/G2333//AND1062/VAX6	41.5	44.8	43.2
Kenya Umoja///G10909/G2333//RWR719/VAX6	41.6	44.1	42.9
Kenya Umoja///MEX54/G2333//AND1062/VAX6	41.2	44.5	42.8
Kenya Umoja///MEX54/G2333//RWR719/VAX6	41.3	42.7	42.0
Mean	40.9	43.8	42.3
LSD (0.05)	2.0		
CV%	2.7		

5.4.3.3 Days to maturity

Male gamete had a significant effect ($P<0.01$) on days-to-maturity. Commercial variety had a significant effect at $P<0.01$ on maturity. Interaction between male gamete and location had significant at $P<0.01$ on maturity. Interaction between commercial variety and location had a significant effect on maturity at $P<0.01$. Interaction between male gamete, commercial variety and location had significant effect at $P<0.01$ on maturity (Appendix 6). In both locations many plants matured in between 60 to 90 days with a majority of plants taking 90 days to mature (Figure 5.12). The range of days taken by the 16 populations to mature was 62 days to about 110 days at Kabete. In Tigoni the populations took slightly longer to mature (64 days to 100 days).



	<60	60-69.9	70-79.9	80-89.9	90-99.9	100-109.9	110-119.9	>=120
Kabete	0	343	236	280	562	71	15	0
Tigoni	0	330	237	230	467	18	0	0

Figure 5.12 Frequency distribution for F_1s days to maturity

Among the parental lines in Kabete, the range for days to maturity was 80 to 90 days (Table 5.7). The parental lines in Tigoni took about 90 days to mature. The mean maturity duration was 83.2 days. In Kabete the mean was 83.7 and in Tigoni it was 82.8 days. Variance for days to maturity was greater than 150 but less than 200 both in Kabete and Tigoni. The populations took between 62 days and 116 days to mature. All the populations took 83 days to mature save for Kenya Umoja populations that took 82 days to mature. In Tigoni the average number of days to maturity for all the populations was 82 days except for G10909/G2333//AND1062/VAX6 that took 83 days to mature. In Kabete

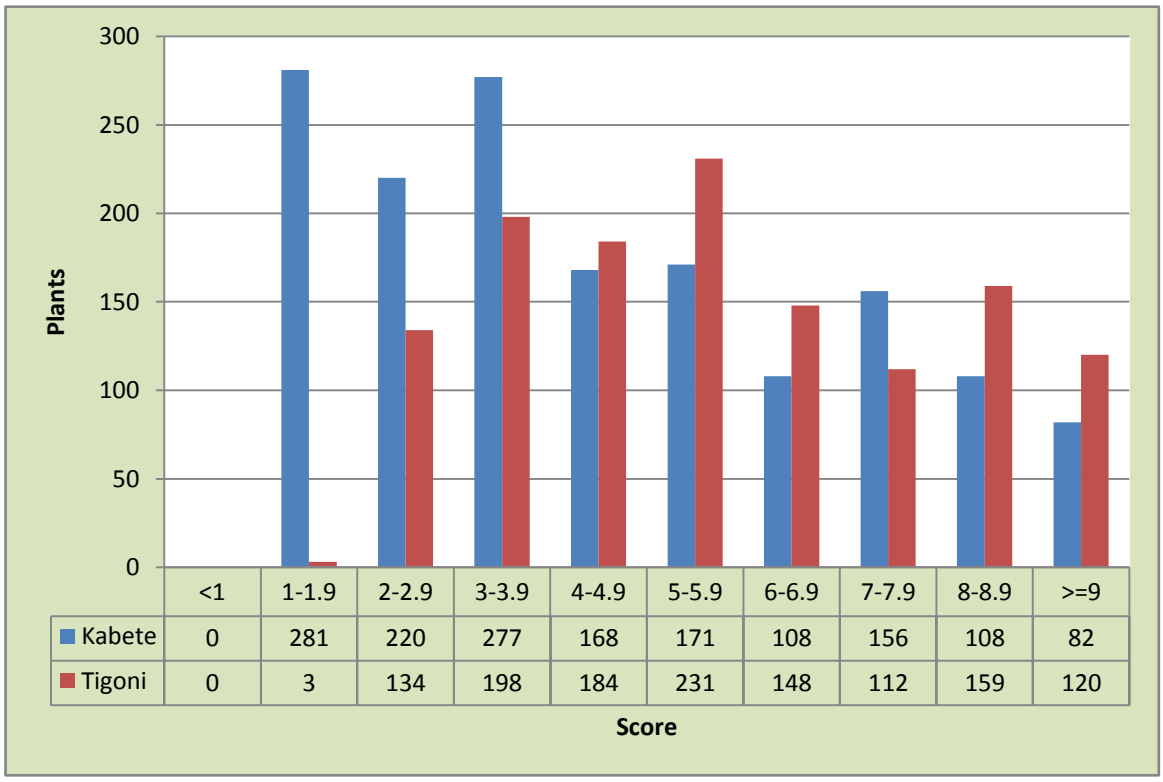
MEX54/G2333//AND1062/VAX6 was the late maturing population taking 85 days to maturity. GLP24 populations were uniformly late maturing across the locations. GLP1004///G10909/G2333//RWR719/VAX6 was the earliest maturing population taking 77.7 days while GLP24///G10909/G2333//RWR719/VAX6 was the late maturing population taking 89.9 days.

Table 5.7 Maturity of F_1 populations grown at Kabete and Tigoni during the 2012 long rain season

Male gamete	KABETE	TIGONI	MEAN
G10909/G2333//AND1062/VAX6	83.5	83.9	83.7
G10909/G2333//RWR719/VAX6	84.1	82.1	83.1
MEX54/G2333//AND1062/VAX6	85.1	82.8	83.9
MEX54/G2333//RWR719/VAX6	82.1	82.3	82.2
Commercial variety			
GLP1004	81.1	82.7	81.9
New Rosecoco	82.4	82.8	82.6
GLP24	86.7	83.0	84.9
Kenya Umoja	84.6	82.5	83.5
F_1			
GLP1004///G10909/G2333//AND1062/VAX6	79.8	84.2	82.0
GLP1004///G10909/G2333//RWR719/VAX6	77.8	77.6	77.7
GLP1004///MEX54/G2333//AND1062/VAX6	83.7	82.6	83.1
GLP1004///MEX54/G2333//RWR719/VAX6	82.8	86.5	84.6
New Rosecoco///G10909/G2333//AND1062/VAX6	87.5	83.7	85.6
New Rosecoco///G10909/G2333//RWR719/VAX6	82.9	83.8	83.3
New Rosecoco///MEX54/G2333//AND1062/VAX6	80.6	83.2	81.9
New Rosecoco///MEX54/G2333//RWR719/VAX6	78.6	80.5	79.5
GLP24///G10909/G2333//AND1062/VAX6	84.9	81.8	83.3
GLP24///G10909/G2333//RWR719/VAX6	89.5	85.3	87.4
GLP24///MEX54/G2333//AND1062/VAX6	85.9	82.3	84.1
GLP24///MEX54/G2333//RWR719/VAX6	86.8	82.7	84.8
Kenya Umoja///G10909/G2333//AND1062/VAX6	81.9	85.8	83.8
Kenya Umoja///G10909/G2333//RWR719/VAX6	86.2	81.7	84.0
Kenya Umoja///MEX54/G2333//AND1062/VAX6	90.1	83.0	86.6
Kenya Umoja///MEX54/G2333//RWR719/VAX6	80.2	79.3	79.8
Mean	83.7	82.8	83.2
LSD (0.05)	3.3		
CV%	2.4		

5.4.3.4 Angular leaf spot

Male gamete had a significant effect at P<0.01 on angular leaf spot. The other sources of variation had no significant effect on angular leaf spot (Appendix 7). Angular leaf spot severity scores among the 16 populations in Kabete ranged from 1 to 9 (Figure 5.12). In Tigoni angular leaf spot scores among the 16 populations ranged from 2 to 9. In Kabete a majority of plants had from resistant reaction to intermediate reaction to angular leaf spot. In Tigoni a majority of the plants had intermediate scores for reaction to angular leaf spot.



	<1	1-1.9	2-2.9	3-3.9	4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	>=9
Kabete	0	281	220	277	168	171	108	156	108	82
Tigoni	0	3	134	198	184	231	148	112	159	120

Figure 5.13 Frequency distribution for F_1s reaction to angular leaf spot.

The parental lines had higher disease scores ranging from 3.3 to 8.3 in Kabete save for MEX 54 and G10909 that had scores of 1 to 1.3 and 1 to 2.7 respectively (Table 5.8). In Tigoni, the parental genotypes had disease scores ranging from 3 to 9. Among the parental lines, MEX54 was the most resistant followed by G10909 in both locations. Kenya Umoja (7.1) and GLP24 (8.1) were the most susceptible genotypes. The mean score for angular leaf spot was 5.0. Angular leaf spot was more severe in Tigoni than Kabete. Angular leaf spot mean score in Kabete was 4.4 and 5.6 in Tigoni. Among the commercial varieties, GLP 24 populations were more susceptible to angular leaf spot in Kabete. However, in Tigoni GLP 24 populations and Kenya Umoja populations were the least susceptible. Populations with G10909/G2333//AND1062/VAX6 male gamete were the least susceptible to angular leaf spot with a mean score of 4.8. Among the 16 populations, New Rosecoco///G10909/G2333//AND1062/VAX6 had the lowest disease reaction to angular leaf spot with a mean score of 4.5. New Rosecoco///MEX54/G2333//AND1062/VAX6 population was the most susceptible for angular leaf spot with a disease reaction mean score of 5.8.

Table 5.8 Angular leaf spot severity of 16 F_1 populations grown at Kabete and Tigoni during the 2012 long rain season

Male gamete	KABETE	TIGONI	MEAN
G10909/G2333//AND1062/VAX6	3.8	5.7	4.8
G10909/G2333//RWR719/VAX6	4.7	5.3	5.0
MEX54/G2333//AND1062/VAX6	4.7	5.5	5.1
MEX54/G2333//RWR719/VAX6	4.3	5.8	5.0
Commercial variety			
GLP1004	4.0	5.8	4.9
New Rosecoco	4.3	5.6	4.9
GLP24	5.0	5.5	5.3
Kenya Umoja	4.1	5.5	4.8
F_1			
GLP1004///G10909/G2333//AND1062/VAX6	4.0	5.7	4.9
GLP1004///G10909/G2333//RWR719/VAX6	3.7	5.8	4.7
GLP1004///MEX54/G2333//AND1062/VAX6	4.8	5.6	5.2
GLP1004///MEX54/G2333//RWR719/VAX6	3.7	6.0	4.8
New Rosecoco///G10909/G2333//AND1062/VAX6	3.2	5.8	4.5
New Rosecoco///G10909/G2333//RWR719/VAX6	4.2	5.3	4.7
New Rosecoco///MEX54/G2333//AND1062/VAX6	5.9	5.7	5.8
New Rosecoco///MEX54/G2333//RWR719/VAX6	3.9	5.5	4.7
GLP24///G10909/G2333//AND1062/VAX6	4.4	5.2	4.8
GLP24///G10909/G2333//RWR719/VAX6	6.2	5.1	5.7
GLP24///MEX54/G2333//AND1062/VAX6	4.1	5.5	4.8
GLP24///MEX54/G2333//RWR719/VAX6	5.2	6.2	5.7
Kenya Umoja///G10909/G2333//AND1062/VAX6	3.6	6.1	4.9
Kenya Umoja///G10909/G2333//RWR719/VAX6	4.6	5.0	4.8
Kenya Umoja///MEX54/G2333//AND1062/VAX6	3.9	5.4	4.7
Kenya Umoja///MEX54/G2333//RWR719/VAX6	4.3	5.4	4.9
Mean	4.4	5.6	5.0
LSD (0.05)	1.0		
CV%	12.3		

For every population grown in Kabete, 144 plants were planted for evaluation. In Tigoni, 108 plants were planted. Due to plants failure to germinate and others dying not all the plants survived until flowering and disease evaluation. For GLP1004///G10909/G2333//AND1062/VAX6 107 plants were evaluated for disease reaction in Kabete of these 56% were resistant to angular leaf spot (Table 5.9). In Kabete 118 plants of Kenya Umoja///MEX54/G2333//AND 1062/VAX6 evaluated, 56.8% were rated resistant to angular leaf spot. The poor performing populations in Kabete were GLP1004///MEX54/G2333//RWR 719/VAX6 and Kenya Umoja///G10909/G2333//AND1062/VAX6 with 69% of 103 and 97 respectively being susceptible to angular leaf spot. The populations with the highest resistance to angular leaf spot In Tigoni were GLP1004///MEX54/G2333//RWR719/VAX6 with 26.5% of 68 plants being resistant and New Rosecoco///MEX54/G2333//RWR719/VAX6 with 25% of its 76 plants being resistant. The most susceptible populations to angular leaf spot were New Rosecoco///MEX54/G2333//AND1062/VAX6 with 87% of 94 plants being susceptible and GLP24///G10909/G2333//RWR719/VAX6 with 86% of its 81 plants being susceptible.

Table 5.9 Percentages of angular leaf spot resistant plants in F_1 populations at Kabete and Tigoni

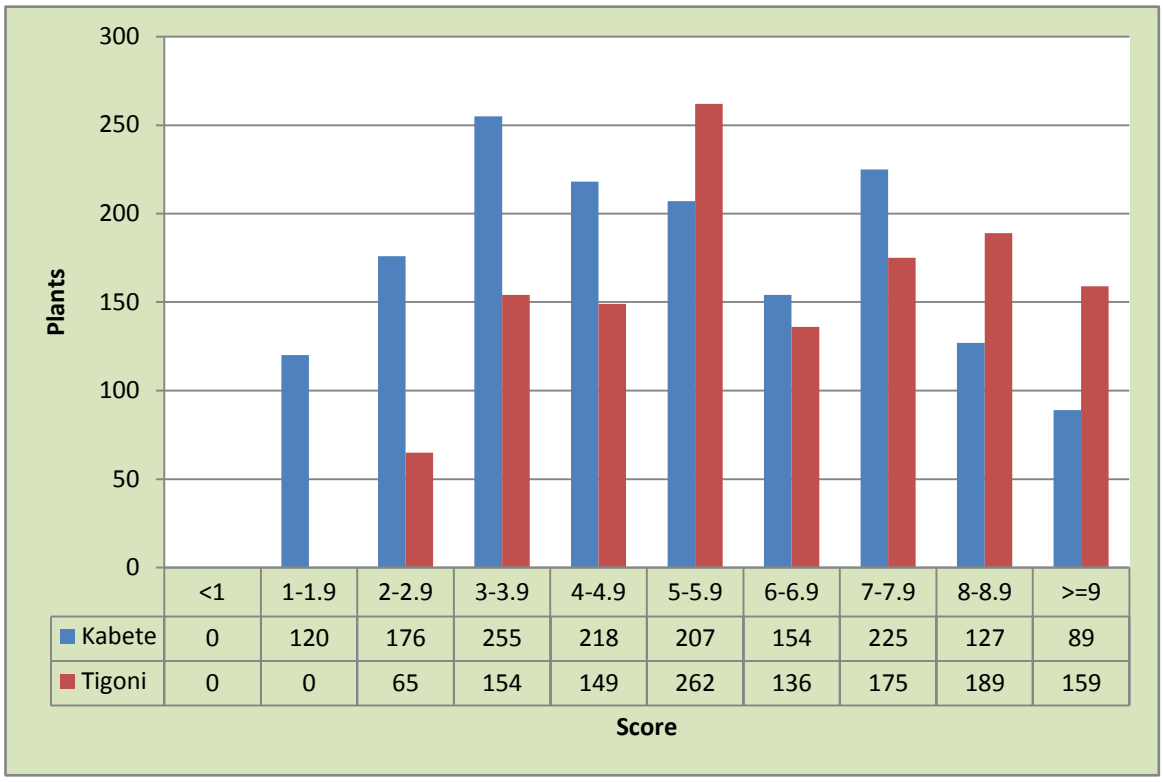
	KABETE		TIGONI	
GENOTYPES	**R**	**S**	**R**	**S**
GLP1004///G10909/G2333//AND1062/VAX6	56.1	43.9	21.1	78.9
GLP1004///G10909/G2333//RWR719/VAX6	47.2	52.8	22.2	77.8
GLP1004///MEX54/G2333//AND1062/VAX6	44.4	55.6	22	78
GLP1004///MEX54/G2333//RWR719/VAX6	30.1	69.9	26.5	73.5
New Rosecoco///G10909/G2333//AND1062/VAX6	34	66	22.4	77.6
New Rosecoco///G10909/G2333//RWR719/VAX6	38	62	17.9	82.1
New Rosecoco///MEX54/G2333//AND1062/VAX6	32.9	67.1	12.8	87.2
New Rosecoco///MEX54/G2333//RWR719/VAX6	49.5	50.5	25	75
GLP24///G10909/G2333//AND1062/VAX6	52.1	47.9	16.3	83.8
GLP24///G10909/G2333//RWR719/VAX6	49.5	50.5	13.6	86.4
GLP24///MEX54/G2333//AND1062/VAX6	38.7	61.3	20.3	79.7
GLP24///MEX54/G2333//RWR719/VAX6	46.3	53.7	17.6	82.4
Kenya Umoja///G10909/G2333//AND1062/VAX6	30.9	69.1	17.3	82.7
Kenya Umoja///G10909/G2333//RWR719/VAX6	40.9	59.1	19	81
Kenya Umoja///MEX54/G2333//AND1062/VAX6	56.8	43.2	20.5	79.5
Kenya Umoja///MEX54/G2333//RWR719/VAX6	54.7	45.3	18.6	81.4

R=resistant, S=susceptible

5.4.3.5 Anthracnose

Male gamete and location had significant effect on anthracnose at P<0.01. Commercial variety had a significant effect at P<0.05 on anthracnose. The two way interaction between male gamete and location had a significant effect on anthracnose at P<0.01. Interaction between commercial variety and location had no significant effect on anthracnose. The three way interaction between male gamete, commercial variety and location had significant effect on anthracnose at P<0.01 (Appendix 7). In Kabete plants had an almost normal distribution with a

majority of plants having an intermediate reaction to *Colletotrichum lindemuthianum,* followed by resistant reaction and much fewer plants having a susceptible reaction (Figure 5.13). In Tigoni we had fewer resistant reactions and more intermediate and susceptible reactions.



	<1	1-1.9	2-2.9	3-3.9	4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	>=9
Kabete	0	120	176	255	218	207	154	225	127	89
Tigoni	0	0	65	154	149	262	136	175	189	159

Figure 5.14 Frequency distribution for F_1s reaction to anthracnose

The range of anthracnose infection in Kabete among the 16 populations was 1-9 (Table 5.10). In Tigoni, disease range for the 16 populations was 2-9. Among the parental lines, anthracnose severity in Kabete and Tigoni ranged from 4 to 9 save for G2333 which had disease scores from 1 to 1.7 in Kabete and Tigoni. In both locations, G2333 was highly resistant (1.2). The differential AND 1062 and

commercial variety GLP 24 were the most susceptible genotypes with scores of 8.1 each. The mean score for anthracnose was 5.5. Anthracnose was severe in Tigoni than Kabete. Anthracnose disease reaction mean score in Kabete was 5.0 and 6.1 in Tigoni. Among the commercial varieties, Kenya Umoja populations were more susceptible to anthracnose. Populations having MEX54/G2333//RWR 719/VAX6 male gamete in there their combination were the least susceptible to anthracnose with a mean score of 5.2. GLP 1004 populations were the least susceptible to anthracnose with a mean disease reaction score of 5.4. In Kabete the least susceptible populations were those of GLP1004 with an average score of 4.7. In Tigoni, GLP24 populations were the least susceptible with an average score of 6.0. Among the 16 populations, the least susceptible population was New Rosecoco///MEX54/G2333//RWR719/VAX6 with a mean score of 5.2. The most susceptible population was New Rosecoco///G10909/G2333//AND 1062/VAX6 with a disease mean score of 6.2.

Table 5.10 Anthracnose severity of 16 F_1 populations grown at Kabete and Tigoni during the 2012 long rain season

Male gamete	KABETE	TIGONI	MEAN
G10909/G2333//AND1062/VAX6	4.9	6.4	5.7
G10909/G2333//RWR719/VAX6	5.2	6.1	5.6
MEX54/G2333//AND1062/VAX6	5.3	6.0	5.6
MEX54/G2333//RWR719/VAX6	4.5	5.9	5.2
Commercial variety			
GLP1004	4.7	6.1	5.4
New Rosecoco	4.9	6.2	5.6
GLP24	5.1	6.0	5.6
Kenya Umoja	5.3	6.2	5.7
F_1			
GLP1004///G10909/G2333//AND1062/VAX6	4.8	6.2	5.5
GLP1004///G10909/G2333//RWR719/VAX6	4.9	6.1	5.5
GLP1004///MEX54/G2333//AND1062/VAX6	4.8	6.6	5.7
GLP1004///MEX54/G2333//RWR719/VAX6	4.2	5.3	4.7
New Rosecoco///G10909/G2333//AND1062/VAX6	5.5	6.9	6.2
New Rosecoco///G10909/G2333//RWR719/VAX6	4.6	6.0	5.3
New Rosecoco///MEX54/G2333//AND1062/VAX6	5.4	5.6	5.5
New Rosecoco///MEX54/G2333//RWR719/VAX6	4.1	6.3	5.2
GLP24///G10909/G2333//AND1062/VAX6	4.5	6.4	5.5
GLP24///G10909/G2333//RWR719/VAX6	5.1	6.4	5.7
GLP24///MEX54/G2333//AND1062/VAX6	5.5	5.5	5.5
GLP24///MEX54/G2333//RWR719/VAX6	5.2	5.9	5.5
Kenya Umoja///G10909/G2333//AND1062/VAX6	5.0	6.2	5.6
Kenya Umoja///G10909/G2333//RWR719/VAX6	6.2	5.8	6.0
Kenya Umoja///MEX54/G2333//AND1062/VAX6	5.3	6.4	5.9
Kenya Umoja///MEX54/G2333//RWR719/VAX6	4.6	6.2	5.4
Mean	5.0	6.1	5.5
LSD (0.05)	1.3		
CV%	13.4		

For every population grown in Kabete, 144 plants were planted for evaluation while in Tigoni, 108 was the number of plants planted. Due to different factors not all the plants made it to disease evaluation. The population that was most resistant to anthracnose in Kabete was GLP24///G10909/G2333//AND 1062/VAX6 with 50% of 79 plants being resistant. The opposite was true for Kenya Umoja///MEX54/G2333//AND1062/VAX6 that had 80.5% of 78 plants being susceptible in Kabete. In Tigoni, the best anthracnose resistance of 74.4% of 78 plants was recorded by Kenya Umoja///MEX54/G2333//AND1062/VAX6. The contrary was true for New Rosecoco///G10909/G2333//AND1062/VAX6 and New Rosecoco///MEX54/G2333//AND1062/VAX6 each scoring 52% susceptibility out of 83 and 93 respectively.

Table 5.11 Percentages of anthracnose resistant plants in F_1 populations at Kabete and Tigoni

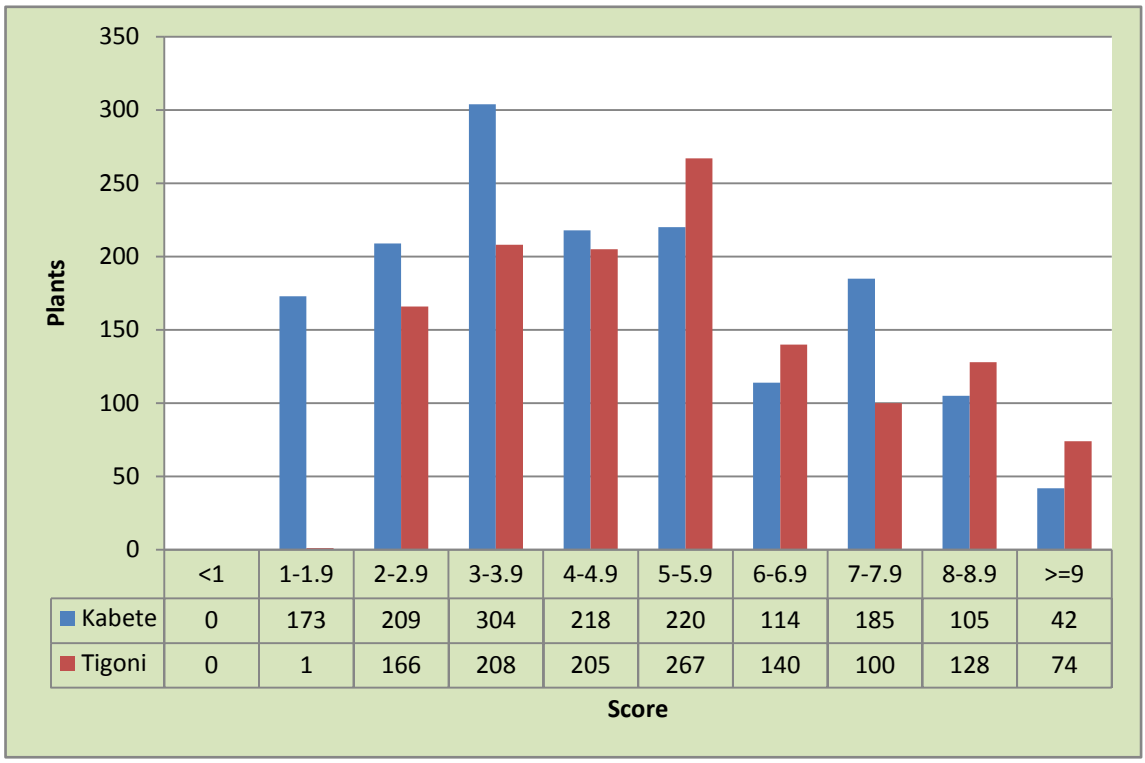
	KABETE		TIGONI	
GENOTYPES	R	S	R	S
GLP1004///G10909/G2333//AND1062/VAX6	34.6	65.4	61.9	38.1
GLP1004///G10909/G2333//RWR719/VAX6	35.5	64.5	57	43
GLP1004///MEX54/G2333//AND1062/VAX6	32	68	58.1	41.9
GLP1004///MEX54/G2333//RWR719/VAX6	26.2	73.8	53.4	46.6
New Rosecoco///G10909/G2333//AND1062/VAX6	38.3	61.7	47.1	52.9
New Rosecoco///G10909/G2333//RWR719/VAX6	26	74	59.4	40.6
New Rosecoco///MEX54/G2333//AND1062/VAX6	36.5	63.5	47.5	52.5
New Rosecoco///MEX54/G2333//RWR719/VAX6	30.9	69.1	61.5	38.5
GLP24///G10909/G2333//AND1062/VAX6	50	50	51	49
GLP24///G10909/G2333//RWR719/VAX6	28	72	63.9	36.1
GLP24///MEX54/G2333//AND1062/VAX6	20.8	79.2	65.1	34.9
GLP24///MEX54/G2333//RWR719/VAX6	28.7	71.3	61.7	38.3
Kenya Umoja///G10909/G2333//AND1062/VAX6	32	68	49.2	50.8
Kenya Umoja///G10909/G2333//RWR719/VAX6	32.7	67.3	55.6	44.4
Kenya Umoja///MEX54/G2333//AND1062/VAX6	19.5	80.5	74.4	25.6
Kenya Umoja///MEX54/G2333//RWR719/VAX6				

R=resistant, S=susceptible

5.4.3.6 Common bacterial blight

Location and male gamete each had a significant effect on common bacterial blight at $P<0.01$. Commercial variety had a significant effect on common bacterial blight infections at $P<0.05$. The two way interactions between male gamete and location and commercial variety and location both had no significant effect on common bacterial blight. The three way interaction between location, commercial variety and male gamete was significant at $P<0.01$ for common bacterial blight (Appendix 7). In both locations Kabete and Tigoni, a majority of

plants had intermediate reaction to *Xanthomonas campestris* pv *phaseoli*, followed by resistant reaction and the least was susceptible reaction (Figure 5.14). The range of infection for common bacterial blight was 1 to 9 for the 16 populations in Kabete. In Tigoni the disease scores for the 16 populations varied from 2 to 9.



	<1	1-1.9	2-2.9	3-3.9	4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	>=9
Kabete	0	173	209	304	218	220	114	185	105	42
Tigoni	0	1	166	208	205	267	140	100	128	74

Figure 5.15 Frequency distribution for F_1s reaction to common bacterial blight

The range of common bacterial blight infection among the parental lines was 3.3-8 in Kabete and 3-9 in Tigoni (Table 5.12). However disease reaction scores for VAX 6 ranged from 1 to 2.7 in Kabete and from 1 to 2 in Tigoni. New Rosecoco was intermediately resistant among the commercial varieties with a

mean score of 5.7. The mean disease reaction score for common bacterial blight was 4.9**.** Common bacterial blight disease reaction mean score in Kabete was 4.5 while in Tigoni it was 5.3. Variance for common bacterial blight for the populations was 3.7-6 in Kabete and 3-4.5 in Tigoni. Among the male gametes, MEX 54/G2333//RWR719/VAX6 was the least susceptible population to common bacterial blight with disease reaction mean score of 4.7. Among the commercial varieties, GLP24 populations were the most susceptible to common bacterial blight with a mean score of 5.0. The 16 populations had an intermediate disease reaction to common bacterial blight with GLP 24///MEX 54/G2333//AND 1062/ VAX6 being the most susceptible with mean score of 6.4.

Table 5.12 Common bacterial blight severity of 16 F_1 populations grown at Kabete and Tigoni during the 2012 long rain season

Male gamete	KABETE	TIGONI	MEAN
G10909/G2333//AND1062/VAX6	4.4	5.3	4.8
G10909/G2333//RWR719/VAX6	4.7	5.4	5.1
MEX54/G2333//AND1062/VAX6	4.7	5.3	5.0
MEX54/G2333//RWR719/VAX6	4.2	5.1	4.7
Commercial variety			
GLP1004	4.4	5.3	4.9
New Rosecoco	4.5	5.2	4.9
GLP24	4.6	5.3	5.0
Kenya Umoja	4.5	5.2	4.8
F_1			
GLP1004///G10909/G2333//AND1062/VAX6	4.5	5.0	4.8
GLP1004///G10909/G2333//RWR719/VAX6	6.0	5.5	5.7
GLP1004///MEX54/G2333//AND1062/VAX6	3.6	5.1	4.4
GLP1004///MEX54/G2333//RWR719/VAX6	3.5	5.7	4.6
New Rosecoco///G10909/G2333//AND1062/VAX6	3.9	5.5	4.7
New Rosecoco///G10909/G2333//RWR719/VAX6	4.4	5.6	5.0
New Rosecoco///MEX54/G2333//AND1062/VAX6	5.4	4.6	5.0
New Rosecoco///MEX54/G2333//RWR719/VAX6	4.3	5.2	4.8
GLP24///G10909/G2333//AND1062/VAX6	4.9	4.8	4.8
GLP24///G10909/G2333//RWR719/VAX6	3.8	5.3	4.5
GLP24///MEX54/G2333//AND1062/VAX6	4.6	6.4	5.5
GLP24///MEX54/G2333//RWR719/VAX6	5.2	4.9	5.0
Kenya Umoja///G10909/G2333//AND1062/VAX6	4.2	5.8	5.0
Kenya Umoja///G10909/G2333//RWR719/VAX6	4.8	5.1	5.0
Kenya Umoja///MEX54/G2333//AND1062/VAX6	5.0	5.2	5.1
Kenya Umoja///MEX54/G2333//RWR719/VAX6	4.0	4.5	4.3
Mean	4.5	5.3	4.9
LSD (0.05)	1.2		
CV%	14.6		

For all the 16 populations grown in Kabete, nine populations had more than 40% common bacterial blight resistance (Table 5.13). Of the nine, GLP1004///G10909/G2333//RWR 719/VAX6 and Kenya Umoja///G10909/G2333 //RWR719/VAX6 were outstanding with 46.7% of 107 and 46.8% of 111 common bacterial blight resistances respectively. In Kabete, New Rosecoco///G10909 /G2333//RWR719/VAX6 and Kenya Umoja///MEX54/G2333//RWR719/VAX6 were the most susceptible plants with scores of 73.7% out of 99, and 70.9% out of 86, respectively. The best performing population in Tigoni was GLP1004///MEX54/G2333//RWR719/VAX6 with 28.4% of its 67 plants being resistant. Trailing it was GLP24///MEX54/G2333//RWR719/VAX6 with 28.2% of 85 plants being resistant. On the other hand New Rosecoco///MEX54 /G2333//RWR719/VAX6 was the most susceptible population to common bacterial blight in Tigoni with 86.8% of its 76 plants being susceptible.

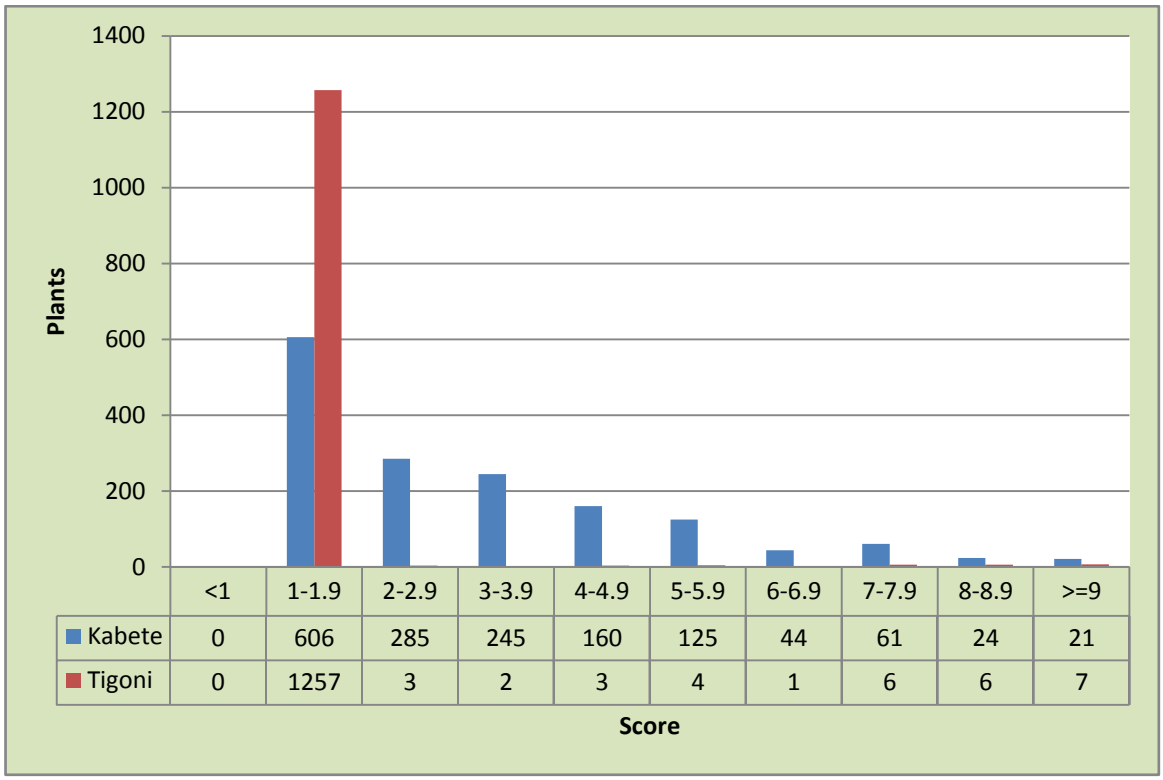
Table 5.13 Percentages of common bacterial blight resistant plants in F_1 populations at Kabete and Tigoni

	KABETE		TIGONI	
GENOTYPES	**R**	**S**	**R**	**S**
GLP1004///G10909/G2333//AND1062/VAX6	44.3	55.7	19.7	80.3
GLP1004///G10909/G2333//RWR719/VAX6	46.7	53.3	24.4	75.6
GLP1004///MEX54/G2333//AND1062/VAX6	42	58	14.6	85.4
GLP1004///MEX54/G2333//RWR719/VAX6	35.9	64.1	28.4	71.6
New Rosecoco///G10909/G2333//AND1062/VAX6	37.2	62.8	18.8	81.2
New Rosecoco///G10909/G2333//RWR719/VAX6	26.3	73.7	23.8	76.2
New Rosecoco///MEX54/G2333//AND1062/VAX6	35.3	64.7	23.4	76.6
New Rosecoco///MEX54/G2333//RWR719/VAX6	46.3	53.7	13.2	86.8
GLP24///G10909/G2333//AND1062/VAX6	41.4	58.6	27.5	72.5
GLP24///G10909/G2333//RWR719/VAX6	37	63	14.8	85.2
GLP24///MEX54/G2333//AND1062/VAX6	33	67	19	81
GLP24///MEX54/G2333//RWR719/VAX6	43.2	56.8	28.2	71.8
Kenya Umoja///G10909/G2333//AND1062/VAX6	43.3	56.7	19.5	80.5
Kenya Umoja///G10909/G2333//RWR719/VAX6	46.8	53.2	21.4	78.6
Kenya Umoja///MEX54/G2333//AND1062/VAX6	43.2	56.8	19.2	80.8
Kenya Umoja///MEX54/G2333//RWR719/VAX6	29.1	70.9	18.8	81.2

R=resistant, S=susceptible

5.4.3.7 *Pythium* root rot

Location and male gamete each had a significant effect (P<0.01) on Pythium root rot disease reaction. Commercial variety had a significant effect at P<0.01 on root rot. The two way interactions between male gamete and location and commercial variety and location both had significant effects (P<0.01) on root rot. The three way interaction between location, commercial variety and male gamete was significant (P<0.01) for root rot (Appendix 7). In locations Kabete and Tigoni, more plants had a resistant reaction to *Pythium* root rot, few plants had intermediate reaction and much fewer plants had susceptible reaction (Figure 5.15).



	<1	1-1.9	2-2.9	3-3.9	4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	>=9
Kabete	0	606	285	245	160	125	44	61	24	21
Tigoni	0	1257	3	2	3	4	1	6	6	7

Figure 5.16 Frequency distribution for F_1s reaction to *Pythium* root rot

Most of the populations in Kabete had a disease reaction of 1-9 for root rot save for three populations New Rosecoco///G10909/G2333//RWR719/VAX6, GLP24///G10909/G2333//RWR719/VAX6, GLP24///MEX54/G2333//AND1062/ VAX6 that had disease reaction range of 1-8.3, 1-6.7, and 1-8.3 (Table 5.14). In Tigoni the disease scores ranged from 1 to 7. Four populations in Tigoni had resistance to root rot namely Kenya Umoja///G10909/G2333//AND1062/VAX6 (1-1.7), GLP1004///G10909/G2333//RWR719/VAX6 (1-1.7), Kenya Umoja///MEX 54/G2333//AND1062/VAX6 (1-4) and New Rosecoco///MEX54/G2333//AND1062/ VAX6 (1-3). All Parental lines showed high resistance to root rot. The mean disease

reaction for root rot was 2.2. Root rot disease reaction mean score in Kabete was 3.0 and 1.4 in Tigoni. Variance for root rot was 1.57-4.63 in Kabete and 0.1-3.5 in Tigoni. For resistance to root rot G10909/G2333//AND1062/VAX6 populations were the least resistant with a mean score of 2.4. Among the commercial varieties, New Rosecoco populations were the least resistant to root rot with disease average score of 2.6. Generally *Pythium* root rot had reactions that can be categorized as resistant (1-3). New Rosecoco///MEX54/G2333//AND1062/VAX6 was the least resistant to root rot with a disease reaction mean score of 2.8.

Table 5.14 *Pythium* root rot severity of 16 F_1 populations grown at Kabete and Tigoni during the 2012 long rain season

Male gamete	KABETE	TIGONI	MEAN
G10909/G2333//AND1062/VAX6	3.4	1.4	2.4
G10909/G2333//RWR719/VAX6	2.6	1.5	2.1
MEX54/G2333//AND1062/VAX6	3.1	1.3	2.2
MEX54/G2333//RWR719/VAX6	3.0	1.3	2.2
Commercial variety			
GLP1004	2.8	1.3	2.0
New Rosecoco	3.6	1.6	2.6
GLP24	3.0	1.4	2.2
Kenya Umoja	2.7	1.2	2.0
F_1			
GLP1004///G10909/G2333//AND1062/VAX6	3.6	1.4	2.5
GLP1004///G10909/G2333//RWR719/VAX6	1.9	1.2	1.6
GLP1004///MEX54/G2333//AND1062/VAX6	2.8	1.2	2.0
GLP1004///MEX54/G2333//RWR719/VAX6	2.8	1.2	2.0
New Rosecoco///G10909/G2333//AND1062/VAX6	4.0	1.3	2.6
New Rosecoco///G10909/G2333//RWR719/VAX6	3.0	2.1	2.6
New Rosecoco///MEX54/G2333//AND1062/VAX6	3.9	1.6	2.8
New Rosecoco///MEX54/G2333//RWR719/VAX6	3.6	1.5	2.6
GLP24///G10909/G2333//AND1062/VAX6	3.2	1.7	2.5
GLP24///G10909/G2333//RWR719/VAX6	2.3	1.3	1.8
GLP24///MEX54/G2333//AND1062/VAX6	3.3	1.3	2.3
GLP24///MEX54/G2333//RWR719/VAX6	3.2	1.2	2.2
Kenya Umoja///G10909/G2333//AND1062/VAX6	2.6	1.2	1.9
Kenya Umoja///G10909/G2333//RWR719/VAX6	3.4	1.3	2.3
Kenya Umoja///MEX54/G2333//AND1062/VAX6	2.3	1.1	1.7
Kenya Umoja///MEX54/G2333//RWR719/VAX6	2.5	1.3	1.9
Mean	3.0	1.4	2.2
LSD (0.05)	1.0		
CV%	31.4		

All the populations in Kabete had more than 60% being resistant *Pythium* root rot save for Kenya Umoja///MEX54/G2333//AND1062/VAX6 that had only 47.5% of its 118 plants being resistance thereby being the most susceptible population with 52.5% of its 118 plants being susceptible (Table 5.15). Among the good performers New Rosecoco///MEX54/G2333//AND1062/VAX6 stood out with 85.9% of its 85 plants showing resistance. In Tigoni recorded resistances were not as high as in Kabete however one population was exceptional, New Rosecoco///MEX54/G2333//AND1062/VAX6 registered *Pythium* root rot resistance of 62.4% out of 94 evaluated plants. In Tigoni New Rosecoco///MEX54/G2333//RWR719/VAX6 was the most susceptible population to *Pythium* root rot with 74.4% of its 76 plants being susceptible.

Table 5.15 Percentages of *Pythium* root rot resistant plants in F_1 populations at Kabete and Tigoni

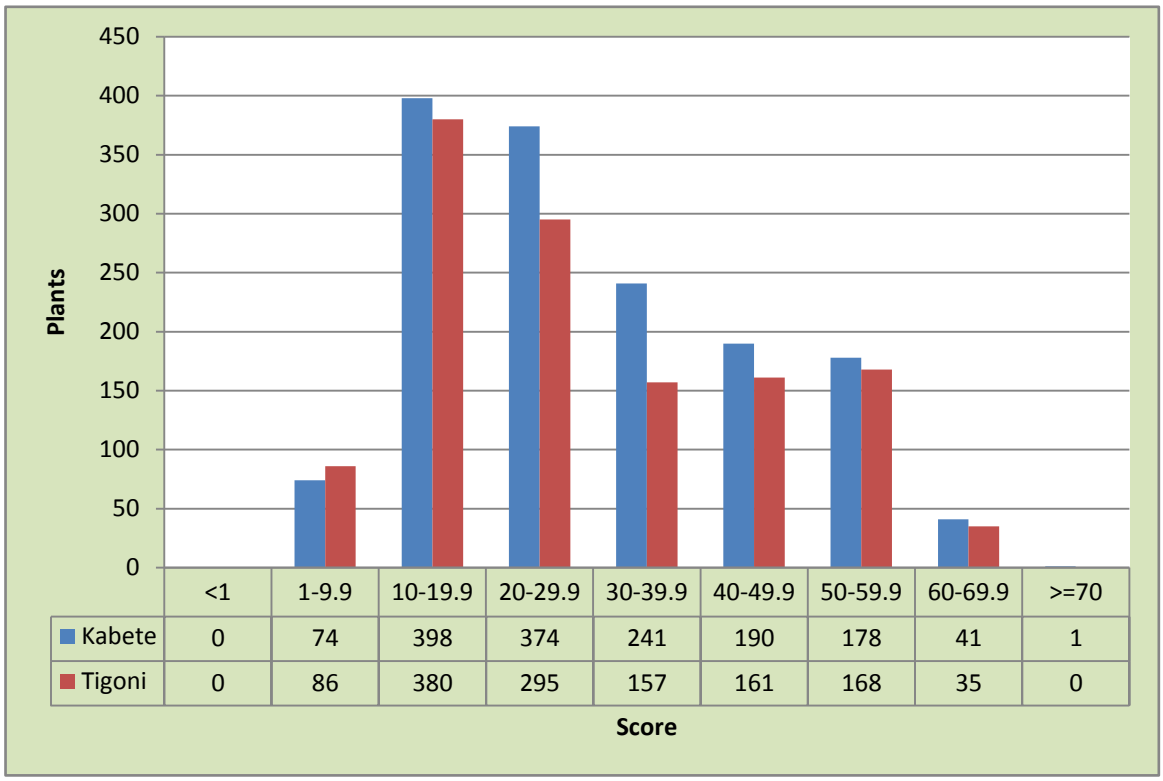
	KABETE		TIGONI	
GENOTYPES	**R**	**S**	**R**	**S**
GLP1004///G10909/G2333//AND1062/VAX6	66	34	36.7	63.3
GLP1004///G10909/G2333//RWR719/VAX6	77.6	22.4	52.1	47.9
GLP1004///MEX54/G2333//AND1062/VAX6	78	22	39.9	60.1
GLP1004///MEX54/G2333//RWR719/VAX6	68.6	31.4	47.5	52.5
New Rosecoco///G10909/G2333//AND1062/VAX6	78.7	21.3	46.9	53.1
New Rosecoco///G10909/G2333//RWR719/VAX6	62	38	38.5	61.5
New Rosecoco///MEX54/G2333//AND1062/VAX6	85.9	14.1	62.4	37.6
New Rosecoco///MEX54/G2333//RWR719/VAX6	61.9	38.1	25.6	74.4
GLP24///G10909/G2333//AND1062/VAX6	67.6	32.4	45.9	54.1
GLP24///G10909/G2333//RWR719/VAX6	64.5	35.5	29.5	70.5
GLP24///MEX54/G2333//AND1062/VAX6	64.2	35.8	34.6	65.4
GLP24///MEX54/G2333//RWR719/VAX6	73.7	26.3	51.8	48.2
Kenya Umoja///G10909/G2333//AND1062/VAX6	64.9	35.1	35.8	64.2
Kenya Umoja///G10909/G2333//RWR719/VAX6	73.9	26.1	45.1	54.9
Kenya Umoja///MEX54/G2333//AND1062/VAX6	47.5	52.5	26.8	73.2
Kenya Umoja///MEX54/G2333//RWR719/VAX6	64	36	34.3	65.7

R=resistant, S=susceptible

5.4.3.8 Pod plant^{-1}

Location had no significant effect on pods plant^{-1}. Male gamete had a significant effect ($P<0.01$) on pods plant^{-1}. Commercial variety had significant effect ($P<0.01$) on pods plant^{-1}. Location by male gamete interaction had a significant effect ($P<0.01$) on pods plant^{-1}. Location by commercial variety interaction had significant effect ($P<0.01$) on pods plant^{-1}. The three way interaction between location, male gamete and commercial variety was significant at ($P<0.01$) on pods plant^{-1} (Appendix 8). Plants in Kabete and Tigoni a majority had 10 to 30

pods plant^{-1} followed by 31 to 60 pods plant^{-1} (Figure 5.16). Across all the 16 populations, pods plant^{-1} ranged from 3 pods plant^{-1} to 89 pods plant^{-1} at Kabete and Tigoni.



	<1	1-9.9	10-19.9	20-29.9	30-39.9	40-49.9	50-59.9	60-69.9	>=70
Kabete	0	74	398	374	241	190	178	41	1
Tigoni	0	86	380	295	157	161	168	35	0

Figure 5.17 Frequency distribution for F_1s number of pods plant^{-1}

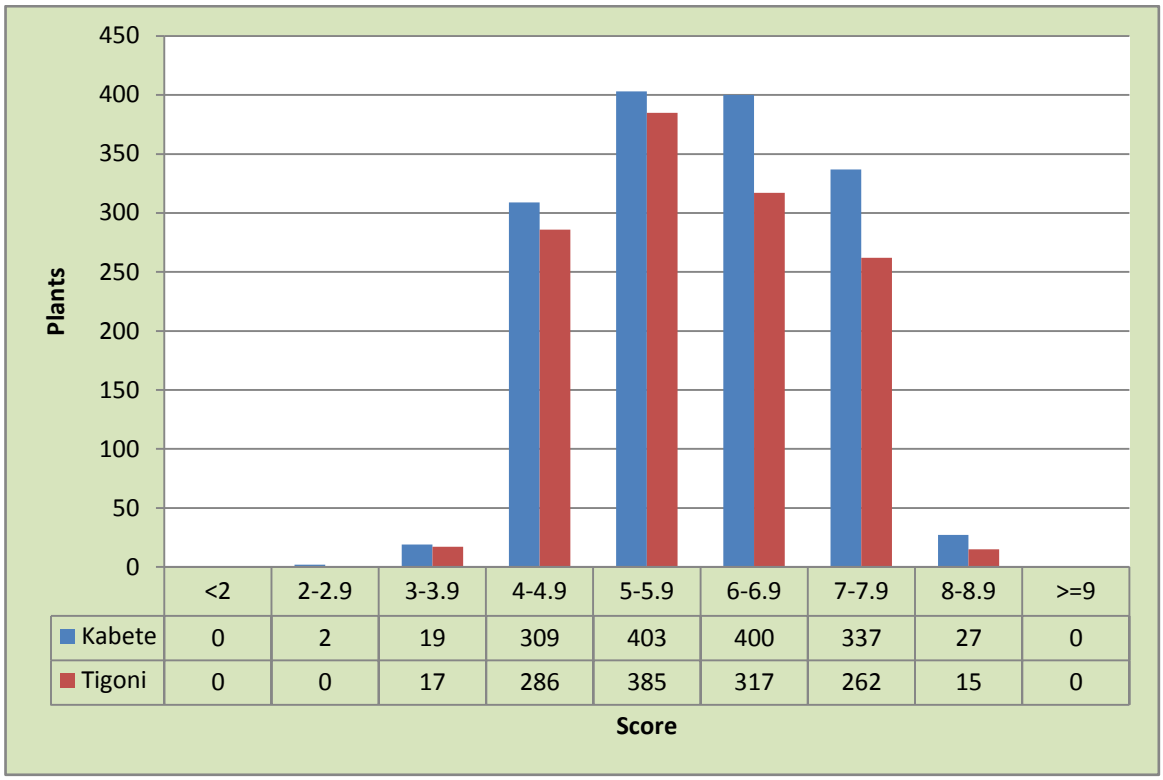
The mean number of pods plant^{-1} was 29.3 with a variance range of between 180 and 280 (Table 5.16). The populations that had the male gamete G10909/G2333//RWR719/VAX6 in their combination had the highest number of pods plant^{-1} (30.3). The populations that had New Rosecoco in their combination had the highest number of pods plant^{-1} at 30.4. Populations in Kabete had an average of 30 pods plant^{-1}.The average number of pods plant^{-1}for the 16-populations at Tigoni was 29. The population with the highest number of pods plant^{-1} was New Rosecoco///G10909/G2333//RWR719/VAX6 with 34.8 pods plant^{-1}.The least bearing population was Kenya Umoja///MEX54/G2333//RWR 719/VAX6 with 26.1 pods plant^{-1}.

Table 5.16 Pods plant^{-1} of 16 F_1 populations grown at Kabete and Tigoni during the 2012 long rain season

Male gamete	KABETE	TIGONI	MEAN
G10909/G2333//AND1062/VAX6	29.5	28.1	28.8
G10909/G2333//RWR719/VAX6	30.9	29.6	30.3
MEX54/G2333//AND1062/VAX6	28.1	30.1	29.1
MEX54/G2333//RWR719/VAX6	29.9	27.9	28.9
Commercial variety			
GLP1004	27.8	28.7	28.3
New Rosecoco	31.7	29.1	30.4
GLP24	29.4	28.8	29.1
Kenya Umoja	29.6	29	29.3
F_1			
GLP1004///G10909/G2333//AND1062/VAX6	31	28.7	29.8
GLP1004///G10909/G2333//RWR719/VAX6	28.6	25.4	27
GLP1004///MEX54/G2333//AND1062/VAX6	25	27.9	26.5
GLP1004///MEX54/G2333//RWR719/VAX6	26.9	32.9	29.9
New Rosecoco///G10909/G2333//AND1062/VAX6	29.2	28.1	28.7
New Rosecoco///G10909/G2333//RWR719/VAX6	36.9	32.7	34.8
New Rosecoco///MEX54/G2333//AND1062/VAX6	28	29.2	28.6
New Rosecoco///MEX54/G2333//RWR719/VAX6	32.6	26.2	29.4
GLP24///G10909/G2333//AND1062/VAX6	30.6	24.1	27.4
GLP24///G10909/G2333//RWR719/VAX6	28	28.6	28.3
GLP24///MEX54/G2333//AND1062/VAX6	27.8	33.2	30.5
GLP24///MEX54/G2333//RWR719/VAX6	31.2	29.3	30.2
Kenya Umoja///G10909/G2333//AND1062/VAX6	27.4	31.3	29.4
Kenya Umoja///G10909/G2333//RWR719/VAX6	30.3	31.5	30.9
Kenya Umoja///MEX54/G2333//AND1062/VAX6	31.6	30.1	30.8
Kenya Umoja///MEX54/G2333//RWR719/VAX6	29	23.1	26.1
Mean	29.6	28.9	29.3
LSD (0.05)			
CV%			

5.4.3.9 Seeds pod^{-1}

Location had a significant effect ($P<0.05$) on seeds pod^{-1}. Male gamete had no significant effect on seeds pod^{-1}. Commercial variety had significant effect ($P<0.01$) on seeds pod^{-1}. Location by male gamete interaction had a significant effect ($P<0.01$) on seeds pod^{-1}. Location by commercial variety interaction had a significant effect ($P<0.01$) on seeds pod^{-1}. The three way interaction between location, male gamete and commercial variety was significant at ($P<0.01$) on seeds pod^{-1} (Appendix 8). A majority of plants in Kabete and Tigoni had 4 to 8 seeds. A few plants had less than 4 seeds or more than 8 seeds (Figure 5.17). Across all the 16 populations, in both locations at Kabete and Tigoni the range of seeds pod^{-1} was 3-8.



	<2	2-2.9	3-3.9	4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	>=9
Kabete	0	2	19	309	403	400	337	27	0
Tigoni	0	0	17	286	385	317	262	15	0

Figure 5.18 Frequency distribution for F_1s seeds pod^{-1}

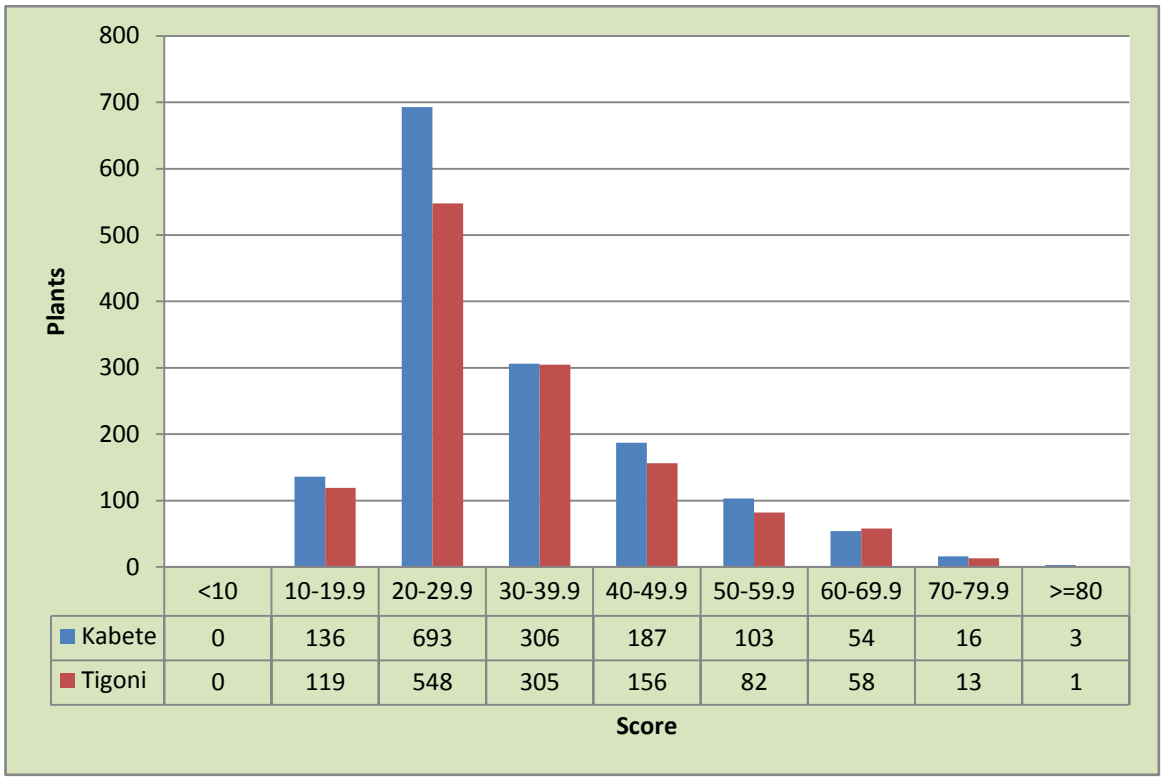
The mean number of seeds pod^{-1} was 5.8 with a variance of 1.09-1.5 (Table 5.17). Seeds pod^{-1} for all the F_1s for all the male gametes were uniform at 5.8. Among the commercial varieties GLP 1004 F_1s had the highest number of seeds pod^{-1} at 5.9. The highest number of seeds pod^{-1}was 6.1 achieved by two populations GLP1004///G10909/G2333//RWR719/VAX6 and New Rosecoco/// G10909/G2333//AND1062/VAX6. The least number of seeds per pod was 5.6 recorded by several populations.

Table 5.17 Seeds pod^{-1} of 16 F_1 populations grown at Kabete and Tigoni during the 2012 long rain season

Male gamete	KABETE	TIGONI	MEAN
G10909/G2333//AND1062/VAX6	5.8	5.8	5.8
G10909/G2333//RWR719/VAX6	6	5.7	5.8
MEX54/G2333//AND1062/VAX6	5.8	5.7	5.8
MEX54/G2333//RWR719/VAX6	5.8	5.7	5.8
Commercial variety			
GLP1004	6.1	5.7	5.9
New Rosecoco	5.8	5.7	5.8
GLP24	5.7	5.8	5.7
Kenya Umoja	5.8	5.7	5.8
F_1			
GLP1004///G10909/G2333//AND1062/VAX6	5.7	5.9	5.8
GLP1004///G10909/G2333//RWR719/VAX6	6.6	5.6	6.1
GLP1004///MEX54/G2333//AND1062/VAX6	6.2	5.7	5.9
GLP1004///MEX54/G2333//RWR719/VAX6	6.1	5.7	5.9
New Rosecoco///G10909/G2333//AND1062/VAX6	6.3	5.9	6.1
New Rosecoco///G10909/G2333//RWR719/VAX6	5.6	5.7	5.6
New Rosecoco///MEX54/G2333//AND1062/VAX6	5.8	5.5	5.6
New Rosecoco///MEX54/G2333//RWR719/VAX6	5.6	5.7	5.7
GLP24///G10909/G2333//AND1062/VAX6	5.6	5.7	5.6
GLP24///G10909/G2333//RWR719/VAX6	5.8	5.7	5.8
GLP24///MEX54/G2333//AND1062/VAX6	5.7	5.8	5.7
GLP24///MEX54/G2333//RWR719/VAX6	5.7	5.8	5.8
Kenya Umoja///G10909/G2333//AND1062/VAX6	5.8	5.6	5.7
Kenya Umoja///G10909/G2333//RWR719/VAX6	6	5.9	5.9
Kenya Umoja///MEX54/G2333//AND1062/VAX6	5.5	5.9	5.7
Kenya Umoja///MEX54/G2333//RWR719/VAX6	5.8	5.6	5.7
Mean	5.9	5.7	5.8
LSD (0.05)			
CV%			

5.4.3.10 100-seed mass

Location had no significant effect on 100-seed mass. Male gamete had no significant effect on 100 seed mass. Commercial variety had significant effect ($P<0.01$) on 100 seed mass. Location by male gamete interaction had no significant effect on 100 seed mass. Location by commercial variety interaction had a significant effect ($P<0.01$) on 100 seed mass. The three way interaction between location, male gamete and commercial variety was significant at ($P<0.01$) on 100 seed weight (Appendix 8). Many of the plants had 100-seed mass of between 10 to 30 followed by 31 to 60 (Figure 5.18). The mass for 100 seeds ranged from 15 to 87 grams per 100 seeds in both locations for all the 16 populations.



	<10	10-19.9	20-29.9	30-39.9	40-49.9	50-59.9	60-69.9	70-79.9	>=80
Kabete	0	136	693	306	187	103	54	16	3
Tigoni	0	119	548	305	156	82	58	13	1

Figure 5.19 Frequency distribution for F_1s 100-seed mass

The mean for mass of 100 seeds was 32.5 with a variance ranging from 116 to 260. Mass of 100 seeds for all the populations for all the male gametes were uniform at 32 (Table 5.18). Among the commercial varieties GLP24 populations at 33.4 grams had the highest mass of 100 seeds. The population with the most weight for 100-seed mass was Kenya Umoja///MEX54/G2333//RWR719/VAX6 at 35.6 grams. The population with the least weight for 100 seeds was GLP1004///MEX54/G2333//RWR719/VAX6 (29.6 g).

Table 5.18 100-seed mass of 16 F_1 populations grown at Kabete and Tigoni during the 2012 long rain season

Male gamete	KABETE	TIGONI	MEAN
G10909/G2333//AND1062/VAX6	32.1	32	32
G10909/G2333//RWR719/VAX6	32.1	32.3	32.2
MEX54/G2333//AND1062/VAX6	31.8	33	32.4
MEX54/G2333//RWR719/VAX6	32.1	32.7	32.4
Commercial variety			
GLP1004	31.1	30.1	30.6
New Rosecoco	31.6	33.3	32.4
GLP24	32.7	34.1	33.4
Kenya Umoja	32.8	32.6	32.7
F_1			
GLP1004///G10909/G2333//AND1062/VAX6	30.3	29.6	30
GLP1004///G10909/G2333//RWR719/VAX6	33.2	30.1	31.6
GLP1004///MEX54/G2333//AND1062/VAX6	31.1	31.1	31.1
GLP1004///MEX54/G2333//RWR719/VAX6	29.7	29.4	29.6
New Rosecoco///G10909/G2333//AND1062/VAX6	30.3	31.6	31
New Rosecoco///G10909/G2333//RWR719/VAX6	34.3	35.1	34.7
New Rosecoco///MEX54/G2333//AND1062/VAX6	29.4	33	31.2
New Rosecoco///MEX54/G2333//RWR719/VAX6	32.3	33.5	32.9
GLP24///G10909/G2333//AND1062/VAX6	32.1	36.1	34.1
GLP24///G10909/G2333//RWR719/VAX6	32.3	32.7	32.5
GLP24///MEX54/G2333//AND1062/VAX6	34.6	36	35.3
GLP24///MEX54/G2333//RWR719/VAX6	31.7	31.5	31.6
Kenya Umoja///G10909/G2333//AND1062/VAX6	35.5	30.7	33.1
Kenya Umoja///G10909/G2333//RWR719/VAX6	28.9	31.2	30
Kenya Umoja///MEX54/G2333//AND1062/VAX6	32	32.1	32.1
Kenya Umoja///MEX54/G2333//RWR719/VAX6	34.7	36.4	35.6
Mean	32	32.5	32.3
LSD (0.05)			
CV%			

Across both locations the number of Mesoamericans (≤40g) among the F_1s was higher than Andeans (Table 5.19).

Table 5.19 Segregation for seed size in F_1 populations

	100 Seed Weight					
	Kabete			Tigoni		
	Mesoamerican		Andean	Mesoamerican		Andean
GENOTYPES	Small	Medium	Large	Small	Medium	Large
GLP1004///G10909/G2333//AND1062/VAX6	49	22	30	37	16	18
GLP1004///G10909/G2333//RWR719/VAX6	54	34	18	41	16	31
GLP1004///MEX54/G2333//AND1062/VAX6	50	24	19	36	25	21
GLP1004///MEX54/G2333//RWR719/VAX6	42	26	26	36	18	14
New Rosecoco///G10909/G2333//AND1062/VAX6	38	31	22	39	21	25
New Rosecoco///G10909/G2333//RWR719/VAX6	35	26	29	35	20	27
New Rosecoco///MEX54/G2333//AND1062/VAX6	30	27	24	52	22	20
New Rosecoco///MEX54/G2333//RWR719/VAX6	46	24	26	35	17	23
GLP24///G10909/G2333//AND1062/VAX6	34	16	20	51	14	14
GLP24///G10909/G2333//RWR719/VAX6	43	19	31	47	19	15
GLP24///MEX54/G2333//AND1062/VAX6	46	33	19	25	20	34
GLP24///MEX54/G2333//RWR719/VAX6	43	26	25	42	20	23
Kenya Umoja///G10909/G2333//AND1062/VAX6	37	31	21	45	15	21
Kenya Umoja///G10909/G2333//RWR719/VAX6	48	29	27	43	20	21
Kenya Umoja///MEX54/G2333//AND1062/VAX6	53	34	26	38	19	21
Kenya Umoja///MEX54/G2333//RWR719/VAX6	39	20	25	38	14	18

Location had significant effect ($P<0.01$) on F_1 yields. Male gamete and the two way interaction between location and male gamete had no significant effect on F_1 yields. Commercial variety, two way interactions between location and commercial variety, male gamete and commercial variety and the three way interaction between location, male gamete and commercial variety all had significant effect at $p<0.05$ on F_1 yields. Male gamete had no significant effect on $F_{1.2}$ yields however, commercial variety as well as the interaction between male gamete and commercial variety had significant effect on the $F_{1.2}$ data at $P<0.01$ (Appendix 8). Grain yield of F_1 populations varied from 2283.25 kg ha $^{-1}$ to 5432.5 kg ha $^{-1}$ with a mean of 3,943.9 kg ha^{-1} over the two locations (Table 5.20). In $F_{1.2}$ the mean yield of $F_{1.2}$ families was 2,671 kg ha^{-1}. G10909/G2333//RWR719/VAX6 cross combination yielded highest with 4,095 kg ha^{-1}. In Tigoni G10909/G2333//RWR719/VAX6 had the highest yield of 4,634.3 kg ha^{-1}. In Kabete the high yielding male gamete was G10909/G2333//AND1062/VAX6 with 3,628.4 kg ha^{-1}. In $F_{1.2}$ cross combinations involving MEX54/G2333//RWR719/VAX6 double cross were the best yielding with 3,202 kg ha^{-1}. On the basis of commercial variety the cross combinations involving New Rosecoco were the best yielding with a mean of 4,194.5 kg ha^{-1}. In Kabete cross combinations involving New Rosecoco yielded highly at 3,936.2 kg ha^{-1}. In Tigoni, Kenya Umoja cross combinations yielded highly with 4,497.4 kg ha^{-1}. With 3,320

kg ha^{-1}, Kenya Umoja cross combinations yielded the highest in $F_{1.2}$. Among the 16 populations, New Rosecoco///MEX54/G2333//AND1062/VAX6 was high yielding with 5,052 kg ha^{-1}. In Kabete, GLP1004///G10909/G2333//AND 1062/VAX6 with yields of 4,513 kg ha^{-1} was the best yielding while in Tigoni New Rosecoco///MEX54/G2333//AND1062/VAX6 attained the highest yields of 5,675.1 kg ha^{-1} . In $F_{1.2}$ the population that attained the highest yields was Kenya Umoja///MEX54/G2333//RWR719/VAX6 with 3,981 kg ha^{-1}.

Table 5.20 Yield of F_1 and $F_{1.2}$ population grown at Kabete and Tigoni during the 2012 long and short rain seasons

	F_1 kg ha^{-1}			$F_{1.2}$ kg ha^{-1}
Male gamete	**Kabete**	**Tigoni**	**Mean**	**Kabete**
G10909/G2333//AND1062/VAX6	3628.4	4241.9	3935.1	2670
G10909/G2333//RWR719/VAX6	3555.7	4634.3	4095.0	2611
MEX54/G2333//AND1062/VAX6	3395.9	4438.1	3917.0	2201
MEX54/G2333//RWR719/VAX6	3305.3	4351.6	3828.5	3202
Commercial variety				
GLP1004	3364.4	4478.3	3921.4	2080
New Rosecoco	3936.2	4452.8	4194.5	2810
GLP24	3520.5	4237.4	3878.9	2473
Kenya Umoja	3064.1	4497.5	3780.8	3320
F_1				
GLP1004///G10909/G2333//AND1062/VAX6	4513.0	4901.4	4707.2	1512
GLP1004///G10909/G2333//RWR719/VAX6	2846.5	4392.8	3619.7	2121
GLP1004///MEX54/G2333//AND1062/VAX6	3093.3	3211.6	3152.5	1908
GLP1004///MEX54/G2333//RWR719/VAX6	3004.9	5407.5	4206.2	2782
New Rosecoco///G10909/G2333//AND1062/VAX6	3354.1	3306.9	3330.5	2897
New Rosecoco///G10909/G2333//RWR719/VAX6	4315.8	5505.6	4910.7	3213
New Rosecoco///MEX54/G2333//AND1062/VAX6	4428.9	5675.1	5052.0	2328
New Rosecoco///MEX54/G2333//RWR719/VAX6	3646.1	3323.4	3484.7	2803
GLP24///G10909/G2333//AND1062/VAX6	4080.3	4576.2	4328.2	2768
GLP24///G10909/G2333//RWR719/VAX6	3215.2	3625.7	3420.4	2177
GLP24///MEX54/G2333//AND1062/VAX6	2696.9	4198.5	3447.7	1705
GLP24///MEX54/G2333//RWR719/VAX6	4089.6	4549.1	4319.3	3241
Kenya Umoja///G10909/G2333//AND1062/VAX6	2566.1	4183.0	3374.6	3502
Kenya Umoja///G10909/G2333//RWR719/VAX6	3845.3	5013.0	4429.1	2933
Kenya Umoja///MEX54/G2333//AND1062/VAX6	3364.3	4667.4	4015.8	2863
Kenya Umoja///MEX54/G2333//RWR719/VAX6	2480.6	4126.5	3303.6	3981
Mean	3471.3	4416.5	3943.9	2671

5.4.4 Summary of findings

Among the four commercial varieties used for introgression, New Rosecoco F_1s showed intermediate resistance to the four diseases of interest in this study (Table 5.21). All the F_1s showed a resistance of 2.2 against *Pythium* root rot. All the populations showed intermediate resistance to anthracnose at 5.5. Resistance to angular leaf spot was scored at 5.0 while resistance to common bacterial blight was scored at 4.9. The background used in this case the different commercial varieties influence the expression of the resistance genes. F_1s of G10909/G2333//RWR719/VAX6 with yields of 4095 kg ha^{-1} were the highest. In the case of commercial varieties, F_1s of New Rosecoco yielded highest with 4194.5 kg ha^{-1}. In $F_{1.2}$ plants of MEX54/G2333//RWR719/VAX6 gave the highest yields of 3202 kg ha^{-1}. Kenya Umoja $F_{1.2}$s among the commercial varieties yielded highly with yields of 3320 kg ha^{-1}.

Table 5.21 Summary of disease reactions and F_1 and $F_{1.2}$ yields for the different male gametes and commercial varieties

	Disease reaction					Yield Kg ha^{-1}	
Male gamete	ALS	ANTH	CBB	RR	MEAN	F_1	$F_{1.2}$
G10909/G2333//AND1062/VAX6	4.8	5.7	4.8	2.4	4.4	3935.1	2670
G10909/G2333//RWR719/VAX6	5.0	5.6	5.1	2.1	4.5	4095.0	2611
MEX54/G2333//AND1062/VAX6	5.1	5.6	5.0	2.2	4.5	3917.0	2201
MEX54/G2333//RWR719/VAX6	5.0	5.2	4.7	2.2	4.3	3828.5	3202
Commercial variety							
GLP1004	4.9	5.4	4.9	2.0	4.3	3921.4	2080
New Rosecoco	4.9	5.6	4.9	2.6	4.5	4194.5	2810
GLP24	5.3	5.6	5.0	2.2	4.5	3878.9	2473
Kenya Umoja	4.8	5.7	4.8	2.0	4.3	3780.8	3320
Mean	5.0	5.5	4.9	2.2	4.4		

5.4 DISCUSSION

Marker assisted selection was effective in selecting plants with resistance genes to be introgressed into commercial varieties. Early generation testing was effective in identifying plants with resistance genes. Of the 89 male gamete plants screened for angular leaf spot, anthracnose, common bacterial blight and *Pythium* root rot, 3 plants had 3 resistance genes, 8 had 2, 36 had 1. Selection for yield and other agronomic traits, including resistance to biotic and abiotic stresses, plant vigour, flowering, and maturity has been extensively utilized by bean breeders to develop cultivars with superior performance or to develop cultivars that are adapted to specific environments and/or cropping systems

(Acquaah *et al*., 1991; Brothers and Kelly, 1993; Kelly *et al.*, 1998; Nienhuis and Singh, 1986; Schneider *et al.*, 1997; Scully *et al.*, 1991; Singh, 1994; Singh and Muñoz, 1999). Field disease reactions for angular leaf spot (page 97, Table 5.8) and anthracnose (page 101, Table 5.10) for some of the commercial varieties were higher (intermediate) than green house disease reactions (resistant) (page 66, Table 4.4). In the green house evaluations were done using pathogen isolates from Kabete. In the field conditions are much hostile and in Tigoni we had presence of other pathotypes other than the Kabete isolates that were used in the green house. Angular leaf spot, anthracnose and common bacterial blight were severe in Tigoni than Kabete. The weather condition in Tigoni is wet almost all year round. This condition is favourable for disease development. Roots rots in Kenya are prevalent in western therefore not much of the disease was witnessed in both locations. Markers were used for selection of MEX/G2333//RWR719/VAX6 resistant plants for introgression. Three plants had 3 resistance genes, 8 had 2, 36 had 1 and 42 had none. This is in consideration that the marker for *Pythium* root rot did not work. This was effective and confirms that markers can be used to select for plants with the resistance gene. Among the four male gametes constituted, G10909/G2333//AND1062/VAX6 F_1s showed intermediate resistance to angular leaf spot (4.4) (Table 5.21). MEX54/G2333//RWR719/VAX6 was the least susceptible to anthracnose (5.2) and

common bacterial blight (4.7). G10909/G2333//RWR719/VAX6 was resistant to root rot (2.1).

5.5 CONCLUSION

Markers are an effective tool in identifying resistant segregants in early generation for population advancement. To improve on disease pressure during field selection inoculations could be performed if the resources allow. Selection under disease pressure is a vital as a proof of concept even if the presence of resistance genes has been confirmed by markers.

CHAPTER 6 : CONCLUSIONS AND RECOMMENDATIONS

6.1 Prevalence of diseases

During the survey of 2010 and 2011 (Figure 2) it was evident that angular leaf spot caused by *Phaeoisariopsis griseola* was the most prevalent disease in most Kenyan farmers' farms. In all the farms visited *P. griseola* had representatively attacked the common bean crop. Bean anthracnose caused by *Colletotrichum lindemuthianum* was second in prevalence across the regions. Common bacterial blight was next in frequency of occurrence in the farms visited. Loitokitok and Njukini regions of the Rift valley were particularly highly infested by *Xanthomonas axonopodis* pv. *Phaseoli* (Xap). *Pythium* root rot was the least occurring with high incidences recorded in Western Kenya and pockets in Taita Taveta. Wortmann *et al* (1998) found angular leaf spot (*Phaeoisariopsis griseola*), anthracnose (*Colletotrichum lindemuthianum*), rust (*Uromyces appendiculatus*), common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*), and bean common mosaic (caused by a virus) to be the major diseases of common bean in Africa and indeed eastern Africa. These diseases are widespread and therefore justify this research effort.

6.2 Resistance in parental lines

The disease reactions (page 64, Table 4.3) corresponded with the marker-selection data (page 66, Table 4.4). The genotypes that were found to have the markers for the resistance genes also showed low disease reactions with the pathogens.

6.3 Diversity of *Colletotrichum lindemuthianum*

Twelve race groups of *Colletotrichum lindemuthianum* were characterized from a total of 31 isolates indicating high variability of the pathogen in the bean growing areas of Kenya. The most widely spread race that could be considered of importance in Kenyan breeding programs in future are races 65 with 8 isolates being characterized and race 73 with 4 isolates. Races 485, 55, 1 and 2 were the least occurring with only one isolate each being designated to them. Of the 12 races identified seven races (17, 2, 38, 23, 1, 55, and 485) had been previously identified while five races were new (81, 65, 73, 87, and 89). Previous studies by Kinyua (1976) and Mwangi (1986) reported races 17, 2, 23, 38, and 55. Another *Colletotrichum lindemuthianum* diversity study was done by Gathuru and Mwangi (1991) and reported races 2, 38, 1, 23 and 17 in Kenya. The latest study was conducted by Ombiri *et al* (1997) and race 485 was reported for the first time in Kenya. In Kenya now a total of 12 races have been identified namely; 2, 1, 38, 23, 17, 55, 81, 65, 73, 87, 89 and 485. This information is going to be

relevant to common bean breeding programs for breeding beans that are resistant to identified races of *Colletotrichum lindemuthianum* in Kenya.

6.4 Gamete selection in breeding for multiple constraints and selection for agronomic traits

Both resistance donor parents (MEX 54, G 10909, G 2333, AND 1062, RWR 719 and VAX 6) and recipient parents (Kenya Umoja, New rosecoco, GLP 1004 and Canadian wonder) were tested for markers of resistance genes before embarking on hybridizations. Thus Mexico 54 and G10909 was tested for $OPE04_{709}$ (*Phg*-1 gene), SH-13 (*Phg*-1 gene) and $SNO2_{890}$; G2333 was used to validate SAB-3 (*Co-5* gene), SAS-13 (*Co-4*^{2} gene) and SBB-14 (*Co-4*^{2} gene) linked to resistance genes. SAP6 and SU91 were validated with VAX 6 plants while RWR719 and AND1062 were tested for PYAA. SAB-3 for anthracnose, SH-13 for angular leaf spot and SU-91 for common bacterial blight were found to be polymorphic among both donor and recipient parents and were selected for use in this study. No positive amplification was observed with the PYAA-19 marker for *Pythium* root rot.

ii) Marker assisted population development

Gamete selection allows screening and selection for desirable dominant and co-dominant alleles during hybridization and immediately after production of the final multiple parent F_1 hybrids (Singh, 1994). After production of the double cross MEX54/G2333//RWR719/VAX6 screening was done for markers of

resistance genes to aid in selection of male plants with the resistance genes before making the final cross between the male plants and the susceptible commercial variety Kenya Umoja. Out of the 89 male plants that were screened 11 (with 2 or 3 resistance genes) were selected for introgression.

6.5 Early generation gamete selection

For desirable dominant and co-dominant alleles and possibly other segregating traits with high heritability, evaluation and selection of the final cross commence in F_1 (Singh, 1994). Of the 89 male gamete plants screened with markers for angular leaf spot, anthracnose, common bacterial blight and *Pythium* root rot, 3 plants had 3 resistance genes, 8 had 2, 36 had 1 and 42 had none. A total of 11 plants with either three or two desired resistance genes were selected for introgression into Kenya Umoja. The F_1 hybrid seed resulting from each pair of plant-to-plant crosses was sown in Kabete and Tigoni. Data on vigor, days-to-flowering, days-to-maturity, 100 seed mass, and disease reactions were recorded.

6.6 Conclusions

Colletotrichum lindemuthianum is very diverse. Race characterisation should be an on-going activity due to emergence of new pathotypes. Marker assisted selection was effective in selecting plants with resistance genes to be

introgressed into commercial varieties. Early generation testing was effective in identifying potentially good plants for advancement therefore avoiding advancing plants with no desirable traits. Gamete selection was successful in simultaneously transferring multiple resistance. Among the four male gametes constituted, G10909/G2333//AND1062/VAX6 F_1s showed the least susceptibility to the four diseases assessed in this study with a mean score of 7.4. Among the four commercial varieties used for introgression, New Rosecoco (New Rosecoco) F_1s showed the least susceptibility to the four diseases of interest in this study.

6.7 Recommendations

Marker assisted selection should be adopted by breeding programs in the region for its efficiency in selecting plants with the resistance genes in early generations thus reducing time for developing resistant varieties. Complete pathogen characterisation of other common bean pathogens in Kenya is recommended. Pathogen characterisation should be carried out often due to the changing importance of different pathotypes. The $F_{1.2}$ families selected from this study need to be evaluated further. In subsequent studies root rots should be tested in western Kenya where they are more prevalent.

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CHAPTER 8 : APPENDICES

Appendix 1: Formula for making 0.5M EDTA pH 8.0 (1 Litre)

186.12 g of EDTA

750ml of ddH_20

Add about 20 g of NaOH pellets

Slowly add more NaOH until the pH is 8.0

Make up the volume to 1000 ml using ddH_20

Sterilize by autoclaving

(**Note**: EDTA will not completely dissolve until the pH is around 8.0)

Appendix 2: Formula 1 M Tris pH 8.0 (1 Litre)

Dissolve 121.1 g of Tris base in 800 ml of H_2O.

Adjust pH to 8.0 by adding 42 ml of concentrated HCL.

Allow the solution to cool to room temperature before

making the final adjustments to the pH.

Adjust the volume to 1 L with H_2O.

Sterilize using an autoclave.

Appendix 3: Formula for making 1X TE buffer (1 Litre)

10 mM Tris (10 ml of 1 M Tris-HCl, pH 8.0)

0.1 mM EDTA (500 µl of 0.5 M EDTA, pH 8.0)

Make up to 950ml with de-ionized water and adjust to pH 8.0 using HCl and adjust total volume to 1 liter. Sterilize by autoclaving

Appendix 4: Formula for making 5x TBE (Tris-Borate EDTA) buffer pH 8.0 (1 Litre)

54 g Tris base

27.5 g Boric acid

20 ml 0.5M EDTA, pH 8.0

Stir, do not adjust the pH

Add sterile distilled water to 1000 ml

Dilute to working concentration of 1x by taking 400 ml

of 5x TBE and diluting it to 2000 ml using 1600 ml of ddH_2O

Appendix 5: Mean maximum and minimum temperature and rainfall at Kabete Field Station, 2010-2012.

Period	2010			2011			2012		
	Temperature (°C)			Temperature (°C)			Temperature (°C)		
	Mean Max	Mean Min	Rainfall (mm)	Mean Max	Mean Min	Rainfall (mm)	Mean Max	Mean Min	Rainfall (mm)
Jan	23.7	14.0	143.5	25.3	13.3	4.2	-	11.9	0.0
Feb	24.9	15.0	73.8	26.5	13.6	66.3	26.4	13.5	16.0
Mar	23.9	14.8	250.3	25.7	14.6	147.7	26.6	13.9	5.0
Apr	23.8	15.5	252.8	24.0	15.3	80.7	23.9	15.0	352.6
May	22.5	14.8	266.1	23.3	14.7	93.9	23.5	14.2	262.0
Jun	21.5	13.5	51.9	23.2	13.5	47.8	22.3	12.8	39.9
Jul	21.1	11.5	2.0	23.4	11.3	14.3	21.4	12.0	23.4
Aug	21.5	11.8	29.9	21.2	12.7	26.9	22.7	11.7	42.4
Sep	23.8	12.0	19.9	23.9	13.2	32.5	24.6	12.2	8.9
Oct	24.8	13.8	64.3	23.9	14.5	154.2	24.6	14.2	241.5
Nov	22.5	14.4	93.3	23.0	14.6	175.7	23.3	14.1	261.8
Dec	23.7	13.8	74.5	23.2	14.0	245.5	22.8	14.1	244.7

Appendix 6: Mean squares for vigour, days to flowering and maturity of 16 F_1 populations grown at Kabete and Tigoni during the 2012 long rain season

Source of variation	df	Vigour	Flowering	Maturity
Replication	2	0.0	2.3	1.6
Location	1	10.7	210.7**	21.6NS
Error (a)	2	0.0	2.6	1.8
Male Gamete	3	1.3**	1.2NS	14.7**
Location*Male Gamete	3	2.3**	1.5NS	11.7**
Error (b)	12	0.0	2.6	1.9
Commercial Variety	3	2.2**	1.8**	40.5**
Location*Commercial Variety	3	0.9**	1.2**	34.8**
Male Gamete*Commercial Variety	9	1.4**	3.0**	48.1**
Location*Male Gamete*Commercial Variety	9	1.0**	2.1**	15.6**
Pooled Error (c)	48	0.0	0.4	0.8
Total	95			

* and ** Significant at 0.05 and 0.01 respectively
NS not significant

Appendix 7: Mean squares for angular leaf spot (ALS), anthracnose (ANTH), common bacterial blight (CBB) and Pythium root rot (RR) of 16 F_1 populations grown at Kabete and Tigoni during the 2012 long rain season

Source of variation	d.f	ALS	ANTH	CBB	RR
Replication	2	0.1	1.1	0.1	0.1
Location	1	35.0**	30.8*	14.0**	64.8**
Error (a)	2	0.1	0.9	0.1	0.1
Male Gamete	3	0.6**	1.1**	0.7**	0.4**
Location*Male Gamete	3	2.0**	0.8**	0.1^{NS}	0.7**
Error (b)	12	0.1	0.1	0.1	0.1
Commercial Variety	3	0.9**	0.5*	0.1*	2.2**
Location*Commercial Variety	3	1.5**	0.4^{NS}	0.1^{NS}	0.4**
Male Gamete*Commercial Variety	9	1.1**	0.5**	1.1**	0.5**
Location*Male Gamete*Commercial Variety	9	1.3**	0.9**	2.0**	0.5**
Pooled Error (c)	48	0.1	0.2	0.0**	0.0
Total	95				

* and ** Significant at 0.05 and 0.01 respectively
NS not significant

Appendix 8: Mean squares for pods plant^{-1}, seeds pod^{-1}, 100-seed mass and yield plot^{-1}of 16 F_1 populations grown at Kabete and Tigoni during the 2012 long rain season

Source of variation	df	Pods plant^{-1}	Seeds pod^{-1}	100 seed wt	Yield kg ha^{-1}
Replication	2	0.1	0.0	0.4	112353.0
Location	1	12.4^{NS}	0.3*	5.6^{NS}	21440518.0**
Error (a)	2	2.1	0.0	6.9	126339.0
Male Gamete	3	10.8**	0.0^{NS}	0.8^{NS}	295633.0^{NS}
Location*Male Gamete	3	20.5**	0.1^{NS}	2.0^{NS}	294939.0^{NS}
Error (b)	12	1.8	0.0	1.2	92710.0
Commercial Variety	3	17.6**	0.2**	34.6**	753147.0**
Location*Commercial Variety	3	12.3**	0.3**	10.0**	1005321.0**
Male Gamete*Commercial Variety	9	35.7**	0.1**	23.6**	3594578.0**
Location*Male Gamete*Commercial Variety	9	29.5**	0.1**	8.0**	962410.0**
Pooled Error (c)	48	2.4	0.0	1.1	117944.0
Total	95				

* and ** Significant at 0.05 and 0.01 respectively
NS not significant

APPENDIX 9: Mean squares for F_2 yield

Source of Variation	df	F_2Yield kgha^{-1}
Replication	2	406283.0
Male Gamete	3	2024157.0^{NS}
Error (a)	6	609241.0
Commercial Variety	3	3312157.0**
Male Gamete X Commercial Variety	9	468914.0**
Pooled Error (b)	24	124012.0
Total	47	

Appendix 10: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 2Pa from Bureti at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	5.7	6.0	7.7	6.5
2	MDRK	A	2	3.3	3.3	3.3	3.3
3	Perry Marrow	A	4	2.3	2.7	3.0	2.7
4	Cornell 49-242	MA	8	1.3	2.0	2.3	1.9
5	Widusa	A	16	2.7	3.3	3.7	3.2
6	Kaboon	A	32	1.7	2.3	3.0	2.3
7	Mexico 222	MA	64	4.7	6.0	6.3	5.7
8	PI 207262	MA	128	1.3	2.0	2.7	2.0
9	TO	MA	256	2.0	3.7	4.3	3.3
10	TU	MA	512	2.7	3.0	3.0	2.9
11	AB 136	MA	1024	3.0	3.3	4.0	3.4
12	G2333	MA	2048	1.0	1.3	2.0	1.4

Appendix 11: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 2Pb from Bureti at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	4.0	5.3	6.7	5.3
2	MDRK	A	2	1.0	1.3	1.7	1.3
3	Perry Marrow	A	4	2.0	2.3	3.3	2.5
4	Cornell 49-242	MA	8	1.0	1.0	1.0	1.0
5	Widusa	A	16	4.7	6.0	7.3	6.0
6	Kaboon	A	32	2.3	3.0	3.0	2.8
7	Mexico 222	MA	64	5.7	6.0	7.0	6.2
8	PI 207262	MA	128	2.0	3.3	3.7	3.0
9	TO	MA	256	1.0	1.3	1.3	1.2
10	TU	MA	512	2.3	2.7	3.0	2.7
11	AB 136	MA	1024	1.3	1.7	2.0	1.7
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 12: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 30Pa from Othaya at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	5.7	6.3	8.3	6.8
2	MDRK	A	2	2.3	3.0	3.0	2.8
3	Perry Marrow	A	4	2.3	3.7	4.3	3.4
4	Cornell 49-242	MA	8	1.0	1.0	1.3	1.1
5	Widusa	A	16	5.7	8.0	9.0	7.6
6	Kaboon	A	32	1.0	1.3	1.7	1.3
7	Mexico 222	MA	64	3.0	3.0	3.3	3.1
8	PI 207262	MA	128	2.3	2.7	3.0	2.7
9	TO	MA	256	1.0	1.7	2.3	1.7
10	TU	MA	512	1.0	1.0	1.3	1.1
11	AB 136	MA	1024	1.0	1.3	1.7	1.3
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 13: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 3Pa from Kakamega at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	2.0	2.3	2.3	2.2
2	MDRK	A	2	5.7	6.0	7.7	6.5
3	Perry Marrow	A	4	5.3	6.7	8.0	6.7
4	Cornell 49-242	MA	8	1.0	1.3	2.0	1.4
5	Widusa	A	16	2.3	2.7	3.0	2.7
6	Kaboon	A	32	6.0	7.3	7.7	7.0
7	Mexico 222	MA	64	3.3	3.3	3.3	3.3
8	PI 207262	MA	128	1.0	1.3	2.0	1.4
9	TO	MA	256	1.0	1.7	2.3	1.7
10	TU	MA	512	1.0	1.0	1.7	1.2
11	AB 136	MA	1024	1.0	1.7	2.0	1.6
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 14: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 11Pa from Nakuru at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	6.0	6.7	8.0	6.9
2	MDRK	A	2	2.0	2.0	2.7	2.2
3	Perry Marrow	A	4	4.0	4.7	6.0	4.9
4	Cornell 49-242	MA	8	1.7	2.0	2.0	1.9
5	Widusa	A	16	2.7	3.3	3.3	3.1
6	Kaboon	A	32	6.3	7.0	8.3	7.2
7	Mexico 222	MA	64	4.7	6.0	6.3	5.7
8	PI 207262	MA	128	5.7	8.0	9.0	7.6
9	TO	MA	256	3.4	3.7	3.7	3.6
10	TU	MA	512	1.7	2.0	2.7	2.1
11	AB 136	MA	1024	1.7	2.0	2.0	1.9
12	G2333	MA	2048	1.0	1.3	2.0	1.4

Appendix 15: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 28Pa from Meru at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	5.3	6.0	7.7	6.3
2	MDRK	A	2	1.3	1.7	2.3	1.8
3	Perry Marrow	A	4	2.0	2.3	3.0	2.4
4	Cornell 49-242	MA	8	2.7	3.7	4.3	3.6
5	Widusa	A	16	6.7	7.3	7.3	7.1
6	Kaboon	A	32	2.3	2.7	3.0	2.7
7	Mexico 222	MA	64	5.7	6.7	7.3	6.6
8	PI 207262	MA	128	1.7	2.3	3.0	2.3
9	TO	MA	256	2.3	2.7	2.7	2.6
10	TU	MA	512	2.7	3.3	3.7	3.2
11	AB 136	MA	1024	1.0	1.3	1.7	1.3
12	G2333	MA	2048	1.0	1.0	1.3	1.1

Appendix 16: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 5Pb from Bomet at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	6.3	6.7	8.0	7.0
2	MDRK	A	2	2.3	2.7	3.0	2.7
3	Perry Marrow	A	4	2.0	3.7	4.3	3.3
4	Cornell 49-242	MA	8	1.3	2.0	2.0	1.8
5	Widusa	A	16	2.7	3.0	3.7	3.1
6	Kaboon	A	32	3.3	3.3	3.7	3.4
7	Mexico 222	MA	64	4.7	6.0	7.0	5.9
8	PI 207262	MA	128	1.7	2.3	3.0	2.3
9	TO	MA	256	2.7	3.3	3.3	3.1
10	TU	MA	512	2.3	3.7	4.3	3.4
11	AB 136	MA	1024	1.3	2.0	2.7	2.0
12	G2333	MA	2048	1.0	1.7	2.3	1.7

Appendix 17: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 22Pa from Njoro at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	6.3	7.3	8.7	7.4
2	MDRK	A	2	3.0	3.3	3.3	3.2
3	Perry Marrow	A	4	2.3	3.0	3.0	2.8
4	Cornell 49-242	MA	8	3.7	4.0	4.0	3.9
5	Widusa	A	16	1.0	4.3	5.0	3.4
6	Kaboon	A	32	2.7	2.7	3.0	2.8
7	Mexico 222	MA	64	5.7	6.3	8.0	6.7
8	PI 207262	MA	128	2.0	2.0	2.0	2.0
9	TO	MA	256	1.0	1.0	1.3	1.1
10	TU	MA	512	1.0	1.0	1.0	1.0
11	AB 136	MA	1024	1.0	1.3	1.7	1.3
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 18: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 35Pa from Kerugoya at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	6.3	6.7	7.3	6.8
2	MDRK	A	2	2.0	3.7	4.3	3.3
3	Perry Marrow	A	4	2.0	2.3	2.3	2.2
4	Cornell 49-242	MA	8	3.3	4.7	6.3	4.8
5	Widusa	A	16	3.3	3.3	3.3	3.3
6	Kaboon	A	32	2.7	3.3	3.7	3.2
7	Mexico 222	MA	64	5.3	6.0	6.3	5.9
8	PI 207262	MA	128	1.0	1.0	1.7	1.2
9	TO	MA	256	1.0	1.0	1.0	1.0
10	TU	MA	512	1.0	1.3	1.7	1.3
11	AB 136	MA	1024	1.0	1.7	2.0	1.6
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 19: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 4Pa from Kericho at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	6.0	6.0	7.7	6.6
2	MDRK	A	2	2.7	3.0	3.3	3.0
3	Perry Marrow	A	4	1.7	2.3	3.0	2.3
4	Cornell 49-242	MA	8	1.0	1.0	1.0	1.0
5	Widusa	A	16	2.0	3.7	4.3	3.3
6	Kaboon	A	32	2.3	2.7	3.0	2.7
7	Mexico 222	MA	64	5.3	5.7	6.0	5.7
8	PI 207262	MA	128	1.0	1.0	1.0	1.0
9	TO	MA	256	1.0	1.3	1.7	1.3
10	TU	MA	512	1.0	1.0	1.0	1.0
11	AB 136	MA	1024	1.0	1.0	1.0	1.0
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 20: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 44Pa from Kabete at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	5.7	6.3	7.0	6.3
2	MDRK	A	2	6.3	7.0	8.3	7.2
3	Perry Marrow	A	4	5.0	5.7	6.3	5.7
4	Cornell 49-242	MA	8	1.0	1.0	1.0	1.0
5	Widusa	A	16	4.7	6.0	7.3	6.0
6	Kaboon	A	32	6.0	6.7	8.0	6.9
7	Mexico 222	MA	64	2.7	3.0	3.3	3.0
8	PI 207262	MA	128	1.0	1.0	1.0	1.0
9	TO	MA	256	2.3	2.7	3.0	2.7
10	TU	MA	512	1.0	1.3	1.7	1.3
11	AB 136	MA	1024	1.3	1.7	2.3	1.8
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 21: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 4Pb from Kericho at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	1.7	2.3	3.0	2.3
2	MDRK	A	2	5.3	6.7	8.3	6.8
3	Perry Marrow	A	4	6.0	7.7	8.7	7.5
4	Cornell 49-242	MA	8	1.0	1.0	1.0	1.0
5	Widusa	A	16	3.0	3.3	4.0	3.4
6	Kaboon	A	32	5.3	6.3	7.7	6.4
7	Mexico 222	MA	64	2.3	2.7	3.0	2.7
8	PI 207262	MA	128	1.0	1.0	1.0	1.0
9	TO	MA	256	1.3	2.3	3.0	2.2
10	TU	MA	512	1.0	1.3	1.7	1.3
11	AB 136	MA	1024	1.3	2.0	2.3	1.9
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 22: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 10Pa from Siaya at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	4.7	6.0	6.3	5.7
2	MDRK	A	2	1.7	2.0	2.3	2.0
3	Perry Marrow	A	4	3.0	3.0	3.3	3.1
4	Cornell 49-242	MA	8	1.0	1.3	1.7	1.3
5	Widusa	A	16	2.3	2.7	3.0	2.7
6	Kaboon	A	32	2.0	3.7	4.3	3.3
7	Mexico 222	MA	64	5.3	6.0	7.0	6.1
8	PI 207262	MA	128	1.0	1.0	1.0	1.0
9	TO	MA	256	1.0	1.7	2.3	1.7
10	TU	MA	512	1.7	2.3	3.0	2.3
11	AB 136	MA	1024	1.0	1.3	2.0	1.4
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 23: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 26Pa from Narumoru at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	5.3	6.3	7.0	6.2
2	MDRK	A	2	5.7	7.0	7.3	6.7
3	Perry Marrow	A	4	5.3	6.7	8.0	6.7
4	Cornell 49-242	MA	8	1.0	1.0	1.0	1.0
5	Widusa	A	16	5.3	6.0	6.7	6.0
6	Kaboon	A	32	2.7	3.7	3.7	3.4
7	Mexico 222	MA	64	4.3	5.7	6.7	5.6
8	PI 207262	MA	128	1.0	1.0	1.0	1.0
9	TO	MA	256	2.3	2.7	3.0	2.7
10	TU	MA	512	2.7	3.3	3.7	3.2
11	AB 136	MA	1024	1.3	2.0	2.3	1.9
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 24: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 16Pb from Gucha at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	5.3	6.0	6.3	5.9
2	MDRK	A	2	3.3	3.3	3.3	3.3
3	Perry Marrow	A	4	2.3	3.0	3.0	2.8
4	Cornell 49-242	MA	8	1.0	1.0	1.7	1.2
5	Widusa	A	16	3.0	3.3	4.0	3.4
6	Kaboon	A	32	2.7	3.3	3.7	3.2
7	Mexico 222	MA	64	4.7	6.0	7.7	6.1
8	PI 207262	MA	128	1.0	1.3	1.7	1.3
9	TO	MA	256	1.7	2.3	3.0	2.3
10	TU	MA	512	1.0	1.3	2.0	1.4
11	AB 136	MA	1024	1.0	1.7	2.0	1.6
12	G2333	MA	2048	1.0	1.0	1.3	1.1

Appendix 25: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 28Pb from Meru at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	5.7	7.0	7.3	6.7
2	MDRK	A	2	1.7	2.3	3.0	2.3
3	Perry Marrow	A	4	2.3	3.0	3.0	2.8
4	Cornell 49-242	MA	8	2.3	3.7	6.3	4.1
5	Widusa	A	16	6.3	7.0	8.3	7.2
6	Kaboon	A	32	3.3	3.3	3.7	3.4
7	Mexico 222	MA	64	5.7	6.3	7.0	6.3
8	PI 207262	MA	128	1.0	1.3	1.7	1.3
9	TO	MA	256	1.0	1.0	1.3	1.1
10	TU	MA	512	1.7	2.0	2.3	2.0
11	AB 136	MA	1024	1.0	1.0	1.0	1.0
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 26: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 5Pc from Bomet at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	5.3	6.3	7.7	6.4
2	MDRK	A	2	1.7	2.3	3.0	2.3
3	Perry Marrow	A	4	2.7	3.3	3.3	3.1
4	Cornell 49-242	MA	8	1.0	1.3	1.3	1.2
5	Widusa	A	16	6.7	7.3	9.0	7.7
6	Kaboon	A	32	3.3	3.3	3.3	3.3
7	Mexico 222	MA	64	5.0	6.0	6.0	5.7
8	PI 207262	MA	128	1.0	1.3	1.7	1.3
9	TO	MA	256	1.7	2.3	2.7	2.2
10	TU	MA	512	1.3	1.7	2.0	1.7
11	AB 136	MA	1024	1.7	2.3	3.0	2.3
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 27: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 25Pa from Thika at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	7.0	7.7	9.0	7.9
2	MDRK	A	2	5.7	6.3	8.0	6.7
3	Perry Marrow	A	4	7.7	9.0	9.0	8.6
4	Cornell 49-242	MA	8	1.0	1.0	1.3	1.1
5	Widusa	A	16	6.3	7.7	9.0	7.7
6	Kaboon	A	32	2.3	3.0	3.3	2.9
7	Mexico 222	MA	64	3.0	3.3	4.0	3.4
8	PI 207262	MA	128	1.0	1.0	1.3	1.1
9	TO	MA	256	1.7	2.3	3.0	2.3
10	TU	MA	512	2.0	2.0	2.3	2.1
11	AB 136	MA	1024	2.3	2.7	3.0	2.7
12	G2333	MA	2048	1.0	1.3	1.3	1.2

Appendix 28: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 31Pc from Murang'a at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	7.7	7.7	8	7.8
2	MDRK	A	2	2.7	2.7	3.0	2.8
3	Perry Marrow	A	4	1.0	1.3	1.7	1.3
4	Cornell 49-242	MA	8	2.0	3.7	4.7	3.5
5	Widusa	A	16	2.0	3.7	4.3	3.3
6	Kaboon	A	32	2.0	2.3	2.3	2.2
7	Mexico 222	MA	64	4.3	7	8.3	6.5
8	PI 207262	MA	128	1.0	1.0	1.0	1.0
9	TO	MA	256	2.3	3.0	3.0	2.8
10	TU	MA	512	1.0	1.3	1.7	1.3
11	AB 136	MA	1024	1.3	1.7	2.0	1.7
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 29: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 27Pa from Maragua at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	6.0	7.3	9.0	7.4
2	MDRK	A	2	1.0	4.3	5.0	3.4
3	Perry Marrow	A	4	1.3	2.0	2.7	2.0
4	Cornell 49-242	MA	8	2.7	3.7	4.3	3.6
5	Widusa	A	16	2.7	3.0	3.7	3.1
6	Kaboon	A	32	2.3	2.7	3.0	2.7
7	Mexico 222	MA	64	4.7	5.3	6.3	5.4
8	PI 207262	MA	128	1.0	1.0	1.3	1.1
9	TO	MA	256	1.0	1.0	1.0	1.0
10	TU	MA	512	1.3	2.0	2.0	1.8
11	AB 136	MA	1024	1.7	2.3	3.0	2.3
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 30: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 30Pc from Othaya at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	4.0	5.7	6.0	5.2
2	MDRK	A	2	2.0	2.0	2.7	2.2
3	Perry Marrow	A	4	2.3	3.0	3.3	2.9
4	Cornell 49-242	MA	8	1.0	1.0	1.0	1.0
5	Widusa	A	16	2.7	3.0	3.7	3.1
6	Kaboon	A	32	2.7	3.3	3.7	3.2
7	Mexico 222	MA	64	5.3	5.7	7.3	6.1
8	PI 207262	MA	128	1.0	1.0	1.0	1.0
9	TO	MA	256	1.7	2.3	3.0	2.3
10	TU	MA	512	1.0	1.3	2.0	1.4
11	AB 136	MA	1024	1.3	1.7	2.3	1.8
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 31: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 10Pb from Siaya at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	3.7	5.0	6.0	4.9
2	MDRK	A	2	2.0	3.7	4.3	3.3
3	Perry Marrow	A	4	2.7	3.0	3.7	3.1
4	Cornell 49-242	MA	8	1.0	1.0	1.0	1.0
5	Widusa	A	16	2.7	3.3	3.7	3.2
6	Kaboon	A	32	1.0	1.7	2.0	1.6
7	Mexico 222	MA	64	2.0	2.3	3.0	2.4
8	PI 207262	MA	128	1.0	1.0	1.0	1.0
9	TO	MA	256	1.0	1.7	2.3	1.7
10	TU	MA	512	1.0	1.0	1.0	1.0
11	AB 136	MA	1024	1.0	1.0	1.0	1.0
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 32: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 2Pe from Bureti at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	6.0	6.7	8.0	6.9
2	MDRK	A	2	5.7	7.0	8.7	7.1
3	Perry Marrow	A	4	5.0	5.7	6.7	5.8
4	Cornell 49-242	MA	8	1.0	1.0	1.3	1.1
5	Widusa	A	16	4.3	7.0	8.3	6.5
6	Kaboon	A	32	2.0	3.7	4.3	3.3
7	Mexico 222	MA	64	5.0	5.7	7.0	5.9
8	PI 207262	MA	128	1.0	1.0	1.7	1.2
9	TO	MA	256	1.7	2.3	3.0	2.3
10	TU	MA	512	1.0	1.0	1.0	1.0
11	AB 136	MA	1024	1.0	1.0	1.7	1.2
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 33: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 27Pb from Maragua at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	6.3	7.7	9.0	7.7
2	MDRK	A	2	2.3	3.7	4.3	3.4
3	Perry Marrow	A	4	2.7	3.0	3.3	3.0
4	Cornell 49-242	MA	8	3.3	3.7	4.7	3.9
5	Widusa	A	16	3.0	4.3	5.3	4.2
6	Kaboon	A	32	2.0	3.7	4.3	3.3
7	Mexico 222	MA	64	6.3	7.3	8.7	7.4
8	PI 207262	MA	128	1.0	1.3	2.0	1.4
9	TO	MA	256	2.3	2.7	3.0	2.7
10	TU	MA	512	1.3	1.7	2.3	1.8
11	AB 136	MA	1024	1.0	1.3	1.7	1.3
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 34: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 29Pa from Kiambu at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	5.3	6.0	7.3	6.2
2	MDRK	A	2	5.7	6.3	8.0	6.7
3	Perry Marrow	A	4	5.3	6.0	6.7	6.0
4	Cornell 49-242	MA	8	2.0	2.3	2.3	2.2
5	Widusa	A	16	5.0	5.7	6.7	5.8
6	Kaboon	A	32	2.7	3.3	3.7	3.2
7	Mexico 222	MA	64	2.3	2.7	3.0	2.7
8	PI 207262	MA	128	1.0	1.3	1.7	1.3
9	TO	MA	256	3.0	3.0	3.3	3.1
10	TU	MA	512	1.0	1.3	1.7	1.3
11	AB 136	MA	1024	1.3	2.0	2.3	1.9
12	G2333	MA	2048	1.0	1.0	1.3	1.1

Appendix 35: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 16Pc from Gucha at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	3.0	3.3	4.0	3.4
2	MDRK	A	2	6.3	7.7	9.0	7.7
3	Perry Marrow	A	4	5.3	6.0	7.7	6.3
4	Cornell 49-242	MA	8	1.7	2.3	3.0	2.3
5	Widusa	A	16	2.0	2.0	2.0	2.0
6	Kaboon	A	32	7.7	8.0	8.7	8.1
7	Mexico 222	MA	64	2.3	2.7	3.0	2.7
8	PI 207262	MA	128	1.0	1.3	1.7	1.3
9	TO	MA	256	1.3	2.0	2.7	2.0
10	TU	MA	512	1.0	1.0	1.0	1.0
11	AB 136	MA	1024	1.0	1.0	1.0	1.0
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 36: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 26Pb from Narumoru at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	5.0	6.3	7.0	6.1
2	MDRK	A	2	2.3	3.0	3.0	2.8
3	Perry Marrow	A	4	2.7	3.3	3.3	3.1
4	Cornell 49-242	MA	8	1.0	1.0	1.0	1.0
5	Widusa	A	16	1.7	2.0	2.7	2.1
6	Kaboon	A	32	1.0	1.3	2.0	1.4
7	Mexico 222	MA	64	5.3	6.7	8.0	6.7
8	PI 207262	MA	128	1.0	1.0	1.0	1.0
9	TO	MA	256	1.0	1.3	1.7	1.3
10	TU	MA	512	1.0	1.0	1.3	1.1
11	AB 136	MA	1024	1.3	1.3	1.7	1.4
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 37: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 31Pd from Murang'a at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	4.3	5.7	7.0	5.7
2	MDRK	A	2	2.3	3.0	3.0	2.8
3	Perry Marrow	A	4	2.7	3.0	3.3	3.0
4	Cornell 49-242	MA	8	1.7	2.3	3.0	2.3
5	Widusa	A	16	2.7	3.0	3.7	3.1
6	Kaboon	A	32	2.3	3.0	3.0	2.8
7	Mexico 222	MA	64	5.3	6.7	8.7	6.9
8	PI 207262	MA	128	1.0	1.3	1.7	1.3
9	TO	MA	256	1.7	2.0	2.0	1.9
10	TU	MA	512	2.0	2.3	2.3	2.2
11	AB 136	MA	1024	2.0	2.0	2.0	2.0
12	G2333	MA	2048	1.0	1.3	1.7	1.3

Appendix 38: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 45Pa from Wundanyi at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	4.7	6.0	7.7	6.1
2	MDRK	A	2	2.3	3.7	4.3	3.4
3	Perry Marrow	A	4	2.3	3.0	3.0	2.8
4	Cornell 49-242	MA	8	1.7	2.0	2.0	1.9
5	Widusa	A	16	5.3	6.7	8.3	6.8
6	Kaboon	A	32	2.7	3.3	3.3	3.1
7	Mexico 222	MA	64	2.3	2.7	3.0	2.7
8	PI 207262	MA	128	1.0	1.0	1.0	1.0
9	TO	MA	256	1.0	1.3	2.0	1.4
10	TU	MA	512	1.0	1.0	1.0	1.0
11	AB 136	MA	1024	1.0	1.0	1.0	1.0
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 39: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 45Pc from Wundanyi at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	2.7	3.3	3.7	3.2
2	MDRK	A	2	4.3	5.3	7.0	5.5
3	Perry Marrow	A	4	2.3	3.7	4.3	3.4
4	Cornell 49-242	MA	8	1.0	1.0	1.0	1.0
5	Widusa	A	16	2.3	3.0	3.0	2.8
6	Kaboon	A	32	1.7	2.0	2.7	2.1
7	Mexico 222	MA	64	2.0	3.7	4.3	3.3
8	PI 207262	MA	128	1.0	1.0	1.0	1.0
9	TO	MA	256	1.0	1.7	2.0	1.6
10	TU	MA	512	1.0	1.0	1.0	1.0
11	AB 136	MA	1024	1.0	1.0	1.0	1.0
12	G2333	MA	2048	1.0	1.0	1.0	1.0

Appendix 40: Reaction of 12 international anthracnose differential cultivars to inoculation with *Colletotrichum lindemuthianum* isolate 10Pd from Siaya at 12, 17 and 21 days after inoculation (DAI).

S. No.	Differential cultivar	Gene Pool	Binary value	Disease score			Average Score
				12 DAI	17 DAI	21 DAI	
1	Michelite	MA	1	5.3	6.7	8.3	6.8
2	MDRK	A	2	5.3	6.0	6.7	6.0
3	Perry Marrow	A	4	5.7	8.0	9.0	7.6
4	Cornell 49-242	MA	8	1.0	1.0	1.0	1.0
5	Widusa	A	16	5.3	6.3	7.7	6.4
6	Kaboon	A	32	3.0	3.0	3.3	3.1
7	Mexico 222	MA	64	5.7	7.0	7.7	6.8
8	PI 207262	MA	128	1.0	1.0	1.0	1.0
9	TO	MA	256	1.0	1.0	1.3	1.1
10	TU	MA	512	1.0	1.3	2.0	1.4
11	AB 136	MA	1024	1.0	1.0	1.0	1.0
12	G2333	MA	2048	1.0	1.0	1.0	1.0