# MARKER ASSISTED GAMETE SELECTION FOR MULTIPLE DISEASE RESISTANCE IN MESOAMERICAN BEAN GENOTYPES AND RACE TYPING OF ANGULAR LEAF SPOT PATHOGEN IN KENYA

**Samuel Mwangi Njuguna**

**(B.Sc. Agriculture, University of Nairobi)**

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN PLANT BREEDING AND BIOTECHNOLOGY**

**DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION**

**FACULTY OF AGRICULTURE**

**UNIVERSITY OF NAIROBI**

**2014**

# DECLARATION

I hereby declare that this is my original work and has not been submitted for a degree or any other award in any university.

**Samuel Mwangi Njuguna**

Signature. ***Samuel Mwangi Njuguna*** Date: 18 July 2014

This thesis has been submitted with our approval as university supervisors

**1) Prof. Rama D. Narla**

Department of Plant Science and Crop Protection

University of Nairobi

**2) Prof. Paul M. Kimani**

Department of Plant Science and Crop Protection

University of Nairobi

# DEDICATION

This work is dedicated to my dear parents, Grace Wangari and James Njuguna Joshua for believing in me and providing the opportunity to be educated.

May God bless them abundantly!

# ACKNOWLEDGEMENT

I am very grateful to the entire academic staff, Faculty of Agriculture, University of Nairobi, for the knowledge they imparted in me, which enabled me to conduct this research.

My deepest and sincere gratitude goes out to my supervisors Prof. Rama D. Narla and Prof. Paul M. Kimani, who tirelessly gave me endless guidance right from proposal development up to the end of this study. They read drafts of this thesis and made valuable suggestions, which sufficiently contributed towards the completion of this study.

I thank all District Agricultural Officers (DAOs) who voluntarily assisted during sample collection in the districts surveyed. I am grateful to Dr. J. Kabira who provided evaluation fields in KARI-Tigoni. I am equally grateful to Ms. S. Muchira and Mr. S. Kariuki, for their technical inputs towards my experiments in molecular and pathology aspects respectively. I am also grateful to Mr. D. Maina, Ms. E. Mweru and Mr. J. Wambugu for their contributions in facilitating and managing my field experiments. My gratitude goes to Ms. Mercy Mbogori, a PhD. student at the Faculty of Agriculture, University of Nairobi. Mercy helped me with information regarding PCR, molecular data analysis and gave me general advice on molecular tools in marker assisted selection. I similarly acknowledge the effort from Ms. A. Musyimi, who continuously encouraged me till the completion of this research. My colleagues: J. Orende, Y. Tadesse, A. Marete and others

contributed a lot, and am very appreciative of their efforts towards the completion of this research. God bless you all.

This work was funded by Kirkhouse Trust (UK), of whom I am very grateful. I am also grateful to Prof. Sir Edwin Southern, Prof. Paul Gepts, Dr. Robert Koebner and Emma Wilmore for their contributions.

Finally, I wish to appreciate the encouragement, moral and material support provided by my entire family throughout the period of my research, and who I am highly indebted.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF APPENDICES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AEZ | Agro-ecological zones |
| ALS | Angular leaf spot |
| ANT | Anthracnose |
| BCMV | Bean common mosaic virus |
| BCMNV | Bean common mosaic necrotic virus |
| BGYM | Bean golden yellow mosaic |
| CBB | Common bacterial blight |
| CIAT | International Centre for Tropical Agriculture |
| DNA | Deoxyribonucleic acid |
| EDTA | Ethylene-diamine-tetra-acetic acid |
| FAO | Food and Agricultural Organization |
| GPS | Global positioning system |
| GS | Gamete selection |
| MAS | Marker assisted selection |
| PCR | Polymerase chain reaction |
| Pg | *P. griseola, Pseudocercospora griseola* |
| QTL | Quantitative trait loci |
| RAPD | Randomly amplified polymorphic DNA |
| RR | Root rot |
| TBE | Tris-Borate EDTA |
| TE | Tris-EDTA |
| TWA | Tap water agar |
| SCAR | Sequence characterized amplified regions |

# ABSTRACT

Productivity of common bean (*Phaseolus vulgaris* L.) is severely constrained by angular leaf spot, anthracnose, bean common mosaic virus and *Pythium* root rot, among other biotic and abiotic constraints. The most effective and appropriate control measure for these diseases is use of host plant resistance. The objectives of this study were to: i) identify races of angular leaf spot pathogen and their distribution in Kenya, ii) use markers to pyramid genes for resistance to angular leaf spot, anthracnose, root rots and bean common mosaic virus into susceptible commercial cultivars and, iii) conduct early generation selection for combined resistance to angular leaf spot, anthracnose, root rots and bean common mosaic virus and other agronomic traits.

A survey was carried out across major bean growing regions of Kenya and 62 samples of angular leaf spot, 55 of anthracnose, 9 of root rots, 121 of common bacterial blight and 32 of bean common mosaic virus diseased materials obtained from the 35 districts visited. From 62 samples collected with angular leaf spot, 57 isolates were obtained and race-typed into 23 physiological races of *Pseudocercospora griseola*. Races 63-55, 63-63, 63-54 and 63-35 were found to be the most dominant races in areas studied. Two new races, 31-31 and 63-31 were reported for the first time in Kenya. The two main pathogen groups; Andean and Mesoamerican, were also reported. Twelve isolates were classified as from the Andean pathotype group while 45 isolates were from the Mesoamerican pathotype group.

Sixteen populations were generated from crosses among six sources of resistance (MEX 54, G10909, G2333, RWR 719, AND 1062 and BRB 191) and four susceptible popular varieties (KAT B1, KAT B9, GLP 585 and GLP 92). Male gametes with requisite resistance genes were identified using markers SAB-3 for anthracnose, SH-13 for angular leaf spot, SW-13 for bean common mosaic virus, and PYAA-19 for *Pythium* root rot, and used to construct the $F_1$ with susceptible varieties following gamete selection breeding method. Four varieties susceptible to angular leaf spot, anthracnose, root rots and bean common mosaic virus were utilized as recipient genotypes. The segregating $F_1$ populations and $F_{1.2}$ families were evaluated for resistance to the four biotic constraints and other agronomic traits in the field at Kabete and Tigoni under natural disease infestation.

Results on molecular marker validation showed that three markers (SAB-3, SH-13 and SW-13) were effective selection tools as they amplified the genes and showed polymorphism among the plants. 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. The three markers were used to screen G10909/G2333//AND 1062/BRB 191 for the three disease constraints. Of the 89 male gamete plants that were screened with three markers five were positive for three markers, 18 for two markers, while all others had one or zero markers present. Significant differences ($P< 0.05$) were detected among the $F_1$ genotypes for angular leaf spot, anthracnose, root rots and bean common mosaic virus. The interaction between genotype and location was also significant ($P< 0.05$) for all the four disease constraints.

The mean yield in Kabete (2256 kg $ha^{-1}$) was lower compared to the mean yield recorded in Tigoni (3391 kg $ha^{-1}$). Genotypes derived from population KGS12-16 recorded the highest yield (4762 kg $ha^{-1}$) while genotypes derived from population KGS12-05 had lowest yield (2490 kg $ha^{-1}$) among the crosses. Yield was strongly correlated with days to 50 % maturity (r=0.4**) and number of pods per plant (r=0.4**) in all genotypes across the two locations. The results on *P. griseola* race distribution will assist in developing targeted-resistant varieties for specific bean growing regions of Kenya. This information can also be utilized by bean breeders and pathologists to develop and improve bean varieties with multiple-resistance to the most prevalent and virulent races found in Kenya.

**Key words:** *Phaseolus vulgaris*, molecular markers, introgression, *Pseudocercospora griseola*.

# CHAPTER 1 INTRODUCTION

## 1.1 Background information

Among the grain legumes cultivated as food crops, common bean (*Phaseolus vulgaris* L.) constitute the largest portion of legume production, and is a major crop in many parts of Africa especially in eastern and southern Africa (Kimani *et al.*, 2005). Common bean is the most important pulse crop in Kenya (FAO, 2013), and assumes an important role as a potential source of dietary protein (~22%), the vitamin-B complex, iron, other minerals (Ca, Cu, Mg, Mn and Zn), carbohydrates and soluble fibre for human diets (Broughton *et al*., 2003).

The common bean is a herbaceous annual leguminous plant that belongs to the genus, *Phaseolus*, with pinnately compound trifoliate large leaves. It is now grown worldwide for its edible bean, popular both as dry grain and as green pods. *Phaseolus vulgaris* is a member of the Fabaceae (or Leguminosae) family, subfamily Faboideae, tribe Phaseoleae and subtribe Phaseolinae. 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.

Worldwide annual production, including both dry and snap bean, exceeds 43 million metric tonnes (FAO, 2013), which represents more than half of the world's total food legume production (Miklas *et al*., 2006a). About 23.3 million tonnes of dry common beans and 20.4 million tonnes of green beans

were grown worldwide in 2011 (FAO, 2013). India and Brazil are the largest producers of dry beans, Kenya coming in at position seven with a production of 0.6 million metric tonnes (FAO, 2013).

Common bean is of high nutritional and economic importance in Kenya. Bean production largely occurs under low input agricultural system on small-scale farms in developing countries. Beans produced by these resource-poor farmers are more vulnerable to attack by disease and insect pests and to abiotic stresses including drought and low soil fertility (Kimani *et al.,* 2005; Miklas *et al*., 2006a). In Kenya, bean is cultivated mainly as intercrop with other crops such as maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), cowpeas (*Vigna unguiculata* (L.) Walp.), cassava (*Manihot esculenta* Crantz) and potatoes (*Solanum tuberosum* L.) (Mwaniki *et al*., 2000).

Observed low yields in the region (about 500 kg $ha^{-1}$) compared to the potential yields (3000 kg $ha^{-1}$) of improved varieties cultivated under reliable environmental conditions, are related to the major diseases of common bean in East Africa (Hillocks *et al*., 2006). Farmers are thus forced to use expensive chemicals and are further constrained by high costs of certified seed. The limitations to bean production in Kenya include land scarcity, inadequate use of fertilizer, low quality planting seeds, pests and diseases and varieties with low yield potential (Kimani *et al.,* 2005). Major abiotic stress factors include low soil fertility and drought (Kimani *et al.,* 2005; Lunze *et al.*, 2011).

Generally, about 200 pathogens (fungal, bacterial and viral) are known to attack the common beans, but less than a dozen can cause substantial economic loss (Mwesigwa, 2009). The major biotic constraints to productivity

include angular leaf spot (*Pseudocercospora griseola* (Sacc.) Crous and Braun, previously known as *Phaeoisariopsis griseola* (Sacc.) Ferr. (Crous *et al*., 2006; Silva *et al*., 2008)), anthracnose (*Colletotrichum lindemuthianum* (Sacc. and Magn.) Scrib.), root rots, bean common mosaic and necrotic viruses (BCMV/BCMNV), and common bacterial blight (CBB) caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye (Kimani *et al*., 2005). These diseases cause severe losses to yield and quality of common bean ranging from 20% to as high as 80-100% (Mahuku *et al*., 2010; Singh and Schwartz, 2010). Control strategies include, mainly, cultural practices, foliar spraying with chemical fungicides and the development of resistant cultivars.

*Pseudocercospora griseola*, the causal pathogen of angular leaf spot is considered one of the most important biotic constraints of *Phaseolus vulgaris* in both tropical and subtropical areas (Aggarwal *et al*., 2004), and the pathogen is known to vary greatly in pathogenicity (Stenglein *et al*., 2003; Sartorato, 2004; Wagara *et al*., 2004; Silva *et al*., 2008). Pathogenic variation of *P. griseola* has also been reported in Kenya (Monda, 1995; Wagara, 1996, 2004). Monda (1995) grouped 19 isolates of *P. griseola* from different areas in Kenya into 14 pathotypes using 11 differential cultivars. Wagara (1996) used 30 bean differential cultivars to group 15 races from 18 isolates collected from 15 districts in Kenya. However, discrepancies in the range of differential cultivars used in these studies make the results incomparable.

The first report of a systematic collection and race-typing of *P. griseola* isolates in Kenya using the international bean differentials identified 44 races from 100 isolates (Wagara, 2004). Pyndji (1992) characterized 21 isolates of

*P. griseola* from the Great Lakes region of Africa into 17 pathogenic groups using 11 differential cultivars. Pathogenic variability in *P. griseola* has also been widely reported across the world. Pastor-Corrales *et al.* (1998) identified 333 races from 433 isolates of *P. griseola* obtained from 11 Latin American and 10 African countries. Mahuku *et al.* (2002) identified 50 pathogenic groups of *P. griseola* from 112 isolates collected from 10 countries in Central America.

Due to the vast pathogenic variability of angular leaf spot pathogen, pyramiding genes for disease resistance requires that virulence patterns of pathogens be monitored and new resistance genes be introgressed into commercial bean cultivars to provide resistance to emerging virulent pathotypes (Sartorato, 2004). The wide virulence diversity in *P. griseola* also means that using a single location to test the resistance of a developed variety or source of angular leaf spot resistance is not sufficient because different pathotypes exist in different locations. It is therefore evident that the information obtained from *P. griseola* characterisation has significant implications for regional angular leaf spot resistance breeding and resistance gene deployment. Furthermore, the information on pathogen population structure and distribution of races found in Kenya will help in identifying sources of resistance and targeting or deploying resistance genes.

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scrib. is one of the most widespread and economically important diseases of common bean (Pastor-Corrales, 2005). Anthracnose can cause complete yield loss when susceptible genotypes are used and is favoured by relatively cool

and humid conditions for disease development (Pastor-Corrales *et al*., 1995). Genetic resistance has been suggested as the most effective management strategy for the control of anthracnose in common bean (Kelly and Vallejo, 2004), but the implementation of resistance is challenged by the recurrent appearance of new virulence phenotypes (Kelly *et al*., 1994).

Studies on pathogenic variation in *C. lindemuthianum* in Kenya have been conducted and the presence of different races of the pathogen identified (Gathuru and Mwangi, 1991). Overcoming the vast virulence diversity of *C. lindemuthianum* requires that bean breeders continually broaden the genetic base of common bean by identifying new sources of resistance and incorporating new anthracnose resistance genes (Pastor-Corrales *et al*., 1995).

Bean common mosaic virus (BCMV) causes a serious seed-borne disease of beans that occurs worldwide (Kapil *et al*., 2011). In Kenya, bean common mosaic disease was first identified by Kulkarni (1973) and then by Buruchara (1979). Strains of this virus have been classified into seven pathogenicity groups based on phenotypic reaction of differential cultivars. The bean common mosaic virus have further been divided into serogroups A (pathogroups III and VI), and B (pathogroups I, II, IV, V and VII) (Kapil *et al*., 2011), which are suggested to correlate with two distinct potyviruses (Huang and Chang, 2005). Omunyin *et al.* (1995) carried out a limited survey for distribution of bean common mosaic virus and identification of pathogenicity groups occurring in Kenya, and was able to differentiate 14 virus isolates into four pathogroups. Host resistance to bean common mosaic virus is the only durable and economic method of managing the viral pathogen

(Kapil *et al*., 2011).

Common bean production is hampered by several constraints among which are bean root rot caused by *Pythium* spp. The disease is considered as being the most damaging in Great Lakes region of Africa where beans are grown intensively (Nzungize *et al*., 2011), and can result in total yield loss on susceptible cultivars if the disease occurs under favourable environmental conditions (Otsyula *et al*., 2003). The use of resistant cultivars is usually considered the most viable option for controlling root rot in common bean (Otsyula *et al*., 2003).

In Kenya, a major part of bean production is by small-scale or subsistence farmers who find application of fungicides costly (Mwaniki *et al*., 2000). As a result, breeding for disease resistance is the most effective, safest, practical and economically accessible strategy to control these diseases. Development of host resistance to these diseases in varieties grown by farmers is thus an appropriate method to improve bean production especially in the developing countries. This will greatly reduce the need for chemicals hence increasing returns on farmers' investment.

Transfer of resistance genes using markers from resistant genotypes has been proposed as an effective means to confer host resistance. These include sequence characterised amplified regions (SCAR) linked to genes for resistance to angular leaf spot, anthracnose, *Pythium* root rot and bean common mosaic virus (Buruchara *et al.,* 2007; Garzon *et al*., 2008), which are available for the proposed work. Many of these SCAR markers including SAB-3 for resistance genes to anthracnose, SH-13 for angular leaf spot, SW-

13 for bean common mosaic virus and PYAA19 for *Pythium* root rot resistance genes are already being utilized in Kenya, Uganda and Tanzania (Buruchara *et al*., 2011). The marker technology presents opportunities to accelerate cultivar development with more precision and reduces duration to release of improved bean varieties (Miklas *et al*., 2006a). Hence, marker assisted gamete selection strategy as proposed by Singh (1994) and Singh *et al*. (1998) will be followed in the development of improved market-preferred bean varieties with resistance to angular leaf spot, anthracnose, *Pythium* root rot and bean common mosaic virus.

## 1.2 Problem statement and justification

Bean production in Kenya is a major farming activity that serves as a source of food usually grown by small scale farmers for subsistence use (Kimani *et al*., 2005). To these farmers, beans in most cases, are their only source of proteins, vitamins and other minerals. Cultivation of common bean is practiced as an intercrop and in some instances as relay intercrop and monocrop, thus serving as a major way of livelihoods for the poor farmers. The major constrains to bean production in Kenya are diseases and pests (Kimani *et al*., 2005). It is therefore important if the production constrains are addressed in order for the farmers to realize the benefits of bean production.

The major biotic constraints that affect productivity include angular leaf spot (*P. griseola*), anthracnose (*C. lindemuthianum*), bean common mosaic virus and root rots (Kimani *et al.*, 2005). Angular leaf spot is considered as the

number one constraint to bean production and can result in yield losses of up to 70%, depending on the susceptibility of the cultivars, environmental conditions and the time of the outbreak of the disease (Sartorato, 2004; Mahuku *et al*., 2010). *Colletotrichum lindemuthianum* causes anthracnose of common bean in all bean growing regions in the world (Melotto and Kelly, 2000), resulting into yield losses that can reach or exceed 90% in susceptible genotypes (Pastor-Corrales *et al*., 1995). Bean common mosaic virus is among the most common and destructive potyviruses known to infect common bean worldwide (Drijfhout, 1978).

Kelly *et al*. (2003) proposes gene pyramiding as an effective strategy in breeding beans for virus resistance against the diverse strains of bean common mosaic virus. The bean root rot disease is currently one of the most destructive diseases affecting common bean in the Great Lakes region of Africa (Wortman *et al*., 1998). As much as 70% reductions in yields of some popular commercial bean cultivars due to bean root rot have been reported in Rwanda and Kenya (Otsyula *et al*., 2003). Use of varieties resistant to *Pythium* root rot would be ideal and some resistant varieties (such as RWR 719) have been identified but are poorly adapted under farmers' conditions thus ineffective in management of bean root rot (Otsyula *et al*., 2003).

Wortmann *et al*. (1998) estimates the annual production losses caused by angular leaf spot at 281,300 t, anthracnose at 247,400 t, root rot at 179,800 t and bean common mosaic virus at 144,600 t are recorded in eastern Africa. Therefore, developing bean varieties with multiple resistance genes 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 (Kimani and Mwang'ombe, 2007).

Although bean breeding at the University of Nairobi started in mid-1970's (Kimani *et al*., 1990), more work on improving marketable bean varieties is needed. Development of improved varieties in Kenya has traditionally followed classical breeding methods (Kimani and Mwang'ombe, 2007), resulting in long periods of cultivar development. Thus, the use of genetic resistance is the most practical and economic way to manage diseases of common bean (Mahuku *et al*., 2002b), and utilization of marker technology presents new opportunities to accelerate cultivar development with more precision and reduce duration to release of improved bean varieties (Miklas *et al*., 2006a). This will greatly assist in the transfer and pyramiding of resistance genes to angular leaf spot, anthracnose, *Pythium* root rot and bean common mosaic virus within a shorter period than was previously possible.

Angular leaf spot is a highly variable pathogen and it is not clear which races are most virulent and widely distributed in bean production regions of Kenya. It is therefore critical to identify the major races of this disease and their distribution in Kenya. This will contribute to the design of an effective breeding strategy for resistance to these races. Proposed race typing of angular leaf spot pathogen will lead to identification of races of *P. griseola* and their distribution in Kenya. This will particularly help in the development of improved varieties with resistance to known races of *P. griseola*.

## 1.3 Objectives

Therefore, the overall objective of this study was to identify the different races of angular leaf spot pathogen and their distribution in Kenya, and to develop $F_1$ derived families of small and medium seeded (Mesoamerican) bean varieties with combined resistance to angular leaf spot, anthracnose, bean common mosaic and *Pythium* root rot.

The specific objectives of the study are:

1. To identify races of angular leaf spot pathogen and their distribution in major bean growing areas in Kenya.

2. Use available markers to pyramid genes for resistance to angular leaf spot, anthracnose, root rots and bean common mosaic virus into susceptible preferred commercial cultivars.

3. Conduct early generation selection for combined resistance to angular leaf spot, anthracnose, root rots and bean common mosaic virus and other agronomic traits.

# CHAPTER 2 REVIEW OF LITERATURE

## 2.1 Origin and distribution of common bean

The genus *Phaseolus* is of American origin and comprises of 30 species (Singh, 2001). Only five of the species, namely *P. acutifolious* A. Gray (tepary bean), *P. coccineus* L. (scarlet runner bean), *P. lunatus* L. (Lima bean), *P. polyanthus* Greenmann (year long bean) and *P. vulgaris* L. (common bean) were domesticated (Broughton *et al*., 2003; Mamidi *et al*., 2011). Domestication of common bean (*Phaseolus vulgaris* L.) occurred in the upland regions of Latin America more than 7000 years ago (Mamidi *et al*., 2011). The domestication occurred simultaneously within two major geographic locations: the Andes region of South America, and in Central America and Mexico, which have been identified as the two centres of origin for this crop (Chacon *et al*., 2005). Thus, genetic diversity of the cultivated common bean is organized into two distinct gene pools; Andean gene pool and Middle American gene pool (Chacon *et al*., 2005). Morphological, biochemical, and molecular attributes differentiate these two gene pools. For example, the Andean pool is comprised of large-seeded beans (>40 g/100-seed mass) while the Middle American pool is constituted of small (<25 g/100-seed mass) and medium-seeded (25–40 g/100-seed mass) beans (Singh, 2001; Asensio-S.-Manzanera *et al*., 2006). It is significant that the diversity of cultivated common bean cultivars parallels the diversity of their wild bean ancestors. While common bean is the most widely grown, occupying more than 85% of production area sown to all *Phaseolus* species in the world

(Singh, 2001), production of the crop is limited by numerous biotic and abiotic constraints.

## 2.2 Botany and cytogenetics of common bean

*Phaseolus vulgaris* L. is a member of the Fabaceae (or Leguminosae) family, subfamily Faboideae, tribe Phaseoleae and subtribe Phaseolinae. Botanically, the common bean is classified as a dicotyledonous legume. Cultivated forms are herbaceous annuals, which are determinate or indeterminate in growth habit. On germination, the plant is initially tap-rooted, but adventitious roots emerge soon thereafter, and dominate the tap root which remains 10-15 cm in length. Papilionaceous flowers are borne in auxillary and terminal racemes. Racemes may be one or many flowered. Flowers are zygomorphic with a bi-petalled keel, two lateral wing petals and a large outwardly-displayed standard petal. Flower colour is genetically independent of seed color, but association between particular flower and seed colours is common. Flowers may be white, pink or purple (also red in *P. coccineus*). The flower contains ten stamens and a single multi-ovuled ovary, and develops into a straight or slightly curved fruit or pod. Seeds may be round, elliptical, somewhat flattened or rounded elongate in shape, and a rich assortment of coat colours and patterns exists. Common bean shows variation in growth habits from determinate bush to indeterminate, extreme climbing types. Common bean is a true diploid (2n = 2x = 22), and from a genomic perspective has a relatively small genome comparable to rice, estimated to be about 450–650 million base pairs per haploid (Broughton *et al*., 2003; McClean *et al*., 2004).

## 2.3 Reproductive biology of common bean

With the exception of specific tropical locations where outcrossing can be significant, *P. vulgaris* is considered a predominantly self-pollinated species. Although most data show outcrossing rates below 5%, recent information shows that occasionally higher rates can be achieved (Ibarra-Perez *et al*., 1997). However, interspecific crossing is rare in nature, although hybridization between *P. vulgaris* and *P. coccineus* does occur (Broughton *et al*., 2003).

## 2.4 Common bean production in East Africa

Common bean is the most important grain legume grown in eastern, central and southern Africa – in terms of both area under production and consumption (CIAT, 2005). It provides an inexpensive source of protein for both rural and urban households in East Africa (Mkandawire *et al*., 2004). It is the most important legume in the pulses category of Kenya's agricultural commodities, and is second only to maize as a food crop (Kimani *et al.*, 2005). 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 genetic potential of improved 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 (Otsyula *et al*., 2003; Kimani *et al*., 2005).

The two major gene pools of *Phaseolus* species are represented in Africa. The large-seeded type's characteristic of the Andean gene pool accounts for 61%

of cultivars; the rest are small and medium-seeded types typical of the Mesoamerican gene pool (CIAT, 2005). There are currently two major commercial classes of common bean, snap or green beans, and dry beans (Singh, 2001). Cultivation of common bean in Africa is widespread. However, approximately 80 percent of African bean production is concentrated in 10 countries (Table 2.1). In terms of area harvested in east Africa, Uganda is the leading producer of common bean followed by Kenya with the lowest being Burundi (Table 2.1). In regard to production quantity the five east African countries i.e. Tanzania, Kenya, Uganda, Rwanda and Burundi were among the 20 highest producers of common bean in the world (Table 2.1). 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).

**Table 2.1:** Bean production rank, value and quantity of countries in Africa and the area harvested for the year 2011.

| Country | Production | | | Area Harvested (ha) |
|---|---|---|---|---|
| | World rank | Value (Int $ 1000) | Quantity (t) | |
| 1. Tanzania | 6 | 386,548 | 675,948 | 737,661 |
| 2. Kenya | 7 | 347,410 | 577,674 | 1,036,740 |
| 3. Uganda | 9 | 244,750 | 464,105 | 1,142,660 |
| 4. Ethiopia | 10 | 197,505 | 340,280 | 237,366 |
| 5. Cameroon | 11 | 209,563 | 366,463 | 296,371 |
| 6. Rwanda | 15 | 192,994 | 331,166 | 341,819 |
| 7. Angola | 16 | 172,599 | 303,521 | 786,906 |
| 8. Burundi | 20 | 109,563 | 200,673 | 264,163 |
| 9. Mozambique | 45 | 88,314 | 200,000 | 700,000 |
| 10. Malawi | 47 | 77,259 | 176,760 | 279,579 |

Source: FAO Statistics Division, 2013

The average bean yield in the region is around 0.5 ton $ha^{-1}$, although the potential yield of 1.5 to 3.0 ton $ha^{-1}$ can be realized with improved varieties, proper crop and land husbandry under reliable rain-fed conditions (Mkandawire *et al*., 2004). Yields in Kenya are still low and unstable, fluctuating between 0.4 and 0.6 ton $ha^{-1}$ (Table 2.2). This is perhaps due to intensification of drought, insect pests and diseases (Katungi *et al*., 2009).

**Table 2.2:** Bean production trends in Kenya for the period between 2002 and 2011

| KENYA | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 |
|---|---|---|---|---|---|---|---|---|---|---|
| [1]Area Harvested (Ha) | 928.7 | 897.0 | 787.1 | 1034.4 | 995.4 | 846.3 | 641.9 | 960.7 | 689.4 | 1036.7 |
| [2]Production (Tonnes) | 480.8 | 428.8 | 277.5 | 382.3 | 531.8 | 429.8 | 265.0 | 465.4 | 390.6 | 577.7 |
| Yield (Ton/Ha) | 0.5 | 0.5 | 0.4 | 0.4 | 0.5 | 0.5 | 0.4 | 0.5 | 0.6 | 0.6 |

Source: FAO Statistics Division, 2013

[1]Area harvested in thousands hectares (Ha)

[2]Production in thousands tonnes

The main areas of bean production in Kenya are the mid and high altitude areas of the country, which experience more reliable rainfall and cooler temperatures. About 75% of the annual cultivation occurs in the Central, Eastern and Western highlands, and parts of Nyanza and Rift Valley. About 74% of common bean area in East Africa, and 57% of bean area in southern Africa (Wortmann *et al*., 1998) are grown under multiple cropping systems, mainly in association with maize, roots and tubers, sorghum or millet (Mwaniki *et al*., 2000).

## 2.5 Major common bean diseases and their pathogenic variation

Common bean is affected by a wide range of constraints both biotic and abiotic that include; insect pests, disease pathogens, nutrient and moisture deficiencies, among others (Table 2.3). The main disease constraints include: angular leaf spot, anthracnose, root rots, bean common mosaic virus (BCMV) and common bacterial blight (CBB) (Kimani *et al*., 2005). Many of these diseases are major causes of yield loss in East Africa. When the environmental conditions are favourable for disease development, crop loss can be as high as 80-100% on susceptible cultivars of beans (Mahuku *et al*., 2010). Studies carried out in different bean growing regions of Kenya have indicated the presence of pathogenic variation to common bean diseases.

**Table 2.3:** Losses due to major constraints to bean production in Sub-Saharan Africa in thousands ton year$^{-1}$.

| Constraint | Eastern Africa | Southern Africa | Sub-Saharan Africa |
|---|---|---|---|
| Angular leaf spot | 281.3 | 93.5 | 384.2 |
| N deficiency | 263.6 | 125.2 | 389.9 |
| Anthracnose | 247.4 | 69.8 | 328.0 |
| P deficiency | 234.2 | 120.4 | 355.9 |
| Bean stem maggot | 194.4 | 96.4 | 297.1 |
| Root rot | 179.8 | 31.0 | 221.1 |
| CBB | 145.9 | 69.8 | 220.4 |
| BCMV | 144.6 | 29.9 | 184.2 |
| Aphids | 136.3 | 58.9 | 196.9 |

N=nitrogen, P=phosphorus, CBB=common bacterial blight, BCMV=bean common mosaic virus
Source: Wortmann *et al*., 1998

### 2.5.1 Angular leaf spot and pathogenic variation in *Pseudocercospora griseola*

Angular leaf spot caused by the fungus *Pseudocercospora griseola*, is considered one of the most important biotic constraints of *P. vulgaris* in both tropical and subtropical areas (Aggarwal *et al*., 2004). The disease attacks all aerial parts of common bean especially the leaves and pods. It causes dark grey to brown lesions on the leaves which are often delimited by the veins, giving them a characteristic angular appearance. The tissue surrounding the lesion may become chlorotic, and under severe infection lesions will coalesce and may lead to premature defoliation. In primary leaves the disease causes circular lesions (Borges *et al*., 2013), although some virulent pathotypes have

been reported to cause circular lesions on the trifoliate leaves (Crous *et al*., 2006). The disease is favoured by intermittent dry-wet and warm-cool weather with an optimum temperature for pathogen development being about 24 ºC (Stenglein *et al*., 2003). Losses can be as high as 80% depending on the cultivar genetic background and the pathogenicity of its causal agent, and under disease favourable environmental conditions (Mahuku *et al*., 2010; Singh and Schwartz, 2010).

Pathogenic variation in *Pseudocercospora griseola* was reported as early as 1950's when Brock (1951) found indications of virulent differences between 13 Australian isolates. Pathogenic diversity in *P. griseola* has also been reported by several authors (Stenglein *et al*., 2003; Sartorato, 2004; Wagara *et al*., 2004; Silva *et al*., 2008). Marin-Villegas (1959) used Colombian isolates to characterise 13 pathotypes. The early findings have however, been found inconclusive as Brock (1951) did not use single spore isolates while the purity of differential cultivars used by Marin-Villegas (1959) is disputed. Buruchara (1983) found variation among 21 Colombian isolates using six differential cultivars and grouped them into seven pathotypes.

Pathogenic variability in *P. griseola* has also been widely reported in other parts of the world. Pastor-Corrales *et al.* (1998) identified 333 races from 433 isolates of *P. griseola* obtained from 11 Latin American and 10 African countries. Mahuku *et al.* (2002) identified 50 pathogenic groups of *P. griseola* from 112 isolates collected from 10 countries in Central America. Pyndji (1992) characterised 21 isolates of *P. griseola* from the Great Lakes region of

Africa into 17 pathogenic groups using 11 differential cultivars. The existence of great pathogenic variation within *P. griseola* in Kenya has also been reported previously (Monda, 1995; Wagara, 1996; Wagara *et al*., 2004). Monda (1995) grouped 19 isolates of *P. griseola* from different areas in Kenya into 14 pathotypes using 11 differential cultivars. Wagara (1996) used 30 bean differential cultivars to group 15 races from 18 isolates collected from 15 districts in Kenya. However, discrepancies in the range of differential cultivars used in these studies make the results incomparable.

The first report of a systematic collection and race-typing of *P. griseola* isolates in Kenya using the international bean differentials identified 44 races from 100 isolates obtained in five districts (Wagara, 2004). This report however does not cover all bean growing regions and no attempts have been made to evaluate the pathogenic variation of angular leaf spot pathogen since 2004 or establish the pathotype distribution in different agro-ecological regions in Kenya. For durable use of genetic resistance to control angular leaf spot in common bean, it is necessary to take into account the race structure of *P. griseola*. This can only be achieved by race typing the pathogen through artificial inoculation onto 12 differential cultivars.

In the past, numerous methodologies were used to determine *P. griseola* physiological specialization (Marin-Villegas, 1959; Alvarez-Ayala and Schwartz, 1979; Buruchara, 1983; Correa-Victoria, 1987). Brock (1951) used four cultivars, namely Brown Beauty, Stringless Black Valentine, a Pinto, and a Red Mexican; while Alvarez-Ayala and Schwartz (1979) used five cultivars

that included, Caraota 260, Alabama 1, Red Kidney, ICA-Duva and Cauca 27. Buruchara (1983) utilized eight cultivars, three of which had previously been used by Alvarez-Ayala and Schwartz (1979) i.e. Alabama 1, Caraota 260, and ICA-Duva, which together with G01805-1P-1C, G02575-1OP-2C and G02858 were selected as the most suitable differential cultivars. Correa-Victoria (1987) used 21 differential cultivars, which included four used by Buruchara (1983), and two used by Alvarez-Ayala and Schwartz (1979). Eight of these cultivars namely Montcalm, Seafarer, BAT 332, Pompadour Checa, G05686, Cornell 49-242, A 339 and BAT 1647, were selected on account of their ability to differentiate between the isolates used (Liebenberg and Pretorius, 1997). Pyndji (1992) used these eight cultivars, plus G02858, Caraota 260 and A 285, and reported Montcalm, A 285, A 339, Caraota 260 and BAT 1647 to be the best indicators of the occurrence of new pathotypes in a given area.

It was not until 1995 that a new standardised set of 12 differential cultivars, namely Don Timoteo, G11796, Bolon Bayo, Montcalm, Amendoin, G05686, Pan 72, G02858, Flor de Mayo, MEX 54, BAT 332 and Cornell 49-242, was established at CIAT, Colombia, in November 1995 (Liebenberg and Pretorius, 1997). This has enabled meaningful comparison of results over time and from different parts of the world (Liebenberg and Pretorius, 1997).

According to Chacon *et al*. (2005), two *P. griseola* pathogenic groups appear to have co-evolved with the Andean and Mesoamerican common bean gene pools, respectively. The variation has led to the classification of two major groups of *P. griseola* that are defined as, 'Andean' (*Pseudocercospora*

*griseola* f. *griseola*) and 'Mesoamerican' (*Pseudocercospora griseola* f. *mesoamericana*) (Crous *et al*., 2006; Saparrat *et al*., 2009). As a result, it was suggested that the strategy for developing new angular leaf spot-resistant bean genotype, requires understanding of the genetic variation of the pathogen and, the incorporation of resistant genes from a given gene pool into cultivars of the other gene pool (Pastor-Corrales and Jara, 1995). Studies have shown that resistance to angular leaf spot is controlled by several independent genes, which possess one or more alleles resistant to several races of the pathogen (Carvalho *et al*., 1998; Mahuku *et al*., 2003; Caixeta *et al*., 2005). Recent studies have also shown that resistance of cultivars AND 277, Cornell 49-242, G10474 and MAR 2 to pathotypes 63-23, 31-17, 63-63 and 63-39 respectively, was each conditioned by a single dominant gene (Carvalho *et al*., 1998; Nietsche *et al*., 2000; Namayanja *et al*., 2006). Cultivars with only one resistance gene or allele can control the disease for only a few years, until the appearance of new races of the fungus (Pastor-Corrales *et al*., 1998; Stenglein *et al*., 2003). Pyramiding of resistance genes assisted by molecular markers has been proposed as a durable solution for this type of problem (Young and Kelly, 1996).

Several sources of resistance genes to angular leaf spot have been described and include A 75, A 140, A 152, A 175, A 229, BAT 76, BAT 431, BAT 1432, BAT 1458 and G05686 (CIAT, 1984). Other cultivars that are resistant sources to *P. griseola* include AND 277 (Carvalho *et al*., 1998), MAR 2 (Pastor-Corrales *et al*., 1998), Cornell 49-242 (Nietsche *et al*., 2000), MEX 54

(Sartorato *et al*., 2000), BAT 332 (Caixeta *et al*., 2003), G10474 (Mahuku *et al*., 2004) and G10909 (Mahuku *et al*., 2010). Following allelism tests a total of nine genes have been identified in some of the resistant cultivars. These genes include *Phg-1*$^{a}$, *Phg-2*$^{2}$, *Phg-3*$^{2}$, and *Phg-4*$^{2}$ for cultivar AND 227, *Phg-2, Phg-5* and *Phg-6* for MEX 54 and MAR 2 has *Phg-4* and *Phg-5* resistance genes (Caixeta *et al*., 2005).

Breeding for disease resistance is the most effective, safest, practical and economically viable strategy to control the bean angular leaf spot disease. However, the durable use of new common bean cultivars resistant to angular leaf spot requires a consideration of the pathogenic variability of the causal pathogen, in order to define suitable strategy of genotype management. The understanding of pathogenic variation and race distribution of *P. griseola* in Kenya will facilitate targeted breeding programs in developing varieties resistant to angular leaf spot.

### 2.5.2 Anthracnose and pathogenic variation in *Colletotrichum lindemuthianum*

Anthracnose (*Colletotrichum lindemuthianum*) is one of the most important seed-borne fungal diseases of common bean in tropical and subtropical regions, and is favoured by relatively cool and humid conditions for disease development (Pastor-Corrales *et al*., 1995). The disease is prevalent throughout bean growing areas of Africa. When infection occurs early in the growth cycle of susceptible cultivars yield loss can be 100% (Fernandez *et al*., 2000).

The pathogen has been reported to possess a very high degree of pathogenic variability in different parts of the world. More than 140 races of the pathogen have been reported the world over (Sharma *et al*., 2007; Padder *et al*., 2009). Pathogenic variability of *C. lindemuthianum* was first reported by Barrus (1911). Since then, several races of this fungus have been described in different parts of the world (Gathuru and Mwangi, 1991; Kelly *et al*., 1994; Sharma *et al*., 2007; Padder *et al*., 2009). Alzate-Marin *et al*. (2007) 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 isolates collected from Andean and Mesoamerican bean varieties and regions.

Gathuru and Mwangi (1991) characterised thirty six isolates collected from nine districts of Kenya, 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, five as epsilon, two as delta and one as alpha. Nine isolates did not fit in any of the known races. The cultivar 'Cornell 49-242' was found resistant to all isolates. The cultivar 'Kaboon' was found susceptible to the majority of isolates. So far, seven races namely 17, 2, 38, 23, 1, 55 and 485 have been reported in Kenya (Gathuru and Mwangi, 1991; Ombiri *et al*., 2002). This follows re-classification of races beta, gamma, epsilon, delta, alpha into 17, 2, 38, 23, 1 and 55 respectively using a set of 12 internationally accepted differential cultivars namely

Michelite, Michigan Dark Red Kidney, Perry Marrow, Cornell 49-242, Widusa, Kaboon, Mexico 222, PI 207262, TO, TU, AB 136 and G 2333, and report of race 485 in Kenya (Ombiri *et al*., 2002).

The new set of 12 differential lines and a binary system classification proposed by researchers at CIAT (1987) is used to characterize anthracnose races. This has facilitated identification and comparison of economically important races of *C. lindemuthianum* in different bean growing regions. It also provided vital information used by bean breeders to pyramid genes for resistance to anthracnose targeting specific races of the pathogen (Balardin and Kelly, 1998). Wide pathogenic diversity reflects the highly dynamic nature of the pathogen indicating its constant evolution in nature. Continuous evolution of races indicates the complexity of pathogen which in turn warrants the scientists to breed new varieties against the most complex/evolved races (Padder *et al*., 2009).

The pathotype identification studies in Kenya have been sporadic, and no systematic characterization of *C. lindemuthianum* isolates has been done. Moreover, the last pathotype identification was done in 2002 where four isolates from Nakuru were classified as race 485 (Ombiri *et al*., 2002). Several strategies can be used to manage anthracnose, but planting genetically resistant cultivars is most effective, least expensive, and easiest for farmers to adopt (Pastor-Corrales *et al*., 1995). Genetic resistance to some pathotypes of *C. lindemuthianum* is conferred by different single, duplicate, or complementary dominant genes (Young and Kelly, 1996), and is available in

numerous germplasm accessions (Pastor-Corrales *et al*., 1995; Fernández *et al*., 2000; Sharma *et al*., 2007). Several sources of resistance to this disease have been found. The well documented sources of anthracnose resistance include G2333, AB136, G2641, PI207262, Cornell 49-242, Mex 222, G811, Mex 227, and Ecuador 299 (Graham and Ranalli, 1997).

The main drawback to resistant cultivars is the possible breakdown of resistance caused by the adaptation of the pathogen to host resistance and that these sources have not been effective against all races of the pathogen (Sharma *et al*., 2007). Anthracnose is a highly variable pathogen (Balardin and Kelly, 1998; Mahuku and Riascos, 2004; Alzate-Marin *et al*., 2007), and there are no resistance genes that are effective against all known races of this pathogen from the same or different regions. The cultivar G2333, which has three resistance genes (*Co-4*$^2$, *Co-5*, and *Co-7*), is resistant to more than 90% of the races that have been described, but it is susceptible to some races (e.g., 3481, 3545, 3977, and 3933) from Costa Rica, Mexico, and Argentina (CIAT, 1995).

The breakdown of resistance in G2333 once thought to be effective against all races reflects on the need for diversification of resistance genes (Mahuku *et al*., 2002a). Given the wide variability of the pathogen and the potential for new virulent races to arise, the use of the genetically resistant cultivars is an effective way to control this disease (Kelly *et al*., 1994). Eleven independent resistance genes (*Co*-genes) have been described in common bean; 10 genes were identified from Mesoamerican germplasm and one from Andean germplasm (Kelly and Vallejo, 2004; Alzate-Marin *et al*., 2007). Resistance to

anthracnose is thus conditioned primarily by nine major independent genes, *Co-1* to *Co-10* as the *Co-3*/*Co-9* genes are allelic. With the exception of the recessive *co-8* gene, all other nine are dominant genes and multiple alleles exist at the *Co-1, Co-3* and *Co-4* loci (Alzate-Marin *et al*., 2007; Garzón *et al*., 2008).

A number of SCAR markers have been developed for the three known anthracnose resistance genes in G2333. The dominant marker SAS13 was the first to be developed and was very tightly linked (0.0 cM) to the *Co-4*$^2$ gene (Garzón *et al*., 2008). The second marker was another dominant SCAR called SAB3 that was linked, but not tightly (12.98 cM), with the *Co-5* gene (Vallejo and Kelly, 2009). Two more recent markers for *Co-4*$^2$ have been developed (SH18 and SBB14) and are more genotype specific but less closely linked to the resistance locus than SAS13 (Garzón *et al*., 2008).

The use of specific resistance genes in breeding has not always provided durable resistance due to the continuous development of new physiological races of the pathogen capable of overcoming the resistant germplasm. Continual evaluation of germplasm for resistance to anthracnose as well as the introgression of diverse resistance genes into existing cultivars is therefore needed (Mahuku *et al*., 2002a). Several markers linked to the anthracnose resistance genes, and include SAS-13, SH-18, SBB-14 and SAB-3, are already being used in Kenya, Uganda and Tanzania (Buruchara *et al*., 2011).

There are however, no reports on the utilisation of anthracnose races information in pyramiding resistance genes within eastern Africa. There is need for bean breeders to characterise existing races of anthracnose in eastern Africa region, and use this information for targeted resistance development on important races.

### 2.5.3 Bean common mosaic disease and pathogenic strains of the virus

Bean common mosaic virus (BCMV) and bean common mosaic necrotic virus (BCMNV) are some of the most common and destructive potyviruses known to infect common bean worldwide (Kapil *et al*., 2011). Both viruses are seed-borne and transmitted by several aphid species in a non-persistent manner (Miklas *et al*., 2000). The virus induces the formation of cylindrical ‘‘pinwheel’’ inclusions in the cytoplasm of infected cells of susceptible cultivars (Mavric and Vozlic, 2004).

Pathogenic strains of the virus, first identified by Drijfhout *et al*., (1978) on international differential set of bean varieties, have been categorised into two serotypes, A and B causing temperature insensitive necrosis and mosaic symptoms on differential cultivars carrying *I* and *ii* resistance genes (Huang and Chang, 2005). These strains have now been reclassified as two separate viral species of potyvirus based on their peptide profiles and nucleotide sequence data and named as BCMV (Serotype B) and BCMNV (Serotype A) (Huang and Chang, 2005).

Both BCMV and BCMNV produce similar type of symptoms in bean plants except ‘‘black root’’ or ‘‘top necrosis’’ caused either by BCMNV (at all temperatures) or necrosis by strains of BCMV (at high temperatures) (Kelly, 1997). The similarity of symptoms produced by some strains of both viruses makes it very difficult to distinguish them in the field (Gibbs *et al*., 2008; Kapil *et al*., 2011). BCMV and its host common bean have been studied systematically in Europe, USA and Africa, and the virus is known to possess high degree of pathogenic variability (Kapil *et al*., 2011).

The different strains of BCMV have been reported in common bean in many parts of the world including India (Kapil *et al*., 2011), Mexico (Flores-Estévez *et al*., 2003), Tanzania (Njau and Lyimo, 2000), Kenya (Omunyin *et al*., 1995), Canada (Tu, 1986), U.S. (Kelly *et al*., 1983), and Europe (Drijfhout, 1978). Omunyin *et al.* (1995) reported on the pathogenicity groups occurring in Kenya, and was able to differentiate 14 virus isolates into four pathogroups. These pathogroups included: i) necrotic strain VI from Kakamega, Naivasha, Nyahururu, Murang’a, Thika and Kabete, ii) non-necrotic strain V from Kabete and, iii) two potentially new groups, one necrotic strain from Nyeri and another non-necrotic strain from Subukia in Nakuru.

Genetic resistance to both potyviruses is conditioned by a series of independent dominant and recessive multi-allelic loci in common bean (Drijfhout, 1978). The dominant inhibitory *I* gene located on B2, conditions a classic hypersensitive resistance response against many strains of BCMV and other potyviruses but is vulnerable to the necrotic strains of BCMNV (Kelly *et*

*al*., 2003). The *I* gene is independent of recessive resistance conditioned by four different *bc* genes. Three strain specific recessive loci *bc-1*, *bc-1*$^2$; *bc-2*, *bc-2*$^2$; and *bc-3* control resistance to BCMV and BCMNV (Mukeshimana *et al*., 2005). The fourth recessive locus conditioning resistance is a strain non-specific *bc-u* gene that is necessary for the full expression of all strain specific recessive genes in the absence of the *I* gene (Mukeshimana *et al*., 2005). The *bc-3* gene is located on B6 (Mukeshimana *et al*., 2005), whereas the *bc-1*$^2$ allele was mapped to B3 (Miklas *et al*., 2000). The non-specific *bc-u* allele, needed for expression of *bc*-2$^2$ resistance, also resides on B3 based on the loose linkage with the *bc-1* locus (Kelly *et al*., 2003).

Several sources of resistance genes to this potyviruses include BelNeb RR-1 and BelNeb RR-2 with the *bc-1*$^2$ and *bc-2*$^2$ genes that provide resistance to BCMV and BCMNV (Mukeshimana *et al*., 2005). Other sources of resistance include BRB 29, BRB 32 and BRB 191 that condition resistance to BCMNV (CIAT, 2003). The independence of the resistance genes provides opportunities to use gene pyramiding as a strategy in breeding for durable resistance. Bean breeders recognize that the combination of the dominant *I* gene with recessive *bc* genes offers durable resistance to all known strains of BCMV and BCMNV (Kelly *et al*., 2003). This is because the two types of genes have distinctly different mechanisms of resistance (Kelly, 1997). The dominant *I* gene is defeated by all necrotic strains, whereas the three most effective recessive genes (*bc-1*$^2$, *bc-2*$^2$, *bc-3*) act constitutively by restricting

virus replication or movement within the plant, probably through the virus movement proteins (Kelly *et al*., 2003).

The knowledge of strain spectrum of a particular pathogen is the first pre-requisite to exploit their management through host resistance, which is the only durable and economic method of managing the viral pathogens.

### 2.5.4 *Pythium* species pathogenic to common bean

Common bean production is hampered by several constraints among which are bean root rots caused by *Pythium* spp. This disease is considered as the most damaging in East and Central Africa including Kenya where beans are grown intensively (Nzungize *et al*., 2011). Bean root rot caused by several *Pythium* species is a relatively recent problem on beans (*P. vulgaris*) in East and Central Africa that is increasing in importance. Yield losses of up to 70% in popular commercial bean cultivars have been reported in Rwanda and Kenya but the pathogen can also lead to total yield losses when susceptible varieties are grown under favourable environmental conditions for the pathogen development (Otsyula *et al*., 2003).

The disease is characterized by above ground symptoms such as poor seedling establishment, uneven growth, damping off and premature defoliation of severely infected plants (Spence, 2003; Abawi and Ludwig, 2005). Infected tissues become elongated, spongy, and water-soaked and discoloured with many cavities. In addition to the previous symptoms, the disease is also

characterized by yellowing of lower leaves (similar to nitrogen deficiency), stunting, leaf browning and plant wilt and death (Ampaire, 2003).

The *Pythium* fungus is known to survive in the soil for several years as oospores that germinates to produce zoospores that infect the root and lower stem (Rusuku *et al*., 1997). The *Pythium* inducing agents produce several zoospores that enable them to rapidly and continuously re-infect growing roots in susceptible cultivars (Rusuku *et al*., 1997). Bean root health is an essential component in managing abiotic stresses as root pathogens aggravate problems of drought or phosphorus acquisition by restricting root systems.

Until recently no resistant genotypes to bean root rot were identified and commercial bean varieties released in Kenya, Uganda and Rwanda are highly susceptible to *Pythium* root rot (Otsyula *et al*., 2003). Previous screen house and field screening has identified few sources of resistance to *Pythium* root rot within *P. vulgaris* (Otsyula *et al*., 2003; Buruchara *et al*., 2007). These sources of resistance genes to *Pythium* root rot include RWR 719, AND 1062 and MLB 49-89A that were selected through a greenhouse evaluation conducted at Kawanda in Uganda and were found to be some of the most resistant (Otsyula *et al*., 2003; Buruchara *et al*., 2007). RWR 719 and MLB 49-89A showed resistance to bean root rots in field evaluation carried out in Rwanda, Kenya and Uganda (CIAT, 2000; Otsyula *et al*., 2003).

For an efficient and practical control of the *Pythium* root rot of bean, the use of resistant varieties is considered as the most viable option in East Africa region

(Otsyula *et al*., 2003; Nzungize *et al*., 2011). However, selection and sustainable use of resistant varieties has to take into account diversity of causal agents. *Pythium* species pathogenic to beans in Kenya have been characterised, which is crucial for effective epidemiological studies. Buruchara *et al*. (2004) characterised 134 *Pythium* isolates obtained from root rot affected areas in Kenya and Rwanda and was able to identify 22 species of *Pythium*. Nineteen of the 22 species were recovered from Rwanda with *Pythium ultimum* being the most frequent (Buruchara *et al*., 2004). In Kenya the isolates were collected from Trans-Nzoia, Kakamega, Vihiga, Kisii, Meru, Embu, Kirinyaga, Murang'a, Kiambu and Nairobi districts and a species distribution map established (Buruchara *et al*., 2004).

The Kenyan isolates were characterised into 15 species with *P. vexans* being the most frequent, followed by *P. torulosum, P. irregular* and *P. sp.* (Buruchara *et al*., 2004). However, *Pythium* agents belonging to various species were reported to cause root rots in Rwanda (Nzungize *et al*., 2011) and include: *Pythium vexans, P. ultimum, P. indigoferae, P. torulosum, P. cucurbitacearum* among others. For an efficient and practical control of the *Pythium* root rot of bean, the use of resistant varieties is considered as the most viable option in East Africa region (Otsyula *et al*., 2003). However, selection and sustainable use of resistant varieties has to take into account diversity of causal agents.

## 2.6 Taxonomic nomenclature of *Pseudocercospora griseola*

Angular leaf spot of common bean is incited by *Pseudocercospora griseola* (Sacc.) Crous and Braun (2006), an imperfect fungus belonging to the phylum Ascomycota, class Dothideomycetes, order Capriodiales, and family Mycosphaerellaceae. It is hemibiotrophic and was first described on *P. vulgaris* in Italy as *Isariopsis griseola* Sacc. in 1878 (Saccardo, 1886). The binomial nomenclature of this pathogen has undergone numerous changes, particularly to its genera, and as such acquired many synonyms. Until recently, the pathogen was previously known as *Phaeoisariopsis griseola* (Sacc.) Ferr., other synonyms include; *Isariopsis laxa* (Ellis) Sacc. (1886), *Cercospora columnaris* Ellis and Everh. (as "*columnare*") (1894), *Lindaumyces griseolus* (Sacc.) Gonz. Frag. (as "*griseola*") (1927), *Cercospora griseola* (Sacc.) Ragunath. and K. Ramakr. (1968), among others.

*Pseudocercospora* was originally introduced by Spegazzini (1910) based on the type species *Pseudocercospora vitis*, a foliar pathogen of grapevines (Crous *et al*., 2013). *Pseudocercospora* was established to accommodate synnematal analogues of *Cercospora,* as well as species that produce pigmented conidiogenous structures and conidia with neither thickened nor darkened conidial hila (Crous *et al*., 2013). Based on comprehensive examinations of the phylogenetic studies and molecular data presented by Crous *et al*. 2006, results showed that this species clusters within the *Pseudocercospora* clade and revealed that the formation of synnematous conidiomata does not play any taxonomic role on generic level within the

*Pseudocercospora* complex (Crous *et al*., 2013). Based on these studies, the necessity arose to conserve *Pseudocercospora* over *Stigmina*, which represented an older generic name (Braun and Crous, 2006). Hence, this species was reallocated to the genus *Pseudocercospora* and *Phaeoisariopsis* was reduced to its synonym (Braun and Crous, 2006). The name *Pseudocercospora griseola* has since been accepted and used by authors such as Silva *et al*. (2008), Saparrat *et al*. (2009) and Pereira *et al*. (2013) among others.

## 2.7 Methods and strategies in breeding beans for disease resistance

The overall goal of a breeding program is to improve specific characteristics of a bean variety without compromising other characteristics that the variety possesses. Common bean breeding has been utilized particularly with emphasis to biotic constraints and has been achieved by use of selective resistance genes from donor parents. Molecular markers are used and permit the development of common bean lines that pyramid genes for disease resistance (Kelly and Miklas, 1998). This strategy is designed to develop common bean lines with more durable resistance (McDonald and Linde, 2002). Breeding programs follow several methods in common bean. This includes, pedigree selection which is a common method used by bean breeders to develop improved cultivars. Another method used is the single seed descent (SSD) procedure that provides a way to maintain genetic variability while advanced-generation lines are produced. When multiple generations are grown

each year, bulk breeding is used to rapidly advance bean populations. This approach is most appropriate for crosses between elite lines within a market class where little segregation for seed type or adaptation would be expected. Other methods used in breeding common bean include recurrent selection, participatory plant breeding and the more recent gamete selection procedure. Gamete selection is a breeding procedure that allows screening and selection of desirable dominant and codominant alleles during hybridization and immediately after production of final multiple-parent $F_1$ hybrids (Singh, 1994). The conventional breeding methods most commonly used such as bulk, pedigree, backcross, single seed descent and their modifications have numerous limitations and are less effective. The methods involve managing and advancing considerable amounts of undesirable genotypes that are eventually discarded resulting in wastage of scarce resources. With an increasing need to simultaneously improve multiple traits in common bean, gamete selection presents a method that permits identification of promising populations and families and reliable yield evaluations in early generations. It also allows selection for multiple desirable traits and maximizes the efficiency, usage and reduces costs of molecular markers. Gamete selection has previously been used; to develop carioca bean with resistance to five diseases and a leaf hopper (Singh *et al*., 1998), in intergene-pool populations for simultaneous improvement of resistance to common and halo bacterial blights (Asensio-S.-Manzanera *et al*., 2006), and to introgress physiological resistance to white mold in two intergene-pool double-cross populations (Terán and Singh, 2009).

## 2.8 Parental genotypes for the proposed work

### 2.8.1 Sources of resistance genes

MEX 54 has medium sized seed, with an indeterminate growth habit and has been previously identified as resistant to most African *P. griseola* races (CIAT, 1996). MEX 54 has been found to contain a single dominant gene for resistance to angular leaf spot (Nietsche *et al*., 2001; Mahuku *et al*., 2004; Namayanja *et al*., 2006). G10909 is a medium red-seeded climbing bean genotype from the highlands of Guatemala that was identified as having high levels of resistance to *P. griseola* under field conditions (Pastor-Corrales *et al*., 1998) and under greenhouse conditions using *P. griseola* pathotypes of diverse origin (Mahuku *et al*., 2003). Studies conducted to elucidate the inheritance of angular leaf spot resistance in the bean accession G10909 and to identify molecular markers linked to these genes have showed that two complementary dominant genes condition angular leaf spot resistance of G10909, and identified four molecular markers that segregate in coupling phase with these resistance genes. This confirms the potential of MEX 54 and G10909 as candidates in improving angular leaf spot resistance in common bean using marker-assisted gamete selection.

The Mexican landrace, G2333 – commonly referred as 'Umubano', has been widely used as a source of resistance to *Colletotrichum lindemuthianum,* the causal agent of anthracnose (Young and Kelly, 1996; Vallejo and Kelly, 2009). G2333 carries three characterized naturally-occurring gene pyramid for anthracnose resistance: *Co-4*$^2$, *Co-5* and *Co-7* (Pastor-Corrales *et al*., 1995;

Young *et al*., 1998). The most effective gene in this pyramid is *Co-4*$^2$, which conferred resistance to 33 out of 34 different races of *Colletotrichum lindemuthianum* collected from 9 different countries in the Americas (Balardin *et al*., 1997). Three SCAR markers linked to *Co-4*$^2$ have been developed to facilitate the use of this gene in anthracnose resistance breeding: SAS-13 at 0.39 cM from the *Co-4*$^2$ gene (Young *et al*., 1998; Melotto and Kelly, 2000), SH-18 at 4.27±2.37 cM from the gene, and SBB-14 at 5.87±1.93 cM from the gene (Awale and Kelly, 2001). SAS13 has successfully been used to introduce *Co-4*$^2$ into highly susceptible pinto bean through marker-assisted backcrossing in the absence of pathogen screening (Miklas and Kelly, 2002).

RWR 719 is a late maturing small red-seeded variety of Mesoamerican gene pool that is resistant to all species of *Pythium* (Otsyula *et al*., 2003; Nzungize *et al*., 2011) while AND 1062 is medium maturing and the only large seeded variety resistant to *Pythium* (Mukalazi *et al*., 2001). These genotypes are known to possess resistance to *Pythium* which is controlled by a single dominant gene (Otsyula *et al*., 2003; Nzungize *et al*., 2011). RWR 719 and AND 1062 have been proposed as donors for resistance against the virulent and predominant *Pythium* spp. in breeding programs to create common bean varieties resistant to bean root rot and adapted to East and Central Africa region (Otsyula *et al*., 2003). The red mottled Andean genotype BRB 191 was utilized due to its *bc*-3 resistance genes that confer resistance to bean common mosaic virus (CIAT, 2003).

### 2.8.2 Susceptible commercial varieties

KAT B1 is a high yielding, early maturing, and determinate variety resistant to rust but susceptible to angular leaf spot and anthracnose (Kimani *et al*., 2012). It is recommended for semi-arid areas where rainfall is below 250 mm per season, preferably at higher altitudes between 1000 m to 1800 m above sea level (Kimani *et al*., 2012). Seeds are bold, round and deep yellow in colour.

KAT B9 is a drought tolerant, compact and bushy genotype with a yield potential of between 1400-1900 kg $ha^{-1}$ (Kimani *et al*., 2012). It is preferred for its dark red seeds, low flatulence and sweet taste (Kimani *et al*., 2012).

GLP 585 'Wairimu' or 'Red haricot bean' is a small-seeded commercial variety with good marketability traits and potentially a high yielder. It has bright red seeds. It is susceptible to angular leaf spot, anthracnose and root rot diseases (Kimani *et al*., 2012).

GLP 92 'Mwitemania' is a late maturing, indeterminate, semi-spreading, high yielding pinto bean. It is resistant to halo blight but susceptible to bean common mosaic virus, rust, anthracnose and angular leaf spot (Kimani *et al*., 2012). It has wide adaptability to various agro-ecological zones of low to high rainfall areas and hence recommended for all bean-growing areas except for those notorious for bean common mosaic virus (Kimani *et al*., 2012). Seeds are round and broad with brown flecks on cream background.

## 2.9 Marker-assisted gamete selection

Singh (1994) proposed the use of gamete selection to simultaneously select common beans for multiple traits. Gamete selection is a breeding procedure that allows screening and selection of dominant and co-dominant alleles during hybridisation and immediately after production of final multiple-parent $F_1$ hybrids and involves early generation testing of $F_1$ derived families for multiple traits. The method permits identification of promising populations and families in early generations. It also allows selection for multiple desirable traits and permits reliable yield evaluations in early generations – populations that do not segregate for desired traits in early generations can be discarded, thus avoiding the loss of valuable time and maximizes the efficiency, usage and reduces costs of molecular markers.

However, Singh *et al*., (1998) noted that labour-intensive nature of gamete selection permits the evaluation of only a few populations and that much care should be taken in the selection of parents that possess the desired traits. 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 utilized gamete selection to develop breeding lines with resistance to common bacterial blight and halo blight.

Molecular markers may facilitate gamete selection in the identification of early-generation populations that continue to possess the desired alleles as proposed by Singh *et al*. (1998), which would permit gametic or genotypic

selection from $F_1$ eliminating the need for growing large populations in the $F_{1.2}$ and subsequent generations (Singh, 1994). Liu *et al*. (2004) found in computer simulations that marker-assisted selection of self-fertilized crops was more advantageous in earlier generations.

Marker-assisted selection (MAS) has become a common tool used in many common bean breeding programs (Kelly *et al*., 2003; Miklas *et al*., 2006a). MAS permit the indirect selection of traits in the absence of selection pressure for the trait. Ideally, a marker for routine screening in a plant breeding program should be reliable, rapid and inexpensive (Garzón *et al*., 2008). Marker-assisted selection in early generations allows the elimination of breeding lines having inferior genotypes while maintaining sufficient variability to produce superior breeding lines in later generations. Molecular markers have been developed for many disease resistance genes and these markers have been successfully used to develop improved common bean cultivars and germplasm (Kelly and Miklas, 1998; Miklas *et al*., 2006a).

Pastor-Corrales *et al*. (2007) used the SW-13 SCAR to confirm the presence of the *I* gene for BCMV resistance in the development of great northern bean germplasm with multiple disease resistance. Miklas *et al*. (2006b) used MAS to identify plants with the SCAR markers SU-91 and SAP-6 in the development of the common bacterial blight resistant dark red kidney germplasm USDK-CBB-15. Miklas *et al*. (2003) used the SCAR marker SAS-13 to develop pinto bean germplasm having the *Co-4*$^2$ gene for anthracnose resistance. Molecular markers have also permitted the development of

common bean lines with pyramid genes for disease resistance (Kelly and Miklas, 1998). The marker-assisted gamete selection strategy is designed to develop common bean lines with more durable disease resistance (McDonald and Linde, 2002). However, marker-assisted selection has not been utilized before in gamete selection to improve common bean varieties against either biotic and/or abiotic constraints.

# CHAPTER 3 PREVALENCE OF BEAN DISEASES AND PATHOGENIC DIVERSITY OF ANGULAR LEAF SPOT OF COMMON BEAN IN KENYA

## 3.1 ABSTRACT

In the recent past, angular leaf spot incidence and severity have increased in many areas where beans are cultivated particularly in Kenya and other Eastern Africa countries. Monitoring pathogenic diversity is critical for breeding programs aiming at genetic resistance. However, a comprehensive survey of bean diseases and race diversity of angular leaf spot in all major bean growing regions has not been conducted. The objective of this study was to conduct a survey of prevalent diseases and identify races of *P. griseola* in Kenya.

Five economically important diseases causing major losses mainly (angular leaf spot, anthracnose, common bacterial blight, root rots and bean common mosaic virus) were sampled in all the agro-ecological zones. A collection of 62 samples of angular leaf spot, 55 of anthracnose, 9 of root rots, 121 of common bacterial blight and 32 of bean common mosaic virus were obtained. The survey covered 12 agro-ecological zones (AEZs) that included Upper Highland (UH2), Lower Highland (LH1, LH2, LH3 and LH4), Upper Midland (UM1, UM2, UM3, UM4 and UM5) and Lower Midland (LM1 and LM2).

The pathogenic variability of 57 isolates of the *P. griseola*, collected from thirty-five districts in the bean growing regions of Kenya, was studied using the current 12 angular leaf spot differentials (Don Timoteo, Bolon Bayo, Montcalm, G05686, Amendoin, G11796, BAT 332, PAN 72, Cornell 49-242,

MEX 54, Flor de Mayo and G02858). The first trifoliate leaf was inoculated with a $2 \times 10^4$ conidia $ml^{-1}$. Plants were maintained at 22–28 °C and ≥ 95% relative humidity for 48 hours. Symptoms were evaluated 12, 17 and 21 days after inoculation.

Twenty-three races of *P. griseola* were identified, 12 of which were represented by only one isolate. Only 11 races were found in two or more districts. Race 63-63 was the most virulent and caused leaf spots on all 12 common-bean differential genotypes, whereas race 63-55 was the most frequent (10 of 57 isolates), being widely distributed among the regions studied. Races 63-55, 63-63, 63-54 and 63-35 were found to be the most dominant races in areas studied. Two new races, 31-31 and 63-31 were reported for the first time in Kenya.

The virulence phenotype indicated that 45 isolates studied were of the Mesoamerican group, with only 12 isolates of Andean group, suggesting co-evolution of the pathogen with *P. vulgaris* in this host-pathogen interaction. The information generated on race diversity and distribution of *P. griseola* pathogen will facilitate targeted development of common bean varieties with resistance to these races and for specific regions.

**Key words:** *Pseudocercospora griseola, Phaseolus vulgaris,* differential cultivars, races, pathogenic variability.

## 3.2 INTRODUCTION

Angular leaf spot, incited by the imperfect fungus *Pseudocercospora griseola* (Sacc.) Crous and Braun, is the most wide spread disease of beans in Africa, particularly in tropical countries such as Kenya, Uganda, Tanzania and the Great lakes region where beans are produced (Stenglein *et al*., 2003; Namayanja *et al*., 2006; Mahuku *et al*., 2010). Angular leaf spot is rated as the second most significant among numerous biotic and abiotic constraints that afflict the bean crop in Africa (Aggarwal *et al*., 2004).

In Kenya, angular leaf spot has been reported to cause significant losses of bean (Wagara *et al*., 2004) resulting in yield losses of up to 80% under favourable environmental conditions (Mahuku *et al*., 2010; Singh and Schwartz, 2010; Wahome *et al*., 2011). The disease affects foliage, petioles and pods throughout the growing season and is particularly destructive in areas where warm, moist conditions are accompanied by abundant inocula (Mahuku *et al*., 2010). Lesions on leaves start as small, brown or grey spots that become angular and necrotic, being confined by leaf veins while pod symptoms consist of circular to elliptical reddish-brown lesions.

In the recent past, angular leaf spot incidence and severity have increased in many areas where beans are cultivated (Stenglein *et al*., 2003), particularly in Kenya and other eastern Africa countries (Mwang'ombe *et al*., 2007). Angular leaf spot is a significant constraint to bean production with annual losses estimated at 281,300 tonnes in eastern Africa (Wortmann *et al*., 1998). The disease also causes severe and premature defoliation resulting in shrivelled

pods, shrunken seeds and reduced seed and pod quality (Stenglein *et al*., 2003). The disease is controlled mainly through the use of resistant varieties derived from monogenic and polygenic genes acting together or separately (Stenglein *et al*., 2003).

Monogenic resistance is typically race-specific and controlled by single genes whereas polygenic resistance is race-non specific and controlled by many genes (Stenglein *et al*., 2003). Most of the qualitative genes such as *Phg*-2, *Phg*-3, *Phg*-5 and *Phg*-6 are dominant or partially dominant and have a great phenotypic effect but might be overcome by virulent genes present in specific races of the pathogen (Caixeta *et al*., 2005). The angular leaf spot pathogen has been reported to have a high degree of pathogenic and genetic variability (Nietsche *et al*., 2001; Sartorato, 2002; Wagara *et al*., 2004; Mahuku *et al*., 2010).

The race composition in any given area is continuously changing, with the emergence and introduction of new races, and needs to be constantly monitored. These races frequently vary in time and space; thus, a bean variety that is resistant in one year or location may be susceptible in another (Aggarwal *et al*., 2004). Pathogenic variation of *P. griseola* is expressed in different geographical and agro-ecological zones and on host genotypes. Hence identification of the *P. griseola* races present in an area and the understanding of their geographical distribution are critical in screening for resistance and deployment of resistant genes. Pathogenicity is differentiated in the symptom variation expressed in test plants as a result of pathogen variability.

Race-typing the *P. griseola* population from Kenya will provide information on the amount of pathogenic variation that is maintained in this pathogen and the geographical distribution of the different pathotypes. Severity and incidence of bean diseases is dynamic and changes with localities and environmental conditions especially those associated with climate changes and variability. However, a comprehensive survey of bean diseases in major bean growing regions has not been conducted. The objective of this study was to conduct a survey of prevalent diseases and identify races of *P. griseola* in bean growing regions of Kenya.

## 3.3 MATERIALS AND METHODS

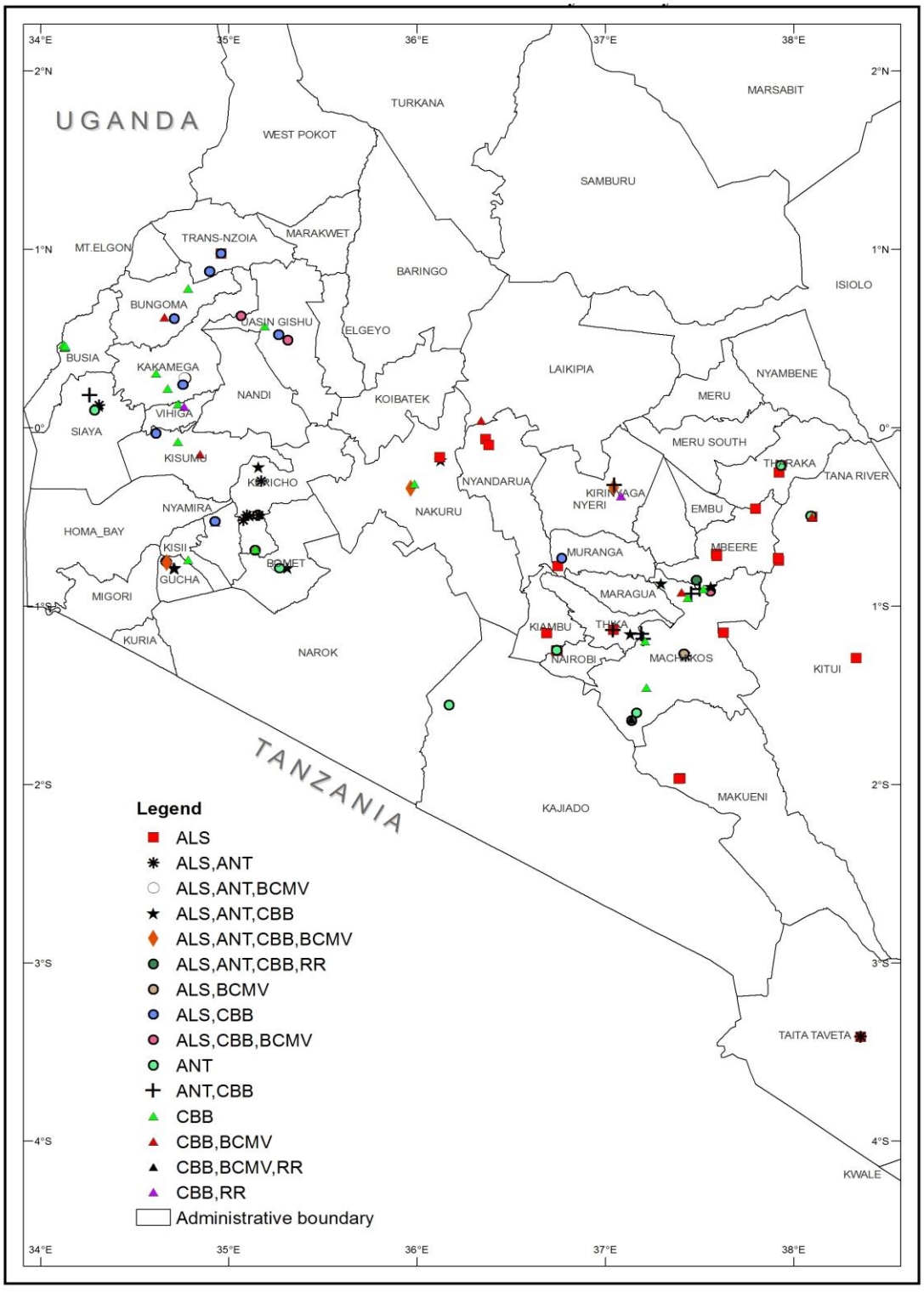
### 3.3.1 Collection of diseased material

To study the prevalence of five economically significant diseases of common bean, a survey was conducted in all major common bean growing regions of Kenya. This survey covered the prevalence of angular leaf spot, anthracnose, common bacterial blight, *Pythium* root rot and bean common mosaic disease. Two surveys were carried out in December 2010 and June 2011 in 35 bean growing districts of Kenya during the short and long rains season, respectively (Table 3.3; Fig. 3.1). Samples were collected from naturally infected common bean cultivars and landraces from the major bean producing agro-ecological regions of Kenya (Table 3.3).

The agro-ecological zones (AEZs) covered were Upper Highland (UH2), Lower Highland (LH1, LH2, LH3 and LH4), Upper Midland (UM1, UM2, UM3, UM4 and UM5) and Lower Midland (LM1 and LM2) (Table 3.4). The five economic important diseases causing major losses mainly (angular leaf spot, anthracnose, common bacterial blight, root rots and bean common mosaic virus) were sampled in all these agro-ecological zones. Diseased leaf samples were preserved neatly between sheets of old newspapers and pressed with a wire mesh to dry and for transportation from the field to the laboratory for analysis.

Leaves symptomatic to bean common mosaic virus were placed in ziploc polythene bags and preserved in ice flakes in cool boxes. Roots sampled with root rot symptoms were carried in khaki size-3 paper bags while soil was

carried in 5 kg size nylon sacs. Seeds obtained from the survey with common bacterial blight symptoms were carried in khaki size-3 bags. The bean gene pools (Andean or Mesoamerican), plant growth habit (climber or bush) and variety identities from which the isolates were obtained were recorded whenever it was possible. The geographic coordinates were recorded for all the points where diseases were sampled using an eTrex® (Garmin™, Taiwan) global-positioning-system (GPS) recorder.




ALS=angular leaf spot, ANT=anthracnose, BCMV=bean common mosaic virus, CBB=common bacterial blight, RR=*Pythium* root rot

**Figure 3.1:** The map of Kenya showing areas surveyed and prevalence of common bean diseases.

### 3.3.2 Pathogen isolation and preservation

Isolation and culturing was done aseptically in the Pathology Laboratory at University of Nairobi. Angular leaf spot pathogen was isolated by picking conidia from well developed and sporulating lesions using a tiny piece of agar placed on the tip of a sterilized dissecting needle, and streaked onto tap water agar (TWA – 15g Agar powder and 1000ml double distilled water) and then incubated in darkness for 24h at 21 °C. The TWA plates streaked with conidia were then observed under a dissecting microscope to identify the germinated conidia.

The individual germinated conidia were then transplanted onto V8 medium (200ml V8 juice, 3g $CaCO_3$, 18g Bacto agar and 800ml $ddH_2O$) to obtain monosporic cultures for each *P. griseola* isolate. Isolates were maintained on V8 juice agar and kept in a dark incubator at 21 °C for up to 21 days to promote sporulation (Fig. 3.2). V8 juice is a trademark name for commercial beverage products made from either eight vegetables or a mixture of vegetables and fruits and whose brand is owned by the Campbell Soup Company-USA.

Anthracnose was isolated using small pieces of symptomatic tissue that were surface sterilized by immersing them in 0.5% NaOCl for 3 minutes. After which they were rinsed in sterile distilled water, blotted dry on sterile filter paper. Inorder to suppress bacterial growth, the antibiotic streptomycin sulphate (40ppm) was added onto the medium before plating.

The surface sterilized tissues were then plated in to potato dextrose agar (PDA), and were incubated for 14 days in darkness at 20-21 °C. Under sterile conditions the cultures were monitored and transferred into new potato dextrose agar plates to increase inocula. Monosporic isolates were characterized using a set of 12 host differential genotypes (A. S. Musyimi, unpubl. data) and were maintained on potato dextrose agar medium at 4 °C for short term storage, or maintained on fungus-colonized filter papers at -20 °C for long term storage.

The inoculum of each bean common mosaic disease collected from farmers' fields was prepared by making sap extracts from severely infected young leaves. This was done by grinding infected leaves with a mortar and pestle in hydrogen phosphate buffer containing 0.1% Tween 20. The supernatant was then sieved to eliminate plant debris.

Healthy seedlings raised under greenhouse were inoculated at primary leaf stage by leaf rub method using carborundum (Kieselguhr) powder as an abrasive. Proper care was taken to avoid the contamination of isolates. Individual isolate was maintained on healthy seedlings of the susceptible variety G10909 and infected leaves collected from the artificially inoculated plants and stored in the refrigerator at -20 °C.

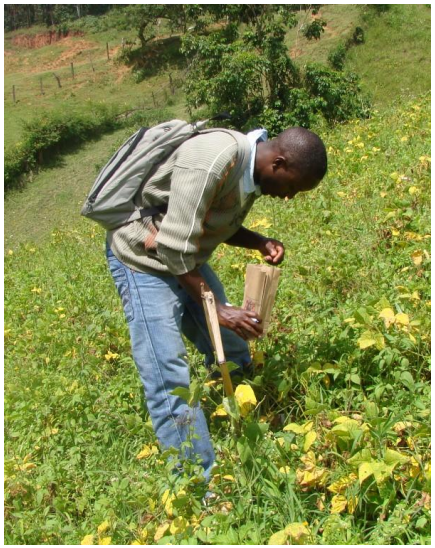
Root rot isolations were accomplished by first washing soil from infected root samples in a jet-stream of tap water, rinsing twice in sterile distilled water, blotting dry on sterile paper towel, and placing infected root pieces cut from expanding lesions on the prepared selective medium (CMA) using flamed forceps. Petri plates with plant samples were observed after incubation for 24-

48 h at room temperature (20-25 °C). The pathogen mycelia developing from the plant tissues were confirmed and transferred onto potato dextrose agar (PDA) slants.

### 3.3.3 Inoculation of test plants

Numerous methodologies were used to determine *P. griseola* physiological specialization in the past (Alvarez-Ayala and Schwartz, 1979; Buruchara, 1983; Correa-Victoria, 1987). In an effort to standardize the methodology for *P. griseola* pathotype identification, a set of 12 common bean differential cultivars was established (CIAT, 1995).

Test plants from these cultivars were used for characterizing the virulence diversity of *P. griseola* in this study (Table 3.1). The differential genotypes consist of six Mesoamerican and six Andean bean lines. Seeds of the angular leaf spot differentials were obtained from CIAT, Kawanda in Uganda and Dr. Merion Liebenberg of Grain Crops Institute, Potchefstroom, South Africa. Some seeds were also obtained from Dr. Isabel Wagara of Egerton University, Kenya.



A. Field collection of diseased

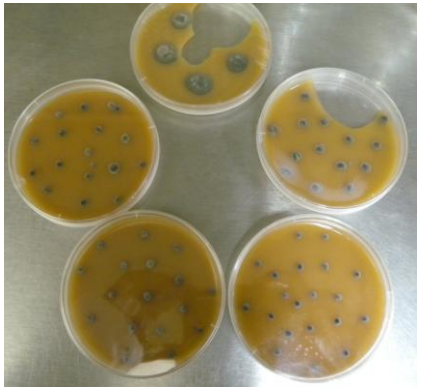
B. Isolation and monitoring of single spore isolates on TWA

C. ALS conidia on TWA as observed under the microscope

D. A group of germinating conidia on TWA after 48 hrs

E. Single conidia ideal for transfer into V8-media

F. Incubation of monosporic cultures at 21-24 ℃ in an incubator



G. Increase of ALS inocula on V8-media for inoculation

**Figure 3.2:** Procedure followed during collection of diseased material, isolation and multiplication of *P. griseola*.

**Table 3.1:** Some characteristics of differential genotypes used to identify races of *P. griseola* isolates collected in bean growing regions in Kenya.

| Genotype | Gene pool | Seed size[1] | Seed colour | Bean race[2] | Binary value[3] | Resistance genes |
|---|---|---|---|---|---|---|
| a. Don Timoteo | Andean | L | Purple | C | 1 | |
| b. G11796 | Andean | L | Yellow | P | 2 | |
| c. Bolon Bayo | Andean | L | Cream-beige | P | 4 | |
| d. Montcalm | Andean | L | Red | NG | 8 | |
| e. Amendoin | Andean | L | Pink | NG | 16 | |
| f. G05686 | Andean | L | Cream-beige | NG | 32 | |
| g. Pan 72 | Mesoamerican | S | White | M | 1 | |
| h. G02858 | Mesoamerican | M | Cream-beige | D | 2 | |
| i. Flor de Mayo | Mesoamerican | S | Pink | J | 4 | |
| j. MEX 54 | Mesoamerican | M | Cream-beige | J | 8 | *Phg*-2, *Phg*-5, *Phg*-6 |
| k. BAT 332 | Mesoamerican | S | Cream-beige | M | 16 | *Phg*-6[2] |
| l. Cornell 49-242 | Mesoamerican | S | Black | M | 32 | *Phg*-3 |

Source: Pastor-Corrales and Jara (1995), Nietsche *et al*. (2001), Mahuku *et al*. (2002b), Miklas *et al*. (2006a).
[1]L=large, M=medium and S=small.
[2]Andean common bean races: C – Chile, P – Peru, NG – Nueva Granada.
Mesoamerican races: M – Mesoamerica, D – Durango, J – Jalisco.
[3]Binary value used to assign isolates of *P. griseola* to races.

A set of 12 differential cultivars were used to classify P. Griseola pathotypes (Table 3.1). Ten seeds per differential were obtained from CIAT-Kawanda and were sowed in the greenhouse and screen house for seed production at a density of 1 seed per pot containing 5 kg of soil plus 10 g of NPK (17:17:17) per pot. After the differential seed increase, three seeds of each differential genotype were sown in a pot filled with sterilized soil in the greenhouse in

Kabete Field Station of University of Nairobi. There were five replicates of one pot each (a total of fifteen plants) for each genotype.

Inoculum of each isolate was increased by culturing the pathogen onto several plates of V8 juice agar medium. Conidia were harvested from 12 day old cultures, suspended in sterile distilled water from petri plates by gently scraping the surface of sporulating colonies with a soft brush. The conidia suspension was filtered through a double layer of cheese cloth to remove mycelia mass. The number of spores was determined using a haemocytometer and concentration adjusted to $2 \times 10^4$ conidia $ml^{-1}$.

The aqueous conidial suspension was misted with a hand sprayer until run off onto both abaxial and adaxial surfaces of bean differential plants at first trifoliate leaf (14-day old seedlings) to induce disease. Inoculated plants were covered with polythene sheets and maintained in the humid chamber for four days after inoculation under high relative humidity (RH $\geq$ 95%). Plants were then uncovered and disease reactions evaluated for angular leaf spot symptoms (Fig. 3.3).

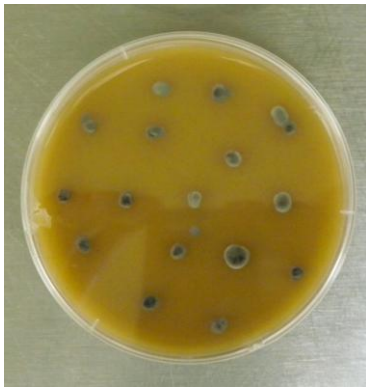
### 3.3.4 Disease evaluation and data analysis

Inoculation of test plants, disease evaluation and data collection was conducted between May 2011 and July 2012. Plants were maintained in greenhouse for 12 days and evaluations for symptoms performed 12, 17 and 21 days after inoculation, using 1 to 9 (Schoonhoven and Pastor-Corrales, 1987) standard scale where 1 = no symptoms; 2 = lesions on up to 3% of leaf

area; 3 = lesions on up to 5% of leaf area, with no sporulation of the pathogen; 4 = lesions and sporulation on up to 10% of leaf area; 5 = lesions and sporulations with 2–3 mm in diameter on 11–15% of leaf area; 6 = lesions and sporulations >3 mm in diameter on 16–20% of leaf area; 7 = lesions and sporulations >3 mm in diameter on 21–25% of leaf area; 8 = lesions and sporulations >3 mm in diameter on 26–30% of leaf area; and 9 = lesions, frequently associated with early loss of leaves and plant death, on 90% of leaf area (Table 3.2 and Fig. 3.3). Plants with scores ≤ 3 were considered resistant, 4 to 6, intermediate and 7 to 9 as susceptible. Reaction type categories were determined according to the averages of these symptom scores attributed for each plant-pathogen combination.

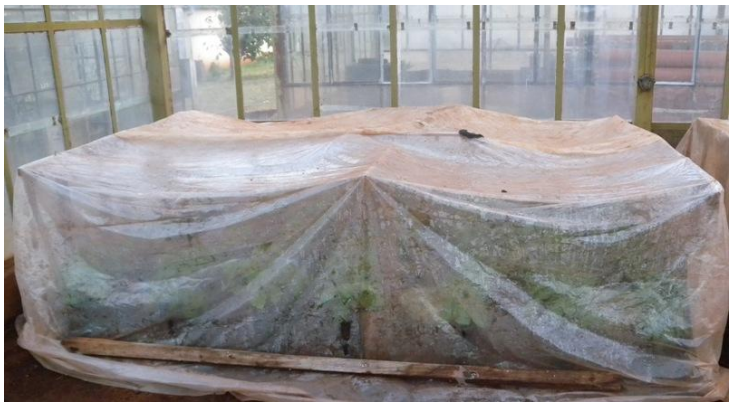
### 3.3.5 Angular leaf spot race identification

For race determination, a scale of binary values proposed by Pastor-Corrales and Jara (1995) was used. The race designation was obtained by adding the binary value for each differential line that presented a compatible reaction. To determine races of isolates, two numbers separated by a dash were used, for example, race 63-31 for isolate Pg01BN from Bungoma (Table 3.6). The first number, 63, was obtained by adding the binary values of the susceptible Andean differential cultivars, each of which was given a (+) sign denoting compatibility; 1 + 2 + 4 + 8 + 16 + 32 = 63. The second number, 31, was obtained by adding the binary values of the susceptible Middle American cultivars: assigned (+) sign; 1 + 2 + 4 + 8 + 16 = 31.



A. Inocula preparation for each isolate

B. Inoculation of 12 test differential cultivars with disease pathogen



C. Incubation of inoculated plants in a humid chamber to promote ALS conidia germination


D. ALS symptom evaluation using the 1-9 CIAT's disease score scale and race identification. D1 is 7, D2 is 9 and D3 is 8 on the 1 to 9 CIAT scale.

**Figure 3.3:** Inoculation and incubation of different plants and scoring for angular leaf spot.

**Table 3.2:** CIAT disease evaluation scale for screening angular leaf spot reaction.

| Rating | Category | Description |
|---|---|---|
| 1 | Resistant | no visible symptom |
| 2 | Resistant | lesions on up to 3% of leaf area |
| 3 | Resistant | lesions on up to5% of leaf area |
| 4 | Intermediate | lesions and sporulation on up to 10% of leaf area |
| 5 | Intermediate | lesions and sporulations with 2–3 mm in diameter on 11–15% of leaf area |
| 6 | Intermediate | lesions and sporulations >3 mm in diameter on 16–20% of leaf area |
| 7 | Susceptible | lesions and sporulations >3 mm in diameter on 21–25% of leaf area |
| 8 | Susceptible | lesions and sporulations >3 mm in diameter on 26–30% of leaf area |
| 9 | Susceptible | lesions, frequently associated with early loss of leaves and plant death, on 90% of leaf area |

Source: Schoonhoven and Pastor-Corrales, 1987

## 3.4 RESULTS

### 3.4.1 Prevalence of major common bean diseases in Kenya

The disease prevalence was high for angular leaf spot, anthracnose, common bacterial blight and bean common mosaic disease, but very low prevalences were recorded for *Pythium* root rot (Table 3.3). Angular leaf spot was highly prevalent in the coastal, central and rift valley regions with a prevalence rate of >96%. The disease was recorded in all fields visited in the coastal region. Angular leaf spot was found to occur from the low altitudes (<1500 m asl) to the high altitudes (>2000 m asl) across all farms visited, and was observed from 1151 to 2371 m asl. Angular leaf spot was particularly more prevalent in the mid altitude (1500-2000 m asl) compared to low and high altitudes (Table 3.3). Anthracnose prevalence was high in central (95.6%), rift valley (72.4%) and coastal (75%) regions with lowest occurrences being reported in eastern region (16.7%). Unlike angular leaf spot, anthracnose was highly prevalent (96.4%) in the high altitude and low prevalence in low altitudes (58.1%). Common bacterial blight was also highly prevalent in the areas surveyed with a total of 121 samples being collected (Table 3.4). The disease was widespread across all the regions and occurred in all farms visited in the rift valley, nyanza and coastal regions. The lowest prevalence of common bacterial blight was observed in the central region (71.5%), and within the high altitude areas (74.5%) compared to the low altitude zones (100%).

Bean common mosaic virus had the highest prevalence in the nyanza region (76.5%) and within the low altitudes (77.4%). The viral disease was however low in the rift valley (62.1%) and the mid altitudes (60.9%) (Table 3.3).

*Pythium* root rot was the least prevalent disease with only western region recording a prevalence of 12.5% with negligible occurrence in all other regions and across altitude range. There *Pythium* root rot was only concentrated in a few hot spots in Kakamega and Busia. The other occurrences of root rot were mostly caused by other pathogenic agents and not the targeted *Pythium* root rot.

**Table 3.3:** Prevalence of major common bean disease in various regions and agro-ecological zones of Kenya.

| | Prevalence (%) | | | | |
|---|---|---|---|---|---|
| | ALS | ANT | CBB | BCMV | RR |
| **Region** | | | | | |
| Central | 97.9 | 95.6 | 71.5 | 63.6 | 9.1 |
| Coastal | 100.0 | 75.0 | 100.0 | 75.0 | 0.0 |
| Eastern | 75.0 | 16.7 | 83.3 | 75.0 | 0.0 |
| Nyanza | 64.7 | 52.9 | 100.0 | 76.5 | 0.0 |
| Rift Valley | 96.2 | 72.4 | 100.0 | 62.1 | 3.4 |
| Western | 68.8 | 56.3 | 93.8 | 75.0 | 12.5 |
| **Altitude** | | | | | |
| Low altitude | 93.7 | 58.1 | 100.0 | 77.4 | 0.1 |
| Mid altitude | 97.1 | 66.7 | 98.1 | 60.9 | 0.1 |
| High altitude | 78.9 | 96.4 | 74.5 | 72.7 | 0.0 |

ALS=angular leaf spot, ANT=anthracnose, BCMV=bean common mosaic virus, CBB=common bacterial blight, RR=*Pythium* root rot

### 3.4.2 Isolation and preservation of *P. griseola*

Fifty-seven monosporic cultures were successfully isolated (Table 3.5) as described in section 3.3.2 above. All the 57 isolated samples were maintained onto V8 medium and preserved at -20 ºC in Pathology Laboratory at University of Nairobi.

**Table 3.4:** The coverage of disease survey and samples of angular leaf spot, anthracnose, common bacterial blight, bean common mosaic virus and root rots collected in Kenya during December 2010 and June 2011.

| District | [1]AEZ | [2]GPS Coordinates | | [3]ALS | ANT | CBB | BCMV | RR |
|---|---|---|---|---|---|---|---|---|
| | | Longitude | Latitude | | | | | |
| Nyeri | UM3 | 37º 95' E | 0º 43' S | + | - | - | - | - |
| | LH1 | 37º 89' E | 0º 43' S | + | - | - | - | - |
| Bureti | UM1 | 35º 04' E | 0º 31' S | + | + | - | - | - |
| | UM4 | 35º 05' E | 0º 29' S | + | + | - | - | - |
| | UM1 | 35º 06' E | 0º 29' S | + | + | + | - | - |
| | UM1 | 35º 08' E | 0º 29' S | + | + | - | - | - |
| | UM2 | 35º 09' E | 0º 29' S | + | - | - | + | - |
| | UM1 | 35º 09' E | 0º 29' S | + | + | - | - | - |
| Bomet | LH2 | 35º 15' E | 0º 46' S | + | + | - | + | - |
| | LH2 | 35º 18' E | 0º 47' S | + | + | + | - | - |
| | LH2 | 35º 16' E | 0º 47' S | - | + | - | - | - |
| Sotik | LH2 | 35º 08' E | 0º 41' S | + | - | + | + | - |
| | LH2 | 35º 08' E | 0º 41' S | - | - | + | - | - |
| Meru | UM2 | 37º 55' E | 0º 12' S | + | - | - | - | - |
| Central | UM1 | 37º 55' E | 0º 12' S | + | - | - | - | - |
| Gucha | UM1 | 34º 42' E | 0º 47' S | + | + | + | - | - |
| | UM1 | 34º 42' E | 0º 47' S | + | + | + | - | - |
| | UM2 | 34º 42' E | 0º 47' S | + | + | + | - | - |
| | UM3 | 34º 42' E | 0º 47' S | + | + | + | - | - |
| | UM2 | 34º 40' E | 0º 44' S | + | - | + | - | - |
| Mugirang | UM1 | 34º 40' E | 0º 45' S | + | - | + | - | - |
| o | UM1 | 34º 40' E | 0º 45' S | + | + | + | + | - |
| Kisumu | LM2 | 34º 36' E | 0º 01' S | + | - | + | - | - |
| | LM2 | 34º 50' E | 0º 08' S | - | - | + | + | - |
| | LM2 | 34º 43' E | 0º 04' S | - | - | + | - | - |
| Meru | UM1 | 37º 65' E | 0º 29' S | + | - | - | - | - |
| | UM2 | 37º 55' E | 0º 12' S | - | + | - | - | - |
| | UM3 | 37º 47' E | 0º 27' S | + | - | - | - | - |
| | UM4 | 37º 65' E | 0º 29' S | - | + | - | - | - |
| Siaya | LM1 | 34º 18' E | 0º 07' N | + | + | - | - | - |
| | LM1 | 34º 18' E | 0º 07' N | + | + | + | - | - |
| | LM1 | 34º 18' E | 0º 07' N | - | + | + | - | - |
| | LM1 | 34º 18' E | 0º 07' N | - | + | - | - | - |
| Mwea | UM1 | 37º 37' E | 0º 68' S | + | - | - | - | - |
| Kakamega | UM1 | 34º 46' E | 0º 16' N | + | - | + | - | - |
| | UM1 | 34º 45' E | 0º 16' N | + | + | - | + | - |
| | UM1 | 34º 45' E | 0º 14' N | + | - | + | - | - |
| Kerugoya | UM3 | 37º 28' E | 0º 51' S | + | + | + | - | + |

| District | [1]AEZ | [2]GPS Coordinates | | [3]ALS | ANT | CBB | BCMV | RR |
|---|---|---|---|---|---|---|---|---|
| | | Longitude | Latitude | | | | | |
| | UM3 | 37º 33’ E | 0º 55’ S | + | - | + | + | - |
| Bungoma | LM2 | 34º 42’ E | 0º 36’ N | + | - | + | - | - |
| | UM1 | 34º 53’ E | 0º 52’ N | + | - | + | - | - |
| | UM1 | 34º 39’ E | 0º 37’ N | - | - | + | + | - |
| | UM2 | 34º 47’ E | 0º 46’ N | - | - | + | - | - |
| Kitale | UM4 | 34º 57’ E | 0º 58’ N | + | - | - | - | - |
| Turbo | UM3 | 35º 15’ E | 0º 31’ N | + | - | + | - | - |
| | UM3 | 35º 03’ E | 0º 37’ N | + | - | + | + | - |
| | UM3 | 35º 11’ E | 0º 34’ N | - | - | + | - | - |
| Kitui | UM1 | 37º 79’ E | 1º 17’ S | + | - | - | - | - |
| Kericho | LH1 | 35º 09’ E | 0º 13’ S | + | + | + | - | - |
| | LH1 | 35º 10’ E | 0º 17’ S | + | + | - | - | - |
| Njoro | LH3 | 35º 57’ E | 0º 20’ S | + | + | + | + | - |
| | LH3 | 35º 59’ E | 0º 18’ S | - | - | + | - | - |
| Nakuru | UM3 | 36º 07’ E | 0º 10’ S | + | + | + | - | - |
| | UM2 | 36º 07’ E | 0º 09’ S | + | - | - | - | - |
| Narumoru | LH4 | 37º 02’ E | 0º 20’ S | + | + | + | + | - |
| | LH4 | 37º 02’ E | 0º 19’ S | - | + | + | - | - |
| | LH4 | 37º 05’ E | 0º 23’ S | - | - | + | - | + |
| Naivasha | UM5 | 36º 21’ E | 0º 46’ S | + | - | - | - | - |
| | UM5 | 36º 21’ E | 0º 46’ S | + | - | + | - | - |
| Wundanyi | LH2 | 38º 21’ E | 3º 24’ S | + | + | - | - | - |
| | UM1 | 38º 21’ E | 3º 24’ S | + | - | - | - | - |
| | UM3 | 38º 21’ E | 3º 24’ S | + | + | - | - | - |
| | UM2 | 38º 21’ E | 3º 24’ S | + | + | - | - | - |
| Kiambu | UM1 | 36º 85’ E | 1º 16’ S | + | + | - | - | - |
| | UM4 | 36º 84’ E | 1º 16’ S | + | - | - | + | - |
| Othaya | LH3 | 36º 93’ E | 0º 53’ S | + | + | + | - | - |
| | LH2 | 36º 87’ E | 0º 55’ S | - | + | + | - | - |
| | LH3 | 36º 90’ E | 0º 54’ S | - | + | + | - | - |
| | LH3 | 36º 84’ E | 0º 55’ S | - | - | + | + | - |
| | LH3 | 36º 86’ E | 0º 57’ S | - | - | + | - | - |
| | LH2 | 36º 91’ E | 0º 54’ S | - | - | + | - | - |
| Thika | UM4 | 37º 08’ E | 0º 98’ S | + | + | + | - | + |
| | UM4 | 37º 13’ E | 0º 87’ S | - | - | + | - | - |
| | UM4 | 37º 08’ E | 0º 98’ S | - | - | + | + | + |
| Tigoni | LH1 | 36º 41’ E | 1º 09’ S | + | - | - | - | - |
| | LH1 | 36º 41’ E | 1º 09’ S | + | - | - | - | - |
| Subukia | UM1 | 36º 20’ E | 0º 02’ N | - | - | + | + | - |
| Kabete | LH3 | 36º 44’ E | 1º 15’ S | + | - | - | - | - |
| | LH3 | 36º 44’ E | 1º 15’ S | + | - | - | - | - |
| | LH3 | 36º 44’ E | 1º 14’ S | + | - | - | - | - |

| District | [1]AEZ | [2]GPS Coordinates | | [3]ALS | ANT | CBB | BCMV | RR |
|---|---|---|---|---|---|---|---|---|
| | | Longitude | Latitude | | | | | |
| | LH3 | 36º 44’ E | 0º 05’ S | - | + | - | - | - |
| Kibirigwi | UM2 | 37º 17’ E | 0º 52’ S | + | + | + | - | - |
| Embu | UM2 | 37º 55’ E | 0º 44’ S | + | - | - | - | - |
| | UM4 | 37º 55’ E | 0º 43’ S | + | - | - | - | - |
| Kangema | LH1 | 37º 02’ E | 0º 68’ S | + | - | - | - | - |
| Machakos | UM4 | 37º 23’ E | 1º 57’ S | + | - | - | - | - |
| | UM4 | 37º 23’ E | 1º 58’ S | + | - | - | - | - |
| Eldoret | LH3 | 35º 18’ E | 0º 29’ N | + | - | + | + | - |
| Murang’a | UM4 | 37º 07’ E | 0º 69’ S | + | + | + | - | - |
| | UM2 | 37º 12’ E | 0º 71’ S | - | + | + | - | - |
| | UM2 | 37º 02’ E | 0º 68’ S | - | + | + | - | - |
| | UM2 | 37º 11’ E | 0º 69’ S | - | + | + | - | - |
| Nyahururu | UH2 | 36º 21’ E | 0º 03’ S | + | - | - | - | - |
| | UH2 | 36º 22’ E | 0º 05’ S | + | - | - | - | - |
| Maragua | UM2 | 37º 09’ E | 0º 95’ S | - | + | - | - | - |
| | UM2 | 36º 10’ E | 0º 95’ S | - | + | - | - | - |
| Kitale | UM4 | 34º 57’ E | 0º 58’ N | + | - | + | - | - |
| Nyamira | UM1 | 34º 55’ E | 0º 31’ S | - | - | + | + | - |
| | UM1 | 34º 55’ E | 0º 31’ S | + | - | + | - | - |
| Mumias | UM1 | 34º 36’ E | 0º 18’ N | - | - | + | - | - |
| Vihiga | UM1 | 34º 43’ E | 0º 07’ N | - | - | + | - | - |
| | UM1 | 34º 40’ E | 0º 13’ N | - | - | + | - | - |
| Sabatia | UM1 | 34º 45’ E | 0º 07’ N | - | - | + | - | + |
| Kisii Central | UM1 | 34º 47’ E | 0º 44’ S | - | - | + | - | - |
| Busia | UM2 | 34º 07’ E | 0º 28’ N | - | - | + | - | - |
| | UM2 | 34º 07’ E | 0º 27’ N | - | - | + | - | - |
| | UM2 | 34º 07’ E | 0º 26’ N | - | - | + | - | + |
| | UM2 | 34º 07’ E | 0º 28’ N | - | - | + | - | - |
| | UM2 | 34º 07’ E | 0º 27’ N | - | - | + | - | - |
| Meru South | UM4 | 37º 65’ E | 0º 29’ S | - | - | + | + | - |
| Murang’a South | UM2 | 37º 12’ E | 0º 71’ S | - | - | + | - | - |
| | | Total | | 62 | 55 | 121 | 32 | 9 |

[1]AEZ=agro-ecological zone
[2]GPS=global positioning system
[3]ALS=angular leaf spot, ANT=anthracnose, BCMV=bean common mosaic virus, CBB=common bacterial blight, RR=*Pythium* root rot
(+) disease symptomatic samples collected
(-) no symptoms collected

**Table 3.5:** Angular leaf spot pathogen collection areas and their geographical coordinates, and samples obtained.

| County | District | District Code | [1]AEZ | Sample Code | [2]GPS Coordinates | | |
|---|---|---|---|---|---|---|---|
| | | | | | Elev. (m) | Longitude | Latitude |
| Nyeri | Nyeri | NY | UM3 | Pg01NY | 1794 | 37º 95' E | 0º 43' S |
| | | | LH1 | Pg02NY | 1900 | 37º 89' E | 0º 43' S |
| | Othaya | TY | LH3 | Pg01TY | 1870 | 36º 93' E | 0º 53' S |
| | Narumoru | NR | LH4 | Pg01NR | 1994 | 37º 02' E | 0º 20' S |
| Kericho | Bureti | BR | UM1 | Pg01BR | 1613 | 35º 04' E | 0º 31' S |
| | | | UM4 | Pg02BR | 1736 | 35º 05' E | 0º 29' S |
| | | | UM1 | Pg03BR | 1727 | 35º 06' E | 0º 29' S |
| | | | UM1 | Pg04BR | 1768 | 35º 08' E | 0º 29' S |
| | | | UM2 | Pg05BR | 1783 | 35º 09' E | 0º 29' S |
| | | | UM1 | Pg06BR | 1784 | 35º 09' E | 0º 29' S |
| | Kericho | KR | LH1 | Pg01KR | 1949 | 35º 09' E | 0º 13' S |
| | | | LH1 | Pg02KR | 1985 | 35º 10' E | 0º 17' S |
| Bomet | Bomet | BM | LH2 | Pg01BM | 1928 | 35º 15' E | 0º 46' S |
| | | | LH2 | Pg02BM | 1964 | 35º 18' E | 0º 47' S |
| | Sotik | ST | LH2 | Pg01ST | 1828 | 35º 08' E | 0º 41' S |
| Meru | Meru Central | MR | UM2 | Pg01MR | 2011 | 37º 55' E | 0º 12' S |
| | | | UM1 | Pg02MR | 2017 | 37º 55' E | 0º 12' S |
| | Meru | MS | UM1 | Pg01MS | 1354 | 37º 65' E | 0º 29' S |
| | | | UM1 | Pg02MS | 1311 | 37º 47' E | 0º 27' S |
| Kisii | Gucha | GC | UM1 | Pg01GC | 1664 | 34º 42' E | 0º 47' S |
| | | | UM1 | Pg02GC | 1687 | 34º 42' E | 0º 47' S |
| | | | UM2 | Pg03GC | 1722 | 34º 42' E | 0º 47' S |
| | | | UM3 | Pg04GC | 1729 | 34º 42' E | 0º 47' S |
| | | | UM2 | Pg05GC | 1706 | 34º 40' E | 0º 44' S |
| | Mugirango | MG | UM1 | Pg01MG | 1715 | 34º 40' E | 0º 45' S |
| | | | UM1 | Pg02MG | 1710 | 34º 40' E | 0º 45' S |
| Kisumu | Kisumu | KS | LM2 | Pg01KS | 1426 | 34º 36' E | 0º 01' S |
| Siaya | Siaya | SY | LM1 | Pg01SY | 1272 | 34º 18' E | 0º 07' N |
| | | | LM1 | Pg02SY | 1288 | 34º 18' E | 0º 07' N |
| Kirinyaga | Mwea | MW | UM1 | Pg01MW | 1151 | 37º 37' E | 0º 68' S |
| | Kerugoya | KY | UM2 | Pg01KY | 1461 | 37º 28' E | 0º 51' S |
| | | | UM1 | Pg02KY | 1327 | 37º 33' E | 0º 55' S |
| | Kibirigwi | KG | UM2 | Pg01KG | 1440 | 37º 17' E | 0º 52' S |
| Kakamega | Kakamega | KK | UM1 | Pg01KK | 1517 | 34º 46' E | 0º 16' N |
| | | | UM1 | Pg02KK | 1552 | 34º 45' E | 0º 16' N |
| | | | UM1 | Pg03KK | 1501 | 34º 45' E | 0º 14' N |

| County | District | District Code | [1]AEZ | Sample Code | [2]GPS Coordinates | | |
|---|---|---|---|---|---|---|---|
| | | | | | Elev. (m) | Longitude | Latitude |
| Bungoma | Bungoma | BN | LM2 | Pg01BN | 1460 | 34º 42' E | 0º 36' N |
| | | | UM1 | Pg02BN | 1783 | 34º 53' E | 0º 52' N |
| Trans Nzoia | Kitale | KL | UM4 | Pg01KL | 1824 | 34º 57' E | 0º 58' N |
| Uasin Gishu | Turbo | TR | UM3 | Pg01TR | 2079 | 35º 15' E | 0º 31' N |
| | | TR | UM3 | Pg02TR | 1840 | 35º 03' E | 0º 37' N |
| | Eldoret | LD | LH3 | Pg01LD | 2171 | 35º 18' E | 0º 29' N |
| Kitui | Kitui | KT | UM1 | Pg01KT | 1217 | 37º 79' E | 1º 17' S |
| Nakuru | Njoro | NJ | LH3 | Pg01NJ | 2139 | 35º 57' E | 0º 20' S |
| | Nakuru | NK | UM3 | Pg01NK | 1944 | 36º 07' E | 0º 10' S |
| | | | UM2 | Pg02NK | 1909 | 36º 07' E | 0º 09' S |
| | Naivasha | NV | UM5 | Pg01NV | 1887 | 36º 21' E | 0º 46' S |
| | | | UM5 | Pg02NV | 1887 | 36º 21' E | 0º 46' S |
| Taita Taveta | Wundanyi | WN | LH2 | Pg01WN | 1468 | 38º 21' E | 3º 24' S |
| | | | UM1 | Pg02WN | 1462 | 38º 21' E | 3º 24' S |
| | | | UM3 | Pg03WN | 1444 | 38º 21' E | 3º 24' S |
| | | | UM2 | Pg04WN | 1420 | 38º 21' E | 3º 24' S |
| Kiambu | Kiambu | KM | UM1 | Pg01KM | 1628 | 36º 85' E | 1º 16' S |
| | | | UM4 | Pg02KM | 1734 | 36º 84' E | 1º 16' S |
| | Tigoni | TG | LH1 | Pg01TG | 2103 | 36º 41' E | 1º 09' S |
| | | | LH1 | Pg02TG | 2095 | 36º 41' E | 1º 09' S |
| | Kabete | KB | LH3 | Pg01KB | 1842 | 36º 44' E | 1º 15' S |
| | | | LH3 | Pg02KB | 1844 | 36º 44' E | 1º 15' S |
| | | | LH3 | Pg03KB | 1844 | 36º 44' E | 1º 14' S |
| | Thika | TH | UM4 | Pg01TH | 1525 | 37º 08' E | 0º 98' S |
| Embu | Embu | MB | UM2 | Pg01MB | 1433 | 37º 55' E | 0º 44' S |
| | | | UM4 | Pg02MB | 1437 | 37º 55' E | 0º 43' S |
| Murang'a | Kangema | KN | LH1 | Pg01KN | 1624 | 37º 02' E | 0º 68' S |
| | Murang'a | MN | UM4 | Pg01MN | 1454 | 37º 07' E | 0º 69' S |
| Machakos | Machakos | MC | UM4 | Pg01MC | 1596 | 37º 23' E | 1º 57' S |
| | | | UM4 | Pg02MC | 1587 | 37º 23' E | 1º 58' S |
| Nyandarua | Nyahururu | NH | UH2 | Pg01NH | 2371 | 36º 21' E | 0º 03' S |
| | | | UH2 | Pg02NH | 2371 | 36º 22' E | 0º 05' S |

[1]AEZ=agro-ecological zone
[2]GPS=global positioning system

### 3.4.3 Production of seed of differential lines

Inorder to obtain sufficient seeds required for race identification, all differential genotypes were planted to increase their seeds. Most of the genotypes flowered and matured normally, except for G11796 and Bolon Bayo that started to flower after 140-150 days after planting. Two bush-type cultivars namely BAT 332 and Cornell 49-242 were observed to have high yields while Montcalm and G05686 had poor seed set, pod load and seed yield (Table 3.6). Two type IV Andean cultivars, G11796 and Bolon Bayo required vernalization to flower. The two cultivars took about 165 to flower and 215 for maturity. They had been planted in January 2011 and continued to grow vegetatively and only began to flower in June/July when minimum temperatures were very low (Appendix 1). Due to the growth habit of G11796 and Bolon Bayo and long vegetative period, the two differential cultivars produced sufficient seeds from the first sowing. A total of 2370 seeds were harvested from BAT 332 which had the highest number of seeds obtained per genotype (Table 3.5). G11796, MEX 54, Pan 72 and Cornell 49-242 all had more than 1000 seeds per genotype. G05686 had the lowest number of seeds (235 seeds) with Montcalm, G02858 and Flor de Mayo each yielding less than 500 seeds per genotype (Table 3.6).

**Table 3.6:** Seed increase for the 12 angular leaf spot standard differential cultivars used in *Pseudocercospora griseola* race identification.

| Genotype | Growth habit | DF | DM | No. of seeds | Remarks |
|---|---|---|---|---|---|
| Don Timoteo | III | 56 | 93 | 631 | Late flowering. |
| G11796 | IV | 165 | 215 | 1467 | Requires vernalization to flower. |
| Bolon Bayo | IV | 165 | 215 | 958 | Requires vernalization to flower. |
| Montcalm | I | 44 | 78 | 331 | Poor pod load. |
| Amendoin | II | 40 | 81 | 544 | High vigour |
| G05686 | I | 39 | 67 | 235 | Very low seed production. |
| Pan 72 | IV | 51 | 89 | 1295 | Late flowering. |
| G02858 | III | 36 | 79 | 485 | Early flowering. |
| Flor de Mayo | IV | 47 | 88 | 460 | Poor pod load |
| MEX 54 | IV | 46 | 78 | 1422 | Highly resistant to ALS. |
| BAT 332 | I | 37 | 65 | 2370 | High yielding. |
| Cornell 49-242 | I | 35 | 69 | 1281 | High yielding. |

DF=days to 50% flowering, DM=days to 50% maturity, ALS = angular leaf spot

### 3.4.4 Races of angular leaf spot in Kenya

Fifty-seven isolates obtained from samples collected in bean growing regions of Kenya were classified based on their virulence reactions into 23 different physiological races of *Pseudocercospora griseola* (Table 3.7; Fig. 3.5). Isolates exhibited a different virulence pattern when inoculated on the 12 bean differential genotypes through expression of symptomatic variation giving

different disease severity scores as shown in Table 3.6. Races 63-55, 63-63, 63-54 and 63-35 were the most prevalent pathotypes observed among 57 isolates studied and were reported in 10, 7, 6 and 4 different locations respectively. Two new races, 31-31 and 63-31 were reported for the first time in Kenya.

The new races included two isolates race-typed as race 31-31 originated from Kibirigwi (UM2) and Turbo (UM3), and two isolates characterised as race 63-31and obtained from Othaya (LH3) and Kakamega (UM1). The lower highland (LH) and upper midland (UM) recorded the highest number of the most virulent races observed.

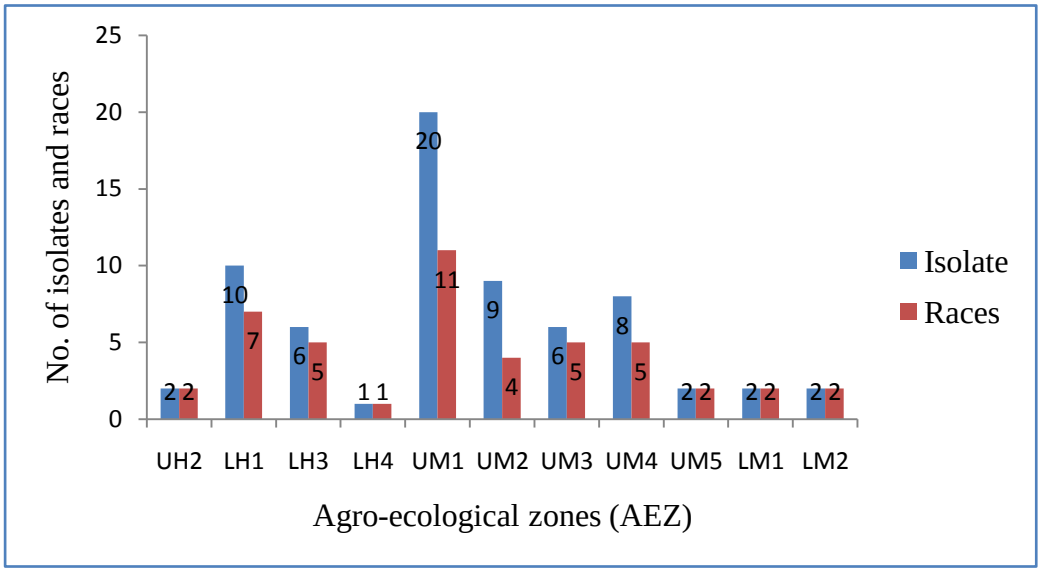



**Figure 3.4:** Distribution of isolates and races of *P. griseola* in major bean growing areas in Kenya.

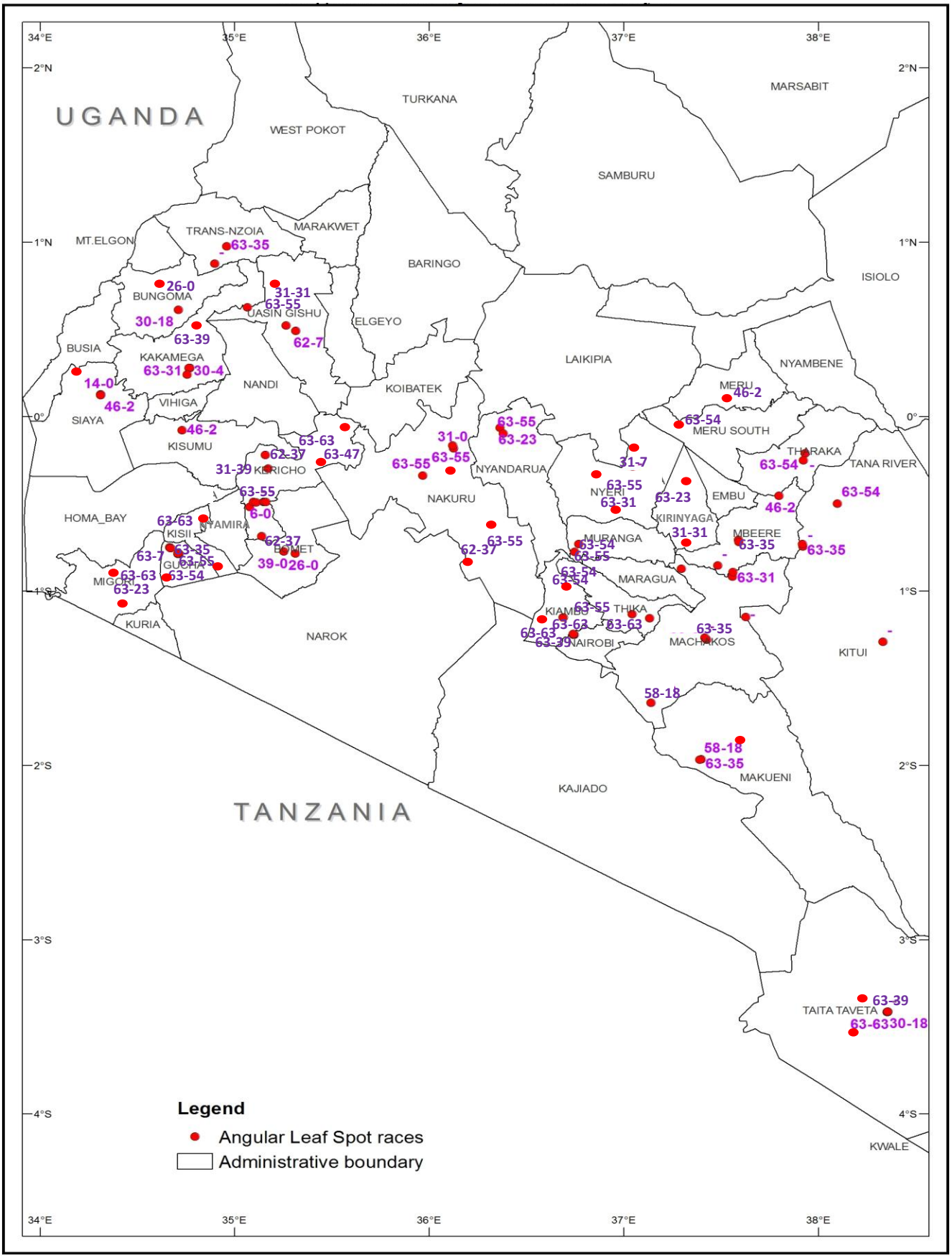



**Figure 3.5:** Pathogenic races of *P. griseola* and their distribution in Kenya.

**Table 3.7:** Angular leaf spot race identification based on the reaction of 12 differential cultivars inoculated with 57 isolates of *Pseudocercospora griseola*.

| | | Differential cultivar[1] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Andean | | | | | | Mesoamerican | | | | | | |
| | | A | B | C | D | E | F | G | H | I | J | K | L | |
| Isolate | Origin | 1[2] | 2 | 4 | 8 | 16 | 32 | 1 | 2 | 4 | 8 | 16 | 32 | Races |
| Pg01NY | Nyeri | +[3] | + | + | + | + | + | + | + | + | -[4] | + | + | 63-55 |
| Pg02NY | Nyeri | + | + | + | + | + | - | + | + | + | - | - | - | 31-7 |
| Pg02BR | Bureti | - | + | + | - | - | - | - | - | - | - | - | - | 6-0 |
| Pg01WN | Wundanyi | + | + | + | + | + | + | + | + | + | + | + | + | 63-63 |
| Pg02KK | Kakamega | - | + | + | + | + | - | - | - | + | - | - | - | 30-4 |
| Pg01KG | Kibirigwi | + | + | + | + | + | - | + | + | + | + | + | - | 31-31 |
| Pg01NK | Nakuru | + | + | + | + | + | - | - | - | - | - | - | - | 31-0 |
| Pg01TY | Othaya | + | + | + | + | + | + | + | + | + | + | + | - | 63-31 |
| Pg02KR | Kericho | - | + | + | + | + | + | + | - | + | - | - | + | 62-37 |
| Pg01NJ | Njoro | + | + | + | + | + | + | + | + | + | - | + | + | 63-55 |
| Pg01GC | Gucha | + | + | + | + | + | + | + | + | + | - | - | - | 63-7 |
| Pg02SY | Siaya | - | + | + | + | - | - | - | - | - | - | - | - | 14-0 |
| Pg02NH | Nyahururu | + | + | + | + | + | + | + | + | + | - | + | + | 63-55 |
| Pg02BN | Bungoma | - | + | - | + | + | - | - | - | - | - | - | - | 26-0 |
| Pg01TH | Thika | + | + | + | + | + | + | + | + | + | + | + | + | 63-63 |
| Pg01MC | Machakos | + | + | + | + | + | + | + | + | - | - | - | + | 63-35 |
| Pg01KS | Kisumu | + | + | + | + | - | + | - | + | - | - | - | - | 46-2 |
| Pg02MG | Mugirango | + | + | + | + | + | + | + | + | + | + | + | + | 63-63 |
| Pg02MR | Meru Central | + | + | + | + | + | + | - | + | + | - | + | + | 63-54 |
| Pg02NK | Nakuru | + | + | + | + | + | + | + | + | + | - | + | + | 63-55 |
| Pg04WN | Wundanyi | - | + | + | + | + | - | - | + | - | - | + | - | 30-18 |
| Pg01KK | Kakamega | + | + | + | + | + | + | + | + | + | - | - | + | 63-39 |
| Pg01MN | Murang'a | + | + | + | + | + | + | + | + | + | - | + | + | 63-55 |
| Pg01KL | Kitale | + | + | + | + | + | + | + | + | - | - | - | + | 63-35 |
| Pg01SY | Siaya | - | + | + | + | - | + | - | + | - | - | - | - | 46-2 |
| Pg01NH | Nyahururu | + | + | + | + | + | + | + | + | + | - | + | - | 63-23 |
| Pg01NV | Naivasha | + | + | + | + | + | + | + | + | + | - | + | + | 63-55 |
| Pg06BR | Bureti | + | + | + | + | + | + | + | + | + | - | + | + | 63-55 |
| Pg01LD | Eldoret | - | + | + | + | + | + | + | + | + | - | - | - | 62-7 |
| Pg02GC | Gucha | + | + | + | + | + | + | - | + | + | - | + | + | 63-54 |
| Pg02MS | Meru | - | + | + | + | - | + | - | + | - | - | - | - | 46-2 |
| Pg04GC | Gucha | + | + | + | + | + | + | + | + | + | + | + | + | 63-63 |
| Pg04BR | Bureti | + | + | + | + | + | + | + | + | + | + | + | + | 63-63 |
| Pg01BM | Bomet | + | + | + | - | - | + | - | - | - | - | - | - | 39-0 |
| Pg02TR | Turbo | + | + | + | + | + | + | + | + | + | - | + | + | 63-55 |

| | | Differential cultivar[1] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Andean | | | | | | Mesoamerican | | | | | | |
| | | A | B | C | D | E | F | G | H | I | J | K | L | |
| Isolate | Origin | 1[2] | 2 | 4 | 8 | 16 | 32 | 1 | 2 | 4 | 8 | 16 | 32 | Races |
| Pg01MS | Meru | + | + | + | + | + | + | - | + | + | - | + | + | 63-54 |
| Pg01ST | Sotik | - | + | + | + | + | + | + | - | + | - | - | + | 62-37 |
| Pg01TR | Turbo | + | + | + | + | + | - | + | + | + | + | + | - | 31-31 |
| Pg01MG | Mugirango | + | + | + | + | + | + | + | + | + | - | + | - | 63-23 |
| Pg01BN | Bungoma | - | + | + | + | + | - | - | + | - | - | + | - | 30-18 |
| Pg03KK | Kakamega | + | + | + | + | + | + | + | + | + | + | + | - | 63-31 |
| Pg02NV | Naivasha | - | + | + | + | + | + | + | - | + | - | - | + | 62-37 |
| Pg01KM | Kiambu | + | + | + | + | + | + | + | + | + | - | + | + | 63-55 |
| Pg02KY | Kerugoya | + | + | + | + | + | + | + | + | + | - | + | - | 63-23 |
| Pg03GC | Gucha | + | + | + | + | + | + | + | + | + | - | + | + | 63-55 |
| Pg02MC | Machakos | - | + | - | + | + | + | - | + | - | - | + | - | 58-18 |
| Pg05GC | Gucha | + | + | + | + | + | + | + | + | - | - | - | + | 63-35 |
| Pg01KR | Kericho | + | + | + | + | + | - | + | + | + | - | - | + | 31-39 |
| Pg01KN | Kangema | + | + | + | + | + | + | - | + | + | - | + | + | 63-54 |
| Pg03WN | Wundanyi | + | + | + | + | + | + | + | + | + | - | - | + | 63-39 |
| Pg02MB | Embu | + | + | + | + | + | + | + | + | - | - | - | + | 63-35 |
| Pg01BR | Bureti | + | + | + | + | + | + | + | + | + | + | - | + | 63-47 |
| Pg01TG | Tigoni | + | + | + | + | + | + | - | + | + | - | + | + | 63-54 |
| Pg02TG | Tigoni | + | + | + | + | + | + | - | + | + | - | + | + | 63-54 |
| Pg01KB | Kabete | + | + | + | + | + | + | + | + | + | + | + | + | 63-63 |
| Pg02KB | Kabete | + | + | + | + | + | + | + | + | + | + | + | + | 63-63 |
| Pg03KB | Kabete | + | + | + | + | + | + | + | + | + | - | - | + | 63-39 |

[1] Andean differential genotypes: A=Don Timoteo; B=G 11796; C=Bolon Bayo; D=Montcalm; E=Amendoin; F=G5686. Mesoamerican differential genotypes: G=PAN 72; H=G2858; I=Flor de Mayo; J=MEX 54; K=BAT 332; L=Cornell 49–242.

[2] Binary values used to classify *Pseudocercospora griseola* races.

[3] Compatible reaction (+)

[4] Incompatible reaction (-)

**Table 3.8:** Distribution of angular leaf spot races based on regions, altitude range and agro-ecological zones of Kenya.

| Region | Races | [1]AEZ | Races | Altitude | Races |
|---|---|---|---|---|---|
| Central | 63-63 | LH1 | 63-54 | Low altitude | 63-63 |
| | 63-55 | | 62-37 | | 63-55 |
| | 63-54 | | 31-7 | | 63-54 |
| | 63-39 | | 31-39 | | 63-39 |
| | 63-31 | LH2 | 63-63 | | 63-35 |
| | 63-23 | | 62-37 | | 63-23 |
| | 31-7 | | 39-0 | | 46-2 |
| | 31-31 | LH3 | 63-63 | | 31-31 |
| Coastal | 63-63 | | 63-55 | | 30-18 |
| | 63-39 | | 63-39 | | 14-0 |
| | 30-18 | | 63-31 | Medium altitude | 63-7 |
| Eastern | 63-54 | | 62-7 | | 63-63 |
| | 63-35 | LM1 | 46-2 | | 63-55 |
| | 58-18 | | 14-0 | | 63-54 |
| | 46-2 | LM2 | 46-2 | | 63-47 |
| Nyanza | 63-7 | | 30-18 | | 63-39 |
| | 63-63 | UH2 | 63-55 | | 63-35 |
| | 63-55 | | 63-23 | | 63-31 |
| | 63-54 | UM1 | 63-7 | | 63-23 |
| | 63-35 | | 63-63 | | 62-37 |
| | 63-23 | | 63-55 | | 58-18 |
| | 46-2 | | 63-54 | | 39-0 |
| | 46-2 | | 63-47 | | 31-7 |
| | 14-0 | | 63-39 | | 31-39 |
| Rift Valley | 63-63 | | 63-31 | | 31-0 |
| | 63-55 | | 63-23 | | 30-4 |
| | 63-47 | | 46-2 | | 26-0 |
| | 63-35 | | 30-4 | | 6-0 |
| | 62-7 | | 26-0 | High altitude | 63-55 |
| | 62-37 | UM2 | 63-55 | | 63-54 |
| | 39-0 | | 63-35 | | 63-23 |
| | 31-39 | | 31-31 | | 62-7 |
| | 31-31 | | 30-18 | | 31-31 |
| | 31-0 | UM3 | 63-63 | | |
| | 6-0 | | 63-55 | | |
| Western | 63-39 | | 63-39 | | |

| Region | Races | [1]AEZ | Races | Altitude | Races |
|---|---|---|---|---|---|
| | 63-31 | | 31-31 | | |
| | 30-4 | | 31-0 | | |
| | 30-18 | UM4 | 63-63 | | |
| | 26-0 | | 63-55 | | |
| | | | 63-35 | | |
| | | | 58-18 | | |
| | | | 6-0 | | |
| | | UM5 | 63-55 | | |
| | | | 62-37 | | |

[1]AEZ = agro-ecological zones

Seven isolates collected in six different locations were classified as race 63-63 (Table 3.7). Race 63-63 was the only race virulent to all the 12 differential cultivars. All the isolates studied in this work presented a pathogenic reaction of compatibility with either/both Andean and Mesoamerican cultivars. The two main pathogen groups; Andean and Mesoamerican, were also identified from this study. Out of the 57 isolates characterised, 12 races were classified as from the Andean pathogenic group while 45 races were from the Mesoamerican pathogenic group. Mesoamerican cultivars MEX 54, Cornell 49-242 and BAT 332, were the most resistant cultivars tested indicating their importance in bean breeding aimed at developing new common bean cultivars resistant to angular leaf spot disease.

Different isolates collected at the same location showed differences in their patterns of virulence. For example, isolates Pg01GC, Pg02GC, Pg03GC, Pg04GC and Pg05GC (Table 3.5, 3.7), collected from Gucha, race-typed into races 63-7, 63-54, 63-55, 63-63 and 63-35 (Table 3.7), and caused compatible

reactions on nine, 10, 11, 12 and nine differential genotypes, respectively. Similar results were observed with isolates collected in other locations (Table 3.7). We detected five isolates that attacked only Andean beans (e.g., Pg01BM, Pg02SY and Pg02BN), although some isolates (e.g., Pg01SY, Pg02KK and Pg04WN) attacked some Middle American beans but were often more virulent on Andean cultivars and thus classified as Andean pathotypes (Table 3.7).

Analysis of virulence phenotype of 57 isolates on a set of 12 differential cultivars using a hierachical cluster analysis method and tree option of Genstat program, generated a dendrogram, which divided all the study samples into two clusters: Mesoamerican and Andean groups (Fig. 3.6). The Mesoamerican cluster was composed of 45 isolates (78.9 %), while the Andean cluster consisted of 12 isolates (21.1 %) (Fig. 3.6). Ten angular leaf spot isolates from Mesoamerican group were observed to share a high pathogenic similarity (Fig. 3.6). Rift valley region had the highest number of different races with 11 pathotypes being realized (Table 3.8).

There were only 3 different races from the coastal region. Rift Valley region was also one of the two regions, the other being central region where a new race (31-31) was identified (Table 3.7). The most virulent pathotype (63-63) of angular leaf spot was reported in Central, Nyanza, Rift Valley and Coastal regions, but was absent in Eastern and Western regions. Mid altitude range had the most diverse races reaching 18 different pathotypes compared to 10 and 5 different races from low and mid altitudes respectively.

The new race was however reported in the low and mid altitudes where

pathogenic diversity was minimal (Table 3.7, 3.8). Eleven races were recorded in the upper midland 1 zone (UM1), with 5 and 4 races being identified in the UM3 and UM2 zones. The highly pathogenic race 63-63 was found in all agro-ecological zones surveyed except zones LM1 and UM2. Race 31-31, which is being reported in Kenya for the first time, was obtained from zones UM2 and UM3 (Table 3.7, 3.8).

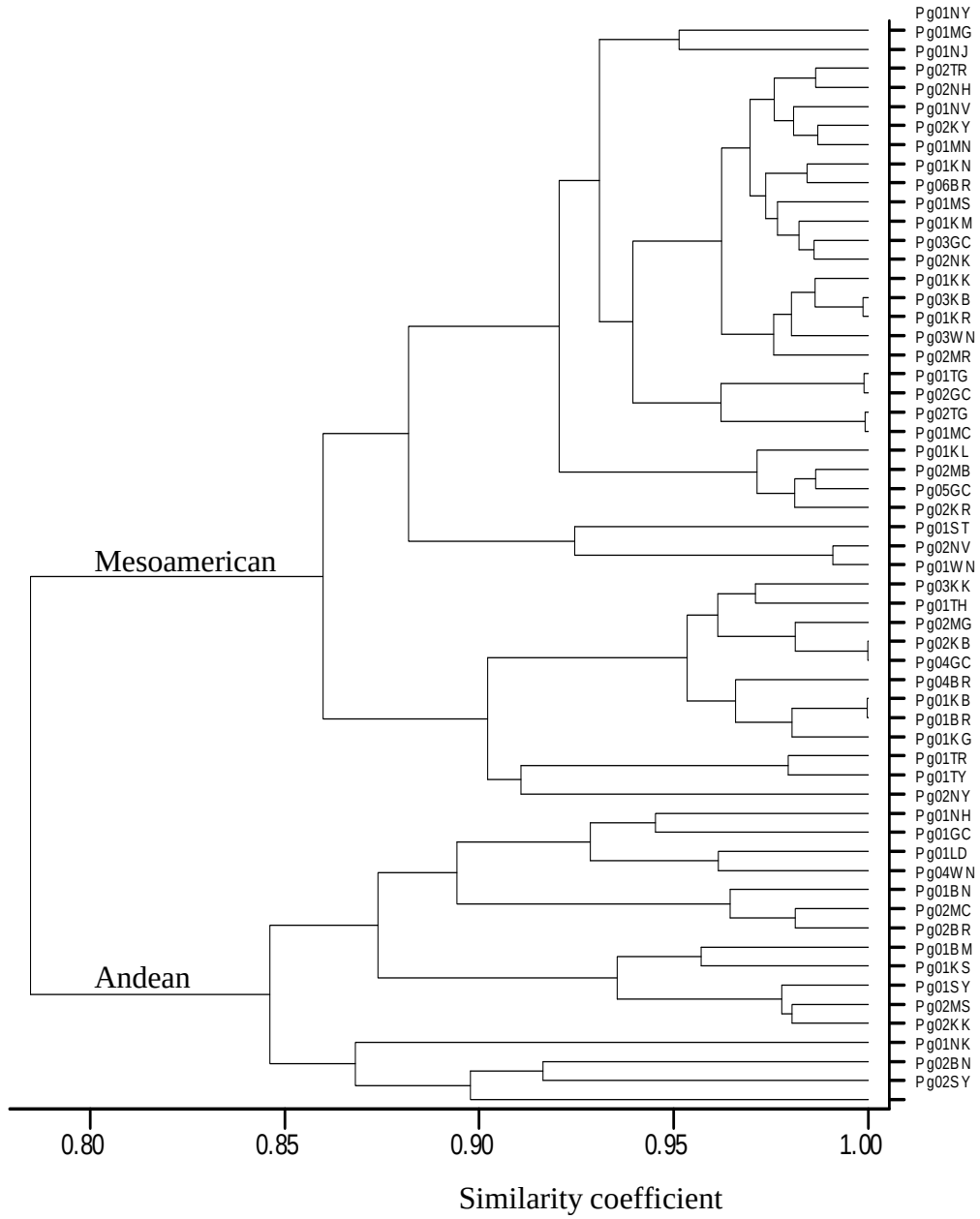



**Figure 3.6:** Virulence clusters of *P. griseola* isolates found in bean growing areas of Kenya.

## 3.5 DISCUSSION

The major diseases of common bean surveyed in bean growing regions of Kenya were observed to be highly prevalent in the regions surveyed. This indicates the necessity of developing multiple disease resistance genotypes to counter the severe losses experienced when they infest susceptible cultivars. Angular leaf spot was observed to occur in all the regions of Kenya where common bean is cultivated. It was highly prevalent in the coastal, central and rift valley regions with a prevalence rate of greater than 96%. The results confirm earlier findings by Mwangombe *et al.* (2007) who reported a prevalence rate of 89%, and observed the occurrence of angular leaf spot in all regions surveyed. Angular leaf spot was also recorded across all altitude ranges from 1151 to 2371 m asl. The prevalence among the altitude ranges differed with high prevalence being recorded in mid altitude (1500-2000 m asl), with lower prevalences in low and high altitude ranges. This is attributable to the warm and moist conditions within the mid altitude and inhibition of growth and development of the pathogen in the low and high altitudes due to high temperatures and low temperatures respectively.

Anthracnose was also highly prevalent across regions but was more prevalent in the high altitudes where cool and humid conditions prevail. Common bacterial blight generally occurred in all regions and altitudes with high prevalence rates, but was dominant in rift valley, nyanza regions and in the low altitudes. There were very low occurrences of *Pythium* root rot in all areas surveyed except a few hot spots in western region. This could partly be

attributed to the nature of the numerous causal agents that incite root rots, and the complexity of distinguishing them symptomatically.

*Pseudocercospora griseola* isolates were examined to evaluate physiological diversity in an attempt to understand the distribution and pathogenic virulence structure of *P. griseola* present in Kenya. The *P. griseola* isolates characterized showed variation in virulence towards differential cultivars demonstrating a high pathogenic variability of the pathogen in Kenya. There were 23 races identified from the 57 isolates obtained from major bean growing regions of Kenya. This occurrence of large pathogenic variation in *P. griseola* populations supports earlier findings by Mahuku *et al*. (2002a), Stenglein *et al*. (2003), Wagara *et al*. (2004) and Silva *et al*. (2008). The broad variation occurred whether isolates were collected from different geographical areas between and within districts or from a given location. Similar observations have been made with the Kenyan isolates by Wagara *et al*. (2004). Pathotypes of angular leaf spot observed adhered to the two *Pseudocercospora griseola* pathotype groups. Twelve isolates were classified as from the Andean pathotype group while 45 isolates were from the Mesoamerican pathotype group. There were different patterns of pathogenic virulence for isolates obtained within close proximity. In the rift valley region 11 different pathotpes were identified each with a divergent pathogenic virulence. This was largely because the range of pathogenic variation among *P. griseola* isolates found at a given location may be a function largely of the variability found among common bean genotypes grown in that location or genetically diverse pathotypes. These results confirmed the existence of

pathogenic diversity within the population of angular leaf spot pathogen in Kenya.

Races 63-55, 63-63, 63-54 and 63-35 were found to be the most prevalent pathotypes in areas studied. These races were observed to possess high pathogenic virulence to the differential cultivars, which may explain their ability to infect and spread widely within common bean growing areas. The identification of these races, particularly race 63-63 which breaks all resistance genes present in all differential cultivars, is of great importance to a breeding programme aiming at the development of angular leaf spot resistant cultivars. Two isolates were identified as the new race 31-31, which had not bee identified in Kenya before. New races have been identified from regions they were absent in the past. This necessitates the regular monitoring and race identification of angular leaf spot from time to time and the development of broad and durable resistance against the *Pseudocercospora griseola*. The results show that both Andean and Mesoamerican bean differential genotypes evaluated in this study are genetically highly variable in response to different races of *P. griseola*. Mesoamerican cultivars MEX 54, Cornell 49-242 and BAT 332, recorded high levels of resistance to majority of isolates characterised indicating their importance in bean breeding programmes aimed at developing new common bean varieties resistant to angular leaf spot disease. These results also show the need to improve local susceptible commercial varieties against the major common bean diseases and other constraints.

Highly pathogenic isolates in both common bean gene pools (Andean and Mesoamerican) were observed. The existence of isolates from Mesoamerican and Andean origins has also been demonstrated in Brazil (Nietsche *et al*., 2001; Sartorato, 2002, 2004; Silva *et al*., 2008). Based on data from the pathogenicity tests, it was observed that all the *P. griseola* isolated in Kenya were pathogenic on common beans. These data confirmed also that the angular leaf spot symptoms previously observed on the sampled materials were due to *P. griseola* agent. Moreover, there was an important variability of the bean differential variety reaction following inoculation with the *P. griseola* isolates. In fact, for a given *P. griseola* race, it was noticed that there were differences in symptom expression and the disease severity level recorded on different bean varieties. There was evidence in variation of symptomatology in any given reaction between isolates and differential cultivars.

The findings of this study further showed the distribution of different races among agro-ecological zones implying that targeted-resistant varieties can be developed for specific bean growing regions of Kenya. For instance, bean breeders in Kenya can now develop a resistant variety to race 63-63 that was found in all agro-ecological zones surveyed except zones LM1 and UM2. In conclusion, the identification of *P. griseola* races in Kenya will enhance control of angular leaf spot disease by using genetic resistance to improve commercial and popular varieties and monitor emerging pathotypes in different regions of Kenya.

# CHAPTER 4 MARKER ASSISTED GAMETE SELECTION FOR PYRAMIDING OF DISEASE RESISTANCE GENES IN SUSCEPTIBLE BEAN CULTIVARS

## 4.1 ABSTRACT

Although gamete selection has been used in breeding common bean for multiple constraint resistance, its efficiency and precision can be enhanced by use of molecular markers. Pathogenic variation is serious threat to sustainable bean productivity in eastern Africa. Angular leaf spot, anthracnose, root rots and bean common mosaic virus pathogens cause combined losses of more than 853,100 t year$^{-1}$ in eastern Africa. Several markers linked to genes for resistance to these diseases are now available to bean breeding programs in this region. The objective of the study was to pyramid genes for resistance to anthracnose, angular leaf spot, bean common mosaic virus and *Pythium* root rot to susceptible cultivars using available markers.

Single crosses were made between G10909 and G2333 (resistant lines to angular leaf spot and anthracnose respectively) and between AND 1062 and BRB 191 (resistant lines to *Pythium* root rot and bean common mosaic virus respectively). Double cross from the two single crosses was crossed to susceptible commercial bean varieties GLP 585 and GLP 92. The single crosses were less successful compared to either double or 5-way cross combinations with the lowest success rate being observed from the cross AND 1062 / BRB 191 (28.8%), and the highest success rate observed was G10909 / G2333 (66.3%). Four double-cross male gametes were developed from crosses

among parental lines with genes for resistance to angular leaf spot, anthracnose, bean common mosaic virus and *Pythium* root rot. The double crosses were the most successful obtaining a median success rate of 84.6% compared to 71.7% generated by 5-way crosses and 46.3% by single crosses.

The resultant multi-parent cross combination resulted into the generation of sixteen gamete populations with pyramided genes for resistance to angular leaf spot, anthracnose, bean common mosaic virus and *Pythium* root rot. Using the molecular markers selected, one male gamete (G10909/G2333//AND 1062/BRB 191) was screened using SAB-3 for anthracnose, SH-13 for angular leaf spot, SW-13 for bean common mosaic virus and PYAA-19 for root rots to identify recombinants for the resistance genes. Male gamete plants positive for two or more markers were crossed to four susceptible female parents to create the final $F_1$. Results showed that only five plants were positive for three markers and 18 for two markers. All other plants had one or zero markers present. No positive amplification was observed with the PYAA-19 marker for *Pythium* root rot.

Twenty-one molecular markers were evaluated for their polymorphism with the parental genotypes' resistance genes. Eleven markers showed polymorphism while 10 of them were non-polymorphic to the disease resistance genes. Fifteen markers tested had positive amplification to the disease resistance genes in at least one parental genotype while 6 markers showed no amplification. Parental genotypes were validated on their reaction to four major common bean diseases present in Kenya by artificial inoculation in the green house and field evaluation. The parental genotypes reacted

differently to specific diseases and were more susceptible in the field than in the green house evaluation.

**Key words:** Marker-assisted selection, gamete selection, common bean, disease resistance, molecular markers.

## 4.2 INTRODUCTION

Diseases can cause significant losses to common bean production (Wortmann *et al*., 1998; Coyne *et al*., 2003; Schwartz *et al*., 2005). Control of biotic constraints using agrochemicals increases production costs and creates potential for contamination of the environment. Resistance represents a valuable disease management tool for improved production of beans especially in the low input cropping systems in developing countries. Therefore, development of cultivars with greater levels of disease resistance is not only safe but also durable and an economically accessible strategy for most bean breeding programs.

Singh (1994) proposed use of gamete selection (GS) to simultaneously select common beans for multiple traits. Use of multiple-parent crosses and gamete selection proved to be successful in development of high-yielding, erect carioca bean lines with resistance to five diseases and leafhoppers (Singh *et al*., 1998). Singh *et al*. (2008) used gamete selection to improve seed yield, seed quality, and resistance to bean common mosaic virus (BCMV) (an aphid-vectored potyvirus) and rust [caused by *Uromyces appendiculatus* (Pers.) Ung.] in pinto beans. Similarly, Asensio-S.-Manzanera *et al*. (2006) used gamete selection to develop breeding lines with resistance to common bacterial blight and halo blight in dry bean.

The effectiveness of gamete selection (Singh, 1994) for introgression of multiple resistance using molecular markers is however not known. Marker-assisted selection (MAS) is an effective and efficient breeding tool for

detecting, tracking, retaining, combining, and pyramiding disease resistance genes (Kelly and Miklas, 1998). For common bean, PCR-based RAPD and SCAR markers linked with more than 20 disease resistance genes have been identified (Miklas *et al*., 2003).

In breeding for multiple traits, gamete selection permits early generation evaluation of potential value of breeding populations. Populations that do not segregate for desired traits in early generations can be discarded, thus avoiding the loss of valuable time and resources. However, Singh *et al*. (1998) noted that labour-intensive nature of gamete selection permits evaluation of only a few populations and that much care should be taken in the selection of parents that possess the desired traits.

Gamete selection may be most effective in pyramiding simply inherited traits or traits that have QTLs with large effects. Molecular markers may facilitate gamete selection in the identification of early-generation populations that continue to possess the desired alleles (Singh *et al*., 1998). Liu *et al*. (2004) found in computer simulations that marker-assisted selection of self-fertilized crops was more advantageous in earlier generations. Marker-assisted selection in early generations allows the elimination of inferior genotypes while maintaining sufficient variability to produce superior breeding lines in later generations.

Pyramiding major genes for resistance may be an effective strategy for pathogen populations that pose a moderate risk of evolving virulent pathotypes

(Beaver and Osorno, 2009). Pyramiding disease resistance genes of Middle American and Andean origin has been used to develop bean germplasm lines with broad and more durable resistance to rust (Pastor-Corrales, 2003). Pyramiding genes for disease resistance requires that virulence patterns of pathogens be monitored and new resistance genes introgressed into commercial bean cultivars to provide resistance to emerging virulent pathotypes (Young and Kelly, 1996).

Pyramiding genes of Mesoamerican and Andean origin may provide the highest and most durable resistance to bean diseases such as rust, angular leaf spot and anthracnose (Miklas *et al*., 2006a). On the other hand, pyramided resistance genes from only one gene pool, usually Middle American, has provided good levels of resistance to certain diseases such as bean golden yellow mosaic and bean common mosaic necrosis viruses (Beaver and Osorno, 2009). Sources of the resistant genes have already been identified, and as indicated in section 2.8 above, different genotypes possess genes with specific resistance to a particular disease. Genotypes Mex 54 and G10909 have been selected as sources of resistance to angular leaf spot, G2333 to anthracnose, AND 1062 and RWR 719 for *Pythium* root rot, and BRB 191 for bean common mosaic disease. The objective of this research was to pyramid multiple disease resistance genes of common bean using six donor parents – with known resistance genes to specific diseases – and introgress the resistance genes into four susceptible commercial cultivars.

## 4.3 MATERIALS AND METHODS

### 4.3.1 Plant materials

**Source of resistance:** Six bean genotypes were utilized as sources of resistance to angular leaf spot, anthracnose, root rots and bean common mosaic virus to develop double cross male gametes in the greenhouse at Kabete Field Station, University of Nairobi between 2010 and 2011. MEX 54 and G10909 were used as sources of resistance to angular leaf spot, G2333 for resistance to anthracnose, AND 1062 and RWR 719 for resistance to *Pythium* root rots and, BRB 191 for resistance to bean common mosaic virus.

**Susceptible parents:** Four commercial varieties susceptible to major diseases of common bean were utilized as recipients of disease resistance genes from the donor parents. These were KAT B1, KAT B9, GLP 585 and GLP 92. The characteristics of the parental genotypes are presented in Table 4.1.

### 4.3.2 Molecular marker polymorphism on parental lines

A total of 21 molecular markers were tested for polymorphism using plant DNA collected from 6 donor parents and 4 recipient commercial parents (Table 4.3).

**Table 4.1:** Some characteristics of parental lines used for population development.

| Genotype | [1]Gene pool | Seed colour | [2]Growth habit | [3]Reaction to diseases | | | | Markers |
|---|---|---|---|---|---|---|---|---|
| | | | | ALS | ANT | RR | BCMV | |
| **Source Parents** | | | | | | | | |
| G2333 | M | Red | IV | R | R | S | S | SAB3, SAS13, SBB14$^{1150/1050}$ |
| MEX 54 | M | Cream beige | IV | R | S | S | S | OPE4$^{708}$ |
| G10909 | M | Red | IV | R | S | S | S | SH13$^{520}$ |
| RWR 719 | M | Red | I | S | S | R | S | PYAA19$^{800}$ |
| AND 1062 | A | Red kidney | I | S | S | R | S | PYAA19$^{800}$ |
| BRB 191 | A | Red mottled | I | S | S | S | R | SW13$^{690}$ |
| **Susceptible Parents** | | | | | | | | |
| GLP 585 | M | Red | I | S | S | S | S | N/A |
| GLP 92 | M | Pinto | II | S | S | S | S | N/A |
| KAT B1 | M | Green | I | S | S | S | S | N/A |
| KAT B9 | M | Red | I | S | S | S | S | N/A |

Source: Otsyula *et al*., 2003; Queiroz *et al*., 2004; Vallejos *et al*., 2006; Mahuku *et al*., 2007 and from this study.

[1]A=Andean, M=Mesoamerican

[2]I=determinate, II=indeterminate bush, erect stem and branches, III=indeterminate bush with weak and prostrate stem and branches, IV=indeterminate climbing habit with weak, long and twisted stem and branches

[3]R=resistant, S=susceptible, ALS=angular leaf spot, ANT=anthracnose, BCMV=bean common mosaic virus, RR=*Pythium* root rot

### 4.3.3 Validation of sources of resistance and susceptibility of recipient parents

All the six donor parents and four selected market class cultivars were subjected to phenotypic evaluation that involved artificially inoculating the selected plants with known races of the pathogens, in the greenhouse. The evaluation of the disease symptoms was done 10, 14 and 21 days after inoculation based on a CIATs 9-point scale (Schoonhoven and Pastor-Corrales, 1987). Plants that scored ≤3.4 were considered resistant, 3.5 to 6.4 as moderately resistant, and ≥6.5 as susceptible.

### 4.3.4 Population development

Single crosses were made between G10909 and G2333 (angular leaf spot and anthracnose resistant lines) and between AND 1062 and BRB 191 (root rots and bean common mosaic virus resistant lines). The two single crosses were then combined into a double cross as: G10909/G2333//AND 1062/BRB 191 to pyramid resistance genes of the four constraints into one background (i.e. male gamete). Commercial bean varieties GLP 585 and GLP 92 were used as susceptible recipient parents of the pyramided disease resistance genes. All the crosses were conducted between 2009 and 2011 in the screen-house at Kabete Field Station (Appendix 1).

Donor parents crossed in pairs to produce single and double cross combinations shown on Table 4.2 and 4.6. Female parents were emasculated and then pollinated following the hooking method described by Bliss (1980).

A fine-tipped curved forceps was used to open and emasculate female buds. The emasculated buds were pollinated by rubbing and hooking the female stigma with a pollen-dusted stigma from the male parent a day before flower opening. The pollinated female flowers were tagged using a small labelled watchmaker tag. Information carried on the labels included type of cross; female and male, date crossed and person who performed the cross. Four combinations of single-way crosses were performed to combine all the donor parents through generation of an $F_1$ (Table 4.2; Fig. 4.1).

The single crosses were then combined to create four, double-cross $F_1$ combination for each male gamete. This crossing design pyramided resistance genes for all four disease constraints into a common genetic background. The resultant double cross male gamete was used to pollinate four susceptible commercial varieties (KAT B1, KAT B9, GLP 585 and GLP 92).

The resultant multi-parent cross combination resulted into the generation of sixteen gamete populations with pyramided genes for resistance to angular leaf spot, anthracnose, bean common mosaic virus and *Pythium* root rot. The design and procedure used to develop the population is presented in Fig. 4.1 and Table 4.2.

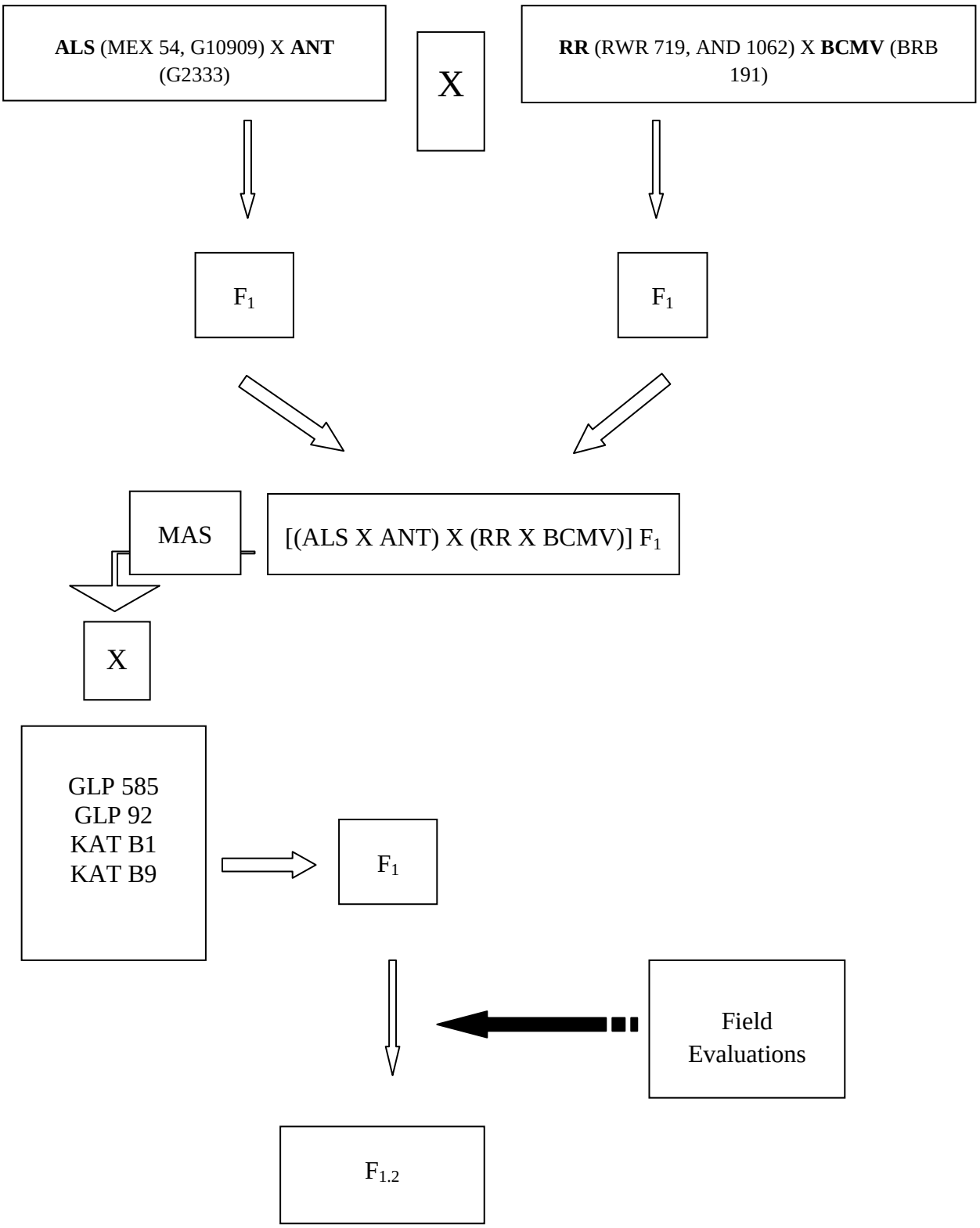



**Figure 4.1:** Crossing scheme followed in population development and early generation selection.

**Table 4.2:** The method of gamete selection for simultaneous improvement of angular leaf spot, anthracnose, *Pythium* root rot and bean common mosaic disease in common bean.

| Generation | Activities |
|---|---|
| Parents | Resistant genotypes were selected for their resistance genes; MEX 54 and G10909 for Angular leaf spot, G2333 for Anthracnose, AND 1062 and RWR 719 for *Pythium* root rot and, BRB 191 for bean common mosaic virus. |
| Single Crosses | Single crosses were conducted between Angular leaf spot and Anthracnose (MEX 54 and G2333, G10909 and G2333), and between *Pythium* root rot and Bean common mosaic virus (AND 1062 and BRB 191, RWR 719 and BRB 191). |
| Double Crosses | Four double crosses were produced by combining two single crosses into; (MEX 54 and G2333) and (AND 1062 and BRB 191), (MEX 54 and G2333) and (RWR 719 and BRB 191), (G10909 and G2333) and (AND 1062 and BRB 191) and, (G10909 and G2333) and (RWR 719 and BRB 191). |
| Parents and Crosses | Male gamete was screened for desirable resistance genes with molecular markers i.e. SH-13 for angular leaf spot, SAB-3 for anthracnose, PYAA-19 for *Pythium* root rot and SW-13 for bean common mosaic virus. Selected single plants were utilized for the production of final multiple-parent crosses with commercial varieties (GLP 585, GLP 92, KAT B1 and KAT B9) using plant-to-plant paired hybridization. |
| $F_1$ | Evaluate the final $F_1$ for successful introgression of resistance genes in the field against angular leaf spot, anthracnose, *Pythium* root rot and bean common mosaic virus and, agronomic traits. |
| $F_{1.2}$ | Evaluate in a multi-location replicated yield trial in contrasting environments. Identifying high yielding populations and discarding undesirable populations. |

*Modified version from Singh, 1994

### 4.3.5 Marker-assisted identification of male gamete plants with resistance genes

#### 4.3.5.1 Establishment and management of study plants

Eighty-nine male gamete plants, donor parents and recipient parents were grown in polythene sleeves in greenhouse at Kabete. Each polythene sleeve was initially sown with two plants, and later thinned to one plant per pot 10 days after germination. The polythene sleeves contained approximately 5 kg of potting media plus 10 g of NPK (17:17:17) each. The potting media comprised of sterilized loam soil and sand in a 3:1 ratio. Crop management was conducted through daily irrigation schedules while weeding was done by hand picking any weeds germinating from the sleeves. Each plant was tagged and staked using nylon strings 14 days after emergence to ensure each plant was independent for ease in DNA harvesting and subsequent use of pollen flowers.

#### 4.3.5.2 DNA extraction

The leaf DNA of double cross $F_1$, donor parents and recipient parents was extracted 15-20 days after germination (Fig. 4.2). DNA extraction was done using FTA® Plantsaver Cards (Whatman®) labelled with the appropriate sample identification. The DNA extraction was done by placing leaves (underside of the leaf facing down) on top of the FTA Matrix Card, and ensuring gloves were worn all times when handling FTA Card to avoid contamination. The cover sheet over FTA Matrix Card was then replaced and

using a porcelain pestle crushed the leaves to burst the cell walls of the plant tissue and allow the leaf extract soak into the paper. When the plant tissue transfer was complete the FTA Card was set to air dry for a minimum one hour at room temperature.

During analysis, FTA Matrix Card was placed on the FTA Sample Mat and a disc punched using a 2.0mm diameter Harris Micro Punch™ Tool from the centre of the dried sample area. The disc was then placed in a 1.5ml eppendorf tube, and washed using 200µl Whatman FTA purification reagent to each tube, giving it moderate manual mixing to disrupt the debris and aid in washing then incubate 4-5minutes at room temperature.

The FTA reagent was pipette up and down twice. The FTA reagent was then removed carefully to retain the disc using a pipettor and discarded from the tube. This was repeated for a total of two FTA reagent washes. Then added 200µl of $TE^{0.1}$ (10mM Tris, 0.1mM EDTA, pH 8.0) to each tube, and gave it moderate manual mixing to disrupt the debris and aid in washing then incubated for 4-5 minutes at room temperature (Appendices 3-5).

The reagent was then pipetted up and down twice, and using a pipettor removed as much $TE^{0.1}$ as possible. This step was then repeated for a total of two FTA reagent washes. The discs in the eppendorf tubes were then allowed to air dry for one hour at room temperature. The discs were then ready for Polymerase Chain Reaction (PCR) amplification within three hours of drying or stored at 4 ºC or -20 ºC in a dark environment.

#### 4.3.5.3 DNA amplification

The amplification of the SCAR markers was done using AccuPower® PCR Premix (Bioneer, Munpyeong-dong, South Korea) in a 20µl reaction mix containing an application specific enzyme in an easy to re-suspend, lyophilized premix of DNA polymerase, dNTPs, a tracking dye, reaction buffer and a stabilizer, freeze-dried into a pellet. Distilled water was added into the AccuPower® PCR tubes to a total volume of 20µl. The lyophilized blue pellet was then dissolved by vortexing, and briefly spinned down. The template DNA (FTA disc) and primer were then added onto the AccuPower® PCR tubes, and PCR amplification of the samples performed. Amplifications were performed in Applied Biosystems 2720 Thermal Cycler with initial DNA denaturation at 94°C for 30s; followed by 40 cycles primer annealing at 60-65°C for 45s; polymerization by DNA polymerase at 72°C for 30s, and a final extension at 72ºC for 20 minutes and a final rest at 15°C. After 40 cycles, the amplification products were separated by gel electrophoresis (2325 Galileo Unit, Galileo Bioscience) on a 1.2% (w/v) agarose gel, stained with 5µl/100ml Ethidium bromide at 100V in 1× TBE buffer for one hour (Appendices 6-9). The DNA bands were visualized under ultraviolet light illuminator and photographed using digital camera (Coolpix P6000, Nikon Corp., Japan) (Fig. 4.2). Selection of the male gamete plants with 4, 3 or 2 resistance gene combination was done by observing amplification of the molecular markers, exhibited by the presence of a band with the right size. Selected single plants were tagged and used as source of pyramided genes that were then introgressed into susceptible recipient parents through artificial pollination.

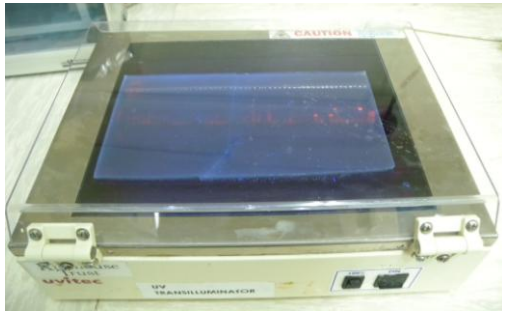
1. Plant DNA sampling in the field

2. DNA extraction using FTA card


3. Processing preserved DNA for amplification


4. Polymerase chain reaction using markers

5. Gel electrophoresis to separate amplified DNA



6. Gel photography using a UV-Transilluminator

**Figure 4.2:** Schematic flow of plant DNA extraction from the field to generation of gel band

## 4.4 RESULTS

### 4.4.1 Polymorphism of molecular markers on parental lines

**Table 4.3:** Molecular marker polymorphism of donor and recipient parental genotypes.

| | | Parental Genotypes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Donor Parents | | | | | | Recipient Parents | | | |
| No. | Marker | MEX 54 | G10909 | G2333 | BRB 191 | AND 1062 | RWR 719 | GLP 92 | GLP 585 | KAT B1 | KAT B9 |
| 1 | SH-13 | + | + | - | - | - | + | + | - | - | - |
| 2 | OPE4 | + | + | + | + | + | + | - | + | - | - |
| 3 | SNO2 | + | + | - | - | + | + | - | + | + | + |
| 4 | SAS-13 | + | + | + | + | + | + | + | + | + | + |
| 5 | SBB-14 | + | - | + | - | - | - | + | + | - | - |
| 6 | SAB3 | - | + | + | - | - | - | - | - | - | - |
| 7 | ROC-11 | - | - | - | - | - | - | - | - | - | - |
| 8 | SW-13 | - | - | - | - | - | - | - | - | - | - |
| 9 | PYAA19 | - | - | - | - | - | - | - | - | - | - |
| 10 | SAP-6 | + | - | - | - | + | + | + | + | - | - |
| 11 | SU-91 | - | - | - | - | - | - | - | - | - | - |
| 12 | elF4E-1 | - | - | - | - | - | - | - | - | - | - |
| 13 | elF4E-2 | + | + | + | + | + | + | + | + | + | + |
| 14 | Bng45 | + | + | + | + | + | + | + | + | + | + |
| 15 | CV542014 | + | + | + | - | - | + | + | + | - | - |
| 16 | TGA-11 | - | - | + | + | + | - | + | + | + | + |
| 17 | PHS | + | + | + | + | + | + | + | + | + | + |
| 18 | Pv97 | + | + | + | + | + | + | + | + | + | + |
| 19 | SHP-1 | - | - | - | + | - | - | - | + | - | - |
| 20 | PVTFL1-Y | - | - | - | - | - | - | - | - | - | + |
| 21 | PVTFL1-Z | - | - | - | - | - | - | - | - | - | - |

(+) presence of amplification and expression of right-sized band
(-) no amplification

A total of 21 molecular markers were evaluated for their polymorphism with the parental genotypes' resistance genes (Table 4.3). Eleven markers showed polymorphism while 10 of them were non-polymorphic to the disease resistance genes (Table 4.3). Fifteen markers tested had positive amplification (+) to the disease resistance genes in at least one parental genotype while 6 markers showed no amplification (-) and therefore no expression of the right-sized DNA band (Table 4.3). Following the ease of molecular markers' optimization, amplification, polymorphism and their expression of DNA bands of the right size for disease resistance genes, one male gamete (G10909/G2333//AND 1062/BRB 191) was screened using SAB-3 for anthracnose, SH-13 for angular leaf spot, SW-13 for bean common mosaic virus and PYAA-19 for *Pythium* root rot to identify recombinants for the resistance genes (Table 4.7).

### 4.4.2 Reaction of parental genotypes to common bean diseases

**Table 4.4:** Validating the parental genotype by evaluation of disease reaction to angular leaf spot, anthracnose, *Pythium* root rot and bean common mosaic virus in greenhouse and field infestation.

| Parental Genotype | Disease Reaction on a 1-9 CIAT scale | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Greenhouse Evaluation | | | | Field Evaluation | | | |
| | ALS | ANT | BCMV | RR | ALS | ANT | BCMV | RR |
| **Donor parents** | | | | | | | | |
| MEX 54 | 1.0 | 1.0 | 6.3 | 5.3 | 1.8 | 6.4 | 6.2 | 2.1 |
| G10909 | 1.0 | 1.0 | 7.9 | 3.9 | 3.0 | 7.6 | 5.4 | 1.8 |
| G2333 | 1.0 | 1.0 | 5.4 | 4.5 | 3.6 | 1.8 | 5.8 | 2.0 |
| AND 1062 | 6.6 | 8.1 | 4.2 | 1.4 | 6.8 | 7.0 | 6.7 | 1.1 |
| RWR 719 | 5.5 | 1.0 | 4.8 | 1.0 | 7.2 | 6.7 | 5.0 | 1.0 |
| BRB 191 | 1.0 | 8.4 | 3.0 | 4.7 | 5.9 | 5.8 | 3.0 | 1.4 |
| **Recipient parents** | | | | | | | | |
| KAT B1 | 6.5 | 9.0 | 6.8 | 7.1 | 6.0 | 7.2 | 5.8 | 2.6 |
| KAT B9 | 7.4 | 8.8 | 6.8 | 6.9 | 6.3 | 7.2 | 5.5 | 2.5 |
| GLP 585 | 5.4 | 4.3 | 3.7 | 5.7 | 6.8 | 7.6 | 5.8 | 2.9 |
| GLP 92 | 6.6 | 2.5 | 5.0 | 6.1 | 7.7 | 8.1 | 6.2 | 2.7 |

ALS=angular leaf spot, ANT=anthracnose, BCMV=bean common mosaic virus, RR=root rot

Parental genotypes were validated on their reaction to four major common bean diseases present in Kenya by artificial inoculation in the greenhouse and field evaluation (Table 4.4). Donor and recipient parental lines were screened in the green house with 57 pathogenic isolates of *Pseudocercospora griseola*, which were grouped into 23 races (Table 4.5). Among the donor parents MEX 54 was resistant to 19 races, and intermediate in only 4 races out of 23 pathotypes (Table 4.5). G10909 had an average score of 2.8 on the disease assessment 1-9 CIAT scale Schoonhoven and Pastor-Corrales (1987), and was resistant to 8 races and intermediate to 15 of the 23 races (Table 4.5).

Four of the donor parents (G2333, AND 1062, RWR 719 and BRB 191) had an overall intermediate reaction to 23 races of angular leaf spot with mean scores of 4.9, 6.4, 4.7 and 6.3 respectively (Table 4.5). All the commercial cultivars (KAT B1, KAT B9, GLP 92 and GLP 585) were susceptible to most races of angular leaf spot indicating the need to improve them through genetic resistance. KAT B1 and KAT B9 were each susceptible to 16 races and intermediate to 7 races of the 23 pathotypes (Table 4.5). GLP 92 was susceptible to 15, intermediate to 7 and resistant to 1 race, while GLP 585 was susceptible to 13, intermediate to 7 and resistant to 3 of the 23 races (Table 4.5). MEX 54 and G10909 were thus confirmed as the most useful sources of resistance to angular leaf spot for bean breeding programs in Kenya (Table 4.4, 4.5).

However, the other donor parents despite being susceptible to some races of angular leaf spot possess resistance genes for other disease constraints for which they were utilized (Table 4.4). The high susceptibility of commercial varieties to all the pathogenic races shows urgent need to improve the market-preferred varieties. Race 63-63 was virulent to all donor and recipient parents except MEX 54 and G10909 which were moderately resistant (Table 4.5).

The parental genotypes reacted differently to specific diseases and were more susceptible in the field than in the greenhouse evaluation (Table 4.4). Reaction to angular leaf spot in the green house showed that only two donor parents (AND 1062 and RWR 719) and all the recipient parents were susceptible to the disease, while in the field evaluation only MEX 54 and G10909 were resistant with all other genotypes being susceptible. This results also showed

that parents reacted differently to anthracnose infection with AND 1062 and BRB 191 being the only donor parents susceptible to the disease in the greenhouse, whereas all the recipient parents showed susceptibility to anthracnose in both greenhouse and field evaluation. However, genotype G2333 was consistently resistant to anthracnose both under artificial inoculation in the greenhouse and natural infestation in the field.

Except BRB 191 donor parent all other parental genotypes were susceptible to bean common mosaic disease both in the greenhouse and field evaluation, although the recipient genotype GLP 585 showed moderate resistance to the disease in the greenhouse. Results on the evaluation of *Pythium* root rot showed the only resistant genotypes were AND 1062 and RWR 719 under artificial inoculation of the pathogen in the greenhouse with all other parental genotypes expressing susceptibility to the disease. Reaction to *Pythium* root rot under field conditions was however different as all donor and recipient genotypes were either resistance or moderately resistance to the disease.

**Table 4.5:** Reaction of parental genotypes to infection by 23 angular leaf spot races.

| | Donor Parents | | | | | | Recipient Parents | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Race | MEX 54 | G10909 | G2333 | AND 1062 | RWR 719 | BRB 191 | KAT B1 | KAT B9 | GLP 585 | GLP 92 |
| 63-55 | 1.0 | 1.0 | 3.7 | 7.0 | 5.9 | 5.4 | 7.3 | 6.8 | 8.2 | 7.9 |
| 31-7 | 1.0 | 1.4 | 2.7 | 6.3 | 5.7 | 5.8 | 6.3 | 6.0 | 7.5 | 6.6 |
| 6-0 | 1.0 | 1.0 | 1.0 | 6.0 | 3.4 | 4.4 | 6.8 | 4.3 | 3.3 | 3.2 |
| 63-63 | 3.6 | 4.0 | 5.4 | 8.2 | 7.3 | 7.2 | 6.3 | 6.6 | 7.7 | 7.0 |
| 30-4 | 1.0 | 1.4 | 2.7 | 8.2 | 5.6 | 5.7 | 8.0 | 7.8 | 4.6 | 3.8 |
| 31-31 | 3.6 | 2.7 | 3.7 | 5.8 | 4.1 | 7.0 | 6.7 | 5.0 | 6.4 | 6.7 |
| 31-0 | 1.0 | 1.0 | 2.2 | 5.8 | 3.7 | 6.9 | 6.3 | 7.1 | 6.3 | 6.5 |
| 63-31 | 3.7 | 3.9 | 6.4 | 7.1 | 5.5 | 7.0 | 6.5 | 7.0 | 6.8 | 7.6 |
| 62-37 | 1.0 | 1.0 | 3.7 | 6.1 | 6.2 | 6.5 | 7.3 | 8.0 | 7.8 | 7.4 |
| 63-7 | 1.0 | 1.3 | 4.1 | 7.4 | 5.4 | 6.5 | 6.8 | 5.9 | 5.3 | 6.0 |
| 14-0 | 1.0 | 1.0 | 1.2 | 6.8 | 3.9 | 7.7 | 5.3 | 6.3 | 5.2 | 5.7 |
| 26-0 | 1.0 | 1.0 | 2.7 | 7.7 | 3.3 | 5.4 | 4.1 | 4.9 | 2.7 | 3.7 |
| 46-2 | 1.0 | 1.0 | 2.3 | 5.3 | 4.0 | 6.8 | 5.4 | 6.3 | 3.7 | 5.2 |
| 63-54 | 1.3 | 3.9 | 5.3 | 7.4 | 6.0 | 6.8 | 4.9 | 4.2 | 6.0 | 6.1 |
| 30-18 | 1.0 | 1.3 | 4.1 | 5.4 | 4.4 | 5.1 | 3.7 | 5.7 | 4.1 | 4.0 |
| 63-39 | 1.3 | 1.1 | 4.3 | 5.1 | 4.0 | 5.2 | 3.9 | 4.8 | 3.4 | 4.1 |
| 63-23 | 1.0 | 1.3 | 4.1 | 6.0 | 4.7 | 6.2 | 5.6 | 5.9 | 7.6 | 6.6 |
| 62-7 | 1.0 | 1.0 | 3.1 | 6.5 | 4.9 | 4.0 | 6.9 | 5.6 | 4.3 | 6.6 |
| 39-0 | 1.0 | 1.0 | 2.5 | 7.2 | 1.0 | 6.8 | 5.8 | 7.0 | 3.4 | 4.1 |
| 58-18 | 1.0 | 2.2 | 3.9 | 7.0 | 5.2 | 6.8 | 5.3 | 6.0 | 6.0 | 5.7 |
| 31-39 | 1.0 | 3.7 | 5.6 | 6.1 | 5.6 | 7.0 | 6.9 | 7.3 | 8.0 | 7.1 |
| 63-35 | 1.0 | 2.3 | 4.3 | 8.0 | 4.1 | 6.1 | 7.9 | 7.2 | 6.7 | 7.6 |
| 63-47 | 3.9 | 5.7 | 6.1 | 7.7 | 3.9 | 8.2 | 7.7 | 6.8 | 7.0 | 7.3 |
| Mean | 1.5 | 2.0 | 3.7 | 6.7 | 4.7 | 6.3 | 6.2 | 6.2 | 5.7 | 5.9 |
| $LSD_{0.05}$ | 0.6 | 2.2 | 2.4 | 2.4 | 2.2 | 2.0 | 2.3 | 2.3 | 3.2 | 2.9 |
| VR | 50.3 | 4.1 | 4.0 | 0.7 | 1.8 | 1.8 | 1.2 | 0.6 | 1.6 | 1.2 |
| CV % | 14.5 | 33.8 | 21.4 | 16.2 | 19.9 | 13.9 | 15.3 | 15.8 | 22.3 | 20.3 |

### 4.4.3 Seed yield of single, double and 5-way cross combinations

The single crosses were less successful compared to either double or 5-way cross combinations with the lowest success rate being observed from the cross AND 1062 / BRB 191 (28.8%), and the highest success rate observed was G10909 / G2333 (66.3%) (Table 4.6). The double crosses were the most successful obtaining a median success rate of 84.6% compared to 71.7% generated by 5-way crosses and 46.3% by single crosses.

The 5-way crosses between the susceptible commercial varieties and the male gamete parents had the highest success rate between KAT B1///MEX 54/G2333//RWR 719/BRB 191 (82.3%) and the lowest success rate was obtained between KAT B9///MEX 54/G2333//RWR 719/BRB 191 at 61.4% (Table 4.6). The multi-parent male gametes were most compatible when crossed with GLP 585, which had the highest success rate (72.6%) followed by KAT B1 (72.4%), KAT B9 (71.1%), and relatively less compatible in crosses with GLP 92 (70.6%). For individual cross combinations, the highest success rate was recorded by GLP 585///G10909/G2333//RWR 719/BRB 191 at 77.4%, and lowest success rate for the cross GLP 585///G10909/G2333//AND 1062/BRB 191 (65.0%).

Among cross with KAT B1 as the female parent, KAT B1///MEX 54/G2333//RWR 719/BRB 191 had the highest success rate (82.3%). KAT B1///MEX 54/G2333//AND 1062/BRB 191 had the lowest success rate (62.6%) (Table 4.6). In the final crosses with KAT B9 as the female parent, the highest success rate was recorded by KAT B9///G10909/G2333//RWR

719/BRB 191 at 81.3%, and lowest success rate was obtained from KAT B9///MEX 54/G2333//RWR 719/BRB 191 at 61.4%. In the final crosses with GLP 92 as the female parent, the highest success rate was recorded for the cross GLP 92///G10909/G2333//AND 1062/BRB 191 at 80.8% and lowest success rate was obtained for the cross GLP 92///G10909/G2333//RWR 719/BRB 191 at 63.1% (Table 4.6).

**Table 4.6:** Pollination success rate (%) of single and complex cross combinations.

| Cross combination | No. of artificial pollinations | No. of successful fertilizations | No. of seeds obtained | Success rate (%) |
|---|---|---|---|---|
| **Single cross gametes** | | | | |
| MEX 54 / G2333 | 76 | 41 | 237 | 53.9 |
| G10909 / G2333 | 86 | 57 | 405 | 66.3 |
| AND 1062 / BRB 191 | 59 | 17 | 83 | 28.8 |
| RWR 719 / BRB 191 | 169 | 71 | 573 | 42.0 |
| **Double cross gametes** | | | | |
| MEX 54 / G2333 // AND 1062 / BRB 191 | 97 | 85 | 401 | 87.6 |
| MEX 54 / G2333 // RWR 719 / BRB 191 | 113 | 104 | 791 | 92.0 |
| G10909 / G2333 // AND 1062 / BRB 191 | 137 | 98 | 626 | 71.5 |
| G10909 / G2333 // RWR 719 / BRB 191 | 116 | 101 | 578 | 87.1 |
| **Final cross with commercial variety** | | | | |
| KAT B1///MEX 54/G2333//AND1062/BRB 191 | 91 | 57 | 417 | 62.6 |
| KAT B9///MEX 54/G2333//AND1062/BRB 191 | 93 | 65 | 376 | 69.9 |
| GLP585///MEX 54/G2333//AND1062/BRB 191 | 102 | 76 | 483 | 74.5 |
| GLP 92///MEX 54/G2333//AND 1062/BRB 191 | 107 | 74 | 451 | 69.1 |
| KAT B1///MEX 54/G2333//RWR 719/BRB 191 | 96 | 79 | 344 | 82.3 |
| KAT B9///MEX 54/G2333//RWR 719/BRB 191 | 83 | 51 | 236 | 61.4 |
| GLP 585///MEX 54/G2333//RWR 719/BRB 191 | 132 | 97 | 711 | 73.5 |
| GLP 92///MEX 54/G2333//RWR 719/BRB 191 | 177 | 123 | 737 | 69.5 |
| KAT B1///G10909/G2333//AND 1062/BRB 191 | 117 | 80 | 349 | 68.4 |
| KAT B9///G10909/G2333//AND 1062/BRB 191 | 99 | 71 | 385 | 71.7 |
| GLP585///G10909/G2333//AND 1062/BRB 191 | 120 | 78 | 463 | 65.0 |
| GLP 92///G10909/G2333//AND 1062/BRB 191 | 78 | 63 | 412 | 80.8 |
| KAT B1///G10909/G2333//RWR 719/BRB 191 | 101 | 77 | 360 | 76.2 |
| KAT B9///G10909/G2333//RWR 719/BRB 191 | 107 | 87 | 328 | 81.3 |
| GLP 585///G10909/G2333//RWR 719/BRB 191 | 93 | 72 | 431 | 77.4 |
| GLP 92///G10909/G2333//RWR 719/BRB 191 | 103 | 65 | 466 | 63.1 |

### 4.4.4 Marker-assisted male gamete selection

Out of the 89 male gamete plants screened for angular leaf spot, anthracnose, root rots and bean common mosaic virus, five plants had three resistance genes, 18 had two, 35 had one, and 36 had none (Table 4.7). Only plants with two or three resistance genes were used for final crossing with susceptible commercial varieties. There was amplification and polymorphism in three markers, SAB-3 for anthracnose; SH-13 for angular leaf spot and SW-13 for bean common mosaic virus. No amplification was observed for PYAA-19 marker, which is linked with the root rot resistance genes (Table 4.7; Fig. 4.3-4.8).

**Table 4.7:** Marker assisted identification of male gamete plants with multiple resistance genes.

| Male gamete | Plant No. | Strip lane | Molecular Markers | | | | No. of Markers |
|---|---|---|---|---|---|---|---|
| | | | ANT | ALS | BCMV | RR | |
| | | | SAB-3 | SH-13 | SW-13 | PYAA-19 | |
| 1 | 1.1 | 1 | - | - | - | - | 0 |
| 2 | 1.2 | 2 | + | - | - | - | 1 |
| 3 | 1.3 | 3 | + | + | - | - | 2 |
| 4 | 1.4 | 4 | + | - | - | - | 1 |
| 5 | 1.5 | 5 | + | + | + | - | 3 |
| 6 | 1.6 | 6 | - | - | - | - | 0 |
| 7 | 1.7 | 7 | + | - | - | - | 1 |
| 8 | 1.8 | 8 | + | - | + | - | 2 |
| 9 | 1.9 | 1 | - | - | - | - | 0 |
| 10 | 2.0 | 2 | + | + | + | - | 3 |
| 11 | 2.1 | 3 | - | - | - | - | 0 |
| 12 | 2.2 | 4 | - | - | - | - | 0 |
| 13 | 2.3 | 5 | - | - | - | - | 0 |
| 14 | 2.4 | 6 | - | + | - | - | 1 |
| 15 | 2.5 | 7 | - | - | + | - | 1 |
| 16 | 2.6 | 8 | - | + | + | - | 2 |
| 17 | 2.7 | 1 | + | - | - | - | 1 |
| 18 | 2.8 | 2 | - | - | - | - | 0 |
| 19 | 2.9 | 3 | + | - | - | - | 1 |
| 20 | 3.0 | 4 | - | - | - | - | 0 |
| 21 | 3.1 | 5 | - | + | - | - | 1 |
| 22 | 3.2 | 6 | - | - | + | - | 1 |
| 23 | 3.3 | 7 | - | - | - | - | 0 |
| 24 | 3.4 | 8 | + | - | - | - | 1 |
| 25 | 3.5 | 1 | + | + | - | - | 2 |
| 26 | 3.6 | 2 | - | - | - | - | 0 |
| 27 | 3.7 | 3 | - | - | + | - | 1 |
| 28 | 3.8 | 4 | - | - | - | - | 0 |
| 29 | 3.9 | 5 | + | - | + | - | 2 |
| 30 | 4.0 | 6 | - | + | + | - | 2 |
| 31 | 4.1 | 7 | - | - | - | - | 0 |
| 32 | 4.2 | 8 | + | + | - | - | 2 |
| 33 | 4.3 | 1 | + | - | - | - | 1 |
| 34 | 4.4 | 2 | + | - | - | - | 1 |
| 35 | 4.5 | 3 | - | - | - | - | 0 |
| 36 | 4.6 | 4 | - | - | - | - | 0 |
| 37 | 4.7 | 5 | - | - | + | - | 1 |

| Male gamete | Plant No. | Strip lane | Molecular Markers | | | | No. of Markers |
|---|---|---|---|---|---|---|---|
| | | | ANT | ALS | BCMV | RR | |
| | | | SAB-3 | SH-13 | SW-13 | PYAA-19 | |
| 38 | 4.8 | 6 | + | - | + | - | 2 |
| 39 | 4.9 | 7 | - | - | - | - | 0 |
| 40 | 5.0 | 8 | - | - | + | - | 1 |
| 41 | 5.1 | 1 | + | + | + | - | 3 |
| 42 | 5.2 | 2 | + | - | - | - | 1 |
| 43 | 5.3 | 3 | + | - | - | - | 1 |
| 44 | 5.4 | 4 | - | - | - | - | 0 |
| 45 | 5.5 | 5 | - | - | - | - | 0 |
| 46 | 5.6 | 6 | + | - | + | - | 2 |
| 47 | 5.7 | 7 | + | + | - | - | 2 |
| 48 | 5.8 | 8 | - | - | - | - | 0 |
| 49 | 5.9 | 1 | + | - | - | - | 1 |
| 50 | 6.0 | 2 | + | - | + | - | 2 |
| 51 | 6.1 | 3 | + | - | - | - | 1 |
| 52 | 6.2 | 4 | + | - | - | - | 1 |
| 53 | 6.3 | 5 | - | + | - | - | 1 |
| 54 | 6.4 | 6 | - | - | - | - | 0 |
| 55 | 6.5 | 7 | + | - | - | - | 1 |
| 56 | 6.6 | 8 | + | + | + | - | 3 |
| 57 | 6.7 | 1 | + | - | + | - | 2 |
| 58 | 6.8 | 2 | - | - | - | - | 0 |
| 59 | 6.9 | 3 | - | - | - | - | 0 |
| 60 | 7.0 | 4 | + | - | - | - | 1 |
| 61 | 7.1 | 5 | - | + | + | - | 2 |
| 62 | 7.2 | 6 | - | - | - | - | 0 |
| 63 | 7.3 | 7 | - | - | + | - | 1 |
| 64 | 7.4 | 8 | - | + | + | - | 2 |
| 65 | 7.5 | 1 | - | - | - | - | 0 |
| 66 | 7.6 | 2 | - | - | - | - | 0 |
| 67 | 7.7 | 3 | + | - | - | - | 1 |
| 68 | 7.8 | 4 | - | - | - | - | 0 |
| 69 | 7.9 | 5 | - | - | - | - | 0 |
| 70 | 8.0 | 6 | - | - | - | - | 0 |
| 71 | 8.1 | 7 | - | + | - | - | 1 |
| 72 | 8.2 | 8 | - | - | - | - | 0 |
| 73 | 8.3 | 1 | + | + | - | - | 2 |
| 74 | 8.4 | 2 | + | - | + | - | 2 |
| 75 | 8.5 | 3 | + | - | - | - | 1 |
| 76 | 8.6 | 4 | + | - | - | - | 1 |
| 77 | 8.7 | 5 | - | - | - | - | 0 |

| Male gamete | Plant No. | Strip lane | Molecular Markers | | | | No. of Markers |
|---|---|---|---|---|---|---|---|
| | | | ANT | ALS | BCMV | RR | |
| | | | SAB-3 | SH-13 | SW-13 | PYAA-19 | |
| 78 | 8.8 | 6 | + | - | + | - | 2 |
| 79 | 8.9 | 7 | - | - | - | - | 0 |
| 80 | 9.0 | 8 | - | - | - | - | 0 |
| 81 | 9.1 | 1 | - | + | - | - | 1 |
| 82 | 9.2 | 2 | + | - | - | - | 1 |
| 83 | 9.3 | 3 | - | - | - | - | 0 |
| 84 | 9.4 | 4 | + | - | + | - | 2 |
| 85 | 9.5 | 5 | - | + | - | - | 1 |
| 86 | 9.6 | 6 | - | - | - | - | 0 |
| 87 | 9.7 | 7 | + | + | + | - | 3 |
| 88 | 9.8 | 8 | - | - | - | - | 0 |
| 89 | 9.9 | 1 | + | - | + | - | 2 |
| G10909 | 10.0 | 2 | - | + | - | - | 1 |
| G2333 | 10.1 | 3 | + | - | - | - | 1 |
| AND 1062 | 10.2 | 4 | - | - | - | - | 0 |
| BRB 191 | 10.3 | 5 | - | - | + | - | 1 |
| GLP 585 | 10.4 | 6 | - | - | - | - | 0 |
| GLP 92 | 10.5 | 7 | - | - | - | - | 0 |
| +ve control | | | + | + | + | - | 3 |
| -ve control | | 8 | - | - | - | - | 0 |
| TOTAL | | | 42 | 21 | 27 | NA | |

ALS=angular leaf spot, ANT=anthracnose, BCMV=bean common mosaic virus, RR=*Pythium* root rot

(+) amplification for the presence of resistance gene

(-) no amplification hence absence of resistance gene

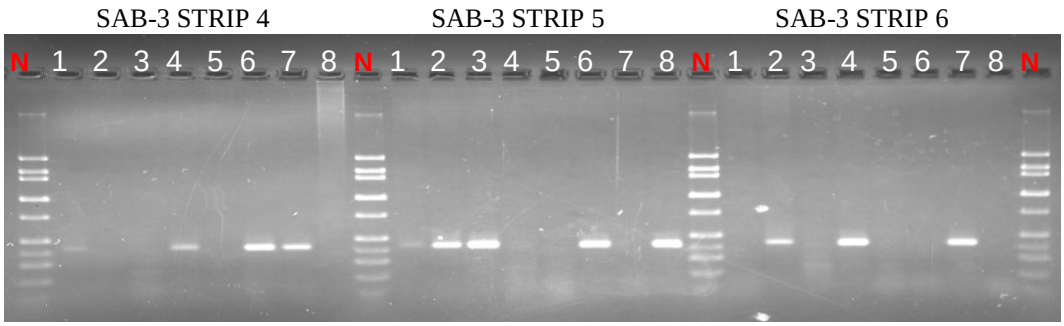



**Figure 4.3:** DNA amplification of male gamete plants using SAB-3 marker (strip 4-6).

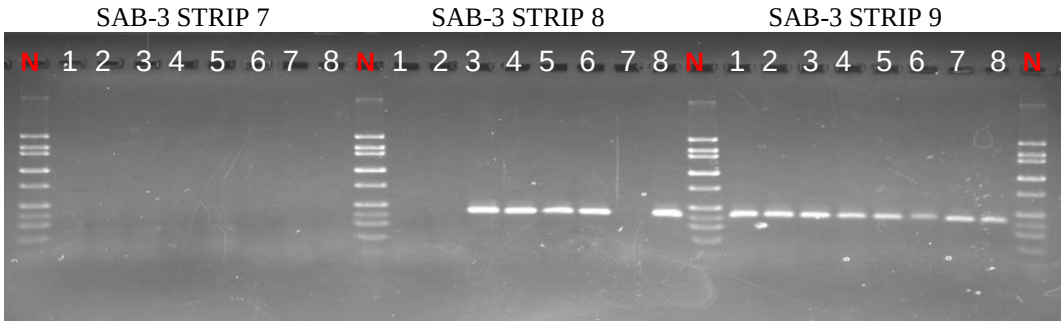



**Figure 4.4:** DNA amplification of male gamete plants using SAB-3 marker (strip 7-9).

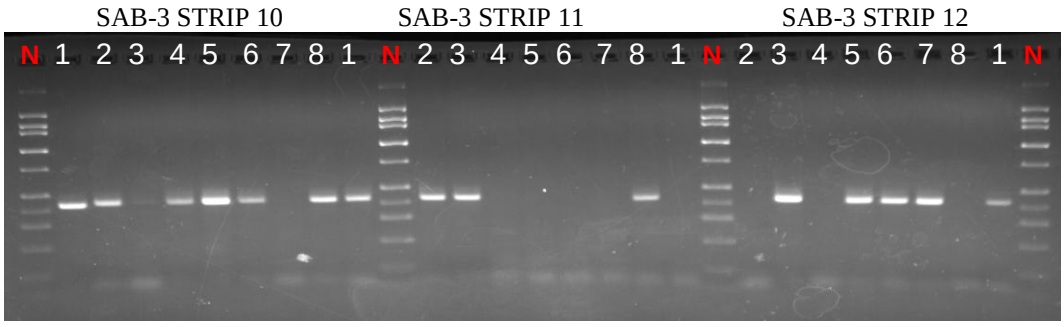



**Figure 4.5:** DNA amplification of male gamete plants using SAB-3 marker (strip 10-12).

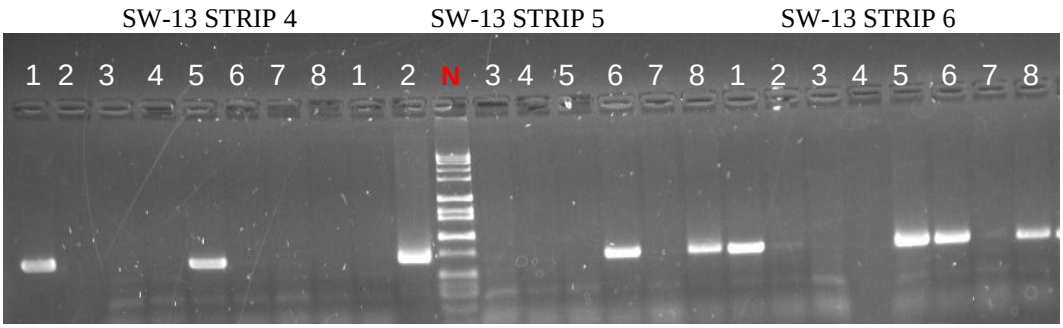



**Figure 4.6:** DNA amplification of male gamete plants using SW-13 marker (strip 4-6).

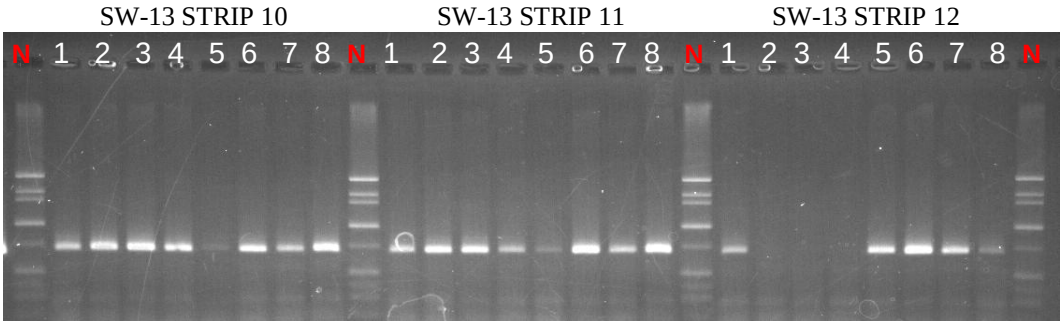



**Figure 4.7:** DNA amplification of male gamete plants using SW-13 marker (strip 10-12).

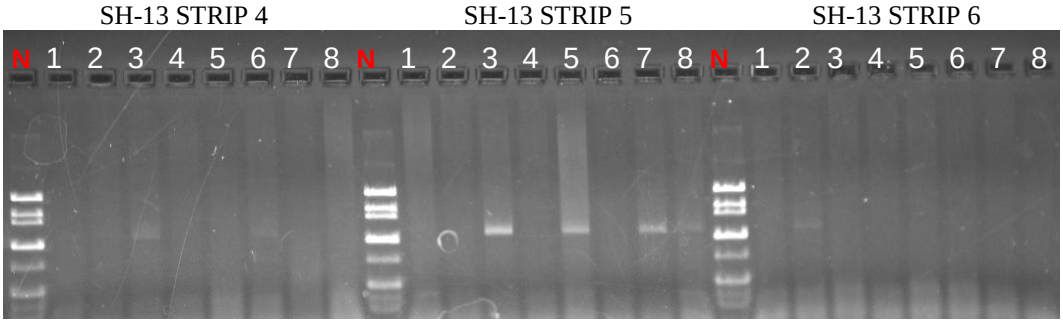



**Figure 4.8:** DNA amplification of male gamete plants using SH-13 marker (strip 4-6).

## 4.5 DISCUSSION

Singh (1994) proposed the use of gamete selection to simultaneously select common beans for multiple traits. Twenty-one markers were subjected to polymorphism tests with the parental genotypes intended as the source of resistance genes. Asensio-S.-Manzanera *et al*. (2006) also used gamete selection to develop breeding lines with resistance to common bacterial blight and halo blight. In breeding for multiple traits, gamete selection permits the early generation evaluation of the potential value of breeding populations. Gamete selection may be most effective in pyramiding simply inherited traits or traits that have QTLs with large effects. Molecular markers may facilitate gamete selection in the identification of early-generation populations that continue to possess the desired alleles (Singh *et al*., 1998).

Fungal diseases are major constraints to bean production throughout the world (Jesus Junior *et al*., 2001). The relative importance of different fungal diseases varies among regions due to differences in soil, climate, crop management practices and degree of susceptibility of cultivars used by bean producers (Mmbaga *et al*., 1996; Boland *et al*., 2004). The degree of virulence among isolates of some fungal pathogens can also vary between regions and over time. This has been observed for many bean pathogens such as angular leaf spot caused by *Pseudocercospora griseola* (Pastor-Corrales *et al*., 1998; Mahuku *et al*., 2002b).

Plant breeders, pathologists and geneticists have made considerable progress in the identification of specific genes and QTL for resistance to anthracnose (Kelly and Vallejo, 2004); angular leaf spot (Caixeta *et al*., 2005); bean

common mosaic virus (Melotto *et al*., 1996; Pastor-Corrales *et al*., 2007). Tarán *et al*. (2003) noted, however, that the efficiency of marker-assisted selection depends on the number of markers available and the degree of linkage of the marker with the desired QTL. Because of this limitation, only few of the most reliable markers are being used routinely by bean breeding programs.

These results clearly support the power and effectiveness of gamete selection for improving multiple disease resistance in common bean. The effectiveness of gamete selection in improvement of qualitative (resistance to BCMV and rust) and quantitative traits (such as seed yield) in pinto bean cultivar '*Shoshone*' for the Western USA (Singh *et al*., 2008), resistance to multiple diseases in carioca bean for Brazil and the eastern plains of Bolivia (Singh *et al*., 2000), and development of resistant dry bean germplasm to halo blight and common bacterial blight in Spain (Asensio-S.-Manzanera *et al*., 2005, 2006) have also been reported.

However, there is currently no report on the utilization of marker assisted gamete selection in common bean from other regions. Breeding lines developed in this study should be used for further improvement of multiple disease resistance in mesoamerican and other market classes of common bean cultivated in Kenya.

# CHAPTER 5 MARKER ASSISTED GAMETE SELECTION FOR MULTIPLE DISEASE RESISTANCE AND AGRONOMIC TRAITS IN INTERGENE POOL AND INTERRACIAL BEAN POPULATIONS

## 5.1 ABSTRACT

Angular leaf spot, anthracnose, bean common mosaic virus and root rots are widespread and major diseases of common bean (*Phaseolus vulgaris* L.) that cause severe yield losses in Kenya. Use of cultivars resistant to these diseases is pivotal for their integrated control and to facilitate production and distribution of pathogen-free seed. The objective was to evaluate and select for multiple resistance to angular leaf spot, anthracnose, bean common mosaic virus, root rots and agronomic traits in $F_1$ and $F_{1.2}$ segregating bean populations. Sixteen bean populations were developed from crosses among four multi-parent male gametes and four commercial varieties. Commercial varieties used as female parents in the final cross were susceptible to the four diseases. $F_1$ and $F_{1.2}$ progenies from each male gamete were evaluated for disease resistance and agronomic traits in field experiments at Kabete and Tigoni for two seasons. There was segregation for growth habit among the sixteen multiple-cross $F_1$ populations which included growth types I, II, III and IV. The range in growth vigour among the 16 five-way crosses ranged from excellent (1) to very poor (9) in the $F_1$ plants. Genotypes flowered two days earlier at Kabete (41.2 days) than at Tigoni (43.2 days). The days to 50% flowering in Kabete ranged from as low as 30 to as high as 56 days whereas

the situation in Tigoni was a low of 33 to a high of 57 days. In Kabete maturity had a range of 56 to 113 days and a mean of 80.8, whereas the range in Tigoni was between 63 to 111 days with an average maturity of 86.7 days. There was significant differences ($P< 0.05$) detected among the genotypes for angular leaf spot, anthracnose, root rot and bean common mosaic virus. Angular leaf spot was less severe at Kabete (score of 5.1) than at Tigoni (5.5) respectively. Similarly, there was on average high disease severity for anthracnose in Kabete than in Tigoni with means of 5.7 and 6.4 respectively. The results on average disease severity for bean common mosaic virus were similar in both Kabete and Tigoni with a mean of 5.1 in the two locations. The average disease severity for root rot was higher in Kabete than was in Tigoni with a means of 2.9 and 1.6 respectively. Grain yield was lower at Kabete (2256 kg $ha^{-1}$) compared to Tigoni (3391 kg $ha^{-1}$). $F_1$ progeny of KAT B9 with the four male gametes gave the best yield of 4360.5 kg $ha^{-1}$ in both locations. Male gamete MEX 54/G2333//RWR 719/BRB 191 had the highest yield at 4383.5 kg $ha^{-1}$ across the locations. Genotypes derived from population KGS12-16 recorded the highest yield (4762 kg $ha^{-1}$) while KGS12-05 had the lowest yield (2490 kg $ha^{-1}$) among the crosses. Yield was strongly correlated to days to 50 % maturity (r=0.4**) and number of pods per plant (r=0.4**) in all genotypes across the two locations.

**Key words:** *Phaseolus vulgaris*, gamete selection, disease resistance, yield.

## 5.2 INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume grown in eastern, central and southern Africa – in terms of both area under production and consumption (CIAT, 2005). It is widely cultivated in tropical and subtropical countries, where it is an important source of proteins, carbohydrates, fiber, vitamins and minerals (Wortmann *et al*., 1998; Kimani *et al.*, 2005). In Kenya, common bean is the most important legume in the pulses category and is second only to maize as a food crop (Kimani *et al.*, 2005).

Despite its importance, bean yields in developing countries are among the lowest in the world, with average of 0.5 tonnes $ha^{-1}$ (FAO, 2013) compared to 1 to 2 tonnes $ha^{-1}$ commonly reported in experimental fields. The low yields are attributed to a number of both biotic and abiotic constraints. One of the factors that accounts for the low yield is the occurrence of several diseases that affect the common bean crop. They include anthracnose, caused by *Colletotrichum lindemuthianum*, angular leaf spot, caused by *Pseudocercospora griseola*, bean common mosaic disease and various root rots. These diseases deserve great attention because of the damage they cause and the high pathogenic variability of the pathogens (Rocha *et al*., 2012). The use of resistant cultivars stands out among the various strategies used to control diseases because it is cheap and generally more efficient.

The identification of molecular markers associated to the resistance genes is an auxiliary tool in common bean breeding for resistance to diseases (Alzate-Marin *et al*., 2007). Molecular markers can enhance the efficiency and

precision of developing new bean lines with resistance alleles to several pathogens (Ragagnin *et al*., 2009; Costa *et al*., 2010). Lines with pyramided genes can be extremely useful in breeding programs aiming to incorporate alleles for resistance to several pathogens in elite lines and commercial cultivars.

However, resistance should be combined with preferred agronomic traits desired by end users. It is for this reason that characters, such as plant architecture, yield and grain type, have received great attention in common bean breeding programs (Cunha *et al*., 2005; Rocha *et al*., 2012). The objective of this study was to evaluate and select early generation families combining multiple disease resistance and agronomic traits.

## 5.3 MATERIALS AND METHODS

### 5.3.1 Plant materials

Sixteen bean populations were developed from crosses among four multi-parent male gametes and four commercial varieties in the greenhouse at Kabete Field Station, University of Nairobi between 2011 and 2012 (Table 5.1). The male gametes were developed from crosses involving six donor parents (MEX 54, G10909, G2333, AND 1062, RWR 719 and BRB 191). In these crosses, MEX 54 and G10909 were used as sources of resistance to angular leaf spot resistance; G2333 to anthracnose resistance; AND 1062 and RWR 719 to *Pythium* root rot; and BRB 191 to bean common mosaic virus.

**Table 5.1:** The sixteen gamete populations derived from five-way multi-parent cross combinations and their respective codes.

| No. | Gamete Population | Population Code |
|---|---|---|
| 1 | GLP 585///G10909/G2333//AND 1062/BRB 191 | KGS12-01[a] |
| 2 | GLP 585///G10909/G2333//RWR 719/BRB 191 | KGS12-02 |
| 3 | GLP 585///MEX 54/G2333//AND 1062/BRB 191 | KGS12-03 |
| 4 | GLP 585///MEX 54/G2333//RWR 719/BRB 191 | KGS12-04 |
| 5 | GLP 92///G10909/G2333//AND 1062/BRB 191 | KGS12-05 |
| 6 | GLP 92///G10909/G2333//RWR 719/BRB 191 | KGS12-06 |
| 7 | GLP 92///MEX 54/G2333//AND 1062/BRB 191 | KGS12-07 |
| 8 | GLP 92///MEX 54/G2333//RWR 719/BRB 191 | KGS12-08 |
| 9 | KAT B1///G10909/G2333//AND 1062/BRB 191 | KGS12-09 |
| 10 | KAT B1///G10909/G2333//RWR 719/BRB 191 | KGS12-10 |
| 11 | KAT B1///MEX 54/G2333//AND 1062/BRB 191 | KGS12-11 |
| 12 | KAT B1///MEX 54/G2333//RWR 719/BRB 191 | KGS12-12 |
| 13 | KAT B9///G10909/G2333//AND 1062/BRB 191 | KGS12-13 |
| 14 | KAT B9///G10909/G2333//RWR 719/BRB 191 | KGS12-14 |
| 15 | KAT B9///MEX 54/G2333//AND 1062/BRB 191 | KGS12-15 |
| 16 | KAT B9///MEX 54/G2333//RWR 719/BRB 191 | KGS12-16 |

a; KGS12-01 codes for Kenya Gamete Selection, 2012, Meso Population 1 etc.

Parental lines were crossed in pairs to produce the $F_1$ single crosses. The $F_1$'s were then crossed to produce four double crosses gametes: MEX 54/G2333//AND 1062/BRB 191, G10909/G2333//RWR 719/BRB 191, MEX 54/G2333//RWR 719/BRB 191 and G10909/G2333//AND 1062/BRB 191. Each double cross combination was subsequently crossed to four susceptible commercial varieties (KAT B1, GLP 585, GLP 92 and KAT B9) as females to produce the final $F_1$. To identify plants with requisite combinations of resistance genes, male gamete plants grown in the greenhouse were screened with four markers: SAB-3 for anthracnose (Vallejo and Kelly, 2009), SH-13 for angular leaf spot (Queiroz *et al*., 2004), SW-13 for bean common mosaic virus (Melotto *et al*., 1996) and PYAA-19 for *Pythium* root rot (Mahuku *et al*., 2007). The markers were obtained from Inqaba Biotech East Africa Ltd., Nairobi, Kenya. Total genomic DNA was extracted from young trifoliate leaves collected from 2-week-old plants in the screen house. The collected leaves were spotted on the FTA plant saver cards following Whatman technologies. The PCR master mix consisted of 0.2 mM of dNTPs, 2 mM MgCl2, 1 µ/25 µl of Taq polymerase, 1× PCR Buffer and 0.4 µM of each primer. The 20µl PCR reaction volume was subjected to 40 amplification cycles in a Applied Biosystems thermal cycler consisting of 1 cycle 94 °C for 5 min, and 34 cycles including each the steps of denaturation at 94 °C for 30 s, annealing at 60-65 °C for 45 s, and extension at 72 °C for 30 seconds. These cycles were followed by a final extension for 20 min at 72 °C and a holding temperature of 15 °C. Amplification products were separated through electrophoresis migration in a 1.2% agarose gel covered by a 1× TBE buffer

under a voltage of 100 V for 1 hour. For the visualization, the gel containing Ethidium bromide (5µl/100ml) was illuminated with ultraviolet light and photographed for scoring (Mahuku *et al*., 2007). $F_1$ and $F_{1.2}$ progenies from each male gamete were evaluated for disease resistance and agronomic traits in field experiments at Kabete and Tigoni during long rain in 2012 and short rain season in 2013 at Kabete.

### 5.3.2 Field experimental site

The field evaluations were conducted at Kabete Field Station and Tigoni Research Station. Kabete Field Station lies at latitudes 1º 15' S and longitudes 36º 44' E within the agro- ecological zone LH3 at 1940m asl (Jaetzold *et al*., 2006). Kabete has bimodal rainfall of 1100 to 1200 mm annually, with a mean minimum temperature of 13.7 ºC and mean maximum temperature of 24.3 ºC (Appendix 1). Kabete Field station was selected as a hot spot for angular leaf spot while Tigoni is particularly highly favourable to anthracnose.

KARI-Tigoni station is located approximately 50 km North-West of Nairobi at an altitude of 2095m asl. The station is found at agro ecological zone LH1 and lies at latitudes 1º 09' S and longitudes 36º 41' E (Jaetzold *et al*., 2006). Tigoni has a bimodal rainfall of 1100 to 1200 mm annually, mean minimum temperature of 11.3 ºC and mean maximum temperature of 22.8 ºC (Appendix 2). The soils within Kabete and Tigoni are moderately acidic and are poor in available phosphorous and sufficient in other available nutrients. The soils are well drained, very deep, dark reddish brown to dark red friable clay soils of

the Nitisols type. The two sites experience short rains during the months of September-November and long rains in April-June.

### 5.3.3 Experimental design and trial management

The experiment was laid out as a split-plot design with three replications. The four male gamete plants used as source of pyramided resistance genes and the four susceptible commercial varieties determined the experimental factors. The male gamete was the main plot while commercial variety was the sub-plot. The commercial varieties used were allocated at random to the subplots within each main-plot. Each entry was sown in 3-meter row plots with a spacing of 10cm within rows and 50cm between rows. A plot consisted of four rows at Kabete and three rows at Tigoni. Planting was carried out at the onset of long rains and was largely rain-fed although supplemental irrigation was provided at Kabete when needed.

Di-ammonium phosphate (DAP) fertilizer was applied during planting at a rate of 2 g per plant. The plots were top dressed with 17:17:17 N.P.K., at the rate of 5 g per plant 3 weeks after emergence, and before flowering. Fertilizer was applied about 5 cm away from the base of the plants to prevent damaging the plant. After fertilizer application the plants were watered well to prevent damage to the roots. Pest control was carried out using Ogor 40EC (Osho Chemical Ltd., Nairobi) insecticide comprising 40% w/v dimethoate. The insecticide was applied at a rate of 30-40ml/20L at 1.5-2L/ha. Weeding was done 14 days after sowing, before flowering and at pod filling stage.

### 5.3.4 Data collection

Data was collected on stand at germination, growth habit, vigour, days to 50% flowering, disease severity for angular leaf spot, anthracnose, bean common mosaic virus and *Pythium* root rot, days to 50% maturity, stand at harvest, pod load, 100-seed mass and yield according to Schoonhoven and Pastor-Corrales (1987). Growth habit was recorded at the flowering stage (R6) to classify plants with a determinate growth habit (Type I), and at physiological maturity (R9) to classify; plants with indeterminate bush habit with erect stem and branches (Type II), plants with indeterminate bush habit with weak and prostrate stem and branches (Type III) and plants with indeterminate climbing habit with weak, long and twisted stem and branches (Type IV). Plant vigour was recorded at pre-flowering (R5) on a scale of 1-9 where; 1 = high vigour and 9 = poor vigour.

Days to flowering was measured in days-after-planting and coinciding with the initiation of flowering (R6) when 50% of the plants had one or more flowers. Disease severity scores were recorded according to the CIAT standard visual scale defined by Schoonhoven and Pastor-Corrales (1987) on a scale of 1-9 where; 1-3 = resistant, 4-6 = intermediate and 7-9 = susceptible. Days to maturity was measured in days-after-planting and coinciding with the initiation of developmental stage R9 when 50% of the plants had reached physiological maturity. The number of pods per plant and number of seeds per pod was counted from plants and pods respectively. The seed size was sampled from the weight of 100 seeds in grams from each plant.

### 5.3.5 Data analysis

Analysis of variance (ANOVA) was performed for a split-plot design using Genstat Release 14.2 (VSN International Ltd.) to determine means, variance, least significant differences at 5% level and coefficient of variation within and between the two sites. The most promising high yielding, diseases resistant families were identified by ranking means.

## 5.4 RESULTS

### 5.4.1 Overview of climatic conditions and performance of the trials

Trials in Kabete showed poor vigour and a high incidence of angular leaf spot and bean common mosaic virus. In Tigoni plants showed high vigour and high frequency of plants exhibiting growth habits III and IV. It was also noted that anthracnose was severe in Tigoni than in Kabete. The yields were also high in Tigoni compared to Kabete. Cooler temperatures and high humidity in Tigoni aided in the sporulation of anthracnose hence limiting the yield potential of the improved genotypes. Yield was however more in Tigoni compared to Kabete where an acute outbreak of fungal diseases infested the crop. Angular leaf spot was more severe in Kabete than in Tigoni as a result of the prevailing warm and humid environment.

### 5.4.2 Growth habit

The growth habit of plants was classified as; determinate growth habit (Type I), indeterminate bush habit with erect stem and branches (Type II), indeterminate bush habit with weak and prostrate stem and branches (Type III) and plants with indeterminate climbing habit with weak, long and twisted stem and branches (Type IV). Parental genotypes utilized to generate the $F_1$ populations possess different growth habits resulting in a wide segregation for growth type genes.

Among the donor parents MEX 54, G10909 and G2333 have growth type IV, whereas AND 1062, RWR 719 and BRB 191 have growth type I. The four recipient parents also show differences in their growth habits with KAT B1,

GLP 585 and KAT B9 have growth type I, while GLP 92 shows a growth type II. There was a high segregation for growth habit among the sixteen multiple-cross $F_1$ populations (Table 5.2). The number of plants with growth habit IV was higher in both Kabete (484) and Tigoni (467). Growth habit I recorded the lowest number of plants in Kabete (289) while growth habit III was the least in Tigoni (215) (Table 5.2).

Population KGS12-02 had the highest number of plants (27) showing growth habit I in Kabete (Table 5.2). KGS12-16 had the highest number of plants showing growth habit II (31) and IV (38) in Kabete, KGS12-04 showed the highest number of plants (32) showing growth habit III in Kabete. In Tigoni, KGS12-14 had the highest number of plants showing growth habit I (28). KGS12-07 had the highest number of plants showing growth habit II (29) in Tigoni. KGS12-09 showed the highest number of plants (25) showing growth habit III in Tigoni. While KGS12-02 had the highest number of plants (37) showing growth habit IV in Tigoni (Table 5.2).

**Table 5.2:** Number of plants with growth types I, II, III and IV of 16 $F_1$ populations evaluated in Kabete and Tigoni during the 2012 long rain season.

| | Growth Habit | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Kabete | | | | Tigoni | | | |
| Population | I | II | III | IV | I | II | III | IV |
| KGS12-01 | 16 | 14 | 23 | 22 | 23 | 18 | 9 | 26 |
| KGS12-02 | 27 | 25 | 26 | 37 | 22 | 13 | 9 | 37 |
| KGS12-03 | 17 | 19 | 19 | 28 | 15 | 15 | 12 | 30 |
| KGS12-04 | 16 | 24 | 32 | 33 | 19 | 19 | 13 | 32 |
| KGS12-05 | 8 | 8 | 14 | 10 | 14 | 12 | 9 | 25 |
| KGS12-06 | 19 | 21 | 25 | 32 | 17 | 12 | 15 | 27 |
| KGS12-07 | 15 | 14 | 19 | 25 | 14 | 29 | 15 | 26 |
| KGS12-08 | 20 | 20 | 27 | 35 | 19 | 18 | 9 | 30 |
| KGS12-09 | 17 | 15 | 15 | 32 | 19 | 22 | 25 | 21 |
| KGS12-10 | 18 | 23 | 25 | 29 | 12 | 14 | 14 | 43 |
| KGS12-11 | 20 | 20 | 18 | 25 | 23 | 25 | 15 | 28 |
| KGS12-12 | 18 | 23 | 26 | 37 | 15 | 17 | 10 | 26 |
| KGS12-13 | 21 | 20 | 20 | 34 | 16 | 21 | 11 | 27 |
| KGS12-14 | 18 | 19 | 17 | 31 | 28 | 17 | 16 | 32 |
| KGS12-15 | 18 | 22 | 27 | 36 | 15 | 28 | 21 | 30 |
| KGS12-16 | 24 | 31 | 30 | 38 | 22 | 19 | 16 | 27 |
| Total | 289 | 318 | 365 | 484 | 293 | 298 | 215 | 467 |

### 5.4.3 Plant vigour

There was significant location effect ($P< 0.05$) on plant vigour among the 16 populations (Appendix 10). There were also significant differences ($P< 0.05$) of male gamete and the commercial varieties used for growth vigour in the sixteen populations (Appendix 10). Two way interactions between location x male gamete, location x commercial variety and male gamete x commercial variety were significant ($P< 0.05$) for the sixteen populations (Appendix 10). The three-way interaction on location x male gamete x commercial variety was also significantly different among the sixteen gamete populations. The

range in growth vigour among the sixteen five-way crosses ranged from excellent (1) to very poor (9) in the $F_1$ plants.

Populations derived from KAT B9 had the highest average vigour (3.7) while those derived from G10909/G2333//RWR 719/BRB 191 male gamete had high vigour at 3.6 (Table 5.3). The plants belonging to KGS12-10 combination were most vigorous (3.2) whereas KGS12-01 had the lowest growth vigour (4.7) (Table 5.3). These genotypes showed high mean growth vigour in Kabete (3.8) and a poorer growth vigour in Tigoni (4.0) (Table 5.3). The frequency among $F_1$ populations indicated a downward trend in the number of plants with highest vigor (score of 1) to the least vigorous (score of 9) across locations (Fig. 5.1).

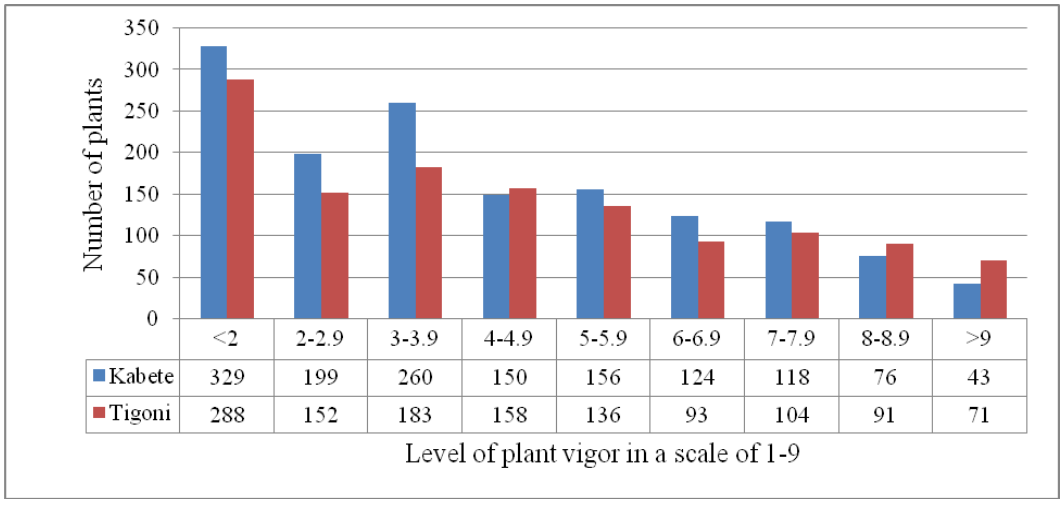



**Figure 5.1:** The frequency of plant vigor among $F_1$ plants evaluated at Kabete and Tigoni during the 2012 long rain season.

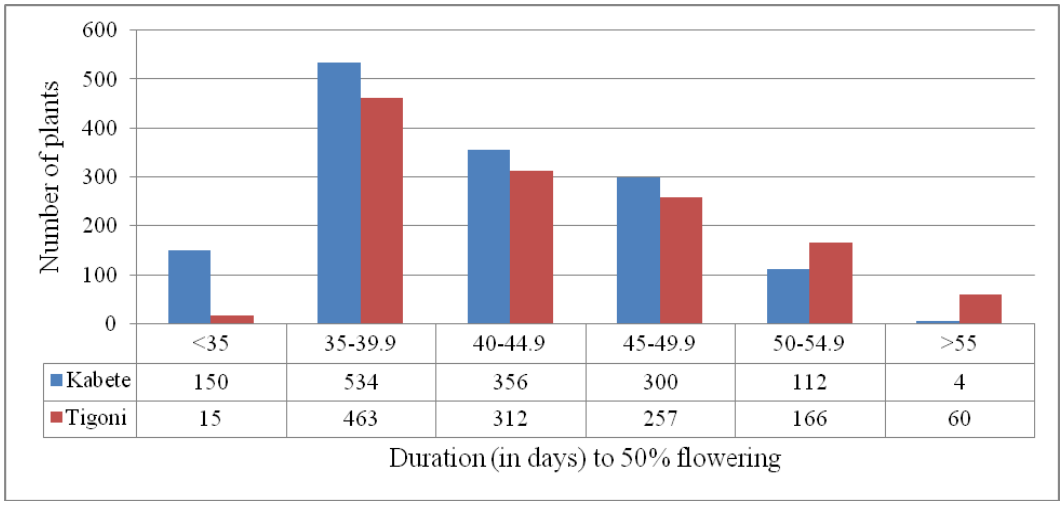
### 5.4.4 Days to 50% flowering

Significant effects ($P< 0.05$) were observed on location, male gamete and commercial variety for the days to 50% flowering among $F_1$ gamete populations. There were significant differences ($P< 0.05$) for the two way interactions between location x male gamete and, male gamete x commercial variety. There was however no significant effect among the populations for the location x commercial variety two way interaction (Appendix 10). The three way interaction between location x male gamete x commercial variety showed significant differences ($P< 0.05$) among the 16 $F_1$ populations.

There were more plants that flowered early (took less than 35 days) in Kabete (150) than Tigoni (15), while more plants had a late flowering (took more than 55 days) in Tigoni (60) compared to Kabete (4) (Fig. 5.2). The $F_1$ populations flowered earlier in Kabete (41.2 days) than in Tigoni (43.2 days) by about two days. The mean duration to 50% flowering was 41.3 in KGS12-05 (early flowering) and 43.8 days for KGS12-15 (late flowering) (Table 5.3). Among the $F_1$ plants evaluated in Kabete, the earliest to flower (30.0 days) was recorded from the cross KGS12-13 while the last to flower (56.0 days) was gamete population KGS12-10.

Populations evaluated in Tigoni had the earliest $F_1$ plants to flower (32.0 days) from the cross KGS12-12 while the last to flower (59.0 days) was an $F_1$ plant from KGS12-07. There was low frequency in number of plants that took less than 35 days to flower, with highest frequency occurring between 35-39.9 days, and frequency gradually reducing upto slightly above 55 days (Fig. 5.2).

Among the check varieties evaluated BRB 191 was the earliest to flower (31.3 days) and RWR 719 was the last to flower (53.4 days) across the two locations (Table 5.3).




| | <35 | 35-39.9 | 40-44.9 | 45-49.9 | 50-54.9 | >55 |
|---|---|---|---|---|---|---|
| Kabete | 150 | 534 | 356 | 300 | 112 | 4 |
| Tigoni | 15 | 463 | 312 | 257 | 166 | 60 |

**Figure 5.2:** Frequency of duration to 50% flowering in $F_1$ plants evaluated at Kabete and Tigoni during the 2012 long rain season.

**Table 5.3:** Vigour, days to 50% flowering and days to 50% maturity of $F_1$ bean populations grown at Kabete and Tigoni during the 2012 long rain season.

| | Vigour | | | Days to Flowering | | | Days to Maturity | | |
|---|---|---|---|---|---|---|---|---|---|
| $F_1$ Populations | Kabete | Tigoni | Mean | Kabete | Tigoni | Mean | Kabete | Tigoni | Mean |
| KGS12-01 | 3.2 | 4.3 | 3.8 | 40.8 | 43.5 | 42.1 | 83.0 | 84.4 | 83.7 |
| KGS12-02 | 3.5 | 3.9 | 3.7 | 41.2 | 42.7 | 42.0 | 79.2 | 84.1 | 81.6 |
| KGS12-03 | 3.6 | 4.1 | 3.9 | 40.1 | 42.9 | 41.5 | 81.3 | 83.1 | 82.2 |
| KGS12-04 | 4.4 | 4.2 | 4.3 | 41.2 | 42.5 | 41.9 | 81.4 | 80.3 | 80.9 |
| KGS12-05 | 3.3 | 3.9 | 3.6 | 39.9 | 42.6 | 41.3 | 79.0 | 79.3 | 79.1 |
| KGS12-06 | 3.6 | 3.5 | 3.6 | 40.8 | 43.9 | 42.4 | 80.7 | 85.1 | 82.9 |
| KGS12-07 | 3.7 | 4.8 | 4.3 | 41.6 | 43.0 | 42.3 | 81.5 | 83.7 | 82.6 |
| KGS12-08 | 4.1 | 3.6 | 3.9 | 42.1 | 43.5 | 42.8 | 82.9 | 86.0 | 84.4 |
| KGS12-09 | 3.9 | 4.6 | 4.3 | 40.7 | 42.6 | 41.7 | 84.3 | 85.9 | 85.1 |
| KGS12-10 | 3.6 | 3.9 | 3.8 | 41.4 | 43.8 | 42.6 | 82.3 | 85.2 | 83.7 |
| KGS12-11 | 3.5 | 4.6 | 4.1 | 40.5 | 42.3 | 41.4 | 78.9 | 82.6 | 80.8 |
| KGS12-12 | 4.1 | 3.8 | 4.0 | 41.0 | 42.8 | 41.9 | 79.3 | 80.8 | 80.0 |
| KGS12-13 | 4.2 | 4.0 | 4.1 | 40.5 | 43.0 | 41.8 | 81.5 | 75.4 | 78.4 |
| KGS12-14 | 3.7 | 3.1 | 3.4 | 40.9 | 42.1 | 41.5 | 79.7 | 80.6 | 80.1 |
| KGS12-15 | 3.6 | 4.3 | 4.0 | 41.7 | 46.0 | 43.8 | 83.6 | 81.9 | 82.8 |
| KGS12-16 | 3.8 | 3.1 | 3.5 | 41.6 | 41.4 | 41.5 | 82.7 | 80.6 | 81.6 |
| **Checks** | | | | | | | | | |
| MEX 54 | 3.8 | 4.2 | 4.0 | 42.5 | 45.5 | 44 | 82.2 | 87.7 | 84.9 |
| G10909 | 3.7 | 4.8 | 4.3 | 43.0 | 38.8 | 40.9 | 77.5 | 79.3 | 78.4 |
| G2333 | 2.7 | 2.9 | 2.8 | 47.3 | 49.8 | 48.5 | 84.9 | 89.8 | 87.4 |
| AND 1062 | 1.5 | 4.7 | 3.1 | 34.3 | 37.7 | 36.0 | 69.0 | 73.3 | 71.2 |
| RWR 719 | 4.2 | 3.8 | 4.0 | 52.2 | 54.7 | 53.4 | 92.2 | 95.8 | 94.0 |
| BRB 191 | 1.0 | 2.2 | 1.6 | 31.8 | 30.7 | 31.3 | 64.9 | 66.0 | 65.5 |
| KAT B1 | 4.3 | 4.4 | 4.3 | 36.9 | 40.0 | 38.5 | 63.6 | 66.8 | 65.2 |
| KAT B9 | 4.3 | 4.2 | 4.2 | 38.3 | 41.8 | 40 | 66.2 | 68.6 | 67.4 |
| GLP 585 | 6.1 | 3.7 | 4.9 | 45.6 | 49.1 | 47.3 | 73.6 | 77.9 | 75.8 |
| GLP 92 | 6.4 | 4.2 | 5.3 | 43.3 | 45.2 | 44.2 | 81.0 | 83.9 | 82.5 |

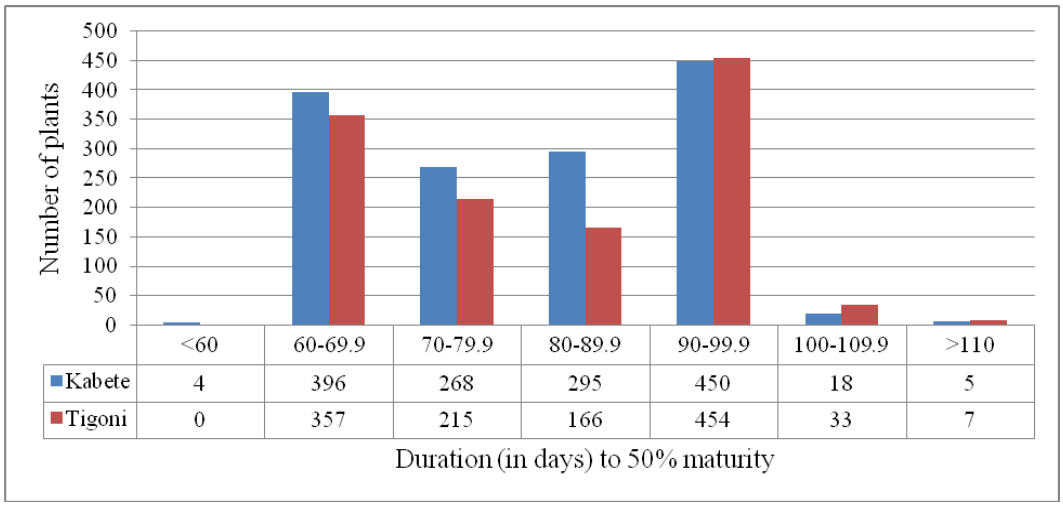
### 5.4.5 Days to 50% maturity

Significant differences ($P< 0.05$) were detected on location and the two way interaction between male gamete x commercial variety among the gamete populations (Appendix 10). However, no significant effects were observed on male gamete and commercial variety, or between location x male gamete, and location x commercial variety. The three way interaction among location x male gamete x commercial variety showed no significant difference for the sixteen gamete populations (Appendix 10).

In Kabete maturity had a range of 56 to 113 days and a mean of 80.8, whereas the range in Tigoni was between 63 to 111 days with an average maturity of 86.7 days (Table 5.3). The populations in Kabete were observed to mature much earlier than in Tigoni with 78.7 and 81.4 days respectively (Table 5.3). Genotypes derived from KGS12-13 matured early at 78.4 days while KGS12-09 had a late maturity rate of 85.1 days (Table 5.3).

Among the $F_1$ plants evaluated in Kabete, the earliest to mature (59.0 days) was recorded from the cross KGS12-09 while the last to flower (115.0 days) was a genotype from the cross KGS12-13. Genotypes evaluated in Tigoni showed the earliest $F_1$ plant to mature (62.0 days) belonged to the cross KGS12-09 while the last to flower (113.0 days) was an $F_1$ plant from the cross KGS12-03. There were high frequencies with number of plants that reached maturity in 90-99.9 days, followed by those that matured in 60-69.9 days. Plant that had an early maturity of >60 days, and those that had late maturity recorded low frequencies in both Kabete and Tigoni (Fig. 5.3).

Among the check varieties evaluated KAT B1 was the earliest to mature (65.2 days) while RWR 719 had a late maturity (94.0 days) across the two locations (Table 5.3).




| | <60 | 60-69.9 | 70-79.9 | 80-89.9 | 90-99.9 | 100-109.9 | >110 |
|---|---|---|---|---|---|---|---|
| Kabete | 4 | 396 | 268 | 295 | 450 | 18 | 5 |
| Tigoni | 0 | 357 | 215 | 166 | 454 | 33 | 7 |

**Figure 5.3:** Frequency of duration to 50% maturity in $F_1$ plants evaluated at Kabete and Tigoni during the 2012 long rain season.

### 5.4.6 Disease evaluation

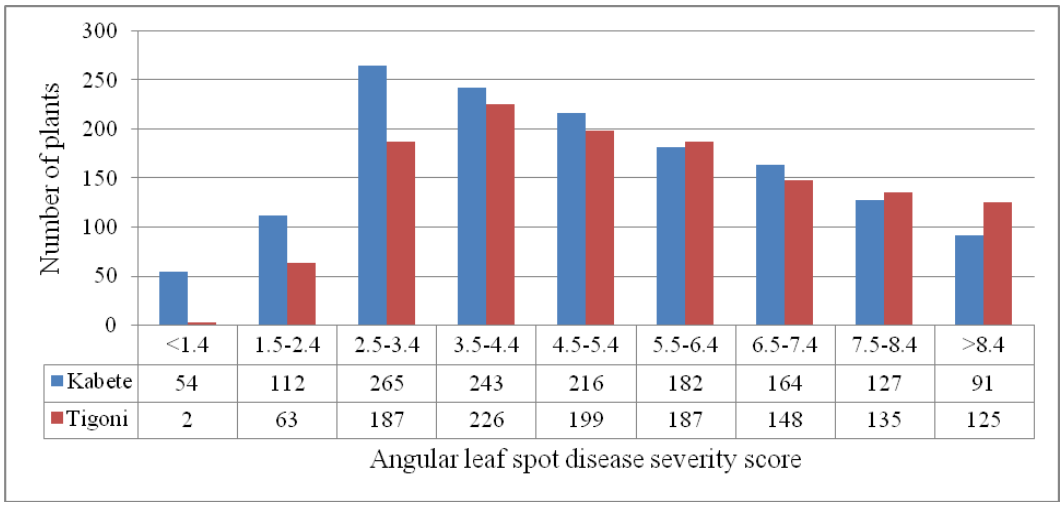
Significant differences ($P< 0.05$) were detected among the gamete populations for angular leaf spot, anthracnose, root rot and bean common mosaic virus on location, male gamete and commercial variety used (Appendix 11). There were also significant differences ($P< 0.05$) detected for the two way interactions between location x male gamete, location x commercial variety and male gamete x commercial variety for angular leaf spot, anthracnose, root rot, and bean common mosaic virus (Appendix 11).

The three way interaction among location x male gamete x commercial variety was significant for all the four disease constraints (Appendix 11). There was on average high disease severity for angular leaf spot in Kabete than Tigoni with means of 5.1 and 5.5 respectively (Table 5.4). Similarly, there was on average high disease severity for anthracnose in Kabete than in Tigoni with means of 5.7 and 6.4 respectively (Table 5.4). The results on average disease severity for bean common mosaic virus were similar in both Kabete and Tigoni with a mean of 5.1 in the two locations (Table 5.7). The average disease severity for root rot was higher in Kabete than was in Tigoni with a means of 2.9 and 1.6 respectively (Table 5.7). The mean value for angular leaf spot across locations indicated KGS12-09 having the lowest disease severity score with a mean of 4.6, whereas KGS12-11 had the highest disease severity score with a mean of 5.7 (Table 5.4).

Genotypes derived from KGS12-11 had the lowest disease severity score for anthracnose with a mean of 5.0, whereas KGS12-12 had the highest anthracnose disease severity score with a mean of 6.4 (Table 5.4). The mean value for bean common mosaic virus across locations indicated KGS12-05 having the lowest disease severity score with a mean of 4.2, whereas KGS12-16 had the highest disease severity score with a mean of 5.8 (Table 5.7). KGS12-07 recorded the lowest root rot disease severity score with a mean of 1.5, while KGS12-13 had the highest root rot disease severity score with a mean of 2.9 (Table 5.7).

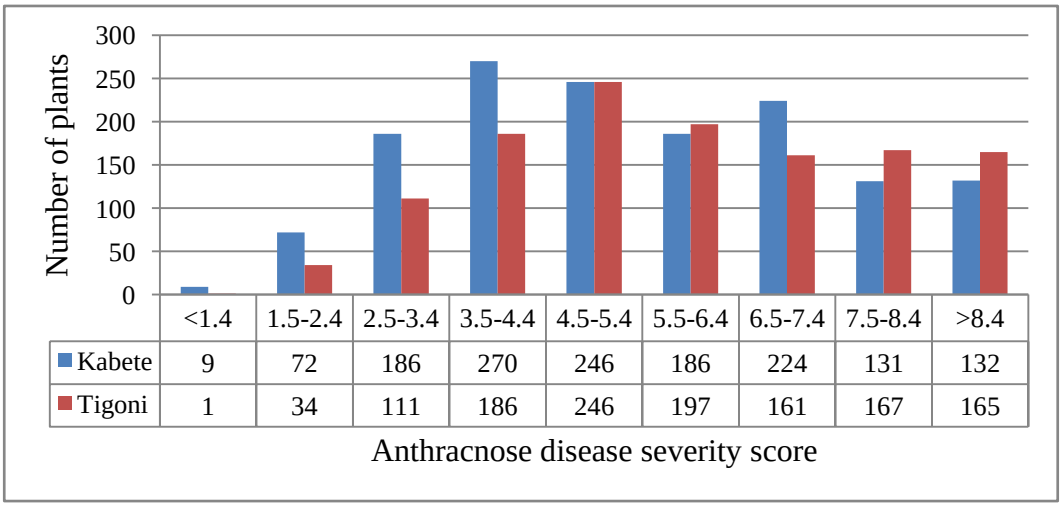
The number of $F_1$ plants that showed resitance (<3.5) to angular leaf spot had a lower frequency when compared to plants with moderate resistance (3.5-6.4)

and the susceptible (>6.4) plants (Fig. 5.4). Similarly, the number of $F_1$ plants with moderate resitance to anthracnose had the highest frequency followed by the susceptible plants, and the resitance plants having the least frequency (Fig. 5.5).




| | <1.4 | 1.5-2.4 | 2.5-3.4 | 3.5-4.4 | 4.5-5.4 | 5.5-6.4 | 6.5-7.4 | 7.5-8.4 | >8.4 |
|---|---|---|---|---|---|---|---|---|---|
| Kabete | 54 | 112 | 265 | 243 | 216 | 182 | 164 | 127 | 91 |
| Tigoni | 2 | 63 | 187 | 226 | 199 | 187 | 148 | 135 | 125 |

**Figure 5.4:** Frequency of angular leaf spot disease severity score in $F_1$ plants evaluated at Kabete and Tigoni during the 2012 long rain season.




| | <1.4 | 1.5-2.4 | 2.5-3.4 | 3.5-4.4 | 4.5-5.4 | 5.5-6.4 | 6.5-7.4 | 7.5-8.4 | >8.4 |
|---|---|---|---|---|---|---|---|---|---|
| Kabete | 9 | 72 | 186 | 270 | 246 | 186 | 224 | 131 | 132 |
| Tigoni | 1 | 34 | 111 | 186 | 246 | 197 | 161 | 167 | 165 |

**Figure 5.5:** Frequency of duration to 50% flowering in $F_1$ plants evaluated at Kabete and Tigoni during the 2012 long rain season.

**Table 5.4:** Disease severity scores of segregating $F_1$ plants grown at Kabete and Tigoni during the 2012 long rain season.

| | Angular leaf spot | | | Anthracnose | | |
|---|---|---|---|---|---|---|
| Populations | Kabete | Tigoni | Mean | Kabete | Tigoni | Mean |
| KGS12-01 | 4.2 | 5.3 | 4.8 | 4.8 | 5.4 | 5.1 |
| KGS12-02 | 4.8 | 5.3 | 5.1 | 5.2 | 6.5 | 5.9 |
| KGS12-03 | 4.6 | 5.9 | 5.2 | 4.8 | 5.2 | 5.0 |
| KGS12-04 | 5.8 | 5.1 | 5.5 | 5.8 | 5.8 | 5.8 |
| KGS12-05 | 4.9 | 5.2 | 5.1 | 6.6 | 5.2 | 5.9 |
| KGS12-06 | 5.3 | 5.5 | 5.4 | 4.4 | 6.3 | 5.3 |
| KGS12-07 | 4.8 | 5.2 | 5.0 | 5.7 | 5.7 | 5.7 |
| KGS12-08 | 4.6 | 5.3 | 4.9 | 5.4 | 5.6 | 5.5 |
| KGS12-09 | 3.8 | 5.3 | 4.6 | 6.2 | 6.4 | 6.3 |
| KGS12-10 | 4.7 | 5.8 | 5.2 | 5.7 | 6.0 | 5.9 |
| KGS12-11 | 5.2 | 6.2 | 5.7 | 5.9 | 5.9 | 5.9 |
| KGS12-12 | 4.8 | 5.3 | 5.1 | 5.8 | 7.0 | 6.4 |
| KGS12-13 | 4.8 | 6.0 | 5.4 | 5.2 | 6.2 | 5.7 |
| KGS12-14 | 5.5 | 5.5 | 5.5 | 5.7 | 5.5 | 5.6 |
| KGS12-15 | 5.7 | 4.9 | 5.3 | 5.5 | 5.9 | 5.7 |
| KGS12-16 | 4.6 | 5.4 | 5.0 | 5.1 | 6.4 | 5.7 |
| **Checks** | | | | | | |
| MEX 54 | 2.1 | 1.6 | 1.8 | 4.6 | 8.2 | 6.4 |
| G10909 | 2.6 | 3.4 | 3.0 | 6.3 | 8.8 | 7.6 |
| G2333 | 3.9 | 3.3 | 3.6 | 1.6 | 2.1 | 1.8 |
| AND 1062 | 7.0 | 6.6 | 6.8 | 6.7 | 7.4 | 7.0 |
| RWR 719 | 7.2 | 7.2 | 7.2 | 6.3 | 7.0 | 6.7 |
| BRB 191 | 5.8 | 5.9 | 5.9 | 5.4 | 6.2 | 5.8 |
| KAT B1 | 6.1 | 6.0 | 6.0 | 6.7 | 7.6 | 7.2 |
| KAT B9 | 5.9 | 6.7 | 6.3 | 6.7 | 7.6 | 7.2 |
| GLP 585 | 6.8 | 6.8 | 6.8 | 7.7 | 7.5 | 7.6 |
| GLP 92 | 7.9 | 7.5 | 7.7 | 8.4 | 7.9 | 8.1 |

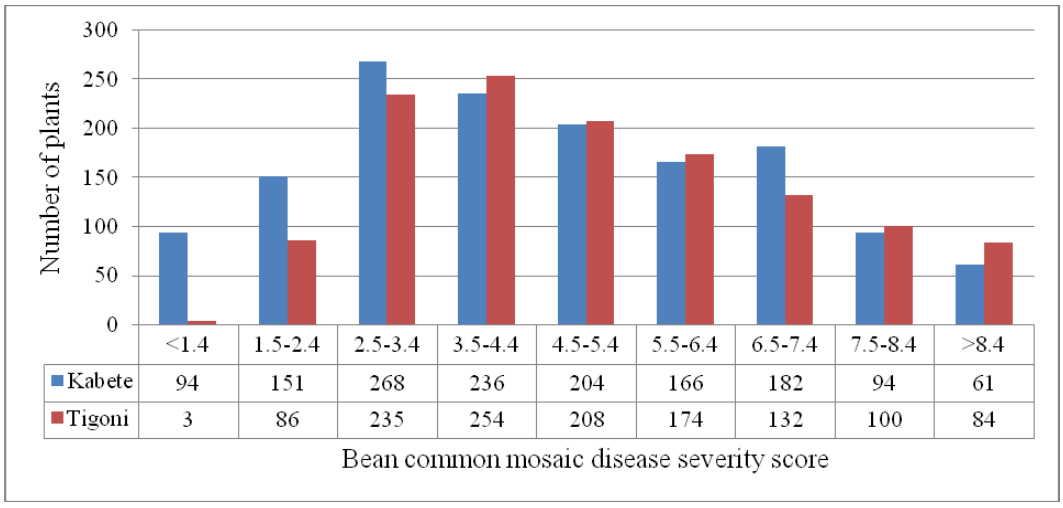
**Table 5.5:** Frequency distribution of resistant and susceptible $F_1$ plants for angular leaf spot and anthracnose in 16 bean populations evaluated at Kabete and Tigoni during the 2012 long rain season.

| | Angular leaf spot | | | Anthracnose | | |
|---|---|---|---|---|---|---|
| | Frequency (%) | | Mean disease | Frequency (%) | | Mean disease |
| Populations | R | S | rating | R | S | rating |
| KGS12-01 | 33.1 | 66.9 | 4.8 | 22.2 | 77.8 | 5.1 |
| KGS12-02 | 25.1 | 74.9 | 5.1 | 15.8 | 84.2 | 5.9 |
| KGS12-03 | 30.1 | 69.9 | 5.2 | 25.2 | 74.8 | 5.0 |
| KGS12-04 | 17.6 | 82.4 | 5.5 | 13.3 | 86.7 | 5.8 |
| KGS12-05 | 20.1 | 79.9 | 5.1 | 11.9 | 88.1 | 5.9 |
| KGS12-06 | 19.7 | 80.3 | 5.4 | 21.8 | 78.2 | 5.3 |
| KGS12-07 | 23.9 | 76.1 | 5.0 | 16.8 | 83.2 | 5.7 |
| KGS12-08 | 26.9 | 73.1 | 4.9 | 14.3 | 85.7 | 5.5 |
| KGS12-09 | 36.2 | 63.8 | 4.6 | 7.4 | 92.6 | 6.3 |
| KGS12-10 | 29.2 | 70.8 | 5.2 | 11.7 | 88.3 | 5.9 |
| KGS12-11 | 20.0 | 80.0 | 5.7 | 11.7 | 88.3 | 5.9 |
| KGS12-12 | 27.8 | 72.2 | 5.1 | 8.8 | 91.2 | 6.4 |
| KGS12-13 | 24.3 | 75.7 | 5.4 | 12.3 | 87.7 | 5.7 |
| KGS12-14 | 21.8 | 78.2 | 5.5 | 13.8 | 86.2 | 5.6 |
| KGS12-15 | 22.7 | 77.3 | 5.3 | 17.4 | 82.6 | 5.7 |
| KGS12-16 | 27.7 | 72.3 | 5.0 | 18.2 | 81.8 | 5.7 |

R = resistant, S = susceptible

Among the check varieties evaluated, the donor parent MEX 54 had the highest level of resistance to angular leaf spot with a mean score of 1.8 while GLP 92 had a score of 8.1 indicating high susceptibility to the disease across the two locations (Table 5.4). All the recipient parents showed high levels of susceptibility to angular leaf spot reaction with a score > 6 both in Kabete and Tigoni (Table 5.4). G2333 showed strong resistance to anthracnose with a score of 1.8 whereas GLP 92 was the most susceptible to anthracnose with a

score of 8.1, recipient parents were highly susceptible to anthracnose each with a score > 7 (Table 5.7). The large red-mottled genotype, BRB 191 showed high resistance to bean common mosaic virus with an average score of 3.0 whereas AND 1062, which is a donor parent for root rot resistance, recorded highest susceptibility to bean common mosaic virus with a score of 6.7 (Table 5.4). The recipient parents had a mean range of 5.5-6.2 on their reaction to bean common mosaic virus. Two donor parents for root rot, RWR 719 and AND 1062 were strongly resistant to the root rot diseases indicating a mean disease score of 1.0 across the two locations (Table 5.4).




| | <1.4 | 1.5-2.4 | 2.5-3.4 | 3.5-4.4 | 4.5-5.4 | 5.5-6.4 | 6.5-7.4 | 7.5-8.4 | >8.4 |
|---|---|---|---|---|---|---|---|---|---|
| Kabete | 94 | 151 | 268 | 236 | 204 | 166 | 182 | 94 | 61 |
| Tigoni | 3 | 86 | 235 | 254 | 208 | 174 | 132 | 100 | 84 |

**Figure 5.6:** Frequency of bean common mosaic disease severity score in $F_1$ plants evaluated at Kabete and Tigoni during the 2012 long rain season.

**Table 5.6:** Frequency distribution of $F_1$ plants resistant or susceptible to bean common mosaic virus and root rots in 16 bean populations at Kabete and Tigoni during the 2012 long rain season.

| | Bean common mosaic virus | | | Root rot | | |
|---|---|---|---|---|---|---|
| | Frequency (%) | | Mean disease | Frequency (%) | | Mean disease |
| Population | R | S | rating | R | S | rating |
| KGS12-01 | 33.3 | 66.7 | 4.8 | 80.7 | 19.3 | 2.2 |
| KGS12-02 | 28.2 | 71.8 | 5.1 | 76.6 | 23.4 | 2.4 |
| KGS12-03 | 17.4 | 82.6 | 5.2 | 82.4 | 17.6 | 2.2 |
| KGS12-04 | 24.6 | 75.4 | 5.1 | 82.2 | 17.8 | 2.0 |
| KGS12-05 | 42.7 | 57.3 | 4.2 | 80.1 | 19.9 | 2.4 |
| KGS12-06 | 29.8 | 70.2 | 4.9 | 70.3 | 29.7 | 2.7 |
| KGS12-07 | 34.3 | 65.7 | 4.6 | 94.8 | 5.2 | 1.5 |
| KGS12-08 | 25.1 | 74.9 | 4.9 | 68.6 | 31.4 | 2.8 |
| KGS12-09 | 37.5 | 62.5 | 4.6 | 69.9 | 30.1 | 2.7 |
| KGS12-10 | 34.9 | 65.1 | 4.5 | 70.8 | 29.2 | 2.7 |
| KGS12-11 | 28.4 | 71.6 | 4.9 | 79.0 | 21.0 | 2.4 |
| KGS12-12 | 32.0 | 68.0 | 4.9 | 73.9 | 26.1 | 2.6 |
| KGS12-13 | 38.5 | 61.5 | 4.3 | 65.5 | 34.5 | 2.9 |
| KGS12-14 | 34.1 | 65.9 | 4.5 | 79.5 | 20.5 | 2.4 |
| KGS12-15 | 26.6 | 73.4 | 5.1 | 78.7 | 21.3 | 2.7 |
| KGS12-16 | 25.1 | 74.9 | 5.8 | 70.0 | 30.0 | 2.6 |

R – Resistant, S - Susceptible

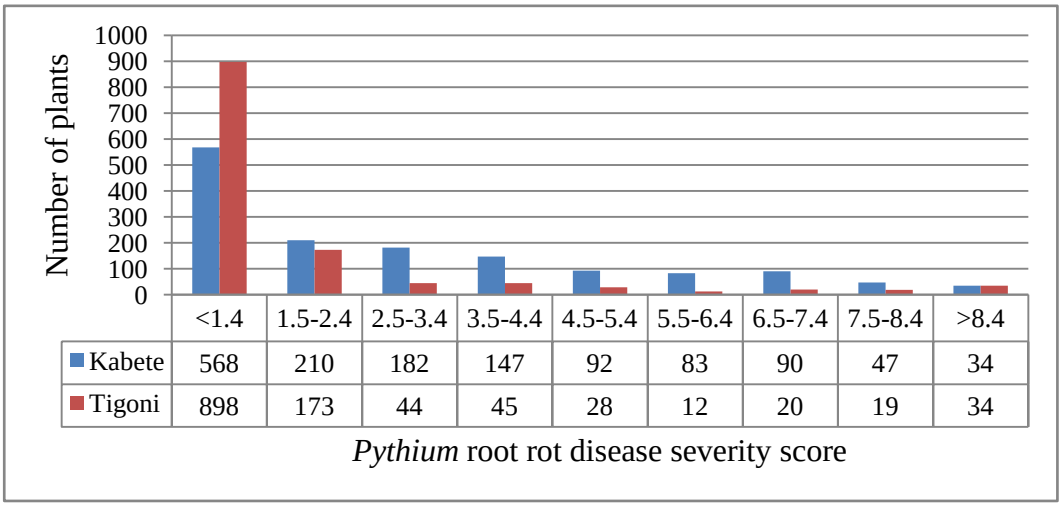



**Figure 5.7:** Frequency of *Pythium* root rot disease severity score in $F_1$ plants evaluated at Kabete and Tigoni during the 2012 long rain season.

**Table 5.7:** Mean disease severity scores for bean common mosaic virus and root rots of $F_1$ bean plants at Kabete and Tigoni during the 2012 long rain season.

| | Bean common mosaic virus | | | Root rot | | |
|---|---|---|---|---|---|---|
| Populations | Kabete | Tigoni | Mean | Kabete | Tigoni | Mean |
| KGS12-01 | 4.5 | 5.1 | 4.8 | 2.3 | 2.2 | 2.2 |
| KGS12-02 | 4.5 | 5.7 | 5.1 | 3.1 | 1.6 | 2.4 |
| KGS12-03 | 5.2 | 5.1 | 5.2 | 2.7 | 1.6 | 2.2 |
| KGS12-04 | 5.1 | 5.1 | 5.1 | 2.7 | 1.3 | 2.0 |
| KGS12-05 | 3.7 | 4.7 | 4.2 | 2.6 | 2.2 | 2.4 |
| KGS12-06 | 4.5 | 5.2 | 4.9 | 3.6 | 1.9 | 2.7 |
| KGS12-07 | 4.5 | 4.7 | 4.6 | 1.8 | 1.1 | 1.5 |
| KGS12-08 | 4.9 | 4.9 | 4.9 | 3.8 | 1.8 | 2.8 |
| KGS12-09 | 4.4 | 4.7 | 4.6 | 4.2 | 1.1 | 2.7 |
| KGS12-10 | 4.7 | 4.4 | 4.5 | 2.9 | 2.6 | 2.7 |
| KGS12-11 | 4.5 | 5.4 | 4.9 | 2.8 | 1.9 | 2.4 |
| KGS12-12 | 4.6 | 5.2 | 4.9 | 3.4 | 1.7 | 2.6 |
| KGS12-13 | 4.2 | 4.5 | 4.3 | 3.3 | 2.4 | 2.9 |
| KGS12-14 | 3.9 | 5.2 | 4.5 | 2.4 | 2.3 | 2.4 |
| KGS12-15 | 5.4 | 4.8 | 5.1 | 2.4 | 3.0 | 2.7 |
| KGS12-16 | 4.8 | 6.8 | 5.8 | 3.6 | 1.7 | 2.6 |
| **Checks** | | | | | | |
| MEX 54 | 5.9 | 6.6 | 6.2 | 3.1 | 1.2 | 2.1 |
| G10909 | 6.2 | 4.6 | 5.4 | 2.1 | 1.5 | 1.8 |
| G2333 | 5.6 | 6.0 | 5.8 | 2.4 | 1.5 | 2.0 |
| AND 1062 | 7.1 | 6.3 | 6.7 | 1.2 | 1.0 | 1.1 |
| RWR 719 | 5.6 | 4.3 | 5.0 | 1.0 | 1.0 | 1.0 |
| BRB 191 | 2.9 | 3.1 | 3.0 | 1.5 | 1.3 | 1.4 |
| KAT B1 | 6.3 | 5.2 | 5.8 | 3.8 | 1.3 | 2.6 |
| KAT B9 | 6.0 | 5.0 | 5.5 | 3.8 | 1.1 | 2.5 |
| GLP 585 | 6.5 | 5.1 | 5.8 | 4.5 | 1.3 | 2.9 |
| GLP 92 | 6.5 | 5.9 | 6.2 | 4.3 | 1.1 | 2.7 |

Results of population KGS12-09 had the highest percent frequency (36.2%) of $F_1$ plants resistant to angular leaf spot while KGS12-04 had the lowest percent frequency (17.6%) of $F_1$ plants resistant to angular leaf spot, in both Kabete and Tigoni (Table 5.5). The cross KGS12-03 showed the highest percent frequency (25.2%) of $F_1$ plants resistant to anthracnose whereas cross KGS12-09 had the lowest percent frequency (7.4%) of $F_1$ plants resistant to anthracnose, in both Kabete and Tigoni (Table 5.5). Genotypes derived from the cross KGS12-05 had the highest percent frequency (42.7%) of $F_1$ plants resistant to bean common mosaic virus while KGS12-03 had the lowest percent frequency (17.4%) of $F_1$ plants resistant to bean common mosaic virus, in both Kabete and Tigoni (Table 5.6).

The cross KGS12-07 showed the highest percent frequency (94.8%) of $F_1$ plants resistant to root rot whereas cross KGS12-13 had the lowest percent frequency (65.5%) of $F_1$ plants resistant to root rot, across the two locations (Table 5.6). Across the two locations, there was a low frequency in the number of $F_1$ plants that expressed resistance to bean common mosaic virus and the highest frequency being recorded by plants with moderate resistance followed by plants showed susceptibility (Fig. 5.6). The frequency in *Pythium* root rot disease severity was highly skewed indicating a large number of plants with a score of less than 2 across Kabete and Tigoni (Fig. 5.7). This may have been indicative of low disease incidence rather than plant resistance to *Pythium* root rot.

### 5.4.7 Pod plant$^{-1}$

There was significant effect ($P< 0.05$) on location and commercial variety for pods plant$^{-1}$ among the $F_1$ populations (Appendix 12). There was however no significant difference due to male gamete. The two way interaction between location x male gamete, location x commercial variety and male gamete x commercial variety were also significant ($P< 0.05$) for all populations (Appendix 12). The three way interaction among location x male gamete x commercial variety was also significantly different ($P< 0.05$). Gamete populations derived from KGS12-04 had a low pod count of 25.0 pods plant$^{-1}$ while KGS12-12 had a high pod count of 30.7 pods plant$^{-1}$ (Table 5.8). Mean pods plant$^{-1}$ at Kabete was higher than at Tigoni. Among the $F_1$ plants evaluated in Kabete, the plant with highest number of pods plant$^{-1}$ (77.0 pods) was recorded from the cross KGS12-01 while the lowest of pods per plant (1 pod plant$^{-1}$) was from the cross KGS12-12 (Table 5.8). Populations evaluated in Tigoni showed the highest number of pods plant$^{-1}$ (69.0 pods) belonged to the cross KGS12-12 whereas the lowest number of pods plant$^{-1}$ (3.0 pods) was an $F_1$ plant from the cross KGS12-08 (Table 5.8). The frequency in the number of pods plant$^{-1}$ indicated that the highest number of plants had about 20-29.9 pods plant$^{-1}$, with more than two-thirds of all $F_1$ plants evaluated in Kabete and Tigoni having a general range of between 10-39.9 pods plant$^{-1}$ (Fig. 5.8). Among the check varieties evaluated KAT B1 had the lowest mean number of pods plant$^{-1}$ (10.5 pods) while G2333 had the highest mean number of pods plant$^{-1}$ (33.7 pods) across the two locations (Table 5.8).

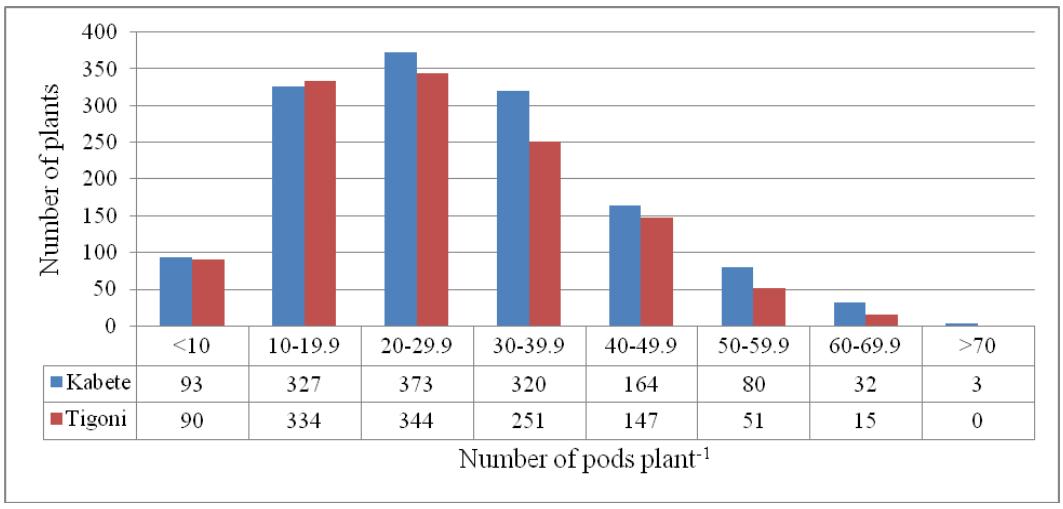


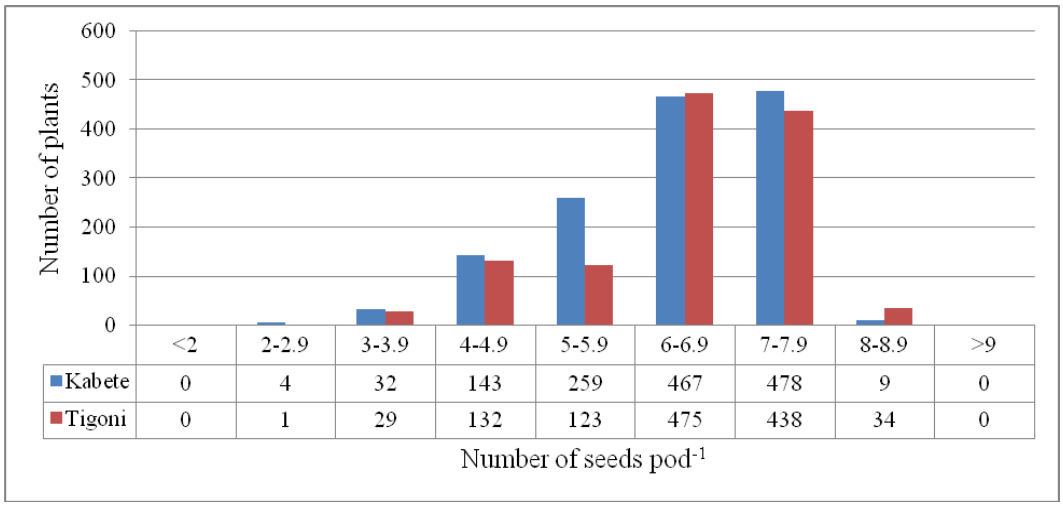

**Figure 5.8:** Frequency of pods plant$^{-1}$ in $F_1$ plants evaluated at Kabete and Tigoni during the 2012 long rain season.

### 5.4.8 Seeds pod$^{-1}$

There were significant genotypic effects ($P< 0.05$) recorded on location, male gamete and commercial variety for the seeds pod$^{-1}$ among the populations (Appendix 12). The two way interactions between location x male gamete, location x commercial variety and male gamete x commercial variety were also significantly different in the sixteen gamete populations (Appendix 12). Significant differences were also recorded for the three way interaction among location x male gamete x commercial variety for the seeds pod$^{-1}$.

The range of seeds per pod in the $F_1$ plants among the sixteen populations ranged from a high of 8.0 seeds pod$^{-1}$ and a low of 2.0 seeds pod$^{-1}$. The average seeds pod$^{-1}$ among the sixteen multiple cross combinations had a mean range of 6.2 to 6.5 seeds pod$^{-1}$ (Table 5.8). The frequency in the number of seeds pod$^{-1}$ indicated the highest number of pods had between 6-7.9 seeds, and a very low frequency in number of pods with seeds above or below that

range (Fig. 5.9). Among the check varieties evaluated G10909 and G2333 had the highest number of seeds pod$^{-1}$ (7.3) while KAT B1 had the lowest number of seeds pod$^{-1}$ (5.0) across the two locations (Table 5.8).




| | <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 | 4 | 32 | 143 | 259 | 467 | 478 | 9 | 0 |
| Tigoni | 0 | 1 | 29 | 132 | 123 | 475 | 438 | 34 | 0 |

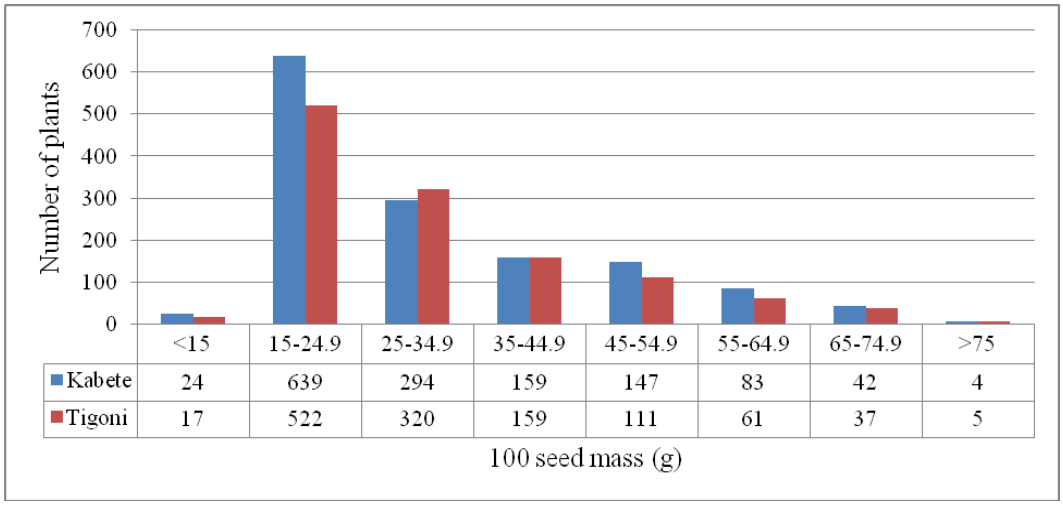
**Figure 5.9:** Frequency of seeds pod$^{-1}$ in $F_1$ plants evaluated at Kabete and Tigoni during the 2012 long rain season.

### 5.4.9 100-seed mass

Results on 100-seed mass showed significant differences ($P< 0.05$) on location, male gamete and commercial variety among the sixteen gamete populations (Appendix 12). There were also significant effects ($P< 0.05$) in the two way interactions between location x male gamete, location x commercial variety and male gamete x commercial variety for the 100-seed mass (Appendix 12). The three way interaction on location x male gamete x commercial variety also showed significant effects ($P< 0.05$) for all the populations. Among the $F_1$ plants evaluated in Kabete, the least number of Mesoamerican genotypes (48.0) was recorded from the cross KGS12-09 while

the highest number of Mesoamerican genotypes (81.0) was observed from the five-way cross KGS12-02 (Table 5.10). Within the genotypes evaluated in Kabete the least number of Andean genotypes (13.0) was recorded from the cross KGS12-04 while the highest number of Andean genotypes (34.0) was observed from the five-way cross KGS12-05 (Table 5.10).

Genotypes evaluated in Tigoni showed the least number of Mesoamerican $F_1$ plants to be 46.0 and belonged to the cross KGS12-09–whereas the highest number of Mesoamerican genotypes was 74.0 $F_1$ plants from the cross KGS12-06 (Table 5.10). The five-way cross KGS12-09 had the highest number of Andean genotypes (26.0) with a low number of Andean genotypes (11.0) being recorded in both KGS12-03 and KGS12-09 cross combinations (Table 5.10). The number of plants with a 100-seed mass of between 15-24.9g had the highest frequency that decreased gradually giving the least frequency in plants with a 100-seed mass at greater than 75g (Fig. 5.10).




| | <15 | 15-24.9 | 25-34.9 | 35-44.9 | 45-54.9 | 55-64.9 | 65-74.9 | >75 |
|---|---|---|---|---|---|---|---|---|
| Kabete | 24 | 639 | 294 | 159 | 147 | 83 | 42 | 4 |
| Tigoni | 17 | 522 | 320 | 159 | 111 | 61 | 37 | 5 |

**Figure 5.10:** Frequency of 100-seed mass in $F_1$ plants evaluated at Kabete and Tigoni during the 2012 long rain season.

**Table 5.8:** Pods plant$^{-1}$ and seeds pod$^{-1}$ of $F_1$ plants in 16 bean populations grown at Kabete and Tigoni during the 2012 long rain season.

| | Pods plant$^{-1}$ | | | Seeds pod$^{-1}$ | | |
|---|---|---|---|---|---|---|
| $F_1$ Populations | Kabete | Tigoni | Mean | Kabete | Tigoni | Mean |
| KGS12-01 | 25.2 | 26.7 | 25.9 | 6.2 | 6.4 | 6.3 |
| KGS12-02 | 28.5 | 29.5 | 29.0 | 6.2 | 6.4 | 6.3 |
| KGS12-03 | 29.1 | 28.4 | 28.8 | 6.3 | 6.5 | 6.4 |
| KGS12-04 | 26.3 | 23.7 | 25.0 | 6.5 | 6.4 | 6.5 |
| KGS12-05 | 30.4 | 27.4 | 28.9 | 6.4 | 6.5 | 6.4 |
| KGS12-06 | 28.4 | 29.0 | 28.7 | 6.2 | 6.4 | 6.3 |
| KGS12-07 | 31.5 | 26.7 | 29.1 | 6.0 | 6.4 | 6.2 |
| KGS12-08 | 27.4 | 25.6 | 26.5 | 6.4 | 6.4 | 6.4 |
| KGS12-09 | 33.5 | 26.6 | 30.1 | 6.1 | 6.6 | 6.3 |
| KGS12-10 | 26.2 | 25.7 | 26.0 | 6.4 | 6.3 | 6.3 |
| KGS12-11 | 24.9 | 26.0 | 25.4 | 6.4 | 6.3 | 6.4 |
| KGS12-12 | 30.0 | 31.3 | 30.7 | 6.2 | 6.3 | 6.3 |
| KGS12-13 | 28.0 | 27.1 | 27.6 | 6.3 | 6.5 | 6.4 |
| KGS12-14 | 28.4 | 28.5 | 28.5 | 6.4 | 6.5 | 6.5 |
| KGS12-15 | 29.3 | 22.9 | 26.1 | 6.3 | 6.7 | 6.5 |
| KGS12-16 | 29.6 | 24.8 | 27.2 | 6.2 | 6.5 | 6.3 |
| **Checks** | | | | | | |
| MEX 54 | 26.8 | 27.3 | 27.1 | 6.5 | 6.3 | 6.4 |
| G10909 | 33.2 | 33.0 | 33.1 | 7.4 | 7.2 | 7.3 |
| G2333 | 34.3 | 33.0 | 33.7 | 7.4 | 7.2 | 7.3 |
| AND 1062 | 13.5 | 12.7 | 13.1 | 5.7 | 5.8 | 5.8 |
| RWR 719 | 18.8 | 20.7 | 19.8 | 6.9 | 6.6 | 6.7 |
| BRB 191 | 12.1 | 13.7 | 12.9 | 5.7 | 6.0 | 5.9 |
| KAT B1 | 10.7 | 10.3 | 10.5 | 4.8 | 5.2 | 5.0 |
| KAT B9 | 12.0 | 13.3 | 12.6 | 4.9 | 4.8 | 4.9 |
| GLP 585 | 23.6 | 23.8 | 23.7 | 6.4 | 6.0 | 6.2 |
| GLP 92 | 27.7 | 27.3 | 27.5 | 6.3 | 6.2 | 6.2 |

**Table 5.9:** Variation for 100-seed mass and grain yield among 16 $F_1$ bean populations at Kabete and Tigoni during the 2012 long rain.

| | 100-seed mass | | | $F_1$ Yield (kg $ha^{-1}$) | | |
|---|---|---|---|---|---|---|
| $F_1$ Populations | Kabete | Tigoni | Mean | Kabete | Tigoni | Mean |
| KGS12-01 | 30.7 | 31.6 | 31.1 | 2640.0 | 3930.0 | 3285.0 |
| KGS12-02 | 30.5 | 29.5 | 30.0 | 4250.0 | 5084.0 | 4667.0 |
| KGS12-03 | 33.0 | 28.4 | 30.7 | 3109.0 | 4073.0 | 3591.0 |
| KGS12-04 | 28.7 | 32.2 | 30.5 | 4123.0 | 4654.0 | 4388.5 |
| KGS12-05 | 36.2 | 29.8 | 33.0 | 1493.0 | 3487.0 | 2490.0 |
| KGS12-06 | 29.5 | 28.8 | 29.1 | 3460.0 | 4347.0 | 3903.5 |
| KGS12-07 | 31.6 | 34.6 | 33.1 | 2388.0 | 4350.0 | 3369.0 |
| KGS12-08 | 30.3 | 32.7 | 31.5 | 3507.0 | 4405.0 | 3956.0 |
| KGS12-09 | 34.1 | 32.7 | 33.4 | 2970.0 | 4151.0 | 3560.5 |
| KGS12-10 | 32.8 | 32.8 | 32.8 | 3401.0 | 4218.0 | 3809.5 |
| KGS12-11 | 30.8 | 37.4 | 34.1 | 2635.0 | 3501.0 | 3068.0 |
| KGS12-12 | 29.2 | 34.3 | 31.8 | 3626.0 | 5228.0 | 4427.0 |
| KGS12-13 | 31.2 | 30.7 | 31.0 | 3331.0 | 4490.0 | 3910.5 |
| KGS12-14 | 33.4 | 33.0 | 33.2 | 2760.0 | 5448.0 | 4104.0 |
| KGS12-15 | 33.8 | 32.8 | 33.3 | 4179.0 | 5150.0 | 4664.5 |
| KGS12-16 | 31.5 | 28.5 | 30.0 | 4804.0 | 4720.0 | 4762.0 |
| **Checks** | | | | | | |
| MEX 54 | 36.2 | 36.9 | 36.5 | 683.0 | 2325.0 | 1504.0 |
| G10909 | 32.6 | 37.5 | 35.1 | 702.0 | 2453.0 | 1577.0 |
| G2333 | 29.7 | 28.5 | 29.1 | 881.0 | 2492.0 | 1686.0 |
| AND 1062 | 50.4 | 53.3 | 51.9 | 683.0 | 1575.0 | 1129.0 |
| RWR 719 | 20.7 | 20.4 | 20.6 | 518.0 | 1542.0 | 1030.0 |
| BRB 191 | 62.4 | 67.0 | 64.7 | 1056.0 | 2102.0 | 1579.0 |
| KAT B1 | 44.2 | 49.0 | 46.6 | 346.0 | 978.0 | 662.0 |
| KAT B9 | 46.1 | 50.8 | 48.5 | 371.0 | 1316.0 | 844.0 |
| GLP 585 | 27.0 | 27.0 | 27.0 | 356.0 | 1215.0 | 785.0 |
| GLP 92 | 30.1 | 32.9 | 31.5 | 377.0 | 919.0 | 648.0 |

**Table 5.10:** Frequency of small, medium and large seeded $F_1$ plants for 100-seed mass in the 16 bean populations evaluated at Kabete and Tigoni during the 2012 long rain season.

| | 100-seed mass (g) | | | | | |
|---|---|---|---|---|---|---|
| | Kabete | | | Tigoni | | |
| | Mesoamerican | | Andean | Mesoamerican | | Andean |
| $F_1$ Population | Small | Medium | Large | Small | Medium | Large |
| KGS12-01 | 53 | 26 | 25 | 35 | 23 | 18 |
| KGS12-02 | 51 | 30 | 21 | 42 | 21 | 13 |
| KGS12-03 | 43 | 25 | 25 | 40 | 19 | 11 |
| KGS12-04 | 42 | 17 | 13 | 32 | 25 | 17 |
| KGS12-05 | 42 | 16 | 34 | 40 | 21 | 14 |
| KGS12-06 | 47 | 16 | 16 | 49 | 25 | 13 |
| KGS12-07 | 38 | 26 | 19 | 28 | 28 | 22 |
| KGS12-08 | 47 | 14 | 22 | 33 | 26 | 17 |
| KGS12-09 | 25 | 23 | 19 | 19 | 27 | 11 |
| KGS12-10 | 41 | 29 | 23 | 34 | 35 | 18 |
| KGS12-11 | 43 | 14 | 17 | 23 | 27 | 26 |
| KGS12-12 | 52 | 23 | 16 | 31 | 26 | 25 |
| KGS12-13 | 50 | 22 | 24 | 43 | 25 | 16 |
| KGS12-14 | 41 | 20 | 24 | 35 | 22 | 23 |
| KGS12-15 | 33 | 21 | 24 | 30 | 32 | 16 |
| KGS12-16 | 47 | 28 | 23 | 43 | 20 | 12 |

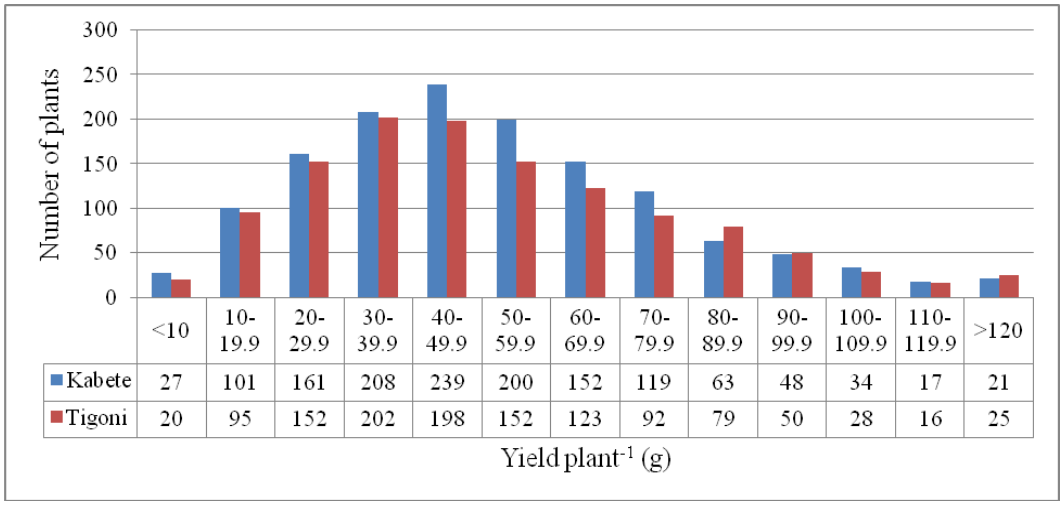



**Figure 5.11:** Frequency of yield plant$^{-1}$ in $F_1$ plants evaluated at Kabete and Tigoni during the 2012 long rain season.

### 5.4.10 Yield evaluation in $F_1$ and $F_{1.2}$ generations

Significant effects ($P< 0.05$) on location, male gamete and commercial variety were observed for yield in all populations (Appendix 12). The two way interactions between location x male gamete and male gamete x commercial variety were also significant ($P< 0.05$) for yield in all populations (Appendix 12). There was however no interaction between location and the commercial variety in the gamete populations. A three way interaction among location x male gamete x commercial variety showed significant effects ($P< 0.05$) for yield in all populations.

The mean yield in Kabete (2256 kg $ha^{-1}$) was lower compared to the mean yield recorded in Tigoni (3391 kg $ha^{-1}$) (Table 5.9; Fig. 5.12). Population KGS12-16 recorded the highest yield (4762 kg $ha^{-1}$) while KGS12-05 had the lowest grain yield (2490 kg $ha^{-1}$) among all the sixteen gamete populations (Table 5.9). Among the $F_1$ plants evaluated in Kabete, the highest yield (4804 kg $ha^{-1}$) was recorded from the cross KGS12-16 while the lowest yield (1493 kg $ha^{-1}$) was a genotype from the cross KGS12-05 (Table 5.9). Populations evaluated in Tigoni showed the highest yield of 5448 kg $ha^{-1}$ which was recorded from KGS12-16 while the lowest yield (3487 kg $ha^{-1}$) was observed from the cross KGS12-05 (Table 5.9).

There was a normal distribution for yield $plant^{-1}$ among $F_1$ plants evaluated in Kabete and Tigoni, and a high frequency of $F_1$ plants with a yield $plant^{-1}$ of between 40-49.9 g was observed across the two locations (Fig. 5.11). Among the check varieties evaluated G2333 had the highest yield (1686 kg $ha^{-1}$) while

GLP 92 had the lowest yield (648 kg $ha^{-1}$) across the two locations. The yield was higher at Tigoni than in Kabete, but there was more variability in Kabete (Fig. 5.12). A high heterosis was expressed in $F_1$ populations across Kabete and Tigoni in comparison to the check varieties that showed very low yields (Table 5.9; Fig. 5.13).

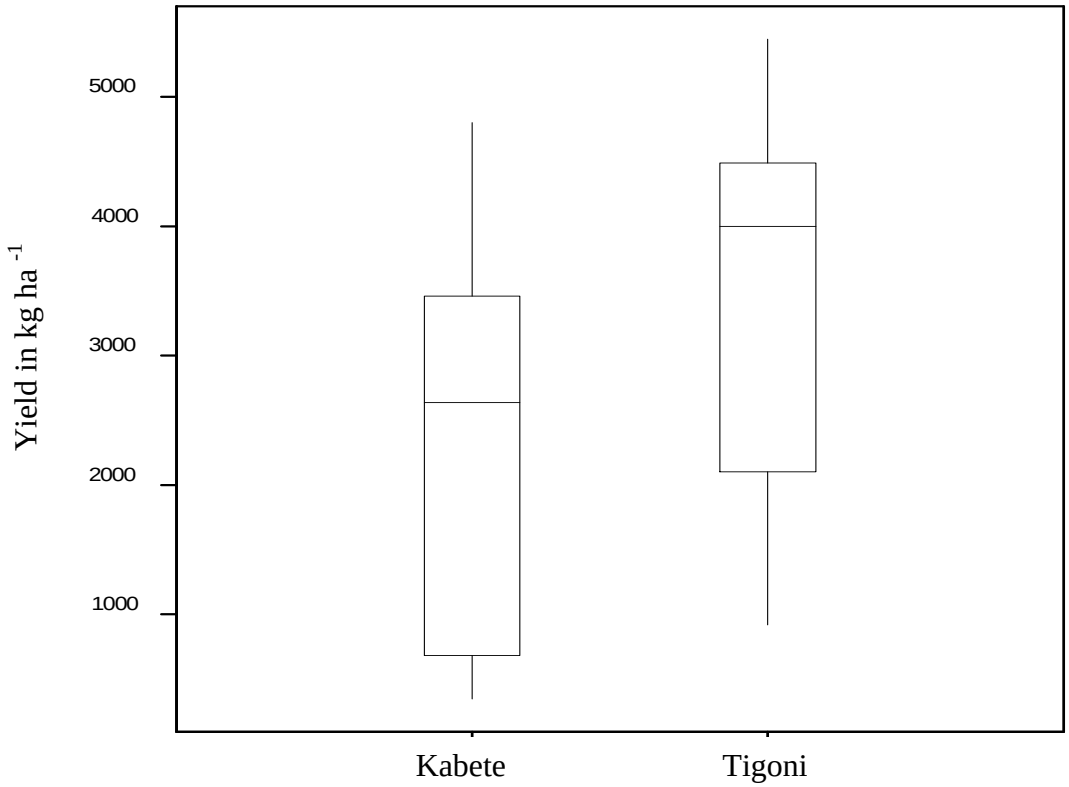



**Figure 5.12:** A box plot of yield in kg $ha^{-1}$ for 16 bean populations and ten check varieties evaluated in Kabete and Tigoni during 2012 long rain season.

The yield in $F_{1.2}$ generation was evaluated for all the sixteen populations and the results showed significant effects ($P< 0.05$) on male gamete and commercial variety for the $F_{1.2}$ yield in all populations (Appendix 13). The

two way interactions between male gamete x commercial variety were also significant ($P< 0.05$) for $F_{1.2}$ yield in all populations (Appendix 13). Population KGS12-16 recorded the highest yield (3388.0 kg $ha^{-1}$) while KGS12-05 had the lowest grain yield (1059.0 kg $ha^{-1}$) among all the sixteen gamete populations (Table 5.11). The $F_{1.2}$ yield results supported the evaluations conducted for yield in $F_1$ but were however much lower than the $F_1$ yields (Table 5.11). This indicated a considerable decrease in heterosis among the $F_{1.2}$ populations, from a mean yield of 3872.3 kg $ha^{-1}$ in $F_1$ populations to a mean yield of 2255.6 kg $ha^{-1}$ in $F_{1.2}$ populations (Table 5.9; Fig. 5.13).

**Table 5.11:** Grain yield among 16 $F_1$ bean populations at Kabete and Tigoni during the 2012 long rain and $F_{1.2}$ populations at Kabete during 2012/2013 short rain seasons.

| $F_1$ Populations | $F_1$ Yield (kg $ha^{-1}$) | $F_{1.2}$ Yield (kg $ha^{-1}$) |
|---|---|---|
| KGS12-01 | 3285.0 | 1856.0 |
| KGS12-02 | 4667.0 | 3064.0 |
| KGS12-03 | 3591.0 | 1939.0 |
| KGS12-04 | 4388.5 | 2592.0 |
| KGS12-05 | 2490.0 | 1059.0 |
| KGS12-06 | 3903.5 | 2022.0 |
| KGS12-07 | 3369.0 | 1780.0 |
| KGS12-08 | 3956.0 | 2381.0 |
| KGS12-09 | 3560.5 | 1829.0 |
| KGS12-10 | 3809.5 | 2200.0 |
| KGS12-11 | 3068.0 | 1447.0 |
| KGS12-12 | 4427.0 | 2783.0 |
| KGS12-13 | 3910.5 | 2238.0 |
| KGS12-14 | 4104.0 | 2524.0 |
| KGS12-15 | 4664.5 | 2988.0 |
| KGS12-16 | 4762.0 | 3388.0 |

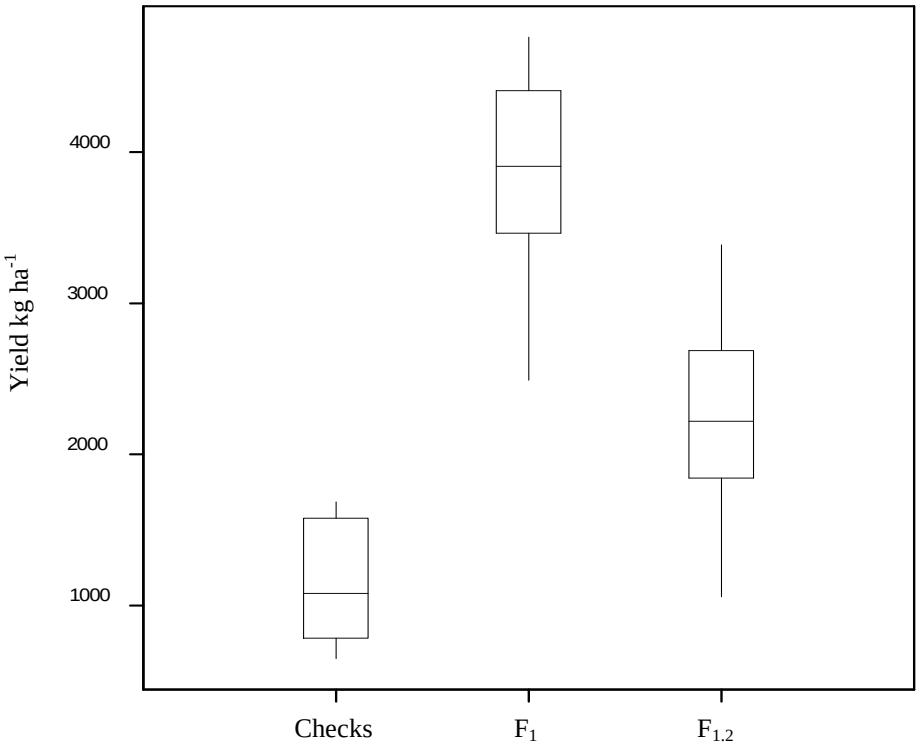



**Figure 5.13:** A box plot of yield for check varieties and $F_1$ populations at Kabete and Tigoni during the 2012 long rain, and $F_{1.2}$ populations at Kabete during 2012/2013 short rain seasons.

### 5.4.11 Correlation between yield and yield components

According to results from Appendices 14-16 both vigour and days to 50% flowering had a negative correlation (r=-0.1) to yield in Kabete and no correlation in Tigoni and across the two locations. Disease severity in angular leaf spot, anthracnose and bean common mosaic virus showed significantly negative correlations (r=-0.2**) to yield in Kabete and Tigoni (Appendices 14-16; Fig. 5.14). The significant negative correlations between disease severity and yield indicate that all these parameters are important measures of disease

resistance as an indicator of yield. Days to 50 % maturity showed a positive correlation of r=0.3** in both Kabete and Tigoni, with a positive correlation of r=0.4** across locations (Appendices 14-16). There was a significantly positive (r=0.5**) correlation between pods plant$^{-1}$ and yield in Kabete and Tigoni, and a significantly positive (r=0.4**) correlation across the locations (Appendices 14-16; Fig. 5.15). The correlation between seeds pod$^{-1}$ and yield were significantly positive at r=0.4** and r=0.3** in Tigoni and across locations respectively, and a positive correlation of r=0.1 in Kabete (Appendices 14-16). As shown in Appendices 14-16 and Figure 5.16, the correlation between 100-seed mass and yield was significantly negative at r=-0.3**, r=-0.4** and r=-0.3** between seeds pod$^{-1}$ and yield in Kabete, Tigoni and across locations respectively. Days to 50 % maturity and number of pods plant$^{-1}$ were the only parameters consistently correlated to grain yield across sites.

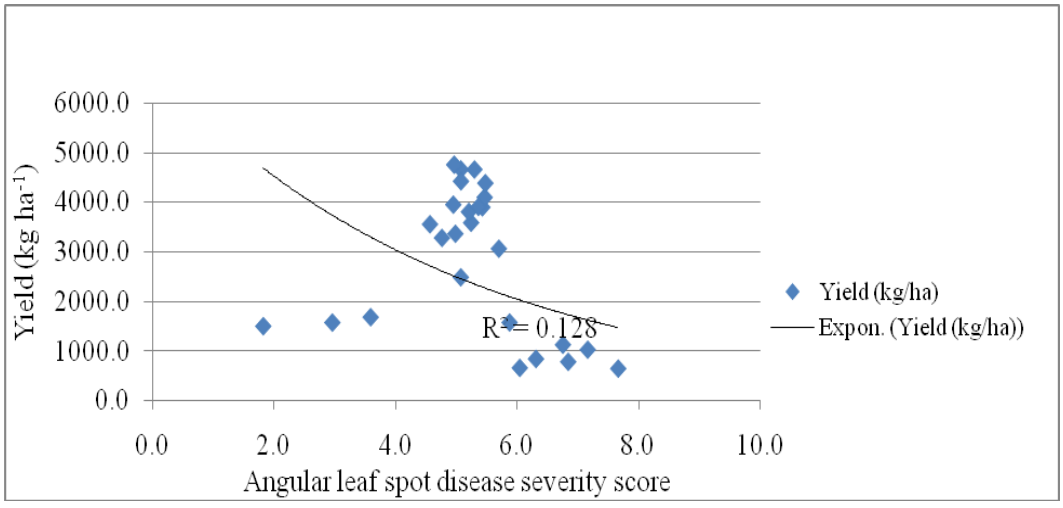



**Figure 5.14:** Correlation between angular leaf spot severity and grain yield at Kabete and Tigoni.

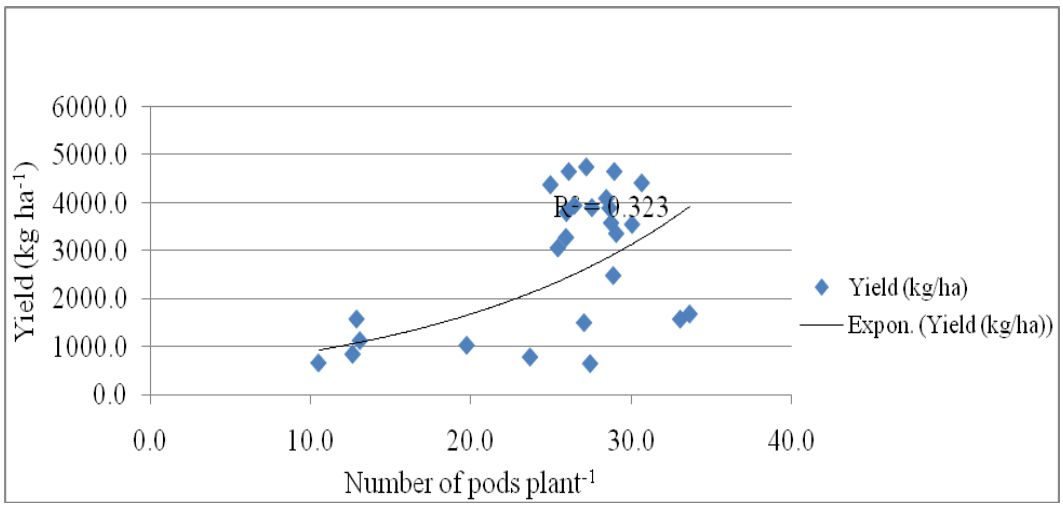



**Figure 5.15:** Correlation between pods plant$^{-1}$ and grain yield at Kabete and Tigoni.

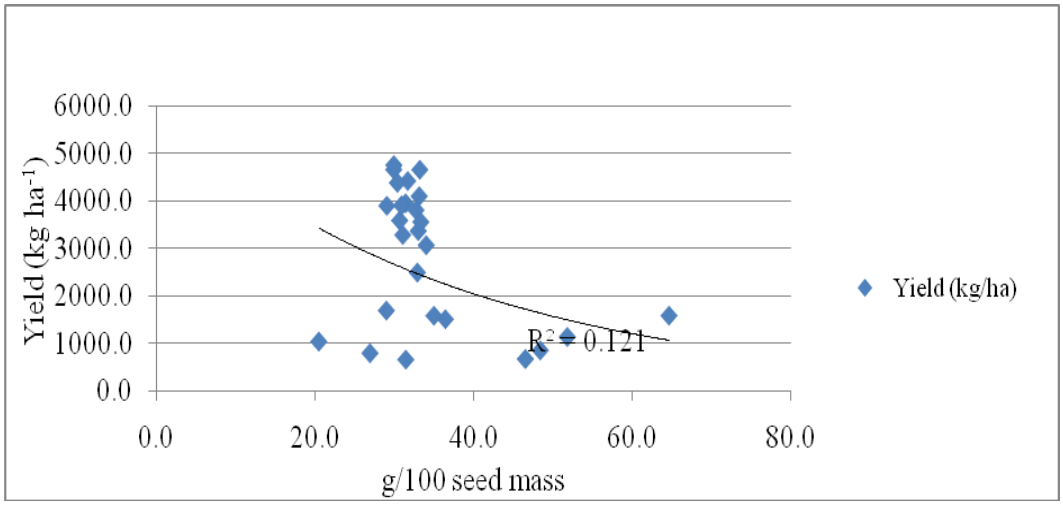



**Figure 5.16:** Correlation between 100-seed mass and grain yield at Kabete and Tigoni.

## 5.5 DISCUSSION

This study revealed that field evaluations on introgressed genotypes provided a selection platform to multiple disease resistance and other qualitative traits desirable in a commercial cultivar. These results clearly support the power and effectiveness of pyramiding and introgression of multiple disease resistance genes for improving angular leaf spot, anthracnose, bean common mosaic virus and root rot resistance and other agronomic traits in common bean. The effectiveness of marker-assisted gamete selection in physiological resistance to white mold (Terán and Singh, 2009), simultaneous resistance to anthracnose, angular leaf spot and rust (Ragagnin *et al*., 2009), improvement of qualitative (resistance to bean common mosaic virus and rust) and quantitative traits (such as seed yield) (Singh *et al*., 2008), development of resistant dry bean germplasm to halo blight and common bacterial blight (Asensio-S.-Manzanera *et al*., 2005, 2006) and resistance to multiple diseases in carioca bean (Singh *et al*., 1998, 2000), have previously been reported. There was a wide variation in the reactions of bean genotypes to the four disease pathogens evaluated. Plants exhibited varied reactions to different diseases with individual plants expressing high resistance to some diseases while others were completely susceptible to some or all the diseases. This indicates a wide segregation of resistance genes introgressed from donor parents. Similar segregations for both common and halo bacterial blights were observed within the $F_1$-derived families by Asensio-S.-Manzanera *et al*., (2006). The improved genotypes also expressed high genotypic variation for

grain yield which ranged from 2490 to 4762 kg $ha^{-1}$. Fifteen of the 16 populations produced yields above 3000 kg $ha^{-1}$ which was above the grand mean of 2824 kg $ha^{-1}$ and exceeded the parental checks by 38.9% whose yield ranged from 648 to 1686 kg $ha^{-1}$. These findings are similar with those of Ragagnin *et al*. (2009) who observed genetic variability for grain yield and other qualitative traits among the 40 multiple parent families' analyzed. These results indicated that, sums of squares due to genotypes, environment and G x E interactions were highly significant (Appendices 9-11). These suggest that broad range of diversity exists among genotypes and among environments and that genotypes performed differently over the environments. This is mainly due to differences in the genes for resistance successfully introgressed, the segregation of these genes and the variation in angular leaf spot, anthracnose, bean common mosaic virus and root rot pathotypes infecting beans. Appearance of significant differences for the diseases severity among the populations shows that variability for resistance existed among the genotypes for angular leaf spot, anthracnose, bean common mosaic virus and root rot. This indicates a possibility of obtaining genotypes with genes for resistance from their donor parents and confirms presence of genes for resistance in the donor parents used in this study as reported by Miklas *et al*. (2003).

# CHAPTER 6 CONCLUSIONS AND RECOMMENDATIONS

## 6.1 CONCLUSIONS

### 6.1.1 Prevalence of common bean diseases in Kenya

Common bean productivity is severely constrained by diseases and prevalence and severity vary considerably from one location to another and from season to season. Fungal diseases are major constraints to bean production throughout the world (Jesus Junior *et al*., 2001). The relative importance of different fungal diseases varies among regions due to differences in soil, climate, crop management practices and degree of susceptibility of cultivars used by bean producers (Mmbaga *et al*., 1996; Boland *et al*., 2004). The degree of virulence among isolates of some fungal pathogens can also vary between regions and over time. This has been observed for many bean pathogens such as angular leaf spot caused by *Pseudocercospora griseola* (Pastor-Corrales *et al*., 1998; Mahuku *et al*., 2002b). In eastern Africa the bean diseases are widespread and reduce yields considerably (Wortmann *et al*., 1998). The major diseases include angular leaf spot, anthracnose, common bacterial blight, root rots and bean common mosaic virus (Wortmann *et al*., 1998). A survey was carried out during flowering and physiological maturity in all the major bean growing areas in Kenya. Plant materials with disease symptoms were collected including angular leaf spots (leaves and pods), anthracnose (pods), root rots (roots and soil), bean common mosaic virus (leaves) and common bacterial blight (seeds and leaves). The survey covered twelve agro-ecological zones (AEZs) that included Upper Highland (UH2), Lower Highland (LH1, LH2,

LH3 and LH4), Upper Midland (UM1, UM2, UM3, UM4 and UM5) and Lower Midland (LM1 and LM2). Angular leaf spot is the most important biotic constraint in eastern and southern Africa (Wortmann *et al*., 1998); and was reported in all major bean growing regions in Kenya where it is favoured by moist, warm conditions with abundant inocula supply. Anthracnose was also relatively widespread but more prevalent at higher altitudes than at lower altitudes. Bean common mosaic virus, root rots and common bacterial blight were also reported in the locations where the survey was conducted. This was in line with previous studies which show that bean diseases are widespread in the region resulting to high yield losses (Wortmann *et al*., 1998).

### 6.1.2 Isolation of bean disease pathogens

All the symptomatic samples collected from the bean growing regions were used to isolate the disease pathogens for further studies. Angular leaf spot pathogen was isolated by picking conidia from well developed and sporulating lesions using a tiny piece of agar placed on the tip of a sterilized dissecting needle, and streaked onto tap water agar (TWA – 15g Agar powder and 1000ml double distilled water) and then incubated in darkness for 24h at 21 °C. The individual germinated conidia were then transplanted onto V8 medium (200ml V-8 juice, 3g $CaCo_3$, 18g Bacto agar and 800ml $ddH_2O$) to obtain monosporic cultures for each *P. griseola* isolate. Isolates were maintained on V8 juice agar and kept in a dark incubator at 21 °C for up to 21 days to promote sporulation. Fifty seven isolates of *P. griseola* were successfully isolated, characterised and preserved.

Anthracnose was isolated using small pieces of necrotic tissue that were surface sterilized by immersing them in 0.5% NaOCl for 3 minutes. After which they were rinsed in sterile distilled water, blotted dry on sterile filter paper, and placed on to potato dextrose agar (PDA). The cultures were incubated for 14 days in darkness at 20-21 °C. Monosporic isolates were characterized using a set of 12 host differential genotypes (A. S. Musyimi, unpubl. data) and were maintained on fungus-colonized filter papers at -20 °C for long term storage.

Thirty two isolates of bean common mosaic virus were prepared by making sap extracts from severely infected young leaves collected from farmers' fields. The supernatant was then sieved to eliminate plant debris. Individual isolate was maintained by inoculation on healthy seedlings of the susceptible variety G10909 and infected leaves collected and stored in the freezer at -20 °C. These pathogens have been preserved for characterisation and further studies on common bean diseases in Kenya.

### 6.1.3 Pathogenic and virulence of *P. griseola* in Kenya

Pathogenic variation in *Pseudocercospora griseola* was reported as early as 1950's when Brock (1951) found indications of virulent differences between 13 Australian isolates. Pathogenic variability in *P. griseola* has also been widely reported in other parts of the world. Pastor-Corrales *et al.* (1998) identified 333 races from 433 isolates of *P. griseola* obtained from 11 Latin American and 10 African countries. Mahuku *et al.* (2002) identified 50 pathogenic groups of *P. griseola* from 112 isolated collected from 10 countries

in Central America. Pyndji (1992) characterised 21 isolates of *P. griseola* from the Great Lakes region of Africa into 17 pathogenic groups using 11 differential cultivars. The existence of great pathogenic variation within *P. griseola* in Kenya has also been reported previously (Monda, 1995; Wagara, 1996; Wagara *et al*., 2004). Monda (1995) grouped 19 isolates of *P. griseola* from different areas in Kenya into 14 pathotypes using 11 differential cultivars. Wagara (1996) used 30 bean differential cultivars to group 15 races from 18 isolates collected from 15 districts in Kenya. The *P. griseola* isolates characterized in this study showed a varied response in the differentials demonstrating a high pathogenic variability of the pathogen present in Kenya. Isolates exhibited a different virulence pattern when inoculated on the 12 bean differential genotypes. The 57 *P. griseola* isolates obtained were classified based on their virulence reactions into 23 different races. The occurrence of large pathogenic variation in *P. griseola* populations supports earlier findings by Sartorato, (2002), Mahuku *et al*., (2002a) and Wagara *et al*., (2004). Races 63-55, 63-63, 63-54 and 63-35 were the most prevalent pathotypes observed among 57 isolates studied and were reported in 10, 7, 6 and 4 different locations respectively. Two new races, 31-31 and 63-31 were reported for the first time in Kenya. The new races included two isolates race-typed as race 31-31 collected from Kibirigwi (UM2) and Turbo (UM3), and two isolates characterised as race 63-31 obtained from Othaya (LH3) and Kakamega (UM1). The lower highland (LH) and upper midland (UM) recorded the highest number of the most virulent races observed. The findings in this study provide new information on the diversity of *P. griseola*, and are the first report

to characterize angular leaf spot isolates obtained from all major bean growing regions in Kenya.

### 6.1.4 Gamete selection for multiple disease constraints

The focus of this research was to utilize gamete selection for multiple disease resistance in Mesoamerican bean genotypes grown in Kenya. This was aimed at setting up a foundation for developing high yielding; market-class bean varieties that are resistant to angular leaf spot, anthracnose, *Pythium* root rot and bean common mosaic virus. Singh (1994) proposed the use of gamete selection to simultaneously select common beans for multiple traits. 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. Resistance represents a valuable disease management tool for improved production of beans. Therefore, development of cultivars with greater levels of disease resistance is not only safe but also durable and an economically accessible strategy for most bean breeding programs. Plant breeders, pathologists and geneticists have therefore made considerable progress in the identification of specific genes and QTL for resistance to anthracnose (Kelly and Vallejo, 2004); angular leaf spot (Caixeta *et al*., 2005); bean common mosaic virus (Melotto *et al*., 1996; Pastor-Corrales *et al*., 2007). A total of 21 molecular markers were evaluated with our parental genotypes and eleven markers showed polymorphism while 10 of them were non-polymorphic to the

disease resistance genes. Fifteen markers tested had positive amplification to the disease resistance genes in at least one parental genotype while 6 markers showed no amplification. Four molecular markers were utilized to confirm the presence of resistance genes in the male gamete plants; SH-13, SAB-3, PYAA-19 and SW-13 linked to the resistance genes present in cultivars G10909, G2333, AND 1062 and BRB 191 respectively were used. There was amplification and polymorphism in three markers, SAB-3 for anthracnose; SH-13 for angular leaf spot and SW-13 for bean common mosaic virus, while no amplification was observed for PYAA-19 marker that is linked with the root rot resistance genes. Out of the 89 male gamete plants screened for angular leaf spot, anthracnose, root rot and BCMV, five had three genes while 18 had two, 35 had one and 36 had none.

### 6.1.5 Early generation gamete selection

Early generation gamete selection was done to evaluate and select for multiple disease resistance to angular leaf spot, anthracnose, bean common mosaic virus, root rots and agronomic traits in $F_1$ and $F_{1.2}$ segregating bean populations. Sixteen bean populations developed from crosses among four multi-parent male gametes and four commercial varieties were used. The field trials were conducted at University of Nairobi-Kabete Field Station and KARI-Tigoni National Potato Research Station. There was high segregation for disease resistance and other agronomic traits among the sixteen multiple-cross $F_1$ populations. The results also expressed high genotypic variation for grain yield which ranged from 2490 to 4762 kg $ha^{-1}$. Fifteen of the sixteen

multiple parent five way crosses produced yields above 3000 kg $ha^{-1}$ which was above the grand mean of 2824 kg $ha^{-1}$ and exceeded the parental checks whose yield ranged from 648 to 1686 kg $ha^{-1}$. The results obtained from evaluation of $F_{1.2}$ generation were similar to those of $F_1$ populations. These findings are similar with those of Ragagnin *et al*. (2009) who observed genetic variability for grain yield and other qualitative traits among the 40 multiple parent families analyzed. Appearance of significant differences for the diseases severity among the populations showed that variability for resistance existed among the genotypes for angular leaf spot, anthracnose, root rot and bean common mosaic virus. This indicates a possibility of obtaining genotypes with genes for resistance from their donor parents and confirms presence genes for resistance in the donor parents used in this study as reported by Miklas *et al*. (2003).

## 6.2 RECOMMENDATIONS

1. Race-typing of common bacterial blight, *Pythium* root rot and bean common mosaic virus collected should be performed to decipher the virulence diversity in these pathogens and their distribution in Kenya.
2. The Kenyan population of *Pseudocercospora griseola* be regularly monitored for emergence of variable pathotypes since the current study revealed new races that were never reported by previous researchers.
3. Molecular markers that can amplify and are polymorphic to resistance genes in bean genotypes with the potential for utilization in developing improved varieties with multiple disease resistance to the major bean diseases present in Kenya should be identified and evaluated.
4. New sources of resistance genes to counter the ever changing nature of pathogenic virulence in common bean diseases should be identified and screened.
5. The segregating bean populations developed in this study should be evaluated for possession of multiple disease resistance genes and with market preferred grain types. This evaluation should be conducted under diverse environments with different pathogen infestation regimes to determine the presence of resistance genes in the segregating generations.
6. The races identified should be considered while developing and deploying genes for resistance to angular leaf spot.

# REFERENCES


**Abawi, G. S. and J. W. Ludwig. 2005.** Effect of three crop rotations with and without deep ploughing on root rot severity and yield of beans. New York, USA, Annul. Rep. BIC, 48: 118-119.

**Aggarwal, V. D., M. A. Pastor-Corrales, R. M. Chirwa and R. A. Buruchara. 2004.** Andean beans (*Phaseolus vulgaris*) with resistance to the angular leaf spot pathogen (*Phaeoisariopsis griseola*) in Southern and Eastern Africa. Euphytica 136: 201–210.

**Alvarez-Ayala, G. and H. F. Schwartz. 1979.** Preliminary investigations of pathogenic variability expressed by *Phaeoisariopsis griseola*. Annu. Rep. Bean Improv. Coop. 22:86-87.

**Alzate-Marin, A. L., K. A. Souza, M. G. Silva, E. J. Oliveira, M. A. Moreira and E. G. Barros. 2007.** Genetic characterization of anthracnose resistance genes *Co-4*$^3$ and *Co*-9 in common bean cultivar Tlalnepantla 64 (PI 207262). Euphytica 154: 1-8.

**Ampaire, E. 2003.** Farmers' indigenous technical knowledge of bean disease management and communication systems in South-western Uganda. Makerere University, Kampala, Uganda. M.Sc. thesis, p. 127.

**Asensio-S.-Manzanera, M. C., C. Asensio and S. P. Singh. 2006.** Gamete selection for resistance to common and halo bacterial blights in dry bean intergene pool populations. Crop Sci. 46: 131–135.

**Asensio-S.-Manzanera, M. C., C. Asensio and S. P. Singh. 2005.** Introgressing resistance to bacterial and viral diseases from the Middle American to Andean common bean. Euphytica 143:223–228.

**Awale, H. E. and J. D. Kelly. 2001.** Development of SCAR markers linked to *Co-4*$^2$ gene in common bean. Ann. Rep. Bean Improv. Coop. 44:119–120.

**Balardin, R. S., A. M. Jarosz and J. D. Kelly. 1997.** Virulence and molecular diversity in *Colletotrichum lindemuthianum* from South,

Central, and North America. Phytopathology 87:1184-1191.

**Balardin, R. S. and J. D. Kelly. 1998.** Interaction between races of *Colletotrichum lindemuthianum* and gene pool diversity in *Phaseolus vulgaris*. J. Am. Soc. Hortic. Sci. 123:1038-1047.

**Balardin, R. S., J. J. Smith and J. D. Kelly. 1999.** Ribosomal DNA polymorphism in *Colletotrichum lindemuthianum*. Myco Res. 103:841-848.

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

**Beaver, J. S. and J. M. Osorno. 2009.** Achievements and limitations of contemporary common bean breeding using conventional and molecular approaches. Euphytica, 168:145–175.

**Bliss, F. A. 1980.** Common Bean. In: *Hybridization of crop plants*. Crop Science Society of America, Madison, Wisconsin. p273-284.

**Boland, G. J., M. S. Melzer, A. Hopkin, V. Higgins and A. Nassuth. 2004.** Climate change and plant diseases in Ontario. Can. J. Plant Pathol 26:335–350.

**Borges L. L., F. A. Santana, I. S. L. Castro, K. M. A. Arruda, H. J. O. Ramos, M. A. Moreira and E. G. Barros. 2013.** Differentially expressed proteins during an incompatible interaction between common bean and the fungus Pseudocercospora griseola. Mol Breeding 32:933–942.

**Braun, U. and P. W. Crous. 2006.** (1732) Proposal to conserve the name *Pseudocercospora* against *Stigmina* and *Phaeoisariopsis* (Hyphomycetes). Taxon 55: 803.

**Brock, R. D. 1951.** Resistance to angular leaf spot among varieties of beans. Journal of the Australian Institute of Agricultural Science 3:25-30.

**Broughton, W. J., G. Hernandez, M. Blair, S. Beebe, P. Gepts and J. Vanderleyden. 2003.** Bean (*Phaseolus* spp.)-model food legumes. Plant Soil 252:55–128.

**Buruchara, R., R. Chirwa, L. Sperling, C. Mukankusi, J. C. Rubyogo, R.**

**Muthoni and M. M. Abang. 2011.** Development and delivery of bean varieties in Africa: The Pan-Africa Bean Reasearch Alliance (PABRA) model. Afr. Cr. Sci. J. 19(4):227-245.

**Buruchara, R., G. Mahuku, J. Mukalazi and A. Levesque. 2007.** *Pythium* species associated with *Pythium* root rot of beans (*Phaseolus vulgaris* L.) in Eastern Africa. Cali, Colombia: CIAT, 42-53.

**Buruchara, R., S. Mayanja, G. Mahuku and A. Levesque. 2004.** Characterization and distribution of *Pythium* spp. Associated with bean root rot in East Africa. Cali, Colombia: CIAT, 345-346.

**Buruchara, R. A. 1983.** Determination of pathogenic variation in *Isariopsis griseola* Sacc. and *Pseudomonas syringae* pv. *phaseolicola* (Burk., 1926) Young, Dye and Wilkie 1978. Ph.D Thesis, University of Nairobi, Kenya.

**Buruchara, R. A. 1979.** Identification of a severe strain of bean common mosaic virus isolate from bean *Phaseolus vulgaris* L. M.Sc. diss. University of Nairobi, Kenya.

**Caixeta, E. T., A. Borém, A. Alzate-Marin, S. Fagundes, M. Silva, E. G. Barros and M. A. Moreira. 2005.** Allelic relationships for genes that confer resistance to angular leaf spot in common bean. Euphytica, 145(3):237-245.

**Caixeta, E. T., A. Borém, S. A. Fagundes, S. Niestche, E. G. Barros and M. A. Moreira. 2003.** Inheritance of angular leaf spot resistance in common bean line BAT 332 and identification of RAPD markers linked to the resistance gene. Euphytica 134:297-303.

**Carvalho, G. A., T. J. Paula, A. L. Alzate-Marin, S. Nietsche, E. G. Barros and M. A. Moreira. 1998.** Inheritance of resistance to angular leaf spot of common bean in AND 277 to race 63-23 of *Phaeoisariopsis griseola* and identification of a RAPD marker linked to the resistance gene. Fitopatol. Bras. 23:482-485.

**Chacon M. I., S. B. Pickersgill and D. G. Debouck. 2005.** Domestication patterns in common bean (*Phaseolus vulgaris* L.) and the origin of

the Mesoamerican and Andean cultivated races. Theor Appl Genet 110: 432–444.

**CIAT. 1984.** A summary of major achievements during the period 1977-1983. Cali, Colombia.

**CIAT. 1987.** Standard system for the evaluation of bean germplasm [van Schoonhoven A, Pastor-Corrales MA. (compilers)]. CIAT, Cali, Colombia, 54 pp.

**CIAT. 1995.** In: *Annual Report of the Bean Program,* 50-53. International Center for Tropical Agriculture, Cali, Colombia.

**CIAT. 1996.** In: *Annual Report of the Bean Program,* 7-20. Working document No. 176. 1998. International Center for Tropical Agriculture, Cali, Colombia.

**CIAT. 2000.** In: *Annual Report of the Bean Program* 52-105. International Center for Tropical Agriculture, Cali, Colombia.

**CIAT. 2003.** In: *Annual Report of the Bean Program* 67-75. International Center for Tropical Agriculture, Cali, Colombia.

**CIAT. 2005.** In: *Annual Report of the Bean Program,* 31-43. International Center for Tropical Agriculture, Cali, Colombia.

**Correa-Victoria, F. J. 1987.** Pathogenic variation, production of toxic metabolites, and isoenzyme analysis in *Phaeoisariopsis griseola* (Sacc.) Ferr. Ph.D. diss. Michigan State University, pp. 154.

**Costa, M. R., J. P. Tanure, K. M. Arruda, J. E. Carneiro, M. A. Moreira and E. G. Barros. 2010.** Development and characterization of common black bean lines resistant to anthracnose, rust and angular leaf spot in Brazil. Euphytica 176: 149-156.

**Coyne, D. R., J. R. Steadman, G. Godoy-Lutz, R. Gilbertson, E. Arnaud-Santana, J. S. Beaver and J. R. Myers. 2003.** Contributions of the bean/cowpea CRSP to management of bean diseases. Field Crops Res., 82: 155–168.

**Crous, P. W., M. M. Liebenberg, U. Braun and J. Z. Groenewald. 2006.** Re-evaluating the taxonomic status of *Phaeoisariopsis griseola*, the

causal agent of angular leaf spot of bean. Studies in Mycology, 55:163-173.

**Crous, P. W., U. Braun, G. C. Hunter, M. J. Wingfield, G. J. M. Verkley, H. D. Shin, C. Nakashima and J. Z. Groenewald. 2013.** Phylogenetic lineages in *Pseudocercospora*. Studies in Mycology, 75: 37–114.

**Cunha, W. G., M. A. P. Ramalho and A. F. B. Abreu. 2005.** Selection aiming at upright growth habit common bean with carioca type grains. Crop Breeding and Applied Biotechnology 5: 379-386.

**Drijfhout, E. 1978.** Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding resistance. Agricultural Research Reports, Vol. 872, pp. 1–98. Centre for Agriculture Publishing and Documentation, Wageningen, The Netherlands.

**Drijfhout, E., M. J. Silbernagel and D. W. Burke. 1978.** Differentiation of strains of bean common mosaic virus. Neth. J. Plant Pathol. 84:13–26.

**FAO. 2013.** Food and Agriculture Organisation of the United Nations, Statistics Division. [Online] Available at http://faostat.fao.org/site/339/default.aspx (accessed 14th July, 2013).

**Fernández, M. T., M. Fernández, A. Casares, R. Rodriguez and M. Fueyo. 2000.** Bean germplasm evaluation for anthracnose resistance and characterization of agronomic traits: A new physiological strain of *Colletotrichum lindemuthianum* infecting *Phaseolus vulgaris* L. in Spain. Euphytica 114:143-149.

**Flores-Estévez, N., J. A. Acosta-Gallegos and L. Silva-Rosales. 2003.** Bean common mosaic virus and bean common mosaic necrosis virus in Mexico. Plant Dis. 87: 21–25.

**Garzón, L. N., G. A. Ligarreto and M. W. Blair. 2008.** Molecular marker-assisted backcrossing of anthracnose resistance into Andean climbing beans (*Phaseolus vulgaris* L.). Crop Sci. 48: 562-570.

**Gathuru, E. M. and S. M. Mwangi. 1991.** Bean anthracnose research in Kenya. In: Buruchara, R. A. (Ed.): Proceedings of the first Pan-African working group meeting on anthracnose of beans. Ambo, Ethiopia. February 17–23, 1991. CIAT African Workshop Series No. 15, 43–50.

**Gibbs, A. J., A. M. Mackenzie, K. J. Wei and M. J. Gibbs. 2008.** The potyviruses of Australia. Arch Virol. 153:1411–1420.

**Graham, P. H. and P. Ranalli. 1997.** Common bean (*Phaseolus vulgaris* L.). Field Crops Res. 53: 131-146.

**Guzman, P., R. L. Gilbertson and R. Nodari. 1995.** Characterization of variability in the fungus *Phaeoisariopsis griseola* suggests coevolution with the common bean (*Phaseolus vulgaris*). Phytopathology 85:600–607.

**Hillocks, R. J., C. S. Madata, R. Chirwa, E. M. Minja and S. Msolla. 2006.** *Phaseolus* bean improvement in Tanzania, 1959-2005. Euphytica 150:215-231.

**Huang, C. H. and Y. C. Chang. 2005.** Identification and molecular characterization of Zantedeschia mild mosaic virus, a new calla lily-infecting potyvirus. Arch. Virol. 150:1221–1230.

**Ibarra-Pérez, F., B. Ehdaie and G. Waines. 1997.** Estimation of outcrossing rate in common bean. Crop Sci. 37:60–65

**Jaetzold, R., H. Schmidt, B. Hornetz and S. Shisanya. 2006.** Farm Management Handbook of Kenya, Second edition. Vol.2. Ministry of Agriculture, Nairobi, Kenya.

**Jesus Junior, W. C., F. X. Vale, R. R. Coelho, B. Hau, L. Zambolim, L. C. Costa and A. B. Filho. 2001.** Effects of angular leaf spot and rust on yield loss of *Phaseolus vulgaris*. Phytopathol 91:1045–1053.

**Kapil, R., P. Sharma, S. K. Sharma, O. P. Sharma, J. B. Dhar and P. N.**

**Sharma. 2011.** Pathogenic and molecular variability in bean common mosaic virus infecting common bean in India. Archives of Phytopathology and Plant Protection, 44:11, 1081-1092.

**Katungi, E., A. Farrow, J. Chianu, L. Sperling and S. Beebe. 2009.** Common bean in Eastern and Southern Africa: a situation and outlook analysis. CIAT, Kampala, Uganda.

**Kelly, J. D. and V. A. Vallejo. 2004.** A comprehensive review of the major genes conditioning resistance to anthracnose in common bean. Hortscience 39:1196–1207.

**Kelly, J. D., P. Gepts, P. N. Miklas and D. P. Coyne. 2003.** Tagging and mapping of genes and QTL and molecular-marker assisted selection for traits of economic importance in bean and cowpea. Field Crops Res 82: 135–154.

**Kelly, J. D. and P. N. Miklas. 1998.** The role of RAPD markers in breeding for disease resistance in common bean. Mol. Breed. 4: 1–11.

**Kelly, J. D. 1997.** A review of varietal response to bean common mosaic potyvirus in *Phaseolus vulgaris*. Plant Variety Seeds 10:1–6.

**Kelly, J. D., L. Afanador and L. Cameron. 1994.** New races of *Colletotrichum lindemuthianum* in Michigan and implications in dry bean resistance breeding. Plant Dis.78:892-894.

**Kelly, J. D., A. W. Saettler and M. W. Adams. 1983.** New necrotic strain of bean common mosaic virus in Michigan. Annul Rep Bean Improv Coop 26: 49–50.

**Kimani, P. M., S. Omondi and Z. Mairura. 2012.** Market and value chain mapping for dry beans in Kenya. A report submitted to Kilimo Trust, pp 87.

**Kimani, P. M. and A. W. Mwang'ombe. 2007.** Yield stability in bean lines with multiple disease resistance. Third Biotechnology, Breeding and Seed Systems Conference, 26-29 March 2007, Maputo, Mozambique. The Rockefeller Foundation.

**Kimani, P. M., R. Buruchara, J. Muthamia, N. Mbikayi, A. Asfaw, A.**

**Namayanja, G. Rachier, R. Otsyula and L. Lubanga. 2005.** Selection on marketable bean lines with improved resistance to angular leaf spot, root rots and yield for smallholder farmers in ECA. Presented at the *Second Biotechnology, Breeding and Seed Systems Conference*, 23-27 January 2005, Nairobi, Kenya Rockefeller Foundation, Nairobi, Kenya.

**Kulkarni, H. Y. 1973.** Notes on East African plant virus diseases. IV. Bean common mosaic virus. East Afr. For. J. 39: 72-76.

**Liebenberg, M. M. and Z. A. Pretorius. 1997.** A review of angular leaf spot of common bean (*Phaseolus vulgaris* L.). African Plant Protection 3(2): 81–106.

**Liu, P., J. Zhu and Y. Lu. 2004.** Marker-assisted selection in segregating generations of self-fertilizing crops. Theor. Appl. Genet. 109: 370–376.

**Lunze, L., R. Buruchara, M. A. Ugen, L. Nabahungu, G. O. Rachier, M. Ngongo, I. Rao and M. M. Abang. 2011.** Integrated soil fertility management in bean-based cropping systems of Eastern, Central and Southern Africa. In: Soil fertility improvement and integrated nutrient management - A global perspective. InTech Education and Publishing, Vienna, Austria. In press.

**Mahuku, G. S., C. E. Jara, C. Cajiao and S. Beebe. 2002a.** Sources of resistance to *Colletotrichum lindemuthianum* in the secondary gene pool of *Phaseolus vulgaris* and in crosses of primary and secondary gene pools. Plant Dis. 86:1383-1387.

**Mahuku, G., C. Jara, J. B. Cuasquer and G. Castellanos. 2002b.** Genetic variability within *Phaeoisariopsis griseola* from Central America and its implications for resistance breeding of common bean. Plant Pathology 51: 594–604.

**Mahuku, G., C. Jara, C. Cajiao and S. Beebe. 2003.** Sources of resistance to angular leaf spot (*Phaeoisariopsis griseola*) in common bean core collection, wild Phaseolus vulgaris and secondary gene pool,

Euphytica 130:303-313.

**Mahuku, S. G. and J. J. Riascos, 2004.** Virulence and molecular diversity within *Colletotrichum lindemuthianum* isolates from Andean and Mesoamerican bean varieties and regions. European Journal of Plant Pathology 110: 253–263.

**Mahuku, G., C. Montoya, M. A. Henríquez, C. Jara, H. Teran and S. Beebe. 2004.** Inheritance and characterization of angular leaf spot resistance gene presence in common bean accession G 10474 and identification of an AFLP marker linked to the resistance gene. Crop Sci. 44:1817-1824.

**Mahuku, G. S., C. Jara, M. A. Henríquez, G. Castellanos and J. Cuasquer. 2006.** Genotypic characterization of the common bean bacterial blight pathogens, *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* by rep-PCR and PCR-RFLP of the ribosomal genes. Phytopathology, 154:35–44.

**Mahuku, G., R. Buruchara, M. Navia and R. Otsyula. 2007.** Development of PCR markers tightly linked to Pyult1, a gene that confers *Pythium* root rots resistance in the common bean genotype AND 1062. Phytopathology, 97:69-79.

**Mahuku, G. S., M. A. Henríquez, C. Montoya, C. Jara, H. Teran and S. Beebe. 2010.** Inheritance and development of molecular markers linked to angular leaf spot resistance genes in the common bean accession G10909. Mol Breeding (Online).

**Mamidi S., M. Rossi, D. Annam, S. Moghaddam, R. Lee, R Papa and P. McClean. 2011.** Investigation of the domestication of common bean (*Phaseolus vulgaris*) using multilocus sequence data. Functional Plant Biology, 38:953–967.

**Marin-Villegas, J. 1959.** Variabilidad del *Isariopsis griseola* Sacc., agente causal de la mancha angular del frijol (*Phaseolus vulgaris* L.). Thesis, Universidad de Caldras (*Abstracts on Field Beans 1979,*

*4:140-141*).

**Mavric, I. and S. J. Vozlic. 2004.** Virus diseases and resistance to bean common mosaic and bean common mosaic necrosis potyvirus in common bean (*Phaseolus vulgaris* L.). Acta Agriculturae Slovenica 83:181–190.

**McClean, P., P. Gepts and J. Kami. 2004.** Genomics and genetic diversity in common bean. In: Wilson R. F., H. T. Stalker and E. C. Brummer. (eds) Legume Crops Genomics. AOCS Press, Champaign, Illinois, pp 60–82.

**McDonald, B. A. and C. Linde. 2002.** Pathogen population genetics, evolutionary potential and durable resistance. Annul. Rev. Phytopathol 40:349–379.

**Melotto, M. and J. D. Kelly. 2000.** An allelic series at the *Co-1* locus conditioning resistance to anthracnose in common bean of Andean origin. Euphytica 116:143–149.

**Melotto, M., L. Afanador and J. D. Kelly. 1996.** Development of a SCAR marker linked to the *I* gene in common bean. Genome 39:1216–1219.

**Michelmore, R. W. 1995.** Molecular approaches to manipulation of disease resistance genes. Annul. Rev. Phytopathol 33:393–427.

**Miklas, P. N., J. D. Kelly, S. E. Beebe and M. W. Blair. 2006a.** Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. Euphytica 147:105–131.

**Miklas, P. N., J. R. Smith and S. R. Singh. 2006b.** Registration of common bacterial blight resistant dark red kidney bean germplasm line USDK-CBB-15. Crop Sci. 46: 1005–1007.

**Miklas, P. N., J. D. Kelly and S. P. Singh. 2003.** Registration of anthracnose-resistant pinto bean germplasm line USPTANT-1. Crop Sci. 43: 1889–1990.

**Miklas, P. N. and J. D. Kelly. 2002.** The use of MAS to develop bean germplasm possessing *Co-4*$^2$ gene for anthracnose resistance. Ann.

Rep. Bean Improv. Coop. 45: 68–69.

**Miklas, P. N., R. C. Larsen, R. Riley and J. D. Kelly. 2000.** Potential marker-assisted selection for *bc-1*$^2$ gene for resistance to bean common mosaic potyvirus in common bean. Euphytica 116: 211–219.

**Mkandawire, A. B., R. B. Mabagala, P. Guzman, P. Gepts and R. L. Gilbertson. 2004.** Genetic diversity and pathogenic variation of common blight bacteria (*Xanthomonas campestris* pv. *Phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) suggests co-evolution with the common bean. Phytopathology 94:593–603.

**Mmbaga, M., J. R. Steadman and J. R. Stavely. 1996.** The use of host resistance in disease management of rust in common bean. Integrated Pest Manag Rev 1: 191–200.

**Monda, E. O. 1995.** Angular leaf spot disease of common bean, pathogenic variation of the causal organism (*Phaeoisariopsis griseola* (Sacc.) Ferr.) in Kenya and its effects on host growth and yield. Ph.D. Thesis, University of Leeds, London.

**Mukalazi, J., R. Buruchara, J. Carder, S. Muthumeenakshi, E. Adipala, F. Opio, G. White, T. Pettitt and N. J. Spence. 2001.** Characterization of *Pythium* spp. pathogenic to common beans in Uganda. Afr. Crop Sci. Conference Lagos, Nigeria. p. 142.

**Mukeshimana, G., A. Paneda, C. Rodriguez, J. J. Ferreira, R. Giraldez and J. D. Kelly. 2005.** Markers linked to the *bc-3* gene conditioning resistance to bean common mosaic potyviruses in common bean. Euphytica 144: 291–299.

**Mwang'ombe, A. W., I. N. Wagara, J. W. Kimenju and R. A. Buruchara. 2007.** Occurrence and severity of angular leaf spot of common bean in Kenya as influenced by geographical location, altitude and agro-ecological zones. Plant Pathol. J., 6 (3): 235-241.

**Mwaniki, A. 2000.** Assessment of bean production constraints and seed quality and health of improved common bean seed. University of

Nairobi, Kenya.

**Mwesigwa J. B., 2009.** Diversity of *Colletotrichum lindemuthianum* and reaction of common bean germplasm to anthracnose disease. MSc thesis. Makerere university, Uganda. pp96.

**Namayanja, A., R. Buruchara, G. Mahuku, P. Rubaihayo, P. Kimani, S. Mayanja and H. Eyedu. 2006.** Inheritance of resistance to angular leaf spot in common bean and validation of the utility of resistance linked markers for marker assisted selection outside the mapping population. Euphytica 151:361–369.

**Nderitu, J. H., R. A. Buruchara and J. K. Ampofo. 1997.** Relationships between Bean Stem Maggot, Bean root rots and soil fertility. African Highland Initiative, Nairobi. pp 16.

**Nietsche, S., A. Borém, G. A. Carvalho, R. C. Rocha, T. J. Paula and E. G. Barros. 2000.** RAPD and SCAR markers linked to a gene conferring resistance to angular leaf spot in common bean. Phytopathol. 148: 117-121.

**Nietsche, S., A. Borém, G. A. Carvalho, T. J. Paula Júnior, C. F. Fontes-Ferreira, E. G. Barros and M. A. Moreira. 2001.** Genetic diversity of *Phaeoisariopsis griseola* in the State of Minas Gerais, Brazil. Euphytica 117: 77-84.

**Njau, P. J. and H. F. Lyimo. 2000.** Incidence of bean common mosaic virus and bean common mosaic necrosis virus in bean (*Phaseolus vulgaris* L.) in the wild legumes seedlots in Tanzanian Seed. Sci Technol 28: 85–92.

**Nzungize, J., P. Gepts, R. Buruchara, A. Male, P. Ragama, J. P. Busogoro and J. P. Baudoin. 2011.** Introgression of *Pythium* root rot resistance gene into Rwandan susceptible common bean cultivars. African Journal of Plant Science, 5(3): 193-200.

**Ombiri, J., V. Zinkernagel, E. M. Gathuru and O. Achwanya. 2002.** First report of race 485 of *Colletotrichum lindemuthianum* in Kenya and its implication in bean resistance breeding. Gartenbauwissenschaft,

67 (3). 81–85.

**Omunyin, M. E., E. M. Gathuru and D. M. Mukunya. 1995.** Pathogenicity groups of bean common mosaic virus isolates in Kenya. Plant Dis. 79:985–989.

**Otsyula, R. M., R. A. Buruchara, G. Mahuku and P. Rubaihayo. 2003.** Inheritance and transfer of root rot (*Pythium*) resistance to bean genotypes. Afr. Crop Sci. Soc., 6: 295-298.

**Otsyula, R. M., S. I. Ajanga, R. A. Buruchara and C. S. Wortmann. 1998.** Development of an integrated bean root rot control strategy for western Kenya. African Crop Science Journal 6:61-67.

**Padder, B. A., P. N. Sharma and O. P. Sharma. 2009.** Virulence and RAPD data – A tool to study the evolutionary trends of *Colletotrichum lindemuthianum* virulences in the North Western Himalayan region of India. Archives of Phytopathology and Plant Protection. 42 (7), 610-617.

**Pastor-Corrales, M. A. and C. E. Jara. 1995.** La evolucio´n de *Phaeoisariopsis griseola* con el frijol comun en America Latina. Fitopatologı´a Colombiana 19:15–25.

**Pastor-Corrales, M. A., M. M. Otoya, A. Molina and S. P. Singh. 1995.** Resistance to *Colletotrichum lindemuthianum* isolates from Middle America and Andean South America in different common bean races. Plant Dis. 79:63-67.

**Pastor-Corrales, M. A., C. Jara and S. Singh. 1998.** Pathogenic variation in, source of, and breeding for resistance to *Phaeoisariopsis griseola* causing angular leaf spot in common bean. Euphytica 103:161–171.

**Pastor-Corrales, M. A. 2003.** Sources, genes for resistance, and pedigrees of 52 rust and mosaic resistant dry bean germplasm lines released by the USDA Beltsville bean project in collaboration with the Michigan, Nebraska and North Dakota agricultural experiment stations. Annul. Rep. Bean Improv. Coop 46: 235–241.

**Pastor-Corrales, M. A. 2005.** Anthracnose. p. 25–27. In H. F. Schwartz, J. R. Steadman, and R. L. Forster (ed.) Compendium of bean diseases. 2nd ed. APS Press, St. Paul, MN.

**Pastor-Corrales, M. A., J. D. Kelly, J. R. Steadman, D. T. Lindgren, J. R. Stavely and D. P. Coyne. 2007.** Registration of six great northern bean germplasm lines with enhanced resistance to rust and bean common mosaic and necrosis potyviruses. J. Plant Registrations 1: 77–79.

**Pereira, R., E. A. Souza, Q. L. Barcelos and A. B. F. Abreu. 2013.** Evaluation of resistance in common bean genotypes to the causal agent of angular leaf spot. Ann. Rep. Bean Improv. Coop., 56:33-34.

**Pyndji, M. M. 1992.** Pathogenic variability of *Phaeoisariopsis griseola* in the Great Lakes Region. In: *Proceedings of the Pan-Africa bean pathology working group meeting*, 7-12 (Eds R. A. Buruchara and U. S. Scheidegger). CIAT Workshop Series No. 23, Thika, Kenya.

**Queiroz, V. T., C. S. Sousa, M. R. Costa, D. A. Sanglard, K. M. Arruda, T. L. Souza, V. A. Ragagnin, E. G. Barros and M. A. Moreira. 2004.** Development of SCAR markers linked to common bean angular leaf spot resistance genes. Annual Report of the Bean Improvement Cooperative, 47:237-238.

**Ragagnin, V. A., T. L. De Souza, D. A. Sanglard, K. M. Arruda, M. R. Costa, A. L. Alzate-Marin, J. E. De S. Carneiro, M. A. Moreira and E. G. Barros. 2009.** Development and agronomic performance of common bean lines simultaneously resistant to anthracnose, angular leaf spot and rust. Plant Breeding 128: 156—163.

**Rocha, G. S., L. P. Pereira, P. C. Carneiro, T. J. Paula Junior and J. E. Carneiro. 2012.** Common bean breeding for resistance to anthracnose and angular leaf spot assisted by SCAR molecular markers. Crop Breeding and Applied Biotechnology 12: 34-42.

**Rusuku, G., R. A. Buruchara, M. Gatabazi and M. A. Pastor-Corrales. 1997.** Occurrence and distribution of soil borne fungi pathogenic to the common bean. Plant Dis., 81: 445-449.

**Saccardo, P. A. 1886.** *Syllage fungorum onmium hucusque cognitorum*, volume 4. Pavia, Italy.

**Sapparat, M. C., G. E. Fermoselle, S. A. Stenglein, M. B. Aulicino and P. A. Balatti. 2009.** *Pseudocercospora griseola* causing angular leaf spot on *Phaseolus vulgaris* produces 1,8-dihydroxynaphthalene-melanin. Mycopathologia, 168(1): 41-47.

**Sartorato, A. 2004.** Pathogenic variability and genetic diversity of *Phaeoisariopsis griseola* isolates from two counties in the State of Goias, Brazil. J. Phytopathol 152: 385-390.

**Sartorato, A. 2002.** Identification of *Phaeoisariopsis griseola* pathotypes from five states of Brazil. *Fitopatol* Bras. 27:78–81.

**Sartorato, A., S. Nietsche, E. G. Barros and M. A. Moreira. 2000.** RAPD and SCAR markers linked to resistance gene to angular leaf spot in common beans. Fitopatologia Bras. 25:637-642.

**Schoonhoven, A. and M. A. Pastor-Corrales. (comps). 1987.** Standard system for the evaluation of bean germplasm. Centro Internacional de Agricultura Tropical, Cali, Colombia.

**Schwartz, H. F., J. R. Steadman, R. Hall and R. L. Forster. 2005.** Compendium of bean diseases, 2nd ed. APS press. St, St. Paul.

**Sharma, P. N., B. A. Padder, O. P. Sharma, A. Pathania and P. Sharma. 2007.** Pathological and molecular diversity in *Colletotrichum lindemuthianum* (bean anthracnose) across Himachal Pradesh, a North Western Himalayan state of India. Australasian Pl. Pathol. 36:191-197.

**Silva, K. J., E. A. Souza, A. Sartorato and C. N. Souza Freire. 2008.** Pathogenic variability of isolates of *Pseudocercospora griseola*, the cause of common bean angular leaf spot, and its implications for resistance breeding. J. Phytopathology 156:602–606.

**Singh, S. P. 1994.** Gamete selection for simultaneous improvement of multiple traits in common bean. Crop Sci. 34:352-355.

**Singh, S. P. 2001.** Broadening the genetic base of common bean cultivars: A Review. Crop Sci. 41:1659–1675.

**Singh, S. P., C. Cardona, F. J. Morales, M. A. Pastor-Corrales and O. Voysest. 1998.** Gamete selection for upright Carioca bean with resistance to five diseases and a leafhopper. Crop Sci. 38: 666–672.

**Singh, S. P., H. Terán, C. G. Munoz and J. C. Takegami. 2000.** Registration of multiple-disease resistant carioca dry bean A 801 and A 804 germplasm. Crop Sci 40:1836–1837.

**Singh, S. P., H. Teran, M. Lema, M. F. Dennis, R. Hayes and C. Robinson. 2008.** Breeding for slow darkening, high-yielding, broadly adapted dry bean pinto '*Kimberly*' and '*Shoshone*'. J. Plant Regist. 2: 180–186.

**Singh, S. P. and H. F. Schwartz. 2010.** Breeding common bean for resistance to diseases: A review. Crop Sci. 50: 2199-2223.

**Spegazzini, C. 1910.** Mycetes Argentinenses (Series V). Anales del Museo Nacional de Historia Natural, Buenos Aires 20: 329–467.

**Spence, N. 2003.** Characterisation and epidemiology of root rot diseases caused by *Fusarium* and *Pythium* spp. Beans in Uganda R7568. Final technical report. CIAT Bean Programme, Cali, Colombia, p. 60.

**Stenglein, S., L. D. Ploper, O. Vizgarra and P. Balatti. 2003.** Angular leaf spot: A disease caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris on *Phaseolus vulgaris* L. Adv. Appl. Microbiol. 52:209–243.

**Tarán, B., T. E. Michaels and K. Peter Pauls. 2003.** Marker-assisted selection for complex trait in common bean (*Phaseolus vulgaris* L.) using QTL-based index. Euphytica 130:423–432.

**Terán, H. and S. P. Singh. 2009.** Gamete selection for improving physiological resistance to white mold in common bean. Euphytica

167:271–280.

**Tu, J. C. 1986.** Isolation and characterization of a new necrotic strain (NL-8) of bean common mosaic virus in South-western Ontario. Can Plant Dis Surv 66: 13–14.

**Vallejo, V. and J. D. Kelly. 2009.** New insights into the anthracnose resistance of common bean land race G 2333. The Open Hort. J. 2:29-33.

**Vallejos, C. E., G. Astua-Monge, V. Jones, T. R. Plyler, N. S. Sakiyama and S. A. Mackenzie. 2006.** Genetic and molecular characterization of the *I* locus of *Phaseolus vulgaris*. Genetics 172(2): 1229-1242.

**Wagara, I. N. 1996.** Pathogenic variability in *Phaeoisariopsis griseola* (Sacc.) Ferr. and resistance of *Phaseolus vulgaris* L. to angular leaf spot. M.Sc. thesis, University of Nairobi, Kenya.

**Wagara, I. N. 2004.** Molecular and virulence characterisation of *Phaeoisariopsis griseola* and reaction of bean germplasm to races of angular leaf spot pathogen. Ph.D. thesis, University of Nairobi, Kenya.

**Wagara, I. N., A. W. Mwang´ombe, J. W. Kimenju, R. A. Buruchara, R. Jamnadass and P. A. Majiwa. 2004.** Genetic diversity of *Phaeoisariopsis griseola* in Kenya as revealed by AFLP and group-specific primers. J. Phytopathol. 152: 1–8.

**Wahome, S. W., P. M. Kimani, J. W. Muthomi, R. D. Narla and R. Buruchara. 2011.** Multiple disease resistance in snap bean genotypes in Kenya. African Crop Science Journal, Vol. 19, No. 4, pp. 289-302.

**Wortmann, C. S., R. A. Kirkby, C. A. Elude and D. J. Allen. 1998.** Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa pg 63. CIAT, Cali, Colombia.

**Young, R. A. and Kelly, J. D. 1996.** Characterization of the genetic resistance to *Colletotrichum lindemuthianum* in common bean

differential cultivars. Plant Dis. 80:650-654.

**Young, R. A., M. Melotto, R. O. Nodari and J. D. Kelly. 1998.** Marker-assisted dissection of the oligogenic anthracnose resistance in the common bean variety, G2333. Theoretical and Applied Genetics 96:87-94.

# APPENDICES

**Appendix 1:** Mean maximum and minimum temperature and rainfall at Kabete Field Station, 2010-2012.

| Period | 2010 | | | 2011 | | | 2012 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Temp. (°C) | | | Temp. (°C) | | | Temp. (°C) | | |
| | Mean Max | Mean Min | Rainfall ($kg/m^2$) | Mean Max | Mean Min | Rainfall ($kg/m^2$) | Mean Max | Mean Min | Rainfall ($kg/m^2$) |
| 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 2:** Mean maximum and minimum temperature and rainfall at National Potato Research Station - Tigoni, 2010-2012.

| Period | 2010 | | | 2011 | | | 2012 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Temp. (°C) | | | Temp. (°C) | | | Temp. (°C) | | |
| | Mean Max | Mean Min | Rainfall ($kg/m^2$) | Mean Max | Mean Min | Rainfall ($kg/m^2$) | Mean Max | Mean Min | Rainfall ($kg/m^2$) |
| Jan | 22.9 | 11.9 | 117.1 | 24.1 | 12.7 | 54.3 | 25.8 | 14.8 | 84.9 |
| Feb | 27.6 | 13.1 | 92.0 | 27.1 | 13.2 | 91.7 | 27.8 | 13.5 | 80.1 |
| Mar | 25.4 | 13.6 | 182.6 | 26.9 | 14.6 | 150.2 | 27.2 | 15.6 | 152.0 |
| Apr | 25.1 | 14.7 | 281.9 | 25.5 | 15.4 | 70.1 | 25.3 | 16.5 | 347.3 |
| May | 25.7 | 12.4 | 262.1 | 24.3 | 14.9 | 97.5 | 24.8 | 14.4 | 266.7 |
| Jun | 20.3 | 10.9 | 74.3 | 21.7 | 12.5 | 55.2 | 22.4 | 13.3 | 84.0 |
| Jul | 23.3 | 11.6 | 27.3 | 23.8 | 10.2 | 33.9 | 24.9 | 11.5 | 35.0 |
| Aug | 24.1 | 10.8 | 31.7 | 23.1 | 11.7 | 42.4 | 23.1 | 12.8 | 38.7 |
| Sep | 25.2 | 12.7 | 23.5 | 25.9 | 13.1 | 24.0 | 27.0 | 14.1 | 35.1 |
| Oct | 24.7 | 14.8 | 120.1 | 26.5 | 15.1 | 175.1 | 26.9 | 14.2 | 113.9 |
| Nov | 22.5 | 15.1 | 234.7 | 23.9 | 15.8 | 284.3 | 25.9 | 15.4 | 254.1 |
| Dec | 22.1 | 14.9 | 201.2 | 21.9 | 13.6 | 251.1 | 24.6 | 14.9 | 138.6 |

**Appendix 3:** Protocol for preparation of 0.5M EDTA.

1. 186.12g of EDTA
2. 750ml of $ddH_20$
3. Add about 20g of NaOH pellets
4. Slowly add more NaOH until the pH is 8.0
5. Make up the volume to 1000ml using $ddH_20$
6. Sterilize by autoclaving

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

**Appendix 4:** Protocol for preparation of 1M Tris.

1. Dissolve 121.1 g of Tris base in 800 ml of $H_2O$.
2. Adjust pH to 8.0 by adding 42 ml of concentrated HCL.
3. Allow the solution to cool to room temperature before making the final adjustments to the pH.
4. Adjust the volume to 1 L with double distilled $H_2O$.
5. Sterilize using an autoclave.

**Appendix 5:** Protocol for preparation of $TE^{0.1}$ buffer.

1. 10 mM Tris (10 ml of 1 M Tris-HCl, pH 8.0)
2. 0.1mM EDTA (500 µl of 0.5 M EDTA, pH 8.0)
3. Make up to 950ml with de-ionized water and adjust to pH 8.0 using HCl and adjust total volume to 1 liter.
4. Sterilize by autoclaving

**Appendix 6:** Protocol for preparation of 5X TBE (Tris-Borate EDTA) buffer.

1. 54 g Tris base
2. 27.5 g Boric acid
3. 20 ml 0.5M EDTA, pH 8.0
4. Stir, do not adjust the pH
5. Add sterile distilled water to 1000 ml
6. 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 7:** Composition of 1% Agarose gel.

1. 1 g Agarose dissolved in 100 ml TBE

**Appendix 8:** Composition of Ethidium bromide.

1. 10 mg/ml Ethidium bromide

(**Note**: Ethidium bromide is highly carcinogenic. Always wear gloves and protective goggles while handling Ethidium bromide. Do not dispose to open environment.)

**Appendix 9:** Protocol for preparation of 10X bromophenol blue dye.

1. 1.6 g of bromophenol blue (40%)
2. 0.16 g of xylenecyanol FF
3. Mix with 50 ml glycerol. Make the volume up to 100 ml.
4. Aliquot to 1.5 ml tubes.
5. Heat the tube for 10 minutes, cool and store at 4 °C.

**Appendix 10:** Mean squares for vigour, days to flowering and maturity traits of 16 $F_1$ bean populations grown at two locations during the 2012 long rain season.

| Source of variation | | Mean Squares | | |
|---|---|---|---|---|
| | | Vigour | Days to Flowering | Days to Maturity |
| Replicates | 2 | 0.0 | 0.4 | 13.7 |
| Location | 1 | 1.7** | 99.7** | 118.8* |
| Error (a) | 2 | 0.0 | 0.1 | 2.3 |
| Male Gamete | 3 | 0.8** | 1.4** | 1.5 $^{NS}$ |
| Location x Male Gamete | 3 | 1.8** | 2.9** | 2.1 $^{NS}$ |
| Error (b) | 12 | 0.0 | 0.2 | 6.4 |
| Commercial Variety | 3 | 0.3** | 0.6* | 14.2 $^{NS}$ |
| Location x Commercial Variety | 3 | 0.6** | 0.0 $^{NS}$ | 0.7 $^{NS}$ |
| Male Gamete x Commercial Variety | 9 | 0.4** | 3.7** | 30.9** |
| Location x Male Gamete x Commercial Variety | 9 | 0.1** | 1.7** | 8.6 $^{NS}$ |
| Pooled Error (c) | 48 | 0.0 | 0.2 | 6.8 |
| Total | 95 | | | |

*, ** Significant at 0.05 and 0.01 probability levels respectively.
$^{NS}$ = Not Significant

**Appendix 11:** Mean squares yield traits of 16 $F_{1.2}$ bean populations grown at Kabete during the 2013 short rain season.

| Source of variation | | Mean Square |
|---|---|---|
| | | Yield (kg $ha^{-1)}$ |
| Blocks | 2 | 4713.0 |
| Male Gamete | 3 | 2506897.0** |
| Error (a) | 6 | 13083.0 |
| Commercial Variety | 3 | 2101977.0** |
| Male Gamete x Commercial Variety | 9 | 383286.0** |
| Pooled Error (b) | 24 | 6862.0 |
| Total | 47 | |

*, ** Significant at 0.05 and 0.01 probability levels respectively.

**Appendix 12:** Mean squares for angular leaf spot, anthracnose, bean common mosaic virus and root rots disease traits of 16 $F_1$ bean populations grown at two locations during the 2012 long rain season.

| Source of variation | | Mean Squares | | | |
|---|---|---|---|---|---|
| | | [1]ALS | ANT | BCMV | RR |
| Replicates | 2 | 0.0 | 0.0 | 0.1 | 0.1 |
| Location | 1 | 7.7** | 5.3** | 6.2** | 28.1** |
| Error (a) | 2 | 0.0 | 0.0 | 0.0 | 0.0 |
| Male Gamete | 3 | 0.7** | 0.3** | 2.1** | 0.8** |
| Location x Male Gamete | 3 | 0.6** | 0.8** | 0.4** | 1.6** |
| Error (b) | 12 | 0.0 | 0.0 | 0.0 | 0.0 |
| Commercial Variety | 3 | 0.1* | 1.8** | 0.8** | 1.0** |
| Location x Commercial Variety | 3 | 0.7** | 0.3** | 0.2* | 0.8** |
| Male Gamete x Commercial Variety | 9 | 0.6** | 0.6** | 0.5** | 0.7** |
| Location x Male Gamete x Commercial Variety | 9 | 0.6** | 1.1** | 0.9** | 1.2** |
| Pooled Error (c) | 48 | 0.0 | 0.0 | 0.1 | 0.0 |
| Total | 95 | | | | |

[1]ALS=angular leaf spot, ANT=anthracnose, BCMV=bean common mosaic virus, RR= *Pythium* root rot
*, ** Significant at 0.05 and 0.01 probability levels respectively.
NS = Not Significant

**Appendix 13:** Mean squares for pods plant$^{-1}$, seeds pod$^{-1}$, 100-seed mass and yield traits of 16 $F_1$ bean populations grown at two locations during the 2012 long rain season.

| Source of variation | | Mean Squares | | | |
|---|---|---|---|---|---|
| | | Pods Plant$^{-1}$ | Seeds Pod$^{-1}$ | 100 Seed Mass | Yield (kg ha$^{-1}$) |
| Replicates | 2 | 0.9 | 0.0 | 0.1 | 198124.0 |
| Location | 1 | 68.2** | 0.5** | 0.6** | 32296450.0** |
| Error (a) | 2 | 0.2 | 0.0 | 0.6 | 11628.0 |
| Male Gamete | 3 | 4.2 $^{NS}$ | 0.0* | 17.1** | 5418775.0** |
| Location x Male Gamete | 3 | 10.7** | 0.2** | 17.2** | 524077.0* |
| Error (b) | 12 | 1.3 | 0.0 | 0.7 | 110500.0 |
| Commercial Variety | 3 | 6.9** | 0.0** | 23.8** | 3767737.0** |
| Location x Commercial Variety | 3 | 8.8** | 0.0** | 16.3** | 286836.0 $^{NS}$ |
| Male Gamete x Commercial Variety | 9 | 26.2** | 0.1** | 8.9** | 955656.0** |
| Location x Male Gamete x Commercial Variety | 9 | 13.0** | 0.2** | 17.6** | 789342.0** |
| Pooled Error (c) | 48 | 1.0 | 0.0 | 0.7 | 164378.0 |
| Total | 95 | | | | |

*, ** Significant at 0.05 and 0.01 probability levels respectively.
$^{NS}$ = Not Significant

**Appendix 14:** Correlation between grain yield and its component in 16 $F_1$ bean populations grown at Kabete during the 2012 long rain season.

| | Vigour | [1]DF (50%) | DM (50%) | ALS | ANT | BCMV | RR | Pods /Plant | Seeds /Pod | 100 Seed mass | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Vigour | - | | | | | | | | | | |
| DF (50%) | 0.4** | - | | | | | | | | | |
| DM (50%) | 0.2 | 0.7** | - | | | | | | | | |
| ALS | 0.2* | -0.1 | -0.3* | - | | | | | | | |
| ANT | 0.5** | -0.1 | -0.3** | 0.5** | - | | | | | | |
| BCMV | 0.2* | 0.2 | -0.1 | 0.2 | 0.3* | - | | | | | |
| RR | 0.4** | 0.0 | -0.1 | 0.0 | 0.2 | 0.2 | - | | | | |
| Pods/Plant | 0.2 | 0.4** | 0.6** | -0.5** | -0.3* | -0.2 | 0.1 | - | | | |
| Seeds/Pod | 0.1 | 0.6** | 0.6** | -0.3** | -0.3* | 0.0 | -0.2* | 0.7** | - | | |
| 100 Seed mass | -0.5* | -0.8** | -0.8** | 0.1 | 0.1 | -0.1 | -0.2 | -0.6** | -0.6** | - | |
| Yield (kg/ha) | -0.1 | -0.1 | 0.3** | -0.2* | -0.3** | -0.5** | 0.1 | 0.5** | 0.1 | -0.3** | - |

[1]DF=days to 50% flowering, DM=days to 50% maturity, ALS=angular leaf spot, ANT=anthracnose, BCMV=bean common mosaic virus, RR=*Pythium* root rot.
*, ** Significant at 0.05 and 0.01 probability levels respectively.

**Appendix 15:** Correlation between yield and yield component in 16 $F_1$ bean populations grown at Tigoni during the 2012 long rain season.

| | Vigour | [1]DF (50%) | DM (50%) | ALS | ANT | BCMV | RR | Pods /Plant | Seeds /Pod | 100 Seed Weight | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Vigour | - | | | | | | | | | | |
| DF (50%) | 0.1 | - | | | | | | | | | |
| DM (50%) | 0.0 | 0.7** | - | | | | | | | | |
| ALS | 0.0 | 0.0 | -0.3* | - | | | | | | | |
| ANT | 0.3* | -0.2 | -0.3** | 0.2 | - | | | | | | |
| BCMV | 0.0 | 0.1 | 0.2 | -0.2* | 0.0 | - | | | | | |
| RR | -0.1 | 0.0 | 0.2 | -0.1 | -0.3** | -0.1 | - | | | | |
| Pods/Plant | 0.1 | 0.3** | 0.6** | -0.4** | -0.3** | 0.1 | 0.3** | - | | | |
| Seeds/Pod | -0.1 | 0.3* | 0.6** | -0.5** | -0.4** | 0.0 | 0.2* | 0.7** | - | | |
| 100 Seed Weight | -0.1 | -0.7** | -0.8** | 0.1 | 0.3* | -0.2 | -0.3* | -0.7** | -0.5** | - | |
| Yield (kg/ha) | 0.0 | 0.0 | 0.3** | -0.2* | -0.4** | 0.0 | 0.6** | 0.5** | 0.4** | -0.4** | - |

[1]DF=days to 50% flowering, DM=days to 50% maturity, ALS=angular leaf spot, ANT=anthracnose, BCMV=bean common mosaic virus, RR=*Pythium* root rot.
*, ** Significant at 0.05 and 0.01 probability levels respectively.

**Appendix 16:** Correlation between yield and yield component in 16 $F_1$ bean populations grown at Kabete and Tigoni during the 2012 long rain season.

| | Vigour | DF (50%) | DM (50%) | ALS | ANT | BCMV | RR | Pods /Plant | Seeds /Pod | 100 Seed Weight | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Vigour | - | | | | | | | | | | |
| DF (50%) | 0.3** | - | | | | | | | | | |
| DM (50%) | 0.1 | 0.7** | - | | | | | | | | |
| ALS | 0.2* | 0.0 | -0.2** | - | | | | | | | |
| ANT | 0.4** | -0.1 | -0.2** | 0.4** | - | | | | | | |
| BCMV | 0.2 | 0.2* | 0.0 | 0.0 | 0.2* | - | | | | | |
| RR | 0.2* | -0.1 | -0.1 | -0.1 | -0.2 | 0.1 | - | | | | |
| Pods/Plant | 0.1 | 0.4** | 0.6** | -0.5** | -0.3** | -0.1 | 0.2* | - | | | |
| Seeds/Pod | 0.0 | 0.4** | 0.6** | -0.4** | -0.3** | 0.0 | -0.1 | 0.7** | - | | |
| 100 Seed Weight | -0.3** | -0.7** | -0.8** | 0.1 | 0.2** | -0.1 | -0.2* | -0.6** | -0.5** | - | |
| Yield (kg/ha) | 0.0 | 0.0 | 0.4** | -0.2* | -0.2** | -0.2** | 0.0 | 0.4** | 0.3** | -0.3** | - |

[1]DF=days to 50% flowering, DM=days to 50% maturity, ALS=angular leaf spot, ANT=anthracnose, BCMV=bean common mosaic virus, RR=*Pythium* root rot.
*, ** Significant at 0.05 and 0.01 probability levels respectively.

**Appendix 17:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01NY from Nyeri at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 6.3 | 7.0 | 7.3 | 6.9 |
| 2. G11796 | A | 2 | 5.7 | 7.3 | 8.0 | 7.0 |
| 3. Bolon Bayo | A | 4 | 7.3 | 8.0 | 8.0 | 7.8 |
| 4. Montcalm | A | 8 | 7.3 | 7.7 | 8.3 | 7.8 |
| 5. Amendoin | A | 16 | 7.7 | 7.7 | 8.0 | 7.8 |
| 6. G05686 | A | 32 | 4.0 | 4.3 | 6.3 | 4.9 |
| 7. Pan 72 | MA | 1 | 5.3 | 5.7 | 7.0 | 6.0 |
| 8. G02858 | MA | 2 | 5.7 | 6.3 | 6.7 | 6.2 |
| 9. Flor de Mayo | MA | 4 | 6.3 | 6.7 | 7.0 | 6.7 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 4.3 | 5.0 | 3.4 |
| 12. Cornell 49-242 | MA | 32 | 2.0 | 3.7 | 4.7 | 3.5 |

A=Andean, MA=Mesoamerican

**Appendix 18:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02NY from Nyeri at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 5.3 | 6.0 | 6.7 | 6.0 |
| 2. G11796 | A | 2 | 6.3 | 6.7 | 7.3 | 6.8 |
| 3. Bolon Bayo | A | 4 | 7.3 | 7.7 | 8.7 | 7.9 |
| 4. Montcalm | A | 8 | 5.0 | 5.7 | 6.3 | 5.7 |
| 5. Amendoin | A | 16 | 4.7 | 5.7 | 7.0 | 5.8 |
| 6. G05686 | A | 32 | 1.7 | 2.3 | 2.7 | 2.2 |
| 7. Pan 72 | MA | 1 | 5.3 | 5.7 | 6.0 | 5.7 |
| 8. G02858 | MA | 2 | 5.0 | 5.3 | 5.7 | 5.3 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 7.0 | 8.3 | 6.5 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.3 | 1.7 | 2.3 | 1.8 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.3 | 1.7 | 1.3 |

A=Andean, MA=Mesoamerican

**Appendix 19:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02BR from Bureti at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 3.0 | 3.3 | 3.3 | 3.2 |
| 2. G11796 | A | 2 | 6.3 | 6.7 | 7.3 | 6.8 |
| 3. Bolon Bayo | A | 4 | 5.7 | 6.0 | 6.7 | 6.1 |
| 4. Montcalm | A | 8 | 2.7 | 3.3 | 3.7 | 3.2 |
| 5. Amendoin | A | 16 | 1.7 | 2.0 | 2.3 | 2.0 |
| 6. G05686 | A | 32 | 1.3 | 1.3 | 1.7 | 1.4 |
| 7. Pan 72 | MA | 1 | 2.7 | 2.7 | 3.0 | 2.8 |
| 8. G02858 | MA | 2 | 2.3 | 2.3 | 2.7 | 2.4 |
| 9. Flor de Mayo | MA | 4 | 2.0 | 2.0 | 2.0 | 2.0 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.0 | 1.0 | 1.0 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.0 | 1.0 | 1.0 |

A=Andean, MA=Mesoamerican

**Appendix 20:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01WN from Wundanyi at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.3 | 4.7 | 4.7 | 4.7 |
| 2. G11796 | A | 2 | 7.7 | 8.0 | 8.7 | 8.1 |
| 3. Bolon Bayo | A | 4 | 6.3 | 7.3 | 7.7 | 7.1 |
| 4. Montcalm | A | 8 | 4.7 | 5.3 | 5.7 | 5.2 |
| 5. Amendoin | A | 16 | 5.7 | 6.3 | 7.0 | 6.3 |
| 6. G05686 | A | 32 | 5.7 | 6.3 | 7.0 | 6.3 |
| 7. Pan 72 | MA | 1 | 7.7 | 8.3 | 8.7 | 8.2 |
| 8. G02858 | MA | 2 | 4.7 | 5.3 | 5.7 | 5.2 |
| 9. Flor de Mayo | MA | 4 | 6.3 | 7.0 | 7.3 | 6.9 |
| 10. Mexico 54 | MA | 8 | 2.7 | 3.7 | 4.3 | 3.6 |
| 11. BAT 332 | MA | 16 | 3.3 | 4.3 | 5.0 | 4.2 |
| 12. Cornell 49-242 | MA | 32 | 3.3 | 3.7 | 4.3 | 3.8 |

A=Andean, MA=Mesoamerican

**Appendix 21:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02KK from Kakamega at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 2.7 | 3.7 | 3.7 | 3.4 |
| 2. G11796 | A | 2 | 7.7 | 8.0 | 8.7 | 8.1 |
| 3. Bolon Bayo | A | 4 | 8.7 | 9.0 | 9.0 | 8.9 |
| 4. Montcalm | A | 8 | 4.3 | 5.7 | 6.3 | 5.4 |
| 5. Amendoin | A | 16 | 6.7 | 7.3 | 7.3 | 7.1 |
| 6. G05686 | A | 32 | 1.0 | 1.3 | 1.7 | 1.3 |
| 7. Pan 72 | MA | 1 | 2.7 | 3.0 | 3.3 | 3.0 |
| 8. G02858 | MA | 2 | 2.0 | 2.3 | 2.3 | 2.2 |
| 9. Flor de Mayo | MA | 4 | 5.7 | 6.3 | 6.7 | 6.2 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.0 | 1.7 | 1.2 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.0 | 1.0 | 1.0 |

A=Andean, MA=Mesoamerican

**Appendix 22:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01KG from Kibirigwi at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.3 | 5.3 | 6.0 | 5.2 |
| 2. G11796 | A | 2 | 7.0 | 7.7 | 8.3 | 7.7 |
| 3. Bolon Bayo | A | 4 | 6.3 | 6.7 | 7.0 | 6.7 |
| 4. Montcalm | A | 8 | 6.0 | 6.7 | 7.7 | 6.8 |
| 5. Amendoin | A | 16 | 5.3 | 5.7 | 6.3 | 5.8 |
| 6. G05686 | A | 32 | 1.0 | 1.3 | 1.7 | 1.3 |
| 7. Pan 72 | MA | 1 | 5.7 | 7.0 | 7.3 | 6.7 |
| 8. G02858 | MA | 2 | 5.0 | 5.7 | 6.7 | 5.8 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 5.7 | 6.3 | 5.4 |
| 10. Mexico 54 | MA | 8 | 2.7 | 3.7 | 4.3 | 3.6 |
| 11. BAT 332 | MA | 16 | 3.0 | 3.7 | 4.7 | 3.8 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.0 | 1.0 | 1.0 |

A=Andean, MA=Mesoamerican

**Appendix 23:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01NK from Nakuru at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.0 | 4.7 | 5.3 | 4.7 |
| 2. G11796 | A | 2 | 6.3 | 7.0 | 8.3 | 7.2 |
| 3. Bolon Bayo | A | 4 | 5.3 | 6.0 | 7.3 | 6.2 |
| 4. Montcalm | A | 8 | 4.0 | 4.7 | 6.0 | 4.9 |
| 5. Amendoin | A | 16 | 7.3 | 7.7 | 8.3 | 7.8 |
| 6. G05686 | A | 32 | 1.0 | 1.3 | 1.7 | 1.3 |
| 7. Pan 72 | MA | 1 | 2.3 | 2.7 | 3.0 | 2.7 |
| 8. G02858 | MA | 2 | 1.3 | 1.7 | 2.3 | 1.8 |
| 9. Flor de Mayo | MA | 4 | 1.7 | 2.0 | 2.3 | 2.0 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.0 | 1.0 | 1.0 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.0 | 1.0 | 1.0 |

A=Andean, MA=Mesoamerican

**Appendix 24:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01TY from Othaya at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 5.7 | 6.3 | 7.0 | 6.3 |
| 2. G11796 | A | 2 | 8.7 | 9.0 | 9.0 | 8.9 |
| 3. Bolon Bayo | A | 4 | 7.7 | 8.0 | 8.7 | 8.1 |
| 4. Montcalm | A | 8 | 2.3 | 3.7 | 6.3 | 4.1 |
| 5. Amendoin | A | 16 | 5.0 | 5.7 | 6.3 | 5.7 |
| 6. G05686 | A | 32 | 4.0 | 4.7 | 6.0 | 4.9 |
| 7. Pan 72 | MA | 1 | 2.7 | 5.3 | 6.7 | 4.9 |
| 8. G02858 | MA | 2 | 4.0 | 4.0 | 5.0 | 4.3 |
| 9. Flor de Mayo | MA | 4 | 4.7 | 5.3 | 6.0 | 5.3 |
| 10. Mexico 54 | MA | 8 | 3.0 | 3.7 | 4.3 | 3.7 |
| 11. BAT 332 | MA | 16 | 3.3 | 4.3 | 5.0 | 4.2 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.3 | 1.7 | 1.3 |

A=Andean, MA=Mesoamerican

**Appendix 25:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02KR from Kericho at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 3.0 | 3.3 | 4.0 | 3.4 |
| 2. G11796 | A | 2 | 7.7 | 9.0 | 9.0 | 8.6 |
| 3. Bolon Bayo | A | 4 | 6.3 | 8.0 | 8.3 | 7.5 |
| 4. Montcalm | A | 8 | 7.0 | 7.7 | 9.0 | 7.9 |
| 5. Amendoin | A | 16 | 5.3 | 6.3 | 7.7 | 6.4 |
| 6. G05686 | A | 32 | 3.0 | 4.3 | 5.7 | 4.3 |
| 7. Pan 72 | MA | 1 | 4.7 | 6.0 | 6.7 | 5.8 |
| 8. G02858 | MA | 2 | 1.7 | 2.3 | 3.0 | 2.3 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 5.7 | 6.3 | 5.4 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.3 | 1.7 | 1.3 |
| 12. Cornell 49-242 | MA | 32 | 3.7 | 4.7 | 6.3 | 4.9 |

A=Andean, MA=Mesoamerican

**Appendix 26:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01NJ from Njoro at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 3.7 | 4.3 | 6.0 | 4.7 |
| 2. G11796 | A | 2 | 6.3 | 8.3 | 8.7 | 7.8 |
| 3. Bolon Bayo | A | 4 | 5.7 | 7.0 | 7.0 | 6.6 |
| 4. Montcalm | A | 8 | 4.7 | 6.3 | 7.7 | 6.2 |
| 5. Amendoin | A | 16 | 6.0 | 6.7 | 7.3 | 6.7 |
| 6. G05686 | A | 32 | 3.3 | 3.7 | 4.3 | 3.8 |
| 7. Pan 72 | MA | 1 | 6.3 | 7.0 | 7.7 | 7.0 |
| 8. G02858 | MA | 2 | 5.0 | 5.3 | 6.0 | 5.4 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 4.7 | 6.0 | 5.0 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 2.7 | 3.7 | 4.0 | 3.5 |
| 12. Cornell 49-242 | MA | 32 | 3.7 | 4.3 | 5.3 | 4.4 |

A=Andean, MA=Mesoamerican

**Appendix 27:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01GC from Gucha at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.0 | 4.3 | 5.3 | 4.5 |
| 2. G11796 | A | 2 | 5.3 | 6.3 | 7.3 | 6.3 |
| 3. Bolon Bayo | A | 4 | 4.7 | 5.3 | 6.0 | 5.3 |
| 4. Montcalm | A | 8 | 5.3 | 5.7 | 7.3 | 6.1 |
| 5. Amendoin | A | 16 | 3.0 | 4.3 | 5.7 | 4.3 |
| 6. G05686 | A | 32 | 2.7 | 4.3 | 4.7 | 3.9 |
| 7. Pan 72 | MA | 1 | 5.3 | 5.7 | 6.0 | 5.7 |
| 8. G02858 | MA | 2 | 4.3 | 5.0 | 5.7 | 5.0 |
| 9. Flor de Mayo | MA | 4 | 2.7 | 3.3 | 5.0 | 3.7 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.3 | 1.3 | 1.2 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.0 | 1.0 | 1.0 |

A=Andean, MA=Mesoamerican

**Appendix 28:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02SY from Siaya at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 2.3 | 2.7 | 2.7 | 2.6 |
| 2. G11796 | A | 2 | 6.7 | 8.3 | 9.0 | 8.0 |
| 3. Bolon Bayo | A | 4 | 5.0 | 6.0 | 6.7 | 5.9 |
| 4. Montcalm | A | 8 | 5.7 | 7.3 | 8.7 | 7.2 |
| 5. Amendoin | A | 16 | 2.0 | 2.3 | 3.0 | 2.4 |
| 6. G05686 | A | 32 | 1.0 | 1.0 | 1.0 | 1.0 |
| 7. Pan 72 | MA | 1 | 3.3 | 3.3 | 3.3 | 3.3 |
| 8. G02858 | MA | 2 | 1.3 | 2.0 | 2.3 | 1.9 |
| 9. Flor de Mayo | MA | 4 | 2.7 | 3.3 | 3.7 | 3.2 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.0 | 1.0 | 1.0 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.0 | 1.0 | 1.0 |

A=Andean, MA=Mesoamerican

**Appendix 29:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02NH from Nyahururu at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.0 | 4.3 | 5.0 | 4.4 |
| 2. G11796 | A | 2 | 6.3 | 6.7 | 8.0 | 7.0 |
| 3. Bolon Bayo | A | 4 | 5.3 | 6.0 | 7.0 | 6.1 |
| 4. Montcalm | A | 8 | 5.7 | 6.3 | 7.3 | 6.4 |
| 5. Amendoin | A | 16 | 4.3 | 5.7 | 7.7 | 5.9 |
| 6. G05686 | A | 32 | 2.7 | 4.0 | 4.7 | 3.8 |
| 7. Pan 72 | MA | 1 | 5.3 | 5.7 | 7.3 | 6.1 |
| 8. G02858 | MA | 2 | 3.3 | 4.3 | 5.3 | 4.3 |
| 9. Flor de Mayo | MA | 4 | 3.7 | 4.3 | 5.7 | 4.6 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 2.3 | 4.0 | 5.3 | 3.9 |
| 12. Cornell 49-242 | MA | 32 | 3.4 | 3.7 | 3.7 | 3.6 |

A=Andean, MA=Mesoamerican

**Appendix 30:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02BN from Bungoma at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 2.0 | 2.0 | 2.3 | 2.1 |
| 2. G11796 | A | 2 | 4.7 | 6.3 | 8.0 | 6.3 |
| 3. Bolon Bayo | A | 4 | 2.3 | 3.0 | 3.3 | 2.9 |
| 4. Montcalm | A | 8 | 6.7 | 7.3 | 8.3 | 7.4 |
| 5. Amendoin | A | 16 | 5.7 | 6.0 | 7.7 | 6.5 |
| 6. G05686 | A | 32 | 1.0 | 1.7 | 2.3 | 1.7 |
| 7. Pan 72 | MA | 1 | 2.7 | 3.3 | 3.7 | 3.2 |
| 8. G02858 | MA | 2 | 1.7 | 2.3 | 2.7 | 2.2 |
| 9. Flor de Mayo | MA | 4 | 1.3 | 2.0 | 2.3 | 1.9 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.0 | 1.7 | 1.2 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.0 | 1.0 | 1.0 |

A=Andean, MA=Mesoamerican

**Appendix 31:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01TH from Thika at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.7 | 6.0 | 7.7 | 6.1 |
| 2. G11796 | A | 2 | 7.3 | 8.0 | 8.7 | 8.0 |
| 3. Bolon Bayo | A | 4 | 5.7 | 6.7 | 7.7 | 6.7 |
| 4. Montcalm | A | 8 | 6.0 | 6.3 | 7.3 | 6.5 |
| 5. Amendoin | A | 16 | 4.3 | 6.0 | 6.0 | 5.4 |
| 6. G05686 | A | 32 | 3.3 | 4.0 | 5.3 | 4.2 |
| 7. Pan 72 | MA | 1 | 5.0 | 6.3 | 7.7 | 6.3 |
| 8. G02858 | MA | 2 | 3.3 | 4.0 | 5.7 | 4.3 |
| 9. Flor de Mayo | MA | 4 | 5.0 | 5.7 | 7.0 | 5.9 |
| 10. Mexico 54 | MA | 8 | 3.7 | 4.0 | 4.0 | 3.9 |
| 11. BAT 332 | MA | 16 | 3.0 | 4.7 | 5.3 | 4.3 |
| 12. Cornell 49-242 | MA | 32 | 2.7 | 3.7 | 4.3 | 3.6 |

A=Andean, MA=Mesoamerican

**Appendix 32:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01MC from Machakos at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 3.7 | 5.0 | 6.3 | 5.0 |
| 2. G11796 | A | 2 | 6.0 | 6.3 | 7.7 | 6.7 |
| 3. Bolon Bayo | A | 4 | 5.7 | 6.7 | 7.3 | 6.6 |
| 4. Montcalm | A | 8 | 5.0 | 5.3 | 6.3 | 5.5 |
| 5. Amendoin | A | 16 | 6.3 | 7.0 | 7.0 | 6.8 |
| 6. G05686 | A | 32 | 3.3 | 3.7 | 4.3 | 3.8 |
| 7. Pan 72 | MA | 1 | 4.7 | 5.0 | 6.3 | 5.3 |
| 8. G02858 | MA | 2 | 3.0 | 3.7 | 5.3 | 4.0 |
| 9. Flor de Mayo | MA | 4 | 1.3 | 2.0 | 2.0 | 1.8 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.3 | 1.3 | 1.2 |
| 12. Cornell 49-242 | MA | 32 | 3.7 | 4.0 | 4.3 | 4.0 |

A=Andean, MA=Mesoamerican

**Appendix 33:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01KS from Kisumu at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 3.4 | 3.7 | 3.7 | 3.6 |
| 2. G11796 | A | 2 | 4.7 | 6.3 | 7.7 | 6.2 |
| 3. Bolon Bayo | A | 4 | 6.0 | 7.7 | 9.0 | 7.6 |
| 4. Montcalm | A | 8 | 4.3 | 4.7 | 5.7 | 4.9 |
| 5. Amendoin | A | 16 | 1.0 | 2.0 | 2.3 | 1.8 |
| 6. G05686 | A | 32 | 3.3 | 4.7 | 6.0 | 4.7 |
| 7. Pan 72 | MA | 1 | 1.3 | 1.7 | 2.3 | 1.8 |
| 8. G02858 | MA | 2 | 3.3 | 4.3 | 5.3 | 4.3 |
| 9. Flor de Mayo | MA | 4 | 2.0 | 2.0 | 2.7 | 2.2 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.7 | 2.0 | 1.6 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.3 | 1.7 | 1.3 |

A=Andean, MA=Mesoamerican

**Appendix 34:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02MG from Mugirango at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 3.7 | 5.3 | 6.3 | 5.1 |
| 2. G11796 | A | 2 | 6.0 | 7.7 | 8.7 | 7.5 |
| 3. Bolon Bayo | A | 4 | 5.7 | 7.0 | 8.3 | 7.0 |
| 4. Montcalm | A | 8 | 4.0 | 4.7 | 6.7 | 5.1 |
| 5. Amendoin | A | 16 | 5.3 | 6.0 | 7.3 | 6.2 |
| 6. G05686 | A | 32 | 2.7 | 3.7 | 5.3 | 3.9 |
| 7. Pan 72 | MA | 1 | 4.7 | 6.0 | 6.3 | 5.7 |
| 8. G02858 | MA | 2 | 3.0 | 3.7 | 4.3 | 3.7 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 5.0 | 6.7 | 5.3 |
| 10. Mexico 54 | MA | 8 | 3.0 | 3.7 | 4.0 | 3.6 |
| 11. BAT 332 | MA | 16 | 3.0 | 4.3 | 5.3 | 4.2 |
| 12. Cornell 49-242 | MA | 32 | 3.0 | 3.7 | 4.3 | 3.7 |

A=Andean, MA=Mesoamerican

**Appendix 35:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02MR from Meru Central at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 5.3 | 6.0 | 7.0 | 6.1 |
| 2. G11796 | A | 2 | 5.7 | 6.3 | 7.3 | 6.4 |
| 3. Bolon Bayo | A | 4 | 6.7 | 8.3 | 9.0 | 8.0 |
| 4. Montcalm | A | 8 | 5.3 | 6.0 | 7.7 | 6.3 |
| 5. Amendoin | A | 16 | 4.3 | 5.7 | 6.0 | 5.3 |
| 6. G05686 | A | 32 | 3.0 | 3.7 | 4.3 | 3.7 |
| 7. Pan 72 | MA | 1 | 2.3 | 3.7 | 4.3 | 3.4 |
| 8. G02858 | MA | 2 | 3.3 | 4.0 | 4.7 | 4.0 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 4.7 | 6.3 | 5.1 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.3 | 1.7 | 1.3 |
| 11. BAT 332 | MA | 16 | 2.7 | 4.0 | 4.7 | 3.8 |
| 12. Cornell 49-242 | MA | 32 | 3.0 | 3.7 | 5.0 | 3.9 |

A=Andean, MA=Mesoamerican

**Appendix 36:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02NK from Nakuru at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.3 | 5.3 | 7.0 | 5.5 |
| 2. G11796 | A | 2 | 6.0 | 7.0 | 8.3 | 7.1 |
| 3. Bolon Bayo | A | 4 | 4.7 | 6.0 | 7.7 | 6.1 |
| 4. Montcalm | A | 8 | 3.7 | 5.0 | 5.7 | 4.8 |
| 5. Amendoin | A | 16 | 4.0 | 5.7 | 7.0 | 5.6 |
| 6. G05686 | A | 32 | 3.3 | 3.7 | 4.3 | 3.8 |
| 7. Pan 72 | MA | 1 | 4.7 | 5.3 | 6.0 | 5.3 |
| 8. G02858 | MA | 2 | 4.0 | 4.7 | 5.3 | 4.7 |
| 9. Flor de Mayo | MA | 4 | 3.3 | 5.0 | 5.0 | 4.4 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.3 | 1.7 | 1.3 |
| 11. BAT 332 | MA | 16 | 3.3 | 3.7 | 4.0 | 3.7 |
| 12. Cornell 49-242 | MA | 32 | 3.0 | 4.0 | 4.0 | 3.7 |

A=Andean, MA=Mesoamerican

**Appendix 37:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg04WN from Wundanyi at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 2.7 | 3.3 | 4.0 | 3.3 |
| 2. G11796 | A | 2 | 6.3 | 7.7 | 8.0 | 7.3 |
| 3. Bolon Bayo | A | 4 | 4.7 | 6.0 | 7.3 | 6.0 |
| 4. Montcalm | A | 8 | 3.7 | 5.3 | 6.7 | 5.2 |
| 5. Amendoin | A | 16 | 4.3 | 5.0 | 6.3 | 5.2 |
| 6. G05686 | A | 32 | 1.0 | 1.7 | 3.0 | 1.9 |
| 7. Pan 72 | MA | 1 | 2.0 | 3.3 | 3.7 | 3.0 |
| 8. G02858 | MA | 2 | 3.3 | 4.0 | 4.7 | 4.0 |
| 9. Flor de Mayo | MA | 4 | 1.7 | 2.3 | 3.3 | 2.4 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 3.0 | 4.7 | 5.0 | 4.2 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.3 | 1.7 | 1.3 |

A=Andean, MA=Mesoamerican

**Appendix 38:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01KK from Kakamega at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.0 | 5.7 | 6.0 | 5.2 |
| 2. G11796 | A | 2 | 6.3 | 7.0 | 8.7 | 7.3 |
| 3. Bolon Bayo | A | 4 | 6.0 | 6.7 | 8.0 | 6.9 |
| 4. Montcalm | A | 8 | 4.0 | 4.3 | 4.7 | 4.3 |
| 5. Amendoin | A | 16 | 4.3 | 6.0 | 6.7 | 5.7 |
| 6. G05686 | A | 32 | 3.3 | 4.0 | 4.7 | 4.0 |
| 7. Pan 72 | MA | 1 | 3.7 | 5.3 | 7.0 | 5.3 |
| 8. G02858 | MA | 2 | 4.3 | 5.3 | 6.3 | 5.3 |
| 9. Flor de Mayo | MA | 4 | 3.7 | 5.0 | 6.0 | 4.9 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.3 | 1.7 | 1.3 |
| 11. BAT 332 | MA | 16 | 1.7 | 2.3 | 3.0 | 2.3 |
| 12. Cornell 49-242 | MA | 32 | 3.0 | 3.7 | 4.3 | 3.7 |

A=Andean, MA=Mesoamerican

**Appendix 39:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01MN from Murang'a at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.7 | 5.3 | 6.7 | 5.6 |
| 2. G11796 | A | 2 | 6.0 | 7.3 | 8.7 | 7.3 |
| 3. Bolon Bayo | A | 4 | 6.3 | 7.7 | 9.0 | 7.7 |
| 4. Montcalm | A | 8 | 4.0 | 4.3 | 6.0 | 4.8 |
| 5. Amendoin | A | 16 | 4.3 | 5.3 | 5.7 | 5.1 |
| 6. G05686 | A | 32 | 3.7 | 4.0 | 4.0 | 3.9 |
| 7. Pan 72 | MA | 1 | 3.3 | 4.3 | 6.0 | 4.5 |
| 8. G02858 | MA | 2 | 5.0 | 5.7 | 7.3 | 6.0 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 6.0 | 7.0 | 5.8 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 3.0 | 3.3 | 4.7 | 3.7 |
| 12. Cornell 49-242 | MA | 32 | 3.3 | 4.3 | 4.7 | 4.1 |

A=Andean, MA=Mesoamerican

**Appendix 40:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01MN from Murang'a at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.3 | 5.7 | 7.3 | 5.8 |
| 2. G11796 | A | 2 | 6.0 | 7.3 | 8.0 | 7.1 |
| 3. Bolon Bayo | A | 4 | 5.3 | 7.0 | 7.7 | 6.7 |
| 4. Montcalm | A | 8 | 4.7 | 6.0 | 6.3 | 5.7 |
| 5. Amendoin | A | 16 | 4.3 | 5.7 | 7.3 | 5.8 |
| 6. G05686 | A | 32 | 3.3 | 4.3 | 5.7 | 4.4 |
| 7. Pan 72 | MA | 1 | 3.7 | 5.3 | 6.7 | 5.2 |
| 8. G02858 | MA | 2 | 4.7 | 5.3 | 6.0 | 5.3 |
| 9. Flor de Mayo | MA | 4 | 2.0 | 2.3 | 3.3 | 2.5 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.3 | 1.7 | 2.0 | 1.7 |
| 12. Cornell 49-242 | MA | 32 | 3.0 | 3.7 | 5.0 | 3.9 |

A=Andean, MA=Mesoamerican

**Appendix 41:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01SY from Siaya at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 2.3 | 3.7 | 4.3 | 3.4 |
| 2. G11796 | A | 2 | 5.0 | 5.7 | 6.7 | 5.8 |
| 3. Bolon Bayo | A | 4 | 4.7 | 6.0 | 7.7 | 6.1 |
| 4. Montcalm | A | 8 | 2.3 | 3.7 | 6.3 | 4.1 |
| 5. Amendoin | A | 16 | 2.7 | 3.3 | 3.7 | 3.2 |
| 6. G05686 | A | 32 | 3.4 | 3.7 | 3.7 | 3.6 |
| 7. Pan 72 | MA | 1 | 2.0 | 3.7 | 4.3 | 3.3 |
| 8. G02858 | MA | 2 | 3.3 | 4.0 | 4.7 | 4.0 |
| 9. Flor de Mayo | MA | 4 | 1.7 | 2.3 | 3.0 | 2.3 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.7 | 2.0 | 1.6 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.3 | 1.3 | 1.2 |

A=Andean, MA=Mesoamerican

**Appendix 42:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01NH from Nyahururu at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.0 | 5.3 | 6.7 | 5.3 |
| 2. G11796 | A | 2 | 5.0 | 6.0 | 6.0 | 5.7 |
| 3. Bolon Bayo | A | 4 | 5.7 | 6.3 | 8.0 | 6.7 |
| 4. Montcalm | A | 8 | 4.3 | 5.0 | 5.0 | 4.8 |
| 5. Amendoin | A | 16 | 5.3 | 7.0 | 7.0 | 6.4 |
| 6. G05686 | A | 32 | 3.7 | 4.0 | 4.3 | 4.0 |
| 7. Pan 72 | MA | 1 | 4.3 | 5.7 | 6.7 | 5.6 |
| 8. G02858 | MA | 2 | 4.7 | 5.3 | 6.0 | 5.3 |
| 9. Flor de Mayo | MA | 4 | 3.7 | 5.7 | 6.3 | 5.2 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 3.3 | 3.7 | 4.3 | 3.8 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.0 | 1.3 | 1.1 |

A=Andean, MA=Mesoamerican

**Appendix 43:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01NV from Naivasha at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 3.7 | 4.7 | 5.7 | 4.7 |
| 2. G11796 | A | 2 | 5.7 | 7.0 | 7.3 | 6.7 |
| 3. Bolon Bayo | A | 4 | 5.3 | 6.7 | 8.0 | 6.7 |
| 4. Montcalm | A | 8 | 4.3 | 5.7 | 7.0 | 5.7 |
| 5. Amendoin | A | 16 | 5.0 | 5.0 | 5.3 | 5.1 |
| 6. G05686 | A | 32 | 4.0 | 4.3 | 5.0 | 4.4 |
| 7. Pan 72 | MA | 1 | 5.0 | 5.3 | 6.0 | 5.4 |
| 8. G02858 | MA | 2 | 4.7 | 6.0 | 6.0 | 5.6 |
| 9. Flor de Mayo | MA | 4 | 3.7 | 4.3 | 4.7 | 4.2 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 3.3 | 3.7 | 4.3 | 3.8 |
| 12. Cornell 49-242 | MA | 32 | 3.3 | 4.0 | 4.0 | 3.8 |

A=Andean, MA=Mesoamerican

**Appendix 44:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg06BR from Bureti at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 5.3 | 6.3 | 7.0 | 6.2 |
| 2. G11796 | A | 2 | 5.7 | 8.0 | 9.0 | 7.6 |
| 3. Bolon Bayo | A | 4 | 5.3 | 6.7 | 8.7 | 6.9 |
| 4. Montcalm | A | 8 | 5.0 | 6.3 | 7.0 | 6.1 |
| 5. Amendoin | A | 16 | 5.0 | 6.0 | 6.3 | 5.8 |
| 6. G05686 | A | 32 | 3.7 | 4.0 | 4.0 | 3.9 |
| 7. Pan 72 | MA | 1 | 4.7 | 6.0 | 6.7 | 5.8 |
| 8. G02858 | MA | 2 | 4.3 | 5.7 | 6.0 | 5.3 |
| 9. Flor de Mayo | MA | 4 | 5.3 | 7.3 | 7.7 | 6.8 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 3.7 | 4.0 | 4.7 | 4.1 |
| 12. Cornell 49-242 | MA | 32 | 3.7 | 4.3 | 4.7 | 4.2 |

A=Andean, MA=Mesoamerican

**Appendix 45:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01LD from Eldoret at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 1.7 | 2.3 | 3.0 | 2.3 |
| 2. G11796 | A | 2 | 5.7 | 7.0 | 7.7 | 6.8 |
| 3. Bolon Bayo | A | 4 | 5.3 | 6.7 | 8.3 | 6.8 |
| 4. Montcalm | A | 8 | 5.0 | 5.3 | 7.0 | 5.8 |
| 5. Amendoin | A | 16 | 5.0 | 6.0 | 6.0 | 5.7 |
| 6. G05686 | A | 32 | 3.3 | 4.0 | 4.3 | 3.9 |
| 7. Pan 72 | MA | 1 | 4.3 | 5.7 | 7.3 | 5.8 |
| 8. G02858 | MA | 2 | 3.3 | 4.7 | 6.0 | 4.7 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 5.7 | 6.3 | 5.4 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.0 | 1.7 | 1.2 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.0 | 1.3 | 1.1 |

A=Andean, MA=Mesoamerican

**Appendix 46:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02GC from Gucha at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.7 | 6.0 | 6.3 | 5.7 |
| 2. G11796 | A | 2 | 5.3 | 6.7 | 8.0 | 6.7 |
| 3. Bolon Bayo | A | 4 | 5.7 | 6.3 | 8.3 | 6.8 |
| 4. Montcalm | A | 8 | 6.3 | 8.0 | 8.7 | 7.7 |
| 5. Amendoin | A | 16 | 4.7 | 5.0 | 5.7 | 5.1 |
| 6. G05686 | A | 32 | 3.7 | 4.3 | 5.7 | 4.6 |
| 7. Pan 72 | MA | 1 | 1.0 | 1.3 | 2.0 | 1.4 |
| 8. G02858 | MA | 2 | 4.3 | 5.7 | 7.0 | 5.7 |
| 9. Flor de Mayo | MA | 4 | 5.3 | 6.7 | 7.3 | 6.4 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 3.3 | 4.0 | 4.0 | 3.8 |
| 12. Cornell 49-242 | MA | 32 | 3.3 | 4.3 | 5.3 | 4.3 |

A=Andean, MA=Mesoamerican

**Appendix 47:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02MS from Meru at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 2.0 | 2.0 | 2.0 | 2.0 |
| 2. G11796 | A | 2 | 4.7 | 5.7 | 6.7 | 5.7 |
| 3. Bolon Bayo | A | 4 | 5.7 | 6.0 | 7.0 | 6.2 |
| 4. Montcalm | A | 8 | 4.3 | 4.7 | 5.3 | 4.8 |
| 5. Amendoin | A | 16 | 1.7 | 2.3 | 3.0 | 2.3 |
| 6. G05686 | A | 32 | 3.3 | 3.7 | 4.0 | 3.7 |
| 7. Pan 72 | MA | 1 | 1.7 | 2.0 | 2.0 | 1.9 |
| 8. G02858 | MA | 2 | 3.7 | 5.3 | 5.7 | 4.9 |
| 9. Flor de Mayo | MA | 4 | 1.7 | 2.0 | 2.7 | 2.1 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.0 | 1.0 | 1.0 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.0 | 1.0 | 1.0 |

A=Andean, MA=Mesoamerican

**Appendix 48:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg04GC from Gucha at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 5.0 | 5.3 | 6.3 | 5.5 |
| 2. G11796 | A | 2 | 7.0 | 8.7 | 9.0 | 8.2 |
| 3. Bolon Bayo | A | 4 | 6.7 | 8.0 | 8.7 | 7.8 |
| 4. Montcalm | A | 8 | 5.3 | 6.7 | 7.3 | 6.4 |
| 5. Amendoin | A | 16 | 5.0 | 6.0 | 6.3 | 5.8 |
| 6. G05686 | A | 32 | 4.3 | 5.7 | 6.0 | 5.3 |
| 7. Pan 72 | MA | 1 | 5.7 | 6.3 | 7.7 | 6.6 |
| 8. G02858 | MA | 2 | 5.3 | 6.3 | 7.3 | 6.3 |
| 9. Flor de Mayo | MA | 4 | 4.7 | 5.0 | 5.3 | 5.0 |
| 10. Mexico 54 | MA | 8 | 3.3 | 3.7 | 4.0 | 3.7 |
| 11. BAT 332 | MA | 16 | 4.0 | 5.3 | 5.7 | 5.0 |
| 12. Cornell 49-242 | MA | 32 | 3.7 | 4.7 | 5.3 | 4.4 |

A=Andean, MA=Mesoamerican

**Appendix 49:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg04BR from Bureti at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 5.3 | 6.7 | 7.0 | 6.3 |
| 2. G11796 | A | 2 | 5.7 | 7.0 | 8.3 | 7.0 |
| 3. Bolon Bayo | A | 4 | 6.3 | 7.3 | 8.7 | 7.4 |
| 4. Montcalm | A | 8 | 6.0 | 7.3 | 7.7 | 7.0 |
| 5. Amendoin | A | 16 | 5.3 | 6.0 | 6.0 | 5.8 |
| 6. G05686 | A | 32 | 4.3 | 5.3 | 6.3 | 5.3 |
| 7. Pan 72 | MA | 1 | 4.7 | 6.0 | 7.3 | 6.0 |
| 8. G02858 | MA | 2 | 5.3 | 6.0 | 6.3 | 5.9 |
| 9. Flor de Mayo | MA | 4 | 5.0 | 5.7 | 6.0 | 5.6 |
| 10. Mexico 54 | MA | 8 | 3.7 | 4.0 | 4.0 | 3.9 |
| 11. BAT 332 | MA | 16 | 3.7 | 4.3 | 4.3 | 4.1 |
| 12. Cornell 49-242 | MA | 32 | 4.0 | 4.3 | 4.7 | 4.3 |

A=Andean, MA=Mesoamerican

**Appendix 50:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01BM from Bomet at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.3 | 5.7 | 6.7 | 5.6 |
| 2. G11796 | A | 2 | 5.3 | 7.0 | 7.3 | 6.5 |
| 3. Bolon Bayo | A | 4 | 4.7 | 6.3 | 7.0 | 6.0 |
| 4. Montcalm | A | 8 | 2.7 | 3.3 | 3.3 | 3.1 |
| 5. Amendoin | A | 16 | 2.3 | 2.7 | 3.0 | 2.7 |
| 6. G05686 | A | 32 | 3.3 | 4.0 | 4.3 | 3.9 |
| 7. Pan 72 | MA | 1 | 2.3 | 3.0 | 3.0 | 2.8 |
| 8. G02858 | MA | 2 | 1.3 | 2.0 | 2.3 | 1.9 |
| 9. Flor de Mayo | MA | 4 | 1.7 | 2.3 | 2.7 | 2.2 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.3 | 2.0 | 1.4 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.3 | 1.7 | 1.3 |

A=Andean, MA=Mesoamerican

**Appendix 51:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02TR from Turbo at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.3 | 5.0 | 6.3 | 5.2 |
| 2. G11796 | A | 2 | 6.7 | 7.3 | 8.7 | 7.6 |
| 3. Bolon Bayo | A | 4 | 6.3 | 8.3 | 9.0 | 7.9 |
| 4. Montcalm | A | 8 | 5.3 | 6.0 | 6.7 | 6.0 |
| 5. Amendoin | A | 16 | 6.3 | 6.7 | 7.3 | 6.8 |
| 6. G05686 | A | 32 | 3.7 | 4.3 | 5.0 | 4.3 |
| 7. Pan 72 | MA | 1 | 4.0 | 5.7 | 7.0 | 5.6 |
| 8. G02858 | MA | 2 | 4.7 | 5.3 | 5.7 | 5.2 |
| 9. Flor de Mayo | MA | 4 | 4.0 | 5.0 | 5.3 | 4.8 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.3 | 1.7 | 1.3 |
| 11. BAT 332 | MA | 16 | 3.3 | 4.0 | 4.3 | 3.9 |
| 12. Cornell 49-242 | MA | 32 | 3.7 | 3.7 | 4.0 | 3.8 |

A=Andean, MA=Mesoamerican

**Appendix 52:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01MS from Meru at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.7 | 6.0 | 6.3 | 5.7 |
| 2. G11796 | A | 2 | 6.7 | 7.3 | 9.0 | 7.7 |
| 3. Bolon Bayo | A | 4 | 5.7 | 6.0 | 7.7 | 6.5 |
| 4. Montcalm | A | 8 | 5.0 | 6.3 | 7.0 | 6.1 |
| 5. Amendoin | A | 16 | 5.3 | 5.7 | 6.0 | 5.7 |
| 6. G05686 | A | 32 | 4.3 | 5.0 | 5.3 | 4.9 |
| 7. Pan 72 | MA | 1 | 3.3 | 3.3 | 3.7 | 3.4 |
| 8. G02858 | MA | 2 | 5.3 | 6.0 | 6.3 | 5.9 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 5.7 | 6.3 | 5.4 |
| 10. Mexico 54 | MA | 8 | 1.3 | 1.7 | 2.0 | 1.7 |
| 11. BAT 332 | MA | 16 | 3.3 | 4.3 | 4.7 | 4.1 |
| 12. Cornell 49-242 | MA | 32 | 3.7 | 4.0 | 4.0 | 3.9 |

A=Andean, MA=Mesoamerican

**Appendix 53:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01ST from Sotik at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 2.3 | 3.0 | 3.0 | 2.8 |
| 2. G11796 | A | 2 | 5.7 | 6.3 | 7.7 | 6.6 |
| 3. Bolon Bayo | A | 4 | 4.7 | 5.0 | 6.3 | 5.3 |
| 4. Montcalm | A | 8 | 5.3 | 6.3 | 8.0 | 6.5 |
| 5. Amendoin | A | 16 | 4.0 | 5.0 | 6.3 | 5.1 |
| 6. G05686 | A | 32 | 3.3 | 4.7 | 6.3 | 4.8 |
| 7. Pan 72 | MA | 1 | 5.0 | 6.0 | 7.7 | 6.2 |
| 8. G02858 | MA | 2 | 2.3 | 2.7 | 3.0 | 2.7 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 5.3 | 6.3 | 5.0 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.3 | 1.7 | 1.3 |
| 12. Cornell 49-242 | MA | 32 | 3.3 | 3.7 | 4.0 | 3.7 |

A=Andean, MA=Mesoamerican

**Appendix 54:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01TR from Turbo at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.7 | 6.0 | 7.7 | 6.1 |
| 2. G11796 | A | 2 | 5.7 | 7.7 | 8.7 | 7.4 |
| 3. Bolon Bayo | A | 4 | 6.7 | 8.0 | 8.3 | 7.7 |
| 4. Montcalm | A | 8 | 5.3 | 6.0 | 6.7 | 6.0 |
| 5. Amendoin | A | 16 | 5.7 | 6.7 | 8.0 | 6.8 |
| 6. G05686 | A | 32 | 1.0 | 1.7 | 2.3 | 1.7 |
| 7. Pan 72 | MA | 1 | 4.0 | 5.3 | 7.0 | 5.4 |
| 8. G02858 | MA | 2 | 4.3 | 5.7 | 6.7 | 5.6 |
| 9. Flor de Mayo | MA | 4 | 5.0 | 6.3 | 7.7 | 6.3 |
| 10. Mexico 54 | MA | 8 | 3.3 | 4.0 | 4.0 | 3.8 |
| 11. BAT 332 | MA | 16 | 3.7 | 4.3 | 5.7 | 4.6 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.0 | 1.3 | 1.1 |

A=Andean, MA=Mesoamerican

**Appendix 55:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01MG from Mugirango at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.7 | 6.0 | 7.3 | 6.0 |
| 2. G11796 | A | 2 | 6.3 | 7.7 | 8.3 | 7.4 |
| 3. Bolon Bayo | A | 4 | 7.0 | 7.3 | 8.0 | 7.4 |
| 4. Montcalm | A | 8 | 5.0 | 6.7 | 6.7 | 6.1 |
| 5. Amendoin | A | 16 | 5.3 | 5.7 | 6.0 | 5.7 |
| 6. G05686 | A | 32 | 4.0 | 5.0 | 7.3 | 5.4 |
| 7. Pan 72 | MA | 1 | 6.0 | 6.7 | 7.7 | 6.8 |
| 8. G02858 | MA | 2 | 5.3 | 6.0 | 6.0 | 5.8 |
| 9. Flor de Mayo | MA | 4 | 6.0 | 6.7 | 8.0 | 6.9 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.3 | 2.0 | 1.4 |
| 11. BAT 332 | MA | 16 | 3.7 | 4.0 | 5.7 | 4.5 |
| 12. Cornell 49-242 | MA | 32 | 1.3 | 1.7 | 2.3 | 1.8 |

A=Andean, MA=Mesoamerican

**Appendix 56:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01BN from Bungoma at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 3.0 | 3.0 | 3.3 | 3.1 |
| 2. G11796 | A | 2 | 5.3 | 6.0 | 7.0 | 6.1 |
| 3. Bolon Bayo | A | 4 | 4.3 | 4.7 | 5.0 | 4.7 |
| 4. Montcalm | A | 8 | 4.0 | 5.0 | 5.3 | 4.8 |
| 5. Amendoin | A | 16 | 4.7 | 4.7 | 6.3 | 5.2 |
| 6. G05686 | A | 32 | 1.7 | 3.0 | 3.0 | 2.8 |
| 7. Pan 72 | MA | 1 | 2.7 | 3.0 | 3.0 | 2.9 |
| 8. G02858 | MA | 2 | 4.0 | 4.3 | 5.7 | 4.7 |
| 9. Flor de Mayo | MA | 4 | 1.7 | 2.3 | 3.0 | 2.3 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 3.3 | 3.7 | 4.7 | 3.9 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.0 | 1.0 | 1.0 |

A=Andean, MA=Mesoamerican

**Appendix 57:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg03KK from Kakamega at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.7 | 6.0 | 6.0 | 5.6 |
| 2. G11796 | A | 2 | 6.7 | 8.0 | 9.0 | 7.9 |
| 3. Bolon Bayo | A | 4 | 6.0 | 7.3 | 8.7 | 7.3 |
| 4. Montcalm | A | 8 | 4.7 | 6.0 | 6.7 | 5.8 |
| 5. Amendoin | A | 16 | 5.3 | 6.0 | 6.0 | 5.8 |
| 6. G05686 | A | 32 | 3.7 | 5.3 | 5.7 | 4.9 |
| 7. Pan 72 | MA | 1 | 5.3 | 7.0 | 7.0 | 6.4 |
| 8. G02858 | MA | 2 | 4.7 | 5.0 | 6.3 | 5.3 |
| 9. Flor de Mayo | MA | 4 | 5.7 | 6.7 | 7.0 | 6.6 |
| 10. Mexico 54 | MA | 8 | 3.3 | 4.0 | 4.0 | 3.8 |
| 11. BAT 332 | MA | 16 | 3.7 | 4.3 | 5.7 | 4.6 |
| 12. Cornell 49-242 | MA | 32 | 2.0 | 2.3 | 3.0 | 2.4 |

A=Andean, MA=Mesoamerican

**Appendix 58:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02NV from Naivasha at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 2.7 | 3.0 | 3.7 | 3.1 |
| 2. G11796 | A | 2 | 5.3 | 5.7 | 7.3 | 6.1 |
| 3. Bolon Bayo | A | 4 | 5.0 | 5.7 | 6.0 | 5.6 |
| 4. Montcalm | A | 8 | 4.7 | 6.3 | 7.0 | 6.0 |
| 5. Amendoin | A | 16 | 5.3 | 6.0 | 6.3 | 5.9 |
| 6. G05686 | A | 32 | 3.7 | 5.0 | 6.0 | 4.9 |
| 7. Pan 72 | MA | 1 | 4.3 | 6.0 | 6.0 | 5.4 |
| 8. G02858 | MA | 2 | 2.3 | 3.0 | 3.0 | 2.8 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 5.0 | 5.0 | 4.8 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.3 | 2.0 | 2.7 | 2.0 |
| 12. Cornell 49-242 | MA | 32 | 3.7 | 4.0 | 4.0 | 3.9 |

A=Andean, MA=Mesoamerican

**Appendix 59:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01KM from Kiambu at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.7 | 6.0 | 6.3 | 5.7 |
| 2. G11796 | A | 2 | 6.0 | 7.7 | 9.0 | 7.6 |
| 3. Bolon Bayo | A | 4 | 5.7 | 7.0 | 8.7 | 7.1 |
| 4. Montcalm | A | 8 | 5.0 | 5.7 | 7.3 | 6.0 |
| 5. Amendoin | A | 16 | 4.0 | 5.7 | 6.0 | 5.2 |
| 6. G05686 | A | 32 | 4.3 | 5.3 | 5.7 | 5.1 |
| 7. Pan 72 | MA | 1 | 5.7 | 6.0 | 7.0 | 6.2 |
| 8. G02858 | MA | 2 | 4.7 | 6.0 | 6.7 | 5.8 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 5.7 | 6.3 | 5.4 |
| 10. Mexico 54 | MA | 8 | 1.7 | 2.0 | 2.0 | 1.9 |
| 11. BAT 332 | MA | 16 | 4.0 | 4.7 | 5.0 | 4.6 |
| 12. Cornell 49-242 | MA | 32 | 3.7 | 4.0 | 4.3 | 4.0 |

A=Andean, MA=Mesoamerican

**Appendix 60:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02KY from Kerugoya at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.3 | 5.0 | 6.3 | 5.2 |
| 2. G11796 | A | 2 | 6.0 | 6.7 | 8.0 | 6.9 |
| 3. Bolon Bayo | A | 4 | 5.7 | 7.0 | 8.3 | 7.0 |
| 4. Montcalm | A | 8 | 5.3 | 6.0 | 6.7 | 6.0 |
| 5. Amendoin | A | 16 | 4.7 | 5.3 | 6.3 | 5.4 |
| 6. G05686 | A | 32 | 4.0 | 4.7 | 5.0 | 4.6 |
| 7. Pan 72 | MA | 1 | 4.3 | 5.3 | 6.0 | 5.2 |
| 8. G02858 | MA | 2 | 4.7 | 6.0 | 6.3 | 5.7 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 5.0 | 6.0 | 5.1 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.3 | 2.0 | 1.4 |
| 11. BAT 332 | MA | 16 | 3.7 | 4.0 | 4.0 | 3.9 |
| 12. Cornell 49-242 | MA | 32 | 2.3 | 2.7 | 3.0 | 2.7 |

A=Andean, MA=Mesoamerican

**Appendix 61:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg03GC from Gucha at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.0 | 5.3 | 6.7 | 5.3 |
| 2. G11796 | A | 2 | 6.3 | 7.7 | 9.0 | 7.7 |
| 3. Bolon Bayo | A | 4 | 5.0 | 6.0 | 7.7 | 6.2 |
| 4. Montcalm | A | 8 | 5.3 | 6.7 | 7.0 | 6.3 |
| 5. Amendoin | A | 16 | 5.0 | 5.0 | 6.7 | 5.6 |
| 6. G05686 | A | 32 | 4.3 | 5.0 | 6.3 | 5.2 |
| 7. Pan 72 | MA | 1 | 4.7 | 6.0 | 7.3 | 6.0 |
| 8. G02858 | MA | 2 | 5.0 | 5.7 | 7.0 | 5.9 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 6.0 | 6.3 | 5.5 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.3 | 1.1 |
| 11. BAT 332 | MA | 16 | 3.3 | 4.0 | 4.0 | 3.8 |
| 12. Cornell 49-242 | MA | 32 | 3.0 | 3.7 | 4.0 | 3.6 |

A=Andean, MA=Mesoamerican

**Appendix 62:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02MC from Machakos at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 2.7 | 3.0 | 3.3 | 3.0 |
| 2. G11796 | A | 2 | 5.3 | 6.3 | 7.7 | 6.4 |
| 3. Bolon Bayo | A | 4 | 3.3 | 3.3 | 3.7 | 3.4 |
| 4. Montcalm | A | 8 | 5.0 | 5.7 | 7.0 | 5.9 |
| 5. Amendoin | A | 16 | 5.0 | 5.3 | 6.0 | 5.4 |
| 6. G05686 | A | 32 | 3.3 | 4.0 | 4.7 | 4.0 |
| 7. Pan 72 | MA | 1 | 3.0 | 3.0 | 3.3 | 3.1 |
| 8. G02858 | MA | 2 | 4.3 | 5.7 | 6.3 | 5.4 |
| 9. Flor de Mayo | MA | 4 | 3.3 | 3.3 | 3.3 | 3.3 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 3.3 | 3.7 | 4.3 | 3.8 |
| 12. Cornell 49-242 | MA | 32 | 1.0 | 1.0 | 1.3 | 1.1 |

A=Andean, MA=Mesoamerican

**Appendix 63:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg05GC from Gucha at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.3 | 5.7 | 6.3 | 5.4 |
| 2. G11796 | A | 2 | 6.0 | 7.3 | 9.0 | 7.4 |
| 3. Bolon Bayo | A | 4 | 5.7 | 8.0 | 8.3 | 7.3 |
| 4. Montcalm | A | 8 | 4.3 | 5.7 | 7.3 | 5.8 |
| 5. Amendoin | A | 16 | 5.3 | 6.0 | 6.7 | 6.0 |
| 6. G05686 | A | 32 | 2.3 | 4.0 | 5.3 | 3.9 |
| 7. Pan 72 | MA | 1 | 4.3 | 5.7 | 6.0 | 5.3 |
| 8. G02858 | MA | 2 | 5.0 | 5.3 | 5.7 | 5.3 |
| 9. Flor de Mayo | MA | 4 | 2.7 | 3.3 | 3.7 | 3.2 |
| 10. Mexico 54 | MA | 8 | 1.3 | 2.0 | 2.3 | 1.9 |
| 11. BAT 332 | MA | 16 | 2.0 | 2.3 | 2.3 | 2.2 |
| 12. Cornell 49-242 | MA | 32 | 3.3 | 4.3 | 4.7 | 4.1 |

A=Andean, MA=Mesoamerican

**Appendix 64:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01KR from Kericho at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 3.7 | 4.3 | 4.7 | 4.2 |
| 2. G11796 | A | 2 | 6.3 | 7.0 | 7.3 | 6.9 |
| 3. Bolon Bayo | A | 4 | 5.0 | 6.3 | 7.7 | 6.3 |
| 4. Montcalm | A | 8 | 3.3 | 4.3 | 5.3 | 4.3 |
| 5. Amendoin | A | 16 | 4.7 | 6.0 | 6.7 | 5.8 |
| 6. G05686 | A | 32 | 2.3 | 2.7 | 3.0 | 2.7 |
| 7. Pan 72 | MA | 1 | 5.0 | 6.0 | 6.0 | 5.7 |
| 8. G02858 | MA | 2 | 4.3 | 6.0 | 6.0 | 5.4 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 5.0 | 5.0 | 4.8 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.3 | 2.0 | 2.3 | 1.9 |
| 12. Cornell 49-242 | MA | 32 | 3.3 | 4.0 | 4.0 | 3.8 |

A=Andean, MA=Mesoamerican

**Appendix 65:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01KN from Kangema at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.3 | 5.7 | 6.3 | 5.4 |
| 2. G11796 | A | 2 | 6.3 | 7.7 | 9.0 | 7.7 |
| 3. Bolon Bayo | A | 4 | 7.7 | 9.0 | 9.0 | 8.6 |
| 4. Montcalm | A | 8 | 5.3 | 6.0 | 6.0 | 5.8 |
| 5. Amendoin | A | 16 | 5.7 | 6.0 | 7.0 | 6.2 |
| 6. G05686 | A | 32 | 2.3 | 3.7 | 6.3 | 4.1 |
| 7. Pan 72 | MA | 1 | 2.0 | 3.7 | 4.3 | 3.3 |
| 8. G02858 | MA | 2 | 5.3 | 6.0 | 7.0 | 6.1 |
| 9. Flor de Mayo | MA | 4 | 5.0 | 5.7 | 6.3 | 5.7 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.3 | 1.3 | 1.2 |
| 11. BAT 332 | MA | 16 | 3.0 | 4.3 | 5.7 | 4.3 |
| 12. Cornell 49-242 | MA | 32 | 3.0 | 3.7 | 4.3 | 3.7 |

A=Andean, MA=Mesoamerican

**Appendix 66:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg03WN from Wundanyi at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.7 | 5.3 | 6.0 | 5.3 |
| 2. G11796 | A | 2 | 6.3 | 7.3 | 8.7 | 7.4 |
| 3. Bolon Bayo | A | 4 | 5.3 | 7.0 | 8.0 | 6.8 |
| 4. Montcalm | A | 8 | 5.0 | 6.0 | 6.3 | 5.8 |
| 5. Amendoin | A | 16 | 4.7 | 5.7 | 6.0 | 5.5 |
| 6. G05686 | A | 32 | 4.0 | 4.7 | 6.3 | 5.0 |
| 7. Pan 72 | MA | 1 | 4.7 | 6.0 | 6.3 | 5.7 |
| 8. G02858 | MA | 2 | 4.3 | 6.0 | 6.0 | 5.4 |
| 9. Flor de Mayo | MA | 4 | 5.0 | 5.3 | 6.7 | 5.7 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.7 | 2.0 | 1.6 |
| 11. BAT 332 | MA | 16 | 1.7 | 2.3 | 3.0 | 2.3 |
| 12. Cornell 49-242 | MA | 32 | 3.7 | 4.0 | 4.0 | 3.9 |

A=Andean, MA=Mesoamerican

**Appendix 67:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02MB from Embu at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 5.7 | 6.3 | 7.0 | 6.3 |
| 2. G11796 | A | 2 | 6.7 | 8.0 | 8.7 | 7.8 |
| 3. Bolon Bayo | A | 4 | 6.0 | 7.3 | 9.0 | 7.4 |
| 4. Montcalm | A | 8 | 6.0 | 6.0 | 7.7 | 6.6 |
| 5. Amendoin | A | 16 | 4.7 | 6.0 | 7.0 | 5.9 |
| 6. G05686 | A | 32 | 4.3 | 5.3 | 6.0 | 5.2 |
| 7. Pan 72 | MA | 1 | 4.0 | 5.0 | 6.7 | 5.2 |
| 8. G02858 | MA | 2 | 4.0 | 4.7 | 5.0 | 4.6 |
| 9. Flor de Mayo | MA | 4 | 2.3 | 2.7 | 3.0 | 2.7 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 1.0 | 1.7 | 2.3 | 1.7 |
| 12. Cornell 49-242 | MA | 32 | 4.0 | 4.0 | 4.7 | 4.2 |

A=Andean, MA=Mesoamerican

**Appendix 68:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01BR from Bureti at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 5.0 | 5.3 | 6.3 | 5.5 |
| 2. G11796 | A | 2 | 5.3 | 6.0 | 7.3 | 6.2 |
| 3. Bolon Bayo | A | 4 | 5.7 | 6.7 | 8.0 | 6.8 |
| 4. Montcalm | A | 8 | 5.3 | 5.7 | 6.0 | 5.7 |
| 5. Amendoin | A | 16 | 5.0 | 5.7 | 7.0 | 5.9 |
| 6. G05686 | A | 32 | 3.7 | 5.0 | 5.7 | 4.8 |
| 7. Pan 72 | MA | 1 | 5.0 | 5.0 | 6.7 | 5.6 |
| 8. G02858 | MA | 2 | 5.3 | 6.0 | 6.7 | 6.0 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 6.0 | 6.0 | 5.4 |
| 10. Mexico 54 | MA | 8 | 3.3 | 3.7 | 4.7 | 3.9 |
| 11. BAT 332 | MA | 16 | 2.0 | 3.7 | 4.3 | 3.3 |
| 12. Cornell 49-242 | MA | 32 | 2.3 | 3.7 | 6.3 | 4.1 |

A=Andean, MA=Mesoamerican

**Appendix 69:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01TG from Tigoni at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 5.3 | 6.3 | 7.0 | 6.2 |
| 2. G11796 | A | 2 | 5.3 | 6.7 | 7.7 | 6.6 |
| 3. Bolon Bayo | A | 4 | 6.7 | 8.3 | 9.0 | 8.0 |
| 4. Montcalm | A | 8 | 5.7 | 6.0 | 8.0 | 6.7 |
| 5. Amendoin | A | 16 | 4.3 | 5.7 | 6.0 | 5.3 |
| 6. G05686 | A | 32 | 3.0 | 3.7 | 4.7 | 3.8 |
| 7. Pan 72 | MA | 1 | 2.3 | 3.7 | 4.3 | 3.4 |
| 8. G02858 | MA | 2 | 3.7 | 4.0 | 4.7 | 4.1 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 4.7 | 6.3 | 5.1 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.3 | 1.7 | 1.3 |
| 11. BAT 332 | MA | 16 | 2.7 | 4.0 | 4.7 | 3.8 |
| 12. Cornell 49-242 | MA | 32 | 3.0 | 3.7 | 5.3 | 4.0 |

A=Andean, MA=Mesoamerican

**Appendix 70:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02TG from Tigoni at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 5.0 | 6.0 | 6.3 | 5.8 |
| 2. G11796 | A | 2 | 5.3 | 6.3 | 8.0 | 6.5 |
| 3. Bolon Bayo | A | 4 | 5.7 | 6.3 | 8.3 | 6.8 |
| 4. Montcalm | A | 8 | 6.3 | 8.0 | 8.7 | 7.7 |
| 5. Amendoin | A | 16 | 5.0 | 5.0 | 5.7 | 5.2 |
| 6. G05686 | A | 32 | 3.7 | 4.3 | 5.7 | 4.6 |
| 7. Pan 72 | MA | 1 | 1.0 | 1.7 | 2.0 | 1.6 |
| 8. G02858 | MA | 2 | 4.3 | 5.7 | 7.0 | 5.7 |
| 9. Flor de Mayo | MA | 4 | 5.3 | 6.7 | 7.7 | 6.7 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.0 | 1.0 | 1.0 |
| 11. BAT 332 | MA | 16 | 3.3 | 4.0 | 4.0 | 3.8 |
| 12. Cornell 49-242 | MA | 32 | 3.3 | 4.3 | 5.3 | 4.3 |

A=Andean, MA=Mesoamerican

**Appendix 71:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg01KB from Kabete at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 5.3 | 6.7 | 7.3 | 6.4 |
| 2. G11796 | A | 2 | 5.7 | 7.0 | 8.3 | 7.0 |
| 3. Bolon Bayo | A | 4 | 6.3 | 7.3 | 8.7 | 7.4 |
| 4. Montcalm | A | 8 | 6.0 | 7.3 | 7.7 | 7.0 |
| 5. Amendoin | A | 16 | 5.3 | 6.3 | 6.3 | 6.0 |
| 6. G05686 | A | 32 | 4.3 | 5.3 | 6.3 | 5.3 |
| 7. Pan 72 | MA | 1 | 4.7 | 6.0 | 7.3 | 6.0 |
| 8. G02858 | MA | 2 | 5.3 | 6.0 | 6.3 | 5.9 |
| 9. Flor de Mayo | MA | 4 | 5.0 | 6.0 | 6.0 | 5.7 |
| 10. Mexico 54 | MA | 8 | 3.7 | 4.0 | 4.0 | 3.9 |
| 11. BAT 332 | MA | 16 | 3.7 | 4.3 | 4.3 | 4.1 |
| 12. Cornell 49-242 | MA | 32 | 4.0 | 4.3 | 5.0 | 4.4 |

A=Andean, MA=Mesoamerican

**Appendix 72:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg02KB from Kabete at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 4.0 | 5.3 | 6.3 | 5.2 |
| 2. G11796 | A | 2 | 6.0 | 7.7 | 8.7 | 7.5 |
| 3. Bolon Bayo | A | 4 | 5.7 | 7.0 | 8.3 | 7.0 |
| 4. Montcalm | A | 8 | 4.0 | 4.7 | 6.7 | 5.1 |
| 5. Amendoin | A | 16 | 5.3 | 6.0 | 7.7 | 6.3 |
| 6. G05686 | A | 32 | 2.7 | 3.7 | 5.3 | 3.9 |
| 7. Pan 72 | MA | 1 | 4.7 | 6.0 | 6.3 | 5.7 |
| 8. G02858 | MA | 2 | 3.0 | 3.7 | 4.3 | 3.7 |
| 9. Flor de Mayo | MA | 4 | 4.3 | 5.0 | 6.7 | 5.3 |
| 10. Mexico 54 | MA | 8 | 3.0 | 3.7 | 4.0 | 3.6 |
| 11. BAT 332 | MA | 16 | 3.0 | 4.3 | 5.3 | 4.2 |
| 12. Cornell 49-242 | MA | 32 | 3.0 | 3.7 | 4.7 | 3.8 |

A=Andean, MA=Mesoamerican

**Appendix 73:** Reaction of 12 international angular leaf spot differential cultivars to inoculation with *P. griseola* isolate Pg03KB from Kabete at 12, 17 and 21 days after inoculation (DAI).

| Differential Genotypes | Gene pool | Binary value | Disease score | | | Average Score |
|---|---|---|---|---|---|---|
| | | | 12 DAI | 17 DAI | 21 DAI | |
| 1. Don Timoteo | A | 1 | 3.7 | 5.7 | 6.0 | 5.1 |
| 2. G11796 | A | 2 | 6.3 | 7.3 | 8.7 | 7.4 |
| 3. Bolon Bayo | A | 4 | 6.0 | 6.7 | 8.0 | 6.9 |
| 4. Montcalm | A | 8 | 4.0 | 4.3 | 4.7 | 4.3 |
| 5. Amendoin | A | 16 | 4.3 | 6.3 | 7.0 | 5.9 |
| 6. G05686 | A | 32 | 3.3 | 4.0 | 4.7 | 4.0 |
| 7. Pan 72 | MA | 1 | 3.7 | 5.7 | 7.3 | 5.7 |
| 8. G02858 | MA | 2 | 4.3 | 5.3 | 6.3 | 5.3 |
| 9. Flor de Mayo | MA | 4 | 3.7 | 5.0 | 6.0 | 4.9 |
| 10. Mexico 54 | MA | 8 | 1.0 | 1.3 | 1.7 | 1.3 |
| 11. BAT 332 | MA | 16 | 1.7 | 2.3 | 3.7 | 2.7 |
| 12. Cornell 49-242 | MA | 32 | 3.0 | 3.7 | 4.3 | 3.7 |

A=Andean, MA=Mesoamerican