CHARACTERIZATION AND SELECTION FOR ANTHRACNOSE AND *PYTHIUM* ROOT ROT RESISTANCE IN COMMON BEAN LANDRACES GROWN IN KENYA

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DECLARATION

This thesis is my original work and has not been presented elsewhere for a degree or any other award

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DEDICATION

To my loving mother Susan Odari for her boundless love and support. My gratitude goes to my family and friends for their unconditional support.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BC	Backcross
Bp	Base pair
BSA	Bovine serum albumin
CABI	Center of Agriculture and Bioscience International
CBB	Common bacterial blight
cM	Centimorgan
CMA	Corn meal agar
CTAB	Cetyl Trimethylammonium Bromide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DSI	Disease severity index
g	Grams
F_1	First filial generation
F_2	Second filial generation
F_3	Third filial generation
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
Ha	Hectare
hPAGE	Horizontal polyacrylamide gel electrophoresis
Kg	Kilogram
LG	Linkage group
MAS	Marker assisted selection
masl	Metres above sea level
ml	Millilitre
mM	Millimolar
mm	Millimeter
µM	Micromolar
µL	Microlitre
ng	Nanogram
PC	Principal component

PCA	Principal component analysis
PIC	Polymorphism information content
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
pH	Potential of hydrogen
PSI	Pounds per square inch
RILs	Recombinant inbred lines
SSR	Simple sequence repeats
SCAR	Sequence characterized amplified region
TWA	Tap water agar
UPGMA	Unweighted pair group method with arithmetic mean

LIST OF SYMBOLS & CHEMICAL FORMULAS

H’	Shannon-Weiner diversity index

ABSTRACT

Common bean (*Phaseolus vulgaris L.*) production in Kenya has not reached its potential yield due to various abiotic and biotic factors. Anthracnose, caused by *Colletotrichum lindemunthianum* and root rots caused by *Pythium* species are major production constraints. Landraces are preferred by farmers in Kenya due to yield stability and tolerance to some stresses. However, there is little information on the genetic diversity of the landraces and the level of resistance to anthracnose and *Pythium* root rot. The current study therefore focused on the following objectives (i) to characterize common bean landraces in Kenya based on morphological and simple sequence repeat (SSR) variations, (ii) screen for *Pythium* root rot and anthracnose resistance among Kenyan landraces and (iii) introgress anthracnose and *Pythium* resistance genes into farmer-preferred landraces. The study landraces were collected from farmers' fields and local markets in western, central and eastern Kenya. The genetic diversity within the landraces was determined based on morphological data that was obtained from field experiments and simple sequence repeats (SSRs) markers. Commercial genotypes were included as checks in the study. The results showed significant differences ($P \leq 0.05$) among the genotypes for days to 50% flowering, days to plant maturity, leaf length, leaf width, number of pods per plant, number of seeds per pod, pod length, plant height, 100 seed weight and seed yield per plot, indicating high variability among the genotypes. In addition, 51 alleles were obtained from a set of 22 SSR markers, with a mean of 2.32 alleles. Through morphological cluster analysis, 4 distinct clusters were identified. The first cluster consisted of 20 large-seeded genotypes, the second cluster included 39 medium-seeded genotypes with a mixture of determinate and semi-determinate growth habits, the third cluster contained six medium-seeded indeterminate genotypes while the fourth cluster comprised of 34 small-seeded genotypes. Molecular analysis grouped the germplasm into three clusters. Cluster 1 was majorly composed of commercial genotypes of all seed sizes. Cluster 2 comprised of medium seeded genotypes while the third cluster comprised a mixture of genotypes with no unique observation within the clusters. Under greenhouse conditions, majority of the landraces were moderately resistant to anthracnose while most of the small seeded landraces were moderately resistant to *Pythium* root rot. None of the landraces possessed the molecular markers that are linked to anthracnose and *Pythium* root rot resistances. Three farmer-preferred landraces were selected for introgression of genes that confer resistance to anthracnose and *Pythium* root rot. The anthracnose differential cultivar, *G2333*, was used as the donor parent for anthracnose resistance in a marker-assisted backcrossing scheme, while *KK 8* was used as the donor parent for *Pythium* root rot resistance. Utilization of molecular markers enabled the development of 99 lines that carried *Co-4* gene (anthracnose) and *Pyult1* gene (*Pythium*). The introgression of disease resistance genes in landraces will ultimately enable bean growers achieve greater yields contributing to high income and improvement of their livelihoods. The information that was obtained from characterization of the landraces will inform breeders on suitable parents to use in breeding programs.

CHAPTER ONE

INTRODUCTION

1.1. Background information

The common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes in the world and also produced in Sub-Saharan Africa (FAOSTAT, 2021). Sixty two percent of production of this crop is in East and Central African countries, hence the most important region for the crop in the African continent (Farrow & Muthoni-Andriatsitohaina, 2020). In Kenya, the crop is a major staple second to maize (*Zea mays*) (One Acre Fund, 2016), serving as a vital source of dietary protein (Messina, 2014). Common bean is rich in protein content (18-32%) with a substantial amount of essential elements such as phosphorus (P), potassium (K), iron (Fe), calcium (Ca) and vitamins (thiamin, riboflavin, niacin, folic acid, B_6) (Tajini et al., 2014).

Bean belongs to the genus *Phaseolus*, family *Leguminosae* and sub-family *Papilionoideae* (OECD, 2016). Diversification of wild beans started in South and Central America leading to their domestication in the Southern and Northern ends of each region giving independent origin to the Andean and Mesoamerican domesticates (Gepts et al., 1988; Angioi et al., 2010; Bitocchi et al., 2017). These later followed parallel pathways of dissemination through the world generating new secondary centers of diversity in Africa and Asia (Cortés, 2013). The common bean was introduced to the highlands of Eastern Africa about 400 years ago which are now serving as a secondary genetic diversity center (CIAT, 1989; Wortmann et al., 1998; Asfaw et al., 2009).

Morphological and molecular characterization are essential tools in plant breeding and genetic research, providing insights into the diversity, traits, and relationships within plant populations (Govindaraj et al., 2015). Characterization combines traditional observational methods with modern molecular techniques like simple sequence repeats (SSRs) (Dutta et al., 2016). Morphological characterization involves the study and measurement of visible traits encompassing a wide range of features, including plant architecture, leaf shape, flower colour, pod length, seed size, and other agronomically important traits (Hegay et al., 2014). Morphological characterization provides breeders with valuable information about the phenotypic diversity present in bean populations aiding in selecting parental lines for breeding programs and identifying desirable traits that contribute to yield, disease resistance and adaptability (Govindaraj et al., 2015).

Molecular characterization explores the genetic makeup of organisms. Simple sequence repeats are short repeated DNA sequences scattered throughout the genome where they exhibit high variability in terms of repeat number and are codominant, making them valuable tools for assessing genetic diversity, population structure, and relationships among individuals or varieties (Vidak et al., 2017). The SSR markers are amplified using polymerase chain reaction (PCR) and the resulting fragment sizes are used to create unique genetic profiles for different bean varieties. The presence or absence of specific SSR alleles allows researchers to distinguish between closely related genotypes and assess their genetic relatedness (Blair et al., 2011). The SSR-based molecular characterization provides a higher resolution of genetic diversity compared to morphological traits alone, enabling researchers to identify unique and potentially valuable genotypes for breeding programs (Blair et al., 2012). The integration of morphological and molecular characterization using SSRs offers a comprehensive approach to understanding the genetic diversity of common bean populations (Scarano et al., 2014).

Small-scale subsistence farmers in Kenya extensively cultivate common beans in regions with moderate to high rainfall and the primary production areas in the Kenya are situated at altitudes ranging from 1000 to 2000 masl (Kimiti et al., 2009). The main types grown can be classified as red haricot types, red/purple mottled (red mottled), pintos or sugars, Canadian Wonder, purple/grey speckled, yellows and blacks (Katungi et al., 2009). The various classes of beans are majorly grown based on farmer and consumer preferences within a region. The growth habits of common beans exhibit variation, ranging from determinate bush types to indeterminate extreme climbers and among these, bush types are the most commonly cultivated in Africa (Buruchara, 2007).

Beans in Kenya are grown under poor agronomic practices, often intercropped with other crops in low fertile soils, periodic water stress and diseases and insect pests infestation (Mutai et al., 2019; Esilaba et al., 2021). This generally results in low grain yields of about 568 kg/ha compared to yields of 1,500 kg/ha-2,500 kg/ha often reported (FAOSTAT, 2021). The major common bean diseases that lead to yield losses in Kenya are angular leaf spot (*Pseudocercospora griseola*), anthracnose (*Colletotrichum lindemuthianum*), rust (*Uromyces appendiculatus*), common bacterial blight (CBB) (*Xanthomonas axonopodis* pv. *phaseoli* (*Xap*)), bean common mosaic virus (BCMV) caused by a *Potyvirus*, fusarium wilt (*Fusarium oxysporum*) and root rots caused by

Rhizoctonia solani, Macrophomina phaseolina, Pythium spp. and *Fusarium solani* (Farrow & Muthoni-Andriatsitohaina, 2020). With most common bean diseases being seed borne, the transmission from the informal seed systems intensifies disease spread.

Bean root rot caused by *Pythium* spp. is a one of the most damaging disease affecting common beans in East and Central Africa (Wortmann *et al.,* 1998). The pathogen causes a wet rot of the seedling, either before or after emergence where the pith of the stem is attacked leading to yellowing of leaves, wilting and eventually death (Bost, 2006). Some *Pythium* species are favoured by cool temperatures, whereas others are favoured by warm temperatures but are all favoured by wet conditions (Owen-Going *et al.,* 2008). Like anthracnose, the use of resistant bean varieties is the most effective, economical and environmentally sustainable strategy to control *Pythium* root rot disease (Papias *et al.,* 2016). Resistance to *Pythium* root rot is conditioned by a single dominant gene (Otsyula *et al.,* 2003; Nzungize *et al.,* 2011). The genotypes RWR 719, AND1062 and SCAM-80-CM/15 have been used as donors for resistance against the virulent and predominant *Pythium* spp. in breeding programs in East and Central Africa region (Otsyula *et al.,* 2003).

Bean anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams. Scrib, can affect all aerial plant parts at all stages of development (Agrios, 2005; Halvorson, 2015). Symptoms appear on aerial parts of the plant causing black shrunken lesions with flesh-coloured spores on pods (Kelly & Vallejo, 2004). The disease is more prevalent in temperate and sub-tropical climates with its development favoured by moderate temperature with excessive humidity (Sharma *et al.,* 2019; Kamiri *et al*., 2021). The use of resistant genotypes is the most successful, efficient and safe approach of managing anthracnose in common beans (Meziadi *et al.*, 2016). However, breakdown of resistance has been observed due to several physiological races as well as diversity within the same pathogen race (Sharma *et al.,* 2019). The pyramiding of different race-specific resistance alleles could be used as a strategy for developing broad and durable resistance to a large number of races (Souza *et al.,* 2014).

Landraces have been preferred by farmers since they can tolerate biotic and abiotic stresses, high yield stability and intermediate yield level (Anunda et al., 2019). The landraces have a long history, more than the ephemeral lifespan of modern genotypes and are related with one specific geographical location, as compared with commercial genotypes which are cultivated in diverse locations after release (Hawkes, 2014). Since landraces are associated with specific locations, they

end up taking the name of the location being cultivated (Villa et al., 2006; Musibau et al., 2018). This study therefore focused on genetic characterization of Kenyan common bean landraces in relation to their resistance to *Pythium* and anthracnose resistances, to facilitate in the establishment of breeding lines that can be advanced for release as new genotypes.

1.2. Statement of the problem

The annual common bean demand in Kenya is estimated at 755,000 tonnes which is below the estimated annual production of about 600,000 tonnes (Duku et al., 2020). Insufficient quality seed supply systems together with poor access to the seed, decline in arable land, unimproved technologies, pests and diseases greatly contribute to poor yields. Farmers majorly rely on farm-saved seed and landraces. A large number of landraces are cultivated in medium to high altitude zones in Kenya where beans have been traditionally grown over decades. Despite the importance of these landraces in Kenyan farming systems, these landraces are often attacked by pests and diseases since they were not improved through organized breeding schemes that focus on specific constraints.

Some major common bean diseases in Kenya like root rots and anthracnose have seriously limited the number of genotypes grown by farmers as most landraces are completely susceptible to these diseases. Yield reduction of up to 70% of some popular landraces and local genotypes due to *Pythium* root rot and anthracnose has been reported in Kenya (Otsyula *et al.,* 2003). Farmers recycle seed resulting in transmission of infections across cropping seasons since the diseases are majorly seed borne. Resource poor farmers are not able to purchase chemicals to control these diseases. In this regard, host plant resistance is the most effective and sustainable management method for *Pythium* root rot and anthracnose diseases of common bean.

Breeders use host plant resistance by exploiting the existing genetic diversity among crop species to create variation, hence the importance of genetic information for any successful plant breeding scheme. The use of molecular markers in genetic diversity studies hastens the genetic characterization process. Conventional breeding comes with some limitations whereby it is a relatively slow process and the probability of missing the targeted gene is high. This study therefore focuses on improving common bean landraces by understanding their genetic diversity and developing breeding populations with resistance to anthracnose and *Pythium* root rots.

1.3. Justification

Landraces are highly preferred in terms of certain properties, such as high quality and nutritional characteristics, local adaptation and low input requirements (Mavromatis et al., 2013). They are believed to have the capacity to sensitively respond to even minor environmental influences (Weigel & Nordborg, 2015). Landraces have been grown by the farmers for a long period of time and they have become part of their livelihood. This is evident from the fact that though up to 85% of farmers have adopted and sown new cultivars (Odendo et al., 2011), farmers still grow landraces on some portions of their land (Kiptabut, 2016). Improving these landraces for disease resistance will therefore increase yield and income for Kenyan small-holder farmers.

As a basic step in crop improvement, genetic diversity studies give details on genotypes of interest hence increasing the probability of obtaining superior genotypes in the progeny (Silva et al., 2008). Plant breeders explore the diversity in plant genetic resources to develop new and improved genotypes with desirable characteristics. Various techniques have been used for genetic diversity studies including morphological traits, biochemical and DNA (or molecular) markers. Molecular markers often complement the other two types of markers because they are robust and have fewer limitations, providing more information about particular germplasm. Various molecular markers are available for genetic diversity studies and for marker-assisted selection (MAS) in plant breeding. However, effectiveness of using molecular markers depends on the cost of the platform, efficiency and the quality of data generated. In this regard, simple sequence repeats (SSRs) are preferred for genetic diversity studies and MAS because of their co-dominant nature and together with high information content contained in the SSR loci (Blair et al., 2003). Understanding the genetic diversity that exists among common bean landraces in Kenya will enable the selection of genetically divergent parents that can be crossed to bring forth the highest heterotic effects and also a wide genetic variability in segregating generations.

The pyramiding of different race-specific resistance alleles could be used as a strategy for developing broad and durable resistance to a large number of anthracnose races and *Pythium* root rot (Souza et al. 2014; Nzungize *et al.*, 2011). A number of genes that confer resistance to anthracnose and one gene for *Pythium* root rot resistance in common bean, have been characterized and molecular markers linked to the genes developed to aid crop improvement. Classical breeding methods have been used previously in Kenya resulting in long periods of cultivar development

(Kimani and Mwang'ombe, 2007). Therefore, incorporating marker technology will enhance cultivar development with high precision while reducing the time taken to release cultivars.

1.4. Objectives

1.4.1. General objective

To improve common bean landraces by developing breeding lines with host resistance to *Pythium* root rot and anthracnose, for medium and high altitude zones of Kenya.

1.4.2. Specific objectives

1. To determine the genetic diversity of common bean landraces grown in medium and high-altitude zones of Kenya based on morphological and simple sequence repeat variations.
2. To screen common bean local genotypes and landraces for *Pythium* root rot and anthracnose resistance under greenhouse conditions and by sequence characterized amplified regions (SCAR) markers.
3. To introgress genes that confer resistance to *Pythium* root rot and anthracnose in common bean landraces using SCAR markers.

1.5. Hypotheses

1. Common bean landraces and genotypes grown in Kenya have no significant variations based on morphological characteristics and molecular markers.
2. There is no significant difference in resistance to anthracnose and *Pythium* root rot among common bean genotypes grown in Kenya.
3. Common bean breeding lines with introgressed genes will not confer resistance to anthracnose and *Pythium* root rot.

CHAPTER TWO

LITERATURE REVIEW

2.1. Common bean production in Kenya

Common bean production in Kenya has been increasing progressively but at a slower rate as compared to Uganda and Tanzania. Total production in the country in 2021 was 666,000 tonnes, a drop from the previous year where it was 774,365,160 tonnes (FAOSTAT, 2021). In Kenya, beans grow well at an elevation range of 1000-2000 masl, however, they may grow satisfactorily outside these limits (Kimiti et al., 2009). Beans grow well in areas experiencing rainfall range of 750-2000 mm annually with 65% of the production estimated to occur in areas with an average rainfall higher than 400 mm (Wortmann et al., 1998). Beans grow well at mean temperatures of 15-23 ºC and on a wide range of soil types but best growth is obtained in soils that are medium-textured, well drained, and high in organic matter and with a pH range of 6.0 to 7.0 (Greenlife, 2019). The common bean production areas in Kenya are found in the Rift Valley, Central, Eastern and Western provinces (Nzuma, 2020).

2.2. Constraints to common bean production in Kenya

Farmers growing beans in Kenya face challenges such as biotic and abiotic stresses, lack of capital, poor yields, inadequate agricultural equipment, lack of knowledge on climate change, poor quality of seeds, declining land area, inadequate and unpredictable markets, taxes, low price of the commodity and shortage of extension services (Mahagayu et al., 2010; Birachi et al., 2011; Odendo et al., 2011; Mukankusi et al., 2018). According to Rodríguez & Creamer (2015), diseases are the principal constraint of common bean production; followed by pests. In addition, adoption rates for improved seed continue to be low with access to disease free seed being a major problem affecting bean farmers since most of the diseases are seed borne (One Acre Fund, 2016).

2.3. Common bean anthracnose

2.3.1. Disease biology

Common bean anthracnose is caused by the fungus of the genus *Colletotrichum*, order *Melanconiales,* family *Melanconiaceae* and section *Hyalosporae*. The fungus is found in nature in a conidial imperfect stage, but can overwinter as mycelia or conidia. The conidia are oval in shape with a dark brown colour. They form masses of conidia on the host packed into the acervuli. *Colletotrichum lindemuthianum* differs from other species in this genus by its growth

characteristics and a dark pigmentation on cultures (Mota et al., 2016). The spores are spread from infected plants to the healthy ones through rain splash, wind-blown rain and the movement of insects, animals and man, mostly when the foliage is moist (Buruchara et al., 2011; LeClair et al., 2015). Anthracnose survives on debris that remain dry since water dilutes and removes the mucilage matrix of the conidia hence reducing the conidial viability within 24 hours (Tu, 1983; Conner et al., 2019).

2.3.2. Symptoms of anthracnose

The early stages of infection usually manifest purplish red discolouration while on the lower leaf surface along the veins. Discolouration on the upper leaf surface appears as brown lesions at advanced stages, with black, brown, or purplish red margins, developing around small veins (Allen et al., 1996; Mohammed, 2013). At advanced stages, vein necrosis appears first, followed by wilting and bleaching which occurs at the tip of the leaflet later spreading to the margin and the center of the blade (Godoy et al., 1997). Infection in the stems is expressed by dark brown eyespots which develop longitudinally along the stems. On the stems, the enlarged spots turn brown and many tiny black specks (Bailey et al., 1992; CABI, 2022). Lodging is common in the young seedlings once the eyespots enlarge, however this rarely occurs in older stems. Pod lesions reach a diameter of 5-8 mm, slightly sunken at the center with a dark brown or purplish brown margin on mature pods. The disease displays brown to light chocolate-coloured spots on the seed coat and lesions may extend into the cotyledons (Mohammed, 2013).

2.3.3. Pathogen survival and spread

Cool temperatures and high humid conditions favour and influence the survival of the pathogen (Musyimi, 2014). Even though crop residues are great contributors to the pathogen's survival and distribution, infected seeds play an important role in the wide distribution of the anthracnose pathogen over long distances (Tesfaye, 2003; Halvorson et al., 2021). This is evident when resource poor farmers continuously exchange and use infected farm saved seed (Mogita, 2014). Seeds are capable of transmitting the disease to the next season, as long as the fungus remains (Bailey et al. 1992; Halvorson et al., 2021).

2.3.4. Disease control

The most common methods used for managing anthracnose are crop rotation, field sanitation, application of fungicides, using certified seed and the use of resistant genotypes (Sileshi et al., 2014). Some of these methods reduce profit accrued by farmers due to their cost, cause environmental pollution and lead to development of fungicide resistant biotypes (Miklas et al., 2006). Host resistance still remains the effective and efficient control method (Sicard et al., 2007), however, this has been met with difficulties due to the occurrence of multiple races of the fungus (Mohammed, 2014). Variability of resistance within the same accession provides the opportunity to select resistant genotypes (Ferreira et al., 2008; Da Costa et al., 2018).

2.3.5. Host plant resistance and marker assisted selection

Resistance to some pathotypes of anthracnose is governed by single, duplicate, or complementary dominant genes in many genotypes (Sharma et al., 2007; Young, 2010). Anthracnose resistance genes are identified by the *Co* symbol (Kelly & Vallejo, 2004). Sources of anthracnose resistance in different backgrounds include *G2333* [*Co-4*^{2}, *Co-5*^{2}, *Co-7*], AB136 [*Co-6*, *Co-8*], Tu [*Co-5*], PI207262 [*Co-4*^{3}, *Co-9*], Cornell 49-242 [*Co-2*], Mex 222 [*Co-3*], Kaboon [*Co-1*^{2}], Mitchelite [*Co-11*], and To [*Co-4*] (Graham & Ranalli, 1997; Kelly & Vallejo, 2004). These resistant genes can breakdown due to pathogen variability and do not confer resistance to all races of the pathogen (Sharma et al., 2007). There are no resistance genes that are effective against all known races of anthracnose occurring in the same or different areas (Mahuku & Riascos, 2004; Alzate-Marin et al., 2007; Mogita, 2013; Kamiri et al., 2021).

The anthracnose differential cultivar *G2333* has resistance genes *Co-4*^{2}, *Co-5*, and *Co-7* making it resistant to more than 90% of the anthracnose races. The molecular markers have been used in screening for sources of resistances among germplasm (Vieira et al., 2018; Kamiri et al., 2021). Sequence characterized amplified regions (SCAR) markers SAS13, SH18 and SBB14 linked to *Co-4*^{2}, and SAB3 linked to the *Co-5* gene were developed for the resistance genes in *G2333* (Garzón et al., 2008; Vallejo & Kelly, 2009). Use of these tightly linked molecular markers to the genes improves efficiency of selection of resistant genotypes in the absence of pathogens (Miklas et al., 2006; Collard & Mackill, 2008; Ferreira et al., 2012; Ndee, 2013; Uwera et al., 2021).

2.4. *Pythium* root rot in common bean

2.4.1. Disease biology

The genus *Pythium* belongs to the family *Pythiaceae*, order *Pythiales*, class *Oomycetes*, Phylum *Oomycota* and kingdom *Chromista* (Kirk et al., 2008). *Pythium* species are microorganisms with a filamentous vegetative body called a mycelium. The mycelium is colourless, sometimes lustrous, and occasionally slightly yellowish or a grayish lilac (Owen-Going et al., 2008). *Pythium* spp. may produce appressoria hyphae with swollen digitate regions which enable the fungus to attach and penetrate the host cells (Lévesque & De Cock, 2004). It can reproduce both asexually and sexually whereby sexual reproduction takes place through the zoosporangia and zoospores while asexual reproduction takes place through the oogonia and antheridia (Maheshwari, 2011). All *Pythium* species are known to survive in the soil for many years as oospores (Abawi & Pastor-Corrales, 1990). Soils in arable land, pastures, forests, nurseries, and marshes, and in water are some of the ecological zones where *Pythium* species can be found (Lévesque & De Cock, 2004). Soil temperature can affect spore germination, germ tube growth and zoospore discharge (Ofek et al., 2012). *Pythium ultimum* and *P. irregular* are favoured by cold temperatures hence making temperatures and moisture content within the soil influence the susceptibility reaction of common bean to root rot diseases (Nzungize et al., 2011; Matthiesen et al., 2016).

Pythium species have been recovered in soils with a pH range of 3.6-7.2. However, high populations have been found in soils with a pH range of 6.8 to 7.2 and in soils with low pH range of 3.6 to 5.5 (Martin & Loper, 1999). This is also evident in other root rots where *Fusarium* root rot and *Rhizoctonia solani* severities varied with pH (Naseri, 2014; Acharya, 2017; Cruz et al., 2019; Pal et al., 2019).

2.4.2. Symptoms of *Pythium* root rot

Pythium symptoms may appear as seed rot before germination, damping-off, root rot, pod rot or foliar blight depending on the time of infection and prevailing environmental conditions (Abawi & Pastor-Corrales, 1990). Early stage symptoms on root and lower stem tissues appear as elongated, water-soaked areas. Tissues under infection tend to become soft brownish, spongy, wet, discoloured with many cavities, sunken and end up collapsing leading to wilting and eventually death. The disease is also characterized by lower leaf yellowing that appears similar to nitrogen deficiency, stunting, leaf browning and plant death (Pankhurst et al., 1995; Ampaire, 2003). The above ground

symptoms are characterized by poor seedling establishment, uneven growth and premature defoliation where the plants are severely infected (Abawi, 2006; Otsyula, 2010).

2.4.3. Occurrence and spread

This disease is considered as the most damaging in East and Central Africa including Kenya where beans are grown intensively (Otsyula, 2010; Nzungize et al., 2011). The occurrence of pathogens associated with root rots and the severity of the disease are based on intensification of land use, inappropriate crop rotations and reduced fallow periods, leading to a decline in soil fertility and a build-up of soil pathogen inoculum (Abawi, 2006; Otsyula, 2010; Marzano, 2012). Long rainfall, intermittent droughts with fluctuation in soil moisture condition promote the occurrence of *Pythium* root rot (You et al., 2017; Sinha et al., 2021). Root rot was predicted to become a serious problem in several parts of Kenya , Uganda , Tanzania, Ethiopia, Malawi and Mozambique due to increase in population density and cultivation intensity therefore reduced soil nutrients which favours *Pythium* root rot (Wortmann et al. 1998). This prediction was supported by Papias et al. (2016) who found out the occurrence of the pathogen greatly affecting production of common bean in Tanzania. Changing environmental conditions, high temperatures and frequent floods have forced root rot diseases patterns to change with severe root rots currently occurring in low to mid-altitude areas (Paparu et al., 2018).

2.4.4. *Pythium* root rot management

The complexity and mechanisms of survival of root rot pathogens in the soil as saprophytes makes it hard to control the diseases (Baysal-Gurel et al., 2012; Divya & Sudini, 2013; Chellemi et al., 2016). Cultural methods, host plant resistance and integrated methods are the most used control measures of *Pythium* root rot (Nzungize & Lyumugabe, 2012; Maria et al., 2017). Cultural control involves crop rotation, timely planting, ridge planting, use of fertilizers and crop rotation keeps *Pythium* oospores low (Nzungize & Lyumugabe, 2012; Marzano, 2012; Panth et al., 2020). These methods deprive the pathogen its host and create conditions that favour the growth and development of microorganisms which tend to be antagonists to plant pathogens (Were, 2019).

Infestation of roots rots such as *Rhizoctonia* root rot, southern blight, *Fusarium* root rot and *Pythium* root rot can be reduced by deep ploughing and use of raised ridges (Otsyula et al., 2011; Maria et al., 2017). Incorporating *Leucaena* spp. leaves, *Calliandra magrantha* twigs and Sesbania green manure two weeks before planting reduces plant mortality and increases bean grain yield (Otsyula

et al., 2011). A study by Pane et al. (2011) found that organic soil amendments application reduce root rot diseases. Recommended rates of fertilizer application enhances crop ability to withstand *Pythium* root rot attack through availability of plant nutrients readily taken up by weakened plant roots (Otsyula et al., 2011). These amendments promote plant growth and vigour hence making the plant tolerant to infection in the presence of pathogens (Mehta et al., 2014).

Chemical treatments may be efficient with specific chemicals such as benzimidazole $C_7H_6N_2$, captan ($C_9H8Cl_3NO_2S$), carboxin ($C_{12}H_{13}NO_2S$), metalaxyl ($C_{15}H_{21}NO_4$), propamocarb hydrochloride ($C_9H_{21}ClN_2O_2$) and etridiazole ($C_5H_5Cl_3N_2OS$) proving to be effective in controlling *Pythium* root rot diseases in beans (Abawi, 2006; El-Mohamedy et al., 2015). Seed coating with fungicides has been observed to be effective in seed protection together with young seedlings for 2 to 3 weeks after planting (Mohamed & Amer, 2014; Belay & Anteneh, 2017; Toribio et al., 2021). Soil fumigants such as methyl bromide (CH_3Br) and chloropicrin (CCl_3NO_2) are highly efficient biocides that kill *Pythium* agents (Abawi, 2006; Arora et al., 2021). The most effective management system of *Pythium* root rot disease however, is not by use of a single control measure but by integrating the control measures available including resistant genotypes (Abawi & Pastor-Corrales, 1990; Wu et al., 2020; Arora et al., 2021; Aydin, 2022). This relies on proper knowledge of the host, pathogens involved and the environmental conditions favouring the infection and development of the disease (Divya & Sudini, 2013; Panth et al., 2020).

Small scale farmers rarely practice crop rotation due to land pressure and the fact that dominant crops like maize and beans are grown according to seasons (Muriungi et al., 2013). The use of resistant genotypes is the most efficient management strategy against root rot diseases which is appropriate for small resource poor farmer as compared to fungicide application. This however requires development of adapted genotypes with resistance to all the major root rot pathogens occurring within a given bean growing region (Abawi, 2006; Were, 2019).

2.4.5. Host plant resistance and marker assisted selection

Bean genotypes *RWR 719, MLB 49-89A, AND 1055, AND 1062* and *SCAM 80-CM/15*, resistant to *Pythium* root rot, are advanced lines from an international breeding nursery maintained by the International Center for Tropical Agriculture (CIAT) in Cali, Colombia. Genotypes *RWR 719, AND 1062* and *MLB 49-89A* have shown high levels of *Pythium* root rot resistance (Otsyula et al., 2003; Buruchara et al., 2007). *RWR 719* is a late maturing but resistant to all species of *Pythium*

(Otsyula et al., 2003; Nzungize et al., 2011), whereas *AND 1062* is medium maturing (Mukalazi et al., 2001). *Pythium* resistance is controlled by dominant gene *Pyult1* (Otsyula et al., 2003; Nzungize et al., 2011). *RWR 719* and *AND 1062* have been used as donors for resistance against the virulent and predominant *Pythium* spp. in breeding programs in East and Central Africa region (Otsyula et al., 2003). It was successfully used for enhancing resistance to *Pythium* root rot in various breeding programs. Marker assisted selection for *Pythium* resistance is being aided by a SCAR marker ($PYAA19_{800}$), that is linked to *Pyult1* in common bean (Mahuku et al., 2007; Nzungize et al., 2011).

2.5. Characterization and diversity of common bean germplasm

There are many sources of germplasm that can be used by a breeder i.e. commercial genotypes, breeding lines, elite genotypes, landraces, wild materials and mutants (Ram, 2014). East Africa is majorly considered as a secondary diversity center of common bean due to a wide range of landraces in the region (Wortmann et al., 1998; Asfaw et al., 2009). Genetic diversity characterization helps in identifying genetically diverse parents for improvement. Genetic resources have to be characterized by morphological and agronomic traits to be useful to breeders (Stoilova et al., 2013). Morphological characterization distinguishes some of the existing similarities in landraces hence showing correlations between agronomic performances and the traits (Karaaĝaç & Balkaya, 2013).

Molecular markers have shown that common bean was domesticated twice, leading to the development of the Mesoamerican (Northern Mexico to Colombia) and the Andean (Southern Peru to Northwestern Argentina) genepools (Gepts & Bliss, 1986; Beebe et al., 2001; Blair et al., 2006). Simple sequence repeats (SSR) or microsatellites are multiallelic and co-dominant molecular markers which contain considerable genetic variation (Blair et al., 2003; Song et al., 2004). High amounts of information content contained in SSR loci and their co-dominant expression, give SSRs their ideal features for gene mapping and high efficiency for linkage studies, cultivar protection, marker-assisted selection and diversity studies (Song et al., 2004; Leal et al., 2010). Results from Fisseha et al. (2018) show that germplasm introduced in Ethiopia had extensive hybridization between the Andean and Mesoamerican gene pools. According to Asfaw et al. (2009) there was a greater genetic divergence in Ethiopian landraces as compared to Kenyan landraces with Mesoamerican genotypes being more diverse than the Andean genotypes.

Commercial genotypes in Kenya and Ethiopia were found to have a relatively narrow genetic basis as compared to local genotypes (Asfaw et al., 2009; Cabral et al., 2011; Anunda et al., 2019).

Genetic diversity may be deduced through quantitative and predictive characteristics. Quantitative genetic diversity is generated from a set of genotypic or phenotypic characters with the of use multivariate analysis (Eticha et al., 2010; Meira et al., 2019). According to Cruz et al. (2012) predictive methods are based on morphological, physiological or molecular difference hence quantification is done using similarity/dissimilarity measurement thus showing the degree of parental genetic diversity. Studies show descriptors linked to seeds are the most discriminant traits of common beans (Hegay et al., 2014). Research work by Rana et al. (2015) shows dominance of a growth habit is related to ecological adaptation and the cropping system. According to Kondo et al. (2004) and Rana et al. (2015) grain yield has negative and significant correlations with days to flowering and number of seeds per pod.

CHAPTER THREE

Genetic diversity of common bean landraces grown in Kenya based on morphological and simple sequence repeat variations

Summary

Common bean landraces are preferred by farmers in Kenya due to yield stability and tolerance to some stresses. However, there is little information on the genetic diversity of these landraces which is essential for breeding. The study focused on characterizing the diversity of 98 common bean (*Phaseolus vulgaris* L.) genotypes using a combination of morphological markers and molecular markers. The field experiments were conducted at two sites, representing different agro-ecological zones in Kenya. Data were collected on qualitative traits such as flower color, growth habit, seed shape and seed coat color. In addition, quantitative traits related to yield and its components were also investigated. The results revealed a substantial diversity in these traits, providing a valuable resource for future breeding programs aimed at developing improved bean varieties. The analysis underscored the importance of both genetic and environmental factors in influencing the quantitative traits. Phylogenetic and principal component analyses were employed to further elucidate the relationships among the genotypes. The analyses grouped the genotypes into distinct clusters based on traits such as days to flowering, days to maturity, growth habit, and seed type, contributing to a comprehensive understanding of the genetic landscape of the studied germplasm. Additionally, simple sequence repeats (SSRs) markers were utilized to assess genotypic diversity. The SSR markers revealed allelic variations among the genotypes generating insights into the genetic makeup of the bean genotypes. The genotypes were grouped into clusters based on these molecular markers, with certain traits showing strong correlations with specific clusters. Characterization of the landraces will enable selection of suitable parents for breeding programs.

3.0. Introduction

Common beans (*Phaseolus vulgaris* L.) are characterized by diverse landraces, registered genotypes and wild relatives within and outside the species limits (Gepts, 2001). The grain legume was domesticated in Mesoamerica and the southern Andes mountains (Chacón et al., 2005; Kwak & Gepts, 2009). Diverse genotypes of common bean exist with many commercial classes having different grain types (Broughton et al., 2003). Common beans are grouped based on the Andean and Mesoamerican genepools. Along with molecular marker polymorphism, distinctions between Andean and Mesoamerican beans are notable with seed size being the major distinguishing trait (Blair et al., 2006; Díaz & Blair, 2006; Nkhata et al., 2020). Investigations into the origins and evolution of this species highlights the structure and organization of its genetic diversity. Such knowledge is a crucial prerequisite for efficient conservation and use of the germplasm for the development of new improved plant genotypes (Bitocchi et al., 2012; Bitocchi et al., 2017).

Characterization of plants based on morphology is a method that focuses on traditional identification of evolutionary and pedigree relations (Nkhata et al., 2020). Genetic variation in morphological characteristics such as growth, seed, pod, and flower characteristics is often observed in beans (Pereira et al., 2019; Catarcione et al., 2023). This variation has been extensively used in breeding programs and diversity studies (Pérez-Vega et al., 2010). Andean groups are associated with large or medium seed morphology while Mesoamerican group consists of small seeds genotypes (Singh, Gepts, & Debouck, 1991; Kwak & Gepts, 2009). Morphological features may change under the influence of ecological conditions hence their use in diversity assessment for phenotypic and agronomic traits may have limitations (Razvi et al., 2018).

Because morpho-agronomic traits are highly influenced by environment, use of molecular markers for estimating genetic diversity complements their use (Ceolin et al., 2007). Simple sequence repeats are one of the most used techniques to study polymorphism between DNA sequences. They rely on PCR-based markers that detect loci variations of repetitive sequences. According to Gonçalves-Vidigal & Rubiano (2011), SSRs present high levels of polymorphism, codominant inheritance, multi-allelism and good genome coverage. The SSRs require low amount of DNA, can be easily automated for high throughput screening, may be exchanged between laboratories, and are highly transferable between populations (Gupta et al., 2003; Shiferaw et al., 2012; Gupta et al., 2014; Chen et al., 2020). Simple sequence repeats markers most of the time co-segregate with the gene in the plant genome (Morgante et al., 2002; Cavagnaro et al., 2010; Kalia et al., 2011; Singh et al., 2016).

The analysis of molecular markers has generated knowledge in comprehending the common bean genetic structure diversity and phylogenetic analysis (Asfaw et al., 2009; Sharma et al., 2013; Mercati et al., 2013; Scarano et al., 2014; Zelalem et al., 2017; Vidak et al., 2017; Fiore et al., 2020; Vidak et al., 2021; Ndlangamandla & Ntuli, 2021). Simple sequence repeat markers have been used for plant genetic analysis in bean such measuring natural selection effects (Leite et al., 2011; Blair et al., 2012), determination of genetic diversity (Dong et al., 2014; Kujane et al., 2019), population structure measurement (Ribeiro *et al*., 2010; Albertini et al., 2011; Haddad et al., 2020), integration of genetic, physical and sequence-based physical maps (McClean et al., 2010; Garcia et al., 2011) and in marker assisted selection (Benchimol et al., 2005; Shanbao et al., 2009; Chen et al., 2011). These markers have been used for mapping of genomic regions ofagricultural interest

(Charcosset & Moreau, 2004; Stasyuk et al., 2017; Ahmad et al., 2020). The objective of this study was to determine the genetic diversity among common bean landraces grown in medium and high-altitude zones of Kenya based on morphological traits and SSR markers, in order to provide options for breeders to develop new genotypes for specific traits from the well adapted landraces.

3.1 Materials and methods

3.1.1 Genotypes

A panel of 98 common bean genotypes including 89 landraces and 9 local commercial genotypes, grown in medium and high altitude areas of Kenya, were used in this study (Figure 3.1 and Table 3.1). These genotypes were collected in counties across a number of agro-ecologies in the administrative counties of Kakamega, Bungoma, Busia, Trans-Nzoia, Kisii, Siaya, Migori, Uasin Gishu, Nakuru, Kiambu, Nyeri, Kirinyaga and Embu. Simple random sampling design was used in germplasm collection. The collection was based on morphological characteristics, local knowledge from the farmers and seed merchants in the local markets.

Figure 3.1: Seed type of the beans evaluated for morphological traits

LR=Landrace

Table 3.1: Colour and shape of common bean genotypes used in this study

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

Source: (IBPGR, 1982)

3.1.2 Experimental site

The common bean landraces were grown and phenotypically characterized at the Kenya Agricultural and Livestock Research Organization (KALRO) Kakamega field stations in the upper midland 1 zone (Ministry of Agriculture, 2011a) and Lukhome in Bungoma found in the lower highland 1 (Ministry of Agriculture, 2011b). The sites were chosen as representatives of medium and high altitude high potential areas of Kenya where over 60% of beans are grown (Kimiti et al., 2009). The characteristics of these sites are described in Table 3.2.

Table 3.2: Characteristics of the sites under study

Site description	Kakamega	Bungoma
Agro-ecological zone	Upper midland 1	Lower highland 1
Location	34°46'E, 0°16'N	34°36'E, 0°46'N
Altitude	1595masl	2100masl
Rainfall	1950 mm per annum	1400-1600 mm per annum
Temperature	25°C- 18°C	18°C -15.2°C
Soils	Well drained dark red clays	Well drained dark red to dark brown volcanic clays

Source: (Ministry of Agriculture, 2011a; Ministry of Agriculture, 2011b; Ministry of Agriculture, 2014)

3.1.3. Experimental procedure

The Kakamega field had initially been under cassava plantation which was harvested and the land left fallow for one year. The Bungoma field had left fallow for $1^1/_2$ years after getting rid of a banana plantation. Primary ploughing was done during the dry season. The secondary ploughing followed on the onset of rains. This was done to a fine tilth. The experimental plots were two rows measuring two meters long. The plants were spaced at 50 cm between the rows and 10 cm between plants. At planting, NPK (23:23:0) fertilizer was applied providing100 kg of N/ha and100 kg of P/ha. The field experiment was laid out in a lattice design with three replicates across the 2 sites. First weeding was three weeks after germination. The second weeding was done at the vegetative stage before flowering. Confidor (imidacloprid as the active ingredient) was used to control insect pests at a rate of 20 ml in a 20 litre knapsack. Rodazim with carbendazim as the active ingredient was used to control fungal diseases at a rate of 20 ml in a 20 litres knapsack. The experiments were

set up in the long rains growing seasons in 2020. Plants for genotypic characterization were raised in the screen house in plastic buckets with sterilized soil mixed with manure and sand in a 3:2:1 ratio.

3.1.4 Data collection

Ten plants were randomly selected from each plot. Data were collected according to the International Board of Plant Genetic Resources (IBPGR, 1982) descriptors for *P. vulgaris*. Data were collected on 23 traits as described in Table 3.3.

Table 3.3: Description of traits used to characterization of common beans during this experiment

Trait	Type	Description
Leaf colour	Q	This is the leaf pigmentation intensity based on a whole plot observation
Leaf hairiness	Q	This was based of hairs protruding from the leaf categorized as hairy or smooth based on the entire plot observation
Leaf length	Q	This was measured in the middle of the terminal leaflet of the third trifoliate leaf from the pulvinus to the leaf tip
Leaf width	Q	This was measured at the broadest point at the base of the terminal leaflet of the third trifoliate leaf
Stem colour	Q	This is the stem pigmentation intensity based on a whole plot observation
Stem colour distribution	Q	This is the stem pigmentation distribution based on a whole plot observation
Stem hairiness	Q	This was based of hairs protruding from the stems categorized as hairy or smooth based on the entire plot observation
Stem determinacy	Q	Categorized as determinate based on strong and erect stems and branches or indeterminate based on weak and prostrate stems and branches
Growth habit	Q	This was evaluated on a I-IV scale whereby; I- Determinate bush II- Indeterminate bush habit with erect stems and branches III- Indeterminate bush habit with weak main stem, prostrate stem and branches IV- Indeterminate climber habit with weak, long twisted stems and branches
Plant height	Qn	This was measured as height in centimeters (cm) from the soil surface and the tip of the central shoot of mature plants
Days to flowering	Qn	This is the time elapsed from sowing until 50% of the plants in a plot showed one open flower
Flower standard colour	Q	Prominent colour of the standard observed on freshly opened flowers
Flower wings colour	Q	Prominent colour of the wings observed on freshly opened flowers
Days to maturity	Q	Number of days from planting to the day when the first pod begins to discolour in 50% of the plants in a plot
Pod colour	Q	This is the pod pigmentation intensity based on a whole plot observation
Pod hairiness	Q	This was based of hairs protruding from the pods categorized as hairy or smooth based on the entire plot observation
Pod length	Q	This was measured from the apex to the peduncle
Number of pods per plant	Qn	This was determined by counting randomly selected pods from ten plants
Numbers of seeds per pod	Qn	This is the average number of seeds per pod from 10 pods, one from each of 10 randomly selected plants
100 seed weight	Qn	This is the weight of 100 mature seeds at 13% moisture content
Seed coat pattern	Q	Distribution of colours on the seed coat
Seed shape	Q	Describes the external form, contours or outline of the seed. Ranges from nearly spherical to flattened, elongated, cuboid, oval, round and kidney shaped
Seed brilliance	Q	Seed shininess or opaqueness at harvest

Q= Qualitative, Qn= Quantitative

Source: (IBPGR, 1982)

3.1.5 DNA extraction

The first trifoliate leaves of fourteen day old plants grown in a greenhouse of the 89 genotypes together with 7 local checks were collected and placed in eppendorf tubes while wearing gloves. Genomic DNA was isolated from the leaves using the cetyltrimethylammonium bromide (CTAB) extraction method (Afanador & Haley, 1993). The DNA was quantified using the agarose gel based quantification method.

3.1.6 Polymerase chain reaction (PCR)

The PCR amplifications were performed in a 10 µL final volume containing 5 ng DNA, 1x buffer, 0.2 µM of each forward and reverse primer, 100 µM of each dNTP, 2.0 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, and 0.5 units of *Taq*-DNA polymerase. Twenty-two SSR markers, from the 11 linkage groups of the bean genome were selected for their broad genomic distribution and high polymorphism information. The sequences and genomic regions of the markers are shown in Table 3.4. The 22 microsatellites were genomic SSRs previously mapped by Campos et al. (2011). The amplification program consisted of an initial denaturation for 3 minutes at 94°C; followed by 30 cycles of denaturation step at 94°C for 10 seconds, 30 seconds annealing step at temperatures specific for each SSR and a 2 minutes 72°C extension step; with a final extension of 5 minutes at 72°C. The amplicons were separated on horizontal polyacrylamide gel at 150 V with a current of 100 A for 120 minutes in Tris-acetate-EDTA (TAE) buffer. The gel was later post-stained with 0.5 µg/mL ethidium bromide. This was visualized on a UV trans-illuminator and the gel image captured. Scoring was done by identifying and marking the positions of DNA bands on the gel image. The DNA markers were used as references to estimate the sizes of the fragments whereby the size of a fragment corresponds to the distance migrated from the well. The gel pictures obtained were scored for each targeted allele. The sizes of the DNA fragments for each sample were tabulated as homozygotes or heterozygotes depending on whether the marker was dominant or codominant.

Table 3.4: Description of SSR markers used in the study

SSR	LG	A Tm	Allele Size (Bp)	SSR	Forward primer	Reverse primer	Reference
BM146	1	50	281	(CTGTTG)4-(CTG)	5'-GAGATGAGTCCTTTCCCTACCC-3'	5'-TGCAGACACAATTTATGAAGGC-3'	(Gaitán-Solís et al., 2002)
BMd-45	1	47	129	(AG)5	5'-GGTTGGGAAGCCTCATACAG-3'	5'-ATCTTCGACCCACCTTGCT-3'	(Blair et al., 2003)
PV-cct001	2	47	149	(CCT)7	5'-CCAACCACATTCTTCCCTACGTC-3'	5'-GCGAGGCAGTTATCTTTAGGAGTG-3'	(Yu et al., 2000)
PV-gccacc001	2	49	95	(GCCACC)5	5'-CGTTAGATCCCGCCCAATAGT-3'	5'-CCGTCCAGGAAGAGCGAGC-3'	(Yu et al., 2000)
BM164	2	52	182	(GT)9(GA)21	5'-CCACCACAAGGAGAAGCAAC-3'	5'-ACCATTCAGGCCGATACTCC-3'	(Gaitán-Solís et al., 2002)
PV-at008	3	49	161	(AT)9	5'-AGTCGCCATAGTTGAAATTTAGGTG-3'	5'-CTTATTAAAACGTGAGCATATGTATCATT-3'	(Yu et al., 2000)
BM172	3	50	107	(GA)23	5'-CTGTAGCTCAAACAGGGCACT-3'	5'-GCAATACCGCCATGAGAGAT-3'	(Gaitán-Solís et al., 2002)
PV-at003	4	47	139	(AT)4(T)2	5'-ACCTAGAGCCTAATCCTTCTGCGT-3'	5'-GAATGTGAATATCAGAAAGCAAATGG-3'	(Yu et al., 2000)
PV-atgc001	4	49	126	(ATGC)4	5'-TGCCACCACAGCTTTCTCCTC-3'	5'-TATGAGAGAAGCGGTTGGCACG-3'	(Yu et al., 2000)
BMd-53	5	47	105	(GTA)5	5'-TGCTGACCAAGGAAATTCAG-3'	5'-GGAGGAGGCTTAAGCACAAA-3'	(Blair et al., 2003)
BM155	5	50	114	(CA)8	5'-GTTCATGTTTGTTTGACAGTTCA-3'	5'-CAGAAGTTAGTGTTGGTTTGATAC-3'	(Gaitán-Solís et al., 2002)
BMd-12	6	47	167	(AGC)7	5'-CATCAACAAGGACAGCCTCA-3'	5'-GCAGCTGGCGGGTAAAACAG-3'	(Blair et al., 2003)
PV-at004	6	49	163	(AT)18	5'-AATCTGCCGAGAGTGGTCCTGCC-3'	5'-GATTGAAATATCAAAGAGAAAGAGAATTGTTAC-3'	(Yu et al., 2000)
BM183	7	50	149	(TC)14	5'-CTCAAATCTATTCACTGGTCAGC-3'	5'-TCTTACAGCCTTGCAGACATC-3'	(Gaitán-Solís et al., 2002)
BMd-44	8	47	135	(AG)5	5'-GGCAGCTTACTAACCCGAAA-3'	5'-TTCCTTCCCCTTTCTTCTCC-3'	(Blair et al., 2003)
BM211	8	49	186	(CT)16	5'-ATACCCACATGCACAAGTTTGG-3'	5'-CCACCATGTGCTCATGAAGAT-3'	(Gaitán-Solís et al., 2002)
BM114	9	50	234	(TA08(GT)10	5'-AGCCTGGTGAAATGCTCATAG-3'	5'-CATGCTTGTTGCCTAACTCTCT-3'	(Gaitán-Solís et al., 2002)
BM141	9	50	218	(GA)29	5'-TGAGGAGGAACAATGGTGGC-3'	5'-CTCACAAACCACAACGCACC-3'	(Gaitán-Solís et al., 2002)
BM212	10	49	214	(CA)13	5'-AGGAAGGGATCCAAAGTCACTC-3'	5'-TGAACTTTCAGGTATTGATGAATGAAG-3'	(Gaitán-Solís et al., 2002)
GATS11	10	50	306	(CT)8CA(CT)2GTTT	5'-CACATTGGTCCTAGTGTCGG-3'	5'-GAACCTGCAAAGCAAAGAGC-3'	(Yu et al., 2000)
PV-ag001	11	47	121	(GA)11	5'-CAATCCTCTCTCTCTCATTTCCAATC-3'	5'-GACCTTGAAGTCGGTGTCGTTT-3'	(Yu et al., 2000)
BMd-41	11	47	250	(ATT)9	5'-CAGTAAATATTGGCGTGGATGA-3'	5'-TGAAAGTGCAGAGTGGTGGA-3'	(Blair et al., 2003)

3.2 Data analysis

Morphological data were subjected to analysis of variance (ANOVA) for individual sites using Agricolae package in R statistical software (Oksanen et al., 2020). The following general linear model was used;

$$Y_{ijkl} = \mu + \pi_i + \beta_j + \tau_k + \varepsilon_{ijkl}$$

Where: Y_{ijkl} = Observation, μ= mean of experiment, π_i= effect due to i^{th} replication, β_j=effect due to j^{th} incomplete block, τ_k= effect due to the k^{th} genotype and ε_{ijkl}= intra-block residual effect.

To compare effects due to the environment between Kakamega and Bungoma, during the long rains, the following statistical model was used.

$$Y_{ijkl} = \mu + \lambda_i + \pi_{(j)i} + \tau_k + \lambda\tau_{ik} + \varepsilon_{ijkl}$$

Where: Y_{ijkl} = Observation, μ= mean of experiment, λ_i = effect due to i^{th} location, $\pi_{(j)i}$ =effect due to j^{th} replication within the i^{th} location, τ_k= effect due to the k^{th} genotype, $\lambda\tau_{ik}$= effect due to interaction of k^{th} genotype and i^{th} location and ε_{ijkl}= intra-block residual effect.

Means of genotypes and locations were separated according to Tukey's multiple comparison method at 95% confidence level using the following formula:

$$HSD = q \cdot \sqrt{\frac{MSE}{n}}$$

Where: *HSD* is the honestly significant difference, *q* is the critical value from the studentized range distribution based on the desired confidence level and the degrees of freedom for the error term, *MSE* is the mean squared error from the analysis of variance (ANOVA), *n* is the number of observations per group.

Further, an unweighted pair group arithmetic mean (UPGMA) tree was constructed using the Euclidean distances between the traits from the quantitative traits data set (Sneath & Sokal, 1973). The *Shannon-Weaver* diversity index (H') was used to quantify the diversity within each trait

category. *Shannon Weaner* diversity index was used to determine the diversity in qualitative traits among the landraces using the following formula:

$$H' = \sum_{i=1}^{s} (P_i \cdot \ln P_i))$$

Where: *H'* represents the Shannon diversity index, *S* is the total number of species in the sample P_iP_i is the proportion of individuals belonging to the *i*th species (relative abundance) in the sample, *ln* represents the natural logarithm.

The PCA process involved the calculation of *eigenvalues* and *eigenvectors* from the covariance matrix of the quantitative traits. The eigenvalues represent the amount of variance explained by each principal component, while the eigenvectors indicate the correlation between the original traits and the principal components. The eigenvectors were used to interpret the relationship between the traits and the principal components.

For molecular analysis, the SSR allele sizes were assigned based on the amplicons migration through the gel in comparison to that of a 50 bp ladder. The average number of alleles, allele frequencies, gene diversity and polymorphism information content were calculated for each SSR locus using PowerMarker v3.25 software (Liu, 2005). An unweighted pair group arithmetic mean (UPGMA) tree was constructed in Vegan R statistical package (Oksanen et al., 2020) using the Jaccard distance. A comparison tree of genotypic and morphological variables was also constructed as explained above.

3.3 Results

3.3.1 Qualitative traits

The analysis of qualitative traits in the evaluated common bean genotypes showed the diversity and distribution of different traits (Table 3.5). Regarding flower colour, the genotypes exhibited three predominant sets, with 33.7% having pink flowers, 11.2% displaying purple flowers, and 55.1% exhibiting white flowers. This was a moderate diversity with *H'* = 0.94 among the genotypes. The growth habit of the genotypes was classified into 4 groups, with type I comprising 31.5%, type II accounting for 53.9%, type III representing 10.1%, and type IV making up 4.5% of the genotypes. There was a relatively higher diversity *H'* = 1.07 in growth habits among the studied genotypes. The seed shapes were categorized as cuboid (13.5%), kidney (16.9%), oval (53.9%),

and round (15.7%). The observed 1.19 *Shannon-Weaver* diversity index (*H'*) for seed shape indicated a high diversity within this trait. Eleven different seed colours were observed, with the red seed colour being the most dominant. This suggests a high diversity (H' = 1.91) in terms of seed colour among the genotypes. Furthermore, 57.3% of the genotypes had a single seed coat colour, while the remaining genotypes exhibited various patterns. *H'* for seed coat colour of 1.39 indicated a notable diversity within this trait. Overall, the results revealed substantial diversity in flower colour, growth habit, seed shape, seed colour, and seed coat colour among the evaluated common bean genotypes.

Table 3.5: Frequencies and Shannon-Weaner diversity index for qualitative traits of the common bean genotypes grown at Kakamega and Bungoma

Trait	Frequency	%	H'	Trait	Frequency	%	H'	Trait	Frequency	%	H'
Flower wings colour			**0.941**	**Stem colour distribution**			**0.435**	**Seed colour**			**1.91167**
Pink	30	33.7		Even	75	84.3		Black	2	2.2	
Purple	10	11.2		Uneven	14	15.7		Brown	14	15.7	
White	49	55.1						Cream-Beige	4	4.5	
Flower standard colour			**0.941**	**Stem hairiness**			**0.665**	Green	2	2.2	
Pink	30	33.7		Hairy	34	38.2		Grey	4	4.5	
Purple	10	11.2		Smooth	55	61.8		Pink	8	9.0	
White	49	55.1		**Stem determinacy**			**0.67**	Purple	4	4.5	
				DT	35	39.3		Red	35	39.3	
Growth habit			**1.068**	NDT	54	60.7		Red & White	1	1.1	
I	28	31.5						White	11	12.4	
II	48	53.9		**Pod colour**			**0.6651**	Yellow	4	4.5	
III	9	10.1		Green	56	62.9		**Seed coat pattern**			**1.39332**
IV	4	4.5		Purple & Green	7	7.9		Absent	51	57.3	
				Red & Green	26	29.2		Broad Spotted	4	4.5	
Leaf colour			**0.682**					Broad Striped	13	14.6	
Dark Green	51	57.3		**Pod hairiness**			**1.1942**	Circular Mottling	1	1.1	
Light Green	38	42.7		Hairy	34	38.2		Rhomboid Spotted	4	4.5	
Leaf hairiness			**0.665**	Smooth	55	61.8		Speckled	6	6.7	
Hairy	34	38.2						Spotted Bicolour	1	1.1	
Smooth	55	61.8		**Seed shape**			**1.1942**	Striped	9	10.1	
Stem colour			**0.82**	Cuboid	12	13.5		**Seed brilliance**			**0.97156**
Dark Green	9	10.1		Kidney	15	16.9		Dull	25	28.1	
Green	61	68.5		Oval	48	53.9		Medium	50	56.2	
Red & Green	19	21.3		Round	14	15.7		Shinny	14	15.7	

3.3.2. Quantitative traits

Significant ($P \leq 0.05$) effects due to genotype, environmental effects and genotype × environment interactions were observed for days to flowering, days to maturity, leaf length, leaf width, number of pods per plant, number of seeds per pod, pod length, plant height, 100 seed weight and yield. The results indicated the existence of a significant effect ($P \leq 0.05$) due to the environment and the genotype × environment interaction for traits related to yield and its components. The combined analysis of variance revealed the presence of significant genotype × season interaction effects ($P \leq 0.05$) for all the traits. The means of the long rains season were significantly higher than in the short rains season. The ANOVAs for various traits are detailed in Appendix iii.

3.3.3. Phylogenetic analysis

The phylogenetic analysis revealed the presence of 4 distinct clusters, each characterized by specific traits. Among the traits considered, days to flowering, days to maturity, growth habit, and seed type were identified as the most informative in differentiating the genotypes (Figure 3.2). The first cluster was predominantly composed of large-seeded released genotypes, which served as reference checks in the study. The second cluster consisted of medium-seeded genotypes displaying a mixture of type I and type II growth habits. This includes the medium-sized seeds and a combination of determinate and semi-determinate growth habits among these genotypes. The third cluster comprised genotypes characterized as Type IV genotypes with medium size seeds. These genotypes exhibited specific morphological traits distinguishing them from other clusters. The fourth cluster was composed of small-seeded genotypes, representing a distinct group within the studied germplasm. These genotypes exhibited a combination of type II and type III growth habits, indicating variations in plant architecture and developmental characteristics.

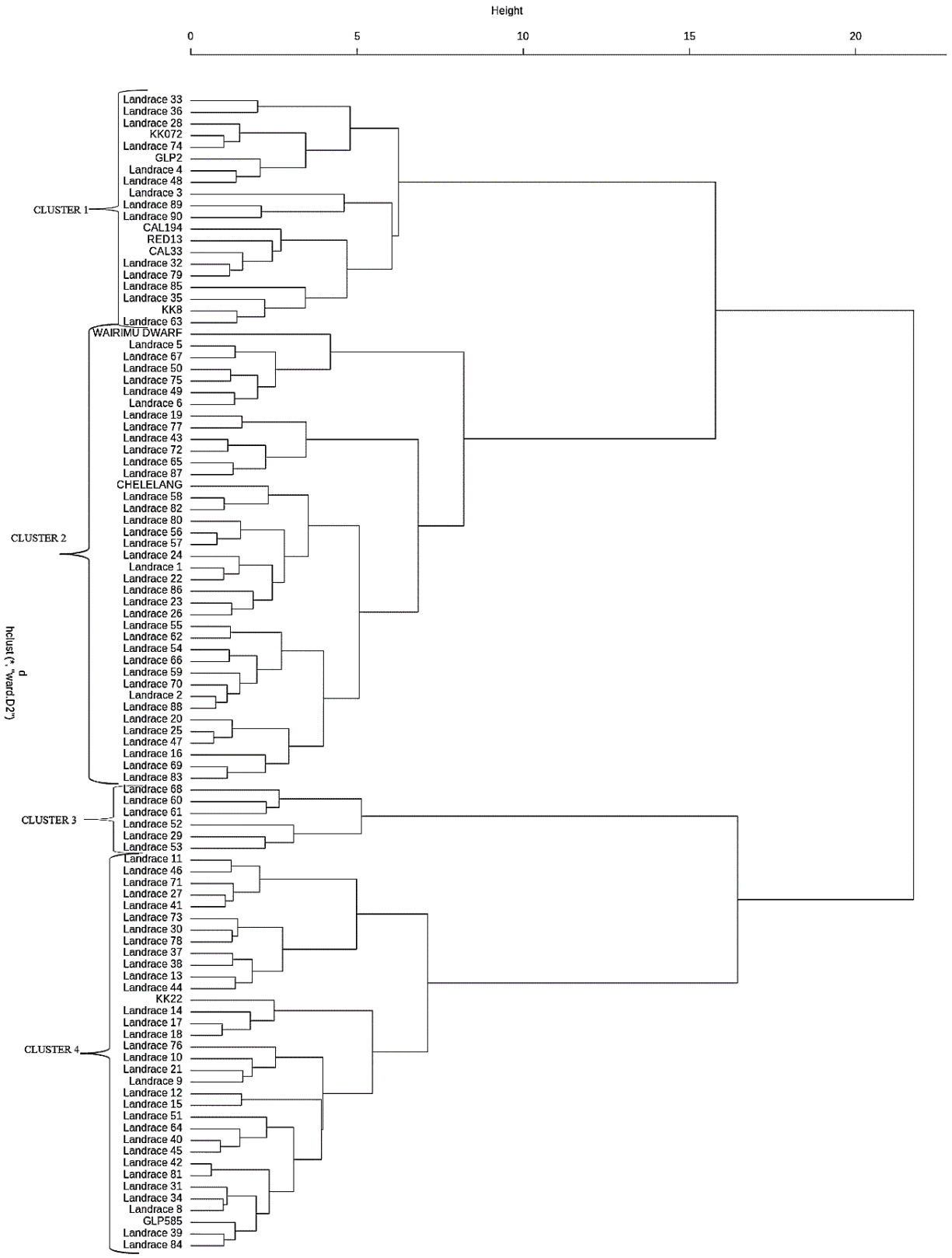

Figure 3.2: Dendrogram illustrating clustering of the common bean genotypes obtained from bean growing regions in Kenya based on agro-morphological traits

3.3.4. Principal component analysis (PCA)

Principal component analyses (PCA) of the quantitative data were conducted to determine the importance of different traits in explaining the variations among the genotypes. The first principal component (PC1) and the second principal component (PC2) accounted for 33.2% and 27.4%, respectively, of the total variation (60.6%). The *Eigen* vectors indicated that PC1 was mainly a positive indicator for days to maturity, yield, plant height, days to flowering and number of seeds per pod. PC2 was mainly a positive indicator of earlier days to flowering, leaf length, leaf width, pod length and characteristics with low harvest index. The components revealed that the germplasm scattered in all the quarters showing the high level of genetic diversity (Figure 3.3).

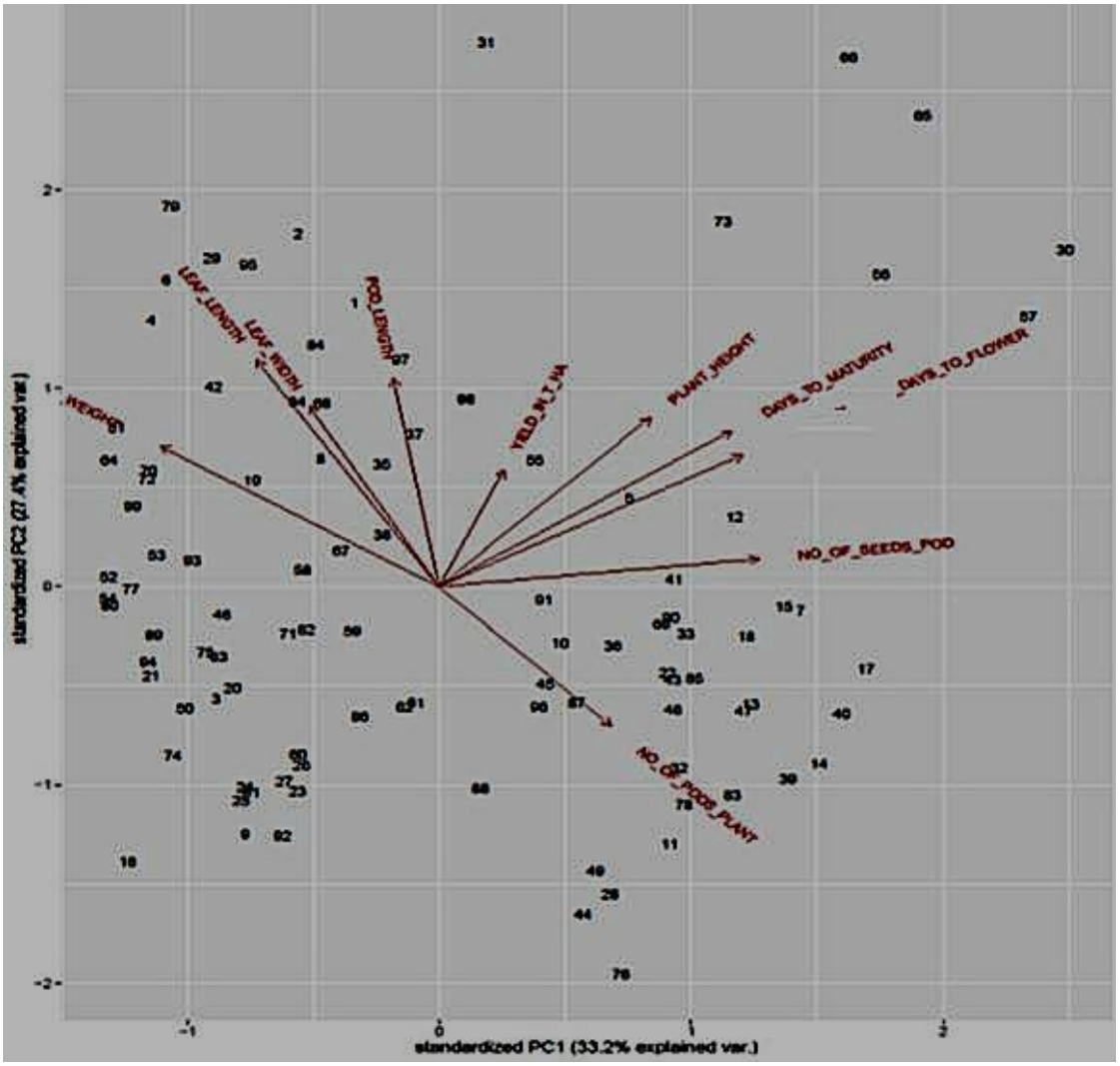

Figure 3.3: Two dimensional ordination of agronomic traits in common bean landraces based on principal component analysis.

3.3.5. Characterization of common bean genotypes using SSR markers

All the 22 SSR markers successfully amplified the DNA samples. The SSRs revealed a total of 51 alleles with a mean of 2.32 alleles per informative locus. The number of alleles per loci varied from 1 to 4. Of the 22 SSRs, 18 markers were di-allelic, one was tri-allelic and three were tetra-allelic. Major allele frequency ranged from 0.45 for marker BMD44 to 0.85 for marker GATS11 respectively, with an average of 0.65. The PIC value ranged from 0.22 for marker GATS11 to 0.59 for marker BM141 with a mean of 0.36. Ninety-one percent of the SSRs were moderately polymorphic i.e. PIC ≥ 25%. Gene diversity levels ranged from 0.25 for marker GATS11 to 0.65 for marker BMD44, with a mean of 0.45. Table 3.6 below shows the polymorphism level in terms of the number of alleles, allele frequency, gene diversity and polymorphism information content indices of the SSR markers. Figure 3.4 shows sample gel photos obtained from the study.

Table 3.6: Major allele frequencies, allele number, gene diversity and PIC based on variation at 22 SSR loci

Marker	Major Allele Frequency	Allele No	Gene Diversity	PIC
BM141	0.4737	4	0.6522	0.5891
BMD41	0.5263	4	0.5977	0.5242
PVGCCACC001	0.6000	2	0.4800	0.3648
BM114	0.6316	2	0.4654	0.3571
PVAT008	0.7579	2	0.3670	0.2996
PVAT004	0.6211	2	0.4707	0.3599
BMD45	0.7895	2	0.3324	0.2772
PVCCT001	0.5684	2	0.4906	0.3703
BMD44	0.4526	4	0.6540	0.5897
BM164	0.6842	3	0.4598	0.3920
BM211	0.7158	2	0.4069	0.3241
BM212	0.6947	2	0.4242	0.3342
BM183	0.6842	2	0.4321	0.3388
BMD12	0.7579	2	0.3670	0.2996
BM172	0.5053	2	0.4999	0.3750
PVAG001	0.5158	2	0.4995	0.3748
BM155	0.8316	2	0.2801	0.2409
PVAT003	0.5474	2	0.4955	0.3727
BM146	0.6211	2	0.4707	0.3599
GATS11	0.8526	2	0.2513	0.2197
BMD53	0.6632	2	0.4468	0.3470
PVATGC001	0.8211	2	0.2939	0.2507
Mean	**0.6507**	**2.3182**	**0.4472**	**0.3619**

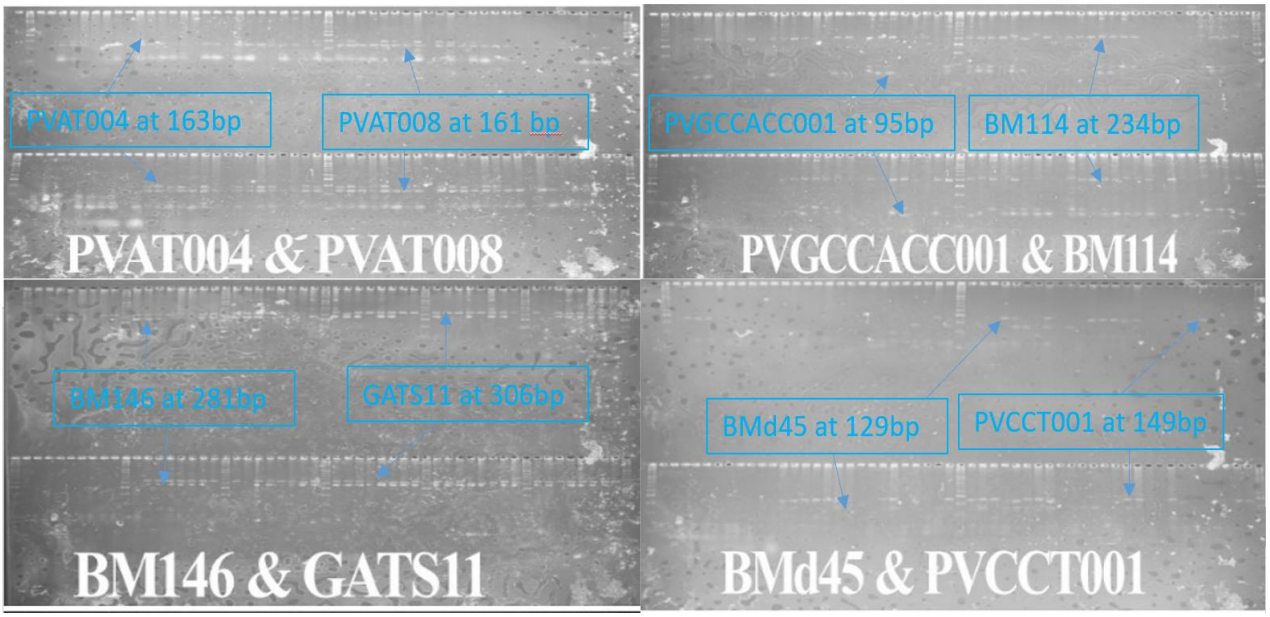

Figure 3.4: Polyacrylamide gel photos of SSR amplifications

The landraces and local genotypes were grouped into three clusters. Cluster 1 composed of released genotypes and landraces showed low genetic diversity due to the markers not tagging any repeats. It is possible that these genotypes and landraces possess other forms of genetic variation that were not captured by the SSR markers used. This cluster comprises of all seed sizes. The second cluster comprised of medium seeded genotypes. Cluster 3 comprised a mixture of traits with no unique observation within the clusters (Figure 3.5).

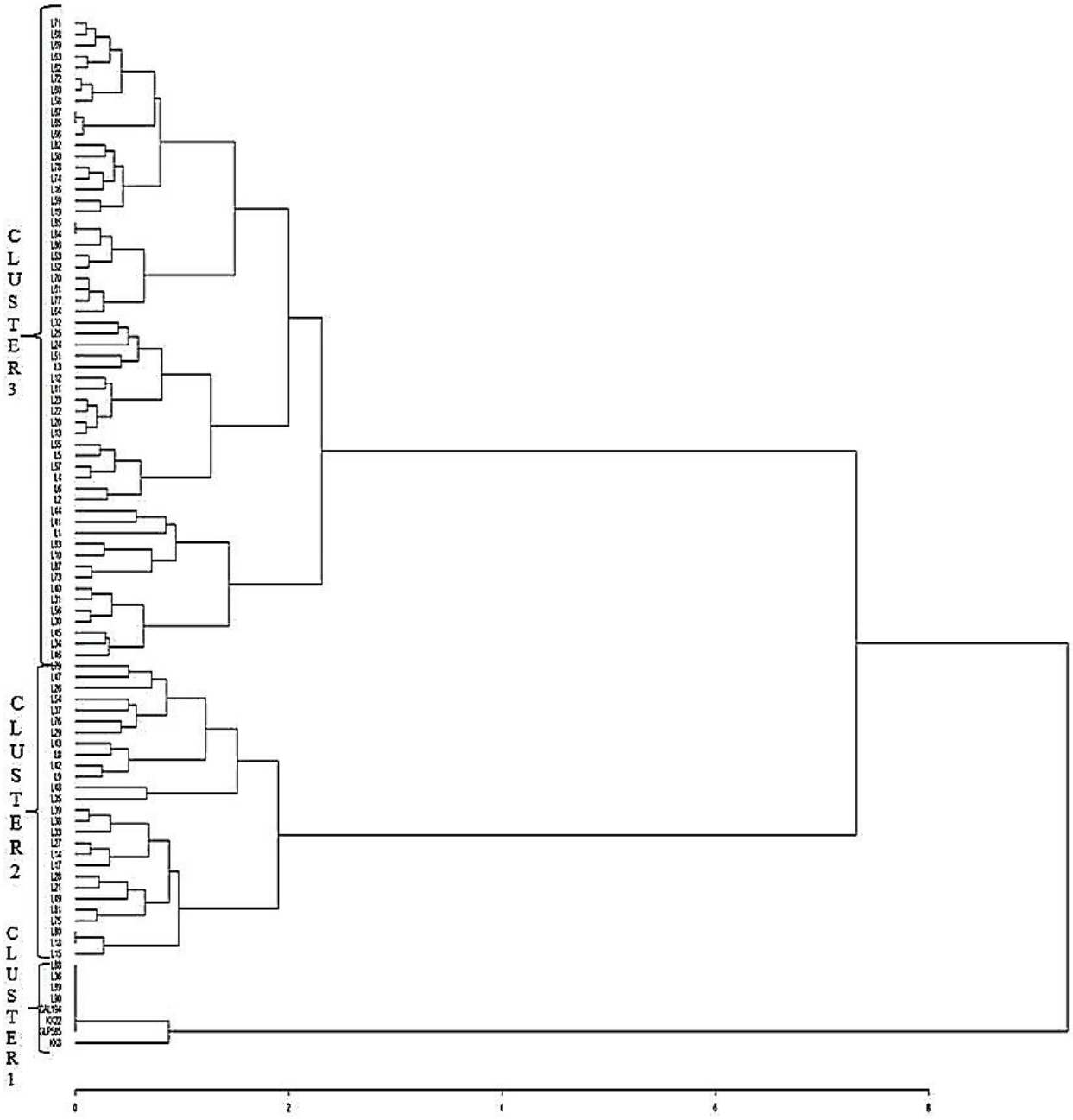

Figure 3.5: Dendrogram showing clustering of the germplasm based on SSRs

3.3.6. Comparison of morphological and molecular cluster analysis

The morphological cluster analysis did not mirror the genotypic clustering. According to Figure 3.6 below, the clustering was dissimilar. This indicates that the patterns of clustering or grouping observed in the analysis of morphological traits were different from those observed in the analysis of genotypic traits. The genetic makeup of the samples may vary significantly even though their physical traits appear similar, or vice versa.

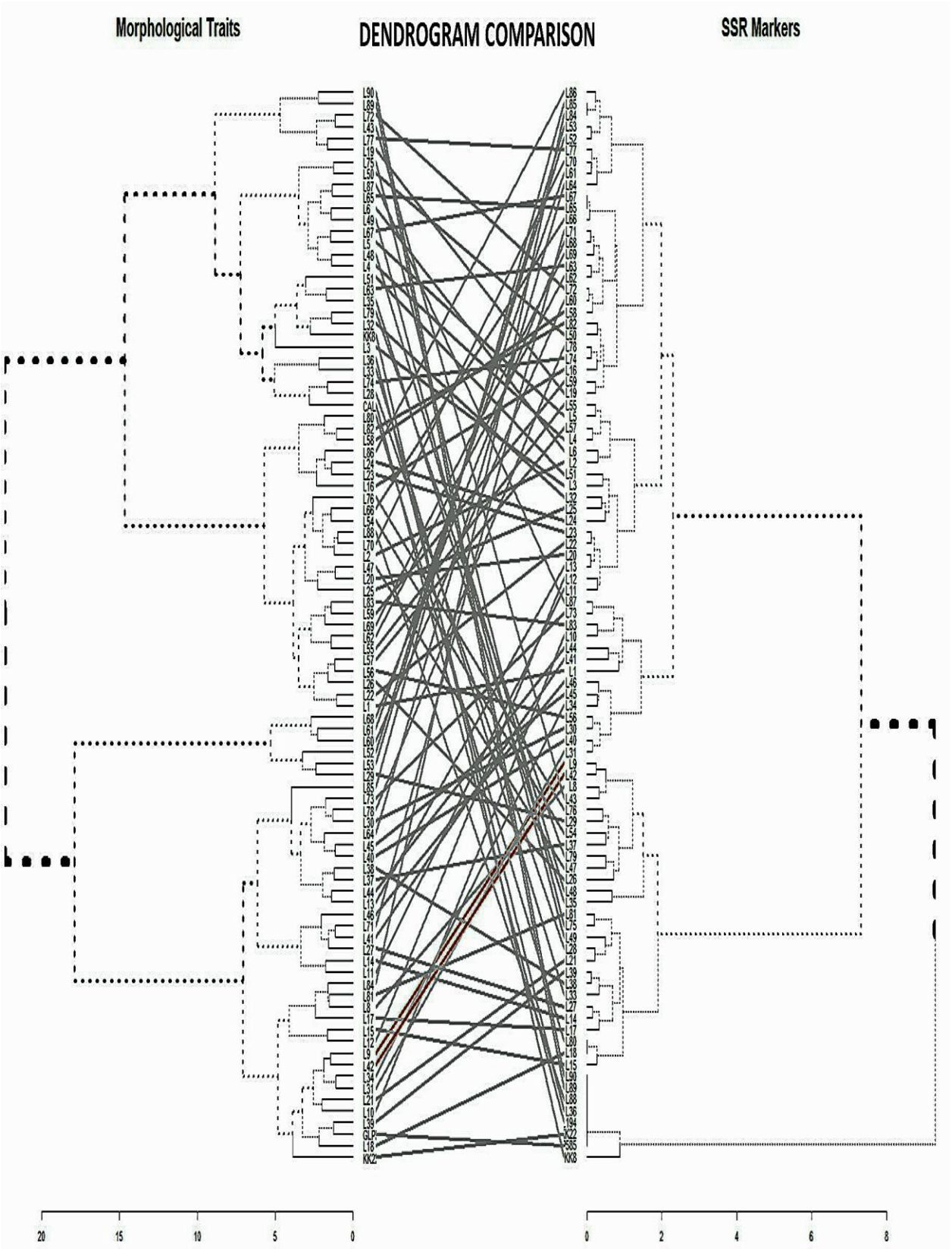

Figure 3.6: Phylogenetic comparison between morphological and genotypic characterization

3.4. Discussion

3.4.1 Morphological characterization

There was high variability in days to maturity, yield, plant height, days to flowering, number of seeds per pod, number of pods per plant and 100 seed weight under study due to the environmental effect. Farmers still cultivated other commercial genotypes in the sample collection regions alongside some of the landraces (Anunda, 2021). This possibly has to do with the large number of production constraints, especially biological, in the high potential bean growing zones (Farrow & Muthoni-Andriatsitohaina, 2020).

A significant variation was revealed among and within the common bean landraces for morphological traits. Flower colour, an index of mutation and a phenotypic marker in all bean genotypes is caused by anthocyanins (Gouveia et al., 2014). In this study, pink purple and white flower colours were observed. Most genotypes produced white flowers corresponding with the finding of Anunda (2021) who observed over 45% of the germplasm screened had white flowers. Flower colower is used by plant breeders as a criterion for determining varietal purity (Blair, 2006).

In Asfaw et al. (2009) study, the genotypes lacked anthocyanin pigmentation of the stem unlike in this study where 15% of the germplasm had pigmentation. Anthocyanin pigmentation in plant tissues, including stems, is often associated with stress responses and environmental adaptation. Anthocyanins can serve as antioxidants and play a role in protecting plant tissues from various environmental stresses, such as high light intensity, UV radiation, cold temperatures, and nutrient deficiencies. The presence of anthocyanin pigmentation in common bean stems may indicate a potential adaptive response to specific environmental conditions or stressors. The growth habit in common beans varies between climbing types to bush beans determined by a combination of factors including determinate verses indeterminate growth types, plant height, degree of branching and internode length. Genotypes with bush growth habit were over 50% corresponding to Anunda (2021) study. This is majorly due to beans being intercropped with maize. Bush beans are well-suited to the diverse agro-ecological conditions found in Kenya making them a versatile choice for farmers across the country.

In this study majority of the genotypes had predominant type I and type II growth as also observed by Asfaw et al. (2009). Bush beans are relatively easy to cultivate compared to other types of beans (Farrow & Muthoni-Andriatsitohaina, 2020). They have a compact growth habit and do not require support structures, which reduces labour and material costs for farmers. They also have a short maturity period, allowing for fast harvests and multiple cropping cycles. Climbing beans thrive in regions with high rainfall and cool temperatures, which are prevalent in certain areas of Uganda, Tanzania, Rwanda, and Burundi (Ronner et al., 2018). These countries have different agro-ecological zones compared to Kenya, where bush beans are suited to the prevailing conditions. Culinary traditions and consumer preferences vary across regions. In some East African countries, climbing beans are preferred for their taste, texture, and cooking properties (Katuuramu et al., 2020). They are often used in traditional dishes and have become an integral part of the local cuisine. In Kenya, bush beans are favoured for their adaptability and higher yields, which align with the farming practices and market demand in the country.

In relation to seed coat colour, this study is in agreement with Asfaw et al. (2009) findings where a majority of the landraces in Kenya were red in colour. Seed size and coat colour have been used to develop a convenient method of seed quality improvement (Blair et al., 2013). Red seed common beans are deeply ingrained in Kenyan culinary traditions. Significant genotypic and phenotypic variations were observed among the tested common bean genotypes across the sites for the quantitative traits. These variations suggest that there is genetic variation among the genotypes tested for various traits. Some of the landraces exhibited intrinsic genetic variation for key quality traits as compared to the released genotypes used as checks. Nkhata et al. (2020) found that the landraces screened are adapted to the regions, resistant to diseases and early maturity as compared to introductions because landraces are heterogenous. The variation in many traits with reference to those related to yield and its components together with flowering and earliness, can be employed to develop genotypes with very different characteristics and adapted to different environments, tailored towards market preferences.

The findings of the phylogenetic analysis based on morphological traits provided information into the genetic relationships and clustering patterns among the studied genotypes into the Andean and Mesoamerican groups, and intercross between the two gene pools. The intercross genotypes were also observed by Nadeem et al. (2018). These groups play crucial roles in determining the

phenotypic characteristics and adaptation of common bean genotypes. This information can be useful for breeders and researchers in selecting appropriate parental lines for hybridization, identifying potential donors of specific traits, and designing breeding strategies for developing improved bean cultivars.

The PC1 was primarily influenced by traits related to maturity and yield, also observed by Khatun et al. (2022). This suggests that genotypes with higher values for these traits are likely to have a positive impact on PC1. The influence of maturity and yield-related traits on PC1 suggests their importance in explaining a substantial portion of the observed variation (Khatun et al., 2022). Traits with positive correlation were also observed in a study by Arteaga et al. (2019). Similarly, the presence of traits related to early maturity, early days to flowering, leaf length, leaf width and pod length on PC2, indicates their contribution to the overall diversity within the germplasm with traits that exhibited a low harvest index, corresponding with a study by Mwangi et al., (2021). Furthermore, these findings highlight the significance of different traits in contributing to the overall variation observed among the genotypes. Understanding the importance of these traits in explaining the variations can assist in selecting desirable genotypes for further improvement of cultivars. By targeting genotypes with positive values on PC1 and PC2, it is possible to develop genotypes with improved maturity, yield potential, and specific morphological traits (Khatun et al., 2022).

3.4.2 Molecular characterization

Out of the twenty-two genomic SSRs used, BM141, BMd41, BMd44 and BM164 were informative. According to Blair et al. (2003) and Zhang et al. (2008), poor informativeness of cDNA-derived SSRs had been noticed in common bean. The low diversity within the germplasm at those particular loci is evident from the low level of polymorphism observed from the SSRs. Consistent low levels of heterozygosity observed is due to the autogamous habit of common bean (Beebe et al., 1997).

The SSR markers were able to distinguish the Mesoamerican and the Andean gene pools effectively than morphological characterization as also observed in a number of studies in common bean (Blair et al., 2006; Asfaw et al., 2009; Hegay et al., 2014; Okii et al., 2014; Zelalem et al., 2017). The findings in this study suggest that the genetic divergence in Kenyan common bean

landraces could be due to original differences in germplasm introduced from the primary centers of origin (Gyang, 2018; Nkhata et al., 2020). Spontaneous out-crossing in fields and farmer selection for adaptations could have also contributed to this divergence (Zelalem et al., 2017).

3.4.3 Comparison of morphological and molecular cluster analyses

Both morphological traits and SSRs were effective in discriminating the genotypes, although there was poor correlation between the two phylogenies. The dissimilarity between the clusters observed in SSR genotyping and those in morphological traits can be attributed to the fact that most plant traits exhibit polygenic inheritance, as highlighted by Arunga et al. (2015), along with the influence of environmental factors on the expression of morphological traits (Ceolin et al., 2007). It was found out by Singh et al. (1991) that differences in clustering based on morphological traits and molecular markers is due to hybridization or mutation.

CHAPTER FOUR

Host plant resistance to *Pythium* root rot and anthracnose among common bean landraces grown in Kenya

Summary

The common bean (*Phaseolus vulgaris L.*) is an important legume crop worldwide, contributing to food security and income generation. However, susceptibility to pathogens such as *Pythium* root rot and anthracnose poses significant challenges. This study evaluated resistance of common bean landraces from Kenya to anthracnose and *Pythium*, utilizing molecular markers and inoculation experiments. Eighty-nine common bean landraces that were collected across common bean growing areas in the country were evaluated. One susceptible check (*CAL 96*) and resistant checks *KK8* and *KK22* for *Pythium* root rot, and resistant check *G2333* for anthracnose, were also included. The MS61 isolate of the *Pythium* pathogen was cultured on CMA and PDA. Inoculum was prepared by fermenting finger millet with agar blocks of the pathogen, and subsequently, sterilized soil was inoculated with the pathogen. Anthracnose samples were collected from farmers' fields and cultured on PDA. The detached leaf method was used for disease screening. Disease evaluations were conducted based on disease severity. Molecular markers linked to resistance genes were used to screen the genotypes, and PCR amplifications were performed to detect the presence of resistance genes. The cultivar *KK8*, developed for *Pythium* root rot resistance, confirmed its resistance. Notably, 42.5% of landraces displayed moderate resistance to *Pythium*. Further, the resistance was assessed using the SCAR marker PYAA19800, which was detected only in the resistant checks, suggesting presence of additional resistance loci in moderately resistant and resistant landraces. Anthracnose resistance showed variability, with some landraces displaying resistance not detected by the SCAR marker that was linked to *C0-4* gene. The detached leaf method facilitated testing across different races. Clusters of resistance genes, including *Co-4* and *Co-5*, showed potential for breeding programs. This study highlights potential resistance sources, promoting sustainable disease management strategies for common bean production.

4.0. Introduction

The common bean (*Phaseolus vulgaris* L.) is the most widely grown edible legume species in the world. It is a member of the family Fabaceae, diploid ($2n = 2x = 22$) legume crop with a genome size of 473 (Schmutz et al., 2014). The crop is cultivated across the world for its leaves, green pods and dry seeds. The consumption of beans is directly related to the grain characteristics, such as colour, shape and size (Swema & Mwinuka, 2021). Despite its importance in addressing food security and a source of income to many rural communities in East Africa, the crop is predisposed to the attack by various pathogens like fungi, bacteria and viruses during favourable environments throughout the growing season (Sharma et al., 2012). Furthermore, low adoption rates for improved seed by the farmers predisposes the crop to some seed borne diseases like *Pythium* root rot and anthracnose (One Acre Fund, 2016). Farm-saved seed is the harvested grain set aside

by the farmer to plant in the following season or exchanged with neighbours or purchased from the informal local grain market, which in most occasions is of poor quality and sometimes infected by seed borne diseases like anthracnose (Ochichi et al., 2018). More than 75% of common bean farmers in Kenya prefer to sow farm-saved seed because it is cheap and readily available (Nzuma et al., 2016; Taban, 2017).

Common bean anthracnose caused by the highly variable Ascomycete, *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib, is a devastating disease of common bean in Kenya (Balardin et al., 1997; Gilio et al., 2020; Kamiri et al., 2021). The seed-borne pathogen is a major production constraint affecting common beans worldwide, especially in areas that experience high relative humidity and moderate temperatures (Singh & Schwartz, 2010; Conner et al., 2019). It may cause yield losses of 100% in cases of ineffective management strategies (Sharma et al., 2007; Mohammed, 2013; Padder et al., 2017). Initial symptoms start as dark brown lesions along the leaf veins on the underside of the leaves that later progress to the leaf petioles and stems and eventual seed infections which are majorly dark, sunken lesions that extend through the seed coat (Chilipa, 2016).

There has been a high prevalence of bean anthracnose in Kenya (Mogita, 2014) and relative variability of the pathogen (Musyimi, 2014; Kamiri et al., 2021). The high variability of the pathogen has been confirmed by the presence of many physiological races characterized through the reaction of a set of twelve differential genotypes when inoculated by different isolates (Ansari et al., 2004; Alzate-Marin & Sartorato, 2004; Sharma et al., 2007; Mota et al., 2016; Gupta et al., 2022). Close to 250 races have been identified where over 74 have been observed in Kenya (Mogita et al., 2013; Musyimi, 2014; De Lima et al., 2017).

The dynamic race structure of *C. lindemuthianum* populations has implications for the deployment of resistance genes, particularly in Africa where the majority of farmers are unable to purchase pathogen-free seeds or fungicides (Otsyula et al., 2004; Mogita et al., 2013). Management of anthracnose can be achieved through the use of certified seed, crop rotation, treatment of seeds with systemic fungicides (metalaxyl-m, carbendazim) and protectant fungicides (captan, thiram) and foliar using systemic fungicides. However, most of these strategies are not followed by marginal farmers having small land and poor resources (Sharma et al., 2012). Under such situations, the deployment of resistant genotypes offers most effective, least expensive and easy to

adopt strategies of managing anthracnose in common bean (Munda et al., 2009; Kiryowa et al., 2010). Generally, host plant resistance to anthracnose in common bean is monogenic and dominant although other patterns of inheritance have been detected (Kelly & Vallejo, 2004; Vallejo & Kelly, 2009; Oblessuc et al., 2014; Oblessuc et al., 2015). The loci *Co-1* to *Co-17* confer resistance to anthracnose and linked molecular markers have been mapped in the common bean chromosomes (Ferreira et al., 2013). The molecular markers are useful for indirect selection of alleles that confer resistance to anthracnose and subsequently, identification of resistant genotypes (Garzón et al., 2007; Beraldo et al., 2009). Sequence characterized amplified regions (SCAR) markers were developed for the resistance genes. The markers SAS13, SH18 and SBB14 are linked to *Co-4*^{2} while SAB3 marker is linked to the *Co-5* gene (Garzón et al., 2008; Vallejo & Kelly, 2009).

Common bean root rots caused by *Pythium* spp, *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Sclerotium rolsfsii* also cause significant yield losses and are widespread in Central and South America, and Africa (Abawi & Corrales, 1990; Buruchara et al., 2015). The most affected areas are highlands where air temperature is low with high relative humidity (Buruchara & Rusuku, 1992; Otsyula, 2010; Paparu et al., 2018), although the diseases have spread to low and mid-altitude areas due to changing environmental conditions (Farrow et al., 2011; Paparu et al., 2018). In particular, *Pythium* root rots can cause a yield reduction of up to 70% of some popular landraces and local genotypes in Kenya (Otsyula, 2010).

The control measures to curb disease spread include biological control by microorganisms such as *Burkholderia cepacia* and *Trichoderma harzianum* that protect the plant from fungal attacks through the production of antifungal metabolites namely Burkholdines Cepacidines, Pyrrolnitrin, Harzianolides, Trichodermin 6-Pentyl-α-pyrone (6PP) and Harzianopyridone (Whipps, 2001; Grosch et al., 2012; Niu et al., 2020; Lazcano et al., 2021). Cultural methods such as crop rotation, deep ploughing and ridging (Otsyula, 2010) have also been deployed to curb the disease. In addition, chemical control with systemic fungicide benomyl, captafol, carboxin, metalaxyl, propamocarb hydrochloride, etridiazole and captan as a protectant fungicide (Nzungize & Baudoin, 2012) are used by farmers. However, due to various challenges of the abovementioned control methods, genetic plant resistance is the most effective, economical and environmentally sustainable strategy to control *Pythium* root rot disease (Papias et al., 2016). *Pythium ultimum* resistance is controlled by a single dominant gene (*Pyult1*), that is marked by a dominant SCAR

marker (Otsyula, 2010). The SCAR marker $PYAA19_{800}$ has an association with *Pythium ultimum* resistance in *RWR 719, MLB 49-89A* and *AND1062,* located 2.7cM from the *Pyult1* gene (Mahuku et al., 2007). Therefore, the objective of this study was to screen common bean landraces for resistance to anthracnose and *Pythium* root rot through inoculation and by use of molecular markers.

4.1. Material and methods

4.1.1. Genotypes

Eighty nine common bean landraces collected across common bean growing areas in the country, one susceptible check (*CAL 96*) (Mukalazi, 2004) and resistant checks *KK8* and *KK22* (Otsyula, 2010) were used for *Pythium* root rot screening, while *G2333* was used as a resistant check for anthracnose resistance (Lobaton et al., 2018, Vallejo & Kelly, 2009; Garzón et al., 2008). The seed characteristics of the genotypes are described in section 3.1.1 in Table 3.1.

4.2. Screening for resistance to *Pythium* root rot

4.2.1. Media preparation

Corn meal agar and potato dextrose agar media were used for culturing the *Pythium* pathogen. Corn meal agar (CMA) was prepared for single tip isolation. This was achieved by suspending 17g of CMA in 1000 ml distilled H_2O. The media was then autoclaved at 121^oC at 103,421.4 pascal for 15 minutes before cooling it under a sterilized laminar flow. The media was later poured into sterilized petri dishes and let to solidify. The CMA offers a slower growing condition for the pathogen hence making it easier to carry out single tip isolation. Potato dextrose agar (PDA) was prepared by mixing 39 g of PDA with 1000 ml of distilled water in a media glass bottle. The media was thereafter autoclaved at a temperature of 121^oC at 103,421.4 pascal for 15 minutes. The media was then cooled in a sterilized laminar flow hood and then poured into sterilized petri dishes.

4.2.2. Pathogen isolation

The inoculum for MS61 isolate was obtained from CIAT, Uganda labouratory. This is the most virulent isolate and has been used in a number of studies (Otsyula, 2010; Mukankusi et al., 2018; Dramadri et al., 2020; Amongi et al., 2020). It had been previously stored on filter pater and was reactivated on CMA and PDA. The inoculum was later sub-cultured on fresh CMA (Figure 4.1) and PDA (Figure 4.2) for sporulation. For subsequent tests the isolate was plated on filter paper and stored at -20°C.

Figure 4.1: MS 61 isolates on CMA

Figure 4.2: MS 61 isolates on PDA

4.2.3. Inoculum preparation

A growth media made of finger millet (*Eleusine coracana*) was prepared by mixing 300 g of finger millet with 200 ml of distilled water in polythene bags. These were sealed and double autoclaved at a temperature of 121^{o}C at 103,421.4 pascal for 30 minutes. The finger millet was left to cool in a sterilized laminar flow hood before mixing each bag with small agar blocks of the sporulating pathogen from 4 petri dishes. The media was left to ferment and colonize the millet for 12 days at room temperature under sterile conditions as shown in Figure 4.3 and 4.4, respectively. The finger millet growth media was thereafter used to inoculate sterilized soil that was placed in 72 cm × 42 cm × 15cm wooden trays. The trays were covered by a polyethylene sheet and incubated for 7 days for the pathogen to colonize the soil. Seeds of the 89 landraces, the resistant check *KK22* and susceptible check *CAL96* were sown in the colonized soil in trays (Figure 4.5) in a completely randomized design (CRD). After germination, the trays were flooded with water for 2 days. The amount of water administered reduced subsequently as the plants grew. After 21 days, the *Pythium* root rot infected plants were uprooted and the roots checked for symptoms (Figure 4.6).

Figure 4.3: Fermentation of inoculated finger millet under sterile conditions

Figure 4.4: Colonized finger millet with *Pythium* MS61 isolate

Figure 4.5: Germplasm on inoculated trays with *Pythium* MS61 isolate

Figure 4.6: Plants growing on *Pythium* inoculated soils at 21 days after sowing

4.2.4. *Pythium* root rot evaluation

Disease rating was based on the incidence and severity 3 weeks after sowing at the first trifoliate stage (Abawi & Pastor-Corrales, 1990). The plants were uprooted and washed carefully under shade and evaluated for disease symptoms (Figure 4.7). Disease incidence was calculated as the percentage number of plants showing symptoms of the disease while severity was measured using a scale of 1-9 (Table 4.1), described by Schoonhoven & Pastor-Corrales (1987).

Figure 4.7: Evaluation of *Pythium* root rot infection on a CIAT 1-9 scale

Table 4.1: *Pythium* root rot screening scale

Reaction rating	Category	Description
1	Resistant	No visible symptom
2	Resistant	Light discolouration either without necrotic lesions or with less than 5% of the hypocotyl and root tissues covered with lesions
3	Resistant	Light discolouration either without necrotic lesions or with approximately 10% of the hypocotyl and root tissues covered with lesions
4	Moderately resistant	Approximately 15% of the hypocotyl and root tissues covered with lesions but tissues remain firm
5	Moderately resistant	Approximately 25% of the hypocotyl and root tissues covered with lesions but tissues remain firm with deterioration of the root system
6	Moderately resistant	Approximately 35% of the hypocotyl and root tissues covered with lesions combined with partial softening and reduction of root system
7	Susceptible	Approximately 50% of the hypocotyl and root tissues covered with lesions combined with considerable softening, rotting and reduction of root system
8	Susceptible	Approximately 60% or more of the hypocotyl and root tissues affected with advanced stages of rotting combined with severe reduction in the root system.
9	Susceptible	Approximately 75% or more of the hypocotyl and root tissues affected with advanced stages of rotting combined with severe reduction in the root system.

Source: (Schoonhoven & Pastor-Corrales, 1987)

4.3. Screening for resistance to anthracnose in common bean

4.3.1. Pathogen isolation and physiological characterization of *C. lindemuthianum*

Anthracnose samples were collected from the farmers' fields in western Kenya through a simple random sampling method. The samples were transferred to KALRO Kakamega labouratory for isolation as described by (Pastor-Corrales, 1998). In summary, 10 *Colletotrichum lindemuthianum* samples collected from the fields were cultured on PDA media. Successful cultures were plated on tap water agar (TWA) for 72 hrs. Single hypha were picked under a stereo microscope for single spore isolation and cultured on new PDA media for sporulation. These plates were incubated at 22°C in alternating 12 hours of light and darkness for 21 days.

Seeds of the 89 landraces and the resistant check (*G2333*) were sown in the screen house in 2 litre 20 cm diameter plastic pots containing 2kgs sterilized soil mixed with manure and sand in the ratio of 3:2:1. The screen house was maintained at an average temperature of 28 ℃. This was done after the single spore isolation to enable plants grow to the first trifoliate leaves by the time the cultures sporulated. Race identification and screening of differential genotypes was carried out in the labouratory using the detached leaf method described by Rezene et al. (2018). The races were

identified based on the susceptibility of each differential cultivar to each isolate using a binary nomenclature as described by Pastor-Corrales (1991). Races of 170, 815, 1286) of *C. lindemuthianum* were identified from the 10 isolates collected and the mixture of the 3 races was used as inoculum for the detached leaf technique.

4.3.2. Inoculation

Inoculum suspension that was obtained from 21 day-old cultures, with a final concentration of 1.2 x 10^6 spores per ml, mixed with Tween-20, was sprayed on the detached leaves with the aid of a 2l hand sprayer. The Tween 20 was added at the rate of 50 µL in every 10 ml of inoculum. The detached leaves from 21 day old plants were placed in petri dishes containing moistened paper towels to create a humid environment inside the petri dish. Two leaves from the trifoliate of each landrace were placed in 1 petri dish and replicated 3 times. The petri dishes were arranged in a randomized complete block design (RCBD) on a labouratory bench and incubated at room temperature (23-27°C) (Figure 4.8). Disease evaluation was done on the 14th and 21st day after inoculation with the later being considered. The CIAT scale of 1-9 (Table 4.2) was used for disease reaction scoring (Schoonhoven & Pastor-Corrales, 1987) whereby scores of 1-3 were considered resistant, 4-6 were moderately resistant and 7-9 were susceptible (Table 4.5).

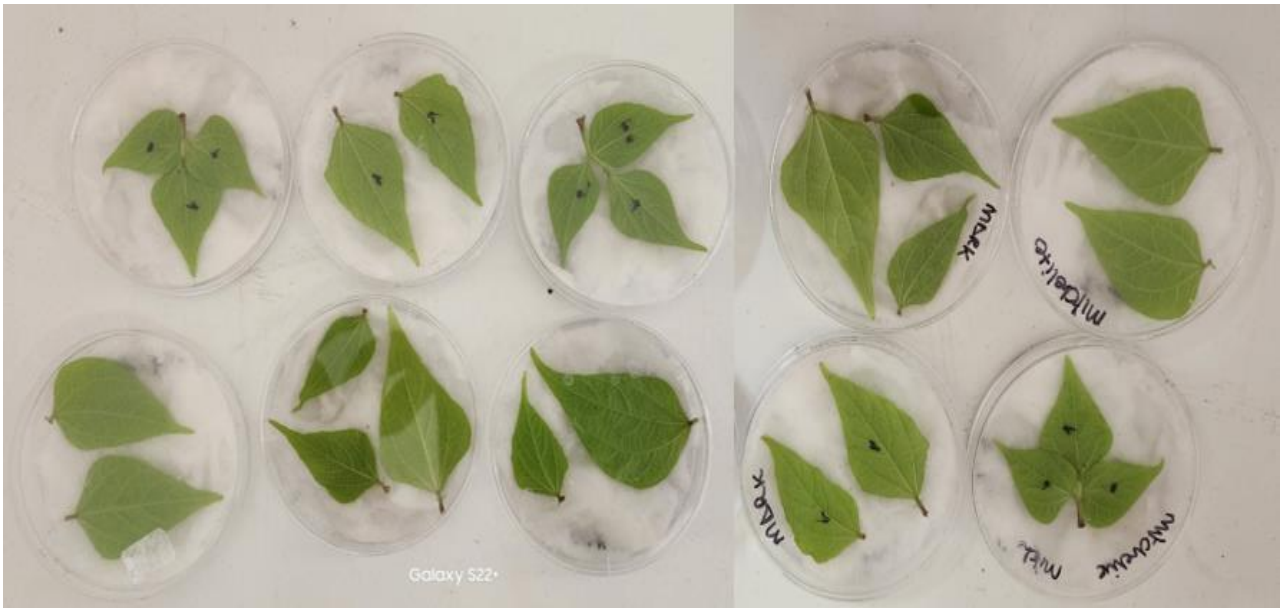

Figure 4. 8: Detached leaf inoculation method

Table 4.2: Description of anthracnose disease scoring scale

Reaction rating	Category	Description
1	Resistant	No visible symptom
2	Resistant	Lesions on up to 3% of leaf area
3	Resistant	Lesions on up to 5% of leaf area
4	Moderately resistant	Lesions and sporulation on up to 10% of leaf area
5	Moderately resistant	Lesions and sporulation with 2–3 mm in diameter on 11–15% of leaf area
6	Moderately resistant	Intermediate lesions and sporulation >3 mm in diameter on 16–20% of leaf area
7	Susceptible	Susceptible lesions and sporulation >3 mm in diameter on 21–25% of leaf area
8	Susceptible	Lesions and sporulation >3 mm in diameter on 26–30% of leaf area
9	Susceptible	Lesions, frequently associated with early loss of leaves and plant

Source: (Schoonhoven & Pastor-Corrales, 1987)

4.4. Screening common bean genotypes using molecular markers linked to anthracnose and *Pythium* resistance genes

The germplasm collected and described in Table 4.1 were planted in 2 litres plastic pots of 20 cm diameter, containing 2 kgs sterilized soil mixed with manure and sand in the ratio of 3:2:1. These were grown in the greenhouse at an average temperature of 28 ℃ for 14 days. One leaf from trifoliates was collected and placed in Eppendorf tubes while wearing clean gloves. Five samples were collected per genotype. DNA was extracted using the CTAB method as described by Afanador & Haley (1993). The DNA was quantified using agarose gel quantification method. DNA from all genotypes were amplified using SCAR markers which are linked to anthracnose and *Pythium* resistance genes. The SCAR marker $PYAA19_{800}$ was used to screen for presence of *Pythium* resistance gene *Pyult1* while SH18, SBB14 and SAB3 were used to detect anthracnose resistance genes *Co-4*^{2} and *Co-5*, respectively (Table 4.5). The PCR amplifications were performed in a 10 µL final volume containing 5 ng DNA, 20 µM of each forward and reverse primer, *puReTaq* Ready- To-Go PCR beads (GE Healthcare) dissolved in 25 µL of molecular water. These beads contain stabilizers, Bovine Serum Albumin (BSA), dNTPs, 2.5 units of *puReTaq* DNA polymerase, and a reaction buffer. When the bead is reconstituted to a 25 µL final volume, the concentration of each dNTP is 200 µm in 10 mM Tris-HCl, 10 mM KCl, and 1.5 mM $MgCl_2$ and 0.5 units of *Taq*-polymerase.

Table 4.3: SCAR markers linked to *Pythium* root rot and anthracnose resistance genes used to evaluate the germplasm for resistance

Marker	Marker origin	Pathogen	Size (Bp)	Primer sequences	Tagged locus	Annealing temperature	Reference
SH18	H18	Anthracnose	1100	F: 5'-CCA GAA GGA GCT GAT AGT ACT CCA CAA C-3' R: 5'-GGT AGG CAC ACT GAT GAA TCT CAT GTT GGG-3'	*Co-4*^{2}	65°C	(Awale & Kelly, 2001; Kelly et al., 2003)
SBB14	BB14	Anthracnose	1150/1050 codominant	F: 5'-GTG GGA CCT GTT CAA GAA TAA TAC-3' R: 5'-GTG GGA CCT GGG TAG TGT AGA AAT-3'	*Co-4*^{2}	67°C	(Awale & Kelly, 2001; Kelly et al., 2003)
SAB3	AB-3	Anthracnose	400	F: 5'-TGG CGC ACA CAT AAG TTC TCA CGG-3' F: 5'-TGG CGC ACA CCA TCA AAA AAG GTT-3'	*Co-5*	65°C	(Vallejo & Kelly, 2001; Campa et al., 2005)
PYAA19	AA19	*Pythium*	800	F: 5'-TTA GGC ATG TTA ATT CAC GTT GG -3' F: 5'-TGA GGC GTG TAA GGT CAG AG-3'	*Pyult1*	63°C	(Mahuku et al., 2007)

The following PCR regimes were used to perform the PCR reactions: A first denaturation step of 94 °C for 10 seconds, 30 cycles of amplification (denaturation at 94 °C for 10 seconds; annealing of primers to the template DNA at temperatures ranging from 30 seconds annealing step of primers to the template DNA at different temperatures ranging from 63 °C to 67 °C for 30 seconds; extension at 72 °C for 2 minutes) and a final extension of 5 minutes at 72 °C. The amplicons were separated by electrophoresis through a 1.2% agarose gel containing 0.5 µg/mL ethidium bromide at 150 V with a current of 100 A for 45 minutes in sodium borate buffer. This was visualized on a UV trans-illuminator and the gel image captured. Scoring was done by identifying and marking the positions of DNA bands on the gel image. The DNA markers were used as references to estimate the sizes of the fragments whereby the size of a fragment corresponds to the distance migrated from the well.

4.5. Data collection and presentation

The gel picture obtained was scored as (1) for the presence or (0) for the absence of the respective band. The data was presented in a table.

4.6. Results

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

Different disease severity levels were observed from the virulence tests. The genotypes reacted differently from each other but consistent across the replications. The germplasm screened were all susceptible to *Pythium* root rot with the exemption of landrace 58, Landrace 75 and the resistant check genotypes *CAL 194, KK8* and *KK22*. Almost half of the germplasm tested (42.5%) were moderately resistant to *Pythium* on a scale of 4 to 6 (Table 4.4). It was observed that a majority of the small seeded genotypes were moderately resistant as compared to the large seeded genotypes.

Table 4.4: Response of common bean genotypes to *Pythium* root rot isolate MS61 under greenhouse conditions

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

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

Anthracnose disease leaf symptoms started appearing on the 7th day after inoculation. Four landraces and the resistant check (*G2333*) showed no reaction to the disease but a majority of the genotypes were moderately resistant to the mixed inoculum on the 21st day after inoculation. A total of 33.7% of the genotypes were resistant to anthracnose, 41.3 % were moderately resistant while 25% were susceptible (Table 4.5).

Table 4.5: Response of common bean genotypes to a mixed isolate of anthracnose

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

Disease symptoms started appearing on the first week after inoculation. Figures 4.9 and 4.10 show a moderately resistant cultivar and a susceptible cultivar, respectively. Most of the genotypes that were screened showed a moderate resistance reaction except for 4 landraces that were considered resistant because they showed a similar disease reaction to the resistant check *G2333* indicating disease resistance. However, all released genotypes that were tested were susceptible compared with the landraces.

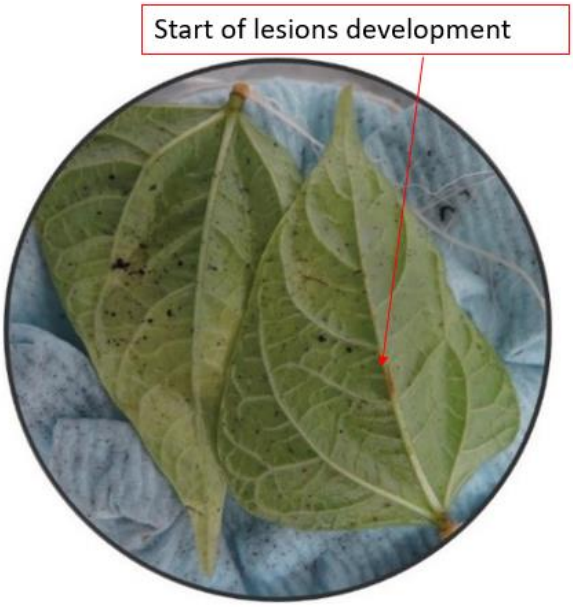

Figure 4. 9: Development of anthracnose lesions on inoculated common bean leaves

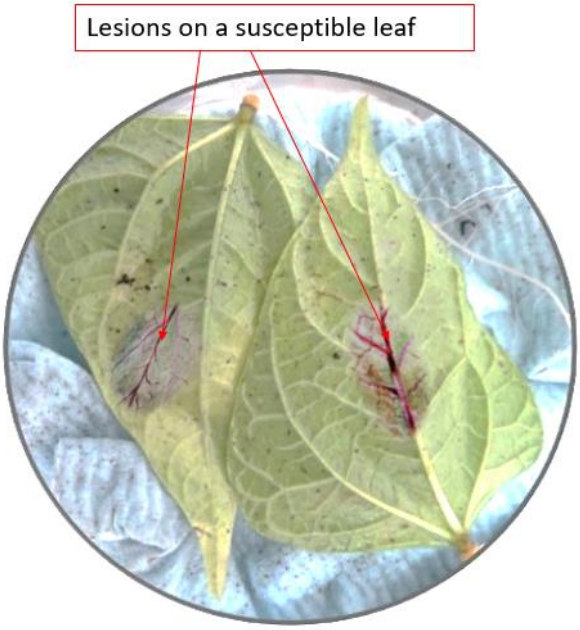

Figure 4.10: Symptoms of anthracnose on detached leaves of susceptible common bean genotypes

4.6.3. Characterization of common bean genotypes using molecular markers linked to *Pythium* and anthracnose resistance

Molecular characterization of the test germplasm showed that none of the landraces possessed the four molecular markers associated to resistance genes. The *Co-4*^{2} gene that was targeted by the SCAR marker $SH18_{1100bps}$ that was only detectable in genotype *G2333* (Figure 4.11). This results were also mirrored by the SCAR marker $SBB\text{-}14_{1050/1150bps}$ a codominant marker also linked to the *Co-4*^{2} gene (Figure 4.11). The *Co-5* gene targeted by $SAB3_{400bps}$ marker was only detectable in the differential cultivar *G2333*. The case was similar for *Pythium* root rot where none of the DNA from landraces was amplified by the SCAR marker $PYAA19_{800}$. This includes three landraces (58, 75 and 8) which had some level of resistance to *Pythium* root rot under greenhouse conditions. The marker was only detected in the control genotypes *KK8* and *KK22* (Figure 4.11).

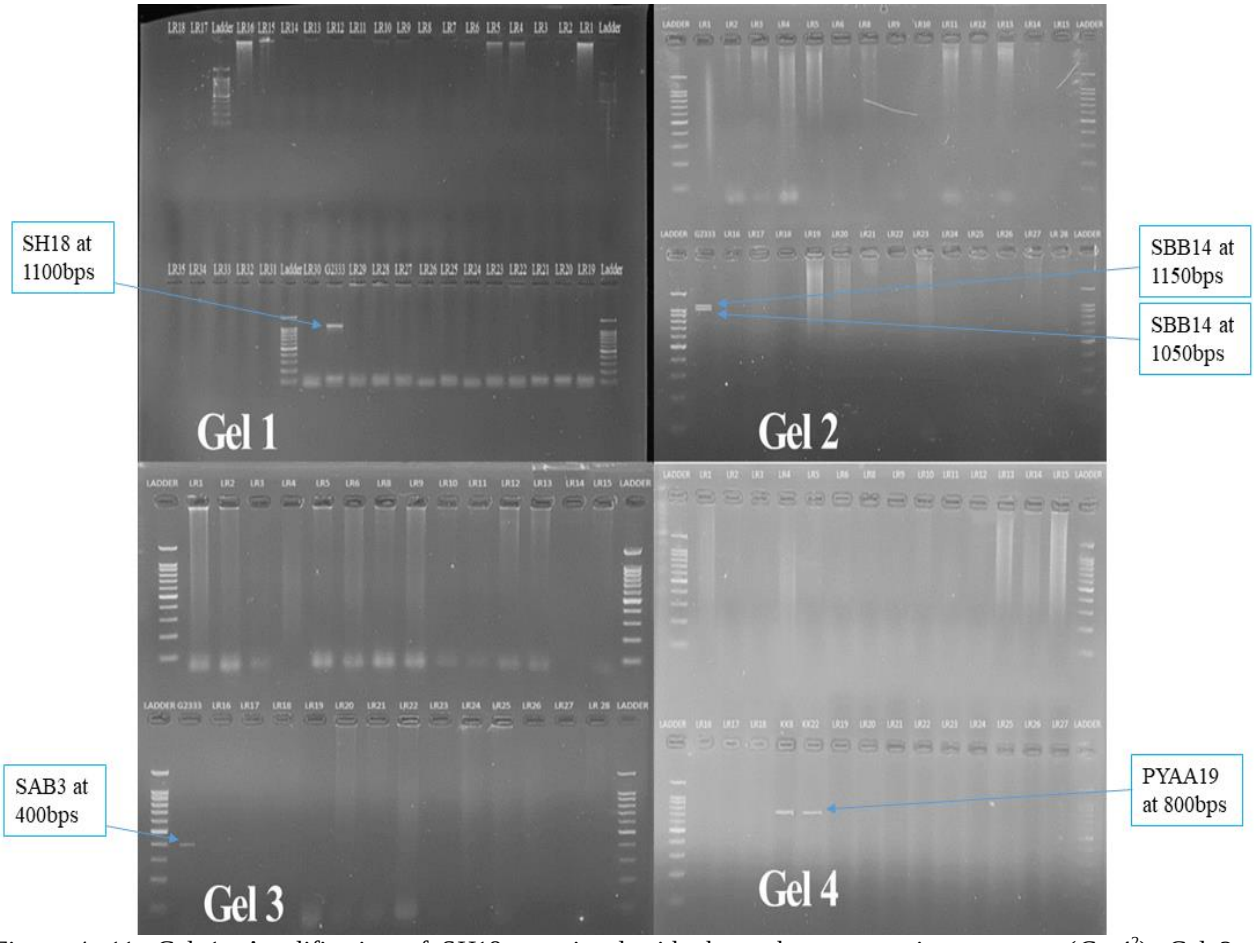

Figure 4. 11: Gel 1- Amplification of SH18 associated with the anthracnose resistance gene (*Co-4*^{2}). Gel 2- Amplification of SBB14 associated with the *Co-4* anthracnose resistance gene. Gel 3- Amplification of SAB3 associated with the *Co-5* anthracnose resistance gene. Gel 4- Amplification of PYAA19800 associated with the (*Pyult1*) *Pythium* root rot resistance gene.

4.7. Discussion

4.7.1 Resistance to *Pythium* root rot

The bean cultivar *KK8* that is resistant to *Pythium* root rot was found to possess the resistance based on greenhouse screening and molecular markers. Most of the large seeded landraces were susceptible to *Pythium* root rot since resistance to the disease is majorly found within the Andean gene pool. Otsyula (2010) similarly indicated that majority of the small seeded landraces showed moderate resistance to *Pythium* root rot. The susceptible bean cultivar *CAL 96* still showed susceptibility as previously reported by Nzungize et al. (2011). In their study, Anunda et al. (2019) identified moderately resistant landraces. However Mukankusi et al. (2010) did not identify moderately resistant landraces in Uganda.

Genetic diversity among landraces and the pathogen population can influence the expression of resistance. Differences in the genetic makeup of landraces and the pathogen strains could contribute to the varying resistance levels observed. Virulence tests showed different disease severity levels depending on the cultivar screened but the same mode of reaction per cultivar was observed across both data points. Normally each cultivar maintains its relative expression of resistance or susceptibility on both roots and hypocotyls across the screening seasons (Li et al., 2014). A study by Dramadri et al. (2020) also found that the resistance or susceptibility of a given bean cultivar was similar at every screening. The use of MS61 isolate in this study was due to previous studies using the isolate to identify general resistance to a wide spectrum of *Pythium* species (Otsyula 2010; Nzungize et al. 2011; Dramadri et al. 2020; Amongi et al. 2020).

To expedite the evaluation process, disease assessments in the greenhouse were conducted using a single isolate of *Pythium* root rot, ensuring consistent and standardized levels of disease inoculum. It is however of importance to conduct further screening of the genotypes under field conditions for resistance to other root rot pathogens because of the possibility of various root rot pathogens occurring in the same field (Paparu et al., 2018). Resistance gene development in bean genotypes and long lasting strategies rely on identifying useful genes and a good understanding of the host – pathogen interaction under field conditions.

The DNA amplification using the SCAR Marker $PYAA19_{800}$ was only observed in the controls *KK22* and *KK8* and not detected in resistant landraces probably because the marker could only detect the presence of the *Pyult1* gene. Namayanja et al. (2014) suggested the presence of other loci conferring resistance to *Pythium ultimum*. The moderately resistant landraces and the two resistant landraces could be possessing other loci that condition *Pythium* resistance. There could be no resistant landraces among the germplasm but high genetic variability exists among the susceptible and moderately resistant genotypes (Anunda et al. (2019). The use of one marker in this study limits the identification of other loci that may be conferring resistance to *Pythium* root rot hence there is need to develop other markers that can tag these specific loci (Maryrose et al., 2015).

4.7.2 Resistance to anthracnose

There was sufficient disease development during the two screening sessions because symptoms of anthracnose were first observed seven days after inoculation. Previous studies have reported that anthracnose develops on inoculated plants within 2-3 weeks after inoculation (Mahuku et al., 2003; Pereira et al., 2014). Characterization of different races occurring in the region will assist in understanding the composition of races hence aiding in breeding for resistance as anthracnose exhibits a vertical/qualitative form of resistance (Miklas et al., 2006). In this study, high variability anthracnose races were identified as 170, 815 and 1286. The use of mixtures of different races could be a viable option as it has been reported to have synergistic effects resulting in increased disease symptoms (Aliyu et al., 2013; Tembo, 2016; Falleiros et al., 2018; Ogunsola et al., 2021). These findings prompted the use of mixed races of 170, 815 and 1286. For individual cultivar performance, there were differences in cultivar performance compared the observations indicated by Kiptoo et al. (2020) studies. For instance, bean cultivars Chelalang and Tasha were reported to be resistant which contradicts the present findings. This was also the case with Tasha where according to their findings, it was tolerant to anthracnose. Nevertheless, crosses between genotypes with complementary resistance spectra could be used to develop lines with wide resistance spectra (Ferreira et al., 2008).

The detached leaf method that was used in this study is advantageous whereby the same plant can be tested using different races avoiding possible interaction among races of the same pathogen or different pathogens during inoculation, reduction in costs and working time (Rezene et al., 2018). Although the technique may be unreliable in very specific race-genotype combinations with predominant symptoms on stems and stalks (Pereira et al., 2014), it can be utilized for procedures where detached leaves can provide satisfactory results.

The SCAR markers that were used in this study did not amplify any of the landraces despite some of them showing resistance phenotypically pointing towards the possibility of other genes that confer resistance to anthracnose. The possibility of exploiting molecular markers that are associated with the gene *Co-5* and the *Co-4*^{2} in improving anthracnose resistance in beans was investigated (Garzón et al., 2008). Gene *Co-4*^{2} was only detectable in cultivar *G2333* by the SCAR markers SH18 (1150 bp) which is linked in coupling phase with *Co-4* locus at 4.27±2.37 cM and SBB14 (150/1050 bp) linked at 5.89±1.93 cM from the *Co-4* gene (Kelly et al., 2003). These were

hence used as flanking markers during the genotypic screening (Awale & Kelly, 1999). The SAB3 (400 bp) maker linked to the *Co-5* gene at a distance of 5.9cM (Vallejo and Kelly, 2001) was amplified in the anthracnose differential cultivar *G2333*. The limited use of *Co-5* in breeding makes the locus important in breeding programs mainly during gene pyramiding (Kamiri et al., 2021). The *Co-4* gene is of importance since it confers a broad resistance to a huge number of races.

Clusters of resistance genes have been identified and described in different plant species (Michelmore & Meyers, 1998). These clustered resistance regions have also been found in the *P. vulgaris* genome (Kelly et al., 2003), hence identification of anthracnose resistance loci consisting of many resistance genes. These findings therefore call for further screening of the germplasm to explore other loci that confer resistance to anthracnose. A combination of *Co-4* and *Co-5* genes show the most effective broad resistance to *C. lindemuthianum* races hence the decision of the study to focus on the two genes. The fact that some of the landraces were resistant shows that they can be valuable sources of genetic stocks in breeding programs. Anthracnose and *Pythium* root rot can cause substantial damage to bean crops, leading to significant economic losses for farmers. By deploying adapted common bean genotypes carrying disease resistance genes, farmers can reduce the reliance on chemical pesticides and adopt more sustainable farming practices.

CHAPTER FIVE

Marker-assisted introgression of *Pythium* root rot and anthracnose resistance genes to improve common bean landraces in Kenya

Summary

Common bean (*Phaseolus vulgaris* L.) is an important legume crop in East Africa for direct consumption and as a source of income to many small-holder farmers. The enhancement of common bean landraces in Kenya holds significant agricultural importance due to their intrinsic genetic diversity and adaptability. However, the prevalence of *Pythium* root rot and anthracnose diseases poses substantial challenges to their productivity. This study aimed to enhance disease resistance in common bean landraces prevalent in Kenya through marker-assisted backcross breeding, targeting anthracnose and *Pythium* root rot. The breeding program involved controlled crosses between resistant donor parents (*G2333* for anthracnose and *KK8* for *Pythium* root rot) and susceptible landraces (Sugar 1, Sugar 2, and Sugar 3). The resultant F_1 progenies were backcrossed iteratively, incorporating resistance alleles through marker-assisted selection. Molecular markers (SH18 for anthracnose and PYAA19800 for *Pythium* root rot) facilitated efficient foreground selection at each generation, allowing for the identification of plants carrying the desired resistance genes. The BC_3F_2 generation, resulting from these crosses, exhibited improved disease resistance. Phenotypic evaluations confirmed that the resistance to anthracnose and *Pythium* root rot was inherited dominantly in the genotypes. Analysis of the genotypic data revealed that out of 99 selected BC_3F_2 lines, 36 lines carried resistance alleles for both diseases, demonstrating the successful introgression of multiple resistance genes. The utilization of marker-assisted breeding demonstrated the efficacy of gene pyramiding, as indicated by the broad and consistent resistance observed in the developed lines. This study highlights the potential of marker-assisted backcross breeding as a rapid and effective approach for incorporating disease resistance genes into common bean landraces. By enhancing resistance to both anthracnose and *Pythium* root rot, the developed genotypes hold promise for sustainable bean production in Kenya.

5.0. Introduction

Anthracnose caused by *Colletotrichum lindemuthianum*, is a seed transmitted pathogen (Halvorson et al., 2021) characterized by dark brown sunken lesions on all above ground parts including the seeds (Mohammed, 2013; Padder et al., 2017). Anthracnose attacks common bean in cool weather with field losses of up to 70% due to seedling, leaf, stem and pod infections under favourable climatic conditions (Vazin et al., 2015). The pathogen survives on plant residue which in turn helps its spread (Conner et al., 2019). With the continuous use of infected seed in the African set up through seed exchange, the disease spreads to wider areas (Mogita, 2014). The pathogen has a high variability evident from the identification of 182 races obtained through

characterization of 1500 isolates using differential genotypes (Padder et al., 2017). The management strategies of *Colletotrichum lindemuthianum* include host resistance, cultural and chemical control methods, with the use of resistant genotypes being the most efficient (Mohammed, 2013; Meziadi et al., 2016).

Pythium root rot disease is a major disease threatening the production of common bean in western Kenya (Otsyula, 2010). Soils with high organic matter and poor drainage favour pathogen survival and inoculum build-up (Nzungize & Lyumugabe, 2012). Root rot symptoms due to the pathogen include a wet rot of the seedling, pre or post-emergence where the pith of the stem is attacked leading to hollowness and yellowing of leaves, wilting and eventually death (Bost, 2006).

The control of anthracnose, a destructive fungal disease, and *Pythium* root rot in common bean crops in East Africa has been achieved through various control measures including chemical, cultural, biological, and genetic approaches (Nzungize & Lyumugabe, 2012; Were, 2019). Among these measures, the utilization of resistant genotypes has proven to be effective and easily adopted. In the case of anthracnose resistance, a specific gene (*Co-4*^{2}) has shown resistance to a number of races. Incorporating gene *Co-4*^{2} into common bean genotypes through marker-assisted selection (MAS) significantly enhances their resistance to anthracnose (Papias et al. 2016). Donor parents, such as the variety *G2333*, carrying the *Co-4*^{2} gene, have been used in breeding programs to introgress the resistance into desired genotypes (Kazimoto et al., 2022). The success of MAS in incorporating anthracnose resistance has been confirmed by the use of the SH18 marker, which serves as a tool for verifying the successful introgression of the resistance gene in each generation.

Similarly, for *Pythium* root rot resistance, previous studies identified a single dominant gene conferring resistance to the disease, present in genotypes *RWR 719*, *AND1062*, and *SCAM-80-CM/15* (Otsyula et al., 2003; Nzungize et al., 2011). The cultivar *KK8*, developed from a breeding program that incorporated the aforementioned resistant genotypes, has been utilized as a donor parent for *Pythium* root rot resistance. The PYAA19$_{800}$ SCAR marker was developed and used for MAS to confirm the successful introgression of *Pythium* resistance in different backgrounds (Mahuku et al., 2007; Nzungize et al., 2011). Marker-assisted selection enables breeders to select for resistance at the seedling stage, reducing the need for extensive resistance tests and allowing identification of resistant genotypes even in the absence of the pathogen (Miklas et al., 2006;

Collard & Mackill, 2008; Ferreira et al., 2012; Ndee, 2013; Uwera et al., 2021). The incorporation of marker-assisted selection in East African bean breeding programs has proven to be successful in introgressing both *Co-4*^{2} and *Pythium* root rot resistance genes. By using specific molecular markers that are tightly linked to the resistance genes, breeders have improved the efficiency and accuracy of selecting for desired traits (Miklas et al., 2006; Collard & Mackill, 2008; Gupta et al., 2010).

Breeding for disease resistance is crucial, particularly when targeting multiple pathogens simultaneously. To achieve broader and long-lasting resistance, a strategy known as gene pyramiding is employed, wherein multiple resistance genes are incorporated into a single genotype (Mondo et al., 2019). The study conducted by Musyimi (2014) confirmed the correlation between disease reactions and marker selection data. Genotypes possessing the resistance markers exhibited low disease reactions against the targeted pathogens, indicating the effectiveness of the transferred genes. Introgression of anthracnose resistance genes by Uwera et al. (2021) proved the success of incorporating specific resistance genes through marker-assisted selection in developing genotypes with enhanced disease resistance. This approach facilitated the development of breeding populations combining resistance to multiple diseases, such as anthracnose and *Pythium* root rot, in common bean genotypes (Luseko et al., 2013; Mondo et al., 2019). The objective of this study therefore, was to develop common bean lines with combined resistance to the anthracnose and *Pythium* root rot.

5.1. Materials and methods

5.1.1. Genotypes

Three common bean landraces; Sugar 1 (Landrace 90), Sugar 2 (Landrace 89) and Sugar 3 (Landrace 88), that are popularly grown in western Kenya, were collected and maintained at KALRO, Kakamega. The landraces were used in this study as recurrent parents while common bean genotypes *G2333* and *KK8* were used as donor parents for anthracnose and *Pythium* root rot resistances, respectively. These landraces were selected based on maturity and farmer preferences and they are cultivated by most farmers in the region (Anunda, 2021; Asfaw et al. 2009) although they are susceptible to *Pythium* root rot and anthracnose as described in Chapter 4, Section 4.5.1 and 4.5.2.

5.1.2. Breeding scheme

A backcross breeding program was used to develop a breeding population combining resistance to anthracnose and *Pythium* root rots. The landraces were hybridized through emasculation and pollen deposited on the stigma (Kalve, 2017). Initially, a three-way crossing scheme was utilized and later progressed in a backcross breeding scheme (Figure 5.1). SCAR markers were used at every generation to confirm successful introgression of the genes. The SCAR marker $PYAA19_{800}$ was used to confirm successful *Pythium* root rot resistance i.e. *Pyult1* (Mahuku et al., 2007) while SH18 was used to confirm anthracnose resistance introgression i.e. *Co-4*^{2} (Awale & Kelly, 2001). The *Pyult1* gene offers broad resistances to root rot diseases while *Co-4*^{2} confers resistance to a wide range of anthracnose races.

The PCR amplifications were performed in a 10 µL final volume containing 2 µL of quantified DNA on agarose gel, 20µM of each forward and reverse primer, *puReTaq* Ready-To-Go PCR beads (GE Healthcare) dissolved in 25 µL of molecular water. These beads contain stabilizers, BSA, dNTPs, 2.5 units of *puReTaq* DNA polymerase and a reaction buffer. When the bead is reconstituted to a 25 µL final volume, the concentration of each dNTP is 200 µm in 10mM Tris-HCl, 10 mM KCl and 1.5 mM $MgCl_2$ and 0.5 units of *Taq*-polyearase. Polymerase chain reaction (PCR) was conducted in a thermocycler in the following regime: initial denaturation (94°C/5 min), followed by 30 cycles of denaturation (94°C/10 s), primer pair-specific annealing step and an extension step (72°C/2 min), and was completed by a final extension step (72°C/5 min). The amplicons were separated by electrophoresis through a 1.2% agarose gel containing 0.5 µg/mL ethidium bromide in sodium bromide buffer tank. The gel picture obtained during genotypic screening for each landrace was scored as (1) for the presence of band or (0) for the absence of band. The selected BC_3F_1 populations were selfed and the resulting BC_3F_2 lines, recurrent parents and the 2 resistance donors were jointly evaluated using 3 *C. lindemuthianum* races (170, 815, 1286) and a mixture of the 3 races. The MS61 isolate was used for screening *Pythium* root rot resistance. Evaluation of breeding lines was conducted as detailed in Section 4.2 and 4.3.

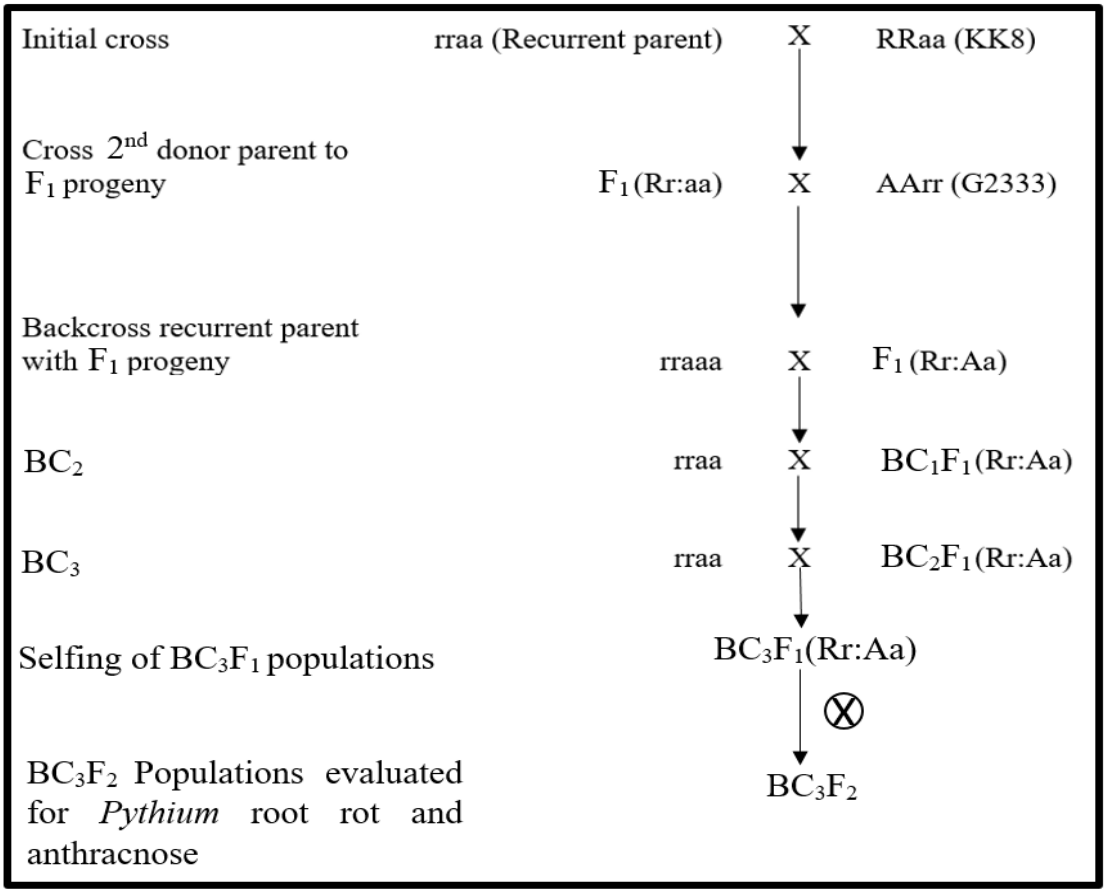

Figure 5.1: Backcrossing scheme for introgression of resistance to *Pythium* root rot and anthracnose into common bean landraces

5.2. Data collection and presentation

The gels images scored as (1) for the presence of band or (0) for the absence of band were presented in a table format as the number of lines carrying both markers. The disease evaluation of the breeding lines was also recorded as resistant and susceptible.

5.3. Results

The first cross involving the anthracnose and *Pythium* root rot resistance donors (F_1) and the recurrent genotypes Sugar 1, Sugar 2 and Sugar 3 yielded 70 F_1 seeds. Subsequently, BC_1 to BC_3 seeds were obtained as detailed in Table 5.1. The lines with varying single genes were not advanced beyond each generation and only plants with the 2 genes were advanced. A total of 20 F_1 plants from SUGAR 1/*KK8*, 20 plants from 'SUGAR 2/*KK8*' and 30 plants from 'SUGAR 3/*KK8*' were used as pollen recipients for a three-way cross with the anthracnose resistance donor parent to obtain SUGAR/*KK8*//*G2333*. The three-way cross yielded 28 seeds that were sown in the greenhouse, in the following season, to develop BC_1 population in a marker assisted backcross

breeding program. The molecular markers $PYAA19_{800}$ and SH18 that were utilized for foreground selection, amplified the expected fragment of 800bp and 1100bp, respectively (Figure 5.2). For the second backcross (BC_2), 33 seeds were obtained including, 14 for Sugar 1, 9 for Sugar 2 and 10 for Sugar 3. From these 33 BC_2 plants, 87 seeds were harvested and sown to develop the BC_3 population. Foreground selection of these plants revealed that 36 BC_3 plants possessed resistance alleles at the two loci and 99 BC_3F_2 plants possessed resistance loci.

Table 5.1: Frequencies of phenotypic and genotypic classes for *Pythium* root rot and anthracnose resistances

	NUMBER OF SEEDS OBTAINED										
CROSS	F_1 Seed	Selected F_1 plants	BC_1 seeds	Selected BC_1 plants	BC_2 Seeds	Selected BC_2 plants	BC_3 Seeds	Selected BC_3 plants	No. of BC_3F_2 plants	No. of R BC_3F_2 plants	No. of S BC_3F_2 plants
SUGAR 1 /*KK8//G2333*	20	11	30	10	22	14	25	12	32	19	13
SUGAR 2 /*KK8//G2333*	20	8	23	13	15	9	24	10	37	21	16
SUGAR 3 /*KK8//G2333*	30	9	35	14	28	10	38	14	105	59	46
TOTAL	70	28	78	37	65	33	87	36	174	99	75

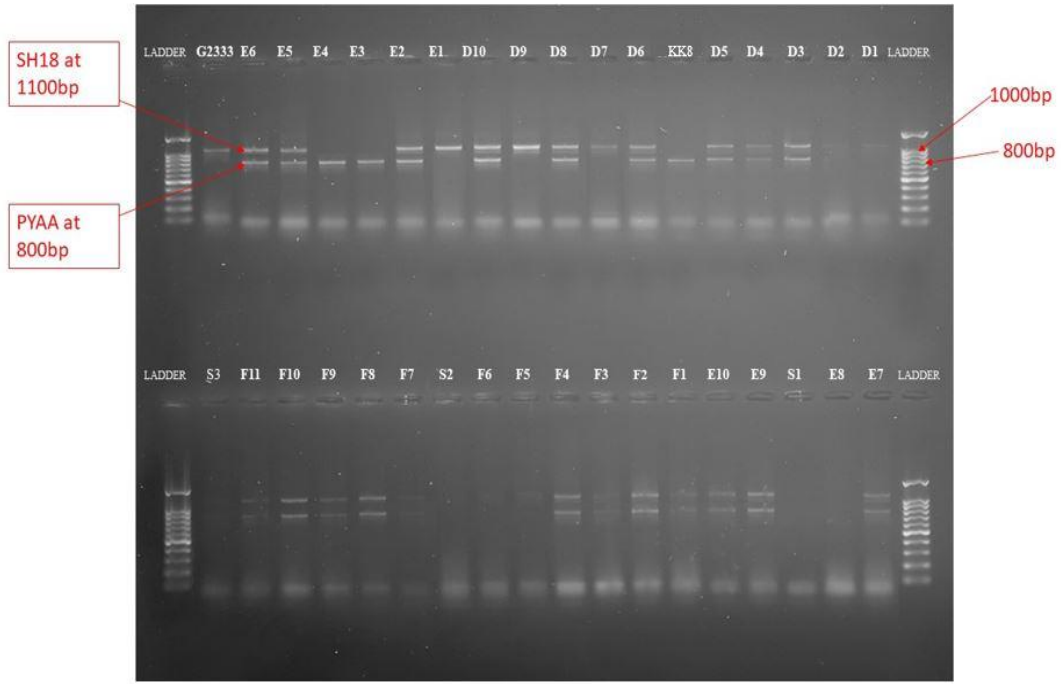

Figure 5.2: Amplification of SH18 and PAA1900 associated with the anthracnose resistance gene *Co-4*^{2} and *Pythium* root rot resistance gene (*Pyult1*) on BC_2F_1 bean populations
KEY: D1-10: Sugar 1 progenies, E1-8: Sugar 2 progenies, F1-11: Sugar 3 progenies, S1: Sugar 1, S2: Sugar 2, S3: Sugar 3

The resulting progenies from the plants were backcrossed to the recurrent parents under each breeding scheme. Selfing was done to the BC_3F_1 populations resulting to BC_3F_2. The BC_3F_2 generation was also screened using molecular markers (Figure 5.3).

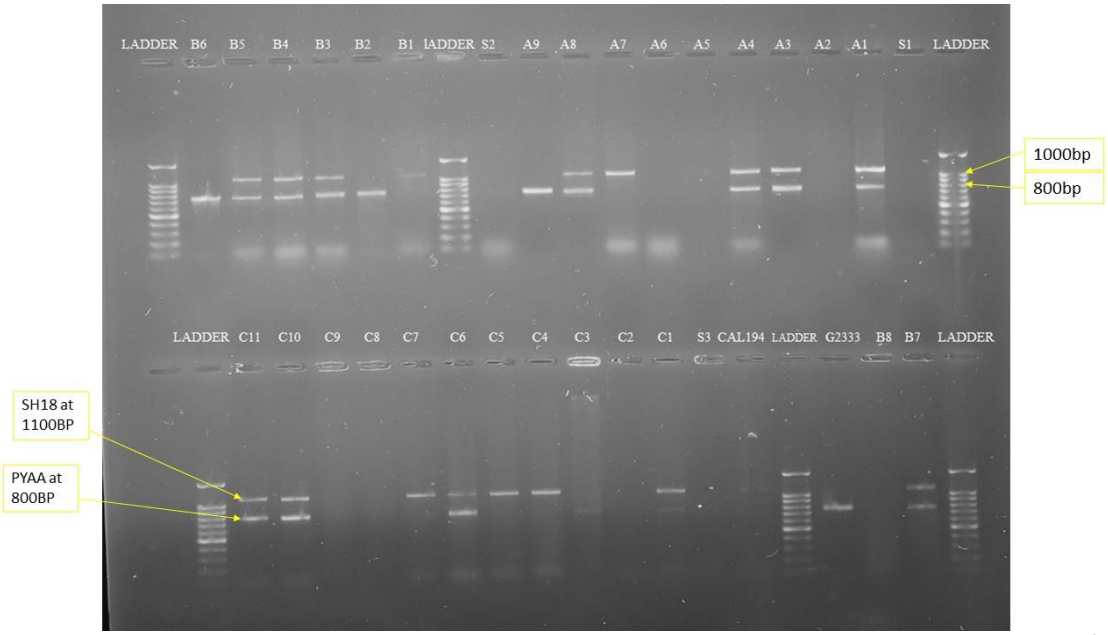

Figure 5.3: Amplification of SH18 and PAA1900 associated with the anthracnose resistance gene (*Co-4*^{2}) and *Pythium* root rot resistance gene (*Pyult1*) in BC_3F_2 populations
KEY: A1-9: Sugar 1 progenies, B1-8: Sugar 2 progenies, C1-11: Sugar 3 progenies, S1: Sugar 1, S2: Sugar 2, S3: Sugar 3

The resistant donor (*G2333*) was resistant to all the three races while cultivar *KK8* was resistant to the MS61 isolate. The BC_3F_2 lines were resistant to all the anthracnose races used although 11 lines were susceptible to the mixture. The genotypic data shows that all the 99 selected lines carried both markers, although 38 lines were susceptible to *Pythium* root rot (Table 5.2).

Table 5.2: Genotypic and phenotypic evaluation of BC_3F_2 populations.

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

S- susceptible, R- Resistant

5.4. Discussion

Root rots and anthracnose have seriously limited the number of genotypes grown by farmers as most landraces are completely susceptible to these diseases in East Africa (Mohammed, 2013; Anunda, 2021). Marker-assisted backcrossing has been proven to be a fast and efficient way to improve one or two traits in preferred common bean genotypes (Muhamba et al., 2013; Okii et al., 2017; Chukwu et al., 2020) as shown in this study. Recombinant inbred lines have been developed that have resistances to both diseases in other countries (Uwera et al., 2021; Paulino et al., 2022). The results of the study demonstrated the successful incorporation of resistance genes, leading to improved resistance levels in the landraces. This research highlights the potential of marker-assisted selection as a valuable tool for enhancing disease resistance in common bean landraces, which can contribute to sustainable production in Kenya.

The BC_3F_2 generation resulting from the crosses suggested that resistance to *Pythium* and anthracnose is inherited as a dominant trait in the genotypes. This corresponds with Namayanja et al. (2014) and Lema et al. (2021). The results further shows that there is a high probability of choosing plants with all the two desirable genes, single gene for each disease or none of the genes in a crossing program as also found by Lema et al. (2021). In their study the *Co-1*^{4}, *Phg-2* and *Phg-1* gene were introgressed in a susceptible cultivar in a backcross breeding program using PCR based markers selection. The two dominant markers used in this study were polymorphic between the resistant and susceptible parents and successfully used to identify bean backcross populations that had the targeted genes. The only challenge that was encountered was the SCAR markers provided limited information at the loci they tag because they are dominant in nature hence it was only possible to tell whether a given allele is present or not at a given locus but could not distinguish the heterozygotes from the homozygotes within the backcross populations. Homozygotes and heterozygotes are able to be differentiated by use of codominant markers at early stages (Piepho & Koch, 2000), hence eliminating the necessity of further genotyping for the fixed allele allowing the breeder to focus on fewer segregating alleles in subsequent generations (Kiryowa, 2015).

The cultivar *G2333* was resistant to all the races of anthracnose used in this study, hence illustrating the value of using *G2333* as a donor which is known to possess *Co-4*^{2}, *Co-5* and *Co-7* genes. Findings by Zuiderveen et al. (2016) showed the cultivar Red Hawk was resistant to 6 races

since it is known to possess the *Co-1* and *Co-2* genes (Kelly et al., 1998). A combination of *Co-5* and *Co-4* provides a broad resistance to *C. lindemuthianum* races as revealed in cultivar *G2333* which comes to agreement with a study conducted by Sousa et al. (2015).

Phenotypic and genotypic screening proved to be effective in confirming the successful pyramiding of genes for disease resistance to the two diseases. Up to 50% of the tested lines had combined resistance to pathogens of the two diseases. The high susceptibility to *Pythium* root rot was due to the high virulence of the MS61 isolate as reported by Otsyula (2010). In the development of inter-gene pool multiple-parent genotypes, Okii et al. (2017) showed the effectiveness of marker-assisted selection in pyramiding of resistance genes together with improved agronomic qualities. The developed lines are meant to yield more than the susceptible parents due to genetic gain from the crossing. This turns out to be advantageous to the farmer. The use of screening methods and marker-assisted selection in developing disease resistant lines with improved agronomic qualities can lead to significant benefits for farmers in terms of increased productivity and enhanced disease resistance in crops.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 Discussion

The integration of morphological and molecular evaluation in the diversity assessment of the germplasm has led to an understanding of the existing diversity among common bean genotypes that are grown in Kenya (Anunda, 2021). This integration has enhanced the identification of important differences for agronomic traits among the existing common bean genotypes. This emphasizes the importance of such an integrated approach in the diversity assessment since the different environments have an effect on the genotype expression (Hegay et al., 2014).

Agricultural productivity increases through plant breeding by the development of new genotypes. This has led to continuous selection of productive and adapted genotypes leading to generally low biological diversity and genetic diversity losses (Gyang, 2018). Subsistence farmers often cultivate for consumption and save selected superior genotypes that are as a result of natural hybridization leading to low biological diversity. There was high variability for agro-morphological traits analyzed indicating the large number of local landraces and genotypes in the medium and high altitude zones of Kenya.

The fact that some of the landraces showed resistance during phenotypic screening, suggests that MAS should not be used alone rather being integrated with phenotypic screening (Govindaraj et al., 2015). More markers that are tightly linked to the resistance genes need to be developed to increase the precision in selection. There could be other loci that condition *Pythium* resistance since both susceptible and moderately resistant genotypes were identified in this study similar to Dramadri et al. (2020). The use of the individual SCAR marker $PYAA19_{800}$ limits the identification of other loci that may be conferring resistance to *Pythium* root rot hence the need to come up with other markers from previous studies that can tag the other specific loci (Clevinger et al., 2021). Inoculation and evaluation of different races of *C. lindemuthianum* can be unfeasible in terms of targeting to get genotypes with durable resistance to anthracnose within a given breeding program due to a high number of physiological races (Ribeiro et al., 2016). Therefore, mixtures of different races could be a viable option since it has synergistic effects resulting in increased disease incidence (Falleiros et al., 2018).

The findings in this study show that incorporation of the two resistance genes in one cultivar could lead to the development of common bean genotypes with multiple disease resistance. *Pythium* root rot resistance was controlled by one gene which has the capabilities of expressing itself in several genetic backgrounds as seen in the set of three recurrent parents used in this study. Such gene action suggests that any resistant genotypes can be used to develop small, medium or large seeded preferred bean genotypes. The developed breeding lines showed a phenotype similar to the recurrent parent in terms of growth habit and seed colour. The other agro-morphological traits however, have to be confirmed through field testing trials, a part where the study did not cover. Field screening should also be an integral part in the selection process due to the genotype by environment interaction.

6.2. Conclusion

The study utilized SSR markers to assess the genetic profiles of a diverse collection of common bean landraces across different regions of Kenya, allowing for a comprehensive understanding of the genetic structure and relationships among these landraces. The results of the study revealed a significant level of genetic diversity among the analyzed landraces, highlighting the rich genetic resources of common bean in Kenya. Such diversity can serve as a valuable genetic pool for the development of improved bean genotypes with desirable traits, including resistance to biotic and abiotic stresses. Furthermore, the knowledge gained from this study can inform breeding efforts aimed at developing improved bean genotypes tailored to the specific needs and challenges of Kenyan farmers, contributing to enhanced productivity, resilience, and food security. Continued research and collaboration are necessary to further explore and exploit the genetic potential of common bean landraces in Kenya.

The study showed the potential for improving the resistance of common bean genotypes to *Pythium* root rot and anthracnose. Through rigorous evaluation and screening, promising common bean genotypes that exhibited resistance to *Pythium* root rot and anthracnose were identified. These findings highlight the genetic diversity present in Kenyan common bean germplasm and provide a valuable resource for breeding programs aimed at developing disease-resistant bean genotypes.

Furthermore, the application of marker-assisted introgression of resistance genes showed its effectiveness in accelerating the breeding process and enhancing the selection of resistant genotypes. By leveraging molecular markers linked to known resistance genes, breeders can make informed decisions during the selection and breeding process, leading to the development of improved common bean genotypes with enhanced resistance traits. Continued efforts in identifying and characterizing novel resistance genes, optimizing marker-assisted selection techniques, and conducting field trials will contribute to the development of durable and high-yielding bean genotypes, ensuring food security and improving the livelihoods of smallholder farmers in Kenya and beyond.

6.3. Recommendations

6.3.1. Recommendations derived from this study

The present study recommends the following:

1. Promotion of conservation of genetic resources in genebanks or germplasm repositories to preserve and safeguard the unique genetic materials identified in this study, to ensure availability for future breeding and research endeavors.
2. Integration of marker-assisted selection techniques in common bean breeding programs to expedite precise development of disease-resistant genotypes. This approach will help enhance the efficiency and precision of selecting genotypes with desirable traits, leading to the development of improved genotypes.
3. The integration of marker assisted selection and phenotypic screening of disease resistance to enhance precision and accuracy of selection in a breeding program.
4. Purification of the landraces that were obtained in the study because they are heterogeneous in nature.
5. Marketing and promotion of the resistant cultivars that were identified in the study.

6.3.2. Recommendations for further research

1. Further screening of germplasm under field conditions to evaluate them for disease reaction in different locations.
2. Further molecular studies on other loci that confer resistance to *Pythium* root rot and anthracnose.

3. Subjecting the developed breeding materials to preliminary and advanced yield trials in order to evaluate their potential compared to the recurrent parents and other commercial genotypes, for potential release.

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APPENDICES

Appendix i: Coordinates of germplasm collection areas

Genotype	Local name	Latitude	Longitude	Genotype	Local name	Latitude	Longitude
	Bungoma County				**Busia County**		
Landrace 1	NP	0.72622	34.63319	Landrace 9	NP	0.43974	34.09966
Landrace 2	Siyingwa	0.63390	34.43439	Landrace 10	NP	0.36195	34.20149
Landrace 8	White	0.95959	34.55346	Landrace 11	NP	0.37925	34.02470
Landrace 15	NP	0.91301	34.57388	Landrace 25	NP	0.50280	34.05661
Landrace 17	NP	0.55920	34.61149	Landrace 26	NP	0.39473	34.08438
Landrace 19	Mabusi	0.90207	34.65491	Landrace 27	NP	0.28489	34.10192
Landrace 20	NP	0.63890	34.46325	Landrace 28	NP	0.42163	34.16491
Landrace 21	NP	0.74271	34.46234	Landrace 29	NP	0.46412	34.14803
Landrace 22	NP	1.01583	34.52193	Landrace 30	NP	0.35503	34.17233
Landrace 23	NP	0.65415	34.53110	Landrace 48	NP	0.41580	34.11421
Landrace 24	NP	0.90364	34.70143	Landrace 57	NP	0.44076	34.13950
Landrace 31	Wairimu	0.69429	34.56372	Landrace 68	Nyembe Rosecoco	0.27868	34.14587
Landrace 32	NP	0.84998	34.65967	Landrace 70	NP	0.43289	34.05778
Landrace 33	NP	0.77780	34.61773		**Embu County**		
Landrace 34	NP	0.95027	34.57516	Landrace 76	Raila	-0.64529	37.64409
Landrace 37	NP	1.07888	34.72463		**Kakamega County**		
Landrace 38	NP	0.81138	34.60695	Landrace 88	NP	0.33087	34.74654
Landrace 39	NP	0.76321	34.51689	Landrace 65	Alulu	0.30322	34.77820
Landrace 40	Kamonge	0.78147	34.52767	Landrace 87	Mukuru wa Oke	0.37483	34.77167
Landrace 42	NP	0.83611	34.52937	Landrace 36	NP	0.40698	34.80015
Landrace 49	Canadian	0.69046	34.60824	Landrace 59	NP	0.29294	34.74454
Landrace 50	NP	0.72928	34.61706	Landrace 66	Alulu	0.34753	34.73070
Landrace 54	NP	0.98962	34.62413	Landrace 41	NP	0.32166	34.71651
Landrace 55	Libya	0.83615	34.58071	Landrace 35	NP	0.36471	34.79213
Landrace 56	Tanzania	0.66691	34.59948	Landrace 90	NP	0.38352	34.73694
Landrace 58	Libya	0.79990	34.55260	Landrace 89	NP	0.31429	34.76789
Landrace 62	NP	0.94359	34.51265	Landrace 63	Alulu	0.29742	34.71194
Landrace 64	Rosecoco	0.83636	34.50945	Landrace 18	Punda	0.39989	34.75022
Landrace 69	NP	0.91916	34.54644	Landrace 61	Alulu	0.34167	34.72277
	Migori County				**Trans-Nzoia County**		
Landrace 43	Canadian	-1.00172	34.47384	Landrace 51	Panadol	1.04732	35.01329
Landrace 67	Sura mbaya	-0.97493	34.48893	Landrace 12	Onkonosia	1.09240	35.11949
Landrace 6	Nyayo Nyeusi	-1.06761	34.57892	Landrace 13	NP	1.06864	35.03990
Landrace 46	Wamboi	-0.93971	34.55081	Landrace 52	NP	1.03689	35.08069

Continuation

Genotype	Local name	Latitude	Longitude	Genotype	Local name	Latitude	Longitude
Kiambu County				**Nyeri County**			
Landrace 72	Kibuu	-1.06835	36.82529	Landrace 75	Gatutura	-0.55258	36.86363
Landrace 77	Giffuu	-1.18049	36.70382	Landrace 83	Kabumbu	-0.45935	36.84017
Landrace 74	Ethuru	-1.05671	36.72468	Landrace 86	NP	-0.50141	36.97922
Landrace 81	Mtoto wa Nyota	-1.23982	36.81708	Landrace 80	Loni	-0.62309	36.83587
Landrace 85	NP	-1.12857	36.80150	Landrace 71	Wairimu royal	-0.56570	36.90400
Nakuru County				Landrace 73	Wairimu Moyale	-0.53391	36.92042
Landrace 3	NP	-0.29584	36.11703	Landrace 79	Njata	-0.42761	36.91716
Landrace 53	NP	-0.36347	36.13750	Landrace 84	Mama safi	-0.45629	36.98232
Landrace 60	NP	-0.33501	36.04237	**Kirinyaga County**			
Uasin-gishu County				Landrace 78	Wairimu Makueni	-0.52736	37.34827
Landrace 5	Rosebella	0.51830	35.29425	Landrace 82	NP	-0.60269	37.42603
Kisii County				**Siaya County**			
Landrace 16	Matumbo ya mbuzi	-0.78371	34.78619	Landrace 4	Nyamango	-0.06255	34.20577
Landrace 47	Ekinawa	-0.75030	34.70762	Landrace 44	NP	-0.11998	34.32480
Landrace 14	Zaire	-0.88405	34.74792	Landrace 45	Wairimu	-0.03129	34.15519

KEY: NP – not provided

Appendix ii: Analyses of variance showing significant differences among common bean genotypes grown in individual sites

	Traits	Source of variation	d.f.	s.s.	m.s.	F value	F pr
KAKAMEGA LONG RAINS (UM1)	50% Days To Flower	Genotype	96	3808.25	39.6693	43.62	<.001
	Days To Maturity	Genotype	96	7644.853	79.6339	239.52	<.001
	Leaf Length	Genotype	96	3209.11	33.43	2.87	<.001
	Leaf Width	Genotype	96	278.784	2.904	10.189	<.001
	No Of Pods Plant	Genotype	96	2413.6	25.142	1.7663	<.001
	No Of Seeds Pod	Genotype	96	173.57	1.808	4.22	<.001
	Pod Length	Genotype	96	498.65	5.1942	3.4117	<.001
	Plant Height	Genotype	96	278614.9	2902.2	5.6	<.001
	100 Seed Weight	Genotype	96	24054.77	250.57	39.23	<.001
	Yield In T/Ha	Genotype	96	20.88352	0.21754	3.38	<.001
KAKAMEGA SHORT RAINS (UM1)	50% Days To Flower	Genotype	88	3079.04	34.989	19.2242	<.001
	Days To Maturity	Genotype	88	5098.5	57.938	5.6067	<.001
	Leaf Length	Genotype	88	375.88	4.2714	4.8339	<.001
	Leaf Width	Genotype	88	209.174	2.377	3.9431	<.001
	No Of Pods Plant	Genotype	88	3914.1	44.478	3.2809	<.001
	No Of Seeds Pod	Genotype	88	261.363	2.97004	4.1417	<.001
	Pod Length	Genotype	88	598.86	6.8053	4.246	<.001
	Plant Height	Genotype	88	301156	3422.2	11.4366	<.001
	100 Seed Weight	Genotype	88	26421.7	300.246	3.3562	<.001
	Yield In T/Ha	Genotype	88	22.835	0.25949	3.5274	<.001
BUNGOMA LONG RAINS (LH1)	50% Days To Flower	Genotype	97	5767.5	59.459	98.0041	<.001
	Days To Maturity	Genotype	97	7502.1	77.341	56.8919	<.001
	Leaf Length	Genotype	97	502.45	5.1799	4.4551	<.001
	Leaf Width	Genotype	97	203.276	2.0956	3.0683	<.001
	No Of Pods Plant	Genotype	97	5604.4	57.777	2.154	<.001
	No Of Seeds Pod	Genotype	97	128.513	1.3249	2.7372	<.001
	Pod Length	Genotype	97	818.64	8.4395	6.608	<.001
	Plant Height	Genotype	97	136790	1410.21	9.9368	<.001
	100 Seed Weight	Genotype	97	44654	460.35	17.443	<.001
	Yield In T/Ha	Genotype	97	31.842	0.3283	3.0926	<.001

Appendix iii: Analyses of variance showing significant differences among common bean genotypes and sites in Kakamega and Bungoma during the long rains

Traits	Source of variation	d.f.	s.s.	m.s.	F value	F pr
50% days to flower	Genotype	97	8830.6	91.038	43.0536	<.001
	Site	1	26.5	26.46	12.5135	<.001
Days to maturity	Genotype	97	9194.2	94.786	7.5149	<.001
	Site	1	1.9	1.927	0.1528	0.6961
Leaf length	Genotype	97	956.22	9.858	5.6195	<.001
	Site	1	5.39	5.3865	3.0706	0.08033
Leaf width	Genotype	97	956.22	9.858	5.6195	<.001
	Site	1	5.39	5.3865	3.0706	0.08033
No of pods plant	Genotype	97	5451.7	56.2	2.5641	<.001
	Site	1	1781.9	1781.93	81.2938	<.001
No of seeds pod	Genotype	97	245.493	2.53085	4.602	<.001
	Site	1	0.282	0.28167	0.5122	0.4745
Pod length	Genotype	97	1124.11	11.589	7.3273	<.001
	Site	1	236.57	236.568	149.575	<.001
Plant height	Genotype	97	371821	3833.2	10.4046	<.001
	Site	1	12515	12515.4	33.9711	<.001
100 seed weight	Genotype	97	64689	666.9	29.081	<.001
	Site	1	7125	7124.9	310.691	<.001
Yield in t/ha	Genotype	97	36.555	0.37686	3.2668	<.001
	Site	1	1.239	1.23897	10.74	<.001

Appendix iv: Means of days to 50% flowering for common bean genotypes grown in different sites

KAKAMEGA LONG RAINS		KAKAMEGA SHORT RAINS		BUNGOMA SHORT RAINS		COMBINED SITES LONG RAINS	
GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN
Landrace 60 & *KK22*	48.667 - 48.000^{a}	Landrace 60 & 61	47.000^{a}	Landrace 52 & 68	50.000^{a}	Landrace 60	47.333^{a}
Landrace 29	44.333^{b}	Landrace 17 18 & 53	46.667^{ab}	Landrace 61	48.000^{ab}	*KK22* Landrace 52 68	47.000 - 46.500^{ab}
Landrace 17 18 52 68	43.000^{bc}	Landrace 21	46.000^{a-c}	*KK22* Landrace 29 & 60	46.000^{bc}	Landrace 61 29	45.333 - 45.167^{a-c}
Landrace 61	42.667^{b-d}	Landrace 38	45.667^{a-d}	Landrace 14	45.667^{bd}	Landrace 18	44.000^{a-d}
GLP585, ,	41.333^{c-e}	Landrace 52	45.333^{a-e}	Landrace 15 18 & 17	45.000 - 44.667^{cd}	Landrace 17	43.833^{a-e}
CAL194 Landrace 14 RED13	41.000 - 40.667^{c-f}	Landrace 14 & 3	45.000^{a-f}	*GLP585*, , Landrace 21 & 39	44.000^{c-e}	Landrace 14	43.333^{b-f}
Landrace 39	40.333^{c-g}	Landrace 29	44.333^{a-g}	Landrace 38 & RED16	43.000^{df}	RED16	43.000^{b-g}
KK8 Landrace 44 & 64	40.000^{c-h}	Landrace 63	43.000^{a-h}	Landrace 45	41.667^{e-g}	*GLP585*, , Landrace 39	42.667 - 42.167^{c-g}
Landrace 31 & 38	39.667^{c-i}	Landrace 15 & 39	42.333^{a-i}	CAL194 Landrace 12 31 62 64 85 & RED13	41.000 - 40.667^{f-h}	Landrace 15	42.000^{c-h}
Landrace 40 & 85	39.333^{d-j}	Landrace 68	42.000^{b-j}	Landrace 89	40.000^{g-i}	Landrace 38	41.333^{d-i}
CAL33 Landrace 13 15 51 63 84	39.000^{e-j}	Landrace 31 & 89	41.667^{c-k}	Landrace 13 30 37 & 84	39.000^{g-j}	CAL194	41.000^{d-j}
Landrace 30 34 35 & 79	38.667^{e-k}	Landrace 12 84 85	41.333^{c-l}	Landrace 63 & 78	38.667^{h-k}	RED13	40.667^{e-j}
Landrace 32 55 62 78 8 81 89	38.333^{e-l}	Landrace 13 30 35 62 64 78 8 90	41.000^{d-m}	Landrace 40 44 46 & 51	38.333^{h-l}	Landrace 64	40.500^{e-k}
CHELALANG *GLP2* Landrace 28 4 58 74 90 WAIRIMU DWARF	38.000^{e-m}	Landrace 37 58 79 82	40.667^{e-n}	Landrace 11 35 58 73 8 & 82	38.000^{i-m}	Landrace 31 85	40.333 - 40.167^{e-l}
Landrace 11 27 82	37.667^{f-n}	Landrace 34 40 42 81	40.333^{f-o}	*KK8* Landrace 32 34 & 79	37.667^{i-n}	Landrace 12 & 62	39.833 - 39.667^{f-m}
KK072 Landrace 42 45	37.333^{g-o}	Landrace 32 & 44	40.000^{g-p}	Landrace 90	37.333^{i-o}	Landrace 21 & 45	39.500^{g-n}
Landrace 24 & 37	37.000^{g-p}	Landrace 27 45 & 55	39.667^{g-q}	CHELALANG Landrace 55 & 9	37.000^{j-p}	Landrace 44 89 13 84 30 40 63 *KK8*	39.167- 38.833^{g-o}
Landrace 73	36.667^{h-q}	Landrace 28 41 & 51	39.333^{h-r}	CAL33 Landrace 42	36.667^{j-q}	Landrace 51	38.667^{g-p}
Landrace 41	36.333^{i-r}	Landrace 73	39.333^{h-r}	Landrace 28	36.333^{j-r}	Landrace 78	38.500^{g-q}
Landrace 10 46 6	36.000^{j-s}	Landrace 74	39.000^{h-s}	KK072 Landrace 4 80 & 81	36.000^{k-s}	Landrace 35	38.333^{h-r}
Landrace 71 80 9	35.333^{k-t}	Landrace 11 71 & 9	38.667^{h-t}	Landrace 10 27 65 & 71	35.667^{l-t}	CAL33 Landrace 34 79 8	38.222 - 38.167^{i-r}
Landrace 19 21 43 77	35.000^{l-u}	Landrace 10 46 & 54	38.333^{h-u}	*GLP2* Landrace 74	35.333^{m-t}	Landrace 32 37 58	38.000^{i-s}

Landrace 1 22 36 54	34.667^{m-v}	Landrace 4 & 33	38.000^{i-u}	Landrace 36	35.000^{n-t}	Landrace 11 & 82	37.833^{j-t}
Landrace 33 & 66	34.333^{n-w}	Landrace 1 19 22 47 56 69 & 77	37.333^{j-u}	Landrace 22 & 41	34.667^{o-t}	Landrace 55 & 90	37.667^{j-u}
Landrace 25 48 65 67	34.000^{o-w}	Landrace 70	37.000^{k-u}	Landrace 24 33 & 76	34.333^{p-t}	CHELALANG & Landrace 73	37.500 - 37.333^{j-v}
Landrace 23 59 83 87	33.667^{p-w}	Landrace 23 36 59 & 66	36.667^{l-u}	Landrace 20 & 6	34.000^{q-t}	Landrace 28 46 81	37.167^{k-w}
Landrace 49 69 76	33.333^{q-w}	Landrace 26 48 & 80	36.333^{m-u}	Landrace 16 2 25 77 WAIRIMU DWARF	33.667^{r-t}	Landrace 4 & 42	37.000^{k-x}
Landrace 26 & 57	33.000^{r-w}	Landrace 25 50 57 6 & 65	36.000^{n-u}	Landrace 19 54 57 & 66	33.333st	*GLP2* KK072 Landrace 27 74	36.667^{l-y}
Landrace 75	32.667^{s-w}	Landrace 2 49 5 72 & 87	35.667^{o-u}	Landrace 1 23 26 43 47 48 49 5 50 56 59 67 69 70 72 75 83 86 87 & 88	33.000^{t}	Landrace 9	36.167^{m-z}
Landrace 2 5 56 70 50 88	32.333 - 32.000^{t-w}	Landrace 43 67 & 83	35.333^{p-u}			Landrace 10	35.833^{n-A}
Landrace 47 72 86	31.66667^{u-w}	Landrace 75 & 86	35.000^{q-u}			Landrace 24 80 41 71	35.667-35.500^{o-A}
Landrace 20	31.33333^{vw}	Landrace 88	34.667^{r-u}			Landrace 6	35.000^{p-A}
Landrace 16	31.00000^{w}	Landrace 20	34.333^{s-u}			Landrace 36 & 65	34.833^{q-A}
		Landrace 24 & 76	34.000^{tu}			Landrace 22	34.667^{r-A}
		Landrace 16	33.667^{u}			Landrace 33 & 77	34.333^{s-A}
						Landrace 19	34.167^{t-A}
						Landrace 43 & 54	34.000^{u-A}
						Landrace 1 25 66 76	33.833^{v-A}
						Landrace 48 & 67	33.500^{w-A}
						Landrace 23 59 83 87	33.333^{x-A}
						Landrace 49 57 69 2 26	33.167^{y-A}
						Landrace 75 20 5 56 70 50 88	32.833^{Za}
						Landrace 16 47 72 86	32.333^{A}

Appendix v: Means of leaf length for common bean genotypes grown in different sites

KAKAMEGA LONG RAINS		KAKAMEGA SHORT RAINS		BUNGOMA SHORT RAINS		COMBINED SITES LONG RAINS	
GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN
Landrace 60 & *KK22*	48.667 - 48.000^{a}	Landrace 60 & 61	47.000^{a}	Landrace 52 & 68	50.000^{a}	Landrace 60	47.333^{a}
Landrace 29	44.333^{b}	Landrace 17 18 & 53	46.667^{ab}	Landrace 61	48.000^{ab}	*KK22* Landrace 52 68	47.000 - 46.500^{ab}
Landrace 17 18 52 68	43.000^{bc}	Landrace 21	46.000^{a-c}	*KK22* Landrace 29 & 60	46.000^{bc}	Landrace 61 29	45.333 - 45.167^{a-c}
Landrace 61	42.667^{b-d}	Landrace 38	45.667^{a-d}	Landrace 14	45.667^{bd}	Landrace 18	44.000^{a-d}
GLP585, ,	41.333^{c-e}	Landrace 52	45.333^{a-e}	Landrace 15 18 & 17	45.000 - 44.667^{cd}	Landrace 17	43.833^{a-e}
CAL194 Landrace 14 RED13	41.000 - 40.667^{c-f}	Landrace 14 & 3	45.000^{a-f}	*GLP585*, , Landrace 21 & 39	44.000^{c-e}	Landrace 14	43.333^{b-f}
Landrace 39	40.333^{c-g}	Landrace 29	44.333^{a-g}	Landrace 38 & RED16	43.000^{df}	RED16	43.000^{b-g}
KK8 Landrace 44 & 64	40.000^{c-h}	Landrace 63	43.000^{a-h}	Landrace 45	41.667^{e-g}	*GLP585*, , Landrace 39	42.667 - 42.167^{c-g}
Landrace 31 & 38	39.667^{c-i}	Landrace 15 & 39	42.333^{a-i}	CAL194 Landrace 12 31 62 64 85 & RED13	41.000 - 40.667^{f-h}	Landrace 15	42.000^{c-h}
Landrace 40 & 85	39.333^{d-j}	Landrace 68	42.000^{b-j}	Landrace 89	40.000^{g-i}	Landrace 38	41.333^{d-i}
CAL33 Landrace 13 15 51 63 84	39.000^{e-j}	Landrace 31 & 89	41.667^{c-k}	Landrace 13 30 37 & 84	39.000^{g-j}	CAL194	41.000^{d-j}
Landrace 30 34 35 & 79	38.667^{e-k}	Landrace 12 84 85	41.333^{c-l}	Landrace 63 & 78	38.667^{h-k}	RED13	40.667^{e-j}
Landrace 32 55 62 78 8 81 89	38.333^{e-l}	Landrace 13 30 35 62 64 78 8 90	41.000^{d-m}	Landrace 40 44 46 & 51	38.333^{h-l}	Landrace 64	40.500^{e-k}
CHELALAN G *GLP2* Landrace 28 4 58 74 90 WAIRIMU DWARF	38.000^{e-m}	Landrace 37 58 79 82	40.667^{e-n}	Landrace 11 35 58 73 8 & 82	38.000^{i-m}	Landrace 31 85	40.333 - 40.167^{e-l}
Landrace 11 27 82	37.667^{f-n}	Landrace 34 40 42 81	40.333^{f-o}	*KK8* Landrace 32 34 & 79	37.667^{i-n}	Landrace 12 & 62	39.833 - 39.667^{f-m}
KK072 Landrace 42 45	37.333^{g-o}	Landrace 32 & 44	40.000^{g-p}	Landrace 90	37.333^{i-o}	Landrace 21 & 45	39.500^{g-n}
Landrace 24 & 37	37.000^{g-p}	Landrace 27 45 & 55	39.667^{g-q}	CHELALAN G Landrace 55 & 9	37.000^{j-p}	Landrace 44 89 13 84 30 40 63 *KK8*	39.167- 38.833^{g-o}
Landrace 73	36.667^{h-q}	Landrace 28 41 & 51	39.333^{h-r}	CAL33 Landrace 42	36.667^{j-q}	Landrace 51	38.667^{g-p}
Landrace 41	36.333^{i-r}	Landrace 73	39.333^{h-r}	Landrace 28	36.333^{j-r}	Landrace 78	38.500^{g-q}
Landrace 10 46 6	36.000^{j-s}	Landrace 74	39.000^{h-s}	KK072 Landrace 4 80 & 81	36.000^{k-s}	Landrace 35	38.333^{h-r}
Landrace 71 80 9	35.333^{k-t}	Landrace 11 71 & 9	38.667^{h-t}	Landrace 10 27 65 & 71	35.667^{l-t}	CAL33 Landrace 34 79 8	38.222 - 38.167^{i-r}
Landrace 19 21 43 77	35.000^{l-u}	Landrace 10 46 & 54	38.333^{h-u}	*GLP2* Landrace 74	35.333^{m-t}	Landrace 32 37 58	38.000^{i-s}
Landrace 1 22 36 54	34.667^{m-v}	Landrace 4 & 33	38.000^{i-u}	Landrace 36	35.000^{n-t}	Landrace 11 & 82	37.833^{i-t}
Landrace 33 & 66	34.333^{n-w}	Landrace 1 19 22 47 56 69 & 77	37.333^{j-u}	Landrace 22 & 41	34.667^{o-t}	Landrace 55 & 90	37.667^{i-u}

Landrace 25 48 65 67	34.000^{o-w}	Landrace 70	37.000^{k-u}	Landrace 24 33 & 76	34.333^{p-t}	CHELALANG & Landrace 73	37.500 - 37.333^{j-v}
Landrace 23 59 83 87	33.667^{p-w}	Landrace 23 36 59 & 66	36.667^{l-u}	Landrace 20 & 6	34.000^{q-t}	Landrace 28 46 81	37.167^{k-w}
Landrace 49 69 76	33.333^{q-w}	Landrace 26 48 & 80	36.333^{m-u}	Landrace 16 2 25 77 WAIRIMU DWARF	33.667^{r-t}	Landrace 4 & 42	37.000^{k-x}
Landrace 26 & 57	33.000^{r-w}	Landrace 25 50 57 6 & 65	36.000^{n-u}	Landrace 19 54 57 & 66	33.333st	*GLP2* KK072 Landrace 27 74	36.667^{l-y}
Landrace 75	32.667^{s-w}	Landrace 2 49 5 72 & 87	35.667^{o-u}	Landrace 1 23 26 43 47 48 49 5 50 56 59 67 69 70 72 75 83 86 87 & 88	33.000^{t}	Landrace 9	36.167^{m-z}
Landrace 2 5 56 70 50 88	32.333 - 32.000^{t-w}	Landrace 43 67 & 83	35.333^{p-u}			Landrace 10	35.833^{n-A}
Landrace 47 72 86	31.667^{u-w}	Landrace 75 & 86	35.000^{q-u}			Landrace 24 80 41 71	35.667-35.500^{o-A}
Landrace 20	31.333^{vw}	Landrace 88	34.667^{r-u}			Landrace 6	35.000^{p-A}
Landrace 16	31.000^{w}	Landrace 20	34.333^{s-u}			Landrace 36 & 65	34.833^{q-A}
		Landrace 24 & 76	34.000^{tu}			Landrace 22	34.667^{r-A}
		Landrace 16	33.667^{u}			Landrace 33 & 77	34.333^{s-A}
						Landrace 19	34.167^{t-A}
						Landrace 43 & 54	34.000^{u-A}
						Landrace 1 25 66 76	33.833^{v-A}
						Landrace 48 & 67	33.500^{w-A}
						Landrace 23 59 83 87	33.333^{x-A}
						Landrace 49 57 69 2 26	33.167^{y-A}
						Landrace 75 20 5 56 70 50 88	32.833^{zA}
						Landrace 16 47 72 86	32.333^{A}

Appendix vi: Means of leaf width for common bean genotypes grown in different sites

KAKAMEGA LONG RAINS		KAKAMEGA SHORT RAINS		BUNGOMA LONG RAINS		COMBINED SITES LONG RAINS	
GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN
Landrace 75	10.300[a]	Landrace 74	9.467[a]	Landrace 49	10.167[a]	Landrace 6	10.008[a]
Landrace 5 66 6 47 74	10.100 - 9.933[ab]	Landrace 28	8.833[ab]	Landrace 6	10.017[ab]	Landrace 74	9.892[ab]
Landrace 33	9.900[a-c]	Landrace 61 29	8.567-8.533[a-c]	KK072 Landrace 12 33	9.950 - 9.867[a-c]	Landrace 33	9.883[a-c]
Landrace 67 & 48	9.733 - 9.700[a-d]	Landrace 33 12 15	8.433-8.400[a-d]	Landrace 72 74	9.850[a-d]	Landrace 5	9.825[a-d]
Landrace 89	9.633[a-e]	Landrace 36	8.300[a-e]	CAL194	9.783[a-e]	Landrace 49	9.733[a-e]
Landrace 50 & 70	9.500[a-f]	Landrace 67 79	8.233[a-f]	Landrace 88 89 5 CHELALANG Landrace 36 48 67 13 16 55 63 65 28 23 20 47 CAL33 *GLP2* Landrace 59 75 10 79 66 83 56 58 *KK8* Landrace 64 70 22 39 42 2 25 62 31 14 4 57 1 26 61 80 90 17 WAIRIMU DWARF RED16 Landrace 81 21 60 34 18 69 84 46 51 85 RED13 Landrace 68 32 9 50 24 76 37 19 87 54 43 *GLP585*, , Landrace 86 82 35	9.583-7.500[a-f]	KK072 Landrace 89 75	9.608-9.567[a-f]
Landrace 4	9.467[a-g]	Landrace 3	8.167[a-g]	Landrace 29 8 *KK22* Landrace 30 45 73 27 40 38 11 44 41	7.483-7.050[b-f]	Landrace 48 67	9.517[a-g]
Landrace 25	9.400[a-h]	Landrace 16 65 68 20 53 51 50 4 76 87 54 9 63 90 21 32 6 31 39 10 18 35 84 34 17 42 72 55 8 60 13 52 2 49 75 62 69	7.833-6.733[a-h]	Landrace 77	6.900[c-f]	Landrace 88 47 66 CAL194	9.458-9.425[a-h]
Landrace 88	9.333[a-i]	Landrace 14 5 73 45 81 11 30 24 25 40 85 26 27 64 78 19 47 66 70 1 88 89 37 48 22 58 44 80	6.700 - 6.100[b-h]	Landrace 52	6.800[d-f]	Landrace 12	9.367[a-i]
Landrace 28 & 49	9.300[a-j]	Landrace 41 86	6.067-6.000[c-h]	Landrace 78	6.733[ef]	Landrace 36 28 70 25 4 79	9.258-8.892[a-j]
KK072	9.267[a-k]	Landrace 46 71 83	5.700[d-h]	Landrace 71	6.633[f]	Landrace 63 83 20 22 65 10 72 15 50 55 59 CAL33 Landrace 81 *GLP2*	8.875-8.617[a-k]
Landrace 36 81 CAL194 Landrace 40 22 79	9.100[a-l]	Landrace 23	5.633[e-h]			Landrace 23 13 WAIRIMU DWARF Landrace 21 2 56 1 32 42 51 26 39 85 84 43 60 RED16 Landrace 57 34	8.592 - 8.200[a-l]

Landrace 43	8.933^{a-m}	Landrace 59 77 82	5.500^{f-h}	RED13 Landrace 64 40 62 58 17 9 *KK8* Landrace 69 14 CHELALANG Landrace 54 61 18 68 46 8 90 86 87	8.158 - 7.792^{b-l}
Landrace 21 83 WAIRIMU DWARF	8.900^{a-n}	Landrace 57	5.433^{gh}	Landrace 24	7.775^{c-l}
Landrace 12 32 10	8.800 - 8.733^{a-o}	Landrace 38 43 56	5.333^{h}	Landrace 35 76 *GLP585*, ,	7.767- 7.742^{d-l}
Landrace 51 63 CAL33 Landrace 20 59 2 65 1 85 84 *GLP2* Landrace 26 60	8.633- 8.333^{a-p}			Landrace 45 31 80 19	7.692- 7.642^{e-l}
Landrace 42 54 55 56 34 86	8.300 - 8.233^{b-q}			Landrace 27 & 38	7.567- 7.517^{f-l}
RED13 & Landrace 23	8.217 - 8.200^{c-q}			Landrace 82	7.408^{g-l}
Landrace 39 9 35 57 13 45	8.133 - 7.967^{d-r}			*KK22*	7.400^{h-l}
GLP585, , Landrace 27 17 69 38 62 68 87	7.900 - 7.800^{d-r}			Landrace 30 37 41	7.275- 7.258^{i-l}
Landrace 18 46 64	7.700 - 7.667^{e-r}			Landrace 44 16 77	7.242- 7.200^{j-l}
Landrace 72 76 24 14 15 61 58	7.633- 7.533^{f-r}			Landrace 29 73 11 78 71	7.050- 6.817^{kl}
Landrace 19 77 90	7.500^{g-r}			Landrace 52	6.483^{l}
Landrace & 41 *KK8*	7.467- 7.433^{h-r}				
Landrace 44	7.400^{i-r}				
KK22	7.333^{j-r}				
Landrace 82	7.300^{k-r}				
Landrace 30 & 78	7.133^{l-s}				
Landrace 71 & 80	7.000^{m-s}				
Landrace 31	6.933^{n-s}				
Landrace 11	6.900^{o-s}				
Landrace 37 & 73	6.733^{p-s}				
Landrace 29 & CHELALANG	6.617- 6.500^{q-s}				
Landrace 52	6.167^{rs}				
Landrace 16	5.300^{s}				

Appendix vii: Means of days to physiological maturity for common bean genotypes grown in different sites

KAKAMEGA LONG RAINS		KAKAMEGA SHORT RAINS		BUNGOMA LONG RAINS		COMBINED SITES LONG RAINS	
GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN
Landrace 60 61 68	100.000[a]	Landrace 61	101.333[a]	Landrace 61 62 69	100.000[a]	Landrace 61	100.000[a]
Landrace 29	93.000[b]	Landrace 53	90.667[ab]	Landrace 30	93.000[b]	Landrace 29	90.250[b]
Landrace 52	92.000[bc]	Landrace 29	90.333[a-c]	Landrace 54	92.000[bc]	Landrace 62	89.833[bc]
Landrace 35	90.000[c]	Landrace 52	88.667[b-d]	Landrace 36	90.000[b-d]	Landrace 68	89.000[b-d]
CAL194 CAL33 *GLP585*, , *KK22* Landrace 13 13 15 17 18 31 37 38 39 40 73 78	85.000[d]	Landrace 3 60	88.000[b-e]	*GLP585*, ,	88.000[c-e]	Landrace 60	88.833[b-e]
Landrace 8 RED13	84.333[de]	Landrace 14 21 35 38 17 10 12 18 73 11 13 30 8 34 15 37 44 89 31 51 45 63 79 85 40 46 64 28 78 39 62 9 90 42 74 68 80 32 41 36 82 27 66 84	85.000-79.000[b-f]	Landrace 29	87.500[de]	Landrace 69	88.500[b-f]
Landrace 45	83.000[d-f]	Landrace 55 65 71 77 81 83	78.667[c-f]	CAL194 CAL194 CHELALANG KK072 *KK8* Landrace13 14 15 16 18 19 32 38 394417479	85.000[ef]	Landrace 52	87.000[b-g]
Landrace 85 9 63 79	82.667-82.333[e-g]	Landrace 19 58 76 25 33 54 69 87 4 47 67 20 24 43 6 70	78.333-77.000[d-f]	Landrace 80 RED16	84.333[e-g]	Landrace 30	86.833[b-h]
KK8 Landrace 11 28 44 51 64	82.000[f-h]	Landrace 16	76.667[ef]	Landrace 46	83.000[f-h]	*GLP585*, ,	86.500[b-i]
Landrace 74 84	81.333[f-i]	Landrace 48 50 56 57 23 22 26 49 5 59 72 75 86 1 2 88	76.000[f]	Landrace 86 90	82.667[f-i]	CAL194 CAL33 Landrace 13 14 15 18 35 38 39 54 RED16 Landrace 79 *KK8* Landrace 12 8	85.000-833[b-j]
Landrace 82	81.000[f-j]			Landrace 64 8 RED13	82.333[f-j]	RED13 Landrace 74 36 31 32 45 80 KK072 *KK22* Landrace 64 85 CHELALANG Landrace 19 78 9 90 46 11 41 63 65 84 4 40 83	83.250[c-j]
Landrace 25 30 32 55 58 80 83	80.667[g-k]			Landrace 1 12 45 52 65	82.000[f-k]	Landrace 16 17 37 51 73 82 66 81	80.500-80.000[d-j]
Landrace 10 34 47 65 66 89 90	80.000[h-l]			Landrace 75 85	81.333[f-l]	Landrace 59	79.833[e-j]
Landrace 62 WAIRIMU DWARF	79.667[i-m]			Landrace 83	81.000[f-m]	WAIRIMU DWARF Landrace 47 86 44	79.667-79.500[f-j]
KK072 Landrace 46 81	79.333[i-n]			Landrace 26 31 33 56 59 81 84	80.667[g-n]	Landrace 28 75 1 55 56 48 67 25 26 33 58 6 77 10 34 89	79.333[g-j]

Landrace 50 59	79.000^{j-o}	Landrace 11 35 48 66 67 9 63 WAIRIMU DWARF *KK22* Landrace 47 82 51 6	80.000-79.000^{h-o}	Landrace 21 76	77.833^{h-j}
CHELALANG Landrace 19	78.667^{k-p}	*GLP2* Landrace 2	78.667^{i-o}	*GLP2* 49 23 42 43 50	77.667-77.500^{ij}
Landrace 23 76 77	78.333^{l-q}	Landrace 24 77 78	78.333^{j-o}	Landrace 2 22 24 20 57 70 5 71 87 27 27 72 88	77.333^{j}
Landrace 21 42 48 67	78.000^{l-r}	Landrace 22 43 49 68	78.000^{k-o}		
Landrace 20 54 56 6	77.667^{m-r}	Landrace 21 55 57 60 5 76	77.667^{l-o}		
Landrace 49 75	77.333^{n-r}	Landrace 42 44 70	77.000^{mno}		
Landrace 41 43 69	77.000^{o-r}	Landrace 23 28 40 71 87	76.667^{no}		
GLP2 Landrace 22 27 4 70 86	76.667^{p-r}	Landrace 10 72 88 17 20 25 27 34 37 50 58 73 89	76.333-76.000^{o}		
Landrace 1 71 87	76.333^{qr}				
Landrace 16 2 24 26 33 36 5 57 72 88	76.000^{r}				

Appendix viii: Means of plant height for common bean genotypes grown in different sites

KAKAMEGA LONG RAIN		KAKAMEGA SHORT RAINS		BUNGOMA LONG RAINS		COMBINED SITES LONG RAINS	
GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN
Landrace 29 61 60 68	179.167-166.667[a]	Landrace 60 68 61 29	190.000-166.000[a]	Landrace 61	203.000[a]	Landrace 61	189.833[a]
Landrace 52	125.667[ab]	Landrace 52	144.333[ab]	Landrace 29	132.717[b]	Landrace 29	155.942[ab]
Landrace 51 84 81 39 14 38 18 42 64 17 59 CAL194 Landrace 74	72.667-51.400[bc]	Landrace 53	132.333[a-c]	Landrace 60	109.333[b-c]	Landrace 60	141.333[a-c]
RED13 Landrace 40 28 45 CAL33 Landrace 34 12 85 21 31 46 79 78 63 58 24 62 83 71 44 15 87 *GLP585*, , Landrace 89 8 82 90 76 54 65 66 13 6 33 20 55 77 80 37 2 25 KKO72 *KK22 KK8* Landrace 73 30 48 43 56 19 72 35 16 32 4 57 9 36 11 67 10 27 WAIRIMU DWARF Landrace 70 41 47 CHELALANG Landrace 26 5 22 *GLP2* Landrace 69 86 50 88 23 49 75 1	51.333-28.400[c]	Landrace 51	99.667[b-d]	Landrace 68	103.567[b-d]	Landrace 68	135.117[bc]
		Landrace 103	87.667-84.000[b-e]	Landrace 64	97.667[b-e]	Landrace 52	104.033[cd]
		Landrace 64	72.000[c-e]	Landrace 52	82.400[c-f]	Landrace 64 51 83 81 39 38 RED16 Landrace 54 28 84 34 35 63 17 12	75.450-57.400[de]
		Landrace 54 76 11 46 9 84 31 81 36 17 45 20 78 34 28 88 21 40 74 42 30 32 79 39 90 12 43 66 15 19 83 35 6 85 18 77 8 22 56 63 71 62 67 13 49 44 57 48 87 14 24 72	63.333-36.333[de]	Landrace 83 54 35 36 37 CAL33 Landrace63 28 38 65 81 34 39	79.200-65.967[c-g]	CAL33 Landrace 74 CAL194 Landrace 36 42 37 65 21 14 62 85 RED13 Landrace 24 46 15 18 58 89 79 82 40 59 10 31 87 78 9 44 8 43 76 33 55 11 45 *KK8* Landrace 90 48 KK072 Landrace 13 *GLP585*, , Landrace 41 20 6 22 66 27 72 16 32 *GLP2* Landrace25 CHELALANG Landrace 77 80 73 19 71 30 2 4 23 56 70 *KK22* Landrace 67 47 57 49 69 26 5 75 50 88 86 WAIRIMU DWARF Landrace 1	56.689-36.117[e]
		Landrace 37 73 65 86 5	36.000-24.667[e]	Landrace 10 12 17 62 9 74	65.333-59.933[d-g]		

	89 59 58 82 55 4 16 70 27 2 69 26 33 23 38 25 50 47 75 80 1	15 24 21 CAL194 RED16 Landrace11 22 41 51	
		Landrace 89 85 82 46 43 *GLP2* Landrace 58 84 27 42 RED13 Landrace 79 55 CHELALANG *KK8* Landrace 33 23 48 87 KK072 Landrace 76 8 14 32 44 16 31 72 13 78 20 90 40 49 6 18 66 59 *GLP585*, , Landrace 25 70 19 73 30 77 47 80 4 75 67 45 69 2 56 57 1 26 71 *KK22* Landrace 50 88 5 86 WAIRIMU DWARF	59.200-57.533^{e-g} 57.133-42.167^{fg} 37.333^{g}

Appendix ix: Means of pod length for common bean genotypes grown in different sites

KAKAMEGA LONG RAINS		KAKAMEGA SHORT RAINS		BUNGOMA LONG RAINS		COMBINED SITES LONG RAINS	
GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN
Landrace 89	14.467[a]	Landrace 90	15.833[a]	Landrace 89 *GLP2* Landrace48	15.567-15.000 [a]	Landrace 89	15.017[a]
Landrace 19 77	13.000-12.933[ab]	Landrace 89	14.667[ab]	Landrace 74	14.867[ab]	*GLP2* Landrace 74	13.900-13.883[ab]
Landrace 74	12.900[a-c]	Landrace 19	13.667[a-c]	Landrace 32 72 90	14.833-14.700[a-c]	Landrace 32	13.450[a-c]
Landrace 28	12.667[a-d]	Landrace 3	13.500[a-d]	Landrace 63	14.500[a-d]	Landrace 90 19 28	13.433-13.400[a-d]
GLP2	12.500[a-e]	Landrace 28	12.333[a-e]	Landrace 28 CAL33	14.133-14.000[a-e]	Landrace 48	13.300[a-e]
KK072 & 90	12.233-12.167[a-f]	Landrace 72 74 29 36 65	11.667 - 11.333[a-f]	Landrace 19	13.800[a-f]	Landrace 72	13.200[a-f]
Landrace 32 4 36 48 5 72 *KK8* CAL33 Landrace 63 35 87 29 79 70 62 33 65 68 43 64 76 84 15 10 12 34 31 44 49 *GLP585*, , Landrace 60 67	12.067-10.067 [a-g]	Landrace 51 43 60 77 79 23 35 52 63 8 32 33 37 48 61	11.167-10.167[b-f]	Landrace 49	13.600[a-g]	Landrace 77 63	12.917-12.817[a-g]
Landrace 38 66 CHELALANG CAL194 Landrace 2 59 80 26 83 56 58 46 42 52 55 85 6 39 45 81 88 57 61 *KK22* Landrace 18 27 54 RED13 Landrace 13 17 21 25 11 8 9 73 78 23 69 37 51 30 47	10.033-8.600[b-g]	Landrace 14 31 4 64 87 15 53 68 2 26 34 40 44 5 58 67 78 82 86 38 12 45 21 54 6 62 88 39	10.000-9.167[c-f]	Landrace 77 50	12.900[a-h]	KK072	12.383[a-h]
Landrace 24	8.500[c-g]	Landrace 11 42 55 56 57 80 84	9.000[d-f]	Landrace 87 43 55 66 65 36 KK072 23 58 33	12.800-12.367[a-i]	CAL33	12.144[b-h]
Landrace 14 82 41 40 20 WAIRIMU DWARF Landrace 71 75	8.433-8.300[d-g]	Landrace 17 10 13 18 30 49 59 81 83 85 73 22 41 46 66 70 71 9 16 20 24 27 50 69 75 76	8.833-7.833[ef]	Landrace 67 80 5 4 62 CHELALANG	12.267-12.033[a-j]	Landrace 36 4 87 49 5	12.083-11.900[b-i]
Landrace 1 86 22	8.200-8.100[e-g]	Landrace 47 1 25	7.667-7.333[f]	Landrace 22 34 83 CAL194 Landrace 64 76 6	11.833 - 11.567[a-k]	Landrace 43 65 33 62 66 29	11.650-11.242[b-j]
Landrace 50	8.000[fg]			Landrace 29 68 24 60 16 26 38 54 *KK8* Landrace 79 59 84 40	11.483-10.867[b-k]	*KK8* Landrace 55 67 58 80 64 79 76 CHELALANG Landrace 34 68 70 83 CAL194	11.183-10.783[b-k]
Landrace 16	7.733[g]			Landrace 2 44 70	10.800[c-k]	Landrace 23 84 60 38 6 26 35 44 59 50 2 *GLP585*, , Landrace 15 10	10.717-10.317[c-k]

	Landrace 52 88 37 *GLP585*, , Landrace 20 42 61 *KK22*	10.633-10.467^{d-k}	Landrace 31	10.300^{d-k}
	Landrace 57 31	10.433-10.400^{e-k}	Landrace 54	10.250^{e-k}
	RED13 Landrace 10 39 86 56 15 9 13 69 51 17 25 35 78 75 81 RED16 Landrace 11 21 85	10.383-9.767^{f-k}	Landrace 52 42	10.150-10.100^{f-k}
	Landrace 14 41 82 47	9.633-9.567^{g-k}	Landrace 88 56 RED16 Landrace 22 39 61 57 24 *KK22*	10.050-9.917^{g-k}
	Landrace 18 45 30 1 46 12 27 73	9.550-8.933^{h-k}	RED13 Landrace 81 85 37 12 13 40 17 25 9 21 11 16 45 78 46 69 51 18 20 86	9.842-9.250^{h-k}
	WAIRIMU DWARF	8.767^{i-k}	Landrace 75 27 30 47 14 82 41 73	9.150-8.950^{i-k}
	Landrace 8	8.267^{jk}	Landrace 1 8 WAIRIMU DWARF	8.717^{jk}
	Landrace 71	7.900^{k}	Landrace 71	8.100^{k}

Appendix x: Means of pods per plants for common bean genotypes grown in different sites

KAKAMEGA LONG RAINS		KAKAMEGA SHORT RAINS		BUNGOMA LONG RAINS		COMBINED SITES LONG RAINS	
GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN
Landrace 45	18.333[a]	Landrace 14 & 8	21.333[a]	Landrace 40	25.333[a]	Landrace 40	19.333[a]
Landraces 25 64 17 73 81 71 18 40 56 11 41 84 *KK8* Landraces 38 14 36 8 32 34 9 21 26 85 37 77 RED13 *KK22* Landraces 12 5 13 15 58 *GLP2* *GLP585*, , Landraces 33 46 66 79 22 27 4 44 48 51 76 2 20 78 KK072 Landraces 49 89 CAL194 Landraces 1 10 31 39 54 88 87 CAL33 Landrace 28 5 50 65 67 74 75 80 86 24 59 6 82 30 47 62 72 42 63 69 70 29 16 23 19 52 57 43 83 90 WAIRIMU DWARF CHELALAN G & Landrace 35	16.000-4.667[a-c]	Landrace 17	20.000[a b]	Landrace 40 8 CAL33 Landrace 14 17 18 11 12 13 63 64 65 9 *KK22* Landrace 27 85 32 54 46 21 31 36 39 42 CAL194 *KK8* Landrace 38 45 55 49 89 23 51 76 6 73 83 57 RED13 CHELALAN G Landraces 52 30 81 10 62 16 28 70 78 35 15 34 56 58 86 37 41 67 90 1 33 22 44 71 *GLP2* Landrace 24 69 79 2 20 25 25 5 82 WAIRIMU DWARF Landrace 29 KK072 landrace 48 75 84 47 66 80 88 RED16 GLP 585 Landrace 19 59 87 50 77 26 43 4	23.000 - 6.333[ab]	Landrace 17 8 14 45	18.000 - 17.000[a b]
Landraces 60 & 61	4.000-3.333[bc]	Landrace 81	18.667[a-c]	Landrace 72, 60 61 74 68	6.000 - 5.000[b]	Landrace 64 & 18	16.833 - 16.667[a-c]

Landraces 68	3[c]	Landrace 11, 84 10 30 42 85 21 12 44 13 15 37 40 78 41 71 73 31 39 4 27 38 69 46 32 66 5 50 2 26 57 33 36 70 75 82 18 9 65 79 25 87 47 49 83 22 34 43 51 54 6 67 76 64 72 74 90 19 23 24 35 53 56 1	17.667 - 8.000[a-d]	Landrace 11 12 9 13 73 32 85 *KK22 KK8* Landrace 36 38 81 21 27 25 46 55 CAL33 Landrace65 54 63 56 31 39 RED13 CAL194 Landrace 51 76 49 71 89 41 34 42 15 37 6 58 78 84 10 23 28 30 33 57 62 83 *GLP2* Landrace 22 44 52 79 70 86 1 16 67 CHELALAN G Landrace 2 20 48 66 77 *GLP585*, , *KK22* Landrace 26 RED16 Landrace 24 5 88 35 75 82 69 80 90 87	16.000 - 8.000[a-d]
		Landraces 4 63 88 20 3 48 68 55 61 86 58	7.667 - 6.667[b-d]	Landrace 29 4 47 59 50 WAIRIMU DWARF Landrace 19 72 74 43	7.833 - 6.167[b-d]
		Landrace 28 52 60 62 29 77 16 59 89	6.333 - 5.667[cd]	Landrace 60	4.833[cd]
		Landrace 80	4.667[d]	Landrace 61 & 68	4.500 - 4.000[d]

Appendix xi: Means of seeds per pod for common bean genotypes grown in different sites

KAKAMEGA LONG RAINS		KAKAMEGA SHORT RAINS		BUNGOMA LONG RAINS		COMBINED SITES LONG RAINS	
GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN
Landrace 29	6.500[a]	Landrace 29	8.333[a]	Landrace 52	6.333[a]	Landrace 52 29	6.167-6.083[a]
Landrace 12 13 15 52 84	6.000[ab]	Landrace 15 53	7.000[ab]	Landrace 36	6.000[ab]	Landrace 36 37 84	5.667[ab]
Landrace 37 38	5.667[a-c]	Landrace 12 37 52	6.667[a-c]	Landrace 29 33 37 64	5.667[a-c]	Landrace 13 33	5.500[a-c]
KK22 Landrace 21 33 36 39 44 45 85 *GLP585*, , Landrace 18 27 31 40 41 64 78 9 10 17 2 34 4 42 5 54 55 59 61 71 76 77	5.333-4.667[a-d]	Landrace 10	6.333[a-d]	*GLP585*, , Landrace 27 39 40 60 84 90 13 18 31 34 44 63 81 89 11 15 21 22 30 35 38 42 46 48 51 54 61 71 86 9 *KK8* Landrace 10 12 14 24 41 45 5 59 62 65 70 76 78 8 WAIRIMU DWARF RED13 RED16 CAL194 CAL33 CHELALANG *GLP2* KK072 Landrace 1 16 17 19 2 20 32 4 49 55 56 58 67 68 72 73 83 85 87 88	5.333-4.000[a-d]	Landrace 15 39 64	5.333[a-d]
KK072 Landrace 11 14 19 26 28 30 32 46 62 68 73 8 80 81 86 89 90 CAL194 *GLP2* *KK8* Landrace 48 51 56 58 60 63 65 66 67 70 74 79 82 88 RED13	4.333-4.000[b-d]	Landrace 38 44 60 68 14 17 30 33 34 39 76 8 90 13 21 31	6.000-5.333[a-e]	*KK22* Landrace 23 26 28 43 50 57 6 66 74 80 82	3.667[b-d]	*GLP585*, , Landrace 12 27 38 40 44 18 21 31 34 45 9 90 41 42 54 60 61 71 78 81 85 89 *KK22* Landrace 10 11 30 46 5 59 63 76 86 14 17 2 4 48 51 55 62 8	5.167-4.333[a-e]
CAL33 Landrace 22 25 35 43 47 57 6 72 75 87 WAIRIMU DWARF CHELALANG Landrace 1 20 23 24 49 50 69 83	3.833-3.333[cd]	Landrace 19 27 43 46 51 61 78 82 84 9 11 36 4 40 42 64 71 73 80 81 85 86 22 23 3 41 45 48 54 58 67 70 72 87 89 18 2 20 24 26 28 56 57 62 66 74 75 77 88	5.000-4.000[b-e]	Landrace 25 47 69 77 79	3.333[cd]	KK072 *KK8* Landrace 19 22 32 35 65 68 70 73 RED13 RED16 CAL194 *GLP2* WAIRIMU DWARF Landrace 26 28 56 58 67 77 80 88	4.167-4.000[b-e]
Landrace 16	3.000[d]	Landrace 1 16 25 32 35 47 49 5 50 63 65 79	3.667[c-e]	Landrace 75	3.000[d]	CAL33 Landrace 24 66 72 74 82 87 1 20 43 49 57 6 79 83 CHELALANG	3.889[c-e]
		Landrace 55 59 6 83	3.333[de]			Landrace 16 23 25 47 50	3.500[de]
		Landrace 69	3.000[e]			Landrace 69 75	3.333[e]

Appendix xii: Means of 100 seed weight for common bean genotypes grown in different sites

KAKAMEGA LONG RAINS		KAKAMEGA SHORT RAINS		BUNGOMA LONG RAINS		COMBINED SITES LONG RAINS	
GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN
Landrace 48	47.333[a]	Landrace 85	66.900[a]	Landrace 43	61.867[a]	Landrace 43	50.933[a]
KK8	46.000[ab]	Landrace 3	50.257[ab]	*GLP2* KK072 Landrace 74	57.000-56.000[ab]	Landrace 74	50.333 [ab]
Landrace 74	44.667[a-c]	Landrace 89 65 87 72 6 43 83 4 28 75 32 63 61 79 48 19 49 77 60 69 57 16 67 54 66 88 59 20 68 2 50 56 24 62 5 70 90 23	45.107-32.400[a-c]	Landrace 89	55.667[a-c]	*GLP2*	50.167[a-c]
Landrace 28 6	44.333[a-d]	Landrace 25 22 35 47 82 80 53 51 58 26 74 52 76 55 29 10 1 64 86 33 13 36 9 40 44 13 42 78 45 30 81 15 73 39 31 84 38 11 34 37 71 27 14	31.857-15.667[bc]	Landrace 83 87 6	55.467-54.667[a-d]	Landrace 6 83	49.500-49.067[a-d]
Landrace 72 *GLP2*	43.433[a-e]	Landrace 41 17 8 18 46 21	15.210-13.453[c]	Landrace 65	54.000[a-e]	Landrace 65 72 KK072 Landrace 87 28 89	48.500-47.833[a-e]
Landrace 19 65	43.000[a-f]			Landrace 72 CHELALANG	53.383-53.333[a-f]	*KK8* Landrace 48 16 49	47.333-46.500[a-f]
Landrace 16 83	42.800-42.667[a-g]			Landrace 49 57 16 67	52.333-51.667[a-g]	Landrace 77 19 56 79	44.873-44.333[a-g]
Landrace 87 47 49	41.000-40.667a-h			Landrace 28	51.537[a-h]	CHELALANG Landrace 4 57	43.880-43.600[a-h]
Landrace 43 75 89 KK072 Landrace 69 77	40.000-39.333[a-i]			Landrace 56	51.000[a-i]	Landrace 69 32 67 5	42.167[a-i]
Landrace 79 4 56 61	39.000[a-j]			Landrace 77 79 23	50.413-49.483[a-j]	Landrace 23 54 47 75 59 CAL33	41.658-40.633[a-j]
Landrace 32 5 63 35 55 80	37.667-37.000[b-k]			Landrace 4 *KK8* CAL33 Landrace 48 54 69	48.767-47.000[a-k]	Landrace 55 35 26 20 58 50 63 90 24 CAL194 Landrace 62	40.000-38.667[a-k]
CAL33 Landrace 54 59 66	36.7833-35.667[c-k]			Landrace 86 19 32 CAL194 Landrace 5 20	46.910-46.000[a-l]	Landrace 25 82	38.542-38.500[b-k]
Landrace 26 58	35.333[d-l]			Landrace 59 90	45.667[a-m]	Landrace 66 88 61 WAIRIMU DWARF	38.167-38.052[b-l]
Landrace 50 57	35.000[e-l]			Landrace 26 58 82 WAIRIMU DWARF Landrace 25 50 55	44.240-43.000[a-n]	Landrace 80	38.000[c-l]
Landrace 24 62 CHELALANG Landrace 2 88	34.795-34.333[e-m]			Landrace 24 62 35 70 75 88	42.800-42.000[b-n]	Landrace 70	37.667[d-l]
Landrace 23 68	33.833-33.720[f-n]			Landrace 47	41.667[b-o]	Landrace 86	36.247[e-m]
Landrace 25	33.667[g-n]			Landrace 63 66	40.667[b-p]	RED16	35.667[e-n]
Landrace 20	33.333[h-o]			RED13	39.383[c-p]	Landrace 2 22	35.500-35.212[f-n]
Landrace 70 82	33.000[h-p]			Landrace 80	39.000[c-q]	RED13 Landrace 68	34.525-33.720[g-n]

Landrace 67 22 WAIRIMU DWARF	32.667-32.000^{h-q}	Landrace 61 22	38.130-38.090^{c-r}	Landrace 51	32.000^{h-o}
Landrace 90 CAL194	31.000^{i-r}	Landrace 2	36.667^{d-s}	Landrace 76 1	30.500-30.025^{i-p}
Landrace 1	30.000^{j-s}	RED16	35.667^{e-s}	Landrace 10 52	29.667-29.500^{j-q}
RED13	29.667^{k-s}	Landrace 51	35.000^{f-s}	Landrace 33	28.000^{k-r}
Landrace 51	29.000^{k-t}	Landrace 68	33.720^{g-s}	Landrace 36 64	26.167-26.000^{l-r}
Landrace 76	28.667^{k-u}	Landrace 10	32.667^{h-s}	Landrace 85 9	25.167-24.500^{m-r}
Landrace 52	27.333^{l-v}	Landrace 76	32.333^{i-s}	Landrace 29 30 45 78 81 40 84 37 73 12 15 39 31 38 34 13 42 *GLP585*, , Landrace 44 60 8	24.382-20.667^{n-r}
Landrace 10	26.667^{l-w}	Landrace 33 52	32.000-31.667^{j-s}	Landrace 46 41	20.500-19.833^{o-r}
Landrace 86	25.583^{m-x}	Landrace 1	30.050^{k-s}	*KK22* Landrace 27 11 71 17	19.350-18.333^{p-r}
Landrace 64	25.000^{n-x}	Landrace 36	28.000^{l-s}	Landrace 14	17.667^{qr}
Landrace 36	24.333^{o-y}	Landrace 64	27.000^{m-s}	Landrace 21 18	16.917-16.833^{r}
Landrace 33	24.000^{p-z}	Landrace 85 30 9 81	26.667-25.333^{n-s}		
Landrace 85	23.667^{q-z}	Landrace 29 78 84 12 15 40 45 13 31 42 37 73 8 34 *GLP585*, ,	25.263-23.067^{o-s}		
Landrace 29 9 45 40 78	23.500-22.333^{r-z}	Landrace 11 38 46 39 44	22.667-22.000^{r-s}		
Landrace 30 37 39 73 81 84 60	21.667-21.000^{s-z}	*KK22* Landrace 27 60 41 71	21.200-20.333^{q-s}		
Landrace 12 38 15 44 34	20.667-20.000^{t-z}	Landrace 14 17	20.000^{rs}		
Landrace 31	19.667^{u-z}	Landrace 18 21	19.000-18.333^{s}		
GLP585, , Landrace 13 41 42 46	19.333-18.333^{v-z}				
Landrace 8 *KK22*	18.000-17.500^{w-z}				
Landrace 27 71	17.000^{x-z}				
Landrace 17	16.667^{x-z}				
Landrace 21 14	15.500-15.333^{yz}				
Landrace 11	15.000^{zA}				
Landrace 18	14.667 ^{A}				

Appendix xiii: Means of tons per hectare for common bean genotypes grown in different sites

KAKAMEGA LONG RAINS		KAKAMEGA SHORT RAINS		BUNGOMA LONG RAINS		COMBINED SITES LONG RAINS	
GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN	GENOTYPE	MEAN
RED13	1.344[a]	Landrace 51	1.473[a]	Landrace 12 49 90 CAL33 *KK8* Landrace 5 79	1.518-1.385[a]	Landrace 79 CAL33	1.323-1.308[a]
Landrace 48 40 73 CAL33 Landrace 6	1.315-1.205[ab]	Landrace 35	1.345[ab]	Landrace 32	1.348[ab]	Landrace 12 48 49 6 33 85 5 32 RED13	1.263-1.105[ab]
Landrace 85	1.173[a-c]	Landrace 3	1.325[a-c]	Landrace 11 89 36 58 10 64 51 50 CAL194 Landrace 48 18 33 67 57 63 74 6 85 KK072 Landrace 23 55 56 4 1 21 59 13 65 27 52 87 45 54 83 20 38 15 35 RED13 Landrace 82 22 31 75 78 26 81 *GLP2* Landrace 62 17 80 43 2 9 28 41 30 14 CHELALANG 84 40 72 25 8 76 42 37 88 39 46 70 16 73 24 66 34 69 71 19 68 RED16 *GLP585*, , Landrace 44 86	1.330-0.370[a-c]	Landrace 74 *KK8* Landrace 90 63 64 56 36 4 50 38 18 55 67 CAL194 Landrace 45 *GLP2* Landrace 40 51 13 KK072 Landrace 58 17 89 59 87 81 10 1 75 22 57 54 21 84 15 65 11 28 83 27 31 8 80 26 2 62 41 73 30 39 23 20 37 25 78 82 35 9 66 43 42 76 *GLP585*, , Landrace 52 88 60 46 14 71 19 72 69 70 34 44 47 CHELALANG	1.072-0.451[a-c]
GLP2 Landrace 33 38	1.160-1.143[a-d]	Landrace 44	1.285[a-d]	Landrace 29 60 WAIRIMU DWARF Landrace 61 47 77	0.309-0.183[bc]	Landrace 77 61 68 24 RED16 Landrace 16	0.449-0.412[bc]
Landrace 45 17 74 12 56 4 63 49 *GLP585*, , Landrace 55 81 84 73 18 60 67 13 32 75 22 28 50 8 64 5 39 66 87 CLA194 Landrace 36 KK072 Landrace 37 59 25 51 41 30 54 15 2 80 *KK8* Landrace 42 77 31 44 76 62 19 1 26 71 83 47 9 65 90 58 78 21 61 27 88 69 43 89 57 82 46 20 35	1.042-0.547a-e	Landrace 64 52	1.228-1.223[a-e]	*KK22*	0.132[c]	Landrace 29 86 *KK22* WAIRIMU DWARF	0.340 -0.243[c]
Landrace 34 10 70 72 14 *KK22* Landrace 68 23	0.530-0.390[b-e]	Landrace 33 32	1.188-1.173[a-f]				
Landrace 29 52 86 11 24 16	0.372-0.240[c-e]	Landrace 78 12 73 90 45 87 84 79 42 29 85 13 65 30 76 72 15 34 75 11 31 39 36 17 4 6 18 54 81 53 49 14 40 8 71 9 5 21 88 57 28 2 50 63 20 37 74 66 43 27 61 89 60 67 16 70 56 80 83	1.160-0.483[a-g]				

WAIRIMU DWARF	0.225^{de}	Landrace 24 19 1 26 38 25 10 62 68 48 59 77 41 69 22	0.473-0.372^{b-g}
CHELALANG	0.188^{e}	Landrace 23	0.333^{c-g}
		Landrace 55 47 58	0.315-0.298^{d-g}

Appendix xiv: Polyacrylamide gel preparation protocol

1. Silane solution is prepared in a 50 ml falcon tube by adding 8ml ethanol, I.8ml distilled water, 200µl acetic acid and 10µl silane (3-trimethoxysilyl-propyl-methacrylate).
2. This is mixed gently by shaking. 1ml of the silane solution was pipetted onto a surface of a full glass plate.
3. The remaining solution was stored at 4°C for later preparation of more gels. The solution was spread equally over the glass plate using a lint-free wipe.
4. The glass plate was covered with lint-free wipes to prevent dust from settling on the plate as it dries for an hour.
5. The plate was marked with a permanent marker to identify the silanised side. Polishing the plate was done after one hour with a lint-free wipe moistened with a small amount of ethanol.
6. The gel mould was position on a lab tray and a level bubble was placed in the mould. The screws at the top of the mould were turned until the bubble was centered.
7. 6% gel was prepared in a conical flask by adding 18.8ml acrylamide-bis, 2.5 ml 50X TAE buffer and 53.7 ml distilled.
8. An APS-TEMED solution was prepared by dissolving two APS-TEMED tablets in 50ml distilled water and later added to the conical flask after dissolving.
9. The gel mix were gently mixed and poured into the mould. This was done by pouring the gel mix first around the gel well notches ensuring no bubbles formed.
10. The glass plate was slotted into the top of the mould with the silanised side facing down. 1ml of the gel mix was pipetted into a microfuge tube to monitor the time taken for the gel to solidify.
11. The gel was left for 60 minutes for complete polymerization. Two gel wedges were used to gently lift the glass plate from the mould. The glass plate was placed gel side up into the gel tank.
12. 1X TAE buffer was poured into the gel tank to until it covered the gel plate.
13. To enable sample loading, the gels wells were visualized by placing a blue mat underneath the gel tank. 1µl of each sample was loaded into each well for all the samples for one set SSR. A second set of SSR was loaded into the well maintaining the loading order.
14. A 50BP ladder was loaded at the ends and center of the gel to help in identification of the band sizes.
15. The power pack was set at 60 Volts and a current of 110 Amp for 200 minutes.
16. The gel was stained in a tray containing ethidium bromide for 60 minutes. The plate was then removed allowing the staining solution to run off into the tray. The excess stain was wiped off from the base of the plate.
17. The gel plate was placed on the trans-illuminator with the gel facing down. The gel was photographed using a Canon camera.

Appendix xv: Coordinates of anthracnose samples collection areas

Sub-County	Location	Village	Longitude	Latitude
Bungoma South	Khalaba	Musemwa	0.530856	34.5586325
Muskoma	Muskoma	Wekelekha	0.5321421	34.5163393
Nambale	Nambale Township	Makina	0.4588187	34.299666
Teso South	Ochude	Kaburbur	0.5231736	34.1456126
Sabatia	Chavogere	Itovo	0.1583122	34.7970132
Nambale	Bukhayo Central	Sidende	0.4437454	34.3448318
Shinyalu	Muranda	Airstrip	0.2720136	34.7794082
Kakamega Central	Lurambi	Milimani	00°16'N	34°45'E
Bungoma South	Kibabii	Tuti	0.54°N	34°54"E
Saboti	Saboti	Khaoya	0.9417286	34.8589866

Appendix xvi: *Pythium* root rot disease reaction among common bean cultivars on a CIAT scale

GENOTYPE	SSN1	SSN2	RXN	GENOTYPE	SSN1	SSN2	RXN	GENOTYPE	SSN1	SSN2	RXN
CAL194	3	2	R	Landrace 36	7	6	S	Landrace 65	9	8	S
CAL96	7	8	S	Landrace 37	9	7	S	Landrace 66	6	6	MR
GLP2	6	6	MR	Landrace 38	5	5	MR	Landrace 67	7	8	S
Landrace 1	7	7	S	Landrace 39	7	6	S	Landrace 68	6	5	MR
Landrace 10	7	7	S	Landrace 4	7	5	S	Landrace 69	8	8	S
Landrace 11	7	6	S	Landrace 40	7	6	S	Landrace 7	5	5	MR
Landrace 12	8	7	S	Landrace 41	6	6	MR	Landrace 70	7	6	S
Landrace 13	9	8	S	Landrace 42	9	8	S	Landrace 71	7	8	S
Landrace 14	8	7	S	Landrace 43	7	6	S	Landrace 72	6	6	MR
Landrace 15	8	8	S	Landrace 44	6	5	MR	Landrace 73	7	8	S
Landrace 16	8	8	S	Landrace 45	6	6	MR	Landrace 74	7	7	S
Landrace 17	8	7	S	Landrace 46	6	5	MR	Landrace 75	3	3	R
Landrace 18	4	5	MR	Landrace 47	7	6	S	Landrace 76	7	6	S
Landrace 19	7	8	S	Landrace 48	6	7	MR	Landrace 77	7	8	S
Landrace 2	7	7	S	Landrace 49	5	4	MR	Landrace 78	6	5	MR
Landrace 20	8	8	S	Landrace 5	5	5	MR	Landrace 79	5	4	MR
Landrace 21	5	5	MR	Landrace 50	6	5	MR	Landrace 8	4	4	MR
Landrace 22	6	6	MR	Landrace 51	8	7	S	Landrace 80	7	8	S
Landrace 23	8	9	S	Landrace 52	5	5	MR	Landrace 81	6	5	MR
Landrace 24	7	6	S	Landrace 53	5	5	MR	Landrace 82	8	9	S
Landrace 25	5	4	MR	Landrace 54	7	8	S	Landrace 83	7	9	S
Landrace 26	7	6	S	Landrace 55	7	8	S	Landrace 84	7	8	S
Landrace 27	6	5	MR	Landrace 56	6	7	MR	Landrace 85	6	5	MR
Landrace 28	8	8	S	Landrace 57	6	7	MR	Landrace 86	5	5	MR
Landrace 29	5	5	MR	Landrace 58	3	3	R	Landrace 87	5	6	MR
Landrace 3	6	6	MR	Landrace 59	6	6	MR	Landrace 88	6	6	MR
Landrace 30	7	6	S	Landrace 6	5	5	MR	Landrace 89	6	6	MR
Landrace 31	6	5	MR	Landrace 60	7	7	S	Landrace 9	8	8	S
Landrace 32	6	4	MR	Landrace 61	7	7	S	Landrace 90	6	6	MR
Landrace 33	7	6	S	Landrace 62	4	5	MR	KK22	1	1	R
Landrace 34	8	8	S	Landrace 63	6	5	MR	KK8	1	2	R
Landrace 35	7	6	S	Landrace 64	7	6	S				

KEY: SSN 1= First screening season, SSN 2= Second screening season, RXN= Reaction, R= Resistant, MR= Moderately Resistant, S=Susceptible

Appendix xvii: Anthracnose disease reaction among common bean cultivars on a CIAT scale

GENOTYPE	SSN1	SSN2	RXN	GENOTYPE	SSN1	SSN2	RXN	GENOTYPE	SSN1	SSN2	RXN
CHELALANG	7	8	S	Landrace 36	4	4	MR	Landrace 64	3	4	MR
G2333	1	1	R	Landrace 37	6	6	S	Landrace 65	3	5	MR
Landrace 1	1	2	R	Landrace 38	1	3	R	Landrace 66	6	7	S
Landrace 10	3	5	MR	Landrace 39	2	2	R	Landrace 67	2	1	R
Landrace 11	2	2	R	Landrace 4	5	7	S	Landrace 68	2	1	R
Landrace 12	1	2	R	Landrace 40	3	5	MR	Landrace 69	5	7	S
Landrace 13	4	7	S	Landrace 41	5	6	MR	Landrace 7	4	5	MR
Landrace 14	4	6	MR	Landrace 42	4	4	MR	Landrace 70	2	2	R
Landrace 15	4	7	MR	Landrace 43	2	2	R	Landrace 71	2	2	R
Landrace 16	2	3	R	Landrace 44	3	4	MR	Landrace 72	2	2	R
Landrace 17	4	5	MR	Landrace 45	3	4	MR	Landrace 73	2	3	R
Landrace 18	2	2	R	Landrace 46	2	2	R	Landrace 74	4	6	MR
Landrace 19	5	7	S	Landrace 47	4	5	MR	Landrace 75	6	8	S
Landrace 2	3	5	MR	Landrace 48	2	1	R	Landrace 76	4	5	MR
Landrace 20	6	6	S	Landrace 49	4	4	MR	Landrace 77	2	2	R
Landrace 21	3	1	R	Landrace 5	6	6	S	Landrace 78	2	2	R
Landrace 22	1	2	R	Landrace 50	2	2	R	Landrace 79	4	6	MR
Landrace 23	4	5	MR	Landrace 51	2	1	R	Landrace 8	2	2	R
Landrace 24	4	4	MR	Landrace 52	2	2	R	Landrace 80	4	6	MR
Landrace 25	2	2	R	Landrace 53	5	7	S	Landrace 81	4	5	MR
Landrace 26	4	5	MR	Landrace 54	5	6	S	Landrace 82	2	2	R
Landrace 27	5	6	MR	Landrace 55	4	5	MR	Landrace 83	6	7	S
Landrace 28	3	4	MR	Landrace 56	4	4	MR	Landrace 84	4	6	MR
Landrace 29	4	6	MR	Landrace 57	4	5	MR	Landrace 85	3	5	MR
Landrace 3	5	6	MR	Landrace 58	2	2	R	Landrace 86	6	7	S
Landrace 30	3	4	MR	Landrace 59	4	5	MR	Landrace 87	4	4	MR
Landrace 31	2	3	R	Landrace 6	4	5	MR	Landrace 88	5	7	S
Landrace 32	6	6	S	Landrace 60	6	7	S	Landrace 89	6	7	S
Landrace 33	5	6	S	Landrace 61	5	7	S	Landrace 9	3	5	MR
Landrace 34	2	2	R	Landrace 62	3	5	MR	Landrace 90	5	6	S
Landrace 35	2	2	R	Landrace 63	4	7	MR	TASHA	7	8	S