MORPHO-AGRONOMIC, GENETIC DIVERSITY AND *PYTHIUM* ROOT ROT RESISTANCE OF SOUTH WESTERN KENYA COMMON BEANS (*PHASEOLUS VULGARIS* L.) LANDRACES

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DECLARATION

I, Anunda HenryNyabuto declare that this thesis is my original work and has not been presented for award of a degree in any other University.

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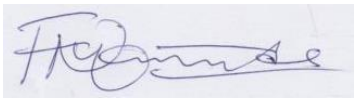
Signature:  ….. Date: 29/04/2021

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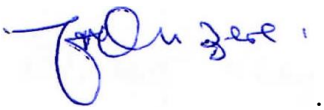
Signature:……  …… Date: 30/04/2021

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DEDICATION

This thesis has been dedicated to my parents: Janet Anunda and the late Alexander Anunda.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFLP	Amplified Fragment Length Polymorphism
AEO	Agricultural Extension Officer
AEZ	Agro Ecological Zone
CIAT	Centro Internacional de Agricultura Tropical
CSA	Climate Smart Agriculture
DAP	Diammonium Phosphate
FAO	Food and Agricultural Organization
GE	Genetic Erosion
GLP	Grain Legume Project
GI	Genetic Integrity
GK	Government of Kenya
GA	Genetic Advance
GAM	Genetic advance as percentage of mean
GBK	Gene Bank of Kenya
GM	Mean of traits
GV	Genotypic Variance
GCV	Genotypic Coefficient of Variation
H_2	Heritability
IBPGR	International Board for Plant Genetic Resources
ISSRs	Inter Simple Sequence Repeats
KALRO	Kenya Agricultural and Livestock Research Organization
LH1	Lower Highland 1
LH2	Lower Highland 2
LM1	Lower Midland 1
LM2	Lower Midland 2
LRC	Landrace
MR	Moderate Resistance
MOA	Ministry of Agriculture
NNP	Name Not Provided

NTSYS	Numerical Taxonomy and Multivariate Analysis System
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
POX	Peroxidase gene-based molecular markers
PCV	Phenotypic Coefficient of Variation
PV	Phenotypic Variance
PIC	Polymorphic Information Content
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphisms
SNPs	Single Nucleotide Polymorphisms
SSR	Simple Sequence Repeats
SW	South Western
UPGMA	Unweighted Pair-Group Method of the Arithmetic Mean
USDA	United States Department of Agriculture

ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is one of the most important legume crops used as a source of protein, vitamins, and other beneficial nutrients among resource-poor populations in Kenya. Common bean landraces derived from local tropical germplasm represent an important source of genetic and phenotypic diversity, which is at present under-exploited by Kenyan crop breeding programs besides being threatened by genetic erosion. Besides, common bean cultivation in Kenya is threatened by many biotic stresses, such as *Pythium* root rot disease. These landraces may also provide important and durable sources of resistance to *Pythium* root rot disease. The main objective of this study was therefore to evaluate the diversity of local common bean landraces from South Western Kenya for *Pythium* root rot resistance using morpho-agronomic characters and peroxidase gene-based (POX) molecular markers. A total of 51 common bean landraces were screened for resistance to Pythium root rot in the screenhouse. The leaves of plants established in the creenhouse were used to evaluate genetic diversity using 5 peroxidase gene (POX) markers. Following infection with *Pythium* spp., 11.77, 54.90 and 33.33% of the landraces were found to be moderate-resistant, susceptible and highly susceptible, respectively. No landrace was found resistance to *Pythium* root rot. A total of 1119 alleles were amplified by the 5 primers, ranging from 3 to 8 alleles per locus, with an average of 4.8. The polymorphism information content (PIC) of the POX markers varied from 0.10 to 0.47, with an average of 0.28. Using genetic similarity coefficients, un-weighted pair group method with arithmetic (UPGMA) grouped the landraces into two main genetic clusters, and the dendrogram did not reveal any unique groupings according to their reaction to *Pythium* root rot disease. Population structure analysis using the Bayesian model-based approach separated the germplasm into 3 genetic groups with low admixture. Population structure analysis showed that all the 3 gene pools contained landraces exhibiting both moderate resistance and susceptible to *Pythium* root rot. For morpho-agronomic characterization, field experiments were conducted using 52 common bean landraces at the Kenya Agricultural and Livestock Research Organization (KALRO). The study was conducted using a randomized complete block design (RCBD) with three replications. Analysis of variance revealed significant differences indicating the existence of genetic variability among the 52 landraces for 14 quantitative traits studied. The genotypic coefficient of variation ranged from 1.00% for biological yield to 84.69% for pod width, while the phenotypic coefficient of variation ranged from 2.34% for biological yield to 84.40% for number of branches. The estimated broad sense heritability ranged from 60.20% for seeds per plant to 87.57% for days to emergence. Estimates of genetic advance as percent of mean ranged from 10.15% for biological yield to 97.45% for number of branches. Positive and highly significant association of plant height, days from planting to 50% flowering, number of pods per plant and biological yield was observed with seed yield per plant hence these traits may be directly attributed for the improvement of seed yield. A survey was also carried out to determine the status genetic erosion of common bean landraces in South Western Kenya. The estimated genetic erosion of the common bean landraces was greater than 50% and the underlying causes were diseases, introduction of new varieties and pests at 100%, 94% and 86%, respectively. The morpho-agronomic and genetic characterization of common bean landraces in this study will give valuable information for breeders and serve as a baseline for efficient development of new cultivars with *Pythium* root rotresistance.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Common beans belong to the Genus *Phaseolus* which has 36 spp. (Buruchara *et al*., 2011) of which *Phaseolus vulgaris* L. is the most popular, globally distributed with a wide range of genetic variation. Common beans (*Phaseolus vulgaris* L.) are the most important grain-legume, second to maize as a food and nutritional security crop in Kenya (FAOSTAT, 2017). Africa produces 17% of the world total production, of which 70% is from Eastern Africa (CIAT, 2015). In Kenya, approximately 400 -1200 kg/ha of common beans is produced mainly from intercropping with maize, bananas, coffee, potatoes and sorghum, among other crops in small-scale farms. In South Western Kenya an average person consumes about 55 kg of beans per year, with average household production of 430 kg/ha (FAOSTAT, 2015).

Beans are an important tropical grain legume; a cheap source of protein and minerals especially iron and zinc (CIAT, 2013). Consumption of common bean provides mankind with several health benefits including cholesterol level reduction in the body (CIAT, 2013), decreasing heart or coronary diseases (Szilagyi *et al*., 2011), favorable effects against cancer (Sicard, 2005) and decreasing diabetes and obesity (Rubyogo *et al*., 2010). Common beans play a very important role in sustaining soil fertility by hosting rhizobium bacteria that fixes atmospheric nitrogen and adds organic matter to the soil (Wortmann *et al.*, 1998). Therefore, it is a multipurpose crop in that it also provides grains as well as fodder for livestock. As a cover crop it is efficient in suppressing weeds and prevents soil erosion (Geil, 1994; Wortmann *et al.*, 1998).

Production of common bean is constrained by various biotic stresses. Insect pests especially pod-borers, aphids, bruchids, bean stem maggots and weevils may cause yield loss of up to 80% (Graham and Ranalli, 1997; Wortmann *et al*., 1998). Other pests include vertebrate pests (moles, porcupines) and nematodes. Moreover, common beans are susceptible to various bacterial, viral and fungal diseases that diminish their yield around the world (Wagara *et al*., 2004; Kimani *et al*., 2005b; Kapil *et al*., 2011; Leitich *et*

al., 2016; Mutuku *et al*., 2016). However, the greatest hindrance to common bean production in South Western Kenya are fungal diseases mainly root rots caused by*Fusarium*, *Rhizoctonia* and *Pythium* that can lead to yield loss of up to 100% (FAOSTAT, 2015). These fungal diseases can also lead to extinction of high yielding common bean landraces. *Pythium* root rot disease alone can cause yield losses of up to 100% in susceptible common bean varieties. Farmers have abandoned these susceptible but high yielding varieties hence resulting to genetic erosion of once elite landraces. The use of resistant bean varieties is the most effective, economical and environmentally sustainable strategy to control *Pythium* root rot disease (Binagwa *et al*., 2016). However, this requires identification of resistant genotypes, and incorporation of the disease resistance into agronomically desirable varieties. There is need to characterize common bean germplasm for *Pythium* root rot resistance in order to identify morphological and molecular markers for selection of resistantvarieties.

Agro-morphological characterization has been applied in the past for various uses including the identification of duplicate genotypes, correlation studies with traits of agronomic and economic importance as well as identification of germplasm resistant to pests and diseases (CIAT, 2013). Common bean exhibits great phenotypic diversity. Understanding the range of phenotypic traits in common bean landraces exhibiting *Pythium* root rot resistance is a valuable step towards efforts aimed at improving the crop for this trait. Genetic differences that exist between accessions can be combined with phenotypic analyses to augment germplasm characterization (FAOSTAT, 2015). Molecular markers permit significant estimation of genetic diversity directly at the DNA level, reducing the interference of environmental variations. Gene-based molecular markers can be associated with economically important traits in Kenyan common bean landraces.

Gene-based molecular markers represent an important resource for characterization of germplasm and elucidation of gene functions. Peroxidase, a key enzyme in metabolic pathways is an example of gene-based molecular marker in plants. Peroxidases (POXs) belong to a multigene family and exhibit high sequence variability with the existence of conserved domains (Oliva *et al*., 2009). Conserved DNA regions of peroxidase share the

same priming site and are distributed across the genomes of different genotypes in different patterns hence polymorphisms can be detected within species. Peroxidases (POXs) are glycoprotein enzymes containing heme cofactor and utilize H_2O_2 in oxidation reactions involving a range of compounds. These enzymes perform diverse roles in plants including detoxification of reactive oxygen species generated during biotic and abiotic stresses (Mittler *et al*., 2004; Gill and Tuteja 2010; Zhang *et al*., 2013), inducing defense response against pathogens (Passardi *et al*., 2005), formation of lignin and suberin (Herrero *et al*. 2013), metabolism of auxin, healing of wounds and plant–microbial symbiosis (Passardi *et al*., 2005). Peroxidases also catalyzes deamination of trans-cinnamic acid in a biosynthesis pathway leading to the formation of phenolic compounds which have many vital activities in plants such as regulation of plant growth differentiation (Vicuna 2005), inhibition of pathogens (Almagro *et al*., 2008), and tolerance to abiotic stresses (Gill and Tuteja 2010; Zhang *et al*., 2014).

It has been shown that the peroxidase gene family possesses highly conserved domains allowing oligonucleotide primers to be designed to amplify DNA sequences coding for peroxidases from many different plants. There are several studies investigating the peroxidase gene polymorphism in a few crops such as buffalo grasses, bermuda grasses, apple, watermelon, Citrus spp., common bean and almond (Gulsen *et al*., 2007, 2009, 2010; Ocal *et al*., 2014; Uzun *et al*., 2014; Nemli *et al*., 2014; Pinar *et al*., 2016). Due to critical roles of peroxidases in plant defense, the peroxidase gene polymorphism analyses may increase the understanding of the relationship among common bean landraces from south Western Kenya and they may give the new perspectives for common bean breeding for *Pythium* root rotresistance.

1.2 Problem statement

Despite being a very vital food for direct human consumption, serving as a good source of calories and proteins for many people around the world; common bean production rates have been declining in developing countries. Production of common bean in South western (SW) Kenya is constrained by various biotic stresses including insect pests especially pod-borers and weevils which may cause yield loss of up to 80%. There is also lack of cultivars with consumer quality attributes such as taste, palatability

andfastcooking. However, the greatest limitation to bean production in SW Kenya are fungal diseases particularly root rots caused by *Fusarium*, *Rhizoctonia* and *Pythium* (Otsyula et al., 2003). *Pythium* root rot disease can cause yield losses of up to 100% in susceptible varieties. Farmers have abandoned these susceptible cultivars even though they produce high yields. Consequently, landraces are being replaced with new varieties, which have led to gradual genetic erosion of once elite landraces.

Genetic characterization provides the key to unraveling disease-resistant accessions in the population. Genetic characterization of common beans has been widely undertaken by various studies including Singh *et al*. (1991) using allozymes, Kumar *et al.* (2008) using AFLP, RAPD, Zargar *et al*. (2016) using RAPD, Velasquez *et al*. (1994) using restriction fragment length polymorphism (RFLP), Svetleva *et al*. (2006) using inter-simple sequence repeat (ISSR), Zargar *et al*. (2016) and Gyang *et al*. (2018) using simple sequence repeats (SSR). However, these markers are not gene-targeted and unable to highlight existing genetic differences that are linked to gene function. Therefore, there is need to explore gene-targeted peroxidase gene (POX) molecular markers for characterization of common bean for *Pythium* root rotresistance.

1.3 Justification

Common bean (*Phaseolus vulgaris* L.) is the second most important source of human dietary proteins and the third most important source of calories (Sharma *et al*., 2013; Stoilova *et al*., 2013; Shaun *et al*., 2012). It has high nutritional value with essential protein contents (~22%), minerals (calcium, copper, iron, magnesium, manganese, zinc selenium, cobalt), and both water and fat soluble vitamins (Rodriguez *et al*., 2016). To provide the much needed food and nutrition security of populations in the low and middle income countries the crop is very essential. However, production is greatly constrained by Pythium root rot disease, causing losses of up to 100%. The use of resistant bean varieties is the most effective, economical and environmentally sustainable strategy to control *Pythium* root rot disease (Binagwa *et al*., 2016). However, this requires identification of resistant genotypes, and incorporation of the disease resistance into agronomically desirable varieties. There is need to characterize common beangermplasm

For*Pythium* root rot resistance in order to identify markers for selection of resistant varieties.

Development of resistant varieties takes time and is made difficult by the genetic nature of common bean. Early identification of resistant common bean would save time in selecting materials for use in breeding programmes. Therefore, use of morphological and molecular marker technology in identifying common bean landraces resistant to Pythium root rot will greatly help in early identification of resistant germplasm and also accelerate the time of developing resistant varieties. In addition, molecular characterization followed by cluster analysis for the national germplasm will lead to selection of representative landraces for conservation. This will promote conservation of *Pythium* root rot resistant germplasm and provide genetic material to restore accessions that may be lost or reduced due to *Pythium* root rotdisease.

Good knowledge of genetic variability and population structure is indispensable to effective management and use of genetic resources (Arunga *et al*., 2015). It provides farmers and plant breeders with options to develop through selection and breeding, new and more productive crops that are resistant to virulent pests and diseases as well as being adapted to changing environments (Nyakio *et al*., 2015). Molecular markers based on sequences of DNA can be great tools in accessing the genetic variability of common bean cultivars. Peroxidase gene molecular markers were used in this study because they are gene-targeted and are able to detect polymorphisms even in closely related genotypes. Peroxidases play an important role in plant self-defence (Nemli *et al*., 2014). They are diverse in plants and therefore can be used as molecular markers to determine genetic diversity and offer information regarding plants defence mechanisms. The knowledge of genetic variation and relationships among genotypes will help breeders in developing appropriate strategies to solve problems of low yield in common beans (Khaidizar *et al*., 2012). Therefore, assessment of genetic diversity in the current common bean landraces would facilitate the development of Pythium root rot resistant by providing an index of parental lines to be used in breedingprograms.

The selection of desirable genotypes is usually based on the genetic variation of agronomic or quantitative traits such as yield and its components. It is therefore necessary to study the relationship between genotype variability and yield components for efficient utilization of common bean genetic resources in improvement programs. Heritability is the degree of genetic control associated with certain heritable important traits (Addissu, 2011). It indicates how much of the genetic variability has a genetic origin and gives necessary information for the selection process (Falconer, 1981). The selection of superior genotypes is proportional to the amount of genetic variability present and the extent to which the characters are inherited (Scarano *et al*., 2014). Therefore, adequate information on the magnitude and type of genetic variability and their corresponding heritability is important in the improvement of grain yield potential of crops in breeding programs.

1.4 Objectives

1.4.1 Generalobjective

The main objective of the study was to evaluate status, genetic variability and characterize common bean landraces from south Western (SW) Kenya for *Pythium* root rot resistance using morpho-agronomic traits and peroxidase gene-based (POX) molecular markers.

1.4.2 Specific objectives

The specific objectives of this study were:

i) To characterize common bean landraces from south Western Kenya for *Pythium* root rot resistance using peroxidase gene-based (POX) markers
ii) To evaluate genetic variability, heritability and genetic advance and correlation for agronomic and yield components among common bean landraces from South WesternKenya
iii) To characterize local common landraces from south Western Kenya based on morpho-agronomic characters for for *Pythium* root rot resistance
iv) To determine the extent of genetic erosion of common bean landraces in south Western Kenya

1.5 Nullhypothesis

(i) There is no genetic variability for Pythium root rot resistance in common bean landraces grown in south Western Kenya

(ii) There is no heritability, genetic advance and correlation for agronomic and components associated with yield among common bean landraces from south Western Kenya.

(iii) There is no variability in morpho-agronomic characters among common bean landraces from south western Kenya

(iv) There is no genetic erosion of common bean landraces in south Western Kenya

CHAPTER TWO

2.0 LITERATUREREVIEW

2.1 Origin and botany of common beans

Available information show that dry beans, along with maize, squash, and amaranth, probably began as weeds in fields planted with cassava and sweet potato in Latin America (Zeven, 1997; CIAT, 2015). Common bean was domesticated more than 7 000 - 8000 years ago in two centers of origin, Mesoamerica (Mexico and Central America) and the Andean region (Negash, 2006; De La Fuente *et al*., 2010; CIAT, 2015). The crop was introduced to Africa by Portuguese traders in the 16th century where it was met with great success in the Great Lakes region. Africa is now regarded as a secondary center of diversity for the crop (Álvarez de Morales, 2002; Bitocchi *et al*., 2012; Spataro and Negri, 2013). Common bean accessions are divided into two major gene pools, namely; the Mesomerican and Andean gene pools (Asfaw *et al*., 2009; Okii *et al*., 2014). Andean gene pool is the predominant common bean accessions in Kenya (Wortmann *et al*., 2006; Mwaipopo *et al*.,2017).

Common bean is a diploid crop with 2n=2×=22 chromosomal number and bears non-endospermic seeds that differ in both size and color. The cultivated forms of common beans are mainly herbaceous annuals with either indeterminate or determinate growth habits. Species with determinate growth pattern are preferred widely due to their short developmental cycle and their ability to adapt to different environmental conditions. Common beans have epigeal germination period of 5-7 days (OECD, 2016). On germination, common beans initially have a tap-root system with lateral roots running down to 15 cm below the soil; the roots are invaded by rhizobium bacteria resulting into the formation of root nodules (Graham and Ranalli, 1997; OECD, 2016).

Flowering in common beans takes 28 among the non-climbing common bean acessions and 42 days or longer among the climbing accessions. Flowers borne are zygomorphic with bi-petal keel, ten stamens and multi-ovuled ovary that is largely self-pollinating and few but observed instances of cross-pollination. Most flowers produced are shed-off; however low temperature or water stress leads to the abortion of young fruits and /ordeveloping seeds (OECD, 2016). Common bean also have a largely varied maturation period, which can be as short as 60 to 65 days after planting among the first growing

varieties used in areas with short growing cycles or as long as 200 days among the climbing varieties in cool upland areas (Graham and Ranalli, 1997; Katungi *et al*., 2009).

2.2 Production and economic importance of common beans

Latin America and Asia are the regions of greatest production, followed by Africa. Common bean is grown in every continent except Antarctica, with Brazil and India being the largest producers, while China produces by far, the largest quantity of green bean. The world production of common bean has been estimated at approximately 23 million tons and it is grown in nearly 150 countries on an estimated 27.7 million hectares (FAOSTAT, 2017). Beans are becoming increasingly commercial with the trends of urbanization and market globalization, with small farmers organizing themselves to tap into opportunities to export in other countries (Beebe *et al*., 2013). As expected, countries with technified agricultural systems present much higher yields than tropical and developing countries. In the USA, average yields in the past decade range from1.64to 1.96 t ha^{-1}, albeit with significant regional differences. Similarly, average yields in Argentina and Colombia are about 1.2 t ha^{-1} due to varietal selection, and in Brazil under intensive management and irrigation, yields average 1.8 t ha^{-1} (Beebe *et al*., 2013).

In Africa, most bean production is found in the eastern and southern highlands, extending from Ethiopia to South Africa, with Kenya, Uganda, Burundi and Tanzania being the largest producers (FAOSTAT, 2017). In West Africa, bean production is localized in specific environments, with Cameroon being the principal producer. Beans are a minor crop in Europe and North Africa, concentrated around the Mediterranean, in Spain, Italy, Morocco, Algeria, and the Balkan states. In Asia, common bean is spread in an extensive band from Turkey through Iran and the Himalayan foothills, and East through Myanmar and China. India is cited as a major producer of common bean, but these figures undoubtedly include other legumes (Beebe *et al*., 2013).

Common bean is the most important and multifaceted legume grown and consumed worldwide due to its nutrition and economic value. It contributes about 65% of the total protein consumption and 32% of the total energy. Common beans are one of the principal staple food in the Eastern and Southern parts of Africa, where they serve as an essential source of dietary protein and calories (Katungi *et al*., 2009). They are grown for their green leaves which are consumed as vegetables, the immature and dry seeds which are consumed as canned or boiled, for the green immature pods used as vegetables and for the bean residues used as fodder for animals. The seeds form the most significant economic part of the bean plant, particularly in the developing world due to its ease of storage and preparation, long storage life, as well as good nutrition properties (Katungi *et al*., 2009).

In Kenya, common bean is the third staple food after maize and wheat, with an annual per capita consumption of 14 kg per person (Katungi *et al*., 2010). Common bean also serve as an affordable alternative protein, rich in essential amino acids such as lysine and tryptophan (Katungi *et al*., 2010). Moreover, the crop is an excellent supplement to the country‘s carbohydrate-rich diet. Due to their short production cycle (Kimani *et al.*, 2014), common beans provide alternative food as other crops mature (Wortmann, 1998; Jones, 1999). Besides, the crop also generates foreign exchange to the country through export and income to small scale farmers who sell the crop to urban residents (Katungi *et al*., 2009; Balete and Bastas, 2017).

Common beans offer significant health benefits due to their low cholesterol, triglycerides and fat content. They are also digested slowly and elicit a sustained increase in the blood sugar. Moreover, common beans are rich in phytochemicals, antioxidants and flavonoids. These factors contribute significantly towards reducing the risk of common diseases such as cancer, diabetes as well as coronary heart disease (Leterme and Munoz, 2002; Katungi *et al*., 2009; Messina, 2014). On the other hand, common bean also combats constipation thus preventing risks of colon cancer (Romera-Arenas *et al*., 2013). Due to their innate ability to fix atmospheric nitrogen, common beans aid in enriching the soil with nitrogen and therefore reducing dependence on the commercial nitrogen fertilizer, which isexpensive for the smallholder farmers. Furthermore, common beans also serve as an

excellent cover crop thus preventing soil erosion (Wortmann *et al.,* 1998).

2.3 Constraints to common beanproduction

Even though common bean is adaptable to different cropping systems and has a short growing cycle, it is susceptible to many abiotic (drought, heat, poor soil fertility) and biotic (insect pests, and diseases including fungal, bacterial and viral) constraints (Rao, 2001; Miklas *et al*., 2006; Beaver and Osorno, 2009; CIAT, 2015).

2.3.1 Abiotic constraints

Abiotic (climate and edhaphic factors) stress factors are major constraints for bean production in many tropical and sub-tropical countres (Rao, 2014; De Ron et al., 2016). Drought, low soil fertility and temperatures are among the abiotic stresses that affect common bean production. Complete crop failures due to drought are very common in arid and semi-arid conditions (Maras *et al*., 2006; Sardana et al., 2007; De Ron et al., 2017). Drought affects more than 60% of bean production regions in Mexico, Central America, parts of the Caribbean, Ethiopia, northern Uganda, eastern Kenya, Tanzania, and southern Africa (Beebe *et al*., 2011; Assefa *et al*., 2013; Ambachew *et al*., 2015; Darkwa *et al*., 2016). Some regions are expected to become progressively drier under climate change, especially Mexico, Central America, and parts of northeast Brazil and eastern and southern Africa (Yadav *et al*., 2011; Vaz Patto and Araújo 2016). Sources of drought tolerance have been reported in common bean varieties in Africa (Asfaw *et al*., 2012; Asfaw and Blair, 2014; Mukeshimana *et al*., 2014). However, most of these varieties are susceptible to major common bean infectingdiseases.

Deficiencies in soil nitrogen, phosphorous (P) and zinc (Zn) causes huge yield loss in common bean production in tropical countries of the world (Newton *et al*., 2010; Ramaekers *et al*., 2010; Beebe *et al*., 2012; Farid *et al*., 2017). Approximately 50% of bean growing regions of the world are affected by low soil P (Nielsen, 2001; Beebe *et al*., 2012). Low P soils are a major constraint to bean production in regions of Africa and Latin America where farmers lack access to sufficient P fertilizer (Wortmann *et al*., 1998;Beebe *et al*., 2012). Phosphorous is essential for germination and early root development and deficiency can lead to retarded growth. Aluminium and manganese

toxins cause delayed maturity, chlorosis and poor yield respectively. Toxicities of aluminium (Al) and manganese associated with soil acidity are particularly disastrous to common bean growth and production, especially in acid soil regions of Africa and Latin America (Rao, 2014; Rao *et al*., 2016).

Common bean is sensitive to low temperatures which affects growth during early stages (Rodino *et al*., 2007). Low temperatures below 15 °C, as well as frost at the beginning and the end of the growing season in the highlands (above 2 000 m elevation) can also reduce yield (Singh, 2001; Coleto *et al*.,2014).

2.3.2 Biotic constraints

Production of common bean is also constrained by various biotic stresses (insect pests and diseases), many of which have co-evolved with the crop (Beebe, 2012; Beebe *et al*., 2013; Girma *et al*., 2017). Among the biotic stresses, many species of insect pests attack beans both before and after harvest. Insect pests especially pod borers and weevils may cause yield loss of up to 100%. The other major insect pests include the bean fly (*Ophiomyia phaseoli*, *O. spencerella*, *O. centrosematis;* Diptera: Agromyzidae), foliage beetles (*Ootheca* sp; Coleoptera: Chrysyomelidae), black aphid (*Aphis fabae*; Homoptera: Aphididae), stripped beetle (*Alcidodes leucogrammus*; Coleoptera: Curculionidae) and flower thrips (*Megalurothrips sjostedti*; Thysanoptera: Thripidae). Other insect pests attacking beans in Kenya include common whitefly (*Bemisa tabaci*; Homoptera: Aleyrodidae), leaf hoppers (*Empoasca* spp.; Homoptera: Cicadelidae), cutworms (*Agrotis* sp and *Spodoptera* sp; Lepidoptera: Noctuidae), blister beetles (*Mylabris* spp. and *Coryna* spp.; Coleoptera: meloidae), pod borer (*Maruca testularis*; lepidoptera: Pyralidae), American bollworm (*Helicoverpa armigera*; Lepidoptera: Noctuidae) and pod-sucking bugs (*Clavigralla* sp., *Anoplocenemis curvipei*, *Nezara viridula*, *Piptortus dentipes*) (Ceccarelli *et al*., 2001; Lévesque and de Cock,2004).

Diseases are major constraints to bean production and may be fungal, bacterial or viral in nature. In Kenya diseases attacking beans include common bacterial blight (*Xanthomonas campestris* pv. *phaseoli* Smith), angular leaf spot [*Phaseoriopsis griseolsa* (Sacc) Ferr.], rust (*Uromyces appendiculatus* Pers), bean common mosaic virus (BCMV), and floury

leaf spot [*Mycovellosiella phaseoli* (Drummond) Deighton], which are more important in the low altitude high temperature areas. Halo blight (*Pseudomonas syringae* pv. *Phaseolica* Burkholder),anthracnose (*Colletotrichum lindemuthianum* Sacc & Magn), aschochyta blight [*Phoma exigua* var. *diversipora* (Bub.) Boerma], and root rots *(Rhizoctonia solani*, *Pythium* spp. and *Fusarium* spp.) are considered more important especially in western region of Kenya (Román-Avilés *et al*., 2004; Kumar*et al*., 2008). In Kenya, especially in the south Western highland regions, *Pythium* root rot is one of the most serious constraints to bean production with significant losses occurring to susceptible varieties. *Pythium* root rot disease can cause yield losses of up to 100% in susceptible varieties (especially Canadian wonder, Rose cocoa, Wairimu and Mwesi moja genotypes). Farmers have abandoned these susceptible but high yielding varieties. This has led to genetic erosion of once elitelandraces.

2.3.2.1 *Pythium* root rotdisease

Root and stem rot diseases caused by the fungus *Pythium* spp. has now become the most important disease in almost all common bean cultivars grown in Eastern, Central and parts of Southern Africa where common bean is under subsistence and large scale production systems (Al-Mahmooli *et al*., 2015; Vasseur, 2005). In susceptible cultivars the fungus infects not only the root and lower stem but also the leaves (Otsyula *et al*., 2003; Brožová, 2002; Schwember *et al*., 2017). Common bean root rot symptoms from the *Pythium* fungus tend to cause seed rot before germination or during germination or damping-off in seedlings but in most cases in growing plants, root rot, foliar blight or pod rot in mature crop (Harvey, 2004; Paul *et al*., 2005 ; Rodiño *et al*., 2006) depending on soil, weather and stage ofgrowth.

Pythium root rot disease constitutes a highly damaging constraint to common bean production in several areas of Eastern and Central Africa. Yield losses of up to 100% in almost all bean cultivars grown have been reported in Kenya and Rwanda (Otsyula *et al*., 2003; Voland *et al*., 2014). In Rwanda, Western Kenya and South Western Uganda, *Pythium* spp. are the fungal pathogens most frequently associated with severe root rot epidemics (Román-Avilés *et al*., 2005; Nzungize *et al*., 2011). In other studies carried out in these countries the following species have been isolated from bean samples affected by

root rot symptoms. In these countries the species isolated from bean samples affected by root rot symptoms included: *Pythium nodosum*, *Pythium echinulatum*, *Pythium pachycaule*, *Pythium oligandrum*, *Pythium acanthicum*, *Pythium chamaehyphon*, *Pythium folliculosum*, *Pythium indigoferae*, *Pythium irregulare*, *Pythium lutarium*, *Pythium macrosporum*, *Pythium myriotylum*, *Pythium paroecandrum*, *Pythium torulosum*, *Pythium vexans*, *Pythium zingiberis*, *Pythium graminicola*, *Pythium spinosum*, *Pythium ultimum*, *Pythium arrhenomanes*, *Pythium catenulatum*, *Pythium diclinum*, *Pythium dissotocum*, *Pythium rostratum*, *Pythium salpingophorum* and *Pythium deliense* (Kageyama, 2005; Buruchara *et al*., 2007; Nzungize *et al*.,2011).

In small-scale farms the most effective method of controlling the disease so far has been the use of tolerant/resistant varieties (Tusiime, 2003; Gichuru, 2008). However, no common bean genotypes resistant to *Pythium* root rot have been identified and released to farmers in Kenya, Uganda and Rwanda (Postman, 2009; Haritha, 2010). Knowledge about sources of disease resistance combined with environmental adaptability is necessary to develop resistant cultivars adopted to bean growing regions of East, Central and Southern Africa (CIAT, 2015).

2.3.2.2 Management of *Pythium* root rotdisease

There are several methods that have been reported for the management of *Pythium* root rot disease in common beans worldwide. One of the commonly used methods is the application of chemical fungicides. Once introduced into the soil, *Pythium* spp. may persist for many years through resistance structures such as oospores, zoospores and sporangia (Schroeder *et al*., 2006; Kirk *et al*., 2008). In these conditions, applying chemical treatments to kill the pathogen may be an efficient method. There are many specific pesticides such as benomyl, captafol, captan, carboxin, metalaxyl, propamocarb hydrochloride and etridiazole, which have already proven to be efficient in controlling *Pythium* root rot diseases on common beans. However, some pesticides, such as benomyl, are only active on growing mycelium, but not during the resting stage of the mycelium.

Soil fumigants such as methyl bromide, chloropicrin and vorlex have been reported to be highly effective biocides that kill *Pythium* agents (Dušková, 1995; Abawi *et al*., 2006). In Latin America and Africa, one of the safest and most economical uses of chemicals to control *Pythium* pathogens consists of coating the seeds of crops. This usually results in effective protection of seeds and young seedlings for about 2 to 3 weeks after sowing (Abawi *et al*., 2006; Schwartz *et al*., 2007; Nekesa et al., 1998). However, given the conditions prevailing in diverse developing countries such as those in Eastern and Central Africa, small-scale farmers with minimal resources cannot afford to use chemical control methods. Moreover, the use of chemical treatments could constitute a source of soil and water contamination, while at the same time exposing farmers to health risks related to handling chemical pesticides.

Microorganisms can protect plants from fungal attacks through the production of antifungal metabolites, competition with the pathogen for nutrients, niche exclusion, parasitism, lysis of the pathogen, and through induction of plant resistance mechanisms (Whipps, 2001; Belbahri *et al*., 2008). Beneficial microorganisms of interest for biological control of plant pathogenic *Pythium* spp. have been identified among fungi and bacteria. Isolates of *Trichoderma* spp. and *Gliocladium* spp. are antagonists of *Pythium*-induced soil-borne diseases and are commercially available for the biological control of *Pythium* root rots (Serraj *et al*., 2004; Fravel, 2005; Leitão *et al*.,2013).

Competition for organic carbon and iron is one of the mechanisms through which some biocontrol agents suppress *Pythium* spp. (Hoitink et al., 1999). Sensitivity of *Pythium* spp. to competition and antagonism during its saprophytic phase of growth is one of the key factors in managing *Pythium* diseases through biological control (Martin et al., 1999). In contrast to this view, it is commonly known that *Pythium* spp. propagules germinate rapidly in response to seed or root exudates and quickly infect seeds or roots, and this complicates the application of biological control (Whipps et al., 1991). It is, therefore, of great importance that the activity of the biological control agent coincides with the period of host susceptibility and it should persist as long as the plant remains susceptible. Insufficient survival of the antagonists may lead to inadequate or partial control of the

pathogen. From a field experiment conducted in Western Kenya, it was concluded that one approach to addressing this limitation is the introduction of a food base, such as compost, which supports the activity of antagonists but does not stimulate the activity of the pathogen (Otsyula et al., 1998; Hoitink et al., 1999). However, the compost must be free of *Pythium* root rot pathogens in order to increase the chance of effectively controlling *Pythium* root rot diseases (Martin et al., 1985).

The use of resistant common bean cultivars is the most efficient management strategy against root rot diseases (Buruchara *et al*., 2007; CIAT, 2015; Vakali *et al*., 2017). This approach is especially appropriate for small farmers with low inputs. However, the strategy requires the development of adapted common bean cultivars with resistance to all the major root rot pathogens that prevail in a given bean growing region (Hangen and Bennink 2003; Abawi *et al*., 2006). Studies by Otsyula *et al*. (2003) and Buruchara *et al*. (2001) identified common bean varieties resistant to *P. ultimum* root rot under screenhouse conditions. Otsyula *et al*. (2003) made crosses between susceptible varieties (GLP 2, GLP 585, CAL 96 and Urugezi) used as female parents and resistant varieties (RWR 719, MLB 49-89A, SCAM 80-CM/15, AND 1055 and AND 1062). TheF1 hybrids were then backcrossed with the recurrent susceptible parents. Results showed that resistance to *P. ultimum* was expressed in all the F1 plants using the resistant genotypes as male parents. This shows that resistance to *P. ultimum* is inherited as a dominant characteristic by common bean (Otsyula *et al*., 2003; Mahuku *et al*., 2007). In order to determine the number of genes necessary for *Pythium*root rot resistance, the segregation of F2 and backcross plants was then analyzed. From the results, it was assumed that resistance to *P. ultimum* is, whatever the genepool origin and the parental genotypes used in the combinations (Buruchara *et al*., 2001; Otsyula *et al*., 2003). Therefore, to speed up the selection process in a breeding program, molecular assisted selection should beapplied.

2.4 Characterization of common bean

Markers for characterization of plant genetic resources are grouped into three main classes: (i) morphological and productive markers which are based on visually evaluated

traits, (ii) biochemical markers which are based on gene product, and (iii) molecular markers which are founded on DNA analysis (Galal *et al*., 2013). Classical methods for characterization of plant germplasm involve the use of morphological and agronomic traits (Homar *et al*., 2011; De Ron *et al*., 2013; Olajide and Ilori, 2018). However, the use of morpho-agronomic traits are influenced by the environment, development stage and do not correctly reflect genetic relatedness between different accessions. To overcome these problems, molecular markers represent a potential tool for effective characterization of genetic diversity and to aid in the management of plant resources (Grisi *et al*., 2007; Laurentin, 2009; Blair *et al*., 2006, 2011). These DNA molecular markers, when closely linked to genes of interest can also be used to select for desirable allele/s in marker-assisted breeding programs (Okii *et al*., 2014; Suso *et al*., 2016; Ismail *et al*., 2017).

2.4.1 Morpho-agronomic characterization of common bean

Equipment and materials for carrying out morphological and/or agronomic characterization experiments are normally cheaper and easily available; one only requires suitable fields of land to perform the experiments. However, it is more labour demanding in addition to being subject to phenotypic plasticity, which can make the technique inaccurate as compared to molecular methods. Although morphological characterization of traits is at the mercy of environmental conditions, on the other hand, it helps in the evaluation of diversity in different environments. Characterization of crop varieties is usually based on the morphology, phenology and agricultural traits that farmers and plant breeders desire (Rodiño *et al*., 2007; 2011). Normally crop varieties with characteristics required for improvement are assessed by their morphological and agronomic qualities (Schneider *et al*., 2001; Opio *et al*., 2001; De la Fuente *et al*., 2012). Morphological diversity of common bean is usually determined by the seed characteristics – texture, shape, size, width, length, colour, appearance, and others. Different bean accessions can also be classified based on their nature of parentage and/or pedigree (Spence, 2003; Mamidi *et al*., 2013; De Ron *et al*., 2014; CIAT, 2015).

Studies have been conducted around the globe to characterize common bean using morpho-agronomic traits. Akram *et al*. (2003) characterized seventy two recombinant

inbred lines and twenty cultivars of common bean (*Phaseolus vulgaris* L.) based on seed yield and 21 morpho-agronomic characters, and demonstrated that seed yield is highly correlated with harvest index, seed number per plant, seed number per pod, pod number per plant, seed length, 50% floweringand poding, pod length and100 seed weight. Carlos *et al*. (2006) evaluated thirteen exotic and local genotypes of common bean (*Phaseolus vulgaris* L.) for various agronomic and morphological characters under rain fed conditions of Islamabad, Pakistan and significant differences among genotypes for grain yield, plant height, 100-seeds weight, number of seeds per pod and number of pods per plant were observed. The correlation coefficients illustrated a positive and significant association of grain yield with flowering duration, number of pods per plant and number of seeds per plant. Therefore, these traits should be considered for genetic improvement through selection.

A study was sought to understand the current state of morphological diversity of 284 common bean accessions in Uganda (Abawi *et al*., 2006). The level of morphological variation estimated with the Shannon Weaver diversity index (H), ranged from 0.47 to 0.58, an indicator of moderate morphological diversity. Asfaw *et al*. (2009) reported the diversity in 82 common bean accessions based on morphological and agronomical characteristics and was able to detect duplicates in the collection.

2.4.2 Genetic diversity and molecular characterization

Molecular markers permit significant estimation of genetic diversity directly at the DNA level, reducing the interference of environmental variations. Genetic variability is a basic pre-requisite for crop improvement as it provides valuable information regarding selection of diverse parents to be used in a hybridization programme. The allelic richness of any crop species or population tends to determine its state for the preservation of genes, either naturally or artificially (Nemli *et al*., 2014). The genetic variability assessment in all organisms requires the examination of DNA using molecular markers (Radosavljević *et al*., 2011; Rodriguez *et al*., 2013). Molecular markers were first used when biochemical markers (storage proteins, isozymes) were discovered in the 1960s

(Matsuoka *et al*., 2002; Kalyebara and Kassozi, 2005). With the discovery of DNA structure in 1950s and with the increase in knowledge on the genetic properties of DNA, many novel molecular methods able to detect polymorphism at DNA level have been discovered (Shadeya *et al*., 2000; Tosti and Negri, 2005). There are many molecular markers used in plant genetic diversity studies including: Random Amplified DNA Polymorphisms (RAPDs), Single Nucleotide Polymorphisms (SNPs), Restriction Fragment Length Polymorphisms (RFLPs), Inter Simple Sequence Repeats (ISSRs), Amplified Fragment Length Polymorphisms (AFLPs) and Simple Sequence Repeats or microsatellites (SSRs). However, molecular marker techniques differ in their efficiency, method of application, and their level of polymorphism (Gaitan-Solis *et al*., 2002; Burger et al., 2008; Schmutz*et al*., 2014; Carvalho *et al*., 2017a).

Genetic diversity in common bean have been studied using different molecular markers such as allozymes (Singh *et al*., 1991; Santalla *et al*., 2002), Amplified Fragment Length Polymorphism, AFLP (Lioi *et al*., 2005; Svetleva *et al*., 2006), Random Amplified Polymorphism, RAPD (Ocampo *et al*., 2005, Martins *et al*., 2006; Marotti *et al*., 2007), Restriction Fragment Length Polymorphism, RFLP (Nodari *et al*., 1992), Inter Simple Sequence Repeats, ISSR (Svetleva *et al*., 2006; Marotti *et al*., 2007), Simple, Sequence Repeats, SSR (Asfaw *et al*., 2009; Okii *et al*., 2014) and gene-based markers such as peroxidase gene (Nemli *et al*, 2014). The utility of SSR markers is highly desirable due to their abundant distribution and high polymorphism in the whole genome, their power to distinguish between closely related genotypes (Khaidizar *et al*., 2012), and because they are easily reproducible, multi-allelic and codominant genetic marker system (Saghai Maroof *et al*., 1994). Considering higher level of sequence diversity in peroxidase gene sequences among plant genotypes (Zhang *et al*., 2001), peroxidase markers can also be used to efficiently assess the genetic relationship among the common bean landraces.

2.4.2.1 Use of peroxidase gene-basedmarkers

Peroxidases are among the highly conserved enzymes in animals, plants and micro-organisms which are conserved (Zhang *et al*., 2001). They are proteins that contain heme

and are capable of oxidizing compounds in the presence of oxygen (O_2) or peroxide (H_2O_2) and consist of three highly conserved motifs namely distal, central and proximal domains (Hiraga *et al*., 2001). Based on their catalytic and structural properties, plant peroxidases are grouped into two major categories. The intracellular peroxidases are related to bacterial peroxidases (class I) (Gulsen *et al*., 2010), the second group of these enzymes (class III) aim the secretary pathway (Welinder, 1992; Felsenstein. 2015). Class III peroxidases are plant-specific heme oxidoreductases made up of c. 300 amino acid residues. More advanced plant species have more peroxidase isoenzymes, encoded by multigene families (Yoshida *et al*., 2003); *Oryza sativa* has 138 (Passardi *et al*., 2004), 73 are found in *Arabidopsis thaliana* (Welinder *et al*., 2002; Felsenstein, 2004) and *Populous trichocarpa* has 93 (Ren *et al*., 2014).

Plant peroxidases serve vital roles in various interactions related to stress tolerance. They also catalyze a wide range of physiological processes such as plant defense, insect tolerance, auxin catabolism, salt tolerance, lignin biosynthesis, cell wall proteins manufacture through deposition of callose, tissue suberization and plant senescence (Gulsen *et al*., 2010; Passardi *et al*., 2005; Tan *et al*., 2016). They act by aiding the deposition of macromolecules on the surface of the cell to strengthen plant tissues, thereby restricting expansion of the cell and invasion of pathogens (Almagro, 2008; Hiraga, 2001; Santalla *et al*., 2002). Peroxidases are also play a role in scavenging reactive oxygen species (ROS), which are partially reduced forms of atmospheric oxygen. They need to be reduced because they are capable of causing oxidative damage to the plant because they are highly reactive (Vicuna, 2005; El-Kholy *et al*., 1997).

Plant peroxidases possess highly conserved domains across different plant species (Collard and Mackill, 2009), thus conserved DNA sequences within the genes can reveal how they function. DNA regions that are conserved and share the same priming site may be spread across the genomes of various germplasms in different ways; therefore it is possible to detect polymorphisms within species (Poczai *et al*., 2013; Svetleva *et al*., 2006). Therefore, degenerate oligonucleotide primers can be employed in amplifying DNA sequences that code for peroxidases from plants using these conserved domains

(Collard and Mackill, 2009). Peroxidase-specific markers have previously been used in detecting polymorphisms of peroxidase genes among accessions of different plant species, including watermelon (*Citrullus lanatus* (Thunb.), apple (*Malus domestica* Borkh.), wheat (*Triticum* spp.), citrus, and beans (*Phaseolus vulgaris* L.); therefore, these markers can be utilized in studying evolutionary relationships and genetic diversity on an inter- and intra-specific level (Gulsen *et al*., 2010; Ceylan 2010; Ocal, 2014; Uzun *et al*., 2014; Nemli *et al*., 2014;).

2.5 Genetic improvement of commonbeans

2.5.1 Conventional breeding of commonbean

Crop breeding methods including mass pedigree and recurrent backcross methods and their modifications have been used for common bean improvement (Singh *et al*., 1991; Kelly *et al*., 1994, 2002; Caproni *et al*., 2018). Congruity backcrossing and single seed descent (SSD) in addition to recurrent and gamete selection methods have also been used. Singh (1991) found that the F2-derived family method of selection was superior to the SSD and bulk methods commonly used for advancing early generation of hybrid seed yield in the early generation of hybrid populations. Singh *et al*. (1991) suggested selection for seed yield in early generations of interracial and intergenepool populations with desirable recombinants. From early generation yield tests (F2-F4), Kelly *et al*. (1994), identified high-yielding and low yielding populations that eventually produced high-yielding and low-yielding advanced generation (F7)varieties.

2.5.2 The role of landraces in common beanbreeding

Fufa *et al*. (2010) described a landraces as variable plant populationsadapted to local agro-climatic conditions which are named, selected and maintained by the traditional farmers to meet their social, economic, cultural and ecological needs‖. De Ron (2016) described landraces as having high stability of their characteristicsand great resistancecapacitytotolerateadverseinfluences‖.Alandracehasalsobeendefinedasa variety with a high potential to tolerate biotic and abiotic stress, resulting in high yield stability and an intermediate yield under low input agricultural systems. A landrace differs from a cultivar since yield stability is the major characteristic of a landrace and a cultivar

is characterized by a high yielding capacity under optimal conditions (Revilla *et al*., 2005; Jose *et al*., 2009; De Ron, 2015). Since the introduction of common bean to the eastern African coast by the Portuguese, farmers have used the crop to develop farming practices that are adapted to local conditions. Hence they have exploited useful alleles in the crop, which have resulted in a wide range of morphologically diverse landraces (Morris *et al*., 2004; Baudoin *et al*., 2001). The genetic diversity helps to broaden the genetic base of new cultivars and hence maximises the available germplasm resources Genetic diversity has been shown to be present in common bean landraces in Italy (Piergiovanni and Lioi, 2010; De Ron et al., 2003), Bulgaria and Portugal (Stoilova *et al*., 2005; Excoffier *et al*., 2005), in Spain, Mexico and Central America, using random amplified polymorphic DNA (RAPD) (Beebe *et al*., 2000), in Nilgiris, India using RAPD analysis (Jose *et al*., 2009) and in Ethiopia and Kenya using micro-satellite marker analysis (Asfaw *et al*., 2009; Bowcock *et al*., 2014). Blair *et al*. (2012) evaluated wild accessions and landraces of common bean using simple sequence repeat markers (SSR) that showed their genetic diversity. In Bulgaria and Portugal, landraces are still important genetic resources that are in use by the small-scale farmers, and have been used in common bean improvement programmes (Santalla *et al*., 2001; Stoilova *et al*., 2005; Vaz Patto *et al*., 2015). In Tanzania, common bean landraces were improved for resistance to angular leaf spot and anthracnose (Santalla *et al*., 2004; Mongi *et al*., 2009). Different regions have specific temperatures, humidity and other production requirements, and hence each landrace may not be grown successfully in regions where they are not traditionally cultivated (Santalla *et al*., 2005; Piergiovanni and Lioi, 2010).

2.5.3 Farmers' participation in conventional breeding of common beans

Plant breeding should be carried out with the participation of farmers to ensure that released varieties meet their demands and are easily adopted. Participatory plant breeding techniques are being used to develop, multiply and distribute seed of improved common bean varieties (Almekinders *et al*., 2007; Roy *et al*., 2016). This approach to plant breeding allows the participation of farmers in the development, evaluation and selection of bean breeding lines (Morris and Bellon, 2004). Morris and Bellon (2004) noted that participatory plant breeding is well suited for the development of a variety that possesses

a unique combination of traits, such as a specific bean type for a niche market. Conventional and centralized plant breeding programmes have been shown to have significant impact in high input areas, but low impact in the marginal and small scale farming sector (Morris and Bellon, 2004; Newton *et al*., 2010). Ceccarelli and Grando (2001) showed that decentralized and demand driven research was essential, especially for the poor farmers in low input farming systems. They reported that this would help farmers choose the varieties that do well in their environmental conditions and hence adopt newly released varieties.

Positive results have been reported with important contributions by farmers, when the farmers are involved during selection in the breeding process. For example, Fufa *et al*., (2010) reported that lines selected by farmers yielded higher than those selected by breeders. Farmers were shown to visually select higher yielding barley lines than the breeders (Ceccarelli *et al*., 2001). Hence involving farmers during selection leads to improvement of the breeding process. In another study, Fufa *et al*. (2010) tested the efficiency of selection by farmers in a barley breeding program. They compared farmers‘ and breeders‘ selection of varieties for different regions and realised that farmers chose varieties that were better adapted to their specific regions, while breeders selected for broad environments. They emphasised the importance of decentralized participatory plant breeding in increasing and stabilizing productivity and maintaining genetic diversity. Courtois *et al*. (2001) carried out a farmer participation study on rain fed rice in eastern India and showed that varietal evaluation (by ranking) on farmers‘ fields was better than when they were evaluated by breeders on the breeding stations. They concluded that combining efforts by farmers and breeders leads to varieties more suitable to the farmers.

Consultation with farmers before or during the breeding process has led to better adoption of newly released varieties. Surveys, interviews, and participatory rural appraisal have been used to determine farmers‘ preferred traits in crops. The information has successfully been used in the breeding of common bean for resistance to bean fly (Ojwang' *et al*., 2009) and resistance to fusarium root rot (Beebe and Pastor-Corrales, 1991; Mukankusi, 2008). Asfaw *et al*. (2012) compared the use of focus group

discussions, interviews and participatory variety selection (PVS) to assess information from farmers, on their preferences for drought tolerant common bean varieties in southern Ethiopia. They found that active selection of drought tolerant genotypes on farmers‘ fields was fast, efficient and accurate. Women play a key role in most farming systems in Africa, since they are involved in production and also in the utilization (cooking). Women smallholder farmers in eastern Ethiopia were able to make significant contributions in identification of superior common bean cultivars when they evaluated them on-farm (Assefa *et al*., 2005; Karapanos *et al*., 2017). Hence farmers can be involved during the breeding process at the beginning, where farmer preferences are evaluated, and also at the end when varieties are tested on the farmers‘fields.

2.6 Genetic erosion in commonbean

The loss of variation has been reported from species populations in regions having diversity or places of origin, characterized by primary and secondary diversification (Negri *et al*., 2001; Luquet *et al*., 2012). In cultivated plants genetic erosion results in the disappearance of specific locally adapted cultivars or landraces. Genetic erosion can be evaluated by comparing the number of landraces being grown at present to their original number in a region. In a narrow sense genetic erosion is the loss of variation as a result of increased homozygosity in a plant species, but in a wider perspective it is the loss of varieties or cultivars (FAOSTAT, 2014; CSA, 2014). (The process is active on wild and cultivated species of plant populations. It can occur naturally and/or artificially. It takes place when there is inbreeding between homozygous individuals in a population that will show lethal recessive gene combinations (FAO, 2008; Negri *et al*., 2013; Tampakaki *et al*., 2017). Genetic erosion causes a population bottleneck as it reduces gene pools or

lowers the genetic diversity of original members of a species. If it occurs naturally, it could lead to loss of heterozygosity and variation that lowers fitness of species populations in its environment (Beebe *et al*., 2000; Negri *et al*., 2001; Blair, 2003).

In cultivated plants, genetic erosion results in the disappearance of diversity in a gene pool leading to increase of homozygosity and consequent uniformity of alleles and genotypes with related gene combinations and phenotypes (Negri *et al*., 2009; Lioi *et al*.,

2012; CSA, 2016). Effective conservation measures can only be achieved by determining causes of genetic erosion. Equally important is the identification of local crop/traditional varieties and closely related wild species that have disappeared or are endangered/threatened, species that are vital in the monitoring of species survival for effective conservation strategies (Moreira *et al*., 2009; Lazaridi *et al*., 2016). Studies on the management and status of cultivated plant species is essential for better utilization of different domesticated plants by researchers, breeders as well as seed suppliers for further quality enhancement and future conservation but more importantly, work on efficient management of crops and their wild relatives, genetic resource studies in marginal areas assists the development of sustainable on-farm conservation strategies.

Assessing changes in genetic diversity within crops can be based on different measures of diversity. There are three types of diversity in crops: a) varietal diversity is easy to measure but does not accurately portray the genetic diversity, especially due to inconsistencies in local variety names; (b) agro-morphological diversity provides an indication of the diversity that could be tapped for agricultural uses, but this is hard to measure in large samples collected under different agro-ecological conditions; and (c) molecular markers can generate repeatable data that is free from environmental interference on genetic diversity, but markers currently available for most species are neutral and do not reflect diversity associated with adaptation (Barry *et al*., 2008; Van de Wuow *et al*., 2010; Polegri and Negri, 2010). Currently, DNA-marker techniques have provided tools to directly measure genetic diversity and hence test for genetic erosion at the allelic level (Barry *et al*., 2008; Karanikolas *et al*., 2017).

Genetic erosion evaluated over time using microsatellite markers at the landrace level in lima bean (*Phaseolus lunatus*) samples collected in 1979 and in 2007 ..showed that the genetic pool from the 1979 lot had a higher genetic diversity than the one for 2007 pool (Nei‘s diversity, H = 0.18 and 0.05, respectively), (Spataro *et al*., 2011). A cluster analysis showed that the alleles present in 1979 were not the same as those found in 2007, indicating an allelic displacement in the genetic pool of the lima bean landraces in the last 30 years. This displacement could be due to the introduction of improved varieties or modern cultivars, resulting in a displacement of local varieties or to changes in the Mayan

criteria for selection of germplasm or both (Hammer *et al*., 2005; Spataro *et al*., 2011; CIAT, 2015; Lazaridi *et al*., 2017). This study showed that the loss of landraces can generate both quantitative and qualitative changes in the genetic pool of the domesticated species. In SW Kenya old varieties, in this case landrases of common beans (in particular Morogi, Richore, Ekebure, Bunda, Ekenagwa, Masaku, Ritinge and Manoa) for modern genotypes especially Rosecocoa, Mwitemania and Canadian Wonder which are more yielding. Such changes are very important to consider when planning ex situ and in situ programs to conserve crop diversity in their domestication areas. No study has been carried out to determine the status of genetic erosion of common bean landraces from south Western Kenya.

CHAPTER THREE

3.0 Determination of genetic variation and population structure of common bean (*Phaseolus vulgaris* L.) landraces from South Western Kenya for resistance to *Pythium* root rot disease

3.1 Introduction

Common beans (*Phaseolus vulgaris* L.), are the most important grain legume, second to maize as a food crop in Kenya. Africa contributes 17% of the world's total yield, of which 70% is from Eastern Africa. Kenya produces 400 - 1200 kg/ha, mainly from intercropping in small scale farms and an average household production of 430 kg/ha in South Western Kenya (FAOSTAT, 2017). Common beans provide a cheap source of protein and minerals (iron and zinc) to humans. In addition, consumption of bean grains provides humans with various health benefits including reduction of cholesterol level and coronary heart diseases (Mattei *et al*., 2011), favorable effects against cancer (Hangen and Bennink, 2002) and decreases diabetes and obesity (Ahn *et al*., 2013). Furthermore, common beans play a very important role in sustaining soil fertility by fixation of atmospheric nitrogen and organic matter to the soil. Therefore, it is a multipurpose crop that also produces grains as well as fodder for livestock. As a cover crop, it is efficient in suppressing weeds and prevents soil erosion.

Production of common bean in South western (SW) Kenya is constrained by various biotic stresses including insect pests especially pod-borers and weevils which may cause yield loss of up to 80%. There is reduced genetic diversity of the common bean in rhe study area, in particular lack of cultivars with consumer quality attributes such as taste, palatability and fast cooking. However, the greatest limitation to bean production in South western Kenya are fungal diseases particularly root rots caused by *Fusarium*, *Rhizoctonia* and *Pythium* (Otsyula *et al*., 2003). *Pythium* root rot disease can cause yield losses of up to 100% in susceptible varieties. Farmers have abandoned these susceptible cultivars even though they produce high yields. This has led to gradual genetic erosion of once elite landraces. The use of resistant bean varieties is the most effective, economical and environmentally sustainable strategy to control *Pythium* root rot disease (Binagwa *et*

al., 2016). However, this requires identification of resistant genotypes,and incorporation of the disease resistance into agronomically desirable varieties. There is need to characterize common bean germplasm for *Pythium* root rot resistance in order to identify markers for selection of resistant varieties. The markers can be used to hasten breeding for high yielding Pythium root rot tolerant lines.

Genetic differences that exist between common bean accessions can be associated with economically important traits and used for germplasm characterization. Molecular markers are useful tools in estimation of genetic diversity and identification of alleles of interest without interference from changes in environmental parameters. Gene-based molecular markers represent an important resource for characterization of germplasm and elucidation of gene functions. Peroxidase, a key enzyme in metabolic pathways is an example of gene-based molecular marker in plants. Peroxidases (POXs) belong to a multigene family and exhibit high sequence variability with the existence of conserved domains (Oliva *et al*., 2009). Conserved DNA regions of peroxidase share the same priming site and are distributed across the genomes of different genotypes in different patterns hence polymorphisms can be detected within species. POXs are glycoprotein enzymes containing heme cofactor and utilize water in oxidation reactions involving a range of compounds. These enzymes perform diverse roles in plants including detoxification of reactive oxygen species generated during biotic and abiotic stresses (Mittler *et al*., 2004; Gill and Tuteja, 2010; Zhang *et al*., 2013), inducing defense response against pathogens (Passardi *et al*., 2005), formation of lignin and suberin (Herrero *et al*., 2013), metabolism of auxin, healing of wounds and plant–microbial symbiosis (Passardi *et al*., 2005). Peroxidases also catalyzes deamination of trans-cinnamic acid in a biosynthesis pathway leading to the formation of phenolic compounds which have many vital activities in plants such as regulation of plant growth differentiation (Vicuna, 2005), inhibition of pathogens (Almagro *et al*., 2008), and tolerance to abiotic stresses (Gill and Tuteja, 2010; Zhang *et al*.,2014).

Peroxidase-specific markers have previously been used in detecting polymorphisms of peroxidase genes among accessions of different plant species including watermelon

(*Citrullus lanatus* (Thunb.), apple (*Malus domestica* Borkh.), wheat (*Triticum* spp.) and beans (*Phaseolus vulgaris* L.); therefore, these markers can be utilized in studying evolutionary relationships and genetic diversity on an inter- and intra-specific level (Gulsen *et al*., 2010; Ceylan 2010; Ocal, 2014; Uzun *et al*., 2014; Nemli *et al*., 2014). There has been limited available data on characterization of Kenyan common bean landraces so far. Therefore molecular studies such as use of peroxidase gene-based markers are required to characterize and identify the genetic relationships among Kenyan germplasm. The objective of this chapter was to characterize common bean landraces from South Western Kenya using peroxidase gene markers for resistance to *Pythium* root rot disease.

3.2 Materials andMethods

3.2.1 Plantmaterials

Fifty one common bean landraces (Table 3.1) were evaluated in the greenhouse for resistance to *Pythium* root rot disease. These comprised of 25 landraces collected from farmers‘ fields (at harvest and from saved, stored seeds) in different agro-climatic zones of south western Kenya and 26 germplasm obtained from the National Gene Bank of Kenya (GBK), Muguga, Kenya in June and July 2015. Landrace LRC002 was not evaluated for pythium root rot resistance. The germplasm from GBK were collected also from South Western Kenya region in 1983 - 1984 and preserved. Disease evaluation was carried in a screen house at KALRO, Kisii station while the laboratory work was done at the Department of Biochemistry, University of Nairobi.

Table 3.1a: Names, codes, local names and source of common bean landraces collected from field used in this study

Entry	**Landrace code**	**Local name**	**Source of genotype**
S1	LRC 006	Esaitoti	Daraja mbili
S2	LRC 008	Chinchae	Kisii Municipality
S3	LRC 018	Richore	Marani

S17	LRC 001	Ekenagwa	Kisii municipality market
S18	LRC005	Egirini	Kisii Municipality
S19	LRC010	Bunda entambe	Daraja mbili
S20	LRC016	Manoa emwamu	Kisii municipality market
S21	LRC015	Ekoko enyenge	Suneka
S22	LRC026	Nyaibu/Bunda enetu	Daraja mbili
S23	LRC011	Ekebure	Daraja mbili
S24	LRC012	Enyamatobu	Kisii municipality market
S25	LRC021	Morogi	Nyacheki
S26	LRC024	Ekoko entambe	Keumbu
S27	LRC019	Manoa endabu	Kisii municipality market
S28	LRC022	Enyamwamu	Daraja mbili
S33	LRC 023	Eosama	Nyamarambe
S34	LRC 009	Eroyoo	Kenyenya
S35	LRC025	Amaika inse	Kisii unicipality market
S36	LRC020	Ritinge	Daraja mbili
S37	LRC 007	Eamini	Nyamache
S38	LRC 014	Esaire	Daraja mbili
S39	LRC 017	Masaku	Marani
S42	LRC 004	Emwetemania	Masimba
S43	LRC 013	Onyoro	Daraja mbili
S51	LRC003	Enchano	Daraja mbili
S52	LRC002	Egiero	Kisii municipality market

Table 3.1a: Names, codes, local names and source of common bean landraces collected from Gene bank used in this study

S4	GK030171	NNP	Gene bank
S5	GK030217	NNP	Gene bank
S6	GK030178	NNP	Gene bank

S7	GK030185	NNP	Gene bank
S8	GK036526	NNP	Gene bank
S9	GK030261	NNP	Gene bank
S10	GK030200	NNP	Gene bank
S11	GK030204	NNP	Gene bank
S12	GK030210	NNP	Gene bank
S13	GK030246	NNP	Gene bank
S14	GK036524	NNP	Gene bank
S15	GK030211	NNP	Gene bank
S16	GK030249	NNP	Gene bank
S29	GK030194	NNP	Gene bank
S30	GK030227	NNP	Gene bank
S31	GK030239	NNP	Gene bank
S32	GK036530	NNP	Gene bank
S40	GK 030244	NNP	Gene bank
S41	GK 036527	NNP	Gene bank
S44	GK036523	NNP	Gene bank
S45	GK030257	NNP	Gene bank
S46	GK036522	NNP	Gene bank
S47	GK030260	NNP	Gene bank
S48	GK030198	NNP	Gene bank
S49	GK030259	NNP	Gene bank
S50	GK030167	NNP	Gene bank

NNP = Name Not Provided

3.2.2 Preparation and inoculation of *Pythium* spp.pathogen

Pythium spp. was isolated from symptomatic bean plants collected from farmers'fields in South Western Kenya. Affected plants exhibited damping off at seedling stage, root and hypocotyl rot, stem cankers (linear or circular reddish-brown sunken lesions delimited by a brown to reddish-brown margin), yellowing, wilting, pod rots among other symptoms.

The bean plants were uprooted washed with running water and stem bases cut off, surface sterilized with 1.5% solution of sodium hypochlorite for 30sec. and then rinsed three times with sterile distilled water and blot dried. The cut stem tissues were plated on Potato Dextrose Agar, PDA (composed of dehydrated potato infusion and dextrose to encourage rapid fungal growth, while the agar was added as a solidifying agent), media supplemented with 50 ppm streptomycin and incubated for 7 days at 24 °C. The *Pythium* spp. *(Pythium ultimum)*.was identified based on morphological and cultural features and confirmed using fungal identification keys as described by Watanabe (2010).

To prepare *Pythium* inocula, three day old, actively growing hyphal regions measuring 4 mm^2 were aseptically cut and grown on autoclaved millet seeds. The culture of *Pythium* spp. was then mixed with pre-sterilized loam soil in a ratio of 1:8 v/v in wooden flats measuring 48 cm × 72 cm and the inoculum was allowed to establish for a period of 14 days in the dark. Fifty one common ben landraces were evaluated in the months of June and July of 2016. Two rows of bean seeds (10 seeds per landrace) were planted in the wooden flats at a spacing of 50cm x 15cm inter and intra row respectively and each treatment replicated thrice. The control experiment contained seeds sown in sterilized loam soil without *Pythium* inoculum. Replications were set using the standard randomized complete block design (RCBD). The experiment was repeated twice in a screen house.

3.2.3 *Pythium* root rot disease assessment in theglasshouse

After germination, bean plants were watered after every three days to ensure optimum growth conditions. Thirty six days after planting, seedlings were uprooted and washed with tap water to remove soil from the roots and the plants were individually assessed for disease severity using the CIAT nine-point severity scale of 1 to 9 (Otsyula *et al*., 2003), where 1 = no root symptoms, 3 = a maximum of 10% of the root tissues have lesions, 5 = approximately 25% of the root tissues have lesions and the root system suffers a considerable decay; 9 = 75% or more of the root tissues have lesions and the root system suffers advanced stages of decay and considerable reduction. Generally, common bean landraces with an average severity score of 1.0 to 1.9, 2.0 to 3.9, 4.0 to 5.9 and greater than 6.0 were considered as resistant, moderately resistant, susceptible and highly

susceptible to *Pythium* root rot disease, respectively. Data on disease severity were subjected to analysis of variance (ANOVA) using the GenStat 11th Edition (VSN International Ltd., 2008) software package. Means were compared using Fisher's protected least significant difference test at 5% probability level (Steel *et al*., 1997).

3.2.4 Genomic DNAextraction

Leaves of common bean landraces planted in plastic pots in the glasshouse were used for DNA isolation. Plant genomic DNA was isolated using cetyltrimethyl ammonium bromide (CTAB) method as described by Gyang *et al*. (2017). Approximately 200 mg of young leaf samples were weighed and crushed in pre-warmed extraction buffer consisting of 700 µl CTAB buffer plus 150 µl 20% sodium dodecyl sulfate (SDS) to form a homogenous paste. The homogenate was transferred into 1.5 ml centrifuge tubes and incubated in a water-bath at 55°C for 20 min. The tubes were gently inverted five times after every five minutes during the incubation period to ensure uniform distribution of the homogenized tissues in the buffer. After incubation, the samples were spun in a micro-centrifuge for 10 min at 13,800 rpm. The debris was discarded and the supernatant transferred to a new 1.5 ml centrifuge tube preceding addition of equal volume of phenol: chloroform: isoamyl alcohol (25:24:1). The contents in the centrifuge tubes were mixed 50 times by inversion to allow proper mixing before spinning for 7 min at 13,800 rpm. The top layer was transferred to new 1.5 ml centrifuge tubes followed by addition of 50 µl of 7.4 M ammonium acetate and 2 volumes of ice cold absolute ethanol. The tubes were incubated at -20°C for 1 h and then centrifuged for 10 min at 10,000 rpm to pellet the precipitated nucleic acids. The supernatant was discarded and 500 µl of a wash solution (75% ethanol and 15 mM ammonium acetate) was added to wash the pellet. The washing step was repeated twice. After every wash, the centrifuge tube was spun for 5 min at 12,000 rpm and the supernatant discarded. The DNA pellet was air dried for 10 min in the fume hood and dissolved in 70 µl TE buffer. Ribonuclease A (3 µl of 10 mg/ml) was added to the dissolved nucleic acids and incubated in a water bath at 37°C for 30 min. The dissolved DNA was stored at -20 °C for subsequent molecularanalysis.

3.2.5 Quantification of DNA

DNA was quantified through spectrophotometric absorbance (A) readings at wavelengths (λ) of 260 and 280 nm. Fifteen microlitres of each sample was added to 735 µl of sterile double distilled water and vortexed to give a 1:50 dilution. A Beckman DU-65 spectrophotometer was used to read the optical density at 260 mm and 280 mm (OD 260/280) so as to determine the concentration of DNA in µg/ml and also to determine the purity of the extracted DNA. DNA has been shown to absorb UV light at 260 mm and one optical density (OD) at 260 nm is equivalent to 50 µg/ml for dsDNA and to 40 µg/ml of ssDNA (Sambrook *et al*., 1989). The DNA concentration was calculated asfollows:-

$$\text{DNA concentration (µg/ml)} = \frac{OD_{260} \text{ X } 50 \text{ (dilution factor) x } 50 \text{ µg/ml}}{1000}$$

(Sambrook *et al*., 1989)

The ratio A_{260}/A_{280} was used to determine the purity of the DNA samples. It has been shown that if the ratio is between 1.8 and 2.0 the absorption is due to nucleic acids. A ratio less that 1.8 indicates that there may be proteins or other UV absorbers in the sample. A ratio higher than 2.0 indicates that samples may be contaminated with chloroform or phenol (Rojas, 1997). After quantification, samples that had a ratio less than 1.8 and higher than 2.0 were reprecipitated with ethanol. The precipitate of DNA, which is allowed to form at low temperature (-20 °C or less) in the presence of moderate concentrations of monovalent cations is recovered by centrifugation and redissolved in an appropriate buffer at the desired concentration. The technique is rapid and is quantitative even with nanogram amounts of DNA.

The volume of the DNA solution was estimated. Three molar sodium acetate solutions was prepared by dissolving 20.412 g of sodium acetate crystals in 50 ml of distilled water and the pH was adjusted to 5.2 by adding a few drops of concentrated hydrochloric acid. 1/10 volume of 3M sodium acetate (pH 5.2) was added to the DNA solution in each tube. The mixture was vortexed briefly. Two volumes of ice-cold absolute ethanol was added to each tube and mixed well by gentle tapping. The mixture was incubated at -20^0C overnight to allow the DNA precipitate to form. The mixture was then spinned at 14,000 rpm for 20 min in a table-top eppendorf centrifuge to pellet the DNA. The supernatant

was carefully removed and the tubes placed in an inverted position on a layer of absorbent paper to allow as much of the supernatant as possible to drain away. Capillary pipettes were used to remove any droplets of fluid that adhered to the walls of the tubes. One milliliter of 70% ethanol was added to each tube to wash the DNA pellet and to remove any solutes that may be trapped in the precipitate. The mixture was vortexed briefly and re-centrifuged as described above. The ethanol was then removed carefully taking care not to loose the DNA pellet. Capillary pipettes were used to remove any droplets of fluid that adhered to the walls of the tubes. The traces of ethanol were removed by brief treatment (2 min) in a vacuum desiccator. The DNA pellet was dissolved in 100 µl of TE buffer (10 mM Tris-HCl pH 8.0, 1mM EDTA). DNA was quantified using a Beckman DU-65 spectrophotometer and the quality was checked by running agarosegels.

3.2.6 Agarose gel electrophoresis

Agarose powder was dissolved in Tris Borate EDTA (TBE) buffer (1% w/v) by slowly boiling in a microwave oven. The agarose was allowed to cool to about 50^0C and ethidium bromide was added to the gel at a concentration of 1 mg/ml. Care was taken because ethidium bromide is a mutagen. While the agarose was cooling, the gel tray was prepared by sealing the open edges of a clean, dry, glass tray with autoclave tape so as to form a mold to avoid leakage and so that the tray could accommodate the desired thickness of the gel. The edges of the gel tray were sealed with a small quantity of the agarose solution using a Pasteur pipette. When the seal was set, the rest of the warm agarose solution was then poured into the gel tray in which a comb was inserted to form sample slots. The gel was allowed to solidify for 30 minutes before removing the autoclave tape, and then the tray/mould was immersed in the electrophoresis tank containing TBE buffer. The combs were removed and 20 µl of each DNA sample containing 2 µl of loading solution was loaded to the wells of the gel to the top. DNA lambda digested with Eco RI and Hind III restriction enzymes was used as a molecular weight marker that was run in parallel i.e. in one lane of each gel. DNA was mixed with sample loading buffer in order to make the solution sink in the gel wells. The gel was run at a constant voltage of 100 V until the bromophenol blue migrated almost to the end of the gel. Resolution was improved by recirculating the buffer every 20 min. The gel was

then removed from the rig, placed in a UV transilluminator and photographed using a digital camera.

3.2.7 Peroxidase-gene-based markers and polymerase chainreactions

Primers (Table 3.2) designed from peroxidase cDNA sequences of *Arabidopsis* and rice (Welinder *et al*., 2002; Gulsen *et al*., 2007; Nemli *et al*., 2014) were used to detect polymorphism in common bean accessions. Polymerase chain reaction (PCR) reactions were done in a total volume of 20 µl, made up of 5 µl 1× GoTaq Mix (Promega Corporation, Madison, USA), 1 µl of each of the forward and reverse POX markers (10 µM), 1 µl genomic DNA (20 ng), 12 µl nuclease-free water. Amplifications were done in an MJ MiniTM Thermal Cycler machine (Bio-Rad, Singapore) as follows: initial denaturation at 94°C for5 mins, for 30se, followed by denaturation(30 s)at 94°C,

annealing at 46 - 56°C (45 s), extension (1 min) at 72°C, then a final extension at 4°C for 7 mins. PCR reaction for each POX primer was done at least twice using DNA extracted from different plants of the same landrace and only clear bands that can be reproduced were used during analysis of data.

Table 3.2: PCR markers used for amplification of peroxidase gene (POX) in common bean landraces

Primer	**Sequence (5' - 3')**		**Fragment size (bp)**
	Forward	**Reverse**	
PM55	TTGTAGATTCTCGCTCGGAA	CTTGGCATAATTGTTATTTGGT	150 - 800
POX1	CTCGACCTACAAGGAC	ATGTAGGCGCTGGTGA	100 - 800
POX8b	CACCATCAAGAGCGTCATAAC	TTGCTAGAGCGAGCTGG	100 - 200
POX11	CCTTCTTCTTGCCATCTTGC	CATATCGCTCCACGACCTTT	150 - 750

POX12b	CTCTCTCCTGGGGGTTCTATGC	GCGAGCGTGGTGATGTC	100 - 750

3.2.8 Analysis of amplified PCR products by agarose gelelectrophoresis

Agarose gel electrophoresis was carried out as outlined in section 3.2.6. To 25 µl of each PCR reaction mix, 3 µl of sample loading buffer was added and mixed by pipetting before loading the resulting mixture in the pre-formed sample wells on the gel. The products of the PCR reactions were ran on a 2% agarose gel for 70 mins at 65 Volts. Amplified DNA bands were photographed using a UV transilluminator and scored for further analysis. The fragment sizes were evaluated base on how they moved through the agarose gel in comparison to a 100-bp DNA ladder (Bioneer, SouthAfrica).

3.2.9 Scoring of alleles and dataanalysis

Each band was scored as present (1) and absent (0) to generate binary matrix for the 5 POX markers. This POX data was used for analysis of genetic diversity and population structure. The polymorphic information content (PIC) was determined for each peroxidase gene locus following the equation PIC = 1 - Σ (pi)2 which was described by Botstein *et al*. (1980) (where *pi* is the population carrying the *i*th allele). The similarity matrix generated using Nei's genetic distance (Nei and Li, 1979) was used to construct a dendrogram using the unweighted pair-group method arithmetic mean (UPGMA) by the use of MVSP 3.1program.

3.2.10 Population structure analysis and analysis of molecular variance (AMOVA)

Population genetic structure analysis was done with a clustering approach of a Bayesian model-base, clustering approach in the STRUCTURE version 2.3.4 program (Pritchard *et al*., 2000). An analysis of all 51 landraces was done using the number of clusters (*K*) ranging from 1-10, and a burn-in period of 5,000 iterations with 50,000 replications of Markov Chain Monte Carlo (MCMC). Results were not significantly affected with longer burn-in periods. The runs showing the maximum posterior probability for each *K* value was used. The *ad hoc* statistic Δ*K* was used to estimate the total sub-populations, and to determine *K* (Evanno *et al*., 2005). Principal component analysis (PCA) was carried out depending on the variation patterns of the POX gene, and a two dimensional

representation of relationships across the 51 common bean landraces using XLSTAT program was generated. Analysis of molecular variance (AMOVA) within and among populations was done with the GenAlEx (v6.5) software (Peakall and Smouse,2012).

3.3 Results

3.3.1 Phenotyping of *Pythium* root rotdisease

Analysis of variance showed highly significant ($p < 0.001$) differences in disease severity among the 51 landraces. Average disease severity of all the landraces ranged from 2.1 to 7.9. Based on the disease severity scores against *Pythium* root rot in the glasshouse, six (11.77%), twenty-eight (54.90%) and seventeen (33.33%) were classified as moderate-resistant, susceptible and highly susceptible, respectively (Table 3.3). The landraces with the lowest *Pythium* root rot disease severity were LRC008, LRC014, LRC016, LRC018, LRC019 and GK030257. None of the 51 landraces was found to be resistant to *Pythium* root rot disease.

Table 3.3: General mean svereity score response of 51 common bean landracesto *Pythium* root rot disease and disease rating

Entry	Landrace code	Disease severity score	Disease rating*
S1	LRC 006	6.2cdefgh	Highly susceptible (HS)
S2	LRC 008	2.1p	Moderately resistant (MR)
S3	LRC 018	3.1no	Moderately resistant (MR)
S4	GK030171	5.4hijkl	Susceptible (S)
S5	GK030217	5.8defgh	Susceptible (S)
S6	GK030178	5.4hijkl	Susceptible (S)
S7	GK030185	6.5bcd	Highly susceptible (HS)
S8	GK036526	7.9a	Highly susceptible (HS)
S9	GK030261	5.2hijkl	Susceptible (S)
S10	GK030200	5.2hijkl	Susceptible (S)

S11	GK030204	4.8jkl	Susceptible (S)
S12	GK030210	4.3lm	Susceptible (S)
S13	GK030246	4.2lm	Susceptible (S)
S14	GK036524	5.6fghi	Susceptible (S)
S15	GK030211	4.8jkl	Susceptible (S)
S16	GK030249	6.3cdef	Highly susceptible (HS)
S17	LRC 001	6.3cdef	Highly susceptible (HS)
S18	LRC005	7.6a	Highly susceptible (HS)
S19	LRC010	5.4hijkl	Susceptible (S)

S20	LRC016	2.4op	Moderately resistant (MR)
S21	LRC015	5.8defgh	Susceptible (S)
S22	LRC026	4.7kl	Susceptible (S)
S23	LRC011	6.6bc	Highly susceptible (HS)
S24	LRC012	7.2ab	Highly susceptible (HS)
S25	LRC021	5.8defgh	Susceptible (S)
S26	LRC024	5.7efgh	Susceptible (S)
S27	LRC019	2.5op	Moderately resistant (MR)
S28	LRC022	5.3hijkl	Susceptible (S)
S29	GK030194	4.2lm	Susceptible (S)
S30	GK030227	6.2cdefgh	Highly susceptible (HS)
S31	GK030239	5.7efgh	Susceptible (S)
S32	GK036530	7.8a	Highly susceptible (HS)
S33	LRC 023	6.5bcd	Highly susceptible (HS)
S34	LRC 009	4.9ijkl	Susceptible (S)
S35	LRC025	4.4l	Susceptible (S)

S36	LRC020	4.8jkl	Susceptible (S)
S37	LRC 007	5.3hijkl	Susceptible (S)
S38	LRC 014	3.6mn	Moderately resistant (MR)
S39	LRC 017	4.2lm	Susceptible (S)
S40	GK 030244	5.5ghij	Susceptible (S)
S41	GK 036527	5.4hijkl	Susceptible (S)
S42	LRC 004	4.8jkl	Susceptible (S)
S43	LRC 013	4.7kl	Susceptible (S)
44	GK036523	6.6bc	Highly susceptible (HS)
S45	GK030257	3.3n	Moderately resistant (MR)
S46	GK036522	6.3cdef	Highly susceptible (HS)
S47	GK030260	4.2lm	Susceptible (S)
S48	GK030198	7.4a	Highly susceptible (HS)
S49	GK030259	6.4cde	Highly susceptible (HS)
S50	GK030167	6.6bc	Highly susceptible (HS)
S51	LRC003	7.7a	Highly susceptible (HS)
S52	LRC002	Not done	Not done

Means within column followed by similar letters are not significantly different (P = 0.5).

*Common bean landraces with an average score of 1.0 to 1.9, 2.0 to 3.9, 4.0 to 5.9 and greater than 6.0 were considered resistant, moderately resistant, susceptible and highly susceptible to *Pythium* root rot disease, respectively.

3.3.2 Polymorphism detected using POXmarkers

Five POX primers were used to characterize 51 common bean landraces based on the amplification of clear banding patterns. The number of bands generated among the 51 common bean landraces using 5 primers was 1119, which ranged from 3 (POX8b and POX55m) to 8 (POX12b), with an average of 4.8 bands/primer. A sample of amplification pattern of primer POX1 using DNA from 51 common bean landraces is

shown in Figure 3.1. Out of the fragments scored, 81% were polymorphic. The percentage polymorphic loci varied from 66.6% (POX 11 and P55m) to 100% (POX 8b) with an average of 80.6% bands /primer (Table 3.4). Primer POX8b gave 100% polymorphism, indicating the capability of POX primers to detect high levels of polymorphism among common bean landraces (Table 3.4).The PIC value ranged from 0.10 (POX11) to 0.47 (POX8b) with an average value of 0.28.

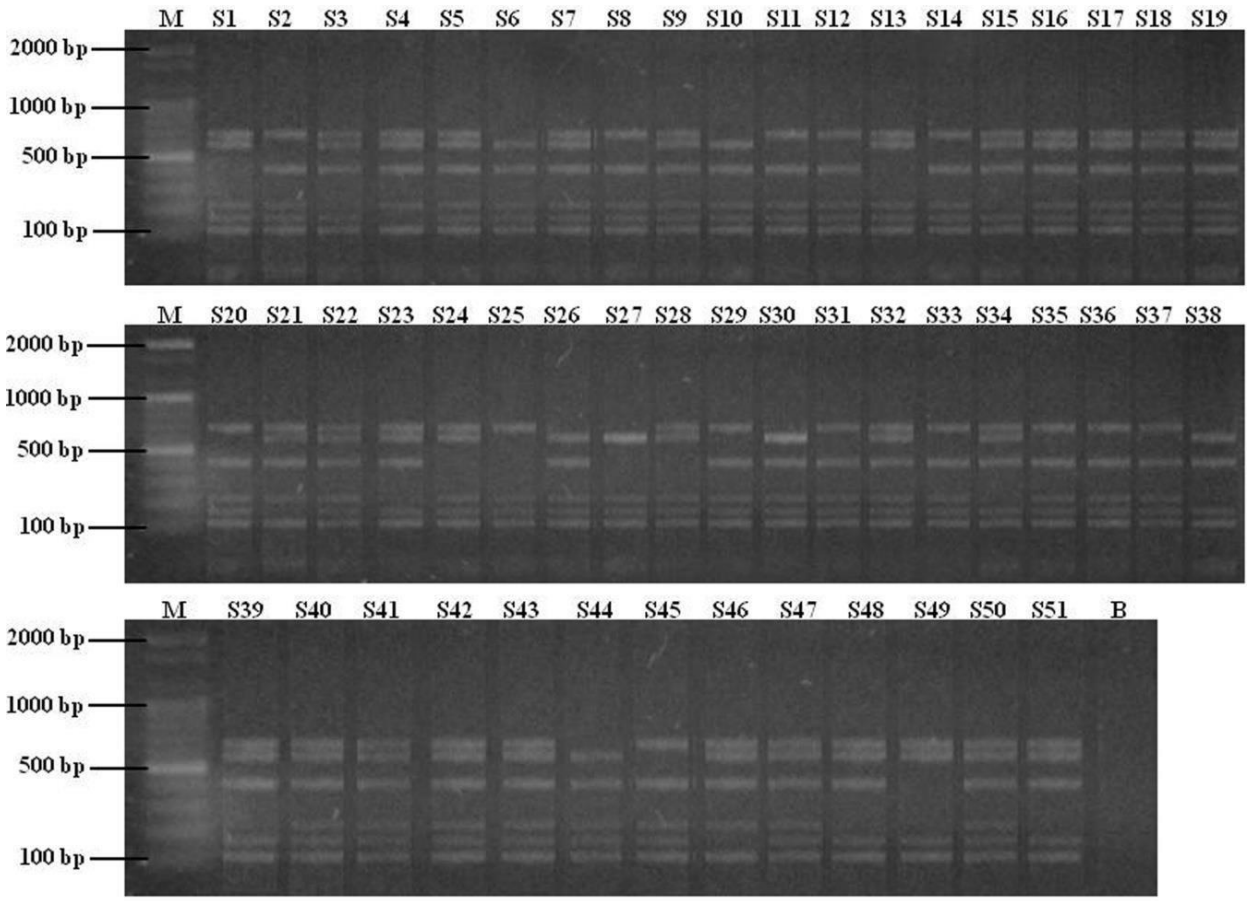

Figure 3.1: POX marker profile of 51 common bean landraces generated by primer POX1. M is marker lane, B contains water

Table 3.4: Peroxidase-gene based markers, numbers of total and polymorphic fragments and PIC values obtained from 51 common bean landraces

POX marker	**Total fragments**	**Polymorphic fragments**	**Polymorphism (%)**	**PIC**
POX1	6	5	83.33	0.23
POX8b	3	3	100	0.47
POX11	6	4	66.67	0.10
POX12b	8	7	87.5	0.34
P55m	3	2	66.67	0.28

3.3.3 Genetic relationships among the common beanlandraces

Pair-wise comparisons among the landraces were used to calculate the genetic similarity coefficient based on the proportion of shared bands. The genetic similarity among the 51 landraces ranged from 0.44 to 1.0, with an average of 0.72. The lowest value of genetic similarity (44%) was observed between S8 (highly susceptible) and S28 (susceptible) common bean landraces. Also low values of genetic similarity was observed between the landraces S4 and S8 (53.8%), S13 and S8 (52%), S24 and S8, (46.2%), S27 and S8 (45.8%) and; S28 and S8 (45.8%). The maximum genetic similarity (100%) was observed between landraces S7 (highly susceptible) and S19 (susceptible). The genetic similarity matrices showed that the 6 landraces that were moderate resistant were not geneticallysimilar.

3.3.4 Phylogenetic analysis

Based on the genetic similarity matrix, a dendrogram was constructed and the 51 common bean landraces were separated into two major Clusters 1 and 2 (Figure 3.2). Cluster 1 was the largest and is divided into 2 sub-clusters (namely 1A and B) which in total contained 42 bean landraces of which 4 (S3, S27, S38 and S45) were moderate resistant. Sub-cluster 1A is the smallest and contains only one (S27) moderate resistant landrace. Sub-cluster 1B was divided into four groups namely I – IV. Groups I and III contained 2 (S3 and S45) and 1 (S38) moderate resistant landraces, respectively, while in Groups II and IV none of the landraces were found to be moderate resistant. Cluster 2 contained 9 landraces of which two (S2 and S20) were moderate resistant. Generally, the landraces form south Western Kenya did not form specific clusters orgroups.

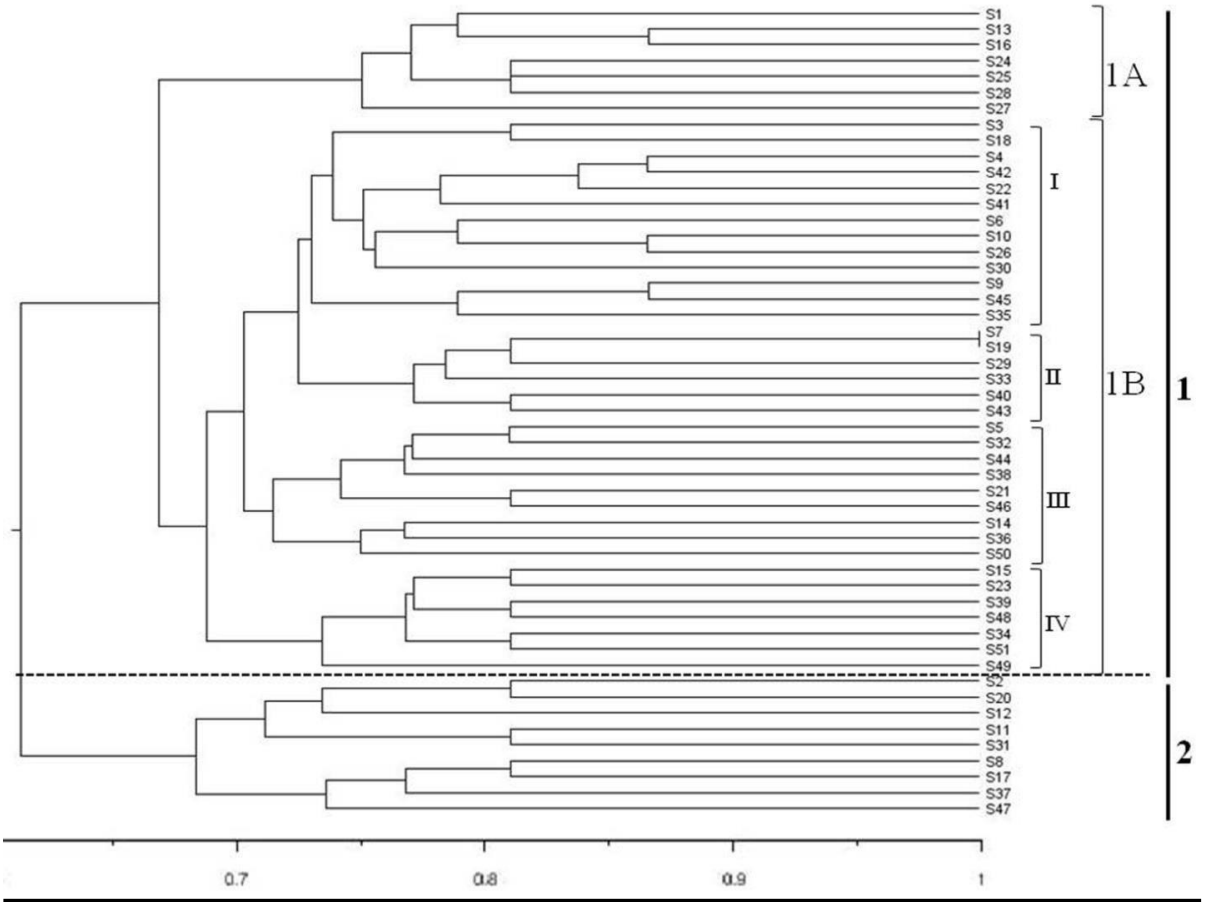

Figure 3.2: UPGMA dendrogram showing genetic relationship among 51 common bean landraces using 5 POX markers. S1 to S51 represent the entries of the common bean landraces as indicated in Table 3.3.

3.3.5 Population structure analysis

The 51 common bean landraces were analyzed for population structure using Bayesian base method without any prior classification to know the highest populations (K). The peak plateau of adhoc measure ΔK was found to be K = 3 (Figure 3.3), which indicated that the entire 51 landraces were distributed into three groups (POP1, POP2 and POP3) (Figure 3.4). The POP2 was the smallest group consisting 12 landraces (23.53%) of which 2, 14 and 6 were found to be moderately resistant, susceptible and highly susceptible, respectively. The POP3 included 17 (33.33%) landraces of which 2, 8 and 7 were found to be resistant, susceptible and highly susceptible, respectively. On the other hand, POP1 was the largest group comprising of 22 (43.14%) landraces of which 2, 6 and

14 were resistant, susceptible and highly susceptible, respectively. There were equal numbers of resistant genotypes (2) in all the three groups. The structure analysis did not differentiate resistant and susceptible landraces into separate groups.

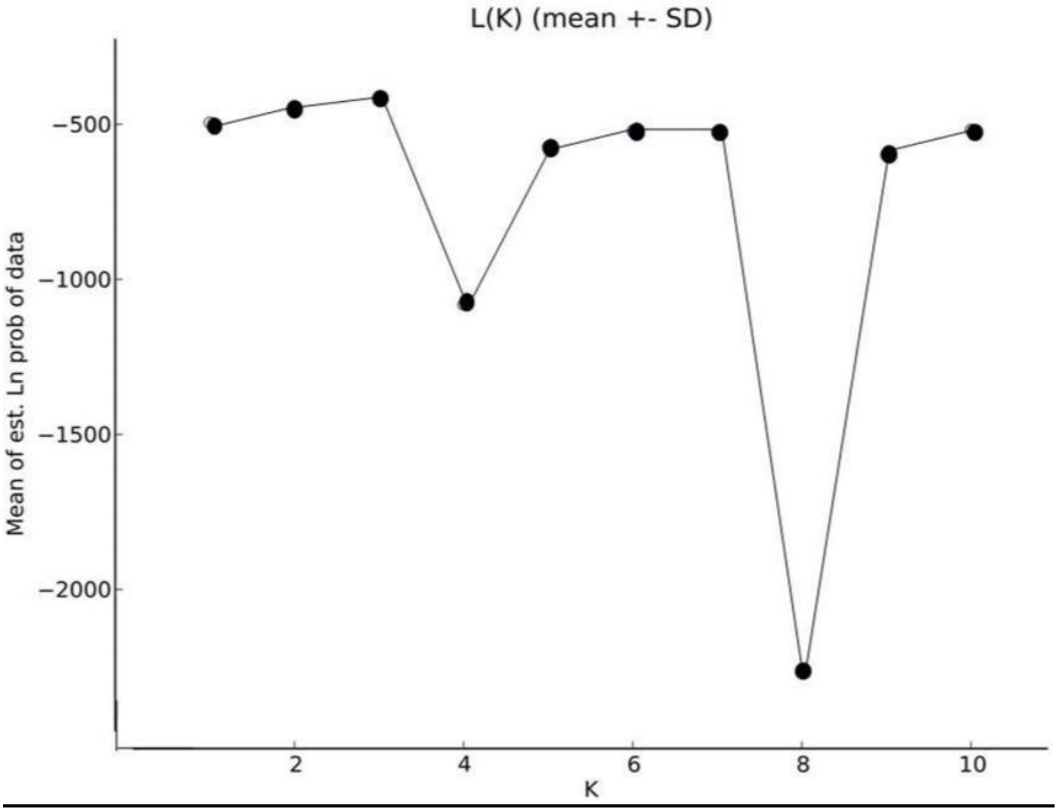

Figure 3.3: STRUCTURE analysis of the total genetic clusters for values of K (K = 1 to 10), using delta K values.

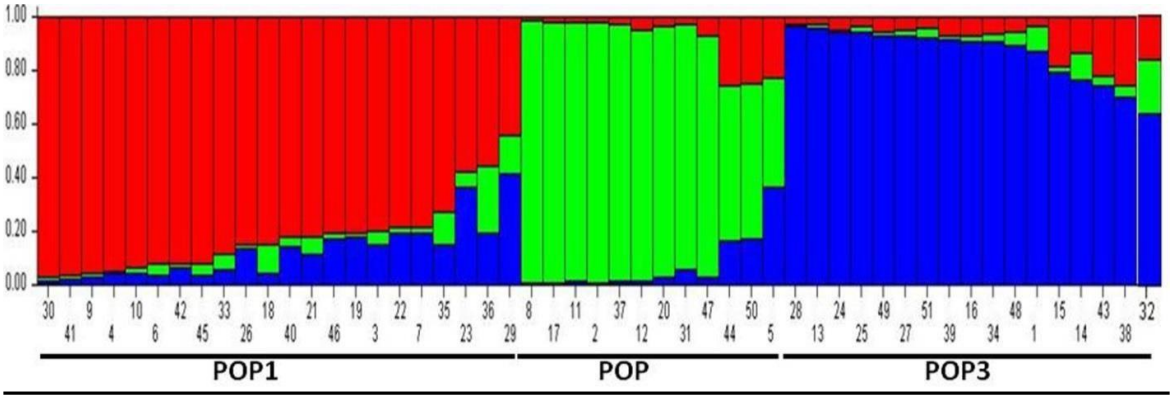

Figure 3.4: Population structure of 51 common bean landraces based on peroxidase-gene (POX) based primers for *K* = 3. The colors represent the 3 single sub-populations as designed by STRUCTURE.

The colors represent single sub-population and the colored segment length indicates the analyzed membership proportion of every sample to designed population. The maximum K value was determined by structure harvest to be 3, which indicates that the entire population of 51 consisted of 3 subgroups. The numbers 1 – 51 represent the entries of the landraces (Table 2) where S isexcluded.

3.3.6 Analysis of molecular variance AMOVA)

Analysis of molecular variance was calculated to estimate the partitioning of genetic variance among and within populations. Within population variance explained 100% and no variance (0%) was observed among population (Table 3.5). All the diversity of common bean landraces from south western Kenya resided within the populations.

Table 3.5: Molecular variance of POXmarkers among and within common bean landraces

Source	**df**	**SS**	**MS**	**Estimated variation**	**Percentage of variation**
Among populations	4	9.628	2.407	0.000	0%
Within populations	46	142.529	3.098	3.098	100%
Total	50	152.157		3.098	100%

df = degrees of freedom, SS = sum of squares, MS = mean of squares, P-Value (<0.001)

3.4 Discussion

The presence of genetic variability in germplasm is a pre-requisite for efficient utilization of available genetic resources for breeding programmes. Understanding genetic differences for *Pythium* root rot resistance is useful for evaluating landraces as breeding parents. Gene based molecular markers such as peroxidase-gene have been used in legumes used to identify genotypes, study genetic diversity and determining the phylogenetic relationships (Nemli *et al*., 2014). This study determined the genetic variations and population structure in common bean landraces from South Western Kenya using peroxidase-gene markers. Plant peroxidase genes serve important roles in providing plants resistance against biotic stresses (Bela *et al*., 2015; Mir *et al*., 2015; Passardi *et al*., 2005), and peroxidase marker patterns might be utilized in defining relationships among plant genotypes in relation to their adaptiveconditions.

In the current study, 51 common bean landraces from South Western Kenya were characterized using five POX loci for resistance to *Pythium* root rot disease. The POX markers are made using conserved motifs of rice and *Arabidopsis* peroxidase (Gulsen *et al*.,2007). The total amplified alleles on each locus varied from 3 - 8, with an average score of 4.8. Similar work has been reported by Nemli *et al*. (2014) who found 1 - 8 alleles on each locus and had an average value of 4.0 when characterizing common bean genotypes from Turkey using POX markers. All the POX markers used in this study were polymorphic. However, the markers had a low average PIC value of 0.28, implying that the landraces used in the present study were closely related. The mean value of PIC observed in this study was lower than that reported by Nemli *et al*. (2014) and Wittayawannakul *et al*., (2010), who estimated variation among common bean genotypes and Garcinia species, respectively, using POX polymorphisms and got mean PIC values of 0.40 and 0.79, respectively. The differences in PIC values could be due to the genotypic differences of the germplasm used in thisstudy.

The mean genetic similarity coefficients ranged from 0.44 to 1.0, with an average of 0.72, which indicates high genetic diversity among the 51 landraces. The high genetic diversity in these landraces is a valuable resource for broadening the genetic base in common bean breeding programs. The genetic differences among moderately resistant landraces to *Pythium* root rot disease as revealed by their clustering into different clusters and groups suggest the presence of different sources and sufficient genetic variation for resistance to *Pythium* root rot. This genetic variability can be exploited for developing cultivars resistant to *Pythium* root rot. In the present study, the dendrogram constructed using UPGMA method suggested occurrence of two major clusters and illustrated no clear pattern of distribution of moderate resistant, susceptible and highly susceptible landraces. The relationship between landraces in the cluster groups could not be attributed to their resistance to *Pythium* root rot. The 51 common bean landraces were divided by STRUCTURE analysis into three groups and did not indicate any distribution pattern in terms of their reaction to *Pythium* root rot. It is therefore predicted that, combining landraces from the different clusters and groups as parents in breeding programmes would result in broadening *Pythium* root rot resistance genes in thepopulation.

Incorporating *Pythium*-moderate resistant landraces which have other desirable agronomic and consumer quality traits such as high iron and zinc content, fast cooking ability, from the different clusters and groups as parents for breeding, would ensure the diversification of resistance to the disease while creating new hybrids. Six common bean landraces from South Western Kenya with the potential moderate resistance to *Pythium* root rot were identified. These landraces would be potential sources to Pythium root rot resistance in common bean breeding programs in Kenya.

CHAPTER FOUR

4.0 Evaluation of genetic variability, heritability, genetic advance and correlation for agronomic and yield components in common bean landraces from South western Kenya

4.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume, second to maize as a stable food crop in Kenya. Africa produces 17% of the world total production, of which 70% is from Eastern Africa. Kenya produces 400-1200 kg/ha, mainly from intercropping with maize by small-scale farmers. Common bean is an important source of protein and minerals especially iron and zinc. It is a dual-purpose crop producing grains as well as fodder for livestock. Consumption of beans confers humans with various health benefits including reduction of cholesterol level, reduction of coronary heart diseases and decreases diabetes and obesity (Broughton *et al*., 2003; Pereira *et al*., 2015). Common beans also play a very important role in sustaining soil fertility by adding atmospheric nitrogen and organic matter to the soil. As a cover crop it is efficient in suppressing weeds and prevents soil erosion (Geil and Anderson, 2014).

Production of common bean in south western Kenya is constrained by various abiotic and biotic stresses. Diseases caused by fungi, bacteria and viruses are considered to be the second biggest constraint to bean production after low soil fertility, causing over 90% or total crop loss (IBPGR, 2012). Insect pests especially pod borers and weevils may also cause yield loss of up to 80%. In many bean producing areas of Kenya, there is lack of clean seed planting materials and varieties grown are often low yielding (Bennick, 2005). There is also a problem of lack of cultivars with market qualities and consumer quality attributes such as fast cooking. These factors have reduced the germplasm sources used in hybridization and have limited the genetic variability available for breeding programs. Development of high yielding cultivars with resistance to major bean diseases is an important breeding priority to reduce impact of diseases and increase common bean production in South- western Kenya. Characterization of plant germplasm using agronomic traits has been used for various purposes including identification of duplicates,

correlation with characteristics of agronomic importance and identification of genotypes resistant to pests and diseases (Smartt, 1988a, and b).

The selection of desirable genotypes is usually based on the genetic variation of agronomic or quantitative traits such as yield and its components. It is therefore necessary to study the relationship between genotype variability and yield components for efficient utilization of common bean genetic resources in improvement programs. Heritability is the degree of genetic control associated with certain heritable important traits (Addissu, 2011). It indicates how much of the genetic variability has a genetic origin and gives necessary information for the selection process (Falconer, 1981). The selection of superior genotypes is proportional to the amount of genetic variability present and the extent to which the characters are inherited (Scarano *et al*., 2014). Therefore, adequate information on the magnitude and type of genetic variability and their corresponding heritability is important in the improvement of grain yield potential of crops in breeding programs.

Yield improvement is an important breeding objective of most crop improvement programs (More and Borkar, 2016). Similar to other crops, yield in common bean is a complex trait comprising of many morphological, physiological and agronomic traits. Seed yield is affected by genotype and environmental factors because of its quantitative properties. Effectiveness of selection is dependent upon the availability of large genetic variability present in the breeding material for the target character and the extent to which it is heritable (Atta *et al*., 2008). It also depends on the direction and magnitude of association between the traits to be improved (More and Borkar, 2016). However, limited attention has been given to studies on genetic variability, heritability and genetic advance of yield and associated traits in common bean landraces to improve the seed yield in south Western Kenya. Therefore, the present study was carried out to assess the extent of genetic variability, heritability and genetic advance among common bean landraces for yield and related traits and examine their correlation with seed yield for efficient design of common bean breedingschemes.

4.2 Materials andMethods

4.2.1 Plantmaterials

A total of 52 common bean landraces were used in this study. Seeds of 26 common bean landraces were collected from farmers‘ fields in different agro-climatic zones and market centers of Kisii County, South western Kenya. The accessions were collected according to the procedure of Plant Genetic Resources International Institute (IBPGR, 2011). The other seeds of 26 accessions were obtained from the National Genebank of Kenya, Muguga, near Nairobi. The germplasm from the Genebank were collected from farmers‘ fields in South western Kenya in earlier and mid 1980s and preserved (Table3.1).

4.2.2 Description of the studysite

Fifty two common bean landraces were planted at the Kenya Agricultural and Livestock Organization (KALRO), Kisii County, South western Kenya (situated at about 0.68° South latitude, 34.77° East longitude, at an elevation of between 1450-2210 m above sea level). The site falls in the Lower Highlands one and two (LH1 and LH2), Upper Midland one (UM1), Lower Midland one and two (LM1 and LM2) Agro-Ecological Zones (AEZs). The soil type was deep, fertile, well-drained red volcanic (nitosols). The county has climatic conditions of average rainfall ranging from 1,400 - 2,600 mm per annum and mean annual temperature ranging from 15 - 28 °C (FAO,2015).

4.2.3 Experimental design and establishment of plants in thefield

The genotypes were evaluated in two consecutive planting seasons namely between March and July, 2015 and repeated in the same period in 2016. The experiment consisted of a randomized complete block design (RCBD) with three plot replications for each landrace. The cultivars were grown in plots measuring 3 m × 4 m with distance between rows of 50 cm. Seeds were sown on raised beds with 40 cm row to row spacing and 15 cm plant to plant spacing at a depth of 5 cm. One teaspoonful of Diammonium phosphate (DAP) was added to each hole at planting. Normal crop management practices were carried out as recommended including weeding, pest/disease checks and topdressing.

4.2.4 Collection of agronomicdata

Fourteen descriptors were surveyed for each common bean landrace at appropriate growth stage. The descriptors developed for *Phaseolus spp.* were used with some modifications (IBPGR, 2013). Data were recorded on a plot basis using ten individual plants selected randomly from the two central rows of each plot. Measurement unit and measurement procedure of each trait are given in Table4.1.

Table 4.1: Fourteen observed common bean quantitative characters, measurement procedures and units

Character	**Code**	**Measurement unit/sampling procedure**
Days from planting to emergence	DTE	Number of days from sowing to the timethe plant emergence was 50% within the centrerows
Days from emergence to 50% flowering	DFSTF	Number of days from the date of emergence to the date on which 50% of the plants on a plot opened a flower
Days from sowing to 95%maturity	DSM	Number of days from sowing to the stage when 90% of the plants in a plot changed the colour of their pods from green to yellowish orange and texture hardened
Number of pods per plant	NPPP	Average number of pods counted at harvest, for 10 plants within plot centre
Number of seeds per pod	NSPPO	Determined from the average number of seeds per 10 pods per 10 sampled plants
Weight of 100 seeds per plant (gm)	WHSPP	Determined from the average 100-seeds mass at physiological maturity (12 - 14%) moisture content of the seed and expressed in grams
Number of seeds per plant	NSPP	Determined from the average number of seeds from 10 pods per 10 sampled plants at physiological maturity
Number of branchesper plant	NOB	Number of shoots arising from the main stem counted and recorded at physiological maturity.

Pod length	PL	Exterior distance of fully matured pod from the pod apex to the peduncle measured in centimeters at physiological maturity froman average of 10 plant within plot centre
Pod width	PW	Average width of 10 pods for each genotype from one end at its widest point of the central pod to the other at physiological maturity,in millimeters
Plant height	PH	The length of the central axis of the stem was measured from the soil surface up to the tip of the main stem at physiologicalmaturity and recorded in centimeters
Biological yield (weight of roots, stalk and leaves)	BY	An average from 10 plants uprooted, cleaned and weighted to get the biological yield perplant in grams
Grain yield/weight of seeds perplant	GY/WSPP	Dried grain yield in grams, obtained from 10 plants within central rows of each plot were harvested, threshed separately and seeds weighted
Harvest index (%)	HI	HI = (GY/BY) × 100

4.2.5 Statisticalanalysis

Analysis of variance: All data were subjected to analysis of variance for the randomized

$$PCV\ (\%) = \frac{\sqrt{Vp}}{Mean}\ X100$$

$$GCV(\%) = \frac{\sqrt{Vg}}{mean}\ X\ 100$$

complete block. Data were analysed using the statistical software Genes (Cruz, 2013). The treatment means were tested at 1% probability levels for significance (LSD). Phenotypic and genotypic coefficient of variation: The estimates of phenotypic and genotypic coefficient of variation were calculated as described on FAOSTAT (2013):

where PCV is phenotypic coefficient of variance, VP is phenotypic variance, GCV is genotypic coefficient of variance, and Vg is genotypic variance. GCV and PCV values were categorized as low (0 – 10%), moderate (10 – 20%), and high (20% and above) as indicated by Subramanian and Nechifor (2011) and SAS(2008). Heritability: It was estimated as the ratio of total genotypic variance to the phenotypic variance according to FAOSTAT (2013):

$$H^2 = \frac{Vg}{vp} \; X \; 100$$

whereH^2 = % Broad sense heritability. The heritability percentage was categorized as low (0 – 30%), moderate (30 – 60%), and high ≥ 60% as described by Nechifor (2011). Genetic advance: The extent of genetic advance expected through selection for the character was calculated as described by Addissu (2011): Genetic Advance (GA): $H \times P \times K$; where $H$is heritability, $P$is phenotypic standard deviation, and K is selection deferential (2.06 at 5%). Genetic Gain (%) = GA × 100; it is categorized as low (0 – 10%), moderate (10 – 20%) and high (20% and above) as described by Nechifor (2011). The genetic advance as a % of the mean (GAM) was calculated as:

$$GAM(\%) = \frac{GA}{X} \; X \; 100$$

Where:

GA = Genetic advance

x = Grand mean of a trait

Clustering and principal component analysis (PCA): Clustering and PCA were carried out to assess the relationships among the common bean landraces based on data from agronomic traits using NTSYS-pc software (version 2.1) (Rohlf, 1997). Data were analyzed based on Euclidian distance method and dissimilarity coefficient. Unweighted pair-group method of the arithmetic (UPGMA) mean and SAHN clustering were used to determine the genetic relationships among the common bean landraces. Principal component analysis (PCA) was calculated using EIGEN module of NTSYS-pc software.

43 Results

4.3.1 Agronomic performance of the common bean landraces

There was significant variation ($P<0.05$) for all the studied traits which also revealed possible amount of variability among the landraces (Table 4.2). Yield component traits including number of pods per plant, number of seeds per pod, number of seeds per plant, 100 seed weight and grain yield significantly varied and ranged from 12 to 244 pods per plant, 0.8 to 11 seeds per pod, 65 to 698, 20 to 113 g per 100 seeds and 26 to 329, respectively (Table 2). The other traits indirectly contributing to agronomic performance varied significantly ($P<0.001$); the number of branches varied from 1 to 17, plant length ranged from 8 to 16 cm and biological yield varied from101 to 2296grams.

The plant height (PH) was highest in genotype LRC008 (185 cm) while the lowest was for GK030198 (40.4 cm). The number of branches (NOB) ranged from 3 (GK030167, GK030246 and LRC004) to 15 (LRC008), while the number of days to emergence (DTE) varied from 5 for cultivar LRC010 to 10 days for cultivars GK030244, GK030204 and GK0302194. The number of days from emergence (DESTF) varied from 95.5 (LRC008) to 34 (LRC019), whereas the length of time to maturity (DSM) ranged from 150 days for genotype LRC008 to 57 for genotype LRC015 (Table 4.2). The table shows that the highest number of pods per plant (NPPP) was obtained from accession LRC008 (238) and the lowest from LRC007 (15), but the average number of seeds per pod (NOSPP) in these genotypes ranged from 4.2 in LRC 005 to 9.7 for LRC011. Accessions LRC016 and LRC 019 recorded the highest weight of hundred seeds per plant (WHSPP) (107 and 103 gm, respectively), compared to genotype LRC 014 which recorded the lowest (21). There was wide variation in the number of seeds per plant (NSPP) ranging from 690 (LRC 008) to 68 (LRC017). Pod length (PL) and pod width (PW) varied from 8.0 cm and 1.2 cm for cultivars LRC009 and GK030249 and GK 036528 as lowest values; while highest values were recorded for landraces LRC016 (14 for PL and 2.5 for PW).The highest biological yield (BY) and grain yield (GY) (234 and 2300) was recorded in accession LRC008. The harvest index ranged from 0.48 to 0.10 of which landraces GK036527 and LRC008 recorded the highest and lowest, respectively (Table4.2).

Table 4.2: Mean performance of 52 common bean landraces evaluated for 14 agronomic traits at KALRO-Kisii field during 2015 and 2016 cropping seasons

Entry	Codeof landrace	Local name of landrace	**PH (cm)**	**NOB**	**DTE**	**DFSTF**	**DSM**	**NPPP**	**NSPP**	**WHSPP (gm)**	**NSPP**	**PL (cm)**	**PW (cm)**	**GY (gm)**	**BY**	**HI**
1	LRC001	Ekenagwa	95.20	4.00	7.70	43.60	68.40	16.60	5.50	67.50	80.10	12.00	1.40	53.60	219.00	0.25
2	LRC002	Egiero	130.00	4.00	7.40	42.00	65.80	21.70	70.00	68.50	147.00	12.00	1.20	99.96	252.10	0.39
3	LRC003	Enchano	50.50	5.00	8.70	34.50	55.60	17.30	6.00	46.60	102.80	13.00	1.50	47.38	217.50	0.22
4	LRC004	Emwetemania	102.70	3.00	8.50	40.00	62.00	23.00	6.00	43.00	138.60	12.00	1.50	58.91	223.00	0.26
5	LRC005	Girini	93.40	4.00	7.00	36.60	58.00	21.00	4.20	42.00	84.40	10.00	1.50	35.28	235.50	0.15
6	LRC006	Esaitoti	45.00	6.00	8.00	35.00	60.90	19.50	4.70	73.70	76.20	10.00	1.50	56.24	234.00	0.24
7	LRC007	Eamini	37.00	15.00	7.90	35.70	60.00	15.00	6.00	69.00	90.00	13.50	1.60	62.10	218.0	0.28
8	LRC008	Echichae	185.00	7.00	6.00	95.50	150.00	238.00	5.00	46.90	690.00	12.00	1.50	324.30	2300.00	0.10
9	LRC009	Eroyoo	79.80	6.00	6.30	37.00	62.7.00	16.00	7.60	27.80	112.00	8.00	1.00	31.36	220.20	0.14
10	LRC010	Bunda entambe	66.00	5.00	5.00	48.40	72.00	32.00	7.00	36.70	224.00	11.00	1.30	82.88	251.40	0.33
11	LRC011	Ekebure	50.30	5.00	8.40	45.00	74.80	35.10	9.40	28.50	315.50	11.00	1.30	88.20	230.00	0.38
12	LRC012	Enyamotobu	43.00	5.00	7.00	35.00	60.00	21.20	5.70	72.40	147.00	15.00	1.80	105.84	240.00	0.44
13	LRC013	Onyoro	148.00	4.00	6.70	40.50	63.00	15.00	7.80	29.50	105.40	13.00	1.40	30.74	220.00	0.14
14	LRC014	Esaire	95.50	6.00	6.00	40.60	65.50	38.30	6.00	21.00	228.00	11.00	1.30	47.88	253.70	0.19
15	LRC015	Ecoco enyenge	74.70	4.00	5.30	38.00	57.00	22.80	5.50	56.80	110.00	10.50	1.50	62.70	234.40	0.27
16	LRC016	Manoa emwamu	118.00	6.00	9.40	35.00	59.90	23.00	4.00	107.00	92.80	14.00	2.50	99.51	300.00	0.33
17	LRC017	Masaku	81.10	5.00	6.70	35.00	61.80	17.00	4.00	66.00	68.40	11.00	1.40	44.88	230.00	0.19
18	LRC018	Richore	50.00	6.00	7.00	43.80	69.00	24.00	4.80	62.40	96.00	12.50	1.50	59.52	235.80	025
19	LRC019	Manoa endabu	120.00	7.00	6.00	34.00	61.00	25.00	3.60	103.00	75.00	13.00	2.50	77.25	334.20	0.23
20	LRC020	Ritinge	64.60	4.00	5.80	36.30	62.00	24.00	6.00	64.80	144.00	14.00	1.50	93.60	218.30	0.43
21	LRC021	Morogi	56.90	6.00	5.70	42.90	73.60	33.00	6.00	31.50	198.60	10.00	1.40	63.36	219.00	0.29
22	LRC022	Enyamwamu	100.00	6.00	5.50	40.00	70.00	36.00	7.00	27.00	252.00	11.50	1.40	68.04	194.00	0.35
23	LRC023	Osama	91.80	5.00	9.00	36.00	68.70	38.00	6.80	32.00	246.00	10.00	1.30	78.72	196.70	0.40
24	LRC024	Ecoco entambe	56.50	7.00	8.00	40.00	70.10	19.00	4.00	57.70	76.60	12.00	1.50	44.08	240.00	0.18
25	LRC025	Amaika inse	84.80	4.00	7.10	42.50	70.00	17.10	6.00	62.00	102.40	12.00	2.00	63.24	270.00	0.23
26	LRC026	Nyaibu (Bunda enetu)	52.00	5.00	8.00	37.70	63.00	21.70	6.00	64.00	126.00	13.50	1.50	80.64	260.00	0.30
27	GK036527	NA	97.30	6.00	9.30	43.80	68.40	28.00	6.30	57.80	168.60	14.00	1.50	97.00	198.40	0.49
28	GK036528	NA	86.40	6.00	6.70	41.40	65.00	25.60	6.70	48.00	150.50	12.40	1.20	72.00	297.00	0.24
29	GK036530	NA	75.20	7.00	6.40	40.30	63.90	36.00	6.80	42.00	216.60	10.00	1.30	91.00	291.00	0.31
30	GK036524	NA	121.10	6.00	7.00	38.90	62.30	37.80	8.70	43.70	296.00	9.40	1.00	130.00	295.00	0.44
31	GK030260	NA	60.50	4.00	9.00	36.00	60.20	36.50	4.00	46.50	144.00	10.00	1.50	67.00	231.00	0.29
32	GK030261	NA	123.00	5.00	7.40	41.90	68.80	33.00	7.80	44.40	231.00	11.00	1.40	102.00	278.60	0.36
33	GK036522	NA	119.00	6.00	8.00	40.20	72.00	33.00	6.60	58.50	198.20	14.00	1.50	117.00	290.40	0.40
34	GK030211	NA	98.3.00	7.00	7.00	40.80	71.70	40.40	8.00	41.00	320.20	11.00	1.50	131.00	271.00	0.48
35	GK030227	NA	65.00	5.00	8.50	44.70	73.80	30.60	6.00	58.60	180.40	12.50	1.50	106.00	269.50	0.39
36	GK030239	NA	40.40	7.00	9.00	38.00	65.60	15.40	5.00	42.10	75.00	11.50	1.40	32.00	216.00	0.15
37	GK030244	NA	67.40	6.00	10.00	37.60	66.10	27.50	5.60	46.00	135.00	10.50	1.50	62.00	263.00	0.23
38	GK030180	NA	78.00	6.00	5.60	36.500	65.00	32.00	6.70	52.00	192.70	14.00	1.50	100.00	248.40	0.40
39	GK030194	NA	65.30	4.00	10.00	43.00	71.00	32.00	5.00	53.00	160.00	10.50	1.50	84.00	251.00	0.33
40	GK030198	NA	40.20	4.00	9.40	37.00	63.20	17.70	6.00	42.00	102.20	12.50	1.50	43.00	233.90	0.18
41	GK030200	NA	61.00	5.00	9.00	40.60	70.20	35.50	4.00	48.50	140.70	10.40	1.40	67.00	260.00	0.26
42	GK030204	NA	82.50	7.00	10,0	41.70	73.30	34.70	6.00	65.00	204.00	150	1.50	132.00	238.00	0.55

					0											
43	GK030210	NA	58.60	4.00	7.30	35.00	60.00	16.30	5.80	38.70	80.60	10.50	1.50	31.00	263.00	0.12
44	GK030167	NA	110.00	3.00	7.80	40.00	68.30	33.00	7.00	43.00	231.80	11.00	1.30	98.00	282.00	0.35
45	GK030171	NA	46.20	5.00	6.20	38.00	68.40	23.30	7.00	54.00	161.90	10.00	1.30	87.00	216.00	0.40
46	GK030178	NA	116.00	5.00	6.0	42.80	73.00	34.00	7.70	42.00	238.60	10.50	1.30	100.00	236.50	0.42
47	GK036523	NA	78.80	6.00	7.60	36.0	67.80	24.00	6.70	38.50	144.00	11.00	1.50	56.00	239.80	0.23
48	GK036526	NA	64.20	7.00	9.70	35.40	63.00	52.00	5.40	41.00	260.00	14.50	1.80	106.00	305.00	0.35
49	GK030246	NA	126.	3.00	8.00	40.70	73.00	22.6.00	8.00	43.00	176.00	11.50	1.40	76.00	273.70	0.28
50	GK030249	NA	121	08	8.3	40.50	70.70	35.00	6.60	37.00	210.00	10.00	1.20	78.00	255.00	0.30
51	GK030257	NA	98.1	05	7.4	36.00	68.40	25.00	6.00	42.00	150.50	10.00	1.40	63.00	267.20	0.23
52	GK030259	NA	58	05	7.4	37.00	63.00	37.10	5.00	48.00	185.10	10.50	1.40	89.00	236.30	0.38
	Mean		83.26	5.50	7.55	40.23	63.00	31.31	6.08	50.45	170.82	11.65	1.47	80.33	247.76	0.30
	CV (%)		37.88	32.71	17.71	21.04	20.04	97.09	21.16	33.41	57.82	13.70	18.04	54.18	12.18	35.00
	LSD (0.05)		0.11	0.10	0.05	0.06	0.06	0.30	0.06	0.10	0.16	0.04	0.05	0.15	0.03	0.10

LRC - landrace; DTE - days from planting to emergency; DFSTF - days from sowing to 50% flowering; DSM - days from sowing to maturity; NPPP - number of pods per plant; NSPP - number of seeds per pod; NSPP - number of seeds per plant; PWPP - pod weight per plant; PH - plant height; NOB - number of branches per plant; PL - pod length; PW - pod width; BY - biological yield; HI - harvest index (%);NA - Not available; GK - Genebank ofKenya.

4.3.2 Analysis of varience on agronomic characteristics of the common bean landraces

The analysis of variance in the present study showed that there were highly significant ($P \leq 0.001$) differences among the common bean landraces for all the 14 agronomic traits (Table 4.3). The coefficients of variation were generally low except for biological yield (60.66). The range and mean values for the 14 traits are presented in Table 4.3.

Table 4.3: Mean values, coefficients of variation, ranges and mean squares from a combined analysis for 14 agronomic traits of 52 common bean landraces

Traits	Mean	Coefficient of variation (%)	Range		Mean square		P value	Least significant difference
			Minimum	Maximum	Between landraces	Error		
PH (cm)	83.26	4.51	36.00	186.00	2983.23	1441.30	<0.001	6.09
NOB	5.50	36.63	3.00	17.00	9.71	414.04	<0.001	3.26
DTE	7.55	14.48	4.60	13.10	5.35	121.65	<0.001	1.76
DFSTF	40.23	7.17	32.00	102.00	215.63	851.84	<0.001	4.70
DSM	63.00	6.57	50.00	154.00	478.41	2018.51	<0.001	7.20
NPPP	31.31	8.91	12.00	244.00	2772.44	793.64	<0.001	4.51
NSPP	6.08	27.64	0.80	11.00	4.95	287.72	<0.001	2.72
WHSPP	50.45	5.86	20.00	113.00	852.66	893.72	<0.001	4.80
NSPP	170.82	3.55	65.00	698.00	29264.55	3755.96	<0.001	9.83
PL	11.65	10.42	8.00	16.00	7.63	150.18	<0.001	1.96
PW	1.47	9.15	0.80	2.7	0.21	1.85	<0.001	0.22
GY	80.33	5.00	26.00	329	5683.92	1645.86	<0.001	6.51
BY	274.76	60.66	101.00	2296	109105.30	2822474.91	<0.001	269.40
HI	0.30	19.82	0.1	0.586	0.03	0.36	<0.001	0.10

PH - plant height; NOB - number of branches per plant; PL - pod length; PW - pod width; PS - plant size; BY - biological yield; GY grain yield; HI - harvest index; NSPP - number of seeds per plant; WHSPP - weight of 100 seeds per plant; NSPP - number of seeds per pod; DFSTF - days from emergence to flowering; DSM - number of days from sowing to maturity; NPPP - number of pods per plant

4.3.3 Phenotypic and genotypic variability and estimation of genotypic and phenotypic coefficient of variation

The extent of variability in respect of phenotypic and genotypic variances and phenotypic and genotypic coefficients of variance (PCV and GCV) for the yield determining quantitative characters studied is represented in Table 4.4. In the present study, the highest genotypic variance were observed for days to maturity (59.44) and number of seeds per plant (31.13) while the lowest genotypic variance were found for pod length (2.57), pod width (2.55) and pods per plant (2.14). The highest phenotypic variances were for days to maturity (76.34) followed by seeds per plant (51.73) while the lowest were for pod width (3.02) and pod length (3.17). The genotypic coefficients of variation (GCV) ranged from 1.00% for biological yield to 84.69% for pod width, while phenotypic coefficients of variation (PCV) ranged from 2.34% for biological yield to

96.68 for pod width. Moreover, moderate GCV and PCV were observed (>10%) in the traits for yield. Moderate (29.46) and high (75) GCV were also recorded for number of seeds per pod and number of branches respectively, while PCV values were 35.12 and 84.40 for number of seeds per pod and number of branches respectively. The lowest GCV were recorded for biological yield (1.00) and grain yield (2.21) while PCV values were 2.34 and 2.72 for the same variables respectively (Table 4.4).

Table 4.4: Estimation of genetic variables of the 14 agronomic traits of 52 common bean landraces

Traits	Mean of trait (GM)	Genotypic variance (GV)	Phenotypic variance (PV)	Genotypic coefficient of variation(%) (GCV)	Phenotypic coefficient of variation(%) (PCV)	Heritability (H^2) (%)	Genetic advance (GA)	Genetic advance as percentage of mean (GAM)
PH	83.25	20.34	24.56	5.41	59.52	81.97	78.13	93.84
NOB	5.50	17.02	21.55	75.00	84.40	78.97	5.36	97.45
DTE	7.54	16.43	18.76	53.75	57.44	87.57	6.78	89.92
DFETF	40.27	12.46	16.0	8.76	10.00	77.87	5.94	14.75
DTM	67.71	59.44	76.34	11.38	13.00	77.86	13.84	20.44
NPPP	31.31	2.14	3.21	4.67	5.72	66.66	16.02	51.16
NSPP	6.10	3.23	4.59	29.46	35.12	70.37	1.78	29.20
WHSPP	50.45	5.44	6.31	4.62	5.00	86.21	39.16	77.62
NSPP	170.81	31.13	51.73	3.26	4.21	60.20	92.71	54.27
PL	11.64	2.57	3.17	14.00	15.29	81.07	2.51	21.56
PW	1.47	1.55	2.02	84.69	96.68	84.43	1.22	83.00
GY	80.33	3.18	4.79	2.21	2.72	66.38	25.37	31.60
BY	274.22	7.43	9.45	1.00	2.34	78.62	27.84	10.15

PH - plant height; NOB - number of branches per plant; PL - pod length; PW - pod width; PS - plant size; BY - biological yield; GY grain yield; HI - harvest index; NSPPL - number of seeds per plant; WHSPP - weight of 100 seeds per plant; NSPP - number of seeds per pod; DFETF - days from emergence to flowering; DSM - number of days from sowing to maturity; NPPP - number of pods per plant.

4.3.4 Heritability and geneticadvance

Broad sense heritability and genetic advance values are presented in Table 4.4. Heritability in broad sense estimates of the 13 quantitative traits ranged from 60.20% for number of seeds per plant to 87.57 for days to emergence. Genetic advance varied from 1.78 for number of seeds per pod to 92.71 for number of seeds per plant. All the traits showed a relatively high heritability values (>60%). However, almost all variables recorded moderate to low genetic advance (<60%) except values for plant height (78.13) and number of seeds per plant (92.71), (Table 4.4).

4.3.5 Association among the agronomic trait components

The genotypic correlation coefficients were significant (Table 4.5). The highest positive correlation (highly significant P≤0.01) was between number of pods per plant (r=0.97) and days from emergency to flowering, closely followed by biological yield (r = 0.96) and days from emergency to flowering. Seed yield per plant showed significant (P≤0.01) positive correlation with plant height (r = 0.68), days from planting to 50% flowering (r=0.68), number of pods per plant (r=0.67), and biological yield (r=0.68). Plant height was observed to have a highly significant (P≤0.01) and positive correlations with days from sowing to flowering (r = 0.68), number of pods per plant (r = 0.67), grain/seed yield (r = 0.68) and biological yield (r = 0.68) but low and non-significant correlation with days to emergence (r = 0.22), weight of hundred seeds per plant (r = 0.34), pod length (r = 0.35) and pod width (r = 0.36) as estimated from the pooled analysis. Number of branches per plant revealed a fairly low to medium correlation with all traits ranging from r = 0.31 for number of seeds per pod to r = 0.47 for pod length. Harvest Index expressed significant (P≤0.01) and positive correlation with number of seeds per pod (r = 0.61) but had low and non-significant correlation with days from emergence to flowering (r = 0.26) and biological yield (r =0.18).

Table 4.5: Associations among the 14 quantitative traits by non-parametric spearmen correlation coefficient

	PH	NOB	DTE	DFETF	DSM	NPP	NSPP	WHSPP	NSPP	PL	PW	BY	HI	GY
PH	1.00	0.27	0.22	0.68**	0.45**	0.67**	0.52**	0.34*	0.41**	0.35*	0.36*	0.68**	0.35*	0.68**
NOB		1.00	0.37*	0.40**	0.34	0.44**	0.31*	0.46**	0.45**	0.47**	0.43**	0.43**	0.38*	0.45**
DTE			1.00	0.23	0.33*	0.26	0.21	0.44**	0.18	0.50**	0.46**	0.25	0.43**	0.30*
DFETF				1.00	0.40**	0.97**	0.38*	0.27	0.46**	0.36*	0.28	0.96**	0.26	0.88**
DSM					1.00	0.43**	0.35*	0.30*	0.35*	0.26	0.32*	0.44**	0.28	0.40**
NPP						1.00	0.32*	0.28	0.52**	0.36*	0.34*	1.00	.281	0.93**
NSPP							1.00	0.01	0.48**	0.25	0.30*	0.27	0.61**	0.43**
WHSPP								1.00	0.27	0.70**	0.83**	0.36*	0.46**	0.43**
NSPP									1.00	0.36*	0.28	0.48**	0.46**	0.55**
PL										1.00	0.71**	0.38*	0.55**	0.50**
PW											1.00	0.40**	0.33*	0.40**
BY												1.00	0.18	0.90**
HI													1.00	0.58**
GY														1.00

* and ** mean significant at P = 5% and 1%; PH - plant height; NOB - number of branches per plant; PL - pod length; PW - pod width; PS - plant size; BY - biological yield; GY - grain yield; HI - harvest index; NSPP - number of seeds per plant; WHSPP -weight of 100 seeds per plant; NSPP - number of seeds per pod; DFETF - days from emergence to flowering; DSM - number of days from sowing to maturity; NPPP - number of pods per plant

4.3.6 Cluster analysis

Cluster analysis based on the 14 agronomic traits grouped the 52 common bean landraces into four distinct clusters (Figure 4.1 and Table 4.6). Cluster I was the largest constituting 36.5% of the total landraces. This cluster consists of landraces with the smallest number of branches and had the minimum number of days to emergence, flowering and maturity. The landraces in cluster I were also characterized by fewer numbers of pods and seeds per plant which resulted in low grain yield compared to other clusters. Clusters II and III constituted 34.6% and 15.38% of the landraces, respectively. The landraces in clusters II and II were characterized by intermediate number of pods per plant and a relatively large number of seeds per pod. However, landraces in cluster II had a higher biological yield and produced more seeds per plant than cluster III (Table 4.6). Landraces with the large seeds, seed weight, pod length and width but a low number of pods per plant were grouped in cluster IV which constituted 11.5% the total number (Table 4.7). Landrace LRC008 was clustered as an outgroup and was characterized with tall and large plants which recorded a higher number of pods per plant and medium seed size but a lower number of seeds per pod. The landrace had the longest period from planting to maturity as well as the largest biomass, although the harvest index (HI) was low.

Figure 4.1: Dendrogram showing relationship among 52 common bean landraces based on 14 agronomic traits using UPGMA method.

Table 4.6: Summary of main characteristics of the clusters of common bean landraces

Cluster number	**Number of landraces (percentage)**	**Landraces**	**Unique agronomic traits of the landraces**
I	19 (36.5%)	LCR003, LCR018, LCR024, LRC006, LCR005, LCR017, LCR001, LRC004, LRC025, LCR 026, GK030260, GK030200, GK030194, GK030244, GK030198, GK030239, GK030210, GK030227, and GK036522	Fewer number of branches, pods, seeds; earlier emergence, flowering and maturity; low yield
II	18 (34.6%)	LCR014, LCR010, LCR021, LCR015, LCR022, LCR002, LCR023, LCR011, GK036528, GK030171, GK030259, GK030178, GK030211, GK036524, GK030261, GK030167, GK030246 and GK030249	Intermediate number of pods per plant; a relatively large number of seeds per pod; high biological yield; a higher number of seeds perplant
III	8 (15.38%)	LCR011, LCR009, LCR013, LRC012, LRC020,GK036527, GK030204 and GK036526	Medium number of pods perplant and a large number of seeds per pod
IV	6 (11.5%)	LRC007, LRC016, LRCO19, GK036530, GK030180 and GK030257	Large sized seeds, seed weight, pod length and width but low number of pods per plant
Outgroup		LRC 008	Long period from planting to maturity, large biomass, high yield, many pods and branches.

Table 4.7: Cluster means for fourteen agronomic traits in 52 common bean landraces

Traits		Means of clusters			
		I	II	III	IV
1	Plant height(PH)	67.82	94.43	78.71	87.71
2	Number of branches (NOB)	4.63	5.23	5.50	7.66
3	Days to emergence (DTE)	8.25	6.85	7.90	7.11
4	Days from sowing to 50% flowering (DFETF)	38.98	40.32	39.33	36.25
5	Days from sowing to maturity (DSM)	65.31	68.03	65.90	65.63
6	Number of pods per plant (NPPP)	23.19	31.53	28.25	26.00
7	Number of seeds per pod (NSPP)	5.22	6.91	6.77	5.52
8	100 seed weight(WHSPP)	53.62	42.00	48.35	69.16
9	Seeds per plant (NSPP)	114.08	208.37	182.06	136.26
10	Pod length (PL)	11.60	10.79	13.06	21.41
11	Pod width (PW)	1.50	1.32	1.47	1.80
12	Grain yield (GY)	60.67	84.58	85.59	82.14
13	Biological yield (BY)	244.34	249.01	233.73	276.46
14	Harvest index (HI)	0.24	0.34	0.36	0.29

4.3.7 Principal component analysis(PCA)

Principal component analysis (PCA) of the quantitative data was performed to determine the importance of different traits in explaining the variations among the landraces (Figures 4.2 and 4.3). In the principal components analyses of the 52 common bean landraces performed using 14 agronomic traits, the first principal component (F1) and the second principal component (F2) accounted for 29.33 and 19.27 %, respectively, of the total variation (48.60%). Trait Eigen vectors indicated that F1 was mainly a positive indicator of biological yield (274.22gm), grain yield (80.33gm), number of pods per plant (31.31), and of characteristics contributing to high to medium-term biological yield and high seed yield (Figure 5.2). F2 was mainly a positive indicator of earlier days to emergency (7.54 days) and maturity (51.31days) and characteristics with low harvest index. Accordingly the first two principal

components revealed that the landraces were scattered in all the quarters (Figure 4.3), which showed the high level of genetic diversity in the evaluated genotypes.

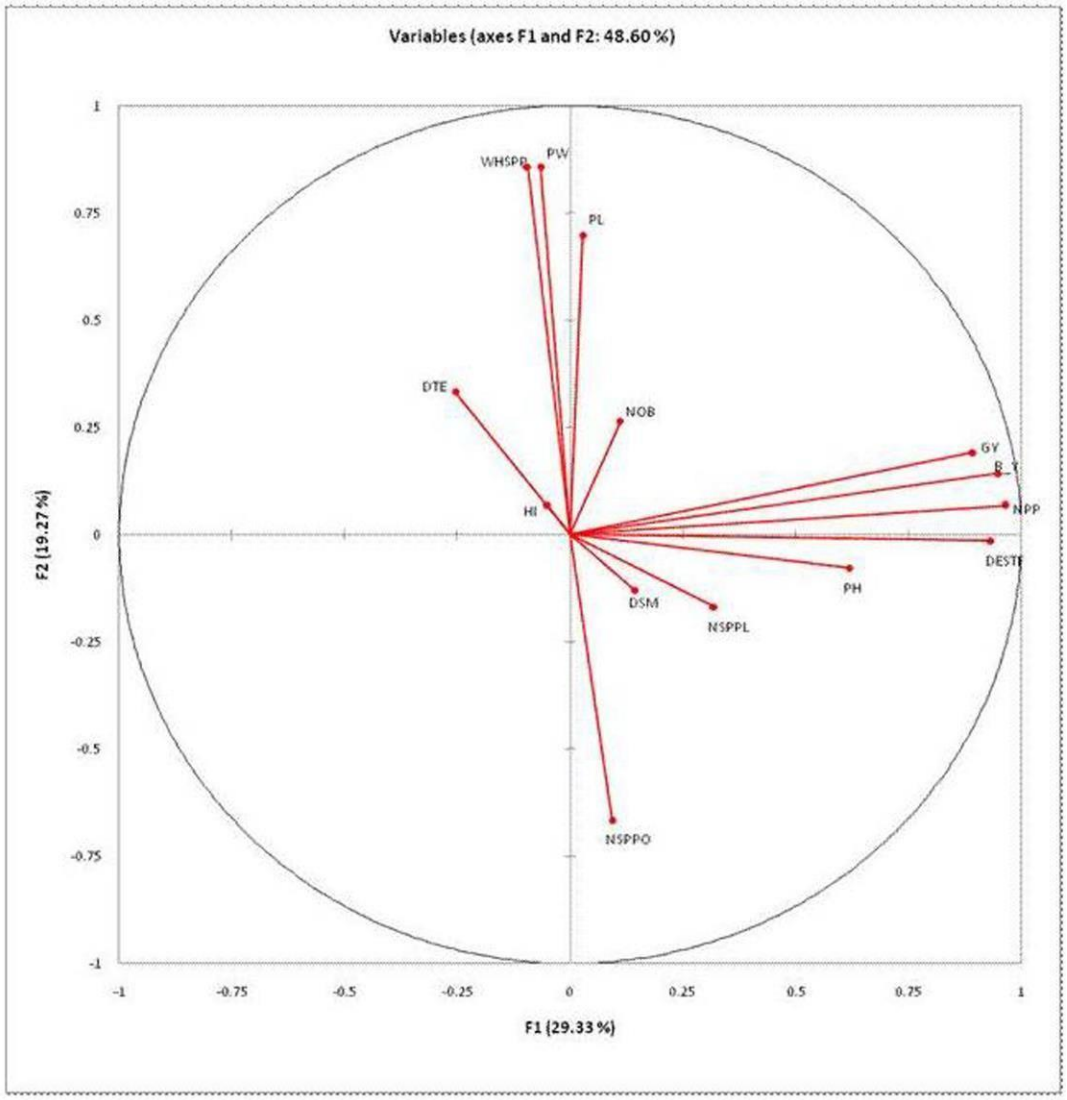

Figure 4.2: Two dimensional ordinationof 14 agronomic traits in common bean landraces based on principal component analysis. PH - plant height; NOB - number of branches per plant; PL - pod length; PW - pod width; PS - plant size; BY - biological yield; GY - grain yield; HI - harvest index; NSPP - number of seeds per plant; WHSPP - weight of 100 seeds per plant; NSPP - number of seeds per pod; DFETF - days from emergence to flowering; DSM - number of days from sowing to maturity; NPPP - number of pods per plant. F1 and F2 = Principal component 1 and 2, respectively.

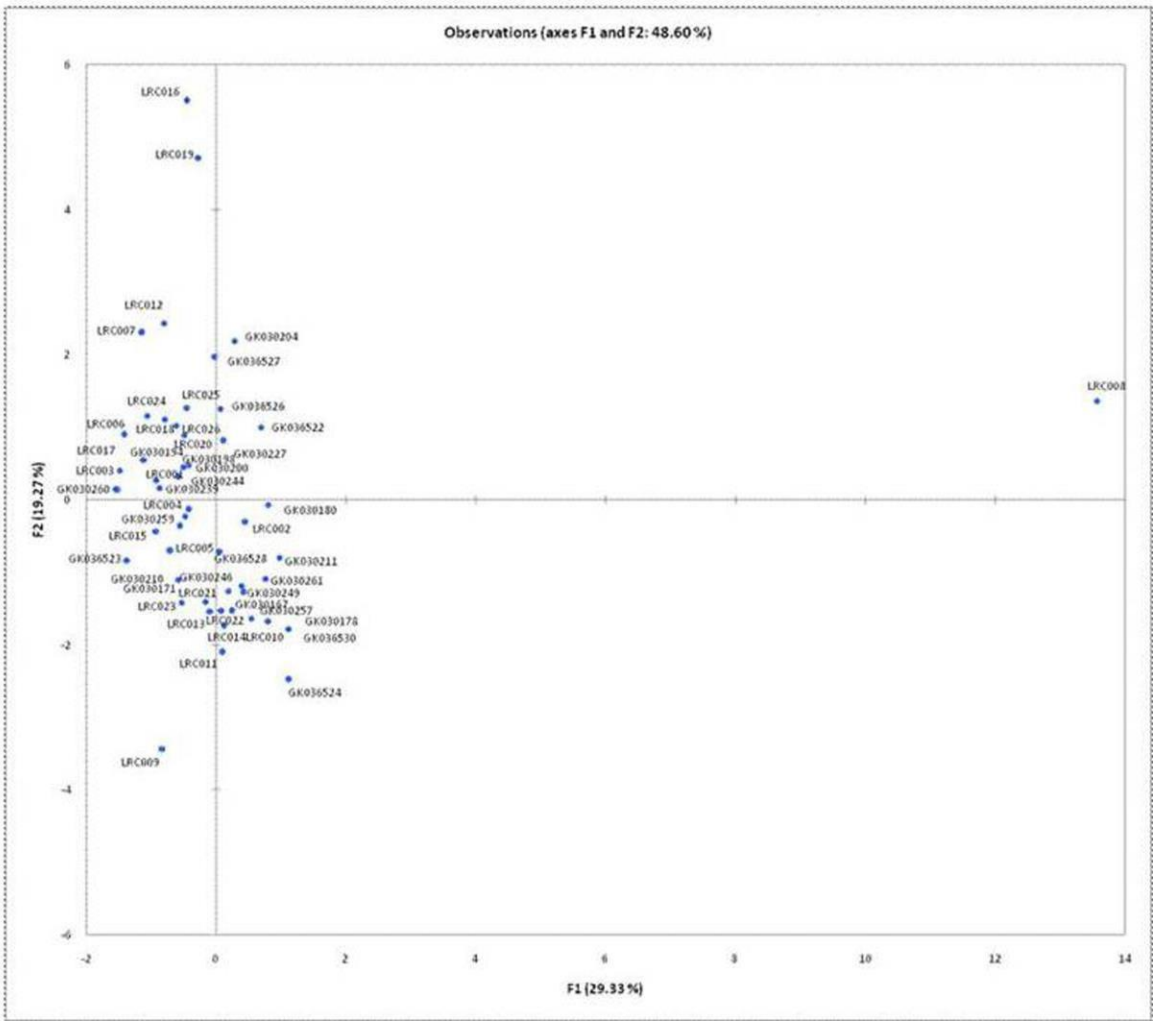

Figure 4.3: Biplot of first and second principal components in 52 common bean landraces

4.4 Discussion

Breeding programs aimed at crop improvement requires heritable variation in important agronomic traits of the crop. The efficacy of selection depends upon the magnitude of genetic variability for yield and yield contributing traits in the breeding material. The knowledge of heritability and genetic advance guides the breeder to select superior parents to initiate an effective and successful crossing program (Johnson *et al*., 1955). Therefore, the available geneticvariation, heritability and expected genetic gain in important agronomic characters are useful to design better and effective breeding strategies in common bean landraces. In the present study, all the fourteen agronomic traits showed highly significant ($P<0.05$) variations indicating the presence of sufficient amount of genetic variability among the landraces for all the studiedtraits.

In common bean genotypes, significant variations have been previously reported for various agronomic traits (Amanulla *et al.,* 2016; Salehi *et al*., 2008; Nechifor *et al*., 2011; Fivano, 2011).

Knowledge about the variability using parameters like genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) is of paramount importance for an effective breeding program in crops like common bean. According to Miklas *et al*. (2006), genotypic and phenotypic coefficients of variation values are categorized as low (<10%), moderate (10–20%), and high (>20%). In this study, based on the classification, high and close values of PCV and GCV were recorded for pods per plant, seeds per pod, 100 seed weight, seeds per plant, grain yield and biomass yield, which suggest the potential variability available in the landraces for these traits for effective selection and improvement as there was minimal influence of environment. Similar results were also reported by Stoilova *et al.* (2004) for clusters per plant, seed yield per plant, and biological yield per plant. Atuahene-Amankwa et al., (1997) also reported high GCV and PCV for plant height, primary number of branches per plant, days to maturity indicating the predominance of additive gene action. Nechifor *et al*. (2011) also reported high genetic variability for numbers of pod per plant and weight of pods per plant in common beans. Stoilova *et al*. (2004) performed a field trial of 42 germplasm of exotic beans at the valley of Kashmir in order to obtain superior genotype of beans under temperate condition and the findings from their study showed medium genetic variability for days of flowering and days of harvesting and low genetic variability for early flowering and earlymaturity.

In the present study, the phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits. This would be due to the fact the variation at the phenotypic level was due to the effect of genotypes and influence of environment as reported by Singh and Chaudhary (2009). Moderate values of GCV and PCV were observed in the present study for some traits including plant height and number of branches. Low GCV and PCV were observed for days to 50% flowering, days to maturity. Phenotypic coefficient of variation was higher than the corresponding genotypic coefficient of variation for all the traits except days to emergence and 100 seed weight. However, the differences between PCV and GCV were small. The narrow differences between PCV and GCV for most of the traits indicate low effect of environmental influence on the expression of these traits. These findings are in agreement with

Salehi *et al*. (2008) who reported narrow differences between PCV and GCV on the study of interrelationship between different traits in common bean.

The heritability estimates help the breeders in selection based on the basis of phenotypic performance. According to Robinson *et al.* (1949), heritability can be classified as low (0-30%), moderate (30-60%) and high (>60%). Most traits showed a high heritability values (>60%) except number of pods per plant and biological yield which were moderate. Similar findings were also reported by Singh *et al.* (2015) in pea crop. Salehi *et al*. (2008) also reported similar results for yield component traits which included number of pods per plant, 100-seed weight and number of seeds per pod in commonbean.

However, when heritability is coupled with genetic advance (GA) together with GCV provides the best prediction of expected gain through phenotypic selection than heritability alone. Estimates of all these parameters help to understand the type of gene action involved in the expression of traits especially for polygenic traits. Johnson *et al*. (1955) suggested genetic advance as percent mean can be categorized as 0 – 10% for low, 10 – 20% for moderate and >20% for high. In the present study, the genetic advance as percent of mean ranged from 10.15% for biological yield to 97.45% for number of branches. High heritability coupled with high genotypic coefficients of variation (GCV) and high genetic advance as percentage of mean were recorded by plant height followed by number of branches and days to emergence which indicates that the traits were simply inherited in nature and possessed additive gene effects. These traits can be considered as favorable for common bean improvement through effective phenotypic selection of these traits and high expected genetic gain from selection for these characters can be achieved. Similar results were reported by Dursum (2007) who tested the variability, heritability and co-relation studies of 40 common bean genotypes. However, high heritability and GA (%) along with low GCV for days to flowering and maturity indicates that expression of these traits is under the involvement of non-additive gene action and phenotypic selection of these traits might not be effective.

Grain yield is a complex character which is as a result of many yield contributing traits, which are in turn influenced by the environment and genotype. Consequently, the direct evaluation and

improvement of grain yield itself may be misleading due to involvement of environmental component. Therefore, to assess the magnitude of correlations for various traits with yield would be immense help in the indirect selection for the improvement of yield. The correlation coefficients of yield and its components determined in the present study indicated that most of the traits studied were positively and significantly correlated with yield. Significant and positive correlation of seed yield/plant was found with plant height, days from sowing to 50% flowering, number of pods/plant and biological yield. These findings are in agreement with previous study in common beans by Dursun (2007) who reported positive and significant correlation of seed yield/plant with number of pods/plant. Valenciano *et al.* (2006) also reported significant positive correlation of pod weight with seed yield and length of pods, number of pods with seed weight/plant, number of pods/plant with number of pod bearing nodes. This study also showed that plant height at maturity was positively and significantly correlated with days from sowing to flowering, number of seeds/plant, seed yield and biological yield. This is in agreement with the findings of Pereira *et al*. (2005) who reported significant positive correlation of plant height with seeds/plant. However, our results contradicts reports by Stoilova *et al*. (2013) and Singh *et al.* (2001) who found negative correlation of plant height with seed yield. This deviation may be attributed to the differences in genotypes and effect of the experimental conditions (Pereira *et al*., (2005).

Cluster analysis based on fourteen agronomic traits grouped 52 common bean landraces into four different clusters indicating that the landraces exhibited notable genetic divergence in terms of agronomic traits. Formation of different clusters using agronomic characters in diverse common bean genotypes has also been reported (Nechif *et al*., 2011). The maximum inter-cluster distance was recorded between cluster I and the out-group (LCR 008) followed cluster II and the out-group, suggesting wide diversity among these groups. On the other hand, the minimum distance between cluster IV and the out-group and cluster I and II indicates their close relationship. Essentially, crossing of genotypes belonging to the same cluster is not expected to generate superior hybrids or segregants, because genotypes grouped in the same cluster diverge little from one another(Nechif *et al*., 2011). However, the larger the divergence between the genotypes, the higher will be the amount of heterosis in F1 progeny and subsequent generations (Negri and Tosti, 2002). It may be useful to produce crosses between genotypes belonging to the clusters separated by large estimated genetic

distances (Negri and Tosti, 2002). Success might therefore be expected through making crosses between the genotypes from cluster II and cluster III, followed by the one between cluster IVand the outgroup. Genotypes from these clusters can be selected for hybridization program that can evolve high heterotic crosses, which might prove potential in isolating superior hybrids. The PCA grouped the accessions into groups over the four quadrants based on the quantitative traits. The accessions remained scattered in all four quadrants, showing large genetic variability for the traits studied.

CHAPTER FIVE

5.0 Assessment of morphological diversity of common bean (*Phaseolus vulgaris* L.) landraces for yield and *Pythium* root rot disease resistance

5.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is an annual multipurpose legume, diploid (2n = 2x = 22), self-pollinating crop (Beebe *et al*., 2001) and the most widely grown pulse crop in eastern and southern Africa (CIAT, 2016). The crop has been essential in the development of several civilizations, forming part of their basic diet as sources of proteins, carbohydrates, etc. (Gómez, 2004). Moreover, common bean has played an important role in agriculture by furnishing much of the available nitrogen in cultivated soils (Beebe *et al*., 2000). It was recorded in pre-Columbian manuscripts that beans, corn, squash, chili and amaranth were subject to taxation in ancient Mexico because of their economic importance (CIAT, 2015). Biochemical evidence shows Mesoamerica to be one of the probable centers of origin and domestication of *Phaseolus*, as does the existence of wild ancestors from northern Mexico to northern Argentina (Beebe *et al*., 2014).

From the 45 species of the genus *Phaseolus*, *P*. v*ulgaris* is the most common because it appears to possess greater plasticity under selection (Nasa, 2012). Wild species and local cultivars of the genus *Phaseolus* are still used for food, medicine, forage, ornament, and fermentation (Papa *et al*., 2007). Mexico harbors the greatest diversity of the genus *Phaseolus*. It has vast genetic variability in the form of varieties tolerant to drought, heat, cold, alkalinity, viruses and pests (Papa *et al*., 2007). Common beans are by far the most important pulse crop in Kenya. The cultivated area is difficult to estimate accurately but is probably about 1 million acres (400 000 hectares), mostly intercropped with maize. In south western Kenya as most parts of Africa, diseases are estimated to be the second most important constraint to bean production after low soil fertility (CIAT, 2013). Most destructive diseases are caused by fungal, bacterial and viral causal agents. Different *Pythium* spp. cause seed decay, pre-emergency and post-emergency on beans genotypes, likewise infected common beans seedlings typically become discolored, chlorotic and soft and decayed even if they germinate and they wilt or even die within 1 - 3 weeks (Nzungize *et al*., 2011). Different screening methods found that beans cultivars with colored seeds had higher

levels of resistance to this pathogen than white seeded cultivars (Lucas and Griffiths, 2004). The most effective, sustainable and environmentally safe methods of control/management of this disease is the identification and use of resistant bean genotypes (Buruchara, 2007).

Morphologically common beans differ in growth habits (Perseguini *et al*., 2011), vegetative characters, flowers, pods and seed traits (Franklin *et al*., 2009) which are useful for selection in breeding programmes. There is great variation in the growth habit; most beans grown in East Africa are determinate, with bush type growth habit, but there are also indeterminate non-climbing, semi-bush types, and indeterminate climbing types. The predominant colour of the flowers is white, although pink, purple, red and yellow are common. The greatest variation occurs in seed characteristics: many shapes, sizes, colours or combinations of colours can be found. Red, brown, purple, black and white are common colours which may occur alone, as stripes or as spots. Beans are nearly 95% self-pollinating under most conditions (Durán *et al*., 2005). The life of the crop varies from 2.5 months for determinate varieties to more than 5 months for indeterminatetypes.

The evaluation of morphological traits is a traditional, important method for the description and the determination of relationship among common bean landraces. The germplasm of different common beans from different regions has been characterized (Piergiovanni et al., 2010; Raggi et al., 2013), but despitethe long history of cultivation, the number of accessions and the presence of some well-known landraces that excel for product quality (e.g. “Onyoro”),the germplasm of the South western Kenya has been poorly investigated. Previous work on morphological characterisation, found that it is possible to deduce that traditional varieties and other selections present in the same area share a recent com-mon ancestor and should be considered a landrace group (Zeven,1997), that is either one landrace derives from another one or the andraces derive from the same parent population. Pairwise anal-ysis of Fst supported this hypothesis, since a lack of a statistically significant genetic differentiation was present only for some com-parisons between landraces with similar bean shape and colour ((Zeven,1997).

In spite of all the observed variations, the landraces grown in East Africa and particularly south Western Kenya are valuable sources of genetic variation for breeding (Blair *et al*., 2010). Unfortunately, this genetic diversity is threatened by pests and diseases (FAOSTAT, 2015; Mukankusi, 2008) and adoption of elite varieties by farmers is at the expense of the un-popular

landraces leading to genetic erosion, consequently narrowing the genetic base of common beans in Kenya. Furthermore, there is scanty documented information on current bean morphological diversity in south Western Kenya region to be used as reference on conservation and breeding programs. Thus, the objective of this study was to evaluate the morphological diversity of common bean landraces in south Western Kenya for *Pythium* root rot disease resistance, effective *In situ* and *Ex situ* conservation as well as their application in the genetic improvement of the landraces.

5.2 Materials andMethods

5.2.1 Plantmaterials

A total of 52 common bean seeds of different landraces were used for the present study; 26 were collected from farmers‘ fields in different agro-climatic zones and market centers of Kisii County, South western Kenya while the other 26 were obtained from the National GeneBank, Muguga, Kiambu (Table 3.1). These accessions were collected according to the procedure of Plant Genetic Resources International Institute (IBPGR, 2014). Collected accessions were stored in cool and dry conditions at Kenya Agricultural and Livestock Research Organization (KALRO), Kisii Station and the University of Nairobi (UoN), awaiting planting in the field for morphological characterization.

5.2.2 Description of studysite

The study area was Kisii county, south Western Kenya. The study area falls in the following Agro-Ecological Zones (AEZs): Lower Highlands one and two (LH1 and LH2), Upper Midland one (UM1), Lower Midland one and two (LM1 and LM2) which are considered agricultural high potential. The altitude range is between 1450-2210 m above sea level. The soil types found in the study area are red volcanic (Nitosols) which are deep, fertile, well-drained and good for farming accounting for 75%; Minisry of Agriculture, MoA (2015). The county has bimodal pattern annual rainfall; long rains from March to July and short rains from September to November without distinct dry spells in between the two seasons sometimes overlapping leading to continuous cropping. The rainfall ranges from 1,400 - 2,600 mm per annum. The mean annual temperature ranges are 20 - 27 °C maximum and 15 - 18 °C minimum Minisry of Agriculture, MoA, (2015), Kenya.

5.2.3 Experimental design and establishment of plants in thefield

The genotypes were evaluated in two consecutive planting seasons/years in 2015 and 2016. Each experiment was laid out as a randomized complete block design (RCBD) with three replications for each entry. The cultivars were grown in plots measuring 4 m x 3 m. Seeds were sown on raised beds with 40 cm row to row spacing and 15 cm plant to plant spacing at a depth of 5 – 7 cm. One teaspoonful of Phosphatic fertilizer, (Diammonium phosphate, DAP) was added to each hole at planting. Other normal management practices were carried out including weed control, pest/disease checks, top-dressing and spraying to control pests and diseases, MoA, (2015), Kenya.

5.2.4 Morphological measurements in fieldexperiment

Nine descriptors of common bean were evaluated according to the International Board for Plant Genetic Resources descriptor list (IBPGR, 2014) for *Phaseoulus vulgaris* L. These data were recorded at different stages of plant growth from plant emergence to seed harvest. Data were recorded on a plot basis, 10 individual plants selected randomly from the two central rows of each plot. Measurement unit and measurement/sampling procedure of each trait are given in Table 5.1. The morphological characteristics measured were: SSH- seed shape, FC-flower colour, GT-growth type, PS-plant size, COP-colour of pod, SSZ- seed size, GH-growth habit, CSC-commercial seed colourandCS seedcolour.The weight of seeds was measured and classified as small seeds for < 25 g, medium seeds for between 25-40 g and large seeds for > 40 g.

Table 5.1: Variable sets and 9 observed common bean morphological characters, measurement procedures and units

Variable (plant organ)	Character	Code	Measurement unit and measurement/ sampling procedure
Seed	Shape of seed	SSH	4 scores from 1 to 4: 1 = Oblong, 2 =Oval, 3 = Cuboid, 4 = Kidney; assessed at maturity,10 seedsper genotype
	Size of seed	SSZ	3 scores from 1 to 3: 1=Small(< 25 gm), 2=Medium (25-40 gm), 3=Large (> 40 gm); assessment done at maturity, 10 seeds per genotype
	Colour of seed	CS	13scoresfrom1to13:1=maroon,2=cream,3= brown, 4 = white, 5 = black, 6 = red, 7 = yellow 8 = green, 9 = orange, 10 = mottled, 11=stripped, 12 = marbled, 13 = speckled;— assessed at harvest, 10 seeds per genotype
	Commercial seed colour	CSC	2 assessment scores, 1-2: 1 = Uniform, 2 = De-uniform; done at maturity, 10 seeds per genotype
Pod	Colour of maturepod	COP	8 scores from 1 to 8: 1 = yellow, 2 = brown, 3 =grey;4 =maroon,5=purple,6=red,7=orange,8=cream — assessed at physiological maturity; 10 plants within plot centre
Flower	Flower colour	FC	5 scores used to assess this trait; 1 = lilac, 2 = white, 3 = violet, 4 = red, 5 = pink; assessed on fully open flowers; 10 plants per genotype
Stem	Growth habit	GH	4 scores from 1 to 3: 1 = Bush, 2 = Erect, 3 = Prostrate, 4 = Climbing; 10 plants per genotype assessed at physiological maturity
	Growth type	GT	3 scores from 1 to 3: 1 = Determinate, 2 = Semi-determinate, 3 = Indeterminate; 10 plants per genotype assessed at physiological maturity
	Plant size	PS	3 scores from 1-3, weight of entire plant/biological yield, assessed at physiological maturity; 1 = small(< 200 gm), 2 = medium (200-300 gm), 3 = large (> 300 gm); 10 plants per genotype

5.2.5 Pythium root rot disease assessment in theglasshouse

The *Pythium* spp. isolate was obtained from symptomatic bean plants collected from farmers' fields in south Western Kenya. The uprooted bean plants were washed with running water and stem bases were cut off, surface sterilized with 1.5% solution of sodium hypochlorite for 30 seconds and then rinsed three times with sterile distilled water and blot dried. The cut stem

tissues were plated on PDA media supplemented with 50 ppm streptomycin and incubated for 7 days at 24 °C. The *Pythium* spp. was identified based on morphological and cultural features and confirmed using fungal identification keys as described by Watanabe (2010).

To prepare *Pythium* inocula, three day old, actively growing hyphal regions measuring 4 mm^2 were aseptically cut and grown on autoclaved millet seeds. The culture of *Pythium* spp. was then mixed with pre-sterilized loam soil in a ratio of 1:8 v/v in wooden flats measuring 48 cm x 72 cm and the inoculum was allowed to establish for a period of 14 days in the dark. Two rows of bean seeds (10 seeds per landrace) were planted in the wooden flats and each treatment replicated thrice. The control experiment contained seeds sown in sterilized loam soil without *Pythium* inoculum. Replications were set using the standard randomized complete block design (RCBD). The experiment was repeated twice.

5.2.6 Statistical dataanalysis

Estimate of Shannon-Weaver diversity index**:** Shannon‘s information index (*H*‘, Shannon, 1949) as a measure of qualitative morphological trait diversity across common bean accessions was calculated for each trait as follows:

Shannon $H' = -\Sigma\, pi \ln pi$

ni– number of individuals in the *i*-th class

pi– proportional abundance of the *i*-th class = ni / N

Cluster analysis: All the morphological observations were grouped by cluster analysis using the unweighted pair group method analysis (UPGMA) based on the similarity matrix of euclidean distances of the morphological data. To trace the relationship among the common bean landraces, the data were standardized before clustering and a dendrogram was constructed. The statistical analyses were performed using MEGA software (Tamura *et al*., 2007). Euclidean or straight-line measure of distance was used for estimating genetic distance (GD) among accessions (Mohammadi and Prasanna,2003).

Principal component analysis: Principal component analysis was performed using SAS procedure PRINCOM (SAS, 2014). Correlations of all traits with the first two principal components were calculated using the SAS procedure CORR, (SAS, 2014) using the Pearson correlationcoefficient.

Disease Evaluation: Root rot severity was measured using a 1 to 9 scale (1= no symptoms, 9=extreme severity). Among the variables taken into account were initial plant stand, number of dying plants two weeks after germination and foliage chlorosis. Plants were evaluated for vigor 17 day after planting and again at flowering.

5.3 Results

5.3.1 Qualitative character variability/phenotypic character distribution

The pattern of variability of the 52 landrace accessions was determined by analyzing the percentages of observed frequencies on a character-by-character basis (Table 5.2). Individual characters differed in their patterns of distribution and amount of variation among the 52 landraces.

Table 5.2: Frequencies of different levels of morphological descriptors in 52 common bean landraces from South Western Kenya

Trait	Labels	Frequency	%
Seed coat colour	1=Maroon	7	13.46
	2=Cream	3	5.76
	3=Brown	5	9.61
	4=White	2	3.84
	5=Black	4	7.78
	6=Red	6	11.53
	7=Yellow	1	1.92
	8=Green	1	1.92
	9=Orange	1	1.92
	10=Mottled	9	17.30

	11=Stripped	5	9.61
	12=Marbled	5	9.61
	13=Speckled	6	11.53
Seed shape	1=Oblong	19	36.53
	2 = Oval	12	23.07
	3 = Cuboid	2	3.84
	4 = Kidney	19	36.53
Flower colour	1=Lilac	20	38.46
	2=White	22	42.30
	3=Violet	4	7.78
	4=Red	1	1.92
	5=Pink	5	9.61
Growth type	1=Determinate	31	59.61
	2=Semi-determinate	15	28.84
	3=Indeterminate	6	11.53
Plant size	1=small	9	17.30
	2= medium	34	65.38
	3=large	9	17.30
Pod colour	1 = yellow	13	25.00
	2 = brown	4	7.78
	3 = grey	3	5.76
	4=maroon	5	9.61
	5=purple	4	7.78
	6=red	9	17.30
	7=orange	5	9.61
	8=cream	9	17.30
Seed size	1=Small	17	32.69
	2=Medium	33	63.46
	3=Large	2	3.84
Growth habit	1=Bush	24	46.15

	2=Erect	6	11.53
	3=Prostrate	8	15.38
	4=Climbing;	14	26.92
Commercial seed colour	1=Uniform	27	51.92
	2=De-uniform	25	48.07

Seed coat colour: Table 5.2 shows that mottled landraces had the highest frequency (17.30%) followed by maroon (13.46%) red and speckled (11.53), while the lowest frequency were from green and yellow landraces (1.92% each).

Seed shape: Oblong and kidney seed shapes were more abundant among the investigated landraces (36.53% each) while 2 landraces with cuboidal shape had the lowest (3.84%).

Flower colour: The 52 landraces produced 5 different coours of petals as shown in Table 5.2. Red flowers were produced by only one landrace (1.92%) and were lowest, while the most abundant flower colours were white (42.30%) and lilac (38.46%).

Seed size: Table 5.2 shows that the majority of landraces were medium sized (33), constituting 63.46% of the total. Large sized seeds were only 2 (3.84%) while small sized landraces were 17 (32.69%).

Growth habit: Most landraces investigated had bush habits (46.15%), while erect and prostrate had the lowest (11.53% and 15.38%, respectively)

Commercial seed colour: The frequency of uniformly coloured seeds was almost equal to those of de-uniform seeds i.e 27 and 25 (51.92% and 51.92%), respectively, (Table5.2).

Pod colour: At physiological maturity, yellow coloured pods were more abundant (25%) among the landraces, while grey and brown were rare (5.76 and 7.78% respectively). The other colours that featured include maroon (9.61%), purple (7.78%), red (17.30%), orange (9.61%), cream (17.30) as shown in Table 5.2.

Plant size: Table 5.2 shows that majority of landraces were medium sized (63.46%). Small and large sized plants were only 17 and 2 (32.69% and 3.84%).

Growth type: There were 3 types of growth as indicated in Table 5.2. Determinate landraces were more abundant (59.61%) followed by semi-indeterminate (28.84%) while indeterminate genotypes were the least abundant (11.53%).

5.3.2 Diversityindex

Estimates of Shannon Weaver diversity index showed high diversity index for the 9 qualitative characters studied (Table 5.3). Generally the diversity indices of all evaluated traits were above 0.400, except for commercial seed colour, (0.321), indicating the presence of adequate variability for these traits among evaluated landraces. Phenotypic diversity was very high for seed color (H′ =0.879 followed by pod colour (0.787) and flower colour (0.721). Table 5.4 also shows that mean diversity index for the morphological traits was relatively high (0.614).

Table 5.3: Shannon-weaver diversity indices for nine qualitative morphological traits of 52 common bean landraces

Serial No.	Trait	Code	i	Trait states	H′
1	Seed coat colour	SC	13	7-3-5-2-4-1-1-1-9-5-5-6	0.879
2	Seed shape	SSH	04	19-12-02-19	0.685
3	Flower colour	FC	05	20-22-4-1-5	0.721
4	Growth type	GT	03	31-15-6	0.514
5	Plant size	PS	03	9-34-9	0.478
6	Pod colour	COP	08	13-4-3-5-4-9-5-9	0.787
7	Seed size	SZ	03	17-33-2	0.454
8	Growth habit	GH	04	24-6-8-14	0.563
9	Commercial seed colour	CSC	02	27-25	0.321
	Mean diversity index (H′)				0.614

5.3.3 Correlation between morphological traits and grainyield

Majority of landraces had grain yield ranging from of 50 – 100g (Table 5.4). The highest yield recorded was 324 grams for genotype LRC 008, which is indeterminate. The lowest yield scores were 30.74, 31.00 and 31.36 grams recorded for genotypes LRC 013, GK030210 and LRC 009, respectively. The table also indicates that indeterminate, prostrate growing landraces had relatively higher yields than other landraces

Table 5.4: Summary of the 9 qualitative characteristics and associated grain yield of the 52 common bean landraces

Entry	Code	Morphological characteristics									
		SSH	**FC**	**GT**	**PS**	**COP**	**SSZ**	**GH**	**CSC**	**SC**	**GY (gm)**
1	LRC 001	Oblong	White	Determinate	Medium	Yellow	Medium	Erect	Uniform	Maroon	53.60
2	LRC 002	Oblong	White	Determinate	Medium	Brown	Medium	Prostrate	Uniform	Yellow	99.96
3	LRC 003	Oval	Lilac	Determinate	Small	Grey	Medium	Erect	Uniform	Orange	47.38
4	LRC 004	Cuboidal	White	Semi determinate	Medium	Maroon	Medium	Prostrate	De-uniform	Speckled	58.91
5	LRC 005	Oval	White	Determinate	Medium	Yellow	Medium	Bush	Uniform	**Green**	35.28
6	LRC 006	Kidney	White	Determinate	Medium	Grey	Medium	Erect	De-uniform	Marbled	56.24
7	LRC 007	Kidney	White	Determinate	Medium	Purple	Medium	Erect	De-uniform	Marbled	62.10
8	LRC 008	Oval	Violet	Indeterminate	Large	Grey	Medium	Climber	De-uniform	Stripped	324.30
9	LRC 009	Oval	White	Semi indeterminate	Medium	Red	Small	Erect	Uniform	Brown	31.36
10	LRC 010	Oblong	Lilac	Determinate	Medium	Yellow	Small	Bush	Uniform	Brown	82.88
11	LRC 011	Oblong	White	Determinate	Medium	Orange	Small	Bush	Uniform	White	88.20
12	LRC 012	Kidney	Lilac	Determinate	Medium	Cream	Medium	Bush	Uniform	Maroon	105.84
13	LRC 013	Kidney	White	Semi determinate	Medium	Maroon	Small	Climber	Uniform	Brown	30.74
14	LRC 014	Oblong	White	Determinate	Small	Yellow	Small	Erect	Uniform	Cream	47.88
15	LRC 015	Kidney	White	Determinate	Medium	Maroon	Medium	Bush	De-uniform	Mottled	62.70
16	LRC 016	Oblong	Red	Indeterminate	Large	Cream	Large	Climber	De-uniform	Mottled	99.51
17	LRC 017	Kidney	Lilac	Determinate	Medium	Maroon	Medium	Bush	De-uniform	Stripped	44.88
18	LRC 018	Kidney	White	Determinate	Medium	Orange	Medium	Bush	De-uniform	Stripped	59.52
19	LRC 019	Oblong	White	Indeterminate	Large	Yellow	Large	Climber	Uniform	white	77.25
20	LRC 020	Kidney	Violet	Determinate	Medium	Maroon	Medium	Climber	De-uniform	Mottled	93.60
21	LRC 021	Oblong	Lilac	Semi determinate	Medium	Purple	Small	Prostrate	Uniform	Black	63.36
22	LRC 022	Oblong	Lilac	Semi determinate	Medium	Purple	Small	Climber	Uniform	Black	68.04
23	LRC 023	Oblong	White	Semi determinate	Small	Red	Small	Bush	De-uniform	Marbled	78.72
24	LRC 024	Kidney	White	Determinate	Medium	Brown	Medium	Bush	De-uniform	Mottled	44.08
25	LRC 025	Oblong	Lilac	Determinate	Medium	Cream	Medium	Bush	De-uniform	Speckled	63.24
26	LRC 026	Oblong	White	Determinate	Medium	Cream	Medium	Bush	De-uniform	Speckled	80.64
27	GK 036527	kidney	Lilac	Determinate	Medium	Orange	Medium	Prostrate	Uniform	Brown	97.00
28	GK 036528	Oblong	Lilac	Semi determinate	Medium	Purple	Small	Climber	Uniform	Black	72.00
29	GK 036530	Oblong	Violet	Determinate	Medium	Cream	Small	Climber	Uniform	Black	91.00
30	GK 036524	Oval	White	Indeterminate	Medium	Red	Small	Climber	Uniform	Red	130.00
31	GK 030260	kidney	Pink	Determinate	Small	Yellow	Medium	Bush	De-uniform	Speckled	67.00
32	GK 030261	Oval	white	Semi determinate	Large	Red	Medium	Climber	Uniform	Maroon	102.00
33	GK 036522	kidney	Pink	Semi determinate	Large	Red	Medium	Bush	Uniform	Cream	117.00
34	GK 030211	Oval	white	Semi determinate	Large	Brown	Small	Climber	Uniform	Maroon	131.00
35	GK 030227	kidney	Lilac	Determinate	Small	Yellow	Medium	Bush	De-uniform	**Mottled**	106.00
36	GK 030239	kidney	Lilac	Determinate	Small	Cream	Medium	Bush	De- uniform	Speckled	32.00
37	GK 030244	Oval	Pink	Determinate	Medium	Red	Medium	Bush	De-uniform	Stripped	62.00
38	GK 030180	kidney	Pink	Semi determinate	Medium	Orange	Medium	Prostrate	Uniform	Maroon	100.00
39	GK 030194	Oval	Lilac	Semi determinate	Medium	Red	Medium	Bush	De-uniform	Mottled	84.00
40	GK 030198	Kidney	Lilac	Determinate	Small	Orange	Medium	Bush	Uniform	Maroon	43.00

41	GK 030200	Kidney	White	Determinate	Medium	Yellow	Medium	Bush	De-uniform	Mottled	67.00
42	GK 030204	Oblong	Lilac	Semi determinate	Medium	Yellow	Medium	Bush	De-uniform	Marbled	132.00
43	GK 030210	Oblong	Lilac	Determinate	Medium	Maroon	Medium	Bush	De-uniform	Mottled	31.00
44	GK 030167	Oblong	White	Indeterminate	Large	Brown	Small	Climber	Uniform	Red	98.00
45	GK 030171	Oval	Violet	Determinate	Small	Yellow	Small	Prostrate	Uniform	Brown	87.00
46	GK 030178	Cuboidal	White	Indeterminate	Large	Yellow	Small	Prostrate	Uniform	Cream	100.00
47	GK 036523	Oblong	Lilac	Determinate	Medium	Red	Medium	Bush	De-uniform	Marbled	56.00
48	GK 036526	Kidney	Pink	Determinate	Medium	Cream	Medium	Bush	Uniform	Maroon	106.00
49	GK 030246	Oval	Lilac	Semi determinate	Medium	Red	Small	Climber	Uniform	Red	76.00
50	GK 030249	Oblong	Lilac	Semi determinate	Large	Cream	Small	Climber	De-uniform	Stripped	78.00
51	GK 030257	Oval	Lilac	Semi determinate	Medium	Yellow	Medium	Prostrate	De-uniform	Speckled	63.00
52	GK 030259	Kidney	Lilac	Determinate	Small	Cream	Medium	Bush	De-uniform	**Mottled**	89.00

Key: **SSH**-seed shape, **FC**-flower colour, **GT**-growth type, **PS**-plant size, **COP**-Colour of pod, **SSZ**- seed size, **GY**-grain yield, **GH**-growth habit, **CSC**-commercial seed colour and **CS**- seed colour.

The results computed for the genetic distance and similarities for the 9 qualitative valuables are presented below (Table 5.5). The highest genetic distance among the morphological traits was 0.95 between growth habit (GH) and seed size (SSZ) while the lowest, 0.21, occurred between seed shape (SSH) and seed colour (SC).

Table 5.5: Genetic distance and similarity among the morphological traits

	CSC	SC	FC	GT	PS	COP	SSH	SSZ	GH
CSC	1.00	0.64	0.53	0.58	0.63	0.57	0.25	0.22	0.54
SC	0.64	1.00	0.68	0.53	0.62	0.35	0.21	0.60	0.57
FC	0.53	0.68	1.00	0.31	0.34	0.50	0.62	0.61	0.75
GT	0.58	0.53	0.31	1.00	0.30	0.32	0.37	0.32	0.65
PS	0.63	0.62	0.34	0.31	1.00	0.55	0.64	0.80	0.80
COP	0.57	0.35	0.50	0.32	0.55	1.00	0.53	0.65	0.74
SSH	0.25	0.21	0.62	0.37	0.64	0.53	1.00	0.63	0.65
SSZ	0.22	0.60	0.61	0.32	0.80	0.65	0.63	1.00	0.95
GA	0.40	0.57	0.75	0.65	0.80	0.74	0.65	0.95	1.00

Also, high values of similarity were recorded between the following pairs of traits: growth habit-GH and plant size-PS, 0.796 (79%), growth habit-GH and flower colour-FC, 0.750 (75%), seed size-SSZ and plant size-PS0.791 (79%). However very low similarity values were also recorded for the seed shape trait and many traits, notably: commercial seed colour-CSC, 0.251 (25%) and seed colour-SC, 0.210 (21%) as well as seed size-SSZ and commercial seed colour-CSC, 0.222 (22%). The correlations between the grain yield and the qualitative traits were not significant except for the correlations between the grain yield and GT and GH (r= 0.454 and 0.328 respectively) (Table 5.6).

Table 5.6: Correlations between the qualitative morphological characters and yield of common bean landraces

Morphological traits	Yield	Significance level
SSH	-0.076	0.592
FC	0.236	0.091
GT	0.454	0.001
PS	-0.262	0.060
COP	-0.036	0.797
SSZ	-0.004	0.975
GH	0.328	0.017
CSC	-0.011	0.935
SC	0.062	0.664

The lowest correlation was found with seed size, SSZ, (r=-0.004). The table indicates that four traits (seed shape SSH, plant size PS, colour of pod COP and commercial seed colour CSC) were negatively correlated. The remaining 5 qualitative traits were positively correlated.

5.3.4 *Pythium* root rot severity score andrating

Table 5.7 shows the recorded findings on the responses of each of the 51 landraces to Pythium root rot disease. The disease severity scores ranged from 2.1, rated as medium resistance for landrace LRC 008 to 7.9 for landrace GK 036526 which is rated as highly susceptible. The majority of landraces, 28 (54.90%) were susceptibe to *Pythium*; a significant number, 17 (33.33%) were highly susceptible while a relatively small number, 6 (11.76%) were exhibiting medium resistance.

Table 5.7: General means score response of 51 common bean landraces to Pythium root rot disease

GenotypeCode	**Local name**	**Disease severity score**	**Disease rating**
LRC 006	Esaitoti	6.2	HS
LRC 008	Cinchae	2.1	MR
LRC 018	Richore	3.1	MR

GK030171	NNP	5.4	S
GK030217	NNP	5.8	S
GK030178	NNP	5.4	S
GK030185	NNP	6.5	HS
GK036526	NNP	7.9	HS
GK030261	NNP	5.2	S
GK030200	NNP	5.2	S
GK030204	NNP	4.8	S
GK030210	NNP	4.3	S
GK030246	NNP	4.2	S
GK036524	NNP	5.6	S
GK030211	NNP	4.8	S
GK030249	NNP	6.3	HS
LRC 001	Ekenagwa	6.3	HS
LRC005	Egirini	7.6	HS
LRC010	Bunda entambe	5.4	S
LRC016	Manoa emwamu	2.4	MR
LRC015	Ekoko enyenge	5.8	S
LRC026	Nyaibu/Bunda enetu	4.7	S
LRC011	Ekebure	6.6	HS
LRC012	Enyamatobu	7.2	HS
LRC021	Morogi	5.8	S
LRC024	Ekoko entambe	5.7	S
LRC019	Manoa endabu	2.5	MR
LRC022	Enyamwamu	5.3	S
GK030194	NNP	4.2	S
GK030227	NNP	6.2	HS
GK030239	NNP	5.7	S
GK036530	NNP	7.8	HS
LRC 023	Eosama	6.5	HS

LRC 009	Eroyoo	4.9	S
LRC025	Amaika inse	4.4	S
LRC020	Ritinge	4.8	S
LRC 007	Eamini	5.3	S
LRC 014	Esaire	3.6	MR
LRC 017	Masaku	4.2	S
GK 030244	NNP	5.5	S
GK 036527	NNP	5.4	S
LRC 004	Emwetemania	4.8	S
LRC 013	Onyoro	4.7	S
GK036523	NNP	6.6	HS
GK030257	NNP	3.3	MR
GK036522	NNP	6.3	HS
GK030260	NNP	4.2	S
GK030198	NNP	7.4	HS
GK030259	NNP	6.4	HS
GK030167	NNP	6.6	HS
LRC003	Enchano	7.7	HS

HS: Highly susceptible, S: Susceptible, MR: Moderately resistant, R: Resistant, NNP: Names Not Provided.

5.3.5 Correlations between the qualitative morphological characters and the *Pythium* root rotdisease

The correlations between the disease rating and the qualitative traits were not significant except for the correlations between the disease rating and growth type (GT) (Table 5.8).

Table 5.8: Correlations between qualitative morphological characters and Pythium root rot disease rating

Morphological traits	Disease rating	Significance level
SSH	-0.0898	0.5439

FC	-0.0397	0.7887
GT	0.3233	0.025
PS	0.0167	0.9103
COP	-0.1776	0.2272
SSZ	-0.1043	0.4807
GH	0.0879	0.5524
CSC	0.2077	0.1566
SC	-0.0109	0.9416

Table 5.8 also shows that seed colour (SC), seed size (SSZ), seed shape (SSH), flower colour (FC) and colour of pod (COP) were negatively correlated with disease rating while growth type (GT), plant size (PS), growth habit (GH), and commercial seed colour (CSC), were positively correlated.

5.3.6 Cluster analysis of common bean landraces based on phenotypictraits

The clustering obtained by UPGMA method is shown in Figure 5.1. The dendrogram divided the52 common bean landraces in 5 main clusters.

Cluster 1 contains the following 13 genotypes; LCR 022, LCR 021, LCR 013, GK036528, GK030180, GK036527, GK036530, GK036524, GK036167, GK030261, GK030211,

GK030171 and GK030246. These genotypes are characterized with oblong or kidneys shaped seeds, have white to lilac flowers, have mainly semi-determinate growth types and are medium sized plants.

Cluster 2 contains the majority of landraces with kidney/oblong or oval shaped seeds, have lilac/violet or white flowers with determinate to semi-indeterminate growth types and are small to medium sized plants. These cultivars include: LCR10, LCR11, LCR25, LCR14, LCR026, LCR012, LRC001, LRC002, LRC005, LRC009, LRC003, GK030178 andGK030198.

Cluster 3 Genotypes in this cluster have small to medium seeds with oblong/cuboid or kidney shapes. They have lilac/violet or white flowers and all are medium sized with mainly bush to semi-determinate growth. Cluster 3 contains landraces LRC023, LRC015, LRC024, LRC007, LRC020, GK030194, GK030257, GK030200, GK030244, GK036522, GK030210, GK030260 and GK030249.

Cluster 4: This group comprise of 8 landraces i.e. LRC017, LRC018, LRC006, GK036523, GK030204, GK030239, GK030259 and GK030227. The chief characteristics of these genotypes included; have mainly kidney shaped seeds, being white flowered, having mostly medium with determinate bush growth, and presence of medium sized seeds that are de-uniform.

Cluster 5 contained only 4 characteristically unique genotypes namely LRC004, LRC008 LRC016 and LRC O19 which are characterized by prominently large oblong shaped seeds and are relatively large plants with indeterminate growth that produce red (LRC 016) or brilliant white flowers (LRC 019). LRC 008 is a relatively larger genotype compared to the others in the cluster, with indeterminate growth, lilac flowers but its oval shaped seeds are medium to small in size.

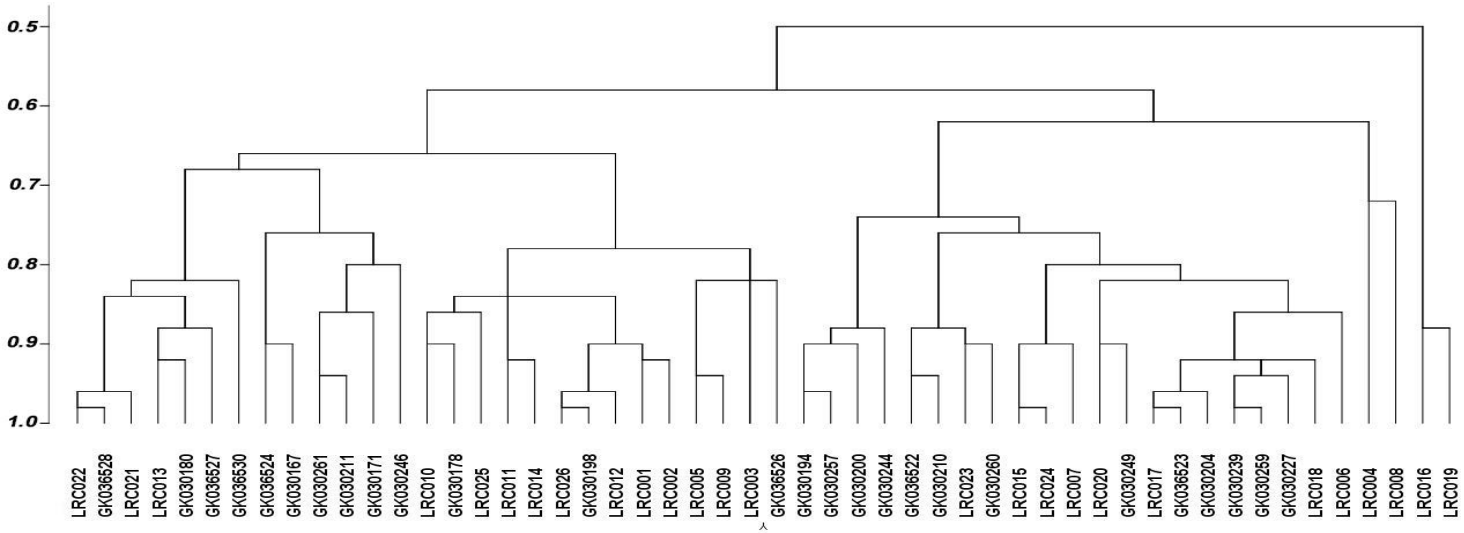

Figure 5.1: UPGMA dendrogram based on elucidation distance coefficients of 52 common bean landraces using morphological characters

5.3.7 Principal component analysis(PCA)

Principal component analysis (PCA) based on the nine qualitative traits revealed that first four components accounted for 70% of the total variance in which PC1 and PC2 (Figure 5.2) accounted for most variation. All the four principal components had Eigen values greater than one; with PC1 being greater than 2 and the largest difference was determined between the first and second Eigen values. The correlations between the first 4 principal components and 9 qualitative traits are shown in Table 5.9. The biplot was constructed by the first two PCs showing common bean landraces and 9 qualitative traits (as vectors) (Figure 5.2).

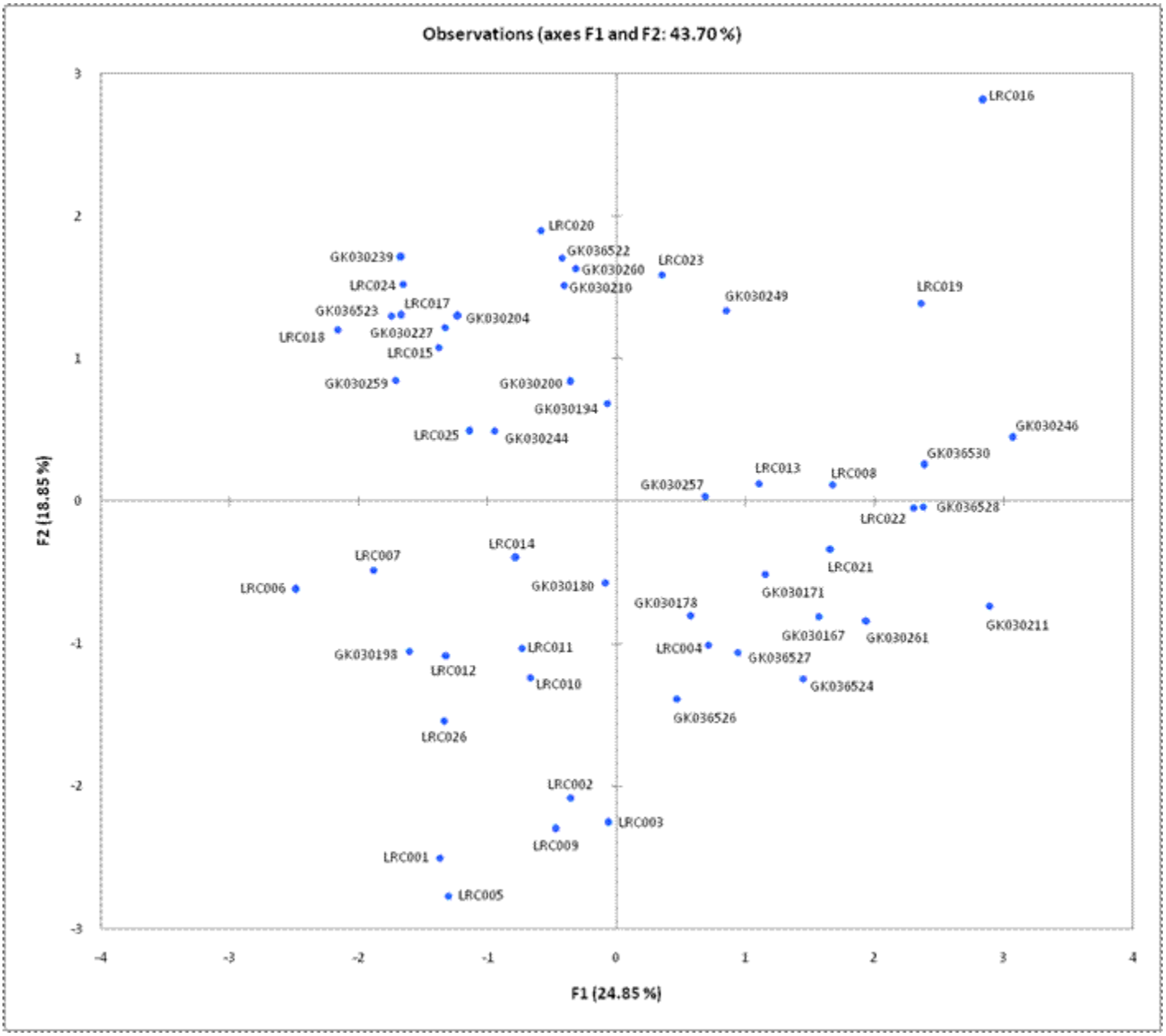

Figure 5.2: Biplot obtained by principal component analysis on the basis of morphological traits in 52 common bean landraces

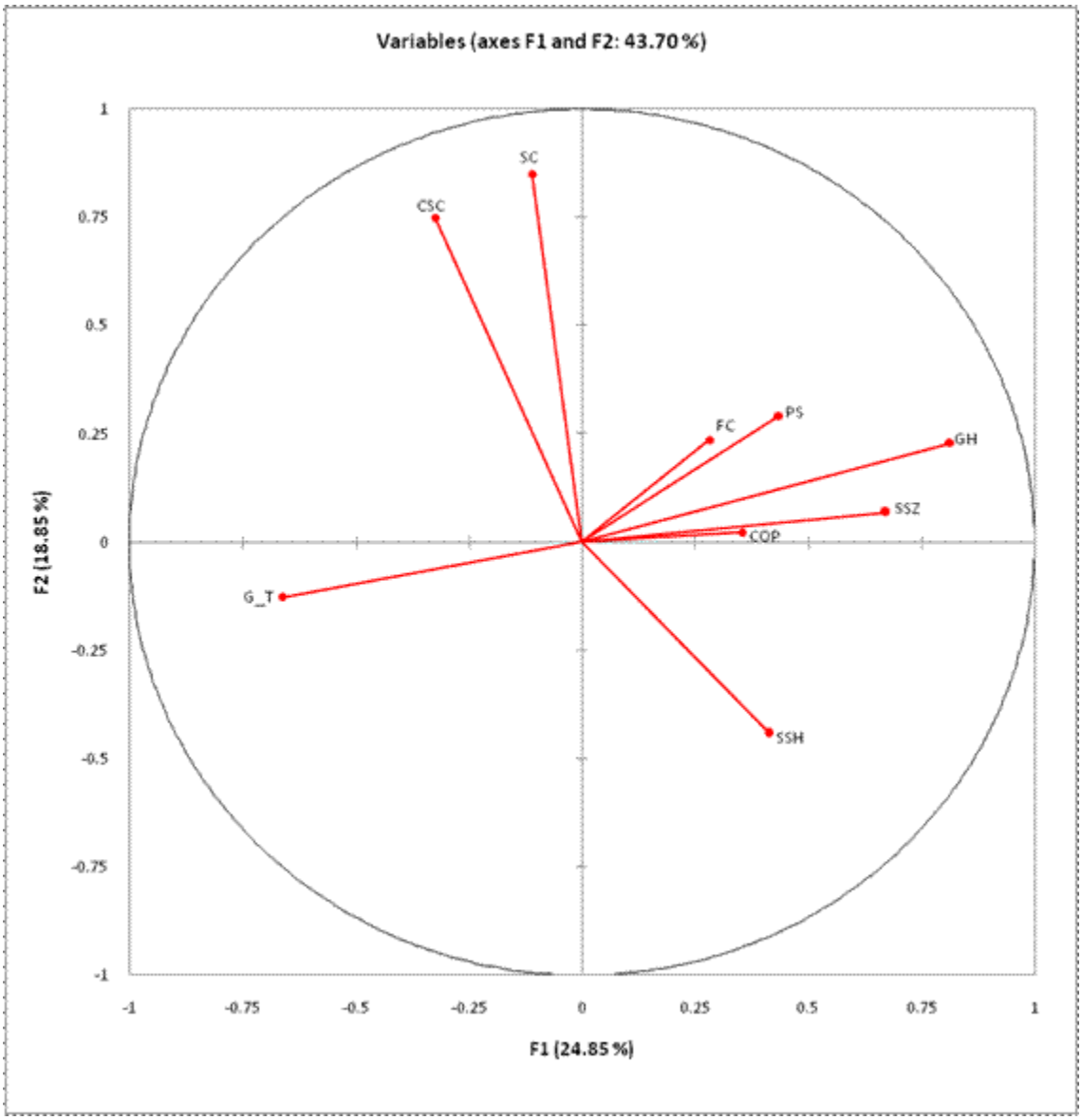

Figure 5.3: Biplot obtained by principal component analysis on the basis of nine qualitative traits in 52 common bean landraces

Table 5.9: Eigen vectors and values, total variance and cumulative variance among 52 common bean landraces based on nine morphological characters

	PC1	PC2	PC3	PC4
Eigen value	2.245	1.6771	1.199	1.156
%Total variance	24.95	18.57	13.32	12.84
%Cumulative	24.95	43.52	56.84	69.68
Traits	**Eigen vectors**			

Shape of seed	0.638	0.026	0.358	-0.264
Size of seed	0.872	-0.067	0.039	-0.1236
Colour of seed	0.205	0.630	-0.526	-0.043
Commercial seed colour	-0.592	0.413	-0.479	-0.164
Colour of mature pod	0.145	0.455	0.372	0.603
Flower colour	0.206	0.456	0.607	-0.121
Growth habit	-0.283	0.720	0.041	0.202
Growth type	-0.675	0.315	-0.043	0.251
Plant size	0.293	-0.259	-0.23	0.746

Two out of nine traits showed a strong positive correlation with the first principal component which explained 24.95% of the total variance (shape of seed, SSH, 0.638 and size of seed SSZ, 0.872). The second principal component accounting for 18.57% of the total variance showed a moderate positive correlation with colour of seed CS (0.630) and growth habit (0.720) (Table 5.9). There was a significant negative correlations in growth habit GH (-0.675) and commercial seed colour CSC (-0.592) for PC1.

5.4 Discussion

The findings revealed a significant variation among and within the common bean landraces based on morphological traits. In the present study, most seeds had oblong shapes followed by kidney, while a significant number were cuboid or oval. Common bean seed shape is one of the most prominent indicators of genetic variability and consumer habits. However, this trait varies with region of domestication (Biswas *et al*., 2010). Red colored kidney shaped and medium sized beans are preferred by many consumers, while cuboid seed shape is popular with farmers in SW Kenya. Most landraces had lilac and white flower colours. The colour of the flower in all bean varieties is caused by anthocyanins (Berber and Yasar, 2011). This trait could be important for the characterization of common bean populations, as flower colour has been documented as an index of mutation and in some cases as a phenotypic marker (Berber and Yasar, 2011). Flower colour has been used by plant breeders, along with other traits, as a criterion for _varietal purity‘ (Blair, 2006).

In the current evaluation most accessions had determinate growth types followed by semi-determinate, few had indeterminate growth. Growth type in common beans is determined by a

series of multiple alleles, where the recessive homozygote (determinate) corresponds to the bush-like, shrub-like, or bushy habit, as mentioned in IBPGR (2014). Previous studies demonstrated that the indeterminate growth habit is dominant over the determinate one (Kwak *et al*., 2009). When the environment is favorable, the potential for expression of all growth habits of these genotypes is likely to increase. Common bean varies in growth habit from aggressive climbing types, prostrate to bush beans. Growth habit is determined by a combination of factors including determinate verses indeterminate growth types, total plant height, degree of branching and internode length. Together, these factors make up bush growth, prostrate or climbing ability. Over 50% of common bean accessions in this study had bush growth habits. Genotypes with bush growth are convenient to weed, harvest as many are alsoindeterminate.

The size of the plant is determined genetically, but seems to be related to plant vigour (environment). It was demonstrated in this study that accessions in the field showed considerable diversity of expression in growth sizes which were grouped into 3 i.e small, medium and large. Almost all the genotypes investigated were mainly medium (majority) or small with the exception of 3 accessions; LRC008, LRC 016 and LRC 019 which were large. A higher yield of branches and hence a larger plant in these genotypes could have been due, in part, to a longer life cycle; in fact, many breeding strategies for yield increase are based on the assumption that increased yield potential depends on an increase in the size of the source, achieved through lengthening the growth cycle (Beebe *et al*., 2000). Increase in crop yield arises out of the interactions of many processes, but it is primarily determined by the amount of solar radiation intercepted; lengthening the life of the canopy will, therefore, tend to increase yield (Kwak *et al*., 2009).

Most genotypes in the current investigation had non uniform colour parttens ranging from yellow, cream, maroon, grey, brown to red. The colour of the pods as that of the hypocotyl, seeds and flowers of all bean varieties is caused by anthocyanin (Gouveia *et al*., 2014) which is genetically determined. These traits could be important for the characterization of common bean landraces, because pod, seed, flower and hypocotyl colour has been documented as an index of mutation and in some cases as a phenotypic marker (Gouveia *et al*.,2014).

In the present study over 65% of seeds investigated were medium sized. Most consumers in Kenya prefer medium to large sized seeds. Seed size is an important characteristic for

distinguishing between hard seeded and soft-seeded varieties of common bean genotypes, given that the seed size of hard-seeded lines is smaller than that of the soft-seeded lines (Pereira *et al*. 2009). In addition, seed size and seed coat colour have been used to develop a convenient method of seed quality improvement for several crop species including common bean cowpea, rapeseed, flax and *Arabidopsis* (Pereira *et al*.,2009).

The present investigation found almost 1:1 ratio of uniform verses de-uniform seeds. Many consumers prefer uniform seeds. Seed coat structure and its colour are important traits for legume species not only to determine the quality and commercial values of seeds (Yang, 2010) but also to reveal seed germination parameters for agricultural applications. Seed coat colour is also a central target in several plant species and any trait that is correlated to. It may be a convenient way to select/deselect desired/undesired plant material in a breeding program. Seed coat attains their specific colour at physiological maturity and seed coat pigmentation has been shown to play an important role in seed dormancy and germination (Debeaujon,2000).

In the present investigation, colours varied considerably from black, red, maroon, cream, brown to mixed or mottled seed coats. Red and white seeded varieties tend to attract consumers. The pigmentation of the seed coat colour is mainly genetically determined by flavonoids and anthocyanins (Dixon and Sumner, 2000). Dark coat colour has higher concentration of anthocyanins and proanthocyanidins than lighter colour or white varieties of beans, offering a valuable source for antioxidants (Stoilova *et al*., 2013). However, the external appearance of the coat colour is also influenced by environmental stimuli such as biotic stress, pests and diseases (Scarano *et al*. 2014) and environment can promote non genetic maternal changes in the seed coat thickness and composition. Previous reports indicated that the coat colour trait was polygenic controlled by several genes in various plant species including legumes such as cowpea, common bean and soybean (CIAT, 2015). Although some reports indicated that testa colour was governed by single gene with complete dominance, others concluded that seed colour was a polymeric character (Atilla *et al*.,2010).

The lowest value of genetic similarity was recorded among seed shape-SSH and seed colour-SC for these common bean traits (r=021), indicating that these traits are highly differentiated genetically. On the other hand, there was a higher genetic similarity recorded among growth habit-GH and seed size-SSZ traits, (r=0.95), which shows little genetic distance between these

accessions (0.05). The high similarity found among these common bean traits indicates that genetic diversity between them is narrow mostly due to their common origin in evolutionary history and in breeding programs. In addition, the near to unit correlations (0.95) of growth habit and seed size suggests that these traits are controlled by one gene or are very closely linked. The traits observed as critical for bean characterization in this study like growth habit and type, seed size and flower colour, were also found to be important in common beans from other parts of Kenya and Ethiopia (Asfaw *et al.*, 2009), which indicates similar diversity manifestation in the Eastern part of African. Blair *et al*. (2010) also observed considerable variations in landraces in Central Africa, in seed size and colour predominated by the red mottled types which was also fairly common in the presentinvestigation.

In this investigation, the Shannon-Weaver diversity indices (H`) were calculated to compare the phenotypic diversity within and among the morphological traits. High H` values indicate a balanced frequency of classes for an individual trait and a rich diversity for the trait and vice versa. In this study Shannon-Weaver diversity values were fairly variable among traits and ranged from 0.321 to 0.879 with a mean value of 0.614. Traits such as seed coat colour, seed shape, flower colour and pod colour were more diverse compared the other traits and the entire morphological diversity. Generally the indices of all evaluated traits were above 0.400, except for commercial seed colour (0.321) indicating the presence of adequate variability for these characteristics among evaluatedlandraces.

In the current investigation, growth type, plant size, growth habit and commercial seed colour were found to be positive correlation with disease severity while the other five (seed shape, flower colour, colour of pod, seed size and seed colour were negatively correlated. These findings are fairly similar to the works done by (Buruchara, 2001) in which 26 common bean cultivars were evaluated for Pythium root rot disease resistance and susceptibility on agro-morphological traits. The symptoms observed on susceptible common bean landraces were similar symptoms as that recorded by Schwartz (2007). Similar results were also obtained by Nzungize *et al*. (2011) and Lucas and Griffiths (2000), who recorded a positive association to *Pythium* with colored seeds. In addition, a number of evaluations of bean cultivars conducted earlier found that colored common bean cultivars had high levels of resistance to *Pythium* than whiteseededbeancultivars.Previousstudieshaveidentifiedsourceofresistanceto*P.ultimum*

from wild accessions of common bean cultivars and their subsequent filial generations obtained through back crossing method (Nzungize *et al*., 2011).

In this study there were positive correlations between four of the morphological traits (Table 5. 7), which can allow for significant selection and use of the related characters among and within the landraces in breeding. These positive correlations among the traits are likely to be controlled by the same genes or are under pleiotropic influence (Miko, 2008). In common bean breeding especially for grain yield, _if two strongly correlated traits are desired, they can both be selected simultaneously basing on one of the traits (Miko, 2008).

Cluster analysis revealed four bigger groups and one smaller group based on morphological features which showed a significant variation the among 52 common bean landraces. These results were almost consistent with the findings of Singh *et al*. (2010) who obtained two major groups and 15 subgroups from 76 common genotypes when applying cluster analysis to determine degree of similarities in common bean cultivars. Similarly, Mavromatis *et al*. (2010) studied genetic diversity of 16 cultivars of common beans grown in Greece and generated dendrogram with four major groups and 9 subgroups emanating from them.

In this study, nine traits were used to differentiate 52 common bean landraces. Different combinations of these nine traits enabled the landraces to be discriminated, but no individual trait distinguished one landrace from the other. A combination of four or more trait, for example plant size, seed coat colour, commercial seed colour, seed shape distinguished some landraces. The results were consistent with the findings of Figliuolo and Spagnoletti (2000) who distinguished 57 common bean cultivars and discovered that no one character can discriminate a cultivar. Similarly, Awan *et al.* (2014) characterized thirteen cultivars of common bean grown in Pakistan and revealed distinguishing morphological characters that led to separation of cultivars. The importance of morphological markers in identifying cultivars is well documented (Stoilova *et al*., 2013; Marzooghian *et al*., 2013). Nduwarugira *et al*. (2016) reported that the cultivars which were morphologically similar had a close genetic relationship. In contrast, Singh *et al*. (2010) argued that the morpho-agronomic characters were phenotypic traits and accessions may be similar morphologically, yet be genetically different. All characters applied in this study were found to influence the separation of landraces in the analysis of principalcomponents.

CHAPTER SIX

6.0 Genetic erosion of cultivated common bean (*Phaseolus vulgaris* L.) landraces in South WesternKenya

6.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is a pulse crop cultivated worldwide andbelongs to the *Fabaceae* family (Kwak *et al*., 2012; Maras *et al*., 2015). It is a highly polymorphic species originating from two gene pools namely Meso-American and Andes (Bellucci *et al.*, 2014). As a result of trade and exchange of goods the gene pools from Meso-America and Andes in the Northern America were moved through other countries to Western Europe (Logozzo, 2007) and Africa (Asfaw et *al*., 2009). Due to adaptation of the varieties to new ecological and man-made conditions, the varieties evolved and changed their morphological features such as growth habits, seed shape, seed size, seed colour, flower colour and growth type (IPGRI, 2001; Kwak and Gepts, 2009; Schmutz *et al*., 2014; Marko *et al.*, 2013). The evolutionary change in morphology resulted in great diversity of common beans (Bellucci *et al*., 2014). There are millions of landraces, modern cultivars as well as hybrids cultivated world-wide and maintained in global gene-bank (CIAT, 2001) and collection centers at regional levels (Rodino *et al*., 2006). Within a species, there are number of cultivars which have been developed to suit particular purposes such as nutritional quality, days to maturity, uniformity at maturity, growth habits and high yield (Smykal *et al*., 2015; Brigide *et al*.,2014).

Crop genetic diversity and its dynamics are believed to be a result of a complex process involving both human and environmental factors. This process has led to a decrease in crop diversity in many farming systems, because traditional varieties are being replaced by modern varieties (FAO, 2015; Anunda *et al*., 2014). However, as landraces are often well adapted to specific environments, they do have a clear advantage in marginal areas. Besides their direct use, these genetic resources have an important potential value in future breeding programs as well (IPGRI, 2014), and therefore need to be conserved. As one of the legume crops cultivated since ancient times in Kenya, common bean has passed through the processes of farming which in turn have been affected by the complex socio-cultural attitudes of communities and the prevailing environmental changes.

Several studies have investigated the loss of varieties in diverse crop systems (De Ron *et al*., 2018), confirming a global pattern of loss of traditional varieties (De Luca *et al*., 2015; Martos *et al*., 2017; Ferreyra *et al*., 2017). Genetic erosion is defined as the loss of genetic diversity within a crop as a result of agricultural modernization. It is the main threat to cultivar breeding programs which are dependent on diversity in pools of potential variety progenitors (Vakali *et al*., 2017). Genetic erosion occurs along with loss of landraces (Hammer *et al*., 1996; Tsegaye and Berg, 2007), though some studies have mitigated concerns about the extent of the genetic loss threat (Angioi *et al*., 2010; Negri *et al*., 2013), suggesting that diversity and variety loss dynamics may stabilize after the transition toward more intensive agriculture has occurred (Van de Wouw *et al*., 2010).

Genetic erosion of crops and their wild relatives is occurring at a high rate because of human activities and climate change in Kenya (FAOSTAT, 2016). However, there is little information about the causes and the degree of genetic erosion on local varieties of crop plant species or list of varieties/ lost in various parts of the country. Knowing the causes of genetic erosion is important for devising conservation measures. In addition, identification of local crop varieties which are on the verge of extinction plays a crucial role in designing and implementation of conservation policies. Although there is a collection of local common bean landraces from south Western Kenya conserved at the National Genebank of Kenya, most of these landraces are no longer used by farmers due to unknown reasons. Therefore, the objectives of the present study were to determine the extent of genetic erosion of common bean landraces in different agro-ecological zones of south Western Kenya and assess the factors that contribute to genetic erosion in common bean landraces. This information will help to develop sustainable on-farm conservationstrategy.

6.2 Materials andMethods

6.2.1 Description of the studyarea

The study area was Kisii County (Figure 6.1) in South Western Kenya. The County was selected because of the following reasons: i) common bean is the most important grain legume, 2nd to maize as a food crop in South West Kenya, (ii) common bean was the dominant legume crop in the area, (iii) improved varieties have been disseminated for over fifteen years that influence on-farm diversity of the common bean landraces, (iv) the production of common bean in the diverse ecologies (altitude, rainfall, soil type, landscape etc.,) helps to assess the impact of environmental

factors on genetic erosion (GE), (v) there are diverse cropping systems namely mono-cropping, intercropping associated with pulses and other cereals, (v) there was no known similar study of any kind that was done before in the study area that could be used as a baseline reference.

The area is characterized by red volcanic soils (nitosols) which are deep and rich in organic matter. The area exhibits a highland equatorial climate resulting into a bimodal rainfall pattern with average annual rainfall of 1600 mm with the long rains between late February and mid-June and the short rains from late September to late November. The County is divided into three topographic zones fall in different Agro-Ecological Zones (AEZs) namely Lower Highlands one and two (LH1 and LH2), Upper Midland one and two (UM1 and UM2) and Lower Midland one (LM1) (Minisry of Agriculture, Kenya, MoA, 2015).

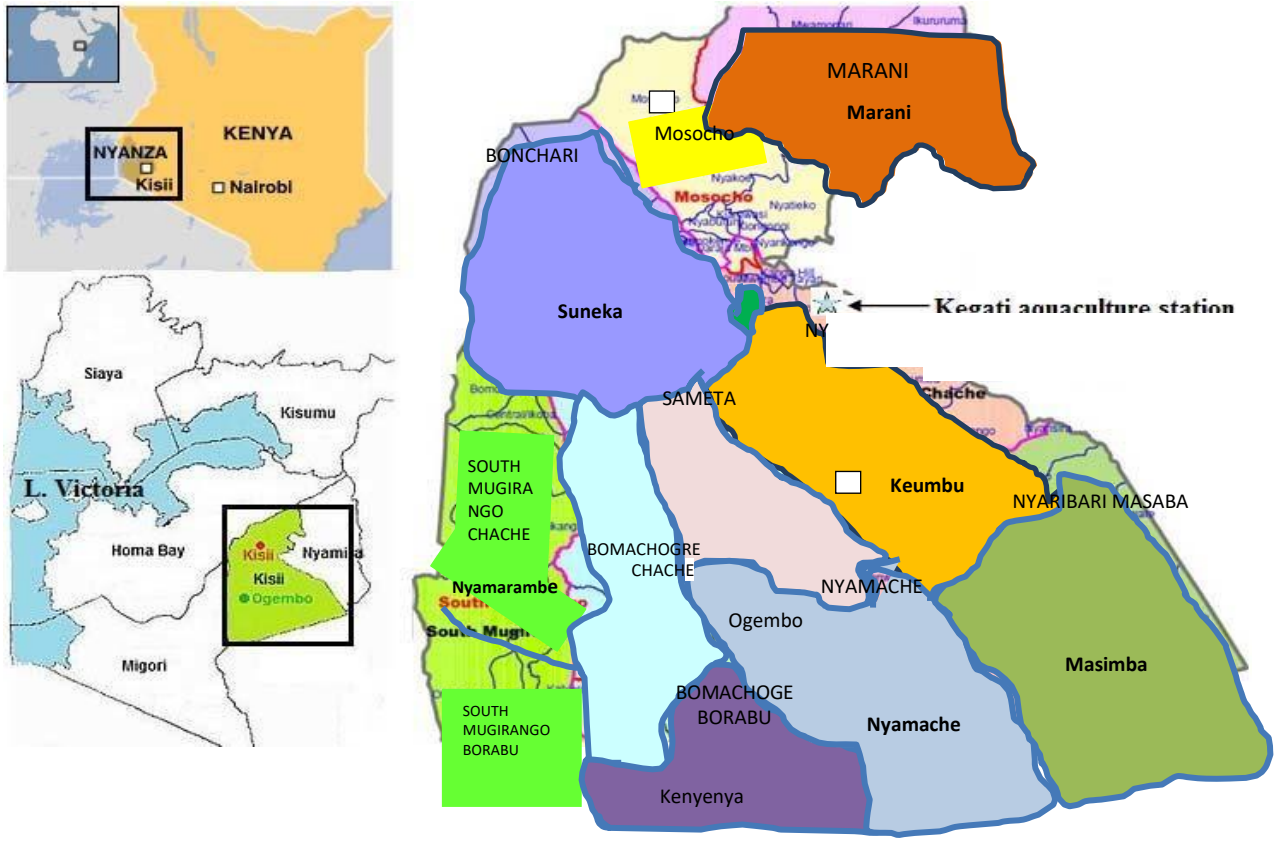

Figure 6.1: A map of south Western Kenya showing the geographical regions where common bean landraces were collected.

6.2.2 Selection of respondents and fieldsurvey

During the survey, leaders of small-scale farmers associations and local government agricultural extension personnel working in the region were involved in the selection of farmers (Snowball, non-random selection). From the list, key informants were randomly selected in order to conduct in-depth interview and discussion. The random sampling permitted all class, sex and age categories to be represented. Fifty randomly selected farmers, that is, 14 males and 36 females were involved in a questionnaire survey. The farmers were selected from different age groups based on their availability, experience, willingness and practical knowledge on common bean genetic resources of the area. The respondents aged between 30 to 84 years with an average of 55 years spread uniformly in all the agro-ecological zones of the region. The level of respondents' education ranged from those with basic education (primary) to those with college (tertiary) education.

The field survey was carried out in sites where common bean germplasm collection was performed in 1985 and preserved in the Genebank of Kenya. This involved interviews with local farmers and/or groups of farmers at each site. Visits were made at the end of growing season of common bean for two consecutive years namely 27^{th} –31^{st} July 2015 and 25^{th} – 29^{th} July 2016. The farmers were interviewed using a semi-structured questionnaire that was pre-tested before the actual survey. The questionnaires were administered to individual farmers with equal representation from each agro-ecological zone (ten farmers per agro-ecological zone) in the County. The questionnaire covered different topics such as information about the study area, landholdings, landraces of common bean commonly grown, introduced improved varieties and specific information on the use and management of common bean. The detailed information focused on cultural practices, the effect of new varieties on local genetic erosion, seed quality of landraces, and types of food prepared, and traditional values of common bean. The respondents were also asked about the status of production of common bean landraces and the possible advantages of growing landraces as compared to the introduced common beanvarieties.

Participatory collection of germplasm was done for comparison with the one performed in 1985 in order to estimate the genetic erosion. Common bean landraces were collected from farmers' fields during the 2015 survey. Focus was placed on sampling different types of common bean from a given farm irrespective of their representation in the mixture. Landraces known by the same name were collected from all agro-ecological zones to monitor within-zone diversity.

6.2.3 Quantification of genetic erosion

Genetic integrity and erosion were calculated as described by Hammer *et al*. (1996). Genetic integrity (GI) is equal to the ratio of the number of collected accessions per crop/ agro-ecological zone where landraces were presented in 1985 and 2015 i.e., $C_{2015}/C_{1985}\times$ 100. Information on landraces collected in 1985 was obtained from the National Gene bank of Kenya (GBK), Muguga, Kiambu. Genetic erosion (GE) was expressed as: GE = 100% - GI. Using this formula, the landraces collected in 1985 were compared with that of the collection made in 2015. Comparison of landraces was made both in number and in name for the purpose of identification and analysis. Besides comparison of landraces, the survey was also used to assess the factors causing genetic erosion, (GE) and agro-ecological distribution and suitability for different genotypes.

The data collected were subjected to descriptive statistics and analyzed with SPSS software version 10.0 for windows for descriptive statistics. To investigate the associations between the variables in the study, percentages and cross tabulations were used.

6.3 Results

6.3.1 Distribution of common bean landraces observed during the survey in South Western Kenya

In all the surveyed areas, 12 landraces (Table 6.1) are currently cultivated by many households in South Western Kenya. The dominant landraces observed in the region were LRC013 (Onyoro) and LRC007 (Eamini) while the most important landrace was LRC024 (Ekoko entambe). The rare landraces in all the agro-ecological zones were LRC005 (Egirini), LRC021 (Morogi) and LRC014 (Esaire).

Table 6.1: Distribution of predominant common bean landraces obtained during field survey in south Western Kenya

Distribution of landraces in production			
Agro-ecological zones	**Dominant**	**Important**	**Rare**
LM_1	LRC007 (Eamini), LRC013 (Onyoro)	LRC023 (Osama), LRC024 (Ekoko entambe), LRC015 (Ekokoenyenge)	LRC012 (Enyamatobu), LRC009 (Eroyoo), LRC023 (Osama), LRC005 (Egirini), LRC021(Morogi)
LH_1	LRC007 (Eamini), LRC013 (Onyoro), LRC014 (Esaire)	LRC024 (Ekoko entambe), LRC014 (Esaire), LRC013 (Onyoro)	LRC004 (Emwetamania), LRC021 (Morogi), LRC023 (Osama), LRC005 (Egirini)
LH_2	LRC006 (Esaitoti), LRC007 (Eamini), LRC013 (Onyoro)	LRC007 (Eamini), LRC009 (Eroyoo)	LRC005 (Egirini), LRC004(Emwetamania), LRC021 (Morogi)
UM_2	LRC015 (Ekoko enyenge), LRC007 (Eamini), LRC013 (Onyoro)	LRC024 (Ekoko entambe), LRC013 (Onyoro)	LRC005 (Egirini), LRC012 (Enyamatobu), LRC021(Morogi)
UM_1	LRC007 (Eamini), LRC013 (Onyoro), LRC024 (Ekoko entambe)	LRC006 (Esaitoti), LRC015 (Ekoko enyenge), LRC012 (Enyamatobu), LRC024 (Ekoko entambe)	LRC014 (Esaire), LRC004 (Emwetamania), LRC023 (Osama), LRC021 (Morogi)

Lower Highlands one and two (LH1 and LH2), Upper Midland one and two (UM1 and UM2) and Lower Midland one (LM1)

6.3.2 Quantification of genetic erosion of common beanlandraces

The overall result shows that out of the 26 varieties identified by the farmers to be cultivated in the past, 14 have gradually been abandoned within the past 15 years (Table 6.1). The remaining 12 varieties are currently under threat from genetic erosion.

The summary in Table 6.2 below, shows that the genetic integrity (GI) and genetic erosion (GE) based on the number of landraces by 1985 was 23 and 76.92 respectively, while the same parameters are 46.15 and 53.84 based on the names of the landraces. The high genetic erosion of 76.92% and 53%, means that more than half of the varieties that were originally grown are now not grown are not available or have been lost.

Table 6.2: Genetic integrity and erosion based on the number and names of landraces collected from South Western Kenya

	Parameter	**Amount (%)**
Based on number of landraces	Total number collected in 1985	52
	Total number collected in 2015	12
	Genetic integrity	23.07
	Genetic erosion	76.92
Based on name of landraces	Total names collected in 1985	26
	Total names collected in 2015	12
	Genetic integrity	46.15
	Genetic erosion	53.84

Thirty eight (38) respondents which translates to (76%) indicated that the production of bean varieties has continued to decrease, 10 (20%) of the respondents indicated that the bean varieties production has remain the same while 2 (4%) of the respondents indicated that beans variety production has increased. These findings are illustrated in the bar chart shown below.

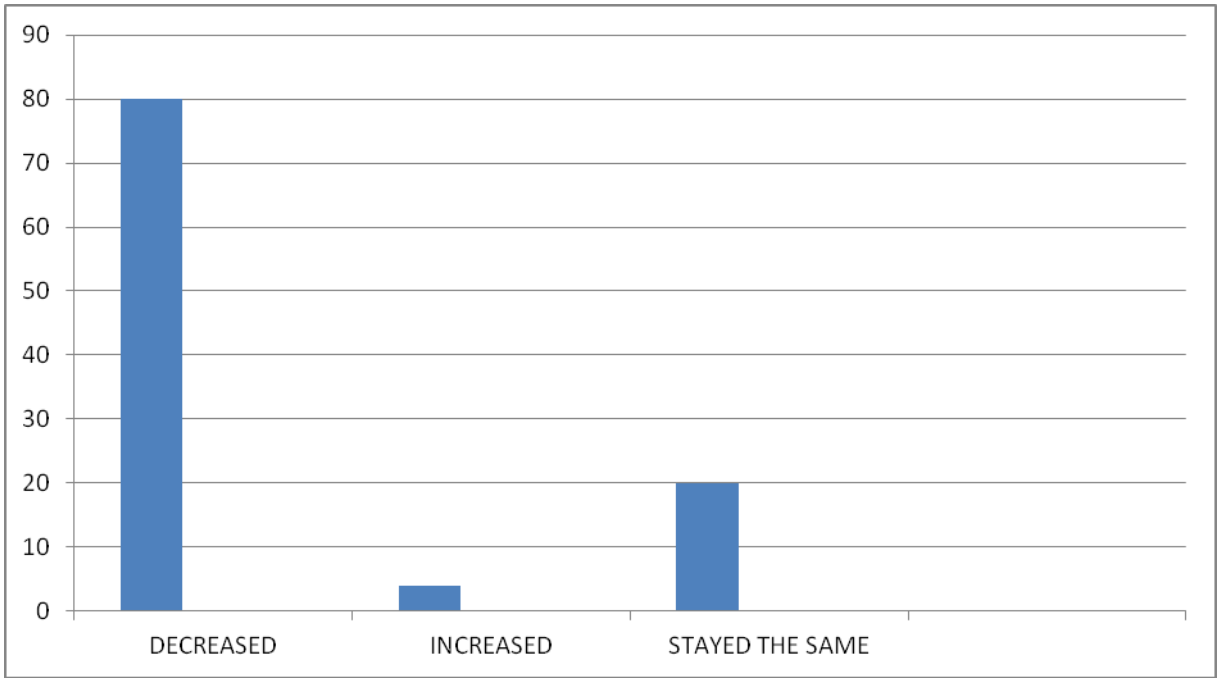

Figure 6.2: Farmers‘ perceptions on the status of common bean landraces production for the last 25 years

Farmers provided various reasons for the decreased or abandonment of each landrace as shown in Table 6.3. The main factors leading to the loss of these varieties are summarized in Table6.4.

Table 6.3: Common bean landraces cultivated in South Western Kenya up to 1985 and factors leading to their abandonment

Code	Local name (s) of landraces cultivated up to 1985	Landraces cultivated in 2015	Landraces that are not cultivated in 2015 (genetic loss)	Reasons for loss of the individual landraces after 1985
LRC 001	Ekenagwa		Ekenagwa	Susceptible to diseases
LRC 002	Egiero		Egiero	Non popular seed colour, taste, poor market
LRC 003	Enchano		Enchano	Non popular seed colour, taste, poor market
LRC 004	Emwetamania	Emwetamania		
LRC 005	Egirini	Egirini		
LRC 006	Esaitoti	Esaitoti		
LRC 007	Emanini	Emanini		
LRC 008	Chinchae		Chinchae	Takes too long to mature, indeterminate, poor taste
LRC 009	Eroyoo	Eroyoo		
LRC 010	Bunda entambe		Bunda entambe	Non popular seed colour, taste, poor market
LRC 011	Ekebure		Ekebure	Low yields, small seeds, taste
LRC 012	Nyamatobu	Nyamatobu		
LRC 013	Onyoro	Onyoro		
LRC 014	Esaire	Esaire		
LRC 015	Ekoko enyenge	Ekoko enyenge		
LRC 016	Manoa emwamu		Manoa emwamu	Non popular seed colour and taste
LRC 017	Masaku		Masaku	Low yields
LRC 018	Richore		Richore	Low yields
LRC 019	Manoa endabu		Manoa endabu	Non popular taste and cooking qualities
LRC 020	Ritinge		Ritinge	Scarcity of certified seed
LRC 021	Morogi		Morogi	Small seeds, Non popular seed colour, poor market
LRC 022	Nyamwamu		Nyamwamu	Small seeds, Non popular seed colour, poor market
LRC 023	Osama	Osama		
LRC 024	Ekoko entambe	Ekoko entambe		
LRC 025	Maika inse		Maika inse	Non popular seed colour, taste, poor market
LRC 026	Nyaibu/Bunda enetu		Nyaibu/Bunda enetu	Non popular seed colour, taste, poor market

Table 6.4: Factors affecting on-farm genetic loss of common bean landraces in south Western Kenya

Factor	N=50	Percentage (%)
Introduction of improved/new varieties	47	94
Poor yield	38	76
Weather variability	26	52
Low market value/Culinary qualities	33	66
Certified seed	18	36
Scarcity of land	21	42
Susceptibility to diseases	50	100
Susceptibility to pests	43	86
Susceptibility to poor soil fertility/soil selectivity	17	34
Labour	06	12
Other	14	28

N=Total number of respondents

The main reasons for abandoning many common bean landraces throughout the surveyed agro-ecological south Western Kenya were quite diverse (Table 6.4). These factors include: the introduction of new varieties with better attributes (94%), susceptibility of many landraces to diseases and pests (100 and 86% respectively), inadequate /expensive certified seed (36%) for planting, loss of soil fertility (34%), labour shortage (12%), shortage of land (42%),change of weather/ hailstones / heavy rains or sudden dry spells(52%) as well as other‘ factors including mixing of varieties at processing / poor selection among other factors (28%).

6.4 Discussion

The most important cause of genetic erosion as mentioned by all farmers (100%) was the susceptibility of many varieties to diseases (Table 6.4). This finding has also been reported by Megersa (2014), Anunda *et al*. (2014), Smykal (2018), and FAO (2015). Diseases, including soil-borne diseases, constitute a serious challenge to common bean production. Of the soil borne diseases, *Pythium* root rot is the major disease constraining production in this region. This factor is closely followed by the introduction of modern/new more yielding varieties (94%). Because of the high influx of many new varieties of common beans (in particular Rose cocoa and the Canadian wonder) which are higher yielding, much genetic erosion has occurred to many traditional landraces (especially Ekenenagwa, Richore and Masaku). Due to superior qualities of modern varieties (high yields and high prices), farmers increasingly replace traditional varieties

with modern varieties in many areas of south west Kenya. This has resulted in reduced diversity of the traditional varieties which are now threatened with extinction. Many respondents (94%) also indicated that pests (both field and storage) have been a major problem in bean production which has forced them to abandon many landraces. The bean varieties that used to be resistant to most of the diseases and pests with time lost the resistance resulting in decline in vigour and yield. Another major suggested cause of genetic erosion was lack of market (66%). Marketability attributes include culinary traits, size, colour and durability. Farmers dropped some varieties due to bad taste, seed color (especially beans which were blue or black), seed size (small ones mostly dropped). This is because majority of the farmers depend mainly on subsistence farming for all their family needs and so they give high priority to varieties that are in high demand and neglect or sometimes drop those with low demand. Those dropped are in most cases traditional varieties (Fu, 2017; Raggi *et al*., 2013; Chávez-Servia *et al*., 2016).Scarcity of land for cultivation (66%) and soil fertility (34%) were other factors mentioned by respondents as causing genetic erosion. Due to high population pressure in the region, there is shortage of land for cultivation and farmers have to utilize their small portions for more productive and high priced crop varieties, usually modern varieties and perennial cash crops like tea. In the process, the poor low yielding varieties, which in most cases are traditional landraces, are dropped and gradually disappear (FAO 2017, Megersa, 2014). Due to frequent cultivation of the land without furrowing, there has been continuous decline in soil fertility and some landraces have been dropped because of their low yield. Many of the farmers cannot afford expensive artificial fertilizers.

There is often a high rate of urban immigration especially with the young generation, (which is still energetic) to look for better paying white collar jobs in urban areas (FAO, 2017). Consequently in many households there is reduced labor force and results in abandonment of crop varieties requiring regular and intense labor like common beans (with climbing and indeterminate growth characterstics) for perennial crops that require less attention like bananas. Mark (2009), Cebolla *et al*. (2007) and Lidder *et al*. (2012) also reported this practice. Participants also mentioned unpredictable weather changes (52%) as another factor causing genetic erosion in the region especially from unreliable rainfall. Some crop varieties including common bean landraces were and are still adapted to the weather conditions of the area and are still high yielding. However, due to climate variation and change, varieties could no longer yield highly and farmers shifted to

other new varieties. Climate change and variation has resulted in diseases and pests that were non-existent in the region. Replacement of landraces by modern cultivars and other more profitable varieties or crops and the trends in diversity reduction as a consequence of modern breeding practices has great impact on the diversity of landraces (Carovic-Stanko *et al*., 2017). Climate change and environmental degradation can also result in changes in cropping patterns and the loss of landraces (Megersa, 2014).

Other causes of genetic erosion (as supported by 28% of the respondents) were plant growth habits, plant suitability to intercropping as well as growth duration. Farmers dropped some varieties due height (tall plants mostly dropped due to lodging), twinning, difficulties to intercrop and long maturity period. The introduction of modern varieties has caused several impacts on the local landraces. There is now low market demand – low consumption for landraces, a threat of having varieties that are not palatable for many consumers, there is reduced production leading to low consumption and low traded volume of traditional varieties. On the other hand, introduction of modern varieties have led to improved bean production per unit area, increased income of families and improved nutrition. Moroever, also in this study, it was also noted during the survey that farmers who adopt and grow modern varieties are rewarded by government or company/NGOs in order to encourage other farmers who will gradually abandon growing landraces. These common bean landraces are vulnerable to serious genetic erosion and the consequences maybe irreversible if the causes identified in this study are not addressed urgently.

CHAPTER SEVEN

7.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General discussion

The molecular characterization of the common bean in this study has shown that there is a significant amount of genetic variability among the local landraces in south Western Kenya. The 5 POX primers used for genetic characterization amplified an average of 5 alleles per locus indicating a significant level of genetic diversity and population structure distribution. The average PIC values (0.3) generated in this study is comparable to those reported in other studies on common bean, an indication that POX primers used are equally suitable in molecular genetic diversity evaluation. Furthermore the study shows that various common bean landraces can be successfully discriminated by POX markers. POX markers have been reported to reveal more polymorphism for the evaluation of genetic variation among common bean accessions (Nemli *et al*., 2014). Regarding population structure obtained, the clustering analysis of the common bean accessions studied, the maximum K value determined by Structure harvest was 3, which indicates that the entire population consisted of 3 subgroups, a significant genetic diversity among thelandraces.

The genetic diversity among and within the common bean landraces evaluated in this study makes them a valuable resource as potential donors of genes for the development, improvement and maintenance of modern varieties in Kenya. This study confirms the existence of a high level of agro-morphological variability of common bean in south west Kenya and this could be a result of several years of natural and artificial selections by local farmers for better adaptation to the area agro-ecological conditions. Among the different traits in this study, seed traits were found to be one of the most important in common bean and major determinants of commercial acceptability of varieties. Seed traits such as size, shape and colour were found to have significant heritability, therefore important in breeding programmes. In this study there were strong correlations between some traits, which could allow for simultaneous selections and use of the related traits interchangeably in selection. These strongly correlated characteristics are possibly under the influence of similar genes or are pleiotropic. To improve these common bean landraces, the strongly correlated traits can be selected simultaneously basing on one of the traits. There were a

significant number of characters with moderate to high heritability which can provide high level of gene transfer during breeding programs. For example, information on heritability in broad sense and genetic advance of yield attributing traits and their association helps plant breeder to identify characters for effective selection. Moderate to high values of genetic advance observed in this study are indicative of additive gene action whereas low values are indicative of non- additive gene action. The estimates of genetic advance can be used in understanding the type of gene action involved in the expression of various polygenic traits. Thus, heritability, (broad sense heritability) and genetic advance are important selection parameters especially in crops with large phenotypic variations like common beans. Furthermore, genetic advance is more useful as a selection tool when considered jointly with heritability estimates.

The results of this study indicated that some of the common bean landraces exhibited good levels of resistance to *Pythium* root rot disease. Their adoption would, therefore, improve common bean production in the region. These landraces can be used as a source of resistance to improve modern or newly introduced high yielding varieties. There is need to speed up common bean breeding programs, using molecular markers associated with resistance to *Pythium* root rot to help in carrying out a rapid screening of large populations. However, the fact that resistance to *Pythium* root rot is under the control of a single gene constitutes a risk factor that can lead to resistance breakdown (Beebe *et al*., 1991). This is complicated by the fact that there is a high diversity in the pathogen populations. Thus, in order to improve the sustainability of this resistance-based control method, it is essential to use diversified sources of resistance and to integrate other control methods, mainly appropriate cropping practices. This will reduce the risk and discourage the rapid build-up of *Pythium* inoculums. For example, ridging and deep tillage increase aeration and drainage, creating less favorable conditions for disease development (CIAT, 2015). This could be done for other common bean diseases as well, in order to succeed further in improving the yield and production common beans in Kenya.

The study found that many common bean landraces in the region have been put at risk of extinction through genetic erosion caused mainly by agricultural modernization. Based on the number of landraces evaluated, the estimated loss accounts for 76.92%. Although no previous comprehensive study has been undertaken to quantify the level of genetic erosion on the

common bean landraces, reports indicate that over the last decade, a lot of genetic erosion has taken place mainly due to replacement of traditional varieties, diseases, pests and other agronomic as well as socio-economic factors. *In situ* and on-farm conservation instruments have unfortunately not been fully utilized to enable the conservation of existing diversity of common bean landraces. *Ex situ* and *in situ* conservation initiatives should be undertaken urgently by the Kenya government, research institutions and NGOs. *Ex situ* conservation could involve the use of specialized facilities such as cold stores or chest freezers. Germplasm materials may also be conserved in special fields, in farmers‘ plots, botanical gardens or arboreta as living collections for future use. Breeding efforts in these threatened common bean landraces should be encouraged so that these crops will keep their place in farming systems and the food chain, while agriculture modernizes in south Western Kenya.

7.2 Conclusions

The following conclusions were made from the research findings:

i) Whereas a majority (54.9%) of the common bean landraces studied were susceptibe to *Pythium* root rot disease, a moderate level of resistance was observed in Six common bean landraces from south Western Kenya.

ii) There were significant levels of genetic variability among the 52 common bean landraces for all the agronomic traits studied based on POX markers. High values of genotypic coefficient of variation, broad sense heritability and genetic advance were recorded for pod width, plant height, number of branches and days to emergence.

iii) Cluster analysis using the fourteen different traits classified the common bean landraces into four separate clusters exhibiting that hybridization of landraces across clusters could lead to an increase in heterosis in progenies.

iv) Based on morphological characterization, seed coat colour was found to be the most polymorphic trait among accessions, having the highest number of categories (13) as well as the highest Shannon,,s information index (0.879), while commercial seed colour trait was the most monomorphic (2 categories, H′= 0.321). Shannon Weaver diversity index showed high diversity index for the qualitative characters studied indicating the presence of adequate variability for these traits among evaluated landraces.

v) The correlations between the *Pythium* root rot disease rating and qualitative traits such as flower colour

were not significant except for the correlations between the disease rating and growth type.

vi) The most dominant and popular landraces in all the agro-ecological zones were LRC013 (Onyoro) and LRC007 (Eamini) while the most important landrace was LRC024 (Ekoko entambe). The rarest landrace in all the agro ecological zones was LRC005 (Egirini), LRC021 (Morogi) and LRC014 (Esaire).

vii) The most important causes of genetic erosion identified were disease, pests and frequent introductions of new improved varieties.

viii) The current level of genetic integrity (GI) was 23%, while genetic integrity and erosion based on the name of the landraces in the study were 46 and 53, respectively.

7.2 Recommendations

Since the POX markers used in the present study were developed to determine genetic diversity, there is need to use markers linked to the genes responsible for resistance to *Pythium* root rot in order to facilitate the effective identification of quantitative trait loci linked to this trait. Incorporating *Pythium*-moderate resistant landraces which have other desirable agronomic and consumer quality traits such as high iron and zinc content, fast cooking ability, from the different clusters and groups as parents for breeding, would ensure the diversification of resistance to the disease while creating new hybrids.

Genetic information on the action of genes on the evaluated morpho-agronomic traits, genetic variability, heritability and genetic advance particularly on trait of economic importance like grain yield and disease resistance should be exploited in common bean improvement breeding programs. Information generated in this study on correlation within and among the landraces can be exploited in the selection of noble quantitative traits to improve the production of common bean landraces in south west Kenya while putting much emphasis on the direction and strength of the trait associations. Many traits in this study are linked to grain yield, hence should be further investigated for other agronomic and breeding values. Despite a low rates of cross-pollination and admixtures, common bean landraces from Kenya comprise an appreciable level of inter- and intra-genotype diversity both at morphological and agronomic levels, a result that needs to be considered when planning conservation strategies or for agro-morphological improvement of these landraces. There is therefore an urgent need to collect, and evaluate all available common bean landraces from south Western Kenya for future utilization and

conservation. There is need to maintain the collected germplasm both at the local and national level, which should serve as gene banks particularly for landraces threatened with genetic erosion. There should be more research by all stakeholders especially relevant research institutions, Universities and international organizations on common bean landraces that are both high yielding, disease and pest tolerant early maturing, fast cooking and those of high nutritive value.

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APPENDICES

Appendix 1: Pairwise Jaccard's genetic similarity index among 51 common bean landraces based on peroxides gene (POX) marker data

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47	S48	S49	S50	S51	S52
S1	1	0.60	0.69	0.76	0.83	0.73	0.77	0.58	0.73	0.73	0.67	0.64	0.91	0.87	0.72	0.87	0.67	0.77	0.77	0.67	0.80	0.84	0.73	0.83	0.82	0.76	0.77	0.82	0.76	0.69	0.74	0.83	0.69	0.75	0.76	0.76	0.64	0.79	0.79	0.69	0.73	0.80	0.76	0.75	0.69	0.80	0.64	0.71	0.79	0.79	0.83	0.73
S2		1.00	0.68	0.62	0.75	0.65	0.69	0.80	0.72	0.65	0.81	0.86	0.60	0.71	0.64	0.64	0.81	0.76	0.69	0.90	0.72	0.69	0.72	0.54	0.58	0.62	0.48	0.52	0.75	0.68	0.73	0.75	0.75	0.60	0.75	0.75	0.86	0.64	0.58	0.68	0.65	0.60	0.68	0.74	0.75	0.65	0.70	0.56	0.58	0.78	0.67	0.72
S3			1.00	0.77	0.77	0.81	0.85	0.60	0.88	0.88	0.68	0.72	0.69	0.73	0.80	0.73	0.68	0.92	0.85	0.68	0.81	0.85	0.88	0.76	0.68	0.84	0.64	0.68	0.77	0.77	0.68	0.77	0.84	0.76	0.77	0.77	0.72	0.80	0.80	0.77	0.88	0.81	0.77	0.76	0.84	0.81	0.65	0.72	0.80	0.76	0.81
S4				1.00	0.77	0.81	0.85	0.54	0.81	0.81	0.62	0.65	0.76	0.80	0.80	0.80	0.62	0.85	0.85	0.62	0.88	0.92	0.81	0.76	0.68	0.84	0.64	0.68	0.77	0.84	0.62	0.77	0.77	0.77	0.76	0.77	0.77	0.84	0.65	0.73	0.80	0.84	0.88	0.96	0.77	0.69	0.77	0.84	0.65	0.72	0.73	0.80	0.76	0.81
S5					1.00	0.81	0.85	0.67	0.81	0.81	0.68	0.79	0.83	0.88	0.73	0.80	0.75	0.85	0.85	0.75	0.88	0.85	0.81	0.76	0.75	0.77	0.71	0.75	0.84	0.77	0.75	0.92	0.77	0.76	0.84	0.84	0.79	0.88	0.80	0.77	0.81	0.76	0.84	0.91	0.77	0.88	0.72	0.72	0.80	0.88	0.83	0.81
S6						1.00	0.89	0.58	0.85	0.92	0.65	0.76	0.73	0.77	0.77	0.77	0.65	0.89	0.89	0.65	0.85	0.89	0.85	0.73	0.65	0.88	0.68	0.65	0.81	0.88	0.65	0.81	0.81	0.73	0.81	0.80	0.81	0.69	0.84	0.77	0.81	0.85	0.85	0.81	0.80	0.81	0.85	0.69	0.69	0.77	0.77	0.73	0.85
S7							1.00	0.62	0.89	0.89	0.69	0.73	0.84	0.81	0.88	0.88	0.69	0.92	1.00	0.69	0.89	0.92	0.89	0.84	0.76	0.85	0.72	0.76	0.92	0.85	0.69	0.92	0.85	0.69	0.85	0.92	0.77	0.77	0.81	0.88	0.92	0.89	0.89	0.92	0.77	0.85	0.89	0.73	0.80	0.88	0.81	0.77	0.89
S8								1.00	0.58	0.58	0.80	0.76	0.52	0.63	0.56	0.56	0.90	0.68	0.62	0.71	0.64	0.62	0.64	0.46	0.50	0.54	0.46	0.44	0.67	0.54	0.71	0.67	0.60	0.52	0.60	0.67	0.85	0.56	0.50	0.60	0.58	0.58	0.60	0.65	0.60	0.58	0.85	0.54	0.50	0.70	0.58	0.71
S9									1.00	0.92	0.65	0.69	0.73	0.77	0.77	0.77	0.65	0.89	0.89	0.72	0.85	0.89	0.85	0.80	0.72	0.88	0.68	0.72	0.81	0.88	0.65	0.81	0.88	0.73	0.88	0.81	0.96	0.85	0.69	0.69	0.77	0.77	0.73	0.85
S10										1.00	0.65	0.69	0.73	0.77	0.77	0.77	0.65	0.89	0.89	0.65	0.85	0.89	0.85	0.80	0.72	0.96	0.75	0.72	0.81	0.88	0.65	0.81	0.81	0.73	0.81	0.80	0.88	0.85	0.69	0.69	0.77	0.77	0.73	0.85
S11											1.00	0.77	0.60	0.71	0.64	0.64	0.81	0.76	0.69	0.81	0.72	0.69	0.72	0.54	0.58	0.62	0.48	0.58	0.75	0.62	0.90	0.75	0.68	0.67	0.68	0.68	0.77	0.64	0.58	0.68	0.65	0.65	0.68	0.67	0.68	0.65	0.70	0.56	0.58	0.71	0.67	0.72
S12												1.00	0.64	0.75	0.68	0.68	0.77	0.80	0.73	0.77	0.76	0.73	0.76	0.58	0.63	0.65	0.52	0.56	0.79	0.65	0.77	0.79	0.72	0.64	0.72	0.79	0.82	0.68	0.62	0.72	0.69	0.69	0.72	0.78	0.72	0.69	0.67	0.60	0.62	0.83	0.71	0.76
S1													1.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.

3													00	87	79	96	60	77	84	67	80	84	73	91	91	76	86	91	83	69	67	83	76	75	76	76	64	79	87	76	73	80	83	75	69	80	58	78	87	79	83	73
S14														1.00	0.76	0.83	0.64	0.81	0.81	0.78	0.84	0.88	0.77	0.79	0.86	0.80	0.74	0.78	0.88	0.73	0.78	0.88	0.73	0.79	0.88	0.88	0.75	0.83	0.83	0.73	0.77	0.84	0.80	0.79	0.80	0.84	0.62	0.75	0.76	0.83	0.87	0.77
S15															1.00	0.83	0.64	0.81	0.88	0.64	0.77	0.88	0.92	0.79	0.71	0.80	0.67	0.71	0.80	0.73	0.64	0.80	0.80	0.87	0.73	0.80	0.62	0.76	0.91	0.88	0.77	0.84	0.88	0.65	0.73	0.77	0.68	0.91	0.83	0.76	0.87	0.84
S16																1.00	0.64	0.81	0.88	0.64	0.84	0.88	0.77	0.87	0.86	0.80	0.82	0.86	0.88	0.73	0.64	0.80	0.80	0.72	0.73	0.80	0.68	0.76	0.83	0.80	0.77	0.84	0.80	0.72	0.73	0.77	0.62	0.75	0.83	0.83	0.79	0.77
S17																	1.00	0.76	0.69	0.73	0.72	0.69	0.72	0.54	0.52	0.62	0.48	0.52	0.68	0.62	0.73	0.75	0.68	0.60	0.62	0.68	0.86	0.64	0.58	0.68	0.65	0.65	0.68	0.74	0.62	0.65	0.86	0.63	0.58	0.78	0.67	0.72
S18																		1.00	0.92	0.76	0.89	0.92	0.89	0.77	0.69	0.85	0.65	0.69	0.85	0.85	0.76	0.85	0.92	0.77	0.85	0.85	0.80	0.81	0.81	0.85	0.89	0.89	0.85	0.84	0.85	0.89	0.73	0.73	0.81	0.88	0.77	0.89
S19																			1.00	0.69	0.89	0.92	0.89	0.84	0.76	0.85	0.72	0.76	0.92	0.85	0.69	0.85	0.92	0.77	0.85	0.85	0.73	0.81	0.88	0.92	0.89	0.89	0.92	0.77	0.85	0.89	0.73	0.80	0.88	0.81	0.77	0.89
S20																				1.00	0.72	0.69	0.72	0.60	0.65	0.62	0.54	0.58	0.75	0.68	0.81	0.83	0.75	0.67	0.83	0.68	0.77	0.71	0.64	0.68	0.65	0.65	0.75	0.74	0.75	0.72	0.63	0.63	0.64	0.71	0.74	0.72
S21																					1.00	0.89	0.85	0.73	0.72	0.81	0.68	0.72	0.88	0.81	0.72	0.88	0.81	0.73	0.81	0.81	0.76	0.84	0.77	0.81	0.85	0.85	0.81	0.80	0.81	0.92	0.69	0.69	0.77	0.84	0.80	0.85
S22																						1.00	0.89	0.84	0.76	0.92	0.72	0.76	0.85	0.85	0.69	0.85	0.85	0.84	0.85	0.92	0.73	0.81	0.88	0.85	0.89	0.96	0.85	0.77	0.85	0.89	0.73	0.80	0.81	0.88	0.84	0.89
S23																							1.00	0.73	0.65	0.81	0.62	0.65	0.81	0.81	0.72	0.88	0.81	0.88	0.81	0.81	0.69	0.84	0.84	0.88	0.85	0.85	0.88	0.73	0.81	0.85	0.76	0.83	0.84	0.77	0.88	0.92
S24																								1.00	0.91	0.83	0.86	0.91	0.76	0.69	0.60	0.76	0.76	0.75	0.76	0.76	0.58	0.72	0.87	0.76	0.80	0.80	0.83	0.68	0.76	0.80	0.58	0.78	0.87	0.72	0.75	0.73
S25																									1.00	0.75	0.85	0.90	0.83	0.62	0.65	0.75	0.68	0.67	0.75	0.75	0.63	0.71	0.78	0.68	0.72	0.72	0.75	0.67	0.75	0.72	0.50	0.70	0.78	0.71	0.74	0.65
S26																										1.00	0.78	0.75	0.77	0.84	0.62	0.77	0.77	0.76	0.77	0.84	0.65	0.80	0.80	0.77	0.88	0.88	0.77	0.76	0.84	0.81	0.65	0.72	0.73	0.80	0.76	0.81
S27																											1.00	0.85	0.71	0.64	0.54	0.71	0.64	0.63	0.64	0.64	0.52	0.74	0.74	0.64	0.68	0.68	0.71	0.70	0.64	0.68	0.52	0.65	0.74	0.67	0.70	0.68
S28																												1.00	0.75	0.62	0.65	0.75	0.68	0.74	0.68	0.68	0.56	0.71	0.78	0.68	0.72	0.72	0.75	0.67	0.68	0.72	0.50	0.70	0.78	0.71	0.74	0.65
S29																													1.00	0.77	0.75	0.84	0.84	0.69	0.84	0.84	0.79	0.80	0.80	0.84	0.81	0.81	0.84	0.76	0.84	0.81	0.65	0.72	0.80	0.80	0.76	0.81

S30																														1.00	0.62	0.77	0.84	0.69	0.84	0.77	0.65	0.80	0.73	0.84	0.88	0.88	0.77	0.76	0.84	0.81	0.65	0.65	0.73	0.73	0.69	0.81
S31																															1.00	0.83	0.68	0.74	0.75	0.68	0.70	0.71	0.64	0.68	0.65	0.65	0.75	0.74	0.68	0.72	0.63	0.63	0.64	0.71	0.74	0.72
S32																																1.00	0.77	0.83	0.84	0.77	0.72	0.88	0.80	0.84	0.81	0.81	0.92	0.83	0.77	0.88	0.72	0.79	0.80	0.80	0.91	0.88
S33																																	1.00	0.69	0.84	0.77	0.72	0.73	0.80	0.84	0.81	0.81	0.84	0.76	0.84	0.81	0.65	0.72	0.80	0.80	0.69	0.81
S34																																		1.00	0.76	0.76	0.58	0.79	0.87	0.76	0.73	0.80	0.83	0.68	0.69	0.80	0.64	0.86	0.79	0.72	0.91	0.80
S35																																			1.00	0.84	0.72	0.80	0.80	0.77	0.81	0.81	0.84	0.76	0.92	0.88	0.65	0.72	0.80	0.73	0.76	0.81
S36																																				1.00	0.79	0.73	0.80	0.77	0.81	0.88	0.77	0.76	0.84	0.81	0.72	0.72	0.73	0.88	0.76	0.81
S37																																					1.00	0.68	0.62	0.65	0.69	0.69	0.65	0.78	0.72	0.69	0.74	0.60	0.62	0.83	0.64	0.69
S38																																						1.00	0.83	0.73	0.77	0.77	0.80	0.87	0.73	0.84	0.62	0.75	0.83	0.76	0.87	0.77
S39																																							1.00	0.80	0.77	0.84	0.88	0.72	0.73	0.84	0.62	0.91	0.91	0.76	0.87	0.77
S40																																								1.00	0.88	0.88	0.92	0.69	0.77	0.81	0.72	0.79	0.80	0.73	0.76	0.88
S41																																									1.00	0.92	0.81	0.73	0.88	0.85	0.69	0.69	0.77	0.77	0.73	0.85
S42																																										1.00	0.81	0.73	0.81	0.85	0.69	0.76	0.77	0.84	0.80	0.85
S43																																											1.00	0.76	0.77	0.88	0.72	0.87	0.88	0.73	0.83	0.88
S44																																												1.00	0.69	0.80	0.64	0.64	0.72	0.87	0.75	0.73
S45																																													1.00	0.81	0.65	0.65	0.73	0.73	0.69	0.81
S4																																														1.	0.	0.	0.	0.	0.	0.

6																																														00	69	76	84	77	80	85
S47																																															1.00	0.67	0.62	0.68	0.64	0.83
S48																																																1.00	0.83	0.68	0.86	0.76
S49																																																	1.00	0.69	0.79	0.77
S50																																																		1.00	0.79	0.77
S51																																																			1.00	0.80
S52																																																				1.00

Common bean landrace ID	Common bean landrace code in the similarity matrix Appendix 1	Common bean landrace ID	Common bean landrace code in the simlarity matrix Appendix 1
LRC006	S1	LRC019	S27
LRC008	S2	LRC022	S28
LRC018	S3	GK030194	S29
GK030171	S4	GK030227	S30
GK030217	S5	GK030239	S31
GK030178	S6	GK036530	S32
GK030185	S7	LRC023	S33
GK036526	S8	LRC009	S34
GK030261	S9	LRC025	S35
GK030200	S10	LRC020	S36
GK030204	S11	LRC007	S37
GK030210	S12	LRC014	S38
GK030246	S13	LRC017	S39
GK036524	S14	GK030244	S40

GK030211	S15	GK036527	S41
GK030249	S16	LRC004	S42
LRC001	S17	LRC013	S43
LRC005	S18	GK036523	S44
LRC010	S19	GK030257	S45
LRC016	S20	GK036522	S46
LRC015	S21	GK030260	S47
LRC026	S22	GK030198	S48
LRC011	S23	GK030259	S49
LRC012	S24	GK030167	S50
LRC021	S25	LRC003	S51
LRC024	S26	LRC002	S52

Appendix 2: Pairwise Jaccard‘s genetic dissimilarity index among 51 common bean landraces based on peroxides gene (POX) marker data

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47	S48	S49	S50	S51
S1	0.00	0.40	0.31	0.24	0.17	0.27	0.23	0.42	0.27	0.27	0.33	0.36	0.09	0.13	0.28	0.13	0.33	0.23	0.23	0.33	0.20	0.16	0.27	0.17	0.18	0.24	0.23	0.18	0.24	0.31	0.26	0.17	0.31	0.25	0.24	0.24	0.36	0.21	0.21	0.31	0.27	0.20	0.24	0.25	0.31	0.20	0.36	0.29	0.21	0.21	0.17
S2		0.00	0.32	0.39	0.25	0.35	0.31	0.20	0.28	0.35	0.19	0.14	0.40	0.29	0.36	0.36	0.19	0.24	0.31	0.10	0.28	0.31	0.28	0.46	0.42	0.39	0.52	0.48	0.25	0.32	0.27	0.25	0.25	0.40	0.25	0.25	0.14	0.36	0.42	0.32	0.35	0.32	0.26	0.25	0.35	0.30	0.44	0.42	0.22	0.33
S3			0.00	0.23	0.23	0.19	0.15	0.40	0.12	0.12	0.32	0.28	0.31	0.27	0.20	0.27	0.32	0.08	0.15	0.32	0.19	0.15	0.12	0.24	0.32	0.16	0.36	0.32	0.23	0.23	0.32	0.23	0.16	0.24	0.23	0.23	0.28	0.20	0.20	0.23	0.12	0.19	0.23	0.24	0.16	0.19	0.35	0.28	0.20	0.20	0.24
S4				0.00	0.23	0.19	0.15	0.46	0.19	0.19	0.39	0.35	0.24	0.20	0.20	0.20	0.39	0.15	0.15	0.39	0.12	0.08	0.19	0.24	0.32	0.16	0.36	0.32	0.23	0.16	0.39	0.23	0.23	0.16	0.24	0.23	0.31	0.23	0.12	0.04	0.23	0.31	0.12	0.28	0.35	0.27	0.20	0.16	0.28	0.28	0.20	0.24
S5					0.00	0.19	0.15	0.33	0.19	0.19	0.32	0.21	0.17	0.13	0.27	0.20	0.25	0.15	0.15	0.25	0.12	0.15	0.19	0.24	0.25	0.23	0.29	0.25	0.16	0.23	0.25	0.19	0.19	0.24	0.16	0.16	0.09	0.23	0.12	0.28	0.28	0.20	0.13	0.20	0.23	0.19	0.12	0.28	0.28	0.20	0.13	0.17
S6						0.00	0.12	0.42	0.15	0.08	0.35	0.24	0.27	0.23	0.23	0.23	0.35	0.12	0.12	0.35	0.15	0.12	0.15	0.27	0.35	0.12	0.32	0.35	0.19	0.12	0.35	0.19	0.19	0.27	0.19	0.19	0.20	0.19	0.15	0.15	0.19	0.20	0.19	0.15	0.31	0.16	0.23	0.23	0.27
S7							0.00	0.39	0.12	0.12	0.31	0.27	0.16	0.19	0.12	0.12	0.31	0.08	0.00	0.31	0.12	0.08	0.12	0.16	0.24	0.15	0.28	0.24	0.08	0.15	0.31	0.15	0.08	0.23	0.15	0.15	0.27	0.19	0.12	0.08	0.12	0.12	0.08	0.23	0.15	0.15	0.27	0.20	0.12	0.19	0.23
S8								0.00	0.42	0.42	0.20	0.24	0.48	0.38	0.44	0.44	0.11	0.32	0.39	0.29	0.36	0.39	0.36	0.54	0.50	0.46	0.54	0.56	0.33	0.46	0.29	0.33	0.40	0.48	0.40	0.33	0.15	0.44	0.50	0.40	0.42	0.42	0.40	0.35	0.40	0.42	0.15	0.46	0.50	0.30	0.42
S9									0.00	0.08	0.35	0.31	0.27	0.23	0.23	0.23	0.35	0.12	0.12	0.28	0.15	0.12	0.15	0.20	0.28	0.12	0.32	0.28	0.19	0.12	0.35	0.19	0.12	0.27	0.12	0.19	0.27	0.12	0.19	0.23	0.23	0.19	0.08	0.15	0.19	0.27	0.04	0.15	0.31	0.23	0.23	0.27
S10										0.00	0.35	0.31	0.27	0.23	0.23	0.23	0.35	0.12	0.12	0.35	0.15	0.12	0.15	0.20	0.28	0.04	0.25	0.28	0.19	0.12	0.35	0.19	0.19	0.27	0.19	0.19	0.31	0.16	0.23	0.19	0.08	0.15	0.19	0.20	0.12	0.15	0.31	0.12	0.23	0.23	0.27
S11											0.00	0.23	0.40	0.29	0.36	0.36	0.19	0.24	0.31	0.19	0.28	0.31	0.28	0.46	0.42	0.39	0.52	0.42	0.25	0.39	0.10	0.25	0.32	0.27	0.25	0.40	0.25	0.25	0.14	0.36	0.42	0.32	0.35	0.32	0.26	0.25	0.35	0.30	0.44	0.42	0.29	0.33
S12												0.00	0.36	0.25	0.32	0.32	0.23	0.20	0.27	0.23	0.24	0.27	0.24	0.42	0.38	0.35	0.48	0.44	0.21	0.35	0.23	0.21	0.28	0.36	0.28	0.21	0.18	0.32	0.39	0.28	0.31	0.31	0.28	0.22	0.28	0.31	0.33	0.40	0.39	0.17	0.29
S13													0.00	0.13	0.21	0.05	0.40	0.23	0.16	0.33	0.20	0.16	0.27	0.09	0.10	0.24	0.14	0.10	0.17	0.31	0.33	0.17	0.24	0.24	0.25	0.24	0.24	0.16	0.36	0.21	0.13	0.24	0.27	0.20	0.17	0.25	0.31	0.20	0.42	0.22	0.13	0.21	0.17
S1														0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.

4														00	24	17	36	19	19	22	16	12	23	21	14	20	26	22	13	27	22	13	27	21	13	13	25	17	17	27	23	16	20	21	20	16	39	25	24	17	13
S15															0.00	0.17	0.36	0.19	0.12	0.36	0.23	0.12	0.08	0.21	0.29	0.20	0.33	0.29	0.20	0.27	0.36	0.20	0.20	0.13	0.27	0.20	0.39	0.24	0.09	0.13	0.23	0.16	0.13	0.35	0.27	0.23	0.32	0.09	0.17	0.24	0.13
S16																0.00	0.36	0.19	0.12	0.36	0.16	0.12	0.23	0.13	0.14	0.20	0.18	0.14	0.13	0.27	0.36	0.20	0.20	0.28	0.27	0.20	0.32	0.24	0.17	0.20	0.23	0.16	0.20	0.28	0.27	0.23	0.39	0.25	0.17	0.17	0.21
S17																	0.00	0.24	0.31	0.27	0.28	0.31	0.28	0.46	0.48	0.39	0.52	0.48	0.32	0.39	0.27	0.25	0.32	0.40	0.39	0.32	0.14	0.36	0.42	0.32	0.35	0.35	0.32	0.26	0.39	0.35	0.14	0.38	0.42	0.22	0.33
S18																		0.00	0.08	0.24	0.12	0.08	0.12	0.23	0.31	0.15	0.35	0.31	0.15	0.15	0.24	0.15	0.08	0.23	0.15	0.15	0.20	0.19	0.19	0.15	0.12	0.12	0.15	0.16	0.15	0.12	0.27	0.27	0.19	0.12	0.23
S19																			0.00	0.31	0.12	0.08	0.12	0.16	0.24	0.15	0.28	0.24	0.08	0.15	0.31	0.15	0.08	0.23	0.15	0.15	0.27	0.19	0.12	0.08	0.12	0.12	0.08	0.23	0.15	0.12	0.27	0.20	0.12	0.19	0.23
S20																				0.00	0.28	0.31	0.28	0.40	0.35	0.39	0.46	0.42	0.25	0.32	0.19	0.17	0.25	0.33	0.17	0.32	0.23	0.29	0.36	0.32	0.35	0.35	0.25	0.26	0.25	0.28	0.38	0.38	0.36	0.29	0.26
S21																					0.00	0.12	0.15	0.27	0.28	0.19	0.32	0.28	0.12	0.19	0.28	0.12	0.19	0.27	0.19	0.19	0.24	0.16	0.23	0.19	0.15	0.15	0.19	0.20	0.19	0.08	0.31	0.31	0.23	0.16	0.20
S22																						0.00	0.12	0.16	0.24	0.08	0.28	0.24	0.15	0.15	0.31	0.15	0.15	0.16	0.15	0.08	0.27	0.19	0.12	0.15	0.12	0.04	0.15	0.23	0.15	0.12	0.27	0.20	0.19	0.12	0.16
S23																							0.00	0.27	0.35	0.19	0.39	0.35	0.19	0.19	0.28	0.12	0.19	0.13	0.19	0.19	0.31	0.16	0.16	0.12	0.15	0.15	0.12	0.27	0.19	0.15	0.24	0.17	0.16	0.23	0.13
S24																								0.00	0.10	0.17	0.14	0.10	0.24	0.31	0.40	0.24	0.24	0.25	0.24	0.24	0.42	0.28	0.13	0.24	0.20	0.20	0.17	0.32	0.24	0.20	0.42	0.22	0.13	0.28	0.25
S25																									0.00	0.25	0.15	0.10	0.17	0.39	0.35	0.25	0.32	0.33	0.25	0.25	0.38	0.29	0.22	0.32	0.28	0.28	0.25	0.33	0.25	0.28	0.50	0.30	0.22	0.29	0.26
S26																										0.00	0.22	0.25	0.23	0.16	0.39	0.23	0.23	0.24	0.23	0.16	0.35	0.20	0.20	0.23	0.12	0.12	0.23	0.24	0.16	0.19	0.35	0.28	0.27	0.20	0.24
S27																											0.00	0.15	0.29	0.36	0.46	0.29	0.36	0.38	0.36	0.36	0.48	0.26	0.26	0.36	0.32	0.32	0.29	0.30	0.36	0.32	0.48	0.35	0.26	0.33	0.30
S28																												0.00	0.25	0.39	0.35	0.25	0.32	0.26	0.32	0.32	0.44	0.29	0.22	0.32	0.28	0.28	0.25	0.33	0.32	0.28	0.50	0.30	0.22	0.29	0.26
S29																													0.00	0.23	0.25	0.16	0.16	0.31	0.16	0.16	0.21	0.20	0.20	0.16	0.19	0.19	0.16	0.24	0.16	0.19	0.35	0.28	0.20	0.20	0.24
S30																														0.00	0.39	0.23	0.16	0.31	0.16	0.23	0.35	0.20	0.27	0.16	0.12	0.12	0.23	0.24	0.16	0.19	0.35	0.35	0.27	0.27	0.31

S31																															0.00	0.17	0.32	0.26	0.25	0.32	0.30	0.29	0.36	0.32	0.35	0.35	0.25	0.26	0.32	0.28	0.38	0.38	0.36	0.29	0.26
S32																																0.00	0.23	0.17	0.16	0.23	0.28	0.13	0.20	0.16	0.19	0.19	0.08	0.17	0.23	0.12	0.28	0.21	0.20	0.20	0.09
S33																																	0.00	0.31	0.16	0.23	0.28	0.27	0.20	0.16	0.19	0.19	0.16	0.24	0.16	0.19	0.35	0.28	0.20	0.20	0.31
S34																																		0.00	0.24	0.24	0.42	0.21	0.13	0.24	0.27	0.20	0.17	0.32	0.31	0.20	0.36	0.14	0.21	0.28	0.09
S35																																			0.00	0.16	0.28	0.20	0.20	0.23	0.19	0.19	0.16	0.24	0.08	0.12	0.35	0.28	0.20	0.27	0.24
S36																																				0.00	0.21	0.27	0.20	0.23	0.19	0.12	0.23	0.24	0.16	0.19	0.28	0.28	0.27	0.13	0.24
S37																																					0.00	0.32	0.39	0.35	0.31	0.31	0.35	0.22	0.28	0.31	0.26	0.40	0.39	0.17	0.36
S38																																						0.00	0.17	0.27	0.23	0.23	0.20	0.13	0.27	0.16	0.39	0.25	0.17	0.24	0.13
S39																																							0.00	0.20	0.23	0.16	0.13	0.28	0.27	0.16	0.39	0.09	0.09	0.24	0.13
S40																																								0.00	0.12	0.12	0.08	0.31	0.23	0.19	0.28	0.21	0.20	0.27	0.24
S41																																									0.00	0.08	0.19	0.27	0.12	0.15	0.31	0.31	0.23	0.23	0.27
S42																																										0.00	0.19	0.27	0.19	0.15	0.31	0.24	0.23	0.16	0.20
S43																																											0.00	0.24	0.23	0.12	0.28	0.13	0.13	0.27	0.17
S44																																												0.00	0.31	0.20	0.36	0.36	0.28	0.13	0.25
S45																																													0.00	0.19	0.35	0.35	0.27	0.27	0.31
S46																																														0.00	0.31	0.24	0.16	0.23	0.20
S4																																															0.	0.	0.	0.	0.

7																																															00	33	39	32	36
S48																																																0.00	0.17	0.32	0.14
S49																																																	0.00	0.31	0.21
S50																																																		0.00	0.21
S51																																																			0.00

Common bean landrace ID	Common bean landrace code in the similarity matrix Appendix 1	Common bean landrace ID	Common bean landrace code in the simlarity matrix Appendix 1
LRC006	S1	LRC019	S27
LRC008	S2	LRC022	S28
LRC018	S3	GK030194	S29
GK030171	S4	GK030227	S30
GK030217	S5	GK030239	S31
GK030178	S6	GK036530	S32
GK030185	S7	LRC023	S33
GK036526	S8	LRC009	S34
GK030261	S9	LRC025	S35
GK030200	S10	LRC020	S36
GK030204	S11	LRC007	S37
GK030210	S12	LRC014	S38
GK030246	S13	LRC017	S39
GK036524	S14	GK030244	S40
GK030211	S15	GK036527	S41
GK030249	S16	LRC004	S42
LRC001	S17	LRC013	S43
LRC005	S18	GK036523	S44

LRC010	S19	GK030257	S45
LRC016	S20	GK036522	S46
LRC015	S21	GK030260	S47
LRC026	S22	GK030198	S48
LRC011	S23	GK030259	S49
LRC012	S24	GK030167	S50
LRC021	S25	LRC003	S51
LRC024	S26	LRC002	S52

APPENDIX 3: Questionnaire on the diversity and status of common bean landraces in Kisii County, South Western Kenya

University of Nairobi

Your answers are completely confidential

Thank you for your time.

A Survey of the Diversity and Genetic Erosion of Common Bean Landraces in Kisii County, South Western Kenya

University of Nairobi

- **Please complete the questionnaire and return it in the postage – paid return envelope.**

Thank you for your help. We hope you will take part and let your views be represented.

Background

There is lack of adequate knowledge and information on the status and risks posed to Plant genetic resources in south western Kenya especially with cultivated crop species and in particular common beans. The increasing market demands and use of modern varieties by many farmers in south western Kenya poses a serious threat of genetic erosion to local/traditional varieties or landraces. Genetic erosion is the loss of variability, crop varieties/cultivars or an entire crop from a region. The loss of landraces/local/traditional varieties results in decrease of the genetic base of the remaining cultivars that may have a consequence on changing climate and/or ecological conditions. Consequently this loss of landraces could be a threat to local, regional, national and international food security and future genetic or plant breeding programs. It is therefore urgent to collect, document, characterize, conserve, monitor and manage the traditional crops and formulate policies that will protect them from further genetic erosion. The aim of this study is to evaluate genetic erosion in common bean cultivars in the south west Kenya region, so that we can develop effective conservation methods and sustainable utilization of plantresources.

The survey seeks to determine your perception regarding the status of local/traditional bean varieties or landraces and that of modern or bred cultivars in the region.

A. Generalinformation

1. Date ofinterview_____
2. Name ofenumerator_____
3. Name of AAO_____
4. Sub-county/District_____
5. Division_____
6. Location_____
7. Sub-Location_____
8. Village_____
9. AEZ_____
10. Name of farmer**……**
11. Age: (i)21-32 (ii) 33-48 (iii) 49-60 (iv) 61-74 (v)>75
12. Sex of farmer: (i) Male (ii)Female
13. Total farmsize…….acres……
14. Educationlevel……..….
15. Years of stay in thearea……

B. Crop diversity on yourfarm

List the main crops grown on your farm and their acreage

C. Bean diversity on yourfarm

Name the varieties of beans that you grow

……

Describe the morphology and physiology of each variety.

……

……

What are the positive and negative attributes for the varieties?

Variety	Positive attributes	Negative attributes

When did you acquire these varieties?

.....

Where did you acquire them from?

.....

What are your main reasons for planting these varieties?

a) Cash
b) Consumption
c) Both
d) Other(specify)

When do you plant beans (month/season)?

.....

D. State what has happened to the varieties of beans in the last 15 - 25years:

In your own farm

a) Has beenincreasing
b) Hasdecreased
c) Has not changed

In yourlocation

a) Has beenincreasing
b) Hasdecreased
c) Has notchanged

E. If the number has been decreasing, please name the varieties that have been lost, when andwhy

Varietylost/decreased When Reason(s)

…….. ……. ……..

F. Any other cause of loss of these varieties, starting from the main to theminor

……

G. State the effects of introduction of new varieties on landraces /local/traditional ones?

H. Is it possible to trace these lost varieties?

……

I. What is your take on the loss of these varieties?

……

J. What are the benefits and problems ofthe

a) New varieties?

……

b) Landraces/local/traditional varieties?

……

K. Any other information/comment you may wish to add here

……

Thank you very much for these information and understanding.

School of Biological Sciences
University of Nairobi
P.O. Box 30197 -00100
NAIROBI

Dear Mr./Mrs./Ms./Dr./Prof…….
We are writing to ask your help in a study of the diversity and status of common bean landraces in Kisii County, SW Kenya.
To better understand your concerns, the School of Biological Sciences, University of Nairobi, is conducting a study of the status of common bean varieties in your county. The enclosed survey is intended to collect information about your observations, beliefs and attitudes about their diversity and current conservationstatus.
The survey will help us and the food industry, government, plant breeders better understand the status of bean cultivars in the area. Yours answer are completely confidential and will bereleased only as summaries in which no individual's answers can be identified. When you return your completed questionnaire, your name will be deleted from mailing list and never connected to your answers in any way. This survey is voluntary, but your response is very important. You can help us very much by taking a few minutes to share your observations on the conservation status of the common bean landlaces in Gusii region. Please take a few minutes to fill out the questionnaire and return it in the postage – paidenvelope.
If you have any questions or comments regarding the survey, we would be happy to talk with you. Our number is Tel.: 0735 – 224 169 / 0723 – 052 664 or Email; hnranunda42@gmail.com.

Thank you very much for helping with this important study.

Sincerely,
Henry N. Anunda

APPENDIX 4: Advantages and disadvantages of modern varities and landraces

Modern varieties

Advantages

- Highproduction
- Ripen moreuniformly
- Fetch moreincome
- Have been researched on to adopt to specificconditions
- Short cookingtime
- Fast growing / earlymaturity
- Some are bred to adapt to change inclimate

Disadvantages

- Susceptible to pests /diseases
- Certified seed forplantingexpensive
- High level ofmanagement
- Prone to rotting /nondorment

Landraces

Advantages

- Tolerant to diseases /pests
- Hardy, tolerant to drought /flood
- Averageyield
- Aredelicious
- Seeds for planting locallyavailable
- Withstand poor soils
- Tolerant to rotting /dormancy

Disadvantages

- Take too long to cook
- Causes acidity /bloating
- Low marketprices
- Low nutritivevalue
- Not attractive in three market e.g. poor colour (omorogi) ataste
- Shatteringhabits

APPENDIX 5: Advantages and disadvantages of most popular landraces

Variety	Advantages	Disadvantages
Rose cocoa	• Better yield/good fruity, colour,taste • Diseasetolerant • Cooks well, consumerpref. • Can be intercropped withmaize • Leaf good asvegetable • Easy tosell • Good colour andflavour	• Diseasessusceptible • Pestendangered • Needs a lot offertilizer • Timely plantingrequired • Pests especially weavils,bruch*
Amini	• Consumer pref./marketable • Prolific • High yielding • Disease resistant/ tolerant comparedto others • Can grow in soils of low fertility • Tolerant to wet conditions / with rainfall	• Takes long tocook • Very acidic • Pods take long to dry • Difficult when shelling • Poor production if planted late • Low demand • Bean fly problem

	• Good cookingcharacteristics • Sheds leaves when dry /mature	
Saitoti	• Maturesquickly	• Requires dryspell • Disease /pests
Nyamatobu	• Large seeds –sweet • Cooks well /faster • Higheryields • Diseasetolerant • Prolific / growsfast • Leaf used asvegetable • Allseasons	• Low colour toappeal • Highacidity
Egiero	• Fastmaturity • Resistant to dieases	• Not liked byconsumers •
Egirini	• Pests and diseasesresistant	• Not verymarketable
Osama	• Very sweet leafvegetable • Very diseaseresistant • Highly aggressivevariety • Allseason	• Goes bad quickly aftercooking • Highly allogenous – easilycross pollinated
Onyoro (morigori)	• Good leafvegetable • Semi – indeterminate – pods startdrying from bottom –up • Highyield • Climber (on maize,intercropping) • Fastermaturity	• Labour demanding delicate being aclimber • Low marketprice • Difficult toharvest

APPENDIX 6: Common bean varieties and Agroecological zones (AEZs) where the varieties are popular

Variety	AEZs	Farmers' number
Amini	LM_1, LH_1, LH_2, UM_2, UM_1	
Onyoro (morigori)	LM_1, LH_1, LH_2, UM_2, UM_1	
Enyamotobu	LM_1, LH_1, LH_2, UM_2, UM_1	
Osama	LM_1, LH_2,	
Ecoco entambe	LM_1, LH_1, LH_2, UM_2, UM_1	
Ecoco enyenge	LM_1, LH_1, LH_2, UM_2, UM_1	
Wairimu	LH_1, UM_2	
Enyerere	LH_1,	
Bunda	UM_2	
Omorogi	UM_2	
Nyamochera	UM_2	
Eroyo	UM_1 UM_2,	