MORPHOLOGICAL AND MOLECULAR GENETIC DIVERSITY OF COMMON BEAN (*Phaseolus vulgaris* L.) VARIETIES AND LANDRACES IN TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MOLECULAR BIOLOGY AND BIOTECHNOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE.

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EXTENDED ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is among the most important crops in Tanzania. The production level has been increasing from 626 340 tonnes to 780 000 tonnes from year 2005 to 2012 respectively. Bean cultivation is highly adopted by small-scale and large-scale farmers. More than one hundred common bean genotypes including varieties and landraces have been collected from different geographical location in Tanzania, and little information is known about their variations. This study characterized sixty-four common bean genotypes by using morpho-agronomic traits and molecular genotyping by using SSR marker. Twenty morpho-agronomic traits were evaluated by using the common bean descriptor of IBPGR. Allelic loci were analyzed by using twenty-five SSR molecular markers. Among the major findings, the Principal component analysis (PCA) for the morpho-agronomic traits accounted 31% of the total variation of the genotypes analyzed; while the PCA from molecular data accounted for 37% cumulative variations for the common bean varieties and landrace analyzed. Results obtained enable the identification of the duplicates in our collection, separating the Mesoamerican gene pool from the Andean gene pool, identifying the relation between the varieties cultivation with their grouped landraces. This information helped in determining the pedigree of the background parent and were able to identify genotypes with important agronomic traits which plant breeders can use them in breeding common bean variety with more desired traits.

This work is expected to be published in Academic Journal of African Journal of Biotechnology (AJB).

DECLARATION

I, DEOGRACIOUS PROTAS, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

13-06-2014

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DEDICATION

I dedicate this work to the ALMIGHTY GOD and to the beloved family of the late Mr. Protas Peter Massawe.

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LIST OF ABBREVIATIONS

ARI	Agriculture Research Institute
BSUA	Bean bank Sokoine University of Agriculture
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
hPAGE	horizontal Polyacrylamide Gels Electrophoresis
IBPGR	International Board for Plant Genetic Resources
IDT	Integrated DNA Technologies
MAS	Marker Assisted Selection
NJ	Neighbour-Joining
NTSYS	Numerical Taxonomy Techniques
PCA	Principal Component Analysis
RCBD	Randomized Complete Block Design
SSR	Simple Sequence Repeats
SUA	Sokoine University of Agriculture
TOSCI	Tanzania Official Seed Certification Agency
UPGMA	Unweighted Pair-Group Mean Arithmetic

CHAPTER ONE

1.0 INTRODUCTION

Common bean (*Phaseolus vulgaris L.*) is the most important legume for direct human consumption in the world (Schoonhoven and Voysest 1991; Córdoba *et al.*, 2010). It has evolved rapidly in Africa and is steadily transforming from a traditional subsistence to a market-oriented crop, with major impacts on household incomes, food and nutritional security and national economies (Buruchara *et al.*, 2011). It provides dietary protein for over 100 million people in rural and poor urban communities, with an annual per *capita* bean consumption in Eastern Africa (50 -60 kg) being the highest in the world (ISAR, 2011). Broughton *et al.* (2003) and Asfaw *et al.* (2009) reported the highland of Africa as a secondary center of diversity for common bean and the crop is important contributor to food security in the region. In Tanzania; common bean together with maize and rice are the major food crops of smallholder farmers and it is estimated that over 75% of rural households in Tanzania depend on beans for daily subsistence (CIAT, 2008).

Common beans are thought to provide up to 65% of protein consumed in the region and to be the second most important source of calories providing 32% of energy supply (Blair *et al.*, 2010). Like other legumes, common bean seeds contain a number of bioactive compounds including enzyme inhibitors, lectins, phytates, oligosaccharides and phenolic compounds, which play metabolic functions in humans or animals (Angela *et al.*, 2010). Valdemiro and Whitaker (1982) reported the daily bean consumption provides significant amount of proteins, calories and micronutrients which reduce the incidence of malnutrition and hunger. Bean proteins have high digestibility with significant nutritional and health advantages for consumers as it's highly correlated with low incidence of

coronary heart diseases and death (Menotti *et al.*, 1999), and Phaseolin being the major protein in the bean seed.

Evaluation of genetic diversity of Tanzanian *P. vulgaris* landraces and varieties is essential since it can enable detection of genetic relationships among different germplasms in seed banks and breeding progenies, identification of duplicates in gene banks, identification of subsets of core accessions with possible utility for specific breeding purposes, search for promising heterotic groups, assessing influx of genetic diversity over time and identification of essentially derived varieties these has been documented also by Prasanna (2005). This work was therefore aimed to study genetic makeup and genetic diversity of common bean landraces and varieties collected from farmer's household in different zones of Tanzania.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background Information

Phaseolus vulgaris L. is a diploid species with 11 chromosomes and a medium-sized genome with estimates ranging from 588 to 637 Mbp (Aramuganathan and Earle, 1991; Bennett and Leitch, 2005). *P. vulgaris* had already diverged into two major gene pools, each with a proper geographical distribution. Domestication of wild common beans occurred independently in Mesoamerica and Andean South America (Kaplan, 1999) and gave rise to two major gene pools also within the cultivated forms (Gepts, 1988).

Common beans have been described and grouped according to two gene pools they belong to; the Andean gene pool originating in the highlands of South America and the Mesoamerican gene pool originating in Central America and Mexico (Gepts, 1988; Singh 1989; Beebe *et al.*, 2000, 2001). Andean beans can be distinguished from Mesoamerican beans on the basis of morphological and biochemical differences such as seed size and seed proteins as well as with various types of molecular markers (Gepts, 1998). The Andean gene pool of common bean (*Phaseolus vulgaris* L.) has high levels of morphological diversity in terms of seed color and size, growth habit and agro-ecological adaptation, but previously was characterized by low levels of molecular marker diversity (Benchimol *et al.*, 2007) Three races have been described within the Andean gene pool: Chile, Nueva Granada and Peru (Blair *et al.*, 2007).

Benchimol *et al.* (2007) reported *P. vulgaris* is widely cultivated in the tropics, subtropics, and temperate regions. Common bean is a predominantly self-pollinated crop (Graham and Ranalli, 1997); however, moderate to high levels of outcrossing have been

reported (Wells *et al.*, 1988 and Gepts, 1993). These may result into improved varieties or formation of new varieties, others with intermediate traits between the genepool as observed also by Blair *et al.* (2010).

2.2 Diversification of Common Bean

Common bean genome has an estimated size of 650 million base pairs (Mb) per haploid genome and cytogenetically, common bean is a true diploid with 11 chromosomes. There is no evidence for poly-ploidisation (Rivkin *et al.*, 1999; Arumuganatham and Earle, 1991; Bennett and Leitch, 1995). *P. vulgaris* is diploid (2n = 2x = 22) and predominantly selfing species, with average outcrossing rates estimated at under 3 % (Ramalho and Abreu, 2006). Broad adaptation, consumer acceptability and genetic diversity have made common bean the most widely grown legume. It's directly consumed by human, with a worldwide distribution and presence in tropical, subtropical and temperate countries and many different environments (Córdoba *et al.*, 2010). Tsvetelina *et al.* (2005) reported the genetic diversity of common bean landraces is of high economic value for global bean biodiversity and is considered of paramount importance for future world production.

2.3 Common Bean Domestication

Within East Africa, the areas bordering the Great Lakes have particularly high per capita common bean consumption rates (above 40 kg/year). The region is intensively farmed due to the high populations with common bean as one of the major crops of each country. The region benefits from bimodal rainfall and therefore common bean can often be planted twice a year and the total production is high (Blair *et al.*, 2010). Frankel *et al.* (1995), commented that landraces are the most diverse populations of cultivated plants and besides being adapted to their natural and man-made environments, landrace genotypes tend to be co-adapted. Hence, genetic variation within a landrace may be

considerable, but is far from random (Qualset *et al.*, 1997). The genetic diversity among and within landraces makes them a valuable resource as potential donors of genes for the development and maintenance of modern crop varieties, and for direct use by farmers (Soleri and Smith, 1995).

2.4 Nutrient Value

The common bean is the world's second most important pulse after soybean. Common bean is a major source of protein and mineral for people in Latin America and Africa (Karen *et al.*, 2005). In addition, common bean seeds are rich in complex carbohydrates, dietary fiber, starch, minerals and vitamins. Blair *et al.* (2009) and Buruchara *et al.* (2011) justified common beans as highly nutritious with almost twice the protein levels of cereals, lower fats as soybean or peanut and has higher amount of lysine, phosphorus, iron, zinc, magnesium, copper and calcium than cereals. Vitamins Biotin is an essential cofactor for a variety of carboxylases and decarboxylases found in diverse metabolic pathways of all organisms (Knowles, 1989). It plays a central role in membrane biogenesis, catabolism of some amino acids and the production of oxaloacetate. Common beans are available in two forms, namely, dry bean and snap bean, which can be consumed as, mature grain, immature seed, as well as a vegetable (both leaves and pods).

It is well known that dry seeds represent an affordable and inexpensive source of proteins despite being deficient in the sulfur amino acids. Like other legumes, common bean seeds contain a number of bioactive compounds including enzyme inhibitors, lectins, phytates, oligosaccharides and phenolic compounds, which play metabolic functions in humans or animals (Angela and Lucia, 2010).

2.5 Importance of Common bean in Tanzania

Dry bean accounts for 57% production of the world food legume, and nearly 80% of dry bean production occurs in the developing countries (FAO, 2003). It is estimated over 75% of households in Tanzania depend on common beans for their daily food (NBS, 2006) since common beans are cheaper source of protein for the poor and one of the most important strategic crops in efforts to eradicate poverty and food insecurity. The government of Tanzania (GOT) initiated a national bean research Program (NBRP) in the early 1980s with the objective of identifying high yielding varieties that are also resistant to diseases and insect pests. It is now more than 30 years since the establishment of NBRP but no farmers preferred bean varieties which have been released which is having most of resistant biotic and abiotic traits. Although CIAT (2008) highlighted that the Common bean (*Phaseolus vulgaris L.*) as one of the major food crops for smallholder farmers in Tanzania while other crops were maize and rice. Hence, improving farmers preferred common bean landraces may made significant positive impacts on the livelihoods of rural farm households in Tanzania. Resulting in improvement of household food security and incomes, poverty reduction, increase in amount of food available during periods of shortage, and savings in fuel wood consumptions.

2.6 Constraints Facing Common Beans

A Biotic and Abiotic stress is another problem that farmers frequently face. Beans require between 200 and 400 mm of rainfall or comparable residual soil moisture during growth and development. It is estimated that up to 73% of the total Latin American and 40% of the total African bean production occurs under micro-climates that have moderate to severe mean water deficits at some time during the cropping season. Recent studies suggest that only 7% of the bean-growing area is well watered (Broughton *et al.*, 2003). Except for a few highland areas with abundant and well-distributed precipitation, and

regions where irrigation is available. Common bean production is exposed to the risk of drought, Soil problems due to toxicities and/or nutritional deficiencies limiting productivity. Small farmers also do not have the capital to solve edaphic limitations through inputs. Moreover, soil problems differ from disease and drought as constraints in the sense that they are largely invariable. Biotic constraint mainly affecting common beans are foliage diseases, root rot diseases, stem diseases which are mainly caused by fungi, bacterial and viral other constraints are field pests and storage pest.

2.7 Importance of Characterizing Common Bean Diversity

In Africa, women farmers, who have little access to fertilizer compared to men farmers, more often grow beans. Intercropping of beans with cereals (maize, millet or sorghum), bananas and plantains or root and tuber crops is common practice. Given these problems, it is not surprising that average yields are low. Much of the bean crop is lost to diseases as well as insect pests or drought, low soil-fertility and other abiotic stresses. Higher-yielding climbing beans have been adopted in some areas of greater population density. Many varieties of beans are grown in Africa, with notable diversity in seed types and adaptation. Local and market preferences as well as the variability in climatic and agronomic conditions generally dictate which varieties are most popular (Broughton *et al.*, 2003).

2.7.1 Why Phenotypic characterization

Common bean landraces usually have local names. They have particular properties, a reputation for adaptation to local climatic conditions and cultural practices, and resistance or tolerance to diseases and pests (Harlan, 1992). Sperling (2001) reported that the phenotypic diversity of common beans is very high in the Great Lakes region due to the fact that much of the common bean crop are grown as varietal mixtures with consumers

accepting a wide range of seed colors; while Vieira *et al.* (1972), reported there were several hundreds of common bean landraces that were cultivated in Brazil emphasizing the importance of such reservoir predominantly, landraces as sources of resistance to diseases.

Common bean have mechanism of coping with environmental variability and with diversifying production in small plots, with early and late maturing components together providing harvestable products over a long period. The cultivated beans are morphologically diverse crop with large variation in growth habit, pigmentation, pod, seed, and phenology (Singh *et al.,* 1991). Oscar *et al.* (2004) on their study they accounted two thirds of the variation within collected Nicaraguan landraces; this was justified earlier by Frankel *et al.* (1995) as landraces are the most diverse populations of cultivated plants with diverse characters which can be used for breeding biotic and abiotic resistant varieties.

2.7.2 Why Molecular marker characterization

Plant improvement implies selection among genetically variable individuals or populations to obtain superior expression of a desired trait. The plant breeder is faced with the challenge of drawing upon and coordinating the use of the many tools developed for the common purpose of improving a crop species for the benefit of the farmer (Broughton *et al.,* 2003). Most traits are still selected by conventional means at field sites where the most important diseases, edaphic constraints and drought are found (Singh *et al.*, 1999). By using molecular marker we can identify important genotypes from our collection having the desired Biotic and abiotic traits.

Tanzania has more than one hundred common bean genotypes, characterizing them is important since, Soleri and Smith (1995), justified the strength of the study as the genetic diversity among and within landraces do make common bean landraces as a valuable resource as potential donors of genes for the development and maintenance of modern crop varieties with good biotic and abiotic factors, and for direct use by farmers. Molecular analyses in conjunction with morphological and agronomic evaluation of germplasms are recommended because these provide complementary information and increase the resolving power of genetic diversity analyses (Singh *et al.,* 1991). Our genotypes can be grouped in the major gene pool, whether Andean beans or Mesoamerican beans on the basis of morphological and biochemical differences such as seed size and seed proteins as well as with various types of molecular markers (Gepts *et al.*, 1998). There is a need for a more comprehensive analysis of genetic diversity and population structure in Tanzania *P. vulgaris* based on a larger sample representative of the major bean growing areas of the crop and a genome-wide sample of markers. This aspect is particularly important for the landrace group, which could be an important reservoir of genetic diversity and rusticity, considering the history of this crop in Tanzania. That is why phenotypic marker and molecular marker has to be used together in order to identify potential common bean genotypes, hence bean breeders can plan for effective bean improvement strategies.

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CHAPTER THREE

3.0 Morphological Characterization of Common Bean Landraces and Varieties in Tanzania

3.1 Abstract

Lack of information about the genetic relatedness in term of morpho-agronomic traits for the common bean landraces and varieties available in Tanzania was the key drive for this study. Twenty morpho-agronomic parameters were accessed among the selected 64 common bean genotypes collected in different regions in Tanzania to assess their similarities and variations. Among all accessions analyzed, 68.9 % were climbing bean while 31.1 % were bush in growth habit. Phylogenetic relationship was developed using scores from morpho-agronomic data, and this demonstrated 2 major clusters separated due to their gene pool. The first cluster was dominated with Mesoamerican genotype while the second cluster with Andean genotypes. Clusters were developed based on the variations of each trait and separated into principal components in PCA where discriminating traits were number of pods per plant, pod length and seed weight each accounting 69.8 %, 21.04 %, and 19.91 % respectively of the total variation. These results managed to group released varieties and landraces together into different sub groups of the major clusters, giving the pedigree information of the original parents for the existing varieties. Also the study determined the important landraces with good quantitative traits which can be used in breeding new varieties with good quantitative and qualitative factors.

3.2 Introduction

Common bean (*Phaseolus vulgaris* L.) is a diverse food legume important to the diet of many people around the world, especially in Latin America and Eastern and Southern Africa (Broughton *et al.*, 2003). Common bean originated and was domesticated in the New World and has two major gene pools, the Andean and the Mesoamerican, based on their centers of origin in South and Central America, respectively (Gepts and Debouck 1991). The diversity in wild accessions of the species can be divided into various sub-populations from specific geographical regions for the species (Chacon *et al.*, 2007 and Miklas *et al.*, 2007). The common bean is by far the most important and widely distributed and has the broadest range of genetic resources (Sing, 1999). It's extremely diverse crop in terms of cultivation methods, uses, the range of environments to which they have been adapted, and on morphological variability. Their genetic resources exist as a complex array of major and minor gene pools, races and intermediate types, with occasional introgression between wild and domesticated-types (Broughton *et al.*, 2003).

In common bean, genetic diversity has been evaluated in the past using both morphological and molecular markers (Galvan *et al.*, 2006). The genetic diversity of landraces is thought to be the economic valuable part of global biodiversity and is considered of paramount importance for future world production (Wood and Lenne, 1997). Morphological differences among groups of wild accessions are not as clear as among races in the cultivated types and rely on differences in seed size, flower coloration, bracteoles size, seed protein (phaseolin) type and in large part on molecular marker evaluations (Kwak and Gepts, 2009; Rossi *et al.*, 2009 and Gepts *et al.*, 1986).

Tanzania has many common bean genotypes constituting new released varieties and local landraces. Tanzania's bean diversity is facing several emerging threats including new persistent diseases, pests, environmental stresses, commercialization, as well as socio-

economic and policy factors. There are more than 100 local landraces which are highly adopted by small scale farmers but their database is not yet officially reported or published (CIAT, 2008). Little information about the released varieties can be found at Agricultural Research Institutes (ARI) and Tanzania Official Seed Certification Agency (TOSCI). Several Researches have only been conducted on morphological characters for the released varieties of *P. vulgaris* found in Tanzania. Estimation of the relationship among landraces and varieties of the common bean in Tanzania is of great interest since breeders can plan for proper management of the common bean germplasms and development of new varieties.

3.2.1 Objective

3.2.1.1 Overall objective

To characterize morpho-agronomic of selected Tanzania common bean landraces and varieties for better understanding of dynamics of the bean genotypes and planning for improvement, conservation, sustainable bean productivity and breeding programs.

3.2.1.2 Specific objective

To characterize morpho-agronomic trait of selected Tanzania common bean landraces and varieties

3.3 Material and Methods

3.3.1 Location of study

This study was conducted at Sokoine university of Agriculture, Morogoro Tanzania during the period of 2010 and 2011 annual season at experimental field of department of Crop Science and Production. The research was performed on 12 commercial varieties and 52 landraces of common bean collected from different geographic zones of Tanzania making the total of 64 bean genotypes as shown on Table 1.

Table 1: Tanzanian common bean varieties and landraces used in this research

No:	Given accession numbers	Local name	Classification	Collection Place
1	BSUA 01	Masai red	Variety	Bashineti – Manyara
2	BSUA 02	Pinto	Variety	Bashineti – Manyara
3	BSUA 03	Maini	Landrace	Itimu – Mbeya
4	BSUA 06	Kisasa	Landrace	Shisonta - Mbeya
5	BSUA 08	Punda	Variety	Bashineti - Manyara
6	BSUA 13	Mekundu	Landrace	Kisanga - Morogoro
7	BSUA 15	Yellow bean (round)	Landrace	Kisanga - Morogoro
8	BSUA 16	White chivoka	Landrace	Iyendwe - Mbeya
9	BSUA 17	Kabumbuli	Landrace	Kisanga - Morogoro
10	BSUA 19	Kalumbugula	Landrace	Kisanga - Morogoro
11	BSUA 23	Kigoma	Landrace	Itimu- Mbeya
12	BSUA 27	Usarago	Landrace	Iringa
13	BSUA 29	Njano ndefu	Variety	Mtumbatu - Morogoro
14	BSUA 31	Rozikoko	Variety	Kilosa - Morogoro
15	BSUA 34	Msafiri	Landrace	Iyendwe - Mbeya
16	BSUA 36	Bwana shamba	Variety	Mamire-Babati-Manyara
17	BSUA 43	Canadian wonder	Landrace	Arusha
18	BSUA 44	Kigoma njano	Landrace	Iyendwe - Mbeya
19	BSUA 53	White bean (nyeupe)	Landrace	Itimu – Mbeya
20	BSUA 56	Kabanima	Variety	Shisonta - Mbeya
21	BSUA 59	Soya Kablanketi	Landrace	Mbeya
22	BSUA 60	Canadian wonder	Variety	Mtumbatu - Morogoro
23	BSUA 78	Grey Iringa	Landrace	Iringa
24	BSUA 79	Ndoga uchee njano	Landrace	Kilolo – Iringa
25	BSUA 85	Lugemba,kiponzelo	Landrace	Iringa
26	BSUA 87	Roba	Variety	Iringa
27	BSUA 100	Rojo	Variety	Morogoro
28	BSUA 111	Rose koko (Nyeusi)	Variety	Arusha
29	BSUA 113	Variagated	Landrace	Arusha
30	BSUA 114	Meusi	Landrace	Arusha
31	BSUA 115	Rose koko	Variety	Arusha
32	BSUA 116	Njano gorori	Landrace	Arusha
33	BSUA 117	Kondoo	Landrace	Arusha
34	BSUA 119	Kiini	Landrace	Geita
35	BSUA 120	Kiratu (Canada)	Landrace	Karagwe
36	BSUA 121	Soworo- Njano	Landrace	Kigoma
37	BSUA 122	Rushahara	Landrace	Bukoba
38	BSUA 123	Njano	Landrace	Ngara
39	BSUA 124	Tumwela Nyeupe	Landrace	Bukoba
40	BSUA 125	Njano - Golori (Rushahara)	Landrace	Bukoba
41	BSUA 127	Rose-tonic	Landrace	Geita
42	BSUA 129	Black-tonic	Landrace	Bukoba
43	BSUA 130	Kaki	Landrace	Geita
44	BSUA 132	Tonic	Landrace	Ngara
45	BSUA 133	Goroli nyekundu	Landrace	Bukoba
46	BSUA 136	Kinyobwa Karanga	Landrace	Karagwe
47	BSUA 137	Sukanywele	Landrace	Ngara
48	BSUA 138	Temakibira	Landrace	Karagwe
49	BSUA 139	Kainja	Landrace	Karagwe
50	BSUA 140	X- nyamizi	Landrace	Geita
51	BSUA 141	Wailimo	Landrace	Bukoba
52	BSUA 142	Tonic	Landrace	Karagwe
53	BSUA 144	Uwondera	Landrace	Bukoba
54	BSUA 145	Soya	Landrace	Bukoba
55	BSUA 146	Gitenge	Landrace	Karagwe
56	BSUA 147	Kayempu	Landrace	Karagwe
57	BSUA 150	Machagga	Landrace	Tanga
58	BSUA 151	Sukanywele	Landrace	Arusha
59	BSUA 152	Sukanywele	Landrace	Arusha
60	BSUA 153	Chombanauroza	Landrace	Bukoba
61	BSUA 154	Mwanamwana	Landrace	Ngara
62	BSUA 155	Karanga	Landrace	Bukoba
63	BSUA 156	Sukanywele	Landrace	Ngara
64	BSUA 158	Sukanywele	Landrace	Geita

Morogoro is characterized by a bimodal rainfall pattern, with unreliable peaks occurring in November / December of some years and reliable ones in February to May. Annual rainfall could be as high as 1500 mm per annum. The average annual temperature in the region's highlands is 18°C but reaches 30°C in the lowland. Rainfall varies from 1200 mm in the highland plateaus to 600 m in lowland (Tryphone *et al.*, 2011).

3.3.2 Experimental design and crop management

A randomized complete block design (RCBD) with three replications was used. Experimental units consisted of two rows of each accession, measuring four meters long with inter- and intra-row spacing of 50 cm X 20 cm respectively. The morphological characterization was scored according to the IBPGR bean descriptors of 1982. Common agricultural management was applied such as weeding and fertilizer application.

3.3.3 Evaluation of *P. vulgaris* morphological traits

Twenty characters were scored using the International Board for Plant Genetic Resources descriptors list (IBPGR, 1982) for *Phaseolus vulgaris* documentation. Two phonological traits; days to emergency (ED) and days to flowering (DTFLO), and 13 qualitative traits including; hypocotyl color (HYP.CLR), emerging cotyledon color (COTCLR), growth habit (Gr.H.), color of standard (CLRSTD), color of wings (CLRWG), pod color (PDCLR), seed coat patterns (SCP), seed coat color (SDCTC), pod curvature (PDCUV), pod cross-section (PDXSC), brilliance of seed (BRLSD), seed shape (SDSHP) and seed size (SSize). Five quantitative traits were evaluated, namely; leaflet length (LFL), number of pods per plant (No. Pod), pod length (PDLG), locules per pod (Loc/Pod), and 100 seeds weight (100Ws). These variables were collected as detailed below:

3.3.3.1 Days to 50 % flowering

This variable was measured as the difference in number of days from the date of planting to date when 50 % of plants in each plot had one or more sets of flower. This stage coincided with the imitation of plant development stage (CIAT, 1987).

3.3.3.2 Leaflet length

This was done by measuring the terminal leaflet of third trifoliate leaf from the pulvinus to the leaf tip on five randomly selected plants in a plot grown under field condition.

3.3.3.3 Color of standard and wings

The color of freshly opened flowers was observed on both color of the standard and on the color of wings. The observed color on each accession was recorded as described in the IBPGR 1982.

3.3.3.4 Pod color

The pod color was recorded from five randomly selected plants per plot from fully expanded immature pod in all accessions by using a color chart.

3.3.3.5 Pod length

Five largest fully expanded immature pods were randomly selected from normal plants, for the measurement of their lengths.

3.3.3.6 Hundred Seed weight

The seed weight for each accession was measured in the seed research laboratory of Department of Crop science and Production by using a weighing balance. This was done by weighing 100 seeds counted randomly from each accession.

3.3.3.7 Number of seeds per pod

The number of seeds per pod was determined by counting the number of locules per pod. This was obtained from 10 randomly selected longest pods from each accession as described in IBPGR (1982).

3.3.3.8 Number of pods per plant

The number of pods per plant was determined as the average number of pods collected randomly from five plants in a plot.

3.3.3.9 Plant type

The grown common bean accession was phenotypically observed for their growth characteristics, whether they were bush type, semi climber, climbers as described on IBPGR (1982) common bean descriptor.

3.3.3.10 Pod cross-section

This was determined from fully expanded immature pod by cutting with a sharp surgical blade and observing whether the cut section was flat, pear, round or figure of eight shaped.

3.3.3.11 Pod curvature

Fully immature expanded pod from five plants in each accession was observed to determine whether their pods were straight, slightly curved, curved or re-curved in shape.

3.3.3.12 Seed coat pattern

The seed coat patterns were scored from harvested seeds in all accessions according to common bean descriptor of 1982 by IBPGR.

3.3.3.13 Seed coat color

Accession used, each expressed its color differently. The seed color from each accession was scored according to the probable genotype as indicated by common bean descriptor by IBPGR 1982.

3.3.3.14 Brilliance of seed

Seeds from each accession were observed to determine whether they were mutt, medium or shiny in appearance by using common bean descriptor IBPGR 1982.

3.3.3.15 Seed shape

The seeds were taken from the middle of the pods and observed for their shapes whether they were round, oval, cuboids, kidney shaped or truncate fastigiated.

3.3.3.16 Emerging cotyledon and hypocotyls color

The color of emerging cotyledon and hypocotyls was scored according to the IBPGR 1982 common bean descriptors.

3.4 Data Analysis for Morpho-agronomic Variables

Twenty morphological data were scored when the common bean accessions start to emerge, attained the physiological maturity, harvested and the weight of 100 seed was taken (Table 2). The scored data was averaged to each characteristic in each accession then subjected to the Gen Stat discovery edition 4 version 10.3 (2011) for their phylogenetic relationship study. SAS statistical software version 8.0 was used to visualize morpho-agronomic traits association among sixty-four common bean varieties and landraces.

3.5 Results

3.5.1 Morpho-agronomic results

Data generated from twenty variables were summarized in Table 2 as shown below.

Table 2: Morpho-agronomic traits in Tanzanian common bean Landraces and varieties

Acc. BSUA	Common bean genotypes	EDate	COTCLR	HYP.CLR	DTFLO	CLRSTD	CLRWG	Gr. IL	LFL (cm)	PDCLR	PDLG (cm)	PDXSC	PDCUV	No. Pods	Loc/Pod	SDCTC	BRLSD	SDSIIP	SCP	SSize	100Ws (g)
1	Masai red - Manyara	5	3	2	31	3	3	5	12.6	7	4.6	2	9	25	5	3	5	3	0	1	28.3
2	Pinto - Manyara	5	3	2	33	3	3	5	9.0	9	7.0	2	7	37	5	4	3	2	2	1	22.1
3	Maini - Mbeya	5	4	2	31	3	3	5	13.6	9	6.1	3	5	32	4	5	5	4	2	2	35.8
6	Kisasa - Mbeya	5	3	2	28	3	3	4	11.6	9	7.7	2	5	24	4	14	7	4	0	3	37.4
8	Punda - Manyara	5	3	2	30	1	1	4	10.6	8	8.5	2	5	14	4	9	5	4	4	3	36.4
13	Mekundu - Morogoro	6	1	2	27	3	3	1	9.5	7	8.0	2	5	23	4	12	7	3	0	3	29.1
15	Yellow bean (round) - Morogoro	6	3	2	28	3	3	1	11.2	8	6.7	2	9	16	4	5	7	2	0	2	30.5
16	White chivoka - Mbeya	5	3	2	32	1	1	5	12.3	7	7.6	2	5	29	5	8	7	2	0	3	30.9
17	Kabumbuli - Morogoro	5	3	2	31	3	3	1	13.4	7	7.0	2	5	13	3	12	7	2	0	2	36.6
19	Kalumbugula - Morogoro	6	3	2	32	3	3	1	13.6	8	6.8	2	5	16	4	12	5	2	0	2	28.2
23	Kigoma - Mbeya	5	3	2	30	1	3	1	10.9	7	7.9	3	5	17	4	5	7	2	0	2	33.2
27	Usarago - Iringa	6	1	2	31	9	8	1	14.7	7	10.5	2	5	18	4	8	7	4	0	3	37.7
29	Njano ndefu - Morogoro	5	1	2	31	3	3	4	11.0	7	4.9	2	7	23	4	5	7	4	0	3	32.5
31	Rozikoko - Morogoro	6	3	2	31	1	1	1	11.2	7	8.3	2	5	30	5	12	5	3	3	3	36.6
34	Msafiri - Mbeya	5	3	2	28	3	3	5	13.0	8	9.6	3	1	31	5	14	5	3	0	3	35.2
36	Bwana shamba - Manyara	6	3	2	32	9	8	1	13.9	7	10.1	1	1	27	5	12	7	3	0	3	36.9
43	Canadian wonder - Arusha	6	3	2	30	3	3	4	13.3	8	9.6	2	1	18	5	12	5	3	0	2	39.0
44	Kigoma njano - Mbeya	6	1	2	31	3	3	4	12.7	7	7.2	2	5	18	3	5	5	3	1	2	38.5
53	White bean (nyeupe) - Mbeya	5	3	2	33	1	1	5	10.5	7	8.0	2	5	32	4	8	5	4	0	3	30.2
56	Kabanima - Mbeya	7	3	2	31	9	8	1	16.9	7	6.8	1	5	6	2	12	5	2	0	3	37.7
59	Soya Kablanketi - Mbeya	6	3	2	31	1	1	5	16.0	8	9.0	2	5	21	3	9	5	2	4	2	37.7
60	Canadian wonder - Morogoro	6	3	2	32	9	8	1	13.0	7	11.0	1	1	29	5	12	7	3	0	3	35.4
78	Grey - Iringa	6	3	1	31	3	3	1	12.9	7	9.3	3	9	15	4	2	5	4	0	2	37.3
79	Ndoga uchee njano - Iringa	5	3	2	30	1	1	5	10.9	9	7.0	2	5	49	4	2	5	3	0	2	29.7
85	Lugemba,kiponzeio - Iringa	6	3	2	31	3	3	5	10.1	8	6.6	2	5	15	4	9	7	4	4	3	34.8
87	Roba - Iringa	6	3	2	32	1	1	5	11.0	7	6.9	2	5	41	4	2	3	3	0	1	12.6
100	Rojo - Morogoro	6	3	2	32	3	3	1	12.4	7	8.6	2	5	9	4	12	7	4	0	2	32.6
111	Rose koko (Nyeusi) - Arusha	5	1	2	26	9	9	1	11.9	3	8.0	2	7	16	5	12	7	2	3	2	36.6
113	Variagated - Arusha	5	4	5	31	9	8	6	10.8	3	6.3	2	1	25	5	6	5	4	2	1	26.8
114	Meusi - Arusha	5	4	5	36	9	9	5	11.2	3	7.7	2	5	44	6	1	5	3	0	1	17.6
115	Rose koko - Arusha	6	1	2	32	1	1	1	10.1	8	6.9	2	5	16	5	9	7	4	3	2	33.3
116	Njano gorori - Arusha	6	3	2	27	3	3	1	9.6	9	6.8	2	5	10	4	5	7	2	0	1	37.6
117	Kondoo - Arusha	5	3	2	33	3	3	4	9.4	9	7.3	2	5	36	4	12	7	4	0	2	36.5
119	Kiini - Geita	5	1	2	37	1	1	6	9.8	7	9.2	2	5	8	5	6	7	3	0	2	25.3
120	Kiratu (Canada) - Karagwe	5	1	2	31	1	1	6	9.4	7	10.5	2	7	28	5	12	7	4	0	3	38.1
121	Soworo- Njano - Kigoma	7	3	2	28	3	3	1	10.1	9	5.1	2	7	22	4	5	5	1	0	2	36.9
122	Rushahara - Bukoba	5	1	2	32	3	3	4	9.4	9	6.8	2	7	34	5	5	7	4	0	3	30.2
123	Njano -Ngara	5	1	2	32	3	3	4	10.4	7	4.9	2	7	23	4	5	7	4	0	3	33.3
124	Tumwela Nyeupe - Bukoba	5	1	2	34	1	1	6	11.2	9	5.2	2	5	23	5	7	5	2	0	1	27.2

Table 2: Continue

Acc. BSUA	Common bean geno types	EDate	COTCLR	HYP.CLR	DTFLO	CLRSTD	CLRWG	Gr. H.	LFL (cm)	PDCLR	PDLG (cm)	PDXSC	PDCUV	No. Pods	Loc/Pod	SDCTC	BRLSD	SDSHP	SCP	SSize	100Ws (g)
125	Njano - Golori (Rushahara) - Bukoba	5	4	2	25	3	3	4	8.6	7	5.8	2	1	16	5	5	7	2	10	1	35.5
127	Rose-tonic - Geita	6	1	2	26	9	9	5	8.9	9	6.2	2	7	30	6	14	5	4	3	3	39.8
129	Black-tonic - Bukoba	6	4	2	30	9	9	5	11.0	8	6.2	2	5	29	6	1	3	3	0	1	20.3
130	Kaki - Geita	6	1	2	25	3	3	1	10.0	7	5.5	2	5	15	5	4	7	3	0	2	29.5
132	Tonic - Ngara	5	4	2	32	9	9	5	9.2	7	7.5	2	5	25	5	14	7	4	8	3	39.9
133	Goroli nyekundu - Bukoba	7	4	2	30	3	3	1	9.3	7	4.8	2	5	22	5	3	7	2	0	2	36.2
136	Kinyobwa Karanga - Karagwe	6	3	2	34	1	3	6	10.0	9	6.3	2	7	26	4	6	5	2	3	2	39.6
137	Sukanywele - Ngara	7	1	2	28	9	3	1	8.1	7	6.4	2	5	19	4	2	5	4	2	3	35.7
138	Temakibira - Karagwe	5	1	2	34	1	1	6	8.9	9	6.7	2	7	21	5	8	7	2	0	1	20.0
139	Kainja - Karagwe	6	1	2	31	1	3	6	10.4	9	7.0	2	7	19	4	3	7	1	0	2	34.5
140	X- nyamizi - Geita	7	1	2	22	11	11	6	11.2	7	6.8	2	5	31	5	4	5	5	3	3	41.1
141	Wailimo - Bukoba	7	4	2	33	9	8	5	9.9	9	6.5	2	5	21	4	12	5	2	0	1	19.9
142	Tonic - Karagwe	7	4	5	30	3	3	4	9.1	9	7.7	2	5	28	5	14	5	4	0	3	40.6
144	Uwondera - Bukoba	6	1	2	27	1	1	6	13.0	7	6.5	2	7	20	3	4	5	2	4	2	28.5
145	Soya - Bukoba	7	1	2	30	3	3	4	9.1	7	6.2	2	5	24	4	4	7	3	4	2	27.9
146	Gitenge - Karagwe	6	1	2	34	3	3	1	9.2	9	8.9	2	5	19	4	12	5	4	3	3	40.7
147	Kayempu - Karagwe	7	1	2	31	3	3	4	7.1	7	5.0	2	1	28	5	4	3	1	1	1	16.8
150	Machagga - Tanga	5	1	2	24	3	3	5	8.2	7	6.7	2	5	32	4	4	5	5	3	3	38.0
151	Sukanywele - Arusha	6	1	2	35	3	3	4	12.8	3	7.8	2	5	13	5	4	5	3	2	2	34.2
152	Sukanywele - Arusha	5	3	2	30	3	3	4	10.8	3	7.6	2	1	23	6	13	7	4	3	2	32.7
153	Chombanauroza - Bukoba	5	1	2	27	1	1	4	9.3	9	6.8	2	5	22	5	2	5	2	0	2	25.9
154	Mwanamwana - Ngara	5	1	2	37	1	1	5	9.9	7	7.4	2	9	24	4	6	5	2	0	1	28.0
155	Karanga - Bukoba	5	4	2	33	3	3	4	8.5	8	4.8	2	5	35	5	2	7	3	2	2	35.6
156	Sukanywele - Ngara	5	1	2	29	3	3	5	11.3	3	5.4	2	7	43	5	6	5	4	2	3	35.4
158	Sukanywele - Geita	6	4	5	30	9	9	6	7.5	7	9.5	3	7	33	5	9	3	4	2	3	40.2

ED (Emergency Days), COTCLR cotyledon color, HYP.CLR (Hypocotyl color), DTFLO (Days to flowering), CLRSTD (Color of standard), CLRWG (Color of wings), Gr.H (growth habit), LFL (Leaflet length), PDCLR (Pod color), PDLG (Pod length), PDXS (Pod cross-section), PDCUV (Pod curvature), No. Pod (Number of Pods per plant), LOC/PD (Locules per pod), SDCTC (Seed coat color), BRLSD (Brilliance of seed), SDSHP (Seed shape), SCP (Seed coat patterns), SSize (Seed size) and 100Ws (100 seeds weight).

3.5.2 Phylogenetic relationship

Morpho-agronomic data scored on Table 2 were subjected for analysis on Gen Stat discovery edition 4 version 10.3 (2011). The hierarchical cluster analysis with complete link in similarity matrix by using the nearest neighbor's algorithm method managed to display a dendogram of the 64 genotypes with comparative high similarity among them ranging from 0.60 to 1.00 coefficients as stipulated on Fig. 1 and supported by Plate 1.

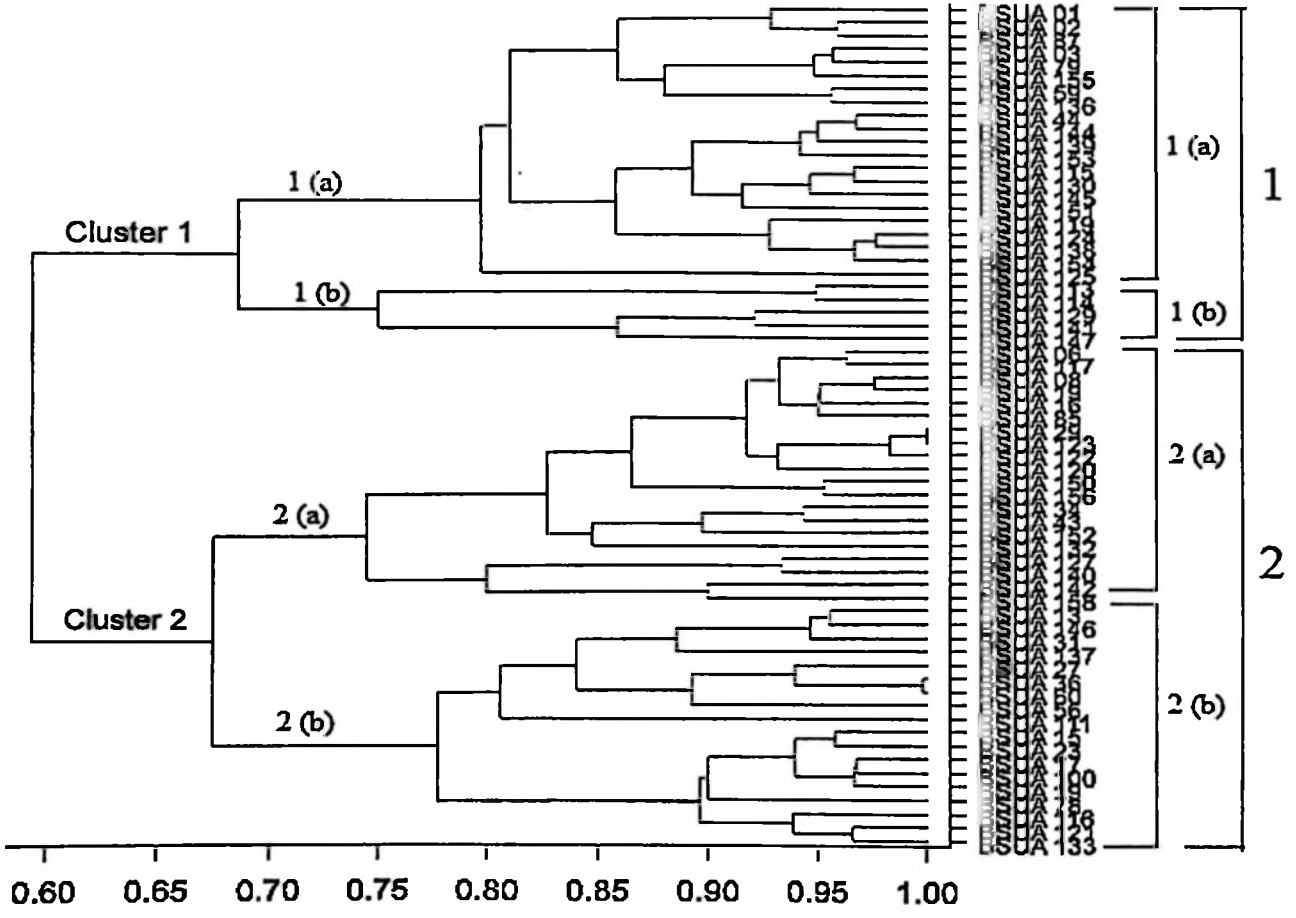
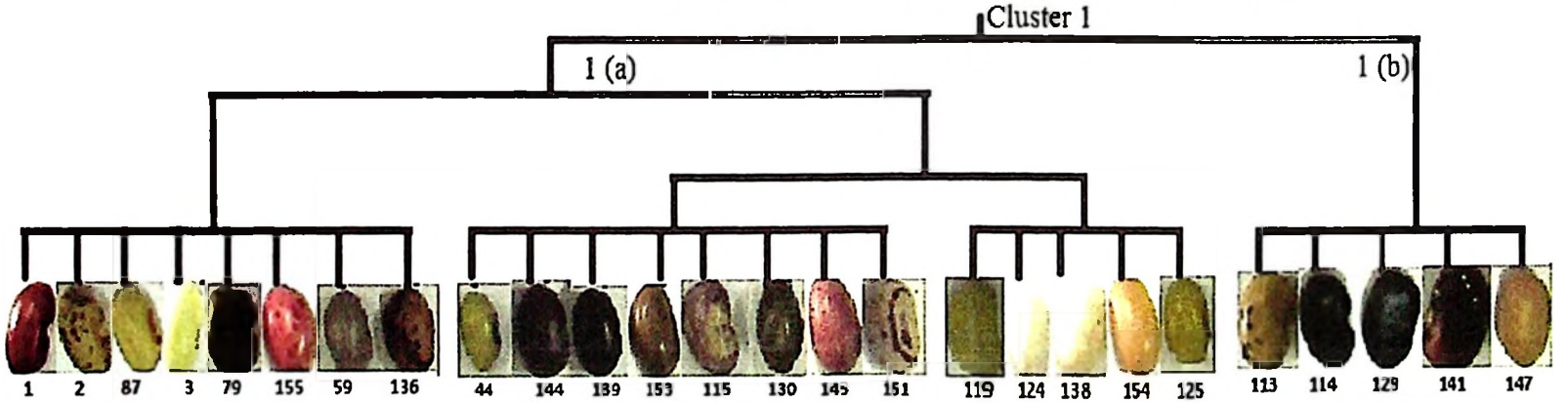


Figure 1: Dendogram of 64 Tanzanian common bean landraces and varieties based on Morpho-agronomic data

Cluster 1: Dominated with genotypes from MesoAmerican gene pool



Cluster 2: Dominated with genotypes from Andean gene pool

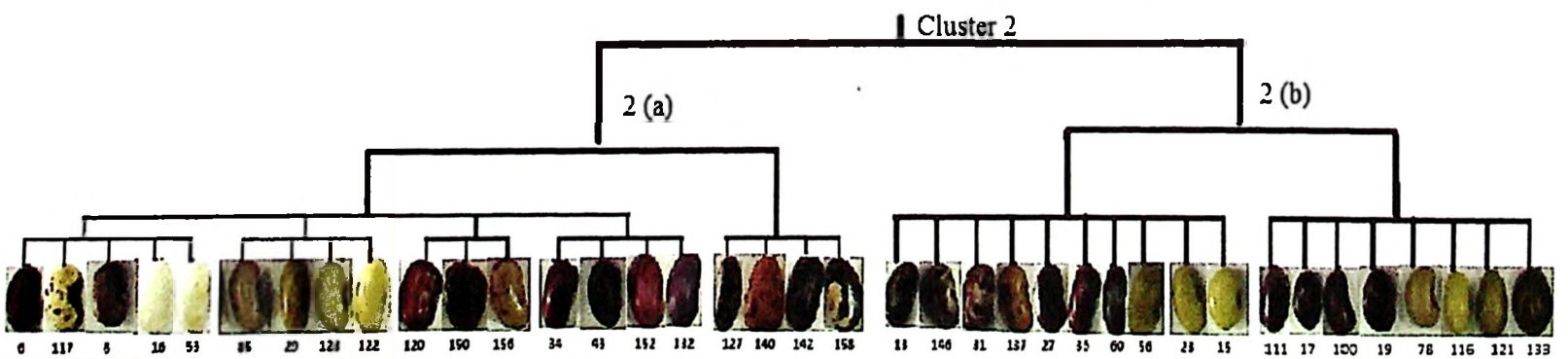


Plate 1: Morphological appearance of the clustered genotypes due to seed color

3.4.3 Principal component analysis

Principal component analysis was performed by using SAS statistical software version 8.0 to visualize morpho-agronomic traits association among sixty-four accessions of common bean landraces and varieties (Table 3).

Table 3: Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
1	3.35861414	0.33128155	0.168	0.1679
2	3.02733259	0.84785236	0.151	0.3193
3	2.17948023	0.54603543	0.109	0.4283
4	1.63344479	0.20866874	0.082	0.5099
5	1.42477605	0.15163892	0.071	0.5812
6	1.27313714	0.17417813	0.064	0.6448
7	1.09895901	0.15270276	0.055	0.6998
8	0.94625625	0.07320411	0.047	0.7471
9	0.87305214	0.06366648	0.044	0.7908
10	0.80938566	0.13434422	0.041	0.8312

3.6 Discussion

3.6.1 Amount and pattern of traits/character distribution among accessions

Tanzanian genotypes used in this study were collected from different agro-ecological zone, the genotype mixture has been postulated to be a mechanism of coping with environmental variability and with diversifying production in small plots, that is why we collected common bean with different phenotypic appearance and others with peculiar characters such as early and late maturing. Blair *et al.* (2010) they highlighted importance of genotype mixture which may be caused due to movements from one agro-ecology zone to another (for example up and down the hillsides or from valley bottom to steep slopes) and where common bean leaves, pods, green seed and dry grain are all consumed at different times of the crop cycle. The cultivated bean is morphologically diverse and has variations in many traits, such as growth habit, phenology, pod pigmentation, and seed pigmentation (Singh *et al.*, 1991).

Phenotypic diversity of common beans is very high in the Great Lakes region due to the fact that much of the common bean crop is grown as varietal mixtures with consumers accepting a wide range of phonological, qualitative and on quantitative traits (Lamb and Hardman 1985; Sperling 2001; Broughton *et al.*, 2003). Basset *et al.* (2002) reported as among of the qualitative traits such as seed coat color and patterns genes lead to the nutritionally important isoflavone. In our study, these characters enabled us to categorize our genotypes due to maximum similarity coefficient of the traits shared among the genotypes that is why we got the two major cluster categorizing the Andean gene pool and the Mesoamerican gene pool.

3.6.1.1 Growth habit as discriminatory factor

Growth habit is usually described as a recessive trait inherited by a single gene, and there is no consensus about the position of the locus (Tatiana *et al.*, 2010). As Growth habit being most variable traits (Oscar *et al.*, 2004); diversifications of our common bean varieties and landraces were clearly observed. Wide variations from bushy to aggressive climbing ability were observed within Tanzania common bean genotypes, though climbing types were widely dominant as have been observed also in the study of Italian genotypes by Piergiovanni *et al.* (2000). Bush types dominated by (31.1%), followed by indeterminate with moderate climbing ability with pods distributed evenly up the plant (28.1%), then indeterminate with semi-climbing main stem with branches (25%) and the least type was indeterminate with aggressive climbing ability with pods mainly on the upper nodes of the plant (15.6%). Angela *et al.* (2010) commented that the predominance of one growth habit type is related to ecological adaptation as well as to the cropping system following their characterization of the Italian common bean landraces. The presence of a different growth habit within these genotypes could be also due to farmer's preferences. Piergiovanni *et al.* (2000) reported the dominant effect of climbing common

bean landraces against bush type while Baldanzi *et al.* (2002) observed that the climbing ability is highly variable as we have also observed in our genotypes.

3.6.1.2 Phonological variations

All accessions emerged five days after planting with the mean days to flowering (DTFLO) of 30.56 accounted by 59.4% of all accessions, while Tsvetelina *et al.* (2005) on characterizing the Bulgarian and Portugal genotypes they find out most of their accessions flowering after 35 days, with mean period to initial flowering of 36.5 days (ranging from 30.3-50.3) days. Meusi from Arusha and Kiini from Geita they flowered after 36 days, these two accessions resemble most of Bulgarian and Portugal genotypes. The remaining 40.6% of the accessions flowered early below 30days after planting, which was below the average.

3.6.1.3 Qualitative characters variation

Breeders provide new cultivars that meet the requirements of farmers and consumers, such as seed color, seed size, maturity, and growth habit (Tatiana *et al.*, 2010). The amount and pattern of distribution in individual morphological trait/character differed significantly among the 64 accessions (Table 2). Most accessions (92.1%) had green hypocotyls color (HYP.CLR), while emerging cotyledon color pre-dominated by green (42.2%) and purple (40.6%) color respectively. A total of 85.9% of all accessions their color of wings resembled the color of standard while the 17.2% of remaining accessions were white in color.

Variation in standard petal color would reflect natural rather than human selection and could be explained by indirect selection of other traits or groups of traits (Go´mez *et al.*, 2004). In freshly opened flower, the predominant color of standard petal (CLRSTD) was

lilalic (50.0%) and others were white (28.1%), purple (20.3%) and light yellow (1.6%), and about 85.9% of all accession the color of wing (CLRWG) resembled the color of standard in exception of 14.1% in variation.

The predominant fully expanded immature pod color (PDCLR) was normal green (48.4%) others were dully green to silver grey (26.6%), shiny green (15.63%) and purple stripe on green (9.4%). The prevalent cross-section of fully expanded immature (PDXSC) was pear shaped (87.5%), where as 7.8% were round elliptic and 4.7% were very flat. Pod curvature (PDCUV) of all accession had slightly curved shape (59.4%), curved shape (21.9%), and straight shape (12.5%) and re-curving pod by 6.2%.

Seed coat pattern (SCPTN) contributed highly in discriminating these accessions (Tsvetelina *et al.*, 2005), whereby about 59.3% of all accession had no patterns the dominant seed coat pattern was rhomboid spotted (14.1%) and stripped by 12.5%, followed by speckled (7.8%), constant mottled (3.1%), pattern around hilum (1.6%) and bicolor coat pattern (1.6%). Piergiovanni *et al.* (2000) reported that the seed coat variation is due to market demand and farmers preference, while the rest had variations in seed coat patterns.

The pigments responsible for seed coat color in common bean are flavonoids and that these compounds have positive health benefits as antioxidants (Angela and Lucia, 2010). Flavonoids have beneficial effects in the human diet as antioxidant, neutralizing free radicals, which damage body tissue and lead to heart disease, stroke and cancer (Encarta, 2008). On our common bean genotypes the predominant seed coat color was red (21.88%) together with yellow to greenish yellow (15.63%) and Grey brownish to greenish by 12.5%. Others were brown pale to dark (10.9%), white purple tinged (7.8%);

pale creamy to buff (7.8%), whitish (6.25%), Black (3.13%) and pink by 1.56%. Tamara, *et al.* (2009) and his group on their study of characterizing the Brazilian common bean landraces they reported how variation in seed color can be counted as one of diversity source on characterizing the bean plant.

3.6.1.4 Quantitative traits variation

Forty point six percent (40.6%) of the accession had high number of pods per plant and many of them associated with high locules per pod, on the data (Table 2) most of these accessions were climbing plant types. There was high range value of 100 seed weight, days to flowering and number of pods per plant indicating a high variability as revealed by these traits, as noted also by Blair *et al.* (2010). The ranges of pod length, number of days a plant to emerge and number of locules per pod were small indicating a low variation of these traits in different bean accession.

3.6.2 Clustering of common bean varieties and landraces

The phylogenetic tree displayed two major clusters at 0.60 coefficients, the first cluster containing 26 genotypes related together at 0.68 coefficients. These genotypes were highly dominated with the Mesoamerican gene pool accessions mixed with intermediate genotypes which were medium in seed size.

The second major cluster was highly dominated with Andean genotypes comprised with 38 genotypes at 0.67 coefficient of similarity as obtained also by Stoilova *et al.* (2005). Sixty point five percent (60.5%) of these genotypes were having large seed size, while remaining 15 genotypes having medium seed size; we identify them as Intermediate genotypes as has been discussed by Blair *et al.* (2010); that may happen due to Inter-gene pool introgression in terms of seed characteristics and alleles. These Andean gene pool

grouped genotypes, were having high levels of morphological diversity in terms of seed color and size, growth habit due to their agro-ecological adaptation as have been observed also by Blair *et al.* (2007).

In the first major cluster with Mesoamerican genotypes, the subgroup formed at 0.85 coefficient of similarity they show to share major morpho-agronomic traits such as Climbing growth habit with late in flower formation (more than 30 days); with slightly curved pods having both small seeded to large seed size genotypes, reported also by Burle *et al.* (2010) when they were characterizing Brazilian landraces they observed Mesoamerican group consisted of two sub-populations with a high level of admixture between them leading to a large proportion of stabilized hybrids not observed in the center of domestication.

While the subgroup formed at 0.75 were having 100% pure Mesoamerican genotypes including BSUA 113, BSUA 114, BSUA 129, BSUA 141, BSUA 147 closely linked with other Mesoamerican genotypes of BSUA 125, BSUA 154, BSUA 138 and BSUA 124. This Mesoamerican genotypes were having aggressive climbing growth habit, with small seed size, their majority weighing less than 28 grams per 100 seeds.

In the second major cluster, two groups were formed at 0.68 coefficients. At 0.83 coefficient, twenty genotypes were clustered together, most of them with early emerging character. These genotypes were having a growth habit of bush to semi climbing character. The genotype in these subgroup having medium to large seed size, with average weight of 35 grams per 100 seeds.

While, the second subgroup clustered at 0.76 were having 18 genotypes. This subgroup segregated independently due to having genotypes with bush growth habit. Other discriminatory character on these genotypes was having long leaflet length more than 10cm, dominated with large seed size with few intermediate medium size seeds. These genotypes most of them were having more than 36 grams per 100 seed weight. Fivao *et al.* (2012) on her research on Tanzania genotypes they observed a positive significant ($P < 0.001$) correlation between yield and pods per plant, 100 seed weight, number of seeds per pod, days to 50% flowering as we have found in this group.

Most of Andean genotypes in cluster 2 were observed to have major important morpho-agronomic traits which have important parameters to be included in future breeding activities especially the genotypes clustered together at 0.84 coefficient; including BSUA 146, BSUA 31 (Rozikoko-Kilosa variety collected in Morogoro), BSUA 137 (Gitenge landrace collected in Karagwe), BSUA 27 (Usarago landrace collected in Iringa), BSUA 36 (Bwana shamba variety collected in Mamire Manyara), BSUA 60 (Canadian wonder variety collected in Mtumbatu Morogoro) and BSUA 56 (Kabanima landrace collected in Mbeya). These accessions produce long pods, many pods per plant with large seed sizes and weights of seed were above 36 grams per weighed 100 seeds in average. These traits in common bean have been studied earlier and represented source of variations that are useful in the crop science breeding programs, particularly in reduction of the vulnerability of improved genotypes (Tamara *et al.*, 2009).

The variation and similarities among the genotypes which enabled these two major clustering to be observed is due to the seed mixtures which are often preferred for home cooking or for local markets. Meanwhile, unique seed colors are only selected for sale to urban populations or export to a niche market. Therefore, as a general rule, production is

not limited to a single seed type. Rather, a wide range of seed colors and sizes are grown, especially for home consumption, (Blair *et al.*, 2010: David and Sperling, 1999).

3.6.3 Variety variation and distribution in the clusters

Among of all analyzed common bean genotypes, only twelve accessions were officially identified as released Varieties from the sixty-four accessions collected from different bean growing areas in our regions. These were Masai red variety collected in Bashineti Manyara (BSUA 01), Pinto variety collected in Bashineti Manyara (BSUA 02), Punda variety collected in Bashineti-Manyara (BSUA 08), Njano ndefu variety collected in Mtumbatu Morogoro (BSUA 29 – Released by ARI Uyole 2008), Rozikoko variety collected in Kilosa Morogoro (BSUA 31), Bwana shamba variety collected in Mamire Babati-Manyara (BSUA 36). Kabanima variety collected in Shisonta Mbeya (BSUA 56 – released by ARI Uyole 1980), Canadian wonder variety collected in Mtumbatu Morogoro (BSUA 60 - released by ARI Selian 1977), Roba variety collected in Iringa (BSUA 87), Rojo variety collected in Morogoro (BSUA 100 released by SUA 1997), Rose koko Nyeusi variety collected in Arusha (BSUA 111) and Rose koko variety collected in Arusha (BSUA 115). Interestingly, these varieties appeared distributed themselves in each subgroup of the formed major clusters. These gave us confidence of saying most of varieties we have, they have some pedigree information with our landraces, as they have been used as the background parents.

3.6.4 Duplicate Identification in our seed bank

Duplication in our seed bank was identified in some of the genotypes we collected from different region referring Fig. 1 and Plate 1. First duplicate were BSUA 36 and BSUA 60 were Identified as Canadian wonder variety, although was collected from different region with different names. Second duplicate was white small bean BSUA 124 and BSUA 138

collected from Bukoba and Karagwe respectively. Third duplicates were Gitenge from karagwe (BSUA 146) and Rozikoko from Morogoro (BSUA 31). Fourth duplicates where medium yellow bean from Mbeya (BSUA 23) and BSUA 15 collected in Morogoro. And the last triplicates were the BSUA 29, BSUA 122 and BSUA 123 was identified as Njano common bean collected from Morogoro, Bukoba and Ngara. Despite of being collected from different ecological zones, with respect to their sub-groups, these genotypes were sharing a lot of morpho-agronomic traits together as revealed in the analysis.

3.6.5 Character associated in different accessions

The SAS statistical software version 8.0 was used to compute the Principal component analysis (PCA) and Pearson product-moment correlations for all morpho-agronomic traits. Principal component analysis was performed to visualize association among sixty-four accessions of common bean landraces and varieties (Table 3).

PCA is a scaling or ordination method that starts with a matrix of similarities between a set of individuals and aims to produce a low-dimensional graphical plot of the data in such a way that distances between points in the plot are close to original (Mohammadi *et al.*, 2003). The result shows that the first seven PC axes recorded eigenvalue greater than 1.09 and explained 69.98% of the total variation. Since the PCs with eigenvalue greater than 1.0 are considered as inherently more informative than any single original variable alone Iezzoni and Pritts, (1991).

The first PC summarizes most of the variability present in the original data relative to all remaining PCs, hence recorded the highest eigenvalue 3.36 and accounted for 16.79% of the total variation, eigenvalues do define the amount of total variation that is displayed on

the PC axes (Mohammadi *et al.*, 2003). Seed weight marked positive and high variations, next was seed coat pattern and seed size (Appendix 2). This axis indicated the most accessions were attributed with positive quantitative traits including also leaflet character, Pod length and high seed weight, complemented with seed coat color, seed size with brilliance trait. This finding justified the statement of Wiley (1981) thus; PCA is a method of data reduction to clarify the relationships between two or more characters and to divide the total variance of the original characters into a limited number of uncorrelated new variables.

The second PC explains most of the variability not summarized by the first PC and uncorrelated with the first (Jolliffe, 1986) and recorded eigenvalue of 3.03 explained by 32% of the total variation, this axis demonstrated explicitly high variation for three qualitative traits which are color of wings, color of standard and on the hypocotyls color. The hypocotyls color marked high variation on PC two, though with negative coefficient on PC 1. The third PC axis demonstrated inverse relationship between Emerging date, cotyledon color, date of flowering, color of flower (standard & wing) and leaflet length.

3.6.8 Variation in gene pool of the analyzed accession

Among of the sixty-four analyzed accessions, 13 accessions were small seeded Mesoamerican type accounting for about 20.31% off all accessions while 79.69% of the remaining accessions were Andean common bean type. Blair *et al.* (2010) on their study to characterize the central Africa inter-gene pool introgression in common bean, they observed seed size was correlated with gene pool identity; Andean group landraces were predominantly medium or large seeded and all Mesoamerican group landraces were either small or medium seeded. In other words, none of the Mesoamerican group genotypes had large seeds as we have found in our studies.

3.7 Conclusion and Recommendations

Findings obtained in this research work justified the existing morpho-agronomic variations among the accessions of common bean landraces and varieties used in this work. The major common bean gene pool were identified the Andean gene pool genotypes, the Mesoamerican genotypes and the intermediate genotypes which are results of gene pool introgression in term of seed characteristics and alleles.

Variety accessions used in this research facilitate the comparison with their resembled landraces grouped together sharing morpho-agronomic traits, hence increased the chance of identifying them as duplicates in our collection which has been given local names by farmers and termed as landraces. This is due to the few varieties we used were widely distributed in different subgroups within the clusters. Other landraces did not resemble any of the varieties used, opening the chances for further studies on their major discriminatory trait by using Molecular markers. Duplicated genotypes in our seed bank were identified, hence narrowing the number of accessions to be maintained; also enabled us to prove the presence of genotype dissemination to the different agro-ecological zones.

Growth habit was one of the morphological traits which discriminate the common bean genotypes analyzed. 68.9% of all accessions had climbing character, while the rest 31.1% had bush plant type. This information revealed that the climbing common bean is highly adopted by bean growers especially in the rural household farmers.

Further studies of morpho-agronomic characterization are recommended to be done to capture higher number of landraces and commercial varieties cultivated in Tanzania and emphasize on multi-location approach for assessing their adaptability to different environments as evaluating their genetic and environment interaction.

Further studies should be conducted to screen for resistance to common bean diseases such as foliage diseases, stem diseases, root rot diseases common bean pests and on abiotic stresses. Since flavonoids and other phenolic compounds are stored in seed coat because of their anti-pathogen and anti-feeding activities in assuring good protection of seeds from external attacks, more research has to be done to identify the landraces and varieties on flavonoid variations and rank their diversity according to seed coat pigmentation.

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CHAPTER FOUR

4.0 Microsatellites diversity among common bean landraces and varieties in Tanzania

4.1 Abstract

Tanzania has more than one hundred common beans (*Phaseolus vulgaris* L.) genotypes including commercially available varieties and landraces. Genotypically little is known about their relatedness and variation that may be well exploited for the genetic improvement of this crop. In this study, Twenty-five common bean microsatellites of Simple Sequence Repeats (SSR) markers were used to characterize different loci on sixty-four Tanzanian common bean landraces and varieties. The genomic DNA of these accessions were amplified using SSR microsatellite markers and results were observed and scored as either monomorphic or polymorphic depending on the number of allelic loci observed per marker. The scored allelic data were subjected to analysis to determine Phylogenetic relationship for clustering using GenStat discovery edition three software. The common bean accessions observed to have mean similarity of coefficient 0.73 and on Principal Component Analysis (PCA) the first eight PC axes recorded eigenvalue greater than 1.00, explained by 75.82% of the total variation among the accessions characterized. These findings justified the existing variation of less than 30% of allelic loci observed among common bean varieties and landraces in Tanzania. This variation existing among the accessions analyzed is important for bean improvement program.

4.2 Introduction

Molecular markers have been used to assist common bean breeding programs in various ways including studies on the origin and diversity of current cultivars (Gepts, 1998; Luiz, 2010). These have demonstrated a potential to detect genetic diversity and to aid in the management of plant genetic resources (Virk *et al.*, 2000). In contrast to morphological traits, molecular markers can reveal differences among genotypes at the DNA level, providing a more direct, reliable and efficient tool for germplasm characterization, conservation and management. Several types of molecular markers are available today but microsatellites are useful for genetic studies because they are co-dominant, high polymorphism/multi-allelic, abundant informative/widely distributed across the genome, convenience of assay by polymerase chain reaction (PCR), require a small amount of DNA for scoring and transferable between different genotypes (Gupta and Varshney, 2000). Information generated by these markers allows comparisons and information exchange between different studies, especially for comparative genetic mapping (Grattapaglia, 2000). Hence, it helps to understand the nature of multilocus association within and between a genome of common bean as a pre-requisite for the identification of associations between genome polymorphisms and qualitative or quantitative traits, such as in association analysis methods for studying this genetic diversity in common bean (Kwak and Gepts, 2009).

Tanzania common bean landraces and varieties have variations that can be useful in breeding programs for purpose of increasing seed quality. Moreover, these genotypes can contribute important characteristics like resistance to biotic and abiotic stresses, diseases, and seed quality, to reduce the vulnerability of improved genotypes. Molecular markers can be used to identify potential genetic traits distributed in different allelic loci of the bean accessions which are associated with resistant to biotic and abiotic constraints.

Hence, by using the SSR marker, we can investigate the level of variations of common bean accessions in Tanzania.

4.3 Objective of the study

The objective of this study was to determine molecular genetic diversity of common bean landraces and varieties found in Tanzania.

4.4 Materials and Methods

4.4.1 Plant materials

Sixty-four genotypes of common bean landraces and varieties were collected from different regions of Tanzania for this research work (Table 1). Five seeds per accession were planted in the screen house for pooled genomic DNA extraction which was used in this research.

4.4.2 Genomic DNA extraction

Genomic DNA was extracted by using modified CTAB protocol by FAO (2009). About 100mg of young leaf tissue was homogenized in 1.5 ml eppendorf tube by using a hand-operated teflon plastic pestle for 60 s, 500 µl of pre heated DNA extraction buffer (0.1M Tris-HCl, pH 8; 0.05 EDTA, pH 8.0; 1.25 % SDS) was added together with 10 µl of RNase (100 mg/ml) and vortexed to mix well. The samples were incubated at 65 °C for 30 min for cell to lyse, and then 300 µl of cooled 6 M ammonium acetate was added, the sample was mixed well by vortexing and then incubated in the fridge at 4 °C for 15 minutes for protein precipitation. The samples were centrifuged for 5 minutes at speed of 13 000 rpm at room temperature. The supernatant (the upper aqueous solution of approximately 700 µl) was transferred to the fresh microfuge tubes and 50 µl CTAB (10 % CTAB, in 0.7 M NaCl) was added to each tube and mixed gently. 700 µl of

chloroform-isoamyl alcohol (24:1) was added and mixed by inverting the tubes upside down to avoid mechanical damage to the DNA. Tubes with samples were centrifuged for 5 minutes at speed of 13 000 rpm. The upper aqueous supernatant was transferred to new eppendorf tube. This upper phase contains the DNA. Five hundred microliter of ice-cold isopropanol was added and mixed gently to avoid mechanical damage to the DNA and DNA was allowed to precipitate for 15 min at -20 °C. Samples were then centrifuged for 20 minutes at 13 000 rpm in order to pellet the DNA. The liquid was carefully drained, and 1000 µl of 70 % ethanol was added and left for 3 minutes to wash the DNA.

Then the sample was centrifuged for 10 minutes at 10 000 rpm and the ethanol drained and 1000 µl of 90 % ethanol was added and centrifuged at 10 000 rpm for 10 min to enable DNA to clump together and easily precipitate at the water/ethanol inter phase. The ethanol was drained and the pellet remaining at the bottom of the centrifuge tube was dried in a flow bench for 15 minutes. The pellet was re-suspended in 100 µl TE and left to dissolve at 4 °C in the refrigerator for 30 minutes followed by centrifuging the tube for 10 min at 13 000 rpm. The supernatant was transferred into a new tube and stored at 4 °C.

4.4.3 Genomic DNA quantitification

The quantity and quality of DNA was evaluated on 1 % (w/v) agarose gels i.e. 1 g of Seakem L. E. Agarose dissolved into 100 ml of 1 X Tris Borate EDTA (0.04 M Tris-Borate, 0.001 M EDTA, pH 8.0). The mixture was heated on a microwave at 95 °C for 3 minutes to allow the mixture to dissolve completely, and allowed to cool to 50 °C, then poured on a gel casting tray fitted with its specific combs to create wells in gel and waited for 25 min at room temperature for gel to polymerize. The polymerized gel on a casting tray was immersed in horizontal electrophoretic tank (Galileo Bioscience, 01-2325, 100 mA) filled with 1 X Tris Borate EDTA and the combs were removed. One microliter of

genomic DNA was mixed with 2 µl of Bromophenol blue loading dye and 3 µl of double distilled water. A total of 6 µl solution was loaded in the wells of prepared 1 % agarose gel in the electrophoretic tank. Loaded samples were separated by 100 V for 1 h. Then the gel was post-stained in 0.5 µg/ml of ethidium bromide solution for 30 min, observed under UV Trans-illuminator, Photographed and results were scored. Bands with good intensity were observed (Plate 2); their sizes were scored in comparison with the reference 25 ng & 50 ng Lamda genomic DNA.

4.4.4 SSR genotyping

4.4.4.1 PCR set up and amplification

Twenty-five common bean SSR markers were supplied by Integrated DNA Technologies (IDT) Company from South Africa (Table 4), and the reagents for Polymerase chain reaction (PCR) were supplied by Qiagen Company of United States of America (USA) with Lot No. 139289388 comprising Taq DNA polymerase, dNTP mix, PCR Buffer and MgCl.

The PCR master mix was prepared in the cold conditions on bench top cooler. A total of 20 µl of PCR master mix with 0.1 U Taq DNA polymerase, 0.2 mM dNTP mix, 10 X PCR Buffer, 1.5 mM MgCl, 1 µM of each primer (forward and reverse), 25 ng genomic DNA and PCR water (DNase free, RNase free water) was mixed according to the required volume. PCR amplification was conducted in 0.2 ml tubes, using an Applied-Biosystem thermal cycler machine (AB 9700). The PCR was set up with these conditions: Denaturation temperature 92.0 ^{0}C for 1 min, annealing temperature was varying due to properties of each primer ranging from 45 ^{0}C to 64 ^{0}C. Extension temperature was 72 ^{0}C for 2 min; final elongation was 72.0 ^{0}C for 2 min and holding temperature was 4.0 ^{0}C.

4.4.4.2 Quantification of PCR products

PCR products were first electrophoresed on 2 % L. E. Agarose to check whether the sample was successfully amplified by a thermal cycler machine and to screen the presence of polymorphism. SSR markers with good amplification were eventually electrophoresed on 6 % non-denaturing horizontal polyacrylamide gels (hPAGE). Compositions of hPAGE were; 40 % Acrylamide/bis-acrylamide (19:1), 10 % (w/v) Ammonium per sulfate, 0.08 % (w/v) TEMED and 10 X TBE. The mixture was poured onto the gel casting plate and the combs were fitted to create wells on the gel. After fifteen minutes the gel was fitted in electrophoretic tank and the tank was filled with 1 X TAE buffer. Six microliters of PCR amplified sample was loaded in the gel together with 5 µl of the reference low molecular weight DNA ladder ranging from 25 bp – 766 bp supplied by BioLabs company of England with Lot. No. 0061005 (Appendix 1).

The loaded samples were run on 1 X TAE buffer at 120 V for 3 h. Thereafter the gel was post-stained with 0.5 µg/ml ethidium bromide solutions for 30 minutes, and destained for 20 minutes on double distilled water. Then gel was placed under UV Trans illuminator (Unitec 500 W), photographed by using a Cannon digital Camera (Power Shot A650 15) and each band was scored by comparing with the reference molecular marker for actual base pair score (Plate 3).

Table 4: SSR markers used in the study

SSR Markers				Scoring		
Code / Accession #	5'-3' Forward SSR sequence	5'-3' Reverse SSR sequence	Tm (°C)	Size (bp)	Size (bp)	Reference:
BM146	GAGATGAGTCCTTTCCCTACCC	TGCAGACACAATTTATGAAGGC	59.6	281	300	Gáitan-Solis *et al.* (2002)
BM172	CTGTAGCTCAAACAGGGCACT	GCAATACCGCCATGAGAGAT	57.6	75	107	Gáitan-Solis *et al.* (2002)
BM199	AAGGAGAATCAGAGAAGCCAAAAG	TGAGGAATGGATGTAGCTCAGG	60	210	280	Gáitan-Solis *et al.* (2002)
BM149	CGATGGATGGATGGTTGCAG	GGGCCGACAAGTTACATCAAATTC	59.8	240	500	Gáitan-Solis *et al.* (2002)
G118	GCATCGCCATTCCCGTCC	GGAAATTGGTAGGGGCAGTGTCTTC	63	140	170	Gáitan-Solis *et al.* (2002)
G2490	GAAGGCATTGATCGTTGAACTTCATAGC	TATTTGTTCACAGGGGTTGGAGTT	61.6	165	200	Gáitan-Solis *et al.* (2002)
G91	GAGTGCGGAAGCGAGTAGAG	TCCGTGTTCCTCTGTCTGTG	60.2	229	250	Gáitan-Solis *et al.* (2002)
G2654	CTATGCTCTCAAGTACCGCAAATTGG	GGTTCCTCCATTTCTTTGTCCAACTC	58.2	190	205	Gáitan-Solis *et al.* (2002)
PV003	TCACGTACGAGTTGAATCTCAGGAT	GGTGTCGGAGAGGTTAAGGTTG	61.7	164	205	Yu *et al.* (2000)
PV004	TTGATGACGTGGATGCATTGC	AAAGGGCTAGGGAGAGTAAGTTGG	61.3	200	300	Yu *et al.* (2000)
BMd45	GGTTGGGAAGCCTCATACAG	ATCTTCGACCCACCTTGCT	55.8	98	130	Blair *et al.* 2003
BMd2	AGCGACAGCAAGAGAACCTC	CAACAAACGGTGATTGACCA	55.5	76	106	Blair *et al.* 2003
BMd3	TGTTTCTTCCTTATGGTTAGGTTG	GTATCCTCCGATCAAATTCACCT	54.4	223	223	Blair *et al.* 2003
BMd7	GGATATGGTGGTGATCAAGGA	CATACCCAATGCCATGTTCTC	54.5	166	330	Blair *et al.* 2003
BMd15	TTGCCATCGTTGCTTAATTG	TTGGAGGAAGCCATGTATGC	53.5	166	180	Blair *et al.* 2003
BMd16	ATGACACCACTGGCCATACA	GCACTGCGACATGAGAGAAA	55.9	136	140	Blair *et al.* 2003
BMd09	TATGACACCACTGGCCATACA	CACTGCGACATGAGAGAAAGA	54.5	135	140	Blair *et al.* 2003
BMd26	CTTGCCTTGTGCTTCCTTCT	TCCATTCCCAACCAAGTTTC	54.3	141	141	Blair *et al.* 2003
BM68	TTCGTTCACAACCTCTTGCATT	TGCTTGTTATCTTGCCCAGTG	56	170	210	Gáitan-Solis *et al.* (2002)
BM143	GGGAAATGAACAGAGGAAA	ATGTTGGGAACTTTTAGTGTG	55	143	180	Gáitan-Solis *et al.* (2002)
BM157	ACTTAACAAGGAATAGCCACACA	GTTAATTGTTTCCAATATCAACCTG	52	100	113	Gáitan-Solis *et al.* (2002)
BMd1	CAAATCGCAACACCTCACAA	GTCGGAGCCATCATCTGTTT	47	165	200	Blair *et al.*, 2003
BMd18	AAAGTTGGACGCACTGTGATT	TCGTGAGGTAGGAGTTTGGTG	47	156	156	Blair *et al.*, 2003
BMd19	GCCAACCACATTCTTCCCTAC	GGAAGCGAGGCAGTTATCTTT	47	154	154	Blair *et al.*, 2003
BMd47	ACCTGGTCCCTCAAACCAAT	CAATGGAGCACCAAAGATCA	47	150	150	Blair *et al.*, 2003

4.5 Data Collection

The observed bands were scored according to allele sizes in base pair (bp) with reference to molecular maker (50-100 bp ladder) and their sizes were recorded in excel sheets. The binary data was also scored on amplified markers (1) as present and (0) as absent meaning no amplification of specific marker on the accession was recorded as discrete variables. The presence of bands was evaluated as dominant genotype as (AA), heterozygous genotype as (Aa) and the homozygous genotype as (aa), these was done according to IPGRI and Cornell University (2003).

4.6 Data analysis for evaluation of genetic diversity of *P. vulgaris*

The Unweighted Pair-Group Mean Arithmetic (UPGMA) dendrogram was analyzed using GenStat discovery edition three software (Payne *et. al.,* 2007), whereas Jaccard's similarity coefficient was used to compute the binary data matrix that was: =NAB/ (NAB+NA+NB) were as NAB is the number of alleles (band) shared by samples, NA represents the fragment amplified in sample A and NB represents the fragment amplified in sample B. GenStat discovery edition three software was used to compute all data scored from PCR products and intrapopulation genetic diversity was measured. The number of variants such as; Polymorphism or rate of polymorphism which demonstrate that microsatellites alleles were showing variation, Proportion of polymorphic loci which expresses the percentage of variable loci in a population, Average number of alleles per locus, Effective number of alleles expected in a locus in each accession, average expected heterozygosity (Nei's genetic diversity) was computed and they estimate the extent of genetic variability in the analyzed genotypes as have been reported by Cavalli–Sforza *et al.,* (1981).The relationship between all genotypes was determined and Clustered on Phylogenetic tree by using Neighbour-Joining method (NJ) specific algorithm for constructing the best tree.

4.7 Results

The results obtained in these objective was impressing since the allelic data we scored, after analysis they justified the similarities and difference among the common bean accessions we have in our collection, and managed to identify the duplicated genotypes in our seed bank.

4.7.1 Quantification of DNA

Quantification of genomic DNA, results shows sharp bands which imply good quality of extracted DNA (Plate 2).

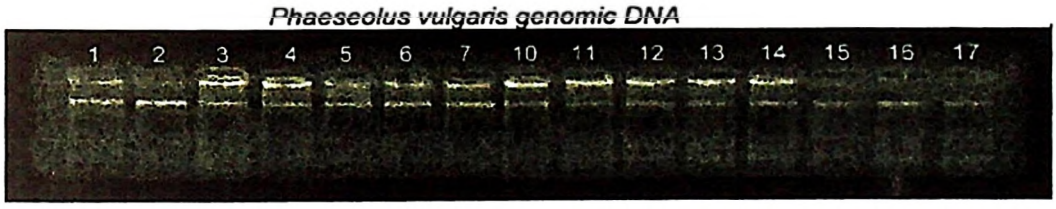

Plate 2: *Phaseolus vulgaris* L. Genomic DNA

4.7.2 PCR products

Allelic loci were scored on the revealed clear separation of 2% L.E. Agarose gel. The Plate 3 below with G91 results is among of the simple sequence repeat used in this study.

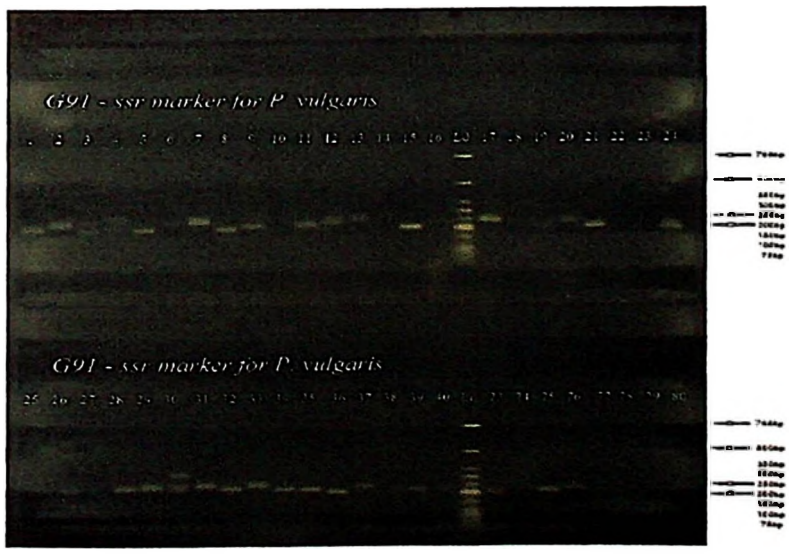

Plate 3: SSR marker separated on 2% Agarose gel

Table 5: Simple Sequence Repeat (SSR) marker score results

Markers	BM146		BM172		BM 199		BM 149			G 118			G2190		G91		G2654		PV 003		PV 004		BMd 45	
Accession	BM146LI	BM146LII	BM172LI	BM172LII	BM199LI	BM199LII	BM149LI	BM149LII	BM149LIII	G118LI	G118LII	G118LIII	G2490LI	G2490LII	G91LI	G91LII	G2654LI	G2654LII	PV003LI	PV003LII	PV004LI	PV004LII	BMd45LI	BMd45LII
BSUA 01	281	281	75	75	280	280	500	240	240	140	140	140	165	165	250	250	190	190	164	164	300	200	98	98
BSUA 02	281	281	75	75	210	280	240	240	240	140	140	140	165	165	250	250	190	190	164	164	303	200	130	130
BSUA 03	281	281	107	107	280	280	240	240	240	170	170	170	165	165	229	229	205	205	285	164	300	200	130	130
BSUA 06	281	281	107	107	0	0	240	240	240	150	150	150	165	165	229	229	0	0	164	164	300	200	130	130
BSUA 08	281	281	75	75	210	280	240	240	240	170	170	170	165	165	229	229	190	190	164	164	300	200	130	130
BSUA 13	281	281	107	107	280	280	240	240	240	170	170	170	0	0	250	250	0	0	205	164	200	200	130	130
BSUA 15	0	0	75	107	280	280	240	240	240	170	170	170	0	0	229	229	0	0	164	164	200	200	130	130
BSUA 16	281	281	75	75	210	280	240	240	240	150	150	150	165	165	250	250	190	190	164	164	200	200	98	98
BSUA 17	281	281	107	107	280	280	240	240	240	140	140	140	165	165	229	229	205	205	205	164	300	200	130	150
BSUA 19	281	281	107	107	210	280	240	240	240	140	140	140	165	165	229	229	190	190	164	164	200	200	130	130
BSUA 23	281	281	107	107	210	280	240	240	240	170	170	170	0	0	229	229	190	190	164	164	300	200	130	130
BSUA 27	281	281	107	107	210	280	500	240	240	140	140	140	165	185	229	229	205	205	205	164	300	200	130	130
BSUA 29	281	281	107	107	210	280	240	240	240	150	150	150	165	165	229	229	190	190	164	164	300	200	130	130
BSUA 31	281	281	107	107	210	280	240	240	240	170	170	170	165	165	229	229	190	190	164	164	300	200	130	130
BSUA 34	281	281	107	107	280	280	240	240	240	170	170	170	165	165	229	229	0	0	164	164	300	200	130	130
BSUA 36	300	281	107	107	210	280	500	240	240	150	150	150	165	165	229	250	205	205	164	164	300	200	130	130
BSUA 43	281	281	107	107	280	280	240	240	240	170	170	170	165	165	229	229	205	205	205	164	300	200	130	130
BSUA 44	281	281	107	107	210	280	240	240	240	170	170	170	165	165	250	250	190	190	205	164	200	200	130	130
BSUA 53	281	281	75	75	210	280	500	240	240	140	140	140	165	165	229	229	190	190	164	164	300	200	98	98
BSUA 56	281	281	107	107	210	280	500	270	240	170	150	150	165	165	229	229	190	190	164	164	300	200	0	0
BSUA 59	281	281	107	107	210	280	240	240	240	170	170	170	165	165	229	229	190	190	164	164	300	200	130	130
BSUA 60	281	281	107	107	280	280	240	240	240	170	170	170	165	165	229	229	190	190	164	164	300	200	130	130
BSUA 78	281	281	107	107	0	0	240	240	240	170	170	170	165	165	229	229	190	190	164	164	300	200	130	130
BSUA 79	281	281	75	75	280	280	240	240	240	150	150	150	165	165	229	229	190	190	205	164	300	200	98	98
BSUA 85	281	281	75	75	280	280	240	240	240	170	170	170	0	0	0	0	190	190	164	164	200	200	130	130
BSUA 87	281	281	75	75	210	280	240	240	240	150	150	150	165	165	250	250	130	190	205	164	300	200	130	130
BSUA 100	281	281	75	75	0	0	240	240	240	170	170	170	165	165	229	229	0	0	0	0	250	250	130	130
BSUA 111	281	281	75	75	280	280	240	240	240	140	140	140	0	0	229	229	205	205	164	164	300	200	130	130
BSUA 113	300	281	75	75	0	0	240	240	240	150	150	150	165	165	229	250	190	190	205	164	300	200	130	130
BSUA 114	300	281	75	75	210	280	240	240	240	140	140	140	165	165	250	250	190	190	205	164	300	200	98	98
BSUA 115	300	281	75	107	210	280	500	270	240	170	150	0	165	165	229	250	0	0	205	164	0	0	130	130
BSUA 116	300	281	75	75	280	280	240	240	240	150	150	150	165	165	229	229	190	190	0	0	300	200	130	130
BSUA 117	281	281	107	107	280	280	500	240	240	150	150	150	165	165	228	250	205	205	164	164	300	200	130	130
BSUA 119	281	281	0	0	0	0	0	0	0	140	140	140	0	0	0	0	0	0	205	164	300	200	130	130
BSUA 120	281	281	75	75	280	280	240	240	240	150	150	150	165	165	250	250	190	190	164	164	300	200	98	98
BSUA 121	281	281	75	75	210	280	240	240	240	170	170	170	165	165	229	229	190	190	164	164	300	200	130	130
BSUA 122	281	281	107	107	280	280	240	240	240	150	150	150	165	165	229	229	205	205	205	164	300	200	130	130
BSUA 123	0	0	0	0	0	0	500	270	240	0	0	0	0	0	0	0	0	0	205	164	200	200	130	130
BSUA 124	281	281	75	75	210	280	240	240	240	140	140	140	165	165	250	250	190	190	205	164	300	200	130	130
BSUA 125	281	281	75	75	280	280	240	240	240	140	140	140	165	165	229	229	190	190	164	164	300	200	0	0
BSUA 127	300	281	75	75	280	280	240	240	240	150	150	150	165	165	250	250	190	190	164	164	200	200	98	98
BSUA 129	281	281	107	107	210	280	240	240	240	170	170	170	165	165	0	0	105	190	164	164	300	200	98	130
BSUA 130	300	281	107	107	280	280	240	240	240	0	0	0	165	165	229	229	205	205	164	164	300	200	130	130
BSUA 132	281	281	107	107	210	280	240	240	240	140	140	140	165	165	0	0	190	190	205	164	300	200	130	130
BSUA 133	281	281	107	107	210	280	240	240	240	150	150	150	165	165	229	229	0	0	210	164	300	200	130	130
BSUA 136	281	281	107	107	210	280	240	240	240	170	170	170	165	165	229	229	190	190	164	164	300	200	130	130
BSUA 137	281	281	107	107	210	280	240	240	240	170	170	170	165	165	229	229	190	190	230	164	300	200	130	130
BSUA 138	281	281	75	75	280	280	240	240	240	140	140	140	165	165	250	250	190	190	164	164	300	200	98	98
BSUA 139	281	281	107	107	0	0	240	240	240	0	0	0	165	165	229	229	0	0	164	164	200	200	130	130
BSUA 140	281	281	0	0	280	280	240	240	240	170	170	170	165	165	250	250	190	190	0	0	200	200	98	98
BSUA 141	281	281	75	75	210	280	240	240	240	150	150	150	165	165	229	229	190	190	230	164	300	200	98	98
BSUA 142	281	281	107	107	210	280	240	240	240	170	170	170	165	165	229	229	190	190	230	164	300	200	130	130
BSUA 144	281	281	75	75	210	280	240	240	240	140	140	140	165	165	250	250	190	190	164	164	300	200	98	98
BSUA 145	0	0	75	75	210	280	240	240	240	150	150	150	165	165	0	0	0	0	164	164	0	0	130	130
BSUA 146	281	281	107	107	280	280	240	240	240	150	150	150	165	165	229	250	190	190	230	164	0	0	130	130
BSUA 147	281	281	75	75	210	280	500	500	240	170	170	170	165	165	250	250	190	190	0	0	300	200	98	98
BSUA 150	281	281	0	0	280	280	240	240	240	170	170	170	165	165	229	229	190	190	164	164	200	200	98	98
BSUA 151	281	281	107	107	280	280	500	240	240	140	140	140	165	165	229	250	190	190	205	164	300	200	130	130
BSUA 152	281	281	75	107	210	280	500	240	240	170	150	0	165	165	229	229	190	205	205	164	0	0	0	0
BSUA 153	281	281	75	75	280	280	240	240	240	140	140	140	165	165	250	250	190	190	164	164	300	200	130	130
BSUA 154	281	281	0	0	280	280	240	240	240	140	140	140	165	165	250	250	190	190	205	164	300	200	98	98
BSUA 155	281	281	107	107	210	280	240	240	240	150	150	150	165	165	229	229	190	190	205	164	300	200	130	130
BSUA 156	300	281	107	107	210	280	240	240	240	150	150	150	165	165	229	229	0	0	164	164	300	200	130	130
BSUA 158	281	281	107	107	210	280	240	240	240	150	150	150	200	165	229	229	190	190	205	164	200	200	130	130

Table 5: Simple Sequence Repeat (SSR) marker score results continue...

Marker	BMd2		BMd3		BMd7		BMd15		BMd16		BMd09		BMd26		BM48		BM143		BM157		BMd1		BMd18		BMd19		BMd47	
Accesion	BMd2LI	BMd2LII	BMd3LI	BMd3LII	BMd7LI	BMd7LII	BMd15LI	BMd15LII	BMd16LI	BMd16LII	BMd09LI	BMd09LII	BMd26LI	BMd26LII	BM48LI	BM48LII	BM143LI	BM143LII	BM157LI	BM157LII	BMd1LI	BMd1LII	BMd18LI	BMd18LII	BMd19LI	BMd19LII	BMd47LI	BMd47LII
BSUA 01	106	106	0	0	166	166	0	0	136	136	135	135	141	141	170	170	0	0	0	0	0	0	0	0	0	0	0	0
BSUA 02	106	106	0	0	166	166	156	166	0	0	0	0	141	141	170	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 05	106	106	0	0	166	330	166	166	136	136	135	135	141	141	170	170	180	180	113	113	200	165	156	156	154	154	150	150
BSUA 06	106	106	0	0	166	166	166	166	136	136	0	0	0	0	170	170	0	0	0	0	165	165	156	156	154	154	0	0
BSUA 08	106	106	223	223	166	330	166	166	136	136	135	135	141	141	210	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 13	106	106	223	223	166	166	166	166	136	136	135	135	141	141	170	170	0	0	0	0	200	165	156	156	154	154	150	150
BSUA 19	106	106	223	223	166	166	166	166	136	136	135	135	141	141	170	170	0	0	0	0	185	165	156	156	154	154	150	150
BSUA 20	106	106	223	223	166	166	0	0	0	0	141	141	170	170	143	143	113	113	165	165	156	156	154	154	150	150		
BSUA 21	106	106	0	0	166	330	166	166	136	136	135	135	141	141	170	170	0	0	0	0	165	165	156	156	154	154	150	150
BSUA 23	106	106	0	0	166	166	0	0	136	136	135	135	141	141	170	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 27	106	106	223	223	166	166	166	166	136	136	135	135	141	141	170	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 29	106	106	223	223	166	330	166	166	136	136	135	135	141	141	170	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 31	106	106	0	0	166	166	166	166	136	136	135	135	141	141	170	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 34	106	106	0	0	166	166	166	166	136	136	0	0	141	141	170	170	0	0	113	113	165	165	156	156	154	154	150	150
BSUA 36	106	106	223	223	166	166	0	0	136	136	135	135	141	141	210	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 43	106	106	223	223	166	330	166	166	136	136	135	135	141	141	170	170	180	180	113	113	200	165	156	156	154	154	150	150
BSUA 44	106	106	223	223	166	166	0	0	136	136	135	135	141	141	170	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 51	106	106	223	223	166	166	0	0	138	138	135	135	141	141	0	0	143	143	113	100	200	165	156	156	154	154	150	150
BSUA 56	106	106	223	223	166	166	0	0	138	138	135	135	141	141	210	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 59	106	106	223	223	0	0	166	166	136	136	135	135	141	141	170	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 60	106	106	223	223	166	166	166	166	136	136	135	135	141	141	170	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 78	106	106	0	0	166	166	166	166	136	136	135	135	141	141	170	170	0	0	113	113	165	165	156	156	154	154	150	150
BSUA 79	106	106	223	223	166	330	166	180	0	0	135	135	141	141	170	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 89	106	106	0	0	166	166	0	0	136	136	135	135	141	141	170	170	0	0	0	0	200	165	156	156	154	154	150	150
BSUA 97	106	106	223	223	166	166	180	180	136	136	135	135	141	141	210	170	143	143	100	100	200	165	156	156	154	154	150	150
BSUA 100	106	106	0	0	0	0	166	166	136	136	135	135	141	141	170	170	180	180	113	113	200	165	156	156	154	154	150	150
BSUA 111	106	106	223	223	166	166	166	166	136	136	135	135	141	141	0	0	0	0	0	0	0	0	156	156	154	154	150	150
BSUA 115	74	106	223	223	166	166	0	0	140	136	135	135	141	141	0	0	143	143	0	0	0	0	0	0	0	0	0	0
BSUA 116	106	106	223	223	166	166	166	166	140	136	140	140	141	141	170	170	141	143	113	113	200	165	156	156	154	154	150	150
BSUA 124	106	106	223	223	166	166	0	0	136	136	135	135	141	141	170	170	180	180	113	113	165	165	156	156	154	154	150	150
BSUA 126	106	106	0	0	166	166	166	166	136	136	135	135	141	141	170	170	180	180	113	113	165	165	156	156	154	154	150	150
BSUA 127	106	106	223	223	166	166	0	0	136	136	135	135	141	141	170	170	143	143	0	0	200	165	156	156	154	154	150	150
BSUA 131	74	106	223	223	166	166	0	0	136	136	135	135	141	141	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BSUA 132	106	106	223	223	166	166	166	180	136	136	135	135	141	141	170	170	143	143	113	113	165	165	0	0	154	154	150	150
BSUA 133	106	106	223	223	0	0	166	166	136	136	135	135	141	141	170	170	180	180	113	113	165	165	156	156	154	154	150	150
BSUA 135	106	106	223	223	166	166	166	166	136	136	135	135	141	141	170	170	143	143	0	0	165	165	156	156	154	154	150	150
BSUA 139	106	106	223	223	166	166	0	0	136	136	135	135	141	141	170	170	143	143	0	0	165	165	156	156	154	154	150	150
BSUA 141	106	106	223	223	166	166	0	0	136	136	135	135	141	141	170	170	143	143	0	0	200	165	156	156	154	154	150	150
BSUA 142	106	106	223	223	166	166	166	166	136	136	135	135	141	141	170	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 144	106	106	223	223	166	166	166	166	136	136	135	135	141	141	170	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 145	0	0	0	0	0	0	0	0	136	136	135	135	141	141	170	170	143	143	0	0	200	165	156	156	154	154	150	150
BSUA 146	106	106	0	0	170	170	166	166	138	136	135	135	141	141	170	170	0	0	0	0	165	165	156	156	154	154	150	150
BSUA 147	106	106	223	223	166	166	166	166	156	136	135	135	141	141	210	170	143	143	100	100	200	165	156	156	154	154	150	150
BSUA 150	106	106	0	0	166	166	0	0	0	0	0	0	141	141	170	170	143	143	100	100	165	165	156	156	154	154	150	150
BSUA 151	106	106	223	223	166	166	0	0	136	136	135	135	141	141	170	170	180	180	113	113	200	165	156	156	154	154	150	150
BSUA 153	0	0	0	0	0	0	0	0	0	0	0	0	0	0	170	170	180	180	113	113	200	165	156	156	0	0	150	150
BSUA 154	106	106	223	223	166	166	0	0	140	138	135	135	141	141	170	170	0	0	0	0	165	165	156	156	154	154	150	150
BSUA 155	106	106	0	0	166	166	166	166	0	0	0	0	141	141	170	170	143	143	113	113	0	0	156	156	154	154	0	0
BSUA 157	106	106	223	223	166	166	166	166	136	136	135	135	141	141	170	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 158	106	106	0	0	166	330	166	166	138	136	135	135	141	141	170	170	143	143	113	113	200	165	156	156	154	154	150	150
BSUA 159	106	106	223	223	166	166	0	0	136	136	135	135	141	141	170	170	143	141	111	111	200	165	156	156	154	154	150	150

Table 6: Summary of Genetic and Allelic frequency showing variation of common bean landraces and varieties in Tanzania with response to SSR marker

Locus	Genotypes $A_1 A_1$	$A_1 A_2$	$A_2 A_2$	Gene frequency A_1	A_2	Allelic frequency f(A1)	f(A2)	Expected Heterozygosity $H_e = 1-(A_1A_1)^2-(A_2A_2)^2$	Allelic frequency summary $=((2*(A_1A_1 + A_2A_2) + (A_1A_2) / (2*64))$	% Homozygosity A_1A_1	% Heterozygote A_1A_2	Total no. of Alleles	Proportion of Polymorphic loci
BM146	0	8	52	0.08	0.48	4	56	0.76	0.88	81.3	12.5	60	0.13
BM172	24	3	32	0.23	0.29	25.5	33.5	0.87	0.9	87.5	4.7	59	0.05
BM199	25	31	0	0.45	0.26	40.5	15.5	0.73	0.63	39.1	48.4	56	0.55
BM149	0	12	50	0.11	0.5	6	56	0.74	0.88	78.1	18.8	62	0.19
G118	22	2	36	0.2	0.31	23	37	0.86	0.92	90.6	3.1	60	0.03
G2490	0	1	54	0.02	0.45	0.5	54.5	0.8	0.85	84.4	1.6	55	0.02
G91	16	6	35	0.19	0.34	19	38	0.85	0.84	79.7	9.4	57	0.11
G2654	9	2	39	0.1	0.34	10	40	0.88	0.77	75	3.1	50	0.04
PV003	0	26	33	0.22	0.48	13	46	0.73	0.72	51.6	40.6	59	0.44
PV004	1	47	11	0.39	0.47	24.5	34.5	0.63	0.55	18.8	73.4	59	0.8
BMd45	46	0	14	0.38	0.13	46	14	0.84	0.94	93.8	0	60	0
BMd2	55	3	2	0.47	0.05	56.5	3.5	0.78	0.91	89.1	4.7	60	0.05
BMd3	40	0	0	0.33	0.02	40	0	0.89	0.63	62.5	0	40	0
BMd7	1	9	48	0.09	0.46	5.5	52.5	0.78	0.84	76.6	14.1	58	0.16
BMd15	2	2	36	0.05	0.31	3	37	0.9	0.61	59.4	3.1	40	0.05
BMd16	1	4	52	0.05	0.45	3	54	0.79	0.86	82.8	6.3	57	0.07
BMd09	4	0	51	0.05	0.41	4	51	0.83	0.86	85.9	0	55	0
BMd26	0	0	60	0.02	0.48	0	60	0.77	0.94	93.8	0	60	0
BM68	0	12	45	0.11	0.46	6	51	0.78	0.8	70.3	18.8	57	0.21
BM143	7	7	35	0.13	0.34	10.5	38.5	0.87	0.71	65.6	10.9	49	0.14
BM157	41	1	6	0.34	0.07	41.5	6.5	0.88	0.74	73.4	1.6	48	0.02
BMd1	0	40	15	0.33	0.45	20	35	0.69	0.55	23.4	62.5	55	0.73
BMd18	0	0	56	0.02	0.45	0	56	0.79	0.88	87.5	0	56	0
BMd19	0	0	56	0.02	0.45	0	56	0.79	0.88	87.5	0	56	0
BMd47	0	0	56	0.02	0.45	0	56	0.79	0.88	87.5	0	56	0

4.8 Cluster Analysis

At 0.55 coefficient of variation on Fig: 2 below; two major clusters were formed. First cluster having the Mesoamerican genotypes while the second major cluster dominated with Andean genotypes. Few intermediate genotypes were observed aggregating together with the Andean and Mesoamerican genotypes while others segregating independently outside of major clusters at 0.3 coefficient.

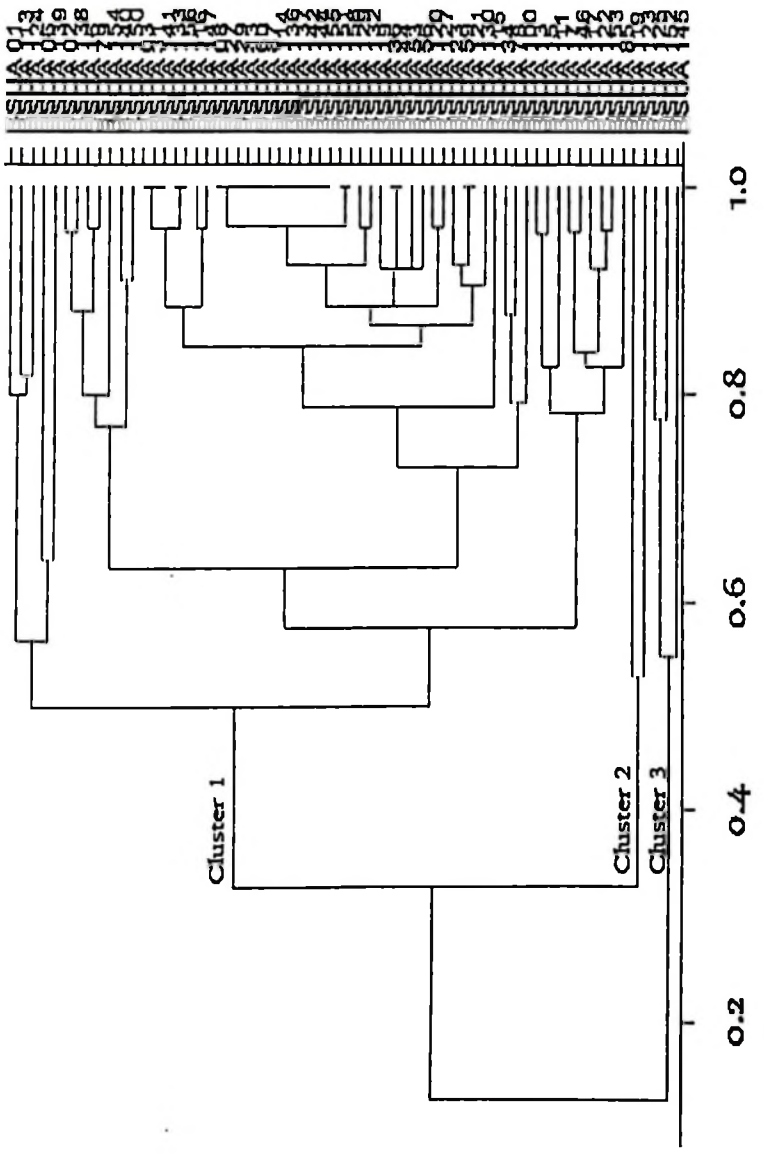

Figure 2: A Phylogenetic tree showing the relatedness of 64 landraces and varieties as characterized by SSR marker

4.9 Principal Component Analysis

Principal component analysis was performed to visualize association of sixty-four accessions of common bean landraces and varieties using molecular markers. The result showed that the first eight PC axes recorded eigenvalue greater than 1.00 and accounted for 75.82 % of the total variation (Table 7).

Table 7: Association of common bean accessions by Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
1	5.14730837	1.0132568	0.2059	0.2059
2	4.13405157	1.64979463	0.1654	0.3713
3	2.48425694	0.57386005	0.0994	0.4706
4	1.91039689	0.20554635	0.0764	0.55
5	1.70485054	0.32463354	0.0682	0.6152
6	1.38021701	0.18865408	0.0552	0.6704
7	1.19156293	0.18861147	0.0477	0.7181
8	1.00295146	0.1554482	0.0401	0.7582
9	0.84750327	0.09558225	0.0339	0.7921
10	0.75192101	0.09084203	0.0301	0.8222

4.10 PCA dimension 1 Versus Dimension 3

The PCA reduced data to clarify the relationships between characters of common bean landraces and varieties in response to twenty-five molecular markers (Fig. 3). The PCA derived a 3 dimensional scatter plot of individuals such that the geometrical distances among individuals in the plot reflected the genetic distances among them with minimal distortion and the aggregations of individuals in such a plot reveals the sets of genetically similar individuals with the respective SSR makers.

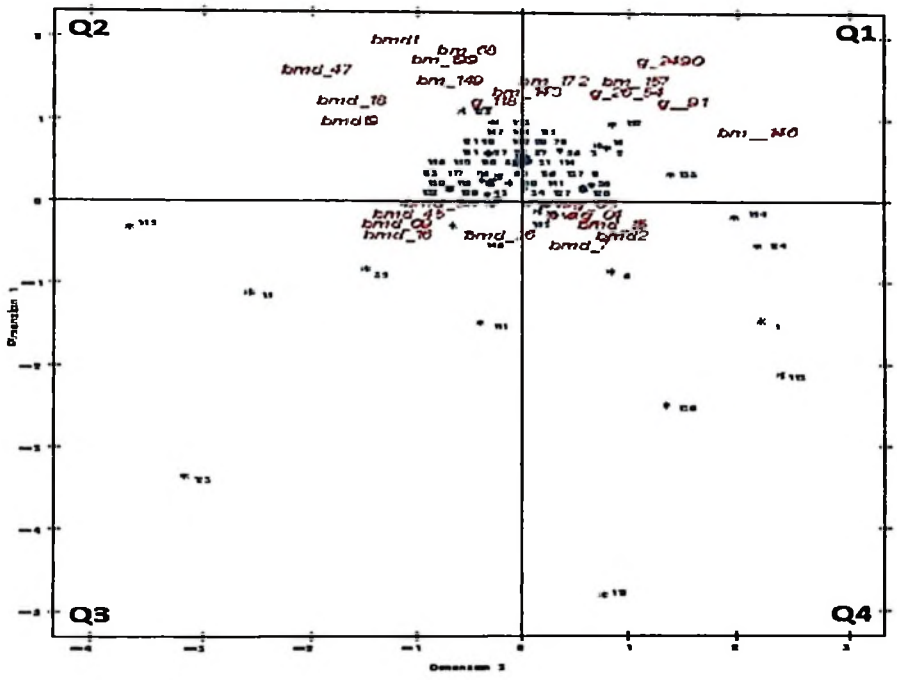

Figure 3: Bi-plot chart showing dispersion of common bean landraces and varieties (in blue color) with response to SSR molecular marker (in red color)

4.11 Discussion

4.11.1 Allelic scoring

The expected total number of genotypes to be fully amplified by the twenty-five SSR marker was sixty-four, but none of the marker used amplified the whole target genotypes as have been observed on marker G91 (Plate 3), this has been reported also by Robinson and Harris (1999) as a specific problem during analysis of genetic diversity in crop plants by using SSR molecular markers, it was concluded that it is often difficult to ascertain whether such lack of amplification is due to null alleles or Microsatellite markers exhibiting higher allelic polymorphism should give more accurate data, although the problem of homoplasy is not completely avoided as stated by Metais *et al.* (2002). The scored results are presented in the Table 5; statistically summarized at table 6 and by cluster analysis on Fig. 2 and by Principle component analysis on Table 7 respectively.

SSR markers used amplified the desired allelic regions in different loci. On documentation base pair results were scored; Genotypic diversification on these diploid plants were observed during scoring, single band in a loci indicated homozygosity of allele on a loci with the same base pair, while double band or more than two band indicated heterozygosity of two alleles with different base pair in different loci. The scored results guided on genetic analysis on analyzing the variation and similarities of common bean landraces and varieties as have been stipulated on Table 6.

4.11.2 Phylogenetic relationship of Common bean landraces and varieties

The dendogram shows the comparative similarities among populations. Common bean genotypes were displayed in cluster ranging from 0.2 to 1.0 coefficient of similarity. A clear separation of genotypes was found although some few Andean genotypes appeared integrated within Mesoamerican group and likewise in sub groups of a dendogram. Blair *et al.* (2010) reported these may happen due to Inter-gene pool introgression in terms of seed characteristics and alleles.

Among of the sixty four genotypes analyzed by using 24 microsatellite markers, 13 genotypes were having the small seed size and we regard them as Mesoamerican genotypes and the remaining 51 genotypes with medium to large seed size and we regarded them as Andean introgressed with intermediate genotypes, resembling results were obtained by Blair *et al.* (2010). These accounted that; off all the genotypes analyzed, 79.7% were Andean genotypes indicating as the most dominant gene pool in Tanzania. Previous studies of southern and East African bean germplasms which were done by Martin and Adams (1987); and Wortmann *et al.* (1998) they find that most of the genotypes analyzed was from the Andean gene pool.

The hierarchical clustering analysis by using Jacard coefficient identified two major clusters at 0.55 coefficient of similarity with few outlier accessions. The first major cluster was dominated with the Mesoamerican genotypes and the second major cluster with more than 38 genotypes were dominated with Andean gene pool accessions by 82% remaining were intermediate genotypes; with exception of the first subgroup in the second major cluster which comprises the Mesoamerican genotypes at 0.79 coefficient and these subgroup were closely related to the Mesoamerican genotypes of the first major cluster by 0.7 coefficient. Blair *et al.* (2006) on their study for their of breeding lines and germplasms accessions they find out that, microsatellite markers uncover high levels of diversity in Andean beans and that this diversity may be higher even than in the Mesoamerican gene pool.

At 0.79 coefficients, nine accessions Andean genotypes grouped together in the second major cluster. These has been observed also by Singh *et al.* (1991a, b) where he predicted that there is less diversity among Andean cultivars than in Mesoamerican cultivars and that compared to wild accessions, the cultivated Andean gene pool underwent a reduction in diversity, however none of the studies could define race structure at the molecular level or define how this structure would agree with the morphological classification. Five accessions segregated independently from the two major clusters at 0.3 coefficients, indicating these accessions they shared few genetic information with the other genotype analyzed and these genotypes and they were landraces, simply intermediate genotypes.

The dendogram were able to identify some of the duplicated genotypes which were from different region with different names but genetically they do resemble and others even phenotypically they resemble. Among of them was BSUA 43 collected in Morogoro and BSUA 60 collected from Arusha which were identified as Canadian wonder varieties by

1.0 coefficient. BSUA 151 and BSUA 158 these were Sukanywele landraces with black striations collected from Arusha and Geita respectively, these genotypes were clustered together by 0.95 coefficients in similarities.

At 0.8 coefficients just below the group of Mesoamerican genotype, two genotypes clustered together which were BSUA 140 and BSUA 150 (X-nyamisi from Geita and Machagga from Tanga respectively). These genotypes they shared a lot of genetic Information with the small seeded (Mesoamerican genotypes) these are landraces which are considered like wild common bean by farmers, morphologically they have a flat kidney shape and having high seed weight, hence more work has to be done in order to be categorised as a real Andean *Phaseolus vulgaris* L. genotypes or as *Phaseolus vulgaris*, Fabaceae.

The Mesoamerican BSUA 129 (Black tonic-Bukoba) and the Andean BSUA 132 (Tonic-Ngara) were clustered together at 0.95 coefficient of variation, indicating these genotypes are sharing a lot of genetic information, hence having the pedigree information among them.

The Mesoamerican genotypes grouped in the first cluster (BSUA 01, BSUA 113, BSUA 124, BSUA 139) together with Mesoamerican genotypes (BSUA 02, BSUA 138, BSUA 154) at first subgroup in the second major cluster despite of sharing a lot of genetic information together, they also show common phenotypic characters such as all are climbing common beans. These was observed also by Blair *et al.* (2010) after characterization of Rwanda genotypes he identified majority of them were Mesoamerican genotypes with climbing growth habit and they observed as most of them were resistant to diseases especially the root rot disease. These genotypes they germinated early within

five days after sowing and attain 50% flowering after 30 days and having long leaflet length more than 9 cm. They make pear shaped pods with pod length more than 20 cm; most accessions have small seed size weighing less than 28 grams of their seed weight after weighing 100 seeds per genotype. Blair *et al.* (2007) he justified in his discussion that there are closely relationship between the detected genes by microsatellite marker with their phenotypic expressions.

The group having 100% Andean genotypes of BSUA 100, BSUA 13, BSUA 15, BSUA 111, BSUA 17, BSUA 146, BSUA 117, BSUA 122 and BSUA 153; despite of sharing lot of genetic information together, they also show phenotypic characters in common such as most of them their growth habit were bush type with late emerging character germinating five days after sowing. These genotypes have pear shaped pods bearing medium to large seed size with average of 33.2 grams for the measured 100 seed weight.

Interestingly, the Twelve Varieties which has been used in this work were successfully distributed in different subgroups of these two major clusters. For example, the Mesoamerican grouped genotypes were clustered together with BSUA 01 and BSUA 02 varieties while the remaining ten varieties (distributed independently in different subgroups of the second major cluster of Andean genotypes. Tohme *et al.* (1995) and *Beebe et al.* (2001) observed the trend of distribution of varieties together with landraces in different clusters during their studies and indicated there was direct sharing of genetic information between genotypes in the same cluster.

Germplasm exchange, gene flow and crossing programs between and within the two gene pools have given rise to introgression between gene pools (Beebe *et al.*, 2001; Islam *et al.*, 2004) and between races (Diaz and Blair, 2006) resulting in intermediate phenotypes

that do not classify well into any single race or gene pool. In our experiment, three intermediate genotypes (BSUA 06, BSUA 16 and BSUA 79) clustered within Mesoamerican group, while seven intermediated genotypes (BSUA 141, BSUA 116, BSUA 147, BSUA 87, BSUA 114, BSUA 129, BSUA 125) clustered in the Andean gene pool accessions. These was observed also by Blair *et al.* (2010) as ten of their genotypes that were intermediate between the gene pools in the neighbour-joining tree and that had mixed seed characteristics, grouped with the Andeans in the UPGMA dendogram, while 22 grouped with the Mesoamerican or Middle American accessions.

Microsatellite markers have generally detected the highest levels of genetic diversity of any nuclear marker type for cultivated common beans reaching as high as 0.64 depending on the germplasms analyzed, the detection system used and whether they were landraces or breeding lines from a specific region or from a wider geographical area (Blair *et al.*, 2006; Hanai *et al.*, 2007; Zhang *et al.*, 2008). While on our studies we Identified 0.55 genetic diversity for the landraces and varieties we have analyzed in Tanzania. That was high compared to genetic diversity analysed by other markers previously used in the world; for example, Becerra-Velazquez and Gepts (1994), analyzing both gene pools and found genetic diversity of 0.38 with RFLP markers, while Paredes-Lopez and Gepts (1995) found a genetic diversity of only 0.16 with isozyme markers and Beebe *et al.* (2001) found even lower values with AFLP markers in Andean beans. In this study microsatellite markers were advantageous because they detected greater genetic polymorphisms compared to other marker types (Diaz and Blair 2006).

4.11.3 Diversification of common bean landraces and varieties

The allelic frequency computed were below 0.95 which indicated there was polymorphism on SSR marker used against the landraces and varieties analyzed, this

demonstrated that the gene were showing variation (Table 6). This has been supported by Cavalli–Sforza, L.L. and W.F. Bodmer, (1981) reported that gene is polymorphic if the frequency of one of its alleles is less than or equal to 0.95 or 0.99. In this work, only 18 markers out of 25 markers used showed the polymorphism hence summarizing the proportion of polymorphic loci for markers used indicating the 72% polymorphism (Table 6). Blair *et al.*, (2009) in their research they find out of all the markers they used, only a single locus were detected, except for one marker which detected two loci distinguished by the pattern of stutter band amplification and size range. While on our work Seven SSR markers including BMd 45, BMd 3, BMd 9, BMd 26, BMd 18, BMd 19 and BMd 47 produced only single band on analyzed accessions indicating homozygous alleles but their loci was scored in different position example loci of BMd 45 were scored at 98 bp and 130 bp, hence the seven markers yield little diversification information of bean landraces and varieties compared to the rest eighteen SSR markers as have been observed on Table 6 results.

Results obtained by SSR markers show that most of our landraces and varieties they do share most important traits, but they do differ on some traits making the diversification of our analyzed landraces and varieties to be high. Loci analyzed with marker BMd1 and PV004 they showed high diversification by 55% followed with marker BMd15, BMd3 and BM199. While loci analyzed with marker BMd45 and BMd26 they showed low diversity among the landraces and varieties analyzed, indicating that allelic loci is highly inherited among common bean accessions analyzed.

4.11.4 Association of molecular marker versus common bean landraces and varieties

The Principal component analysis provided variable independence and balanced weighting of traits, which leads to an effective contribution of different characters on the

basis of respective variation with respective to SSR molecular marker we have used. The Principal components was applied since the correlations among the characters were near significant on a molecular marker dendrograms by 0.73 coefficient, as Goodman, (1972) and Everitt, (1980) justified the importance of constructing the Principal component analysis when the correlations among the characters are significant on the dendrogram. Since, PCA can also be used to determine the optimum number of clusters in a study (Mohammadi *et al.*, 2003).

The first PC axis recorded the highest eigenvalue 5.15 and accounted for 20.59% of the total variation. Marker BMd1 and BM68 marked positive with high variations. The second PC axis recorded eigenvalue of 4.13 and explained 37.13% of the total variation; this axis demonstrated explicitly high variation for marker BMd 2, BMd 26, BMd 45, BMd 15 and BMd 7 respectively though they all have negative coefficient on PC 1. The third PC axis was recorded with 2.48 eigenvalue which was accounted by 47.06 of total variation and demonstrated by marker g91, g2490 and g2654. The g2490 had negative coefficient on PC2; while the rest was having positive coefficient on all PC axes.

The PCA derived from a 3-dimensional scatter plot were based on 75.82% of the total variation on first eight PC axes. The first ordination of PC axis dimension one versus three were having twenty six accessions which were highly categorized by seven molecular markers with positive coefficients. Four markers bm146, g91, g2490 and g2654 show high variations on accessions distributed in these ordinations in quadrant 1, though marker bm146 and g91 contributed positively on PC 2. Most of these accessions they shared some common characteristic of these markers except some few accessions which were BSUA 152, BSUA 15, BSUA 133, BSUA 2, BSUA 120, BSUA 56, and

BSUA 35. Twenty accessions at ordination two were highly characterized with eight molecular markers. Most of the accessions were clustered together with exception of BSUA 125 which shows variation with other accession members have perculiar character such as being small seeded, early flowering charcter with medium seed weigh as per weighed 100 seed weight.

Ordination in the third quadrant has eight accessions accounting for 12.5% of all accessions which were widely distributed in the quadrant sharing markers which had negative coefficient in both first and second PC axes. Phenotypically there were large seeded accessions such as BSUA 123 (Njano ndefu), BSUA 15 (Yellow bean), BSUA 145 (Soya ndefu) and BSUA 111 (rozikoko) which show discriminatory effect with other accessions as stipulated by molecular markers. Nine accessions were highly characterized by five molecular markers in this ordination of quadrant four. Phenotypically were small seeded to medium size.

4.12 Conclusion

Sixty-four common bean genotype accessed were dominated with 79.69 % Andean genotypes mixed with intermediate genotypes; while only 13 accessions (20.31%) identified to be Mesoamerican type. Andean type was widely distributed in the second major clusters while the Mesoamerican genotypes clustered in the first major cluster.

Eighteen SSR markers were found to be more informative in this study out of all twenty-five SSR markers used; out of them five markers show high polymorphisms than the rest including marker BMd1, PV004, BMd15, BMd3 and BM199. The SSR markers used, managed to group the common bean accession into two major Clusters at 0.55 coefficients with few accessions forming outside the cutoff coefficient.

The microsatellite markers enabled the clustering of different groups within the major cluster which were highly sharing most of their allelic loci. Presence of variety in our study help us to identify landraces which were highly associated with the variety, hence we can know the Pedigree of the variety from their background parents. Since; twelve officially Identified varieties (Table: 1) used in this study were widely distributed in this two clusters and enabled some of the general link with their co-aggregated group members corresponding with their coefficient of similarities. The microsatellite markers managed to identify duplicated genotypes in our seed bank especially varieties which have given different names by farmers in different agro-ecological zones.

This study it covered very few common bean genotypes only sixty-four accessions with only twenty-five SSR marker due to limited resources. We therefore recommend further study to be done on more common bean genotype by using advanced molecular techniques in order to identify more potential genotypes existing in our country for future breeding programs.

Also we recommend the use of marker associated gene specific such as linked to good quantitative traits, qualitative traits, biotic resistant trait and abiotic resistant characters to be used specifically to characterize *Phaseolus vulgaris* L. accessions we have in Tanzania.

4.13 References

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CHAPTER FIVE

5.0 Extended Conclusion and Recommendations

Characterization of common bean genotypes collected from different bean growing areas in Tanzania was done by studying both morpho-agronomic traits of all accession followed by molecular fingerprinting of this accessions by using SSR molecular markers. In this study we used sixty-four accessions whereby twelve of them were officially released varieties and the rest were landraces.

The study identified variations existing between these accessions and their commonly shared traits. In both analysis the characters used / allelic loci were able to differentiate the major common bean gene pool of Mesoamerican and the Andean genotypes.

Varieties used in this study distributed in different sub-groups of the formed major cluster, and enabled as to relate them with the landraces grouped together by comparing the evaluated traits. Interestingly we find that the Pinto and Roba varieties grouping them self with other small seeded genotypes in both two studies. Indicating that despite of Mesoamerican genotypes sharing the morpho-agronomic traits; they also share some important allelic loci in their genome.

Andean genotypes were dominant accessions in this study being distributed in different major clusters, some varieties of Andean genotype morpho-agronomically resembled some of collected landraces indicating it can be the same variety adopted locally by farmers example Rozikoko variety (BSUA 111) collected in Arusha resembled with Gitenge landrace (BSUA 146) from Karagwe. Genotypically the SSR molecular marker identified some of the Andean genotype sharing most of the allelic loci with the landraces in different sub-groups due to specific coefficient of their similarities.

Hence; by using information obtained in this study we can identify duplicates in common bean collections in our bean seed bank by identifying the named landraces which are varieties, also plant breeders can use this information for planning proper breeding strategies by easily identifying the pedigree of released varieties with their co-assisted background parents. Also plant breeders can use identified seeds in this study with desired morpho-agronomic traits as have been discussed to plan proper breeding strategy for new varieties with more desired traits.

We recommend further study to be conducted on most of common bean genotypes available in our country and to study many (more than twenty we evaluate) morpho-agronomic traits associated between them, biotic and abiotic constraints existing among them and multi-location trials to be conducted in different common bean growing areas to evaluate their adaptation due to genetic and environment interaction (G & E). Genotyping by using advanced molecular techniques should be done to characterize our genotypes, and identify more duplicates in common bean bank. By using specific molecular markers, potential traits can be identified associated with biotic and abiotic resistant / tolerant traits.

APPENDICES

Appendix 1: Bio-lab ladder

Low Molecular Weight DNA Ladder

1-800-632-7799
info@neb.com
www.neb.com

N3233S

100 gel lanes (50 µg) Lot: 0061005 Exp: 5/12
500 µg/ml Store at –20°C

1.5 ml Gel Loading Dye, Blue (6X) Store at 25°C

Description: A proprietary plasmid is digested to completion with appropriate restriction enzymes to yield 11 bands suitable for use as molecular weight standards for both agarose and polyacrylamide gel electrophoresis. This digested DNA includes fragments ranging from 25–766 base pairs. The 200 base pair band has increased intensity to serve as a reference point.

Supplied in: 10 mM Tris-HCl (pH 8.0), 1 mM EDTA.

Reagents supplied:
6X Gel Loading Dye, Blue

1X Gel Loading Dye, Blue:
2.5% Ficoll-400
11 mM EDTA
3.3 mM Tris-HCl (pH 8.0@25°C)
0.017% SDS
0.015% bromophenol blue

Preparation: Double-stranded DNA is digested to completion with the appropriate restriction enzymes, phenol extracted, ethanol precipitated and equilibrated to 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA.

Usage Recommendation: We recommend loading 0.5 µg of the Low Molecular Weight DNA Ladder diluted in sample buffer. This ladder was not designed for precise quantification of DNA mass but can be used for approximating the mass of DNA in comparably intense samples of similar size. The approximate mass of DNA in each of the bands in our Low Molecular Weight DNA Ladder is as follows (assuming a 0.5 µg loading):

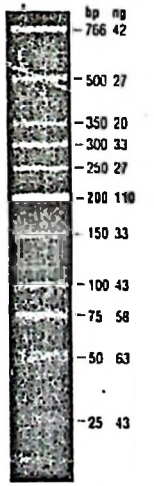
Fragment	Base Pairs	DNA Mass
1	766	42 ng
2	500	27 ng
3	350	20 ng
4	300	33 ng
5	250	27 ng
6	200	110 ng
7	150	33 ng
8	100	43 ng
9	75	58 ng
10	50	63 ng
11	25	43 ng

Notes: All ends have 5′ overhangs that can be end labeled using T4 Polynucleotide Kinase (NEB #M0201) or filled-in using DNA Polymerase I, Klenow Fragment (NEB #M0210) (1). Use α-[^{32}P] dCTP or α-[^{32}P] dGTP for the fill-in reaction.

DNA ladders are stable for at least 3 months at 4°C.

For long term storage, store at –20°C. If samples need to be diluted, use TE or other buffer of minimal ionic strength. DNA may denature if diluted in dH_2O.

LMW DNA Ladder visualized by ethidium bromide staining on a 1.8% TBE agarose gel. Mass values are for 0.5 µg/lane.



(see other side)

CERTIFICATE OF ANALYSIS

Appendix 2: Correlation matrix of Morpho-agrononomic traits

The PRINCOMP Procedure

Observations 64
Variables 20

Simple Statistics

	SSize	ED	COTCLR	HYP_CLR	DTFLO
Mean	2.190476190	5.650793651	2.380952381	2.174603175	30.69841270
StD	0.737413589	0.699279126	1.183605365	0.752194264	2.73964279

Simple Statistics

	CLRSTD	CLRWG	Gr__H_	LFL	PDCLR
Mean	3.666666667	3.571428571	3.619047619	10.87611757	7.317460317
StD	2.873684832	2.557180187	1.904397046	1.98548654	1.644398084

Simple Statistics

	PDLG	PDXSC	PDCUV	No__Pod	Loc_Pod
Mean	7.216243386	2.031746032	5.190476190	23.74365079	4.437129630
StD	1.528073405	0.357782326	1.990762075	8.93465748	0.742040738

Simple Statistics

	SDCTC	BRLSD	SDSHP	SCP	_00Ws
Mean	7.396825397	5.761904762	3.000000000	1.269841270	32.50162619
StD	4.062176766	1.266367496	0.967204152	2.001663415	6.46138883

Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
1	3.35861414	0.33128155	0.1679	0.1679
2	3.02733259	0.84785236	0.1514	0.3193
3	2.17948023	0.54603543	0.1090	0.4283
4	1.63344479	0.20866874	0.0817	0.5099
5	1.42477605	0.15163892	0.0712	0.5812
6	1.27313714	0.17417813	0.0637	0.6448
7	1.09895901	0.15270276	0.0549	0.6998
8	0.94625625	0.07320411	0.0473	0.7471
9	0.87305214	0.06366648	0.0437	0.7908
10	0.80938566	0.13434422	0.0405	0.8312
11	0.67504144	0.10693753	0.0338	0.8650
12	0.56810391	0.09369780	0.0284	0.8934
13	0.47440611	0.07999237	0.0237	0.9171
14	0.39441374	0.07641152	0.0197	0.9368
15	0.31800222	0.01415264	0.0159	0.9527
16	0.30384958	0.05269401	0.0152	0.9679

Eigenvectors

		Prin1	Prin2	Prin3	Prin4	Prin5	Prin6
SSize	SSize	0.357685	-.045381	0.351828	0.001559	0.078759	0.282245
ED	ED	0.128205	-.041140	-.407929	-.216894	0.307824	0.205695
COTCLR	COTCLR	0.023829	0.250878	-.123095	0.111892	0.383077	-.415071
HYP_CLR	HYPSCLR	-.046881	0.407394	0.022387	0.047743	0.157474	0.055786
DTFLO	DTFLO	-.122036	0.036132	-.041318	0.552938	-.091373	0.083902
CLRSTD	CLRSTD	0.226508	0.408802	-.224681	-.130149	-.004533	0.224212
CLRWG	CLRWG	0.206156	0.419782	-.211027	-.102195	0.009494	0.177083
Gr__H_	Gr# H#	-.308251	0.147133	0.260329	0.151021	0.067303	-.115778
LFL	LFL	0.231566	-.084371	-.217518	0.406384	0.008172	-.113816
PDCLR	PDCLR	-.048315	-.254763	0.013592	-.005589	0.459207	0.027835
PDLG	PDLG	0.301933	0.071664	0.114670	0.412610	0.059124	-.036165
PDXSC	PDXSC	-.155687	-.010337	0.266941	-.047906	0.367497	-.099663
PDCUV	PDCUV	-.144680	-.200223	0.106516	-.045049	0.135244	0.513609
No__Pod	NoofPod	-.233284	0.271502	0.234537	0.106388	0.113995	0.141533
Loc_Pod	Loc/Pod	-.170795	0.364999	0.117503	-.103215	-.282797	0.027045
SDCTC	SDCTC	0.377099	0.058649	0.111295	0.173986	0.050613	-.155412
BRLSD	BRLSD	0.225912	-.170829	0.117650	-.070911	-.455668	-.086432
SDSHP	SDSHP	0.164074	0.174686	0.447831	-.004320	0.005254	0.195386
SCP	SCP	0.058932	0.084315	0.170493	-.401861	-.012920	-.467818
100Ws	100Ws	0.386813	-.080686	0.248497	-.153924	0.208252	-.048782